Tamarillo: potential high value nutrition ingredient for functional foods with yoghurt as an example

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Declaration

"I declare that the intellectual content of this thesis is my own work. This thesis does not contain materials previously published or written by another person, nor material which to a substantial extent has been accepted for any degree or diploma at AUT or any other educational institution, except where except where due to reference or acknowledgement is made explicitly in the text. Any contribution made to the research by others, with whom I have worked at AUT or elsewhere, is explicitly acknowledged in the thesis. I also declare that I have permission from journals for reusing my own full articles in this thesis."

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Date .....14 March 2022.....

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Abstract

Tamarillo is a fruit that contains diverse nutrients, antioxidants and anthocyanins; bioactive compounds with potential for well-being, though research about development of tamarillo-derived foods or exploration of the potential of tamarillo as a functional ingredient is scarce. Three tamarillo cultivars grown in New Zealand (NZ) include the 'Amber', 'Laird's Large' and 'Mulligan' varieties. Tamarillo pulp and skin were freeze-dried and powdered to increase shelf-life and deactivate enzymes allow testing. This project, therefore, aimed to explore the physicochemical characteristics and potential for health benefits of the New Zealand grown tamarillo (Part I) and to formulate and test the properties, bioactivities and digestibility of yoghurts fortified with tamarillo powder and cubosome-encapsulated tamarillo powder (Part II).

From literature, the nutritional adequacy scores of tamarillo has been calculated as 7.9 and 7.4 for gold and red varieties, respectively. The pulp of three NZ tamarillo cultivars all contained ~3% dietary fibre which is higher than other fruits (peach, banana, mango, orange, grape and pineapple) as well as high contents of γ-aminobutyric acid (GABA) which are relatively similar to tomato, one of the greatest sources of GABA. Compared with standard serves of common New Zealand fruits, tamarillos had high α-tocopherol (16-23% AI/serve) and ascorbic acid (67-75% RDI/serve) as well as the highest β-carotene (9-20% RDI/serve). Tamarillos were rich sources of phenolics including anthocyanins and possessed high antioxidant activity. Chlorogenic acid was the most dominant phenolic compound in both peel and pulp of three cultivars, while the main anthocyanin in pulp was delphinidin 3-rutinoside. Tamarillo pulp showed higher total anthocyanins compared to the pulp of strawberry, grape and cranberry fruits. Antioxidant activity of tamarillo exhibited relatively higher values than apples, oranges, red grapes, kiwi fruit, pineapple and were strongly correlated with high total phenolic content. A total of 121 volatiles were found in peel and pulp of three cultivars and principal component analysis clearly showed obvious separation among volatile profiles of different cultivars and tissues of tamarillo. Fifteen volatile compounds had relative odour activity values greater than 1 which means that it could be detected by the human nose, and the key contributor to the overall smell of tamarillo was methional being characterized by tomato-like flavour notes. This could help explain the original name of tree tomato.

The results from Part I showed that tamarillo fruit has the potential to be a functional food or a functional ingredient for reformulation of foods such as yoghurt. In part II, yoghurts fortified with tamarillo powder made from pulp were produced either pre- or post-fermentation process. Evidence was produced that tamarillo yoghurt offers the potential for the development of a high-value nutrition product that could be a good dietary source of vitamin C and a source of vitamin E and β-carotene and maintained the volatiles that give tamarillo its distinctive odour and flavour. Addition of tamarillo powder both before and after fermentation increased the acidity, fibre, protein and lactic acid contents of yoghurts. Pre-fermentation produced higher consistency and α-tocopherol concentrations of yoghurt than post-fermentation. Higher elastic modulus, polyunsaturated fatty acids, pro-vitamin A content and retention of vitamin C were observed for post-fermentation samples than pre-fermentation samples. High nutrient contents in terms of essential amino acids, GABA and phenols were observed in yoghurts fortified with tamarillo powder. Addition of tamarillo powder led to a dose-dependent increase in free amino acid content, especially GABA as well as the total essential amino acids of yoghurts in which the pre-fermentation samples showed higher amount of total free amino acids and total essential free amino acids than the post-fermentation ones. The antioxidant capacity of fortified yoghurts from both fermentation processes increased under the influence of *in vitro* intestinal digestion, probably due to chemical changes of the polyphenols and presence of bioactive amino acids or peptides. Tamarillo bioactives could be effectively encapsulated by cubosome nanoparticles with encapsulation efficiency for most polyphenols was over 50%. Compared to the unencapsulated extract, cubosomal encapsulation provided a protective effect on the tamarillo phenols under simulated gastrointestinal conditions, exhibiting good and sustained release characteristics. The encapsulated tamarillo phenols were almost completely released in alkaline pH conditions (intestinal phase). It was observed that stability of phenols in yoghurt samples showed great potentials after *in vitro* digestion regarding total phenolics and antioxidant capacity. In addition, each individual bioactive compound of yoghurts in cubosomal system showed the good stability after *in vitro* digestion. Cubosomes can, therefore, be considered for use to increase the bioavailability of the unique constituents of tamarillo and for use in functional yoghurts. Outcomes in the present study could also have implications for industry to produce value-added yoghurts which are as an important source of dietary bioaccessible phenols. Further work is required to assess the acceptability of these

yoghurts to the consumer, the cost of production and viability of sales of the product, shelf life as well as the interaction between fibres and stater culture (viability, total colony counts). Also, interaction between yoghurt components (mainly protein), starter culture and encapsulated bioactives should be evaluated in the future.

Publications

The published papers based on my PhD thesis are listed below. All these publications are in international peer-reviewed journals.

Peer-reviewed journal papers:

1. ***Diep, T. T.***, Pook, C., & Yoo, M. J. Y. (2020). Physicochemical properties and proximate composition of tamarillo (*Solanum betaceum* Cav.) fruits from New Zealand. *Journal of Food Composition and Analysis*, 103563. **IF: 4.556 – Chapter 2.**

2. ***Diep, T. T.***, Pook, C., Rush, E. C., & Yoo, M. J. Y. (2020). Quantification of Carotenoids, α-Tocopherol, and Ascorbic Acid in Amber, Mulligan, and Laird's Large Cultivars of New Zealand Tamarillos (*Solanum betaceum* Cav.). *Foods*, *9*(6), 769. **IF 4.350 – Chapter 2.**

3. ***Diep, T.***, Pook, C., & Yoo, M. (2020). Phenolic and Anthocyanin Compounds and Antioxidant Activity of Tamarillo (*Solanum betaceum* Cav.). *Antioxidants*, *9*(2), 169. **IF 6.312 – Chapter 2.**

4. ***Diep, T. T.***, Yoo, M. J. Y., & Rush, E. C. (2022). Effect of tamarillo fortification and fermentation process on physicochemical properties and nutrient and volatiles content of yoghurt. *Foods*, *11*(1), 79. **IF 4.350 – Chapter 4.**

5. ***Diep, T. T.***, Yoo, M. J. Y., & Rush, E. (2022). Effect of In Vitro Gastrointestinal Digestion on Amino Acids, Polyphenols and Antioxidant Capacity of Tamarillo Yoghurts. *International Journal of Molecular Sciences*, *23*(5), 2526. **IF 5.924 – Chapter 5.**

6. ***Diep, T. T.***, Yoo, M. J. Y., & Rush, E. C. (2022). Cubosome encapsulation of tamarillo extract, fortification of yoghurt and effects of *in vitro* digestion. *Antioxidants, 11*(3), *520.* **IF 6.312 – Chapter 7.**

Publications listed above have been published by the journals. The author of this thesis contributed at least 80% to these manuscripts.

Tung Diep Michelle Yoo Elaine Rush Chris Pook

Other publications based on PhD project:

1. ***Diep, T. T.***, Rush, E. C., & Yoo, M. J. Y. (2020). Tamarillo (*Solanum betaceum* Cav.): A Review of Physicochemical and Bioactive Properties and Potential Applications. *Food Reviews International*, 1-25. **IF 6.478 – author of this thesis contributed 60%.**
2. ***Diep, T. T.***, Yoo, M. J. Y., Pook, C., Sadooghy-Saraby, S., Gite, A., & Rush, E. (2021). Volatile Components and Preliminary Antibacterial Activity of Tamarillo (*Solanum betaceum* Cav.). *Foods*, *10*(9), 2212. **IF 4.350.**

Other publication related to tamarillo:

1. Pook, C., ***Diep, T. T.***, & Yoo, M. J. Y. (2022). Simultaneous Quantification of Organic Acids in Tamarillo (*Solanum betaceum*) and Untargeted Chemotyping Using Methyl Chloroformate Derivatisation and GC-MS. *Molecules*, *27*(4), 1314. **IF 4.412.**

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List of Abbreviations

Abs	Absorbance
Accutag	6-aminoquinolyl-N-hydroxysuccinimidyl carbamate
AI	Adequate Intake
ANOVA	Analysis of variance
APCI	Atmospheric pressure chemical ionisation
C	Celsius
CUB	Cubosomes
CUPRAC	Cupric ion Reducing Antioxidant Capacity
CUBTAM	Tamarillo polyphenol loaded-cubosomes
DAD	Diode array detector
DLS	Dynamic light scattering
DMSO	Dimethyl sulfoxide
DW	Dry weight
ESI	Electrospray ionization
FAA	Free amino acid
FFA	Free fatty acid
FLD	Fluorescence detection
FRAP	Ferric reducing antioxidant power
FW	Fresh weight
g	Gram
GABA	γ-Amino-n-butyric acid
GAE	Gallic acid equivalent
GC-MS/MS	Gas chromatography-mass spectrometry/mass spectrometry
h	Hour
kDa	Kilo Dalton
LC-MS/MS	Liquid chromatography-mass spectrometry/mass spectrometry
LLC	Lyotropic liquid crystals
LOQ	Limit of quantitation
LOD	Limit of detection
M	Molar
m	Micro
MCF	Methyl chloroformate

MeCN	Acetonitrile
MeOH	Methanol
min	Minute
mL	Milliliter
MMI	Multimode inlet
MRM	Multiple reaction monitoring
MW	Molecular weight
m/z	mass-to-charge ratio
NaOH	Sodium hydroxide
OAV	Odour activity value
PCA	Principal component analysis
PMP	Phenylmethylpyrazolone
PLM	Polarized light microscopy
RCF	Relative centrifugal force
RDA	Recommended daily allowance
RDI	Recommended dietary intake
RPM	Revolutions Per Minute
RT	Retention time
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	Scanning electron microscopy
SPME	Solid-phase microextraction
SSF	Simulated Salivary Fluid
SGF	Simulated Gastric Fluid
SIF	Simulated Intestinal Fluid
TBP	Tetrabromophenyl porphyrin
TD	Thermal desorption
TEAC	Trolox equivalent antioxidant capacity
TPC	Total Phenolic Content
TPTZ	2,4,6-Tri(2-pyridyl)-s-triazine
Trolox	6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid
TSS	Total soluble solids
V	Volt

W	Watt

Chapter 1: Introduction

1.1 Background of research

Globally chronic nutrition-related non-communicable diseases such as type 2 diabetes mellitus, cardiovascular disease, obesity and some cancers impact on public health and increase the burden on the health care systems (Benziger, Roth, & Moran, 2016). To cope with these chronic disorders, medical service should not be the only strategy. Therefore, the consumption of healthy foods and nutraceuticals with disease-preventing or minimizing functions should play a major role in national food strategies and added-value foods produced by the food industry (Bigliardi & Galati, 2013). Subsequently, the production of sustainable, nutrient dense foods to provide essential nutrients and diverse foods that have health-benefits is increasing. In recent years, interest in fruit-based foods that contain high levels of phytochemicals has increased substantially (Sun-Waterhouse, 2011). Liu (2013) has defined phytochemicals as bioactive non-nutrient plant compounds in fruits, vegetables, whole grains, and other plant foods that have been hypothesized to reduce the risk of major chronic diseases.

Phytochemicals present in dried fruit have potential to contribute bioactive properties to functional foods, nutraceuticals and pharmaceutical products, and provide various opportunities for adding value in the food production and manufacturing chains (Chang, Alasalvar, & Shahidi, 2016). In addition, drying of fruits increases shelf-life and reduces food waste. Over the last few decades, numerous projects have been implemented in this area and, subsequently, a lot of food components, such as dietary fibre, carotenoids, phenolics including anthocyanins have been explored to supply a variety of biological activities that may have beneficial effects on human health (Akin, Temelli, & Köseoğlu, 2012).

In the literature, an increasing number of publications year by year, report on functional roles of fruit-derived ingredients or fruit-based foods for disease prohibition and health promotion (Chang, Alasalvar, & Shahidi, 2019). According to the Ministry of Health, malnutrition in New Zealand is high. Two-thirds of adults in New Zealand are either overweight or obese (Ministry of Health, 2019b) and 40% live in food insecure households (Ministry of Health, 2019a). The proportion of the population who have micronutrient deficiencies (hidden hunger) is not known. Also, only half (54%) of the adult population meet the daily recommended intake of fruit (at least two

servings of fruit per day). Due to the change in lifestyle over the years, the consumption of fresh fruit has become a not-so-easy option for those who are busy, want to reduce food waste and do not have enough money to buy the food that they need. It would be advantageous to develop food products using ingredients derived from fruit in order to enhance the nutritional value and to make consumption of fruit easier and more accessible.

1.2 Rationale and significance of the study

In New Zealand, kiwifruit value-added fruit-based products such as kiwifruit ice cream (Sun-Waterhouse, Edmonds, Wadhwa, & Wibisono, 2013a), kiwifruit-banana smoothie (Sun-Waterhouse & Zhou, 2010) or kiwifruit extract-enhanced gluten-free bread (Sun-Waterhouse et al., 2009) have been researched in depth. However, despite an extensive literature search, very little use has been made of tamarillo despite it containing similar or higher concentrations of nutrients particularly vitamin C, dietary fibre and β-carotene. According to Skinner and Hunter (2013), tamarillo is a New Zealand grown fruit which owns a wide range of functional bioactives that may benefit the human health. New Zealand is one of the leading producers and exporters of tamarillo with the yield of approximately 450 tonnes per year (Aitken & Warrington, 2018). Tamarillo has a long production season, and it is available from March through to November. It is eaten fresh, cooked in jams, pickles and sauces, blended with milk or water, prepared as pickles and chutney or consumed as part of salads (Ghosal, Chhetri, Ghosh, & Mandal, 2013; Skinner & Hunter, 2013). Although New Zealand produces a high amount of tamarillo, only a few tamarillo products are available in the market, in the form of frozen or canned tamarillo, vinegar, puree and juice (Schotsmans, East, & Woolf, 2011).

There is a high consumption of fermented milk products in New Zealand. Anecdotally, tamarillo is not easy to eat due to its texture and strong flavour, hence an attempt to make new product from tamarillo with potential high-value-nutrition should be investigated. Numerous studies have reported that the attractive taste of yoghurt and its nutritional properties can be enhanced by addition of fruits (Coïsson, Travaglia, Piana, Capasso, & Arlorio, 2005; Karaaslan, Ozden, Vardin, & Turkoglu, 2011; Wallace & Giusti, 2008) that also meets the demand of consumers as well as guidelines

of the Ministry of Health for New Zealanders with at least 2 servings of fruit and 1 pottle of yoghurt every day. The development of new functional foods requires technologies for incorporating health-promoting compounds into matrices without reducing their bio-functionality, protecting them from degradation and maintaining their bioavailability. Martínez-Ballesta, Gil-Izquierdo, García-Viguera, and Domínguez-Perles (2018) stated that as a result of the gastrointestinal digestion, bioactive compounds from nutrients (bioactive peptides, minerals and vitamins) and non-nutrients (phytochemicals) can be degraded. Hence, encapsulation of bioactive compounds will limit general metabolism and degradation due to chemical and enzymatic impacts of gastrointestinal tract. As a result, an increase of absorption and bioavailability of bioactives is achieved.

Therefore, this study aims to evaluate the nutrient contents, especially bioactive compounds (polyphenols and related antioxidants) of tamarillo that may be beneficial for the human health. Characterisation of tamarillo will make more available information to health and nutrition experts as well as the food companies and export market. And then, there is reasonable potential for producing milk products from tamarillo such as yoghurts that provide opportunities for novel dairy products fortified with fruit-derived ingredients and health-promoting effect from tamarillo. Additionally, encapsulation of bioactives from tamarillo will be evaluated through stability and functionality of polyphenols in fortified yoghurt during *in vitro* digestion.

1.3 Research question (s)

In New Zealand, tamarillo (or tree tomato) is consumed mostly in the form of fresh fruit and in the months March to November. Tamarillo is highly nutritious known to be a source of dietary fibre, potassium, vitamins A, B6 and E and a good source of vitamin C, all bioactive compounds with potential for well-being (Ministry of Health & New Zealand Institute for Plant and Food Research Limited, 2021), though research about developing tamarillo-derived foods is still limited and as a functional ingredient, the potential of tamarillo remains largely unexplored. The overall questions asked in this thesis are:

- What are the main components and bioactive compounds in tamarillos that may benefit human health?

- What is the effect of fortification of yoghurt with dried tamarillo powder and the fermentation process on the physicochemical properties and nutrient and volatiles content of yoghurt?
- What are the effects of *in vitro* gastrointestinal digestion on amino acids, polyphenols and antioxidant capacity of tamarillo yoghurts?
- Does the encapsulation of tamarillo polyphenol extract enhance the stability and bio-accessibility of bioactive compounds in tamarillo yoghurt and maintain texture compared to yoghurt fortified with unencapsulated tamarillo polyphenol extract?

1.4 Objectives

Main objective of this thesis is to explore the nutrient and bioactive profile (polyphenols and related antioxidants) of New Zealand grown tamarillo and use yoghurt as a vehicle for oral delivery of these compounds which may have potential health benefits. Using natural sources in the development and formulation of yoghurt products will contribute to increase in research and industrial application of fruits. Figure 1.1 summarizes schematically the structure of this research including main aims, research questions and objectives. The project investigation designed with six objectives:

<u>Part I: Tamarillo fruit</u>

- Objective 1 – Chapter 2: To explore physicochemical properties, volatile compounds, bioactive compounds (phenolics, anthocyanins and antioxidant vitamins), and antioxidant activity in both endo- and mesocarp (pulp) and exocarp (peel) of ‘Amber’ (yellow), ‘Laird’s Large’ (red) and ‘Mulligan’ (purple-red) tamarillo cultivars.

<u>Part II: Yoghurt fortified with tamarillo</u>

- Objective 2 – Chapter 4: To investigate effect of fermentation process (before and after fermentation) and tamarillo powder concentrations (5%, 10% and 15%) on physicochemical properties, volatile component and nutritional value of tamarillo yoghurt.
- Objective 3 – Chapter 5: To determine amino acid and polyphenols components as well as antioxidant activity of tamarillo yoghurt.

- Objective 4 – Chapter 5: To investigate bioavailability and bio-accessibility of amino acids, major polyphenol classes including anthocyanins and antioxidant activity from tamarillo yoghurt using *in-vitro* digestion model.
- Objective 5 – Chapter 7: To explore potentials of liquid crystalline nanoparticles (cubosomes) to encapsulate polyphenols from tamarillo through identification of encapsulation yield.
- Objective 6 – Chapter 7: To investigate *in-vitro* gastrointestinal behaviour of yoghurt enriched with tamarillo bioactive-loaded nanoparticles and comparing with yoghurts fortified only with tamarillo powder.

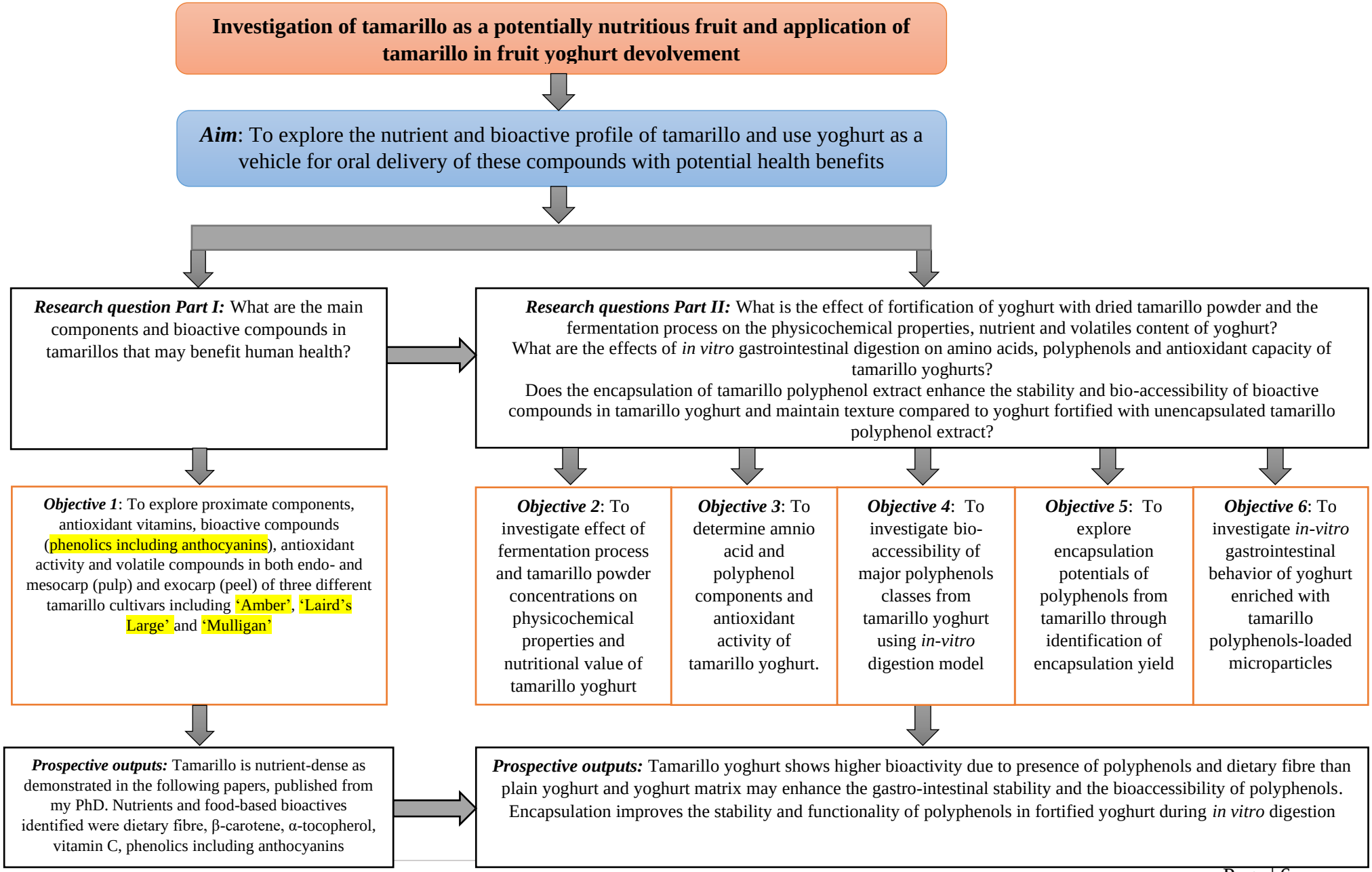

Figure 1.1 General workflow of the project.

1.5 Outline of thesis

To achieve the overall aims of this thesis, two main parts were conducted together with a general introduction (Chapter 1). Part I or Chapter 2 aims to investigate compositions, nutritional value and health benefits of tamarillo. This part is started with a review of the relevant literature focusing on publications in the last two decades about tamarillo such as physiochemical composition, volatiles, bioactive compounds, health benefits and application. Then, all proximate compositions and physiochemical properties of tamarillo were presented. The following section applied LC-MS to quantify dietary antioxidant sources including β-carotene, ascorbic acid, α-tocopherol and carotenoids as well as phenolics including anthocyanins content. The last section of this chapter investigated the volatiles contents by using advanced technique (thermal desorption unit) coupled with GC-MS.

In Part II, application of tamarillo in fruit yoghurt devolvement is extensively addressed. Chapter 3 follows with the review of literature concerning fruit-fortified yoghurts. Properties of tamarillo yoghurt including proximate components, flavour, rheology, texture and nutritional values affected by the concentration of tamarillo powder added and if the addition is pre or post the fermentation are reported in Chapter 4. Chapter 5 describes during *in vitro* digestion of tamarillo yoghurts how protein is digested to amino acids and bioactive compounds (polyphenols), antioxidant activity and bioactives are released. What is known about cubosome encapsulation of bioactive compounds and the use of bioactive-loaded particles in food products is reviewed in Chapter 6. The final experimental chapter (chapter 7) reports cubosome encapsulation of tamarillo extract, fortification of yoghurt and effects of *in vitro* digestion. Finally, chapter 8 discusses the major findings and conclusions reached in this body of work and makes recommendations for future research.

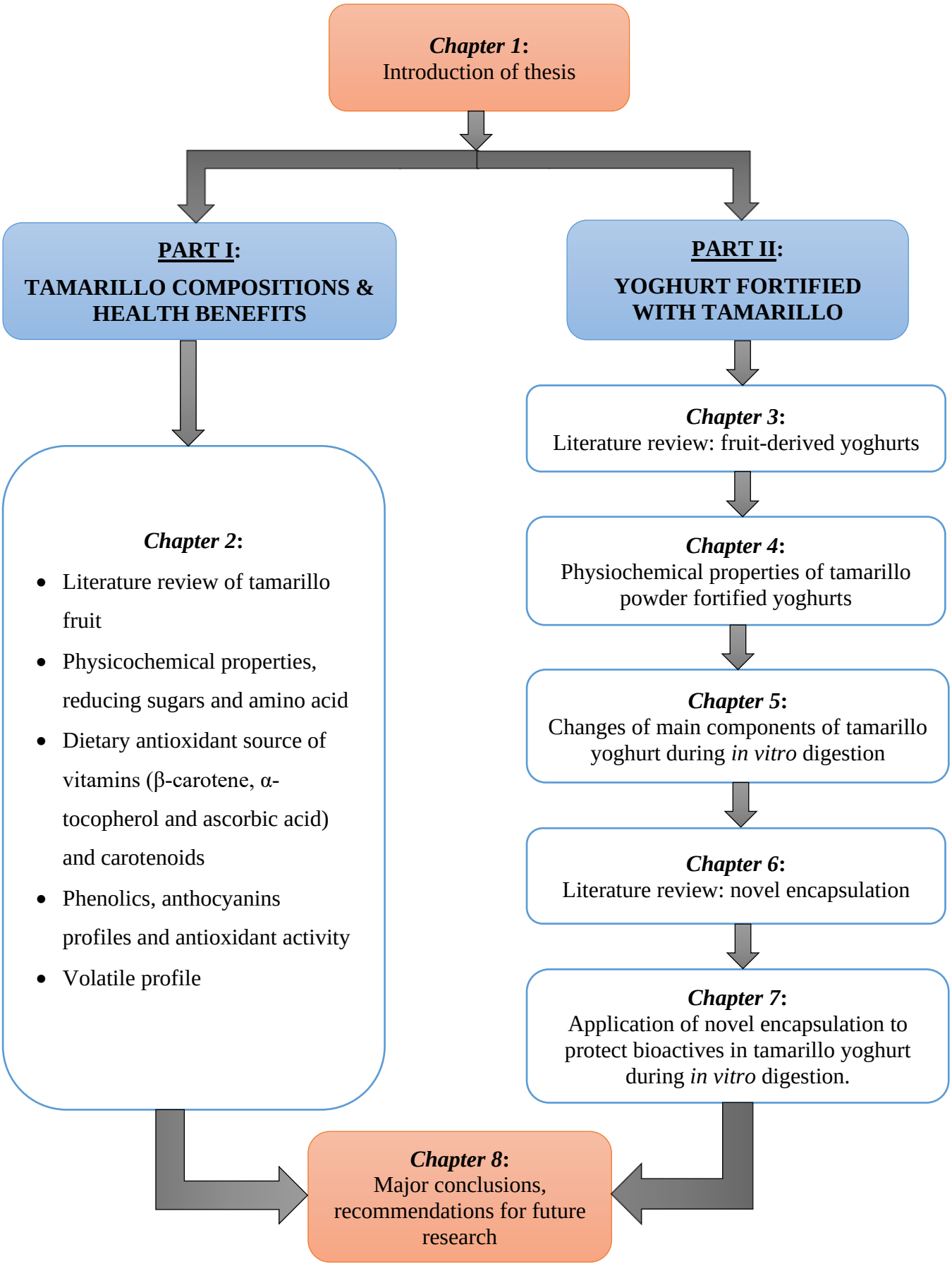

Figure 1.2 Outline of this research.

PART I:
TAMARILLO COMPOSITION
&
HEALTH BENEFITS

Chapter 2: Exploration of tamarillo (*Solanum betaceum* Cav.) as a nutrient and phytochemical dense fruit

This chapter is reproduced from three published papers: "Physicochemical properties and proximate composition of tamarillo (*Solanum betaceum* Cav.) fruits from New Zealand"; "Quantification of Carotenoids, α-Tocopherol, and Ascorbic Acid in Amber, Mulligan, and Laird's Large Cultivars of New Zealand Tamarillos (*Solanum betaceum* Cav.)" and "Phenolic and Anthocyanin Compounds and Antioxidant Activity of Tamarillo (*Solanum betaceum* Cav.)" published in Journal of Food Composition and Analysis; Foods and Antioxidants, respectively with permission.

This chapter summarizes physiochemical compositions and bioactive properties of tamarillo from literature as well as presents main findings about proximate compositions, bioactive compounds, antioxidant vitamins and antioxidant activity of three tamarillo cultivars ('Amber, Laird's Large and Mulligan') from New Zealand which have not been explored in previous studies. Tamarillo (*Solanum betaceum* Cav.) is a sub-tropical fruit with unique flavor and color, known to be highly nutritious. Tamarillo has nutrient adequacy scores ranging from 7.4 (red type) to 7.9 (gold type). The pulp of all three New Zealand cultivars was approximately 3% of dietary fibre and contained a relatively high content of GABA which is similar to tomato (*Solanum lycoperscium*). Highest β-carotene (9-20% RDI/serve), high ascorbic acid (67-75% RDI/serve) and α-tocopherol (16-23% AI/serve) were observed in tamarillos when comparing to standard serves of common New Zealand fruits. The most dominant phenolic compound in both peel and pulp of all cultivars was chlorogenic acid. Delphinidin 3-rutinoside was the main anthocyanin in pulp of all cultivars, whereas cyanidin 3-rutinoside dominated both 'Laird's Large' and 'Mulligan' peels. A higher antioxidant activity determined by CUPRAC and FRAP assays than kiwifruit was explored, although a lesser amount of phenolics were the present. A total of 121 volatile compounds were detected in peel and pulp of tamarillo using an advanced technique (thermal desorption – TD) coupled with GC-MS. The main contributor to the overall flavour of lyophilized tamarillo was methional, regardless of the cultivars and tissues.

This chapter is organized as follows: Section 2.1 is intended to provide a valuable source for knowledge about tamarillo based on current studies and to discuss some

suggestions for future investigations. Materials including tamarillo samples preparation and the experimental works are given in Section 2.2. Proximate compositions and physiochemical properties in three tamarillo cultivars being grown in New Zealand are given in Section 2.3. Section 2.4 and 2.5 describe antioxidant vitamins compositions as well as main bioactive compounds and antioxidant activity of tamarillos, respectively. Section 2.6 characterises volatile component of 'Amber', 'Laird's Large' and 'Mulligan' cultivars, separated into peel and pulp, using TD-GC-MS. Finally, a brief summary of potential applications of this fruit is given in Section 2.7.

2.1 Literature review: what is known about tamarillo fruit as a food

2.1.1 Introduction

Tamarillo, also known as tree tomato (*Solanum betacea* or *Cyphomandra betacea* Cav.), is categorised in the family *Solanaceae*, genus *Solanum* together with tomato, capsicum and eggplant (Figure 2.1) (Bohs, 1995). The egg-shaped fruit has purple-red to golden-yellow skin and small seeds (Romero-Rodriguez, Vazquez-Oderiz, Lopez-Hernandez, & Simal-Lozano, 1994). The exact origin of this fruit is unknown, but wild cultivars exist in South American countries including Bolivia, Chile, Ecuador and Peru (Morton, 1987). In the late 19th century, the fruit was globally introduced to Oceania (Australia and New Zealand), South-East Asia (India, Malaysia, Thailand, Indonesia and Vietnam), Europe (Italy, Germany, Spain, Portugal, France and Netherlands) as well as Africa (South Africa, Uganda and Rwanda) (Bohs, 1995; Lim, 2013; Morton, 1987; Prohens & Nuez, 2001). At present, only three countries: Australia, Colombia and New Zealand commercially grow tamarillo (New Zealand Horticulture Export Authority).

In New Zealand, the yellow and purple cultivars were developed by Hay & Sons in the late 1800's (Morton, 1987), and the purple-red type was developed by an Auckland nurseryman from South America around 1920 (Prohens & Nuez, 2001). In 1967, the New Zealand name "tamarillo" was coined by combing "tama", a Maori word with meaning of leadership, and "rillo", a Spanish word. The new name "tamarillo" was more marketable than tree tomato, the original name (Hewett, 1993). According to Schotsmans et al. (2011), tamarillo successfully grew in New Zealand through

improvements in species cultivation and storage conditions. As a sub-tropical fruit, tamarillo is mainly grown in warmer and sheltered area of North Island of New Zealand, producing 100 tonnes of tamarillo from about 100 ha, with a value of $2.4 million in domestic sales and $100,000 in export sales from New Zealand (Aitken & Hewett, 2016). As a new crop, New Zealand has been recognised as the world' largest producer of tamarillo (Acosta-Quezada, Martínez-Laborde, & Prohens, 2011). America, Australia, Hong Kong, Singapore, Japan and Pacific Islands are the main export markets for tamarillo from New Zealand (Schotsmans et al., 2011).

Figure 2.1 Tamarillo plants and fruits (Source: New Zealand Tamarillo Growers Association).

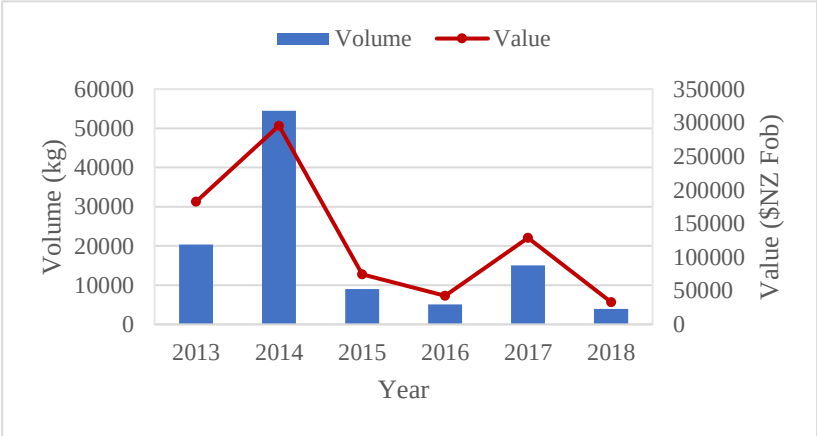

Figure 2.2 Tamarillo Export Statistics from 2013 to 2018 (Source: Statistics New Zealand).

Table 2.1 Tamarillo export market volume and value from 2014 to 2016 (year ending June, tonnes and $NZ FOB) (Source: Statistics New Zealand).

Market	2014		2015		2016	
	Volume	Value	Volume	Value	Volume	Value

USA	12	100,036	11	74,318	8	60,475
Pacific Islands	0.01	98	0	0	0.01	52
Australia	42	182,239	0	0	0	0
Japan	0.05	753	0	0	0	0
Fiji	0	0	0.01	80	0	0
New Caledonia	0	0	0.002	19	0	0
Thailand	4	12,392	0	0	0	0
Total	**59**	**$295,518**	**11**	**$74,417**	**8**	**$60,52**

2.1.2 Cultivars of tamarillo

Tamarillo cultivars are broadly distinguished by the colours of purple, red and yellow (Figure 2.3). These three tamarillo cultivars have similar nutrient profiles and a Brix level between 9.3 and 13.6 but differ in anthocyanin content, which is the highest in the red and purple types (Vasco, Ruales, & Kamal-Eldin, 2008). The oval-shaped purple pulp and vivid dark red peel cultivar is also recognized as dark-red or black with the weight of 60 to 100 g. 'Holmes', 'Kaitaia', 'Rothamer', 'Ruby Red' (Prohens & Nuez, 2001) and 'Mulligan' are cultivars of purple colour.

The red cultivar is the most commonly available type with the weight of 50 to 80 g. This is the most common grown cultivar in New Zealand. Owning dark red pigmentation around the seeds and deep red skin, this tamarillo type is preferred for fresh consumption due to stronger and more acid flavour. Various cultivars within the red group include 'Andys Sweet Red', 'Ecuadorian Orange', 'Oratia Red', 'Secombes Red', 'Solid Gold', 'Red Beam', 'Red Beau', 'Red Delight' and 'Laird's Large'. In New Zealand, the red type accounts for over 80% of exports. It is known as an excellent source of antioxidants and full of natural vitamins and minerals (Prohens & Nuez, 2001; Schotsmans et al., 2011).

Yellow type has a mild flavour with bright yellow skin and the weight of 50 to 70 g. This fruit has less acidic taste which is suitable for canning or making preserves. Cultivars in yellow type include 'Egmont Gold', 'Goldmine', 'Inca Gold' (Prohens & Nuez, 2001; Schotsmans et al., 2011) and 'Amber'.

Figure 2.3 Three tamarillo cultivars: yellow (left), red (centre) and purple (right). (Source: https://www.edible.co.nz/varieties.php?fruitid=14_Tamarillo)

2.1.3 Nutritional compositions

The sugar and acid concentrations also vary depending on the cultivars. Acosta-Quezada et al. (2015) reported that the concentrations of glucose and fructose were relatively similar, and these were lower than sucrose concentration within each tamarillo cultivar (orange, orange pointed, red, red conical and purple) (Figure 2.4A). As with both individual sugars and total sugars, the orange groups had higher values than the red and purple groups. The global means of glucose, fructose, sucrose and the total sugars in tamarillo were reported as 9.1, 9.3, 23.5 and 41.9 g/100 g dry weight (DW), respectively (Acosta-Quezada et al., 2015). Also, the concentrations of mannose, ribose, rhamnose, galactose, xylose, arabinose and uronic acids had been reported in Malaysian tamarillo puree (Gannasin, Adzahan, Hamzah, Mustafa, & Muhammad, 2015a) and in tamarillo mucilage from Brazil (Do Nascimento, Iacomini, & Cordeiro, 2016a) (except for ribose).

According to Ramirez and Kallarackal (2019), there is a decrease of the organic acids in tamarillo over the 25 weeks of development. Malic and citric acids have been identified as two main organic acids in tamarillo, in which the average citric acid concentration was significantly higher than malic acid in most cultivars (Figure 2.4B) (Acosta-Quezada et al., 2015). The red and purple groups had a higher concentration of malic acid than the orange groups which could account for their less astringent flavor. For citric acid, the highest and the lowest average values were found in orange and red cultivars with 6.87 and 5.32 mg/100 g DW, respectively. The global mean of the sugars : acids ratio of tamarillo was 6.43 and this value varied from 5.72 (red conical) to 7.16 (red cultivar) depending on the cultivars (Acosta-Quezada et al., 2015). For example, the Secombes Red cultivar had a lower sugar : acid ratio than

Andys Sweet Red variety (6.6 compared with 10.1), while the Goldmine cultivar had an average ratio of sugar : acid of 6.9 (Boyes & Strübi, 1997).

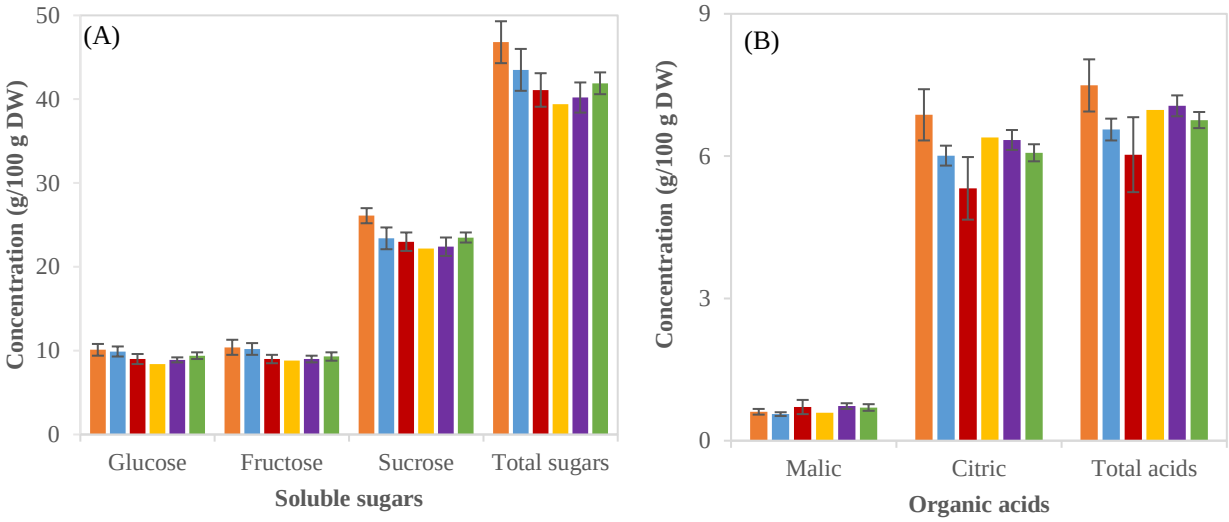

Figure 2.4 Concentrations of soluble sugars (A) and organic acids (B) in five different types of tamarillo.

Used with permission (Diep, Rush, & Yoo, 2020d)

Orange; Orange pointed; Red; Red conical; Purple; Global. Data was presented as mean (g/100 g DW) and error bar (standard deviation) ($n \geq 3$)

Regular consumption of dietary fibre in fruit, vegetables and whole grains is known to reduce blood cholesterol, reduce hyperglycaemia and improve the health of the microbiome and laxation. Tamarillo has significant amount of dietary fibre (more than 4 g/100 g FW) (Vasco, Avila, Ruales, Svanberg, & Kamal-Eldin, 2009). The dietary fibre content of gold and red tamarillo cultivars from New Zealand was 3.1 and 3.6 g/100 g FW, respectively (Lister, Morrison, Kerkhofs, & Wright, 2005), which is higher than that of apple or even kiwifruit (Table 2.2) analysed in the same way. A recent report from the New Zealand Institute for Plant and Food Research has shown the dietary fibre of 3.2 – 3.3% in tamarillo, contributing to 11% of Daily Intakes (DI) (New Zealand Food Composition Database, 2019) (Table 2.2). Mutalib, Rahmat, Ali, Othman, and Ramasamy (2017) reported the total dietary fibre content in Malaysian tamarillo being 6.0 g/100 g edible portion.

Consumption of tamarillo has been reported to significantly contribute to the daily recommended intake of many vitamins and minerals (Table 2.2) (New Zealand Food Composition Database, 2019). The potassium contents in different tamarillo groups

(orange, orange pointed, red, red conical and purple) from different sources ranges are reported as from 1868 to 2789 mg/100 g DW, and the purple cultivar has the highest concentration of this mineral (Acosta-Quezada et al., 2015). Potassium in the red and gold tamarillos sourced from New Zealand was 321 – 495 and 292 – 450 mg/100 g FW, respectively (Lister et al., 2005; New Zealand Food Composition Database, 2019) and in purple-red and golden-yellow sourced from Ecuador, 379 and 398 mg/100 g FW, was found, respectively (Vasco et al., 2009). Malaysian tamarillo also contained an average potassium concentration of 410 mg/100 g edible portion (Mutalib et al., 2017). The concentration of potassium in tamarillo have been reported to be similar or even higher than that reported in banana, with 358 mg/100 g edible portion (USDA, 2018). Potassium is vital for the proper function of nervous cells. Also, tamarillo is a source of the essential minerals: copper, manganese and magnesium which are involved as cofactors in numerous metabolic pathways. Lister et al. (2005) showed single serve of tamarillo (approximately 60 g) can satisfy the recommended daily intake (RDI) of copper (6.5 % for both gold and red tamarillo), manganese (6.5 and 5.0 % for gold and red tamarillos, respectively) and potassium (7.2 and 8.0 % for gold and red tamarillos, respectively).

Ascorbic acid (known as Vitamin C) is a well-known antioxidant and contributes towards production of collagen and increases the absorption of iron. The concentration of ascorbic acid in both purple-red and golden-yellow tamarillos from Ecuador ranged from 16 to 24 mg/100 g FW (Vasco et al., 2008). Higher content was found in gold (24.7 and 31 mg/100 g FW) and red (34.3 and 29.8 mg/100 g FW) tamarillo cultivars from New Zealand, as identified by Lister et al. (2005) and by New Zealand Food Composition Database (2019), respectively. Ordóñez, Cardozo, Zampini, and Isla (2009) analysed the concentrations of vitamin C in different extractive forms of tamarillo from Argentina. They reported the average ascorbic acid contents in fruit, maceration, decoction, juice and pomace were 153, 48, 26, 15 and 13 mg/100 g FW, respectively. These values were converted from the reported values of 1.53, 0.48, 0.26, 0.15 and 0.13 mg/g FW, respectively. The vitamin C concentration in Malaysian tamarillo had also been determined by Mutalib et al. (2017) with 55.9 mg/100 g DW. According to Lim (2013), the concentration of vitamin C was 4.5 times higher in the tamarillo seed jelly than in the pulp. Despite containing a similar amount of vitamin C to orange, tamarillo showed remarkably higher total oxygen scavenging capacity

(TOSC) value against peroxyl radicals than the orange (Gordon, Rodrigues, Marx, & Papagiannopoulos, 2007). The vitamin C, known as natural free radical scavenger, also illustrated a moderate antioxidant activity against peroxynitrite.

The concentrations of pyridoxine or vitamin B_6 identified in gold and red tamarillos from New Zealand were 0.38 – 0.52 and 0.2 – 0.58 mg/100 g FW, respectively (Lister et al., 2005; New Zealand Food Composition Database, 2019). This vitamin plays an important role in supporting the nervous function, generating red blood cells, protein digestion as well as enhancing the immune system to against several diseases (Intakes, 2005). In gold and red tamarillo cultivars from New Zealand, 1.9 – 3.5 and 1.8 – 1.94 mg of vitamin E per 100 g FW were found, respectively (Lister et al., 2005; New Zealand Food Composition Database, 2019) which were higher than the vitamin E content in other fruits (Table 2.2). In this group, α-tocopherol as an antioxidant may have the ability to reduce the risk of several cancers and heart disease (Lister et al., 2005). Another fat-soluble vitamin, vitamin A, had been determined in Malaysian tamarillo with the content of 4.8 mg/100 g DW (Mutalib et al., 2017). Compared with kiwifruit, tomato, orange and apple, tamarillo had a significantly higher concentration of vitamin A (Table 2.2). This vitamin also owns antioxidant activity and is able to react with free radicals and peroxyl radicals. According to Lister et al. (2005), the % RDI values from a serving size of one gold tamarillo for vitamin A, B_6, C and E were 13.62, 18.91, 19.76 and 12.35, respectively, whereas theses values from red cultivar for vitamin A, B6, C and E were 13.15, 21.09, 27.44 and 6.35 %, respectively. Therefore, 60 g of tamarillo pulp (one fruit) can meet the RDI of these vitamins (FSANZ labelling).

Table 2.2 Compositions of yellow and red tamarillo cultivars from New Zealand (per 100 g FW) and comparison to other fruits

Used with permission (Diep et al., 2020d).

Component /100 g	Yellow tamarillo	Red tamarillo	Kiwifruit	Banana	Tomato	Orange	Apple	Strawberry
Moisture (%)	86.3	86.1	83.8	74.9	94.5	86.8	85.56	90.95
Energy (kJ)	139 (2 %)	165 (2 %)	241	371	74	197	218	136
Protein (g)	1.9 (4 %)	2 (4 %)	1.06	1.09	0.88	0.94	0.26	0.67
Fat (g)	0.5 (1 %)	0.4 (1 %)	0.44	0.33	0.2	0.12	0.17	0.3
Dietary fibre (g)	3.2 (11 %)	3.3 (11 %)	3.0	2.6	1.2	2.4	2.4	2.0
Available carbohydrate (g)	3.7 (1 %)	3.8 (1 %)	9.1	20.8	2.7	8.5	10.8	6.6
Total sugars (g)	3.4 (4 %)	3.5 (4 %)	9.0	12.2	2.63	8.5	10.5	6.5
Fructose (g)	0.9	0.9	4.35	4.85	–	–	–	2.44
Glucose (g)	0.8	0.8	4.11	4.98	–	–	–	1.99
Sucrose (g)	1.6	1.7	0.15	2.39	–	–	–	0.47
Vitamin A, retinol equivalent (µg)	127	190	4	3	42	11	3	1
Vitamin B_6 (mg)	0.38 (24 %)	0.2 (12 %)	0.063	0.4	0.046	0.06	0.041	0.047
Vitamin C (mg)	31 (78 %)	29.8 (74 %)	93	8.7	14	53.2	4.6	58.8
Vitamin E (mg)	1.9 (19 %)	1.94 (19 %)	1.46	0.1	0.54	0.18	0.18	0.29
Folate (µg)	4 (2 %)	4 (2 %)	25	20	15	30	3	24
Calcium (mg)	11 (1 %)	11 (1 %)	34	5	13	40	6	16
Copper (mg)	0.06 (2 %)	0.05 (2 %)	0.13	0.078	0.059	0.045	0.027	0.048
Iron (mg)	0.44 (4 %)	0.57 (5 %)	0.31	0.26	0.36	0.1	0.12	0.41
Magnesium (mg)	20 (6 %)	21 (6 %)	17	27	11	10	5	13
Manganese (mg)	0.185 (4 %)	0.114 (2 %)	0.098	0.27	0.114	0.025	0.035	0.386
Phosphorus (mg)	40 (4 %)	39 (4 %)	34	22	24	14	11	24
Potassium (mg)	292	321	312	358	237	181	107	154
Zinc (mg)	0.17 (1 %)	0.15 (1 %)	0.14	0.15	0.07	0.07	0.04	0.14
Total phenolics (mg GAE/100 g FW)[a]	117	191	258.55	120	425[b]	39	187	240
Total anthocyanins (mg/100 g FW)[a]	0	82	–	0	–	0	0	28-70
Antioxidant activity (µmol TEAC/100 g FW)[a]	1002	1659	800	64	–	874	500	1850

FW = Fresh Weight; GAE = Gallic acid equivalent; TEAC = Trolox equivalent antioxidant capacity

% DI (Daily Intakes) are shown in brackets

[a] Data retrieved from Lister et al. (2005)

[c] Data retrieved from Noor Atiqah, Maisarah, and Asmah (2014) and expressed as mg GAE/100 g edible portion on dry weight basis

2.1.4 Nutrient adequacy score

The nutritional adequacy score for tamarillo sourced from New Zealand were calculated based on the method of Darmon, Darmon, Maillot, and Drewnowski (2005) and nutritional values from Athar, McLaughlin, and Taylor (2003). The scores were 7.9 and 7.4 for gold and red cultivars, respectively (Table 2.3), which were relatively similar to orange (8.1) and strawberry (8.0) and even higher than lemon (7.0), banana (4.9), pomegranate (4.8) and blueberry (2.9) (Figure 2.5). These significant scores indicated that tamarillo was on the top of the nutritional value table meaning richness in nutrients. The nutrient richness of tamarillo mainly comes from the high amount of vitamin C, vitamin B_6, vitamin A, fibre and probably potassium. The combination of these attributes from tamarillo if regularly consumed would provide significant health benefits. The nutritional profile and nutritional adequacy score of fruit pulp shows the considerable potential of tamarillo to broaden fruit choice and to enhance nutritional status of expanding world population.

Table 2.3 Nutrient adequacy score of gold and red tamarillos from New Zealand.

Nutrient	Unit	DV[a]	Gold tamarillo[b]	Red tamarillo[b]
Protein	g	60	1.9	2
Fibre	g	25	3.2	3.3
Vitamin A	ug	700	127	190
Thiamin	mg	1.2	0.09	0.04
Riboflavin	mg	1.55	0.01	0.02
Niacin	mg	12.5	0.57	0.27
Pantothenic acid	mg	5	0.04	0.04
Vitamin B6	mg	1.65	0.38	0.2
Folate	ug	315	4	4
Vitamin B12	ug	2.4	0	0
Vitamin C	mg	110	31	29.8
Vitamin E	mg	12	1.9	1.94
Vitamin D	ug	5	0	0
Calcium	mg	900	11	11
Iron	mg	12.5	0.44	0.57
Magnesium	mg	390	20.4	21
Nutritional adequacy score			***7.9***	***7.4***

[a] DV = Frenche-recommended Daily Value

[b] Data retrieved from New Zealand Food Composition Database (2019)

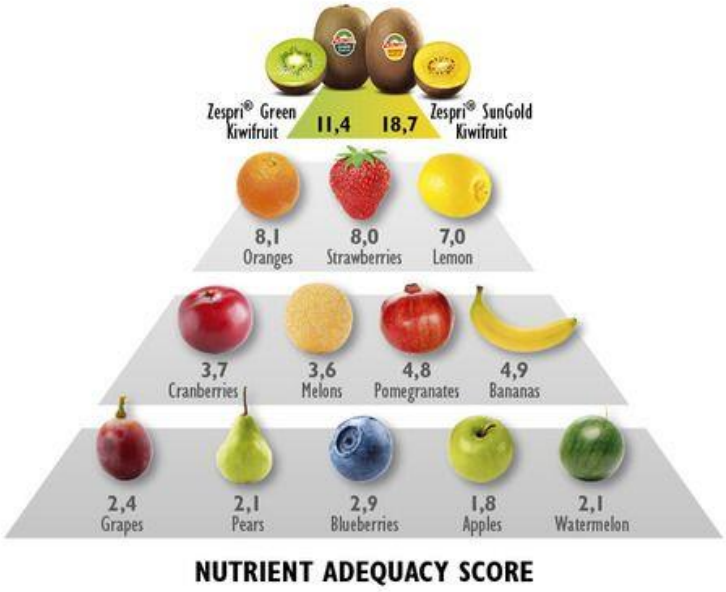

Figure 2.5 Nutrient adequacy score of common fruits.

(Source: https://www.zespri.eu/en/happy-healthy/nutrient-richness-kiwi)

2.1.5 Volatile profile

In red tamarillo sourced from Colombia, Torrado et al. (1995) determined 46 aroma compounds. Five dominant components were methyl hexanoate, eugenol, (E)-hex-2-enal, (Z)-hex-3-en-1-ol, and 4-allyl-2,6-dimethoxyphenol. From the results, a difference in volatile profile was found between tamarillo and other Solanaceae family fruits including tomato (*Solanum lycopersicum* L.) or lulo (*(Solanum vestissimum* D.). Methyl hexanoate, α-terpineol and 4-allyl-2,6-dimethoxyphenol were dominant compounds in volatile profile of tamarillo with the concentration of above 300 μg/kg (~ 30 μg/100 g) (Torrado et al., 1995) but were not detected in tomato (Buttery, Teranishi, Ling, & Turnbaugh, 1990) or lulo (Suárez et al., 1993). Eugenol was an abundant flavour compound in tamarillo (> 500 μg/kg ~ 50 μg/100 g), but it showed low content in lulo fruit (< 200 μg/kg ~ 20 μg/100 g) (Suárez et al., 1993) and not determined in tomato (Buttery et al., 1990). Butan-2-ol, methyl 3-hydroxybutanoate, α-terpineol and 4-terpineol were also determined (Torrado et al., 1995). Butan-2-ol is associated with sweet and apricot odour, while methyl hexanoate has fruity note and eugenol is characterised by sweet note. Both (E)-hex-2-enal and (Z)-hex-3-en-1-ol have green note.

In red Malaysian tamarillo, 49 volatile compounds were identified (Wong & Wong, 1997). Non-terpenoid alcohols constituted 44.7% in Malaysian tamarillo with the most dominant compound of (Z)-3-hexenol (26.6%), followed by (E)-3-hexenol (7.7%) and (E)-2-hexenal (7.3%). Esters consisted of 37.4% of the total flavour components with ethyl butyrate (14.8%) which were not detected in Columbian fruit, methyl butyrate (12.0%), methyl hexanoate (8.6%) and ethyl hexanoate (1.0%). Sixteen terpenoids and two phenolic compounds were also observed in tamarillo from Malaysia (Wong & Wong, 1997). Eugenol and 4-allyl-2,6-dimethoxyphenol were not detected in tamarillo from Malaysia but showed high amounts in tamarillo from Columbia with > 500 for eugenol and 300 – 500 μg/kg (~ 30 – 50 μg/100 g) for 4-allyl-2,6-dimethoxyphenol. Both ethyl butyrate and methyl butyrate are associated with fruity and juicy odour.

The volatile profiles with a total of 70 compounds of reddish-purple and the golden-yellow tamarillo cultivars from Panama studied by Durant et al. (2013) has shown that the flavour of golden-yellow cultivars was characterized by terpenoids (33%) and esters (32%) with the major components of α-terpineol, α-phellandren-8-ol, terpinene-4-ol, β-ionone, ρ-cymenene, methyl hexanoate, methyl octanoate, ethyl octanoate, ethyl hexanoate, ethyl benzoate, methyl eugenol, 2,6-nonadienal, decanal, 1,8-cineole, and naphthalene. Terpenoids (30%) and aromatics (29%) were strongly responsible for the organoleptic characteristics of the reddish-purple type with the main components of α-cedrol, α-phellandren-8-ol, β-ionone, nonanal, decanal, naphthalene, methyl hexanoate, ethyl hexanoate, ethyl butanoate and ethanol (Durant et al., 2013). α-terpineol was not observed in reddish-purple tamarillo although this was the main compound in the golden-yellow type with relative amount of 12.7%. Comparing to the previous work, methyl hexanoate, ethyl hexanoate and terpinene-4-ol were newly found in tamarillo fruit from Colombia, Malaysia and Panama. Methyl hexanoate showed significant contents of > 500 μg/kg (~ 50 μg/100 g), 258 μg/kg (~ 25.8 μg/100 g) (converted from the reported value of 8.6 %) and 46 – 84 mg/g (~ 4600 – 8400 mg/100 g) (converted from the reported value of 4.6 – 8.4 %) in tamarillo from Colombia, Malaysia and Panama, respectively. The concentrations or relative amount of ethyl hexanoate in Colombian, Malaysian and Panamanian grown tamarillos were < 100 μg/kg (~ 10 μg/100 g), 30 μg/kg (~ 30 μg/100 g) (converted from the reported value of 1.0 %) and 5.4 – 5.9 mg/g (converted from the reported value of 5.4 – 5.9 %),

respectively. Tamarillos from three countries contained terpinene-4-ol at concentration of < 100 μg/kg (~ 10 μg/100 g) (Colombia), 12 μg/kg (~ 12 μg/100 g) (converted from the reported value of 0.4 %) (Malaysia) and 16 – 29 mg/g (converted from the reported value of 1.6 – 2.9 %) (Panama). Both methyl hexanoate and ethyl hexanoate have fruity note, while terpinene-4-ol shows sweet odour.

Eleven odour-active volatile compounds had been detected in solvent-assisted flavour evaporation (SAFE) extract of yellow tamarillo from Colombia by Garcia, Prieto, Guevara, Malagon, and Osorio (2016). A combination of SAFE, gas chromatography-mass spectrometry (GC-MS), gas chromatography-olfactometry (GC-O) and aroma extract dilution analysis (AEDA), known as sensomics technique, were used in this investigation. The key volatile in Colombian yellow tree tomato were (*Z*)-3-hexenal, hexanal and ethyl butanoate with odour activity value (OAV) of 19061, 2262 and 1021, respectively (Garcia et al., 2016). The volatiles which were responsible for fruity, herbal-green and fresh-mint aromas of this cultivar were aliphatic esters (ethyl butanoate and methyl butanoate); C_6-aliphatic compounds ((*Z*)-3-hexenal and hexanal); and terpenols (1,8-cineole), respectively. This research also reported rosmarinic acid as a bitter compound in yellow tree tomato from Colombia. The concentration of rosmarinic acid in the fruit was 46.17 ± 1.20 mg/100 g DW, which was higher than the odour threshold (37.00 ± 1.25 mg/L). Hence, it would be perceived as the bitter residual taste (Garcia et al., 2016).

Although initial profiling of volatile compounds of tamarillo have been reported by several studies above, there is still limited data on flavour changes during fruit ripeness, on variability among different tamarillo sources, cultivars and tissues (peel and pulp). Therefore, further research should be carried out to identify factors affecting volatile compositions of tamarillo such as genotype, tissue, maturity stage, climatic environment, postharvest handling and storage. These factors should be optimised to maintain the characteristic flavour of tamarillo. Also, the volatile composition of tamarillo is complex and there is still a lack of validation method to profile volatile compounds. Application of some advanced methods such as GC-MS coupled with thermal desorption (TD) unit and/or Dynamic Headspace (DHS) could be used to analyse volatile compounds in tamarillo. Although these flavour compounds above are dominant in volatile profile of tamarillo, odour threshold and odour activity value (OAV) of these volatiles are relatively lacking. The OAV concept does help

understand what a consumer will be able to sense and indicates contribution of each volatile to the distinctive flavour of any food type. Hence, determination of odour threshold and odour activity value of volatiles in tamarillo should be carried out. From this, knowledge of tamarillo flavour compounds could be improved which would have wider acceptance from costumers and develop new markets for this fruit.

2.1.6 Bioactive compounds

Pulp of tamarillo fruit is a great source of phenolics, carotenoids and anthocyanins compounds (De Rosso & Mercadante, 2007; Mertz et al., 2009). Phytochemical and nutritional studies show the presence of various bioactive compounds in the tamarillo pulp. Bioactive components in tamarillo are complicated and different according to source, cultivar, environmental condition during fruit maturity, and extraction method. At the moment, approximately 42 – 48 bioactives have been reported in tamarillo, including 15 phenolics, 20 – 26 carotenoids and 7 anthocyanins. Extracts of tamarillo may be utilized to produce functional and colourful food products with polyphenols, fruit fibre and anthocyanins with potential health benefits.

Phenolics are found to be important bioactive group of tamarillo which may contribute to the antioxidant activity of this fruit. Eight hydroxycinnamic acids, 2 hydroxybenzoic acids, 3 phenolic glycosides, 1 flavonol and 1 flavanone, were determined in both yellow and purple-red cultivars of tamarillo though the concentration varied (Table 2.4). The major hydroxycinnamic acid in tamarillo from Ecuador and New Zealand was 3-*O*-caffeoylquinic acid, followed by rosmarinic acid (Espin et al., 2016; Mertz et al., 2009). Tamarillo sourced from New Zealand showed higher concentrations of hydroxycinnamic acids and phenolic glycosides than the tree tomato from Ecuador and Colombia (for rosmarinic acid). Hydroxycinnamic acids and rosmarinic acid have stronger free radical scavenging activity than ascorbic acid and tocopherol, respectively (Alamed, Chaiyasit, McClements, & Decker, 2009). For Malaysian tamarillo, caffeic, gallic and vanillic acids were identified with higher concentrations while p-coumaric, ferulic and trans-ferulic acids were determined with lower concentrations (Table 2.4) (Mutalib, Ali, Othman, Ramasamy, & Rahmat, 2016). Also, naringin (33.2 mg/100 g DW) and kaempferol (5 mg/100 g DW) were only detected in tamarillo from Malaysia. The concentration of naringin in Malaysian

tamarillo was significantly higher than that in candied shaddock (*Citrus grandis* Obseck) with 11.9 mg/100 g (Zhou, 2012). Mamdouh and Monira (2004) had reported the ability of this compound to enhance the immune system avoid tissues injury and disease. Phenolic compounds contribute to the colour of the fruit, and may possess biological, therapeutic and protective effects against inflammation, cardiovascular disease and metabolic dysfunction (De Rosso & Mercadante, 2007). Flavonol and flavanone compounds have been acknowledged to have significant anticancer activity toward cancer cells in breast, stomach, blood and liver (Joshi et al., 2011; Patil, Chidambara Murthy, Jayaprakasha, Chetti, & Patil, 2009). These studies provide convincing evidence that tamarillo is a good source of phenolic compounds with potential health benefits. However, the synergistic effects of phenolic compounds from tamarillo have not been described and requires further investigation.

According to Vasco et al. (2009), anthocyanins have been only present in the purple and red tamarillos (Table 2.5). Three anthocyanins in tree tomato from Brazil were detected by using HPLC-PDA-MS: delphinidin 3-rutinoside of 5.26 mg/100 g FW (~ 4.63 mg/100 g DW) (62%), pelargonidin 3-glucoside-5-rhamnoside with 2.67 mg/100 g FW (~ 2.35 mg/100 g DW) (31.5%) and cyanidin 3-rutinoside of 0.55 mg/100 g FW (~ 0.48 mg/100 g DW) (6.5%) (De Rosso & Mercadante, 2007). The total concentration of anthocyanins was 8.5 mg/100 g FW (~ 7.48 mg/100 g DW) (De Rosso & Mercadante, 2007). Also, four anthocyanins including cyanidin 3-rutinoside, delphinidin 3-glucosyl-rutinoside, delphinidin 3-rutinoside and pelargonidin 3-rutinoside were detected in Ecuadorian tamarillos with the total contents of 165.1 mg/100 g DW (Mertz et al., 2009) (Table 2.5). Using UV-Vis, HPLC, LC-MS and 1D/2D-NMR analysis, Osorio et al. (2012) investigated the novel anthocyanin, delphinidin 3-O-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside-3′-O-β-D-glucopyranoside, in tamarillo from Colombia as a minor component with concentration of 138.0 mg delphinidin 3-rutinoside /100 g anthocyanin-rich extracts. Other known anthocyanins including cyanidin 3-rutinoside, delphinidin 3-rutinoside and pelargonidin 3-rutinoside were also determined with the concentrations of 438, 4934 and 2276 mg delphinidin 3-rutinoside /100 g anthocyanin-rich extracts, respectively. The total contents of anthocyanins in Ecuadorian and New Zealand purple cultivars were 102.35 and 168.88 mg/100 g DW, respectively (Espin et al., 2016). The major anthocyanin in Ecuadorian tamarillo was pelargonidin 3-rutinoside

with the concentration of 115 mg/100 g DW (Mertz et al., 2009) or 78.07 mg/100 g DW (Espin et al., 2016) while delphinidin 3-rutinoside (87.43 mg/100 g DW) was dominant in the New Zealand cultivar (Espin et al., 2016) (Table 2.5). The other anthocyanins detected in tree tomato from Ecuador were cyanidin 3-rutinoside, delphinidin 3-rutinoside and delphinidin 3-glucosyl-rutinoside with concentrations of 12.1, 32.7 and 5.3 mg/100 g DW, respectively (Mertz et al., 2009). Reported by Espin et al. (2016), the concentrations of cyanidin 3-rutinoside and delphinidin 3-rutinoside in Ecuadorian tamarillo were 2.49 and 21.79 mg/100 g DW, respectively. Tamarillo from New Zealand also contained cyanidin 3-rutinoside (4.49 mg/100 g DW) and pelargonidin 3-rutinoside (76.96 mg/100 g DW) (Espin et al., 2016). Hurtado, Morales, Gonzalez-Miret, Escudero-Gilete, and Heredia (2009) concluded that the anthocyanins in peeling extract of Colombian tamarillo were more stable to pH changes than that in jelly extract. This could be explained by the greater polymeric anthocyanin content as well as potential presence of several colour stabilising compounds such as organic acids, phenolic acids, flavonols and flavanols in the peel of tamarillo (Hurtado et al., 2009). These authors also demonstrated greater antioxidant activity in aqueous solution (pH 5.2) of these anthocyanin rutinosides extracted from tamarillo when comparing to ascorbic acid.

To date, 20 – 26 carotenoids in both yellow and purple-red tree tomatoes with comparative difference due to the origin, variety of fruit and analytical methods have been explored (De Rosso & Mercadante, 2007; Mertz, Brat, Caris-Veyrat, & Gunata, 2010; Mertz et al., 2009; Vasco et al., 2009). The total carotenoid contents in tamarillo from Brazil, Australia and USA were 3.1 – 5.9, 1.5 and 0.8 – 1.4 mg/100 g FW, respectively (De Rosso & Mercadante, 2007; Homnava, Rogers, & Eitenmiller, 1990; Rodriguez-Amaya, Bobbio, & Bobbio, 1983; Wills, Lim, & Greenfield, 1986), whereas 1.77 and 1.71 mg/100 g FW were quantified for New Zealand gold and red tamarillos, respectively (Lister et al., 2005). The average carotenoid values of orange, orange pointed, red, red conical and purple were 3.95, 4.7, 3.93, 7.35, and 5.94 mg/100 g DW, respectively (Acosta-Quezada et al., 2015). The global mean of carotenoid content in tamarillo was 3.78 mg/100 g DW. With higher total carotenoids component than other fruits such as papaya (2.436 mg/100 g DW), persimmon (1.683 mg/100 g DW), peach (1.489 mg/100 g DW) and orange (1.248 mg/100 g DW) (Breithaupt & Bamedi, 2001); tamarillo has a functionally interesting potential (Ordóñez et al., 2009)

for improving immune system and reducing the risk of diseases (Breithaupt & Bamedi, 2001). The major carotenoid compounds in tamarillo from various sources are shown in Table 2.5. β-cryptoxanthin (45.3%) and β-carotene (26.1%) were two most abundant carotenoids in tamarillo from Brazil, followed by zeaxanthin (5.1%), antheraxanthin (4.0%) and lutein (2.8%) (De Rosso & Mercadante, 2007). For both tamarillo varieties from Ecuador, two main carotenoids were also β-cryptoxanthin and β-carotene (Mertz et al., 2009). Nevertheless, the β-cryptoxanthin content in Brazilian tamarillo was higher than β-carotene, in which the opposite results had been captured in tamarillo from Ecuador. The yellow varieties did not have higher carotenoid content than the red and purple cultivars, on average (Mertz et al., 2009; Vasco et al., 2009). Tamarillo may be considered as a solution to overcome vitamin A deficiency in susceptible communities where food sources are not available.

Generally, the number of bioactive compounds identified in tamarillo increases, meanwhile, the relationship between structure and activity of these compounds and detailed quantitative analysis on different chemical groups of bioactive and on different sources and cultivars of tamarillo remain unexplored. Also, the determination and quantification of bioactive compounds in tamarillo by-products (peel) are completely lacking. Therefore, further investigation should be carried out to fill these gaps. Also, information about absorption, distribution, metabolism and excretion of tamarillo bioactive compounds in human body is still unknown. Hence, more research should be implemented to identify the metabolites of these compounds in humans, then more qualitatively well-correlated results with clinical studies will be achieved. From this, more specified data will be used to illustrate the biological activities of bioactives in terms of nutrition and human health.

Table 2.4 Concentration (mg/100 g DW) of diverse phenolic compounds in different tamarillo cultivars of different sources. Used with permission (Diep et al., 2020d).

Phenolic compounds	Ecuadorian yellow cultivar[a]	Ecuadorian yellow cultivar (Chaltura region)[b]	Ecuadorian yellow cultivar (Pelileo region)[b]	Colombian yellow cultivar[c]	Ecuadorian red cultivar[a]	Ecuadorian Giant purple cultivar[b]	New Zealand purple cultivar[b]	Malaysian cultivar[d]
Hydroxycinnamic acids								
Dicaffeoylquinic acid	17.1 ± 0.2	–	–		21.0 ± 0.3	–	–	–
Dehydrodiferulic acid	–	7.62 ± 1.152	8.52 ± 2.015		–	13.63 ± 1.861	43.23 ± 12.266	–
Caffeoylquinic acid	32.8 ± 0.2	26.31 ± 2.643	45.35 ± 8.26		54.8 ± 0.4	50.84 ± 4.381	165.59 ± 10.886	–
Rosmarinic acid		12.22 ± 1.956	32.85 ± 6.998	46.17 ± 1.20		29.57 ± 2.571	121.89 ± 11.067	–
p-coumaric acid	–	–	–		–	–	–	0.041 ± 0.047
Caffeic acid	–	–	–		–	–	–	0.165 ± 0.072
Ferulic acid	–	–	–		–	–	–	0.005
Trans-ferulic acid	–	–	–		–	–	–	0.049 ± 0.051
Hydroxybenzoic acids								
Gallic acid	–	–	–		–	–	–	0.302 ± 0.136
Vanillic acid	–	–	–		–	–	–	0.111 ± 0.182
Phenolic glycosides								
Caffeoyl glucoside	3.7 ± 0.01	1.35 ± 0.317	3.90 ± 1.054		9.7 ± 0.1	3.64 ± 0.412	29.26 ± 0.471	–
Feruloyl glucoside	6.3 ± 0.05	1.44 ± 0.303	3.01 ± 0.680		9.8 ± 0.1	0.21 ± 0.040	0.40 ± 0.566	–
Rosmarinic acid glucoside		11.31 ± 1.253	16.6 ± 3.918			34.68 ± 1.641	64.18 ± 11.962	–
Flavonols								
Kaempferol	–	–	–		–	–	–	0.05 ± 0.08
Flavanones								

Naringin	–	–	–	–	–	–	0.332 ± 0.15

DW: Dry Weight

[a] Data retrieved from Mertz et al. (2009) and expressed as mg chlorogenic acid equivalents/100 g DW;

[b] Data retrieved from Espin et al. (2016);

[c] Data retrieved from Garcia et al. (2016);

[d] Data retrieved from Mutalib et al. (2016) which converted from μg/g DW

Table 2.5 Concentrations (mg/100 g DW) of main carotenoids and anthocyanins in pulp extracts of tamarillo sourced from different cultivars and countries.

Used with permission (Diep et al., 2020d)

Carotenoid compounds	Golden-yellow cultivars			Purple-red cultivars				
	Ecuador[a]	Ecuador[b]	New Zealand[c]	Ecuador[a]	Ecuador[b]	New Zealand[c]	Brazil[d]	Malaysia[e]
β-carotene	24.29 ± 2.14	3.68 ± 0.24	5.57	65 ± 6.25	4.08 ± 0.24	4.31	10.25	4.8 ± 0.1
β-cryptoxanthin	–	0.88 ± 0.08		–	1.2 ± 0.06	–	17.50	–
Zeaxanthin	–	0.08 ± 0.02		–	0.24 ± 0.05	–	1.83	–
Antheraxanthin	–	–		–	–	–	1.50	–
Lutein	–	–		–	–	–	1.08	–

Anthocyanin compounds	Purple-red cultivars				
	Brazil[d]	Colombia[f]	Ecuador[g]	Ecuador[h]	New Zealand[h]
Cyanidin 3-rutinoside	4.58	438.0 ± 21.9	12.1 ± 0.2[g]	2.49 ± 0.2	4.49 ± 0.53
Delphinidin 3-rutinoside	43.83	4934.0 ± 98.7	32.7 ± 0.4	21.79 ± 0.13	87.43 ± 2.36
Delphinidin glucosyl rutinoside	–	–	5.3 ± 0.2	–	–
Delphinidin 3-O-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside 3′-O-β-D-glucopyranoside	–	138.0 ± 17.3		–	–
Pelargonidin 3-rutinoside	–	2276.0 ± 56.9	115.0 ± 1.3	78.07 ± 1.47	76.96 ± 5.09
Pelargonidin 3-coumaroyl-rutinoside	–	32.0 ± 0.6	–	–	–
Pelargonidin 3-glucoside-5-rhamnoside	22.25	–	–	–	–

DW: Dry Weight

[a] Data retrieved from Vasco et al. (2009) which converted from mg/100 g FW with moisture content of 86% and 92% for gold-yellow and purple-red tamarillo, respectively.

[b] Data retrieved from Mertz et al. (2009) which converted from μg/g FW with moisture content of 87.5% for both yellow and red tamarillos

[c] Data retrieved from Athar et al. (2003) which converted from mg/100 g edible portion with moisture content of 86.3% and 86.08% for gold-yellow and purple-red tamarillo, respectively.
[d] Data retrieved from De Rosso and Mercadante (2007) which converted from mg/100 g FW with assumed moisture content of 88%
[e] Data retrieved from Mutalib et al. (2017)
[f] Data retrieved from Osorio et al. (2012) and expressed as mg Delphinidin 3-rutinoside /100 g anthocyanin-rich extracts
[g] Data retrieved from Mertz et al. (2009). [h] Data retrieved from Espin et al. (2016)

2.1.7 Total phenolic content (TPC) and antioxidant activity

Tamarillo has been reported as new crop with greater total phenolic content identified by Folin-Ciocalteu assay when compared to those known to have high phenolic content fruits such as apple, red grape, black plum and cherries (73.96, 80.28, 88.28 and 114.56 mg GAE/100 g FW, respectively) (Fu et al., 2011). Great variation in the TPC of tamarillo were reported in the literature, arising from difference in tissues, cultivars, sources, and the extraction method (Table 2.6).

The TPCs of purple-red and yellow tamarillos from Ecuador extracted with 50% methanol were 113 and 78 mg GAE/100 g FW (~ 1413 and 557 mg GAE /100 g DW), respectively (Vasco et al., 2009). When extracting with acetone, the TPCs of Ecuadorian purple-red and yellow were 570 and 308 mg GAE /100 g DW, respectively (Mertz et al., 2009). Lister et al. (2005) reported the TPCs of 190.8 and 116.6 mg GAE/100 g FW (~ 1060 and 1563 mg GAE/100 g DW) in the gold and red tamarillo from New Zealand, respectively. Ordóñez et al. (2009) identified the phenolic contents in fruit, maceration, decoction, juice and pomace of tree tomato from Argentina which were 3.24, 1.39, 1.83, 2.05 and 0.72 mg GAE/g FW (~ 2314, 993, 1307, 1464 and 514 mg GAE/100 g DW with average moisture content of 86 %), respectively (Table 2.6). The total phenolic content of Malaysian tamarillo extracted by ethanol (91.56 mg GAE/100 g FW ~ 763 mg GAE/100 g DW with assumed moisture content of 88 %) was higher than that of cherry tomato (56.1 mg GAE/100 g FW ~ 467.5 mg GAE/100 g DW with assumed moisture content of 88 %) and tomato (23.4 mg GAE/g FW ~ 425 mg GAE/100 g DW with moisture content of 94.5%) (Noor Atiqah et al., 2014). In another study of tamarillo from Malaysia, TPC of crude ethanol extract was 2.53 mg GAE/g DW (253 mg GAE/100 g DW), whereas the values for n-butanol, ethyl acetate and water fraction were 2.1, 1.77 and 1.49 mg GAE/g DW (210, 177 and 149 mg GAE/100 g DW), respectively (Mutalib et al., 2017). For tamarillo from Taiwan, Kou et al. (2009) investigated the TPC of crude ethanol extract as well as ethyl acetate, n-butanol and water fractions were 2880, 6110, 2310, 1350 mg Catechin Equivalents/100 g DW, respectively. These values were converted from the actual values expressed as mg Catechin Equivalents/g DW (Table 2.6). There was a difference about TPC between different fractions of tamarillos from Malaysia and Taiwan. For Malaysian tamarillo, n-butanol fraction showed the highest total phenolic content, whereas the highest of this value was observed in ethyl acetate fraction of

Taiwanese tamarillo. A recently reported total phenolic content of orange, orange pointed, red, red conical and purple tamarillos were 4160, 3620, 3750, 3650 and 3980 mg chlorogenic acid equivalents/100 g DW, respectively (Acosta-Quezada et al., 2015).

However, this photometric method only provides quick information about the overall component of phenolics without any given compounds. Therefore, the content of individual bioactive should be parallelly identified to achieve broader information. Also, this method is not applicable for hydrophobic antioxidants (tocopherols and carotenoids) (Karadag, Ozcelik, & Saner, 2009) being dominant in tamarillo as well as is limited to general identification by using a single reference standard, commonly gallic acid. Several other reference standards have been recommended such as caffeic acid, chlorogenic acid, ferulic acid, and catechin equivalents. However, the choice of standard for this assay is also a critical control point since the more reacting OH group in molecular structure of standard, the higher absorbance value; and therefore, the measured value of sample may be low (Karadag et al., 2009). Also, lack of standardization can result in various modifications of this method.

Table 2.6 Total phenolic content (mg/100 g DW) in various pulp extracts of tamarillo sourced from different cultivars and countries. Used with permission (Diep et al., 2020d)

Tamarillo sources	Extraction method	Total phenolic content
Golden-yellow cultivars		
Ecuador[a]	50% aqueous methanol for 1 hour and then 70% aqueous acetone for 1 hour	557 ± 14
Ecuador[b]	Acetone	308
New Zealand[c]	80% aqueous acetone for 4 hours in dark at 10°C	1060
Purple-red cultivars		
Ecuador[a]	50% aqueous methanol for 1h and then 70% aqueous acetone for 1h	1413 ± 50
Ecuador[b]	Acetone	570
New Zealand[c]	80% aqueous acetone for 4 hours in dark at 10°C	1564
Argentina[d]	Fruit extracted with 1% HCl in ethanol for 1 hour at room temperature	2314 ± 357
	Maceration	993 ± 143
	Decoction	1307 ± 179
	Juice	1464 ± 357
	Pomace	514 ± 36
Malaysia[e]	70% aqueous ethanol for 1 hour at 50°C	763 ± 37
	Water for 1 hour at room temperature	183 ± 50
Malaysia[f]	Crude ethanol extract for 24 hours at room temperature	253
	n-butanol fraction	210
	Ethyl acetate fraction	177
	Water fraction	149
Taiwan[g]	Crude ethanol extract for 24 hours at room temperature	2880 ± 10[g]
	Ethyl acetate fraction	6110 ± 10[g]
	n-butanol fraction	2310 ± 10[g]
	Water fraction	1350 ± 10[g]

DW: Dry Weight; FW: Fresh Weight; GAE: Gallic Acid Equivalent

[a] Data retrieved from Vasco et al. (2009) which converted from mg GAE/100 g FW with moisture content of 86% and 92% for gold-yellow and purple-red tamarillo, respectively.

[b] Data retrieved from Mertz et al. (2009)

[c] Data retrieved from Lister et al. (2005) which converted from mg GAE/100 g FW with moisture content of 89% and 87.8% for gold-yellow and purple-red tamarillo, respectively.

[d] Data retrieved from Ordóñez et al. (2009) which converted from mg GAE/g FW with average moisture content of 86%

[e] Data retrieved from Noor Atiqah et al. (2014) which converted from mg GAE/g edible portion on dry weight basis and expressed as mg/100 g edible portion on dry weight basis

[f] Data retrieved from Mutalib et al. (2017) which converted from mg GAE/g DW

[g] Data retrieved from Kou et al. (2009) which converted from mg catechin equivalents/g DW to mg catechin equivalents/100 g DW

Comparing to antioxidant-rich fruits such as kiwifruit, orange and grape, tamarillo shows higher antioxidant activity, though the total phenolic content in tamarillo is similar or even lower than these fruits (Table 2.2) (Espin et al., 2016). This may be due to tamarillo being rich in various types of antioxidants (ascorbic acid, carotenoids and phenolics) which strengthen the overall antioxidant activity of the fruit (Acosta-Quezada et al., 2015). The purple-red tamarillo from Ecuador showed better anti-DPPH radical activity than the yellow one in all peel, pulp and seed-jelly. Also, the peel of both tamarillo cultivars displayed the highest antiradical efficacy, followed by the seed-jelly and pulp (Vasco et al., 2009). This was because higher concentrations of total phenolics were found in the peel than the in pulp and seed-jelly. The antioxidant activity of purple-red tree tomato in peel, seed-jelly and pulp was 40, 9.3 and 3 μmol Trolox/g FW, respectively, while for the yellow variety, 22, 3.8 and 2.3 μmol Trolox/g FW were found, respectively (Vasco et al., 2009). These results indicated that tamarillo peels can be utilized as a functional ingredients containing high antioxidant activity. Noor Atiqah et al. (2014) reported considerably higher antioxidant activity of tamarillo extract than cherry tomato and tomato extracts using FRAP method. The FRAP values of ethanol extracted tamarillo, cherry tomato and tomato were 12.17, 7.435 and 6.58 μM Fe (II)/g of sample, respectively. Tamarillo also exhibited the highest antioxidant capacity than cherry tomato and tomato when extracted with water. The high content of phenolic compounds has been associated with greater reducing power measured by Fe^{3+} to Fe^{2+} transformation (Dragovic-Uzelac, Levaj, Mrkic, Bursac, & Boras, 2007).

The ORAC value of yellow and red Ecuadorian tamarillo crude extracts were 6.5 and 10.0 μmol Trolox Equivalents (TE)/g FW, which were significantly higher than the widely consumed fruit juices of red grape, apple and tomato with 4.0, 1.9 and 1.6 μmol TE/g FW, respectively (Mertz et al., 2009; Wang, Cao, & Prior, 1996). When extracted with 70% acetone, both yellow and red tamarillo types showed better antioxidant activity than that of the crude extracts with ORAC value of 8.1 and 14.8 μmol TE/g FW, respectively (Mertz et al., 2009). These ORAC values of the acetone extracts increased by 25% for the yellow type and 48% for the red one, when compared with the crude extracts. This was because the use of 70% acetone notably enhanced the extraction process of anthocyanins as well as for the phenolic compounds that are

bound to the insoluble cell wall. The ORAC values of tamarillo extracted by hexane were extremely low, with 0.14 and 0.18 μmol TE/g FW for yellow and purple varieties, respectively (Mertz et al., 2009).

The ethyl acetate fraction of Taiwanese tamarillo had the highest TEAC value of 56.73 mg TE/g DW, followed by n-butanol fraction and water fraction. The ethyl acetate fraction exhibited the most robust free radical scavenging activity (Kou et al., 2009). Hence, the higher the polarity of partition solvent, the lower the antioxidant capacity of its partitioned fractions was shown (Kou et al., 2009). Espin et al. (2016) presented that New Zealand purple type owned the highest antioxidant activity, compared with the yellow and purple cultivars from Ecuador in FRAP, ABTS, ORAC assays (Figure 2.6). The FRAP, ABTS, ORAC values of tamarillo extracted by 75% methanol were 50, 89 and 325 μmol TE/g dry pulp, respectively. The phenolic constituent and content could be related to the large variation of antioxidant capacity between various tamarillo cultivars (Espin et al., 2016).

Currently, a simple, validated and well applicable method for identification of antioxidant activity of tamarillo is still unavailable. All of these existing assays still have some drawbacks. For example, the ABTS method may take a longer time to reach end point of reaction, hence low values of TEAC will occur if the reaction end point is taken at short duration (4 – 6 mins). DPPH is limited in the evaluation of hydrophilic antioxidants since this chemical cannot be dissolved in aqueous media. For the FRAP assay, the absorbance is recorded within 4 and 6 min after the reaction is finished. However, the absorption at specific wavelength of caffeic acid, ferulic acid, and especially ascorbic acid being main phenolics in tamarillo slowly increases after several hours. Therefore, it is crucial to develop a rapid, accurate and standardized analysis approach and present the result as standard equivalents. This will lead to possible comparison between different results from various studies (Karadag et al., 2009).

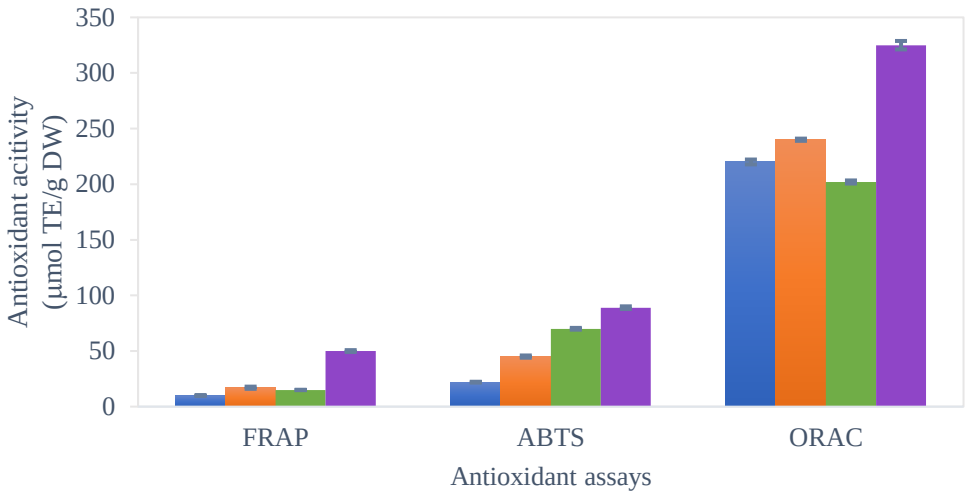

Figure 2.6 Antioxidant activity of pulp extracts of tamarillo sourced from Ecuador and New Zealand.

Used with permission (Diep et al., 2020d)

■ Ecuadorian yellow Giant cultivar from Chaltura region; ■ Ecuadorian yellow Giant cultivar from Pelileo region; ■ Ecuadorian Giant purple cultivar; ■ New Zealand purple cultivar. Data are presented as mean (µmol TE/g DW) and error bar (standard deviation) (n = 6)

2.1.8 Potentials of tamarillo in health and food applications

Several studies had reported the health benefits of tamarillo and tamarillo extracts including antioxidation, antioxidative stress, anti-obesity and anticancer properties (Table 2.7). The polyphenol-rich fractions of tamarillo extract have been shown to have an inhibitory impact on the oxidation of low-density lipoprotein (LDL) and oxidative stress-induced cell death in the neuronal PC12 cells (Kou et al., 2009). The IC_{50} and relative electrophoretic mobility (REM) values of ethyl acetate fraction were better than that of the DL-α-tocopherol. From their findings, ethyl acetate fraction of the phenolic compounds in tamarillo prevented copper-induced LDL oxidation equally to or more efficiently than DL-α-tocopherol.

The inhibition of high-fat-diet-induced obesity has been observed in Sprague-Dawley Rats, demonstrating the potential application of tamarillo for weight control (Abdul Kadir, Rahmat, & Jaafar, 2015). The obese rats treated with tree tomato extracts at different doses of 150, 200 and 300 mg per kg showed considerable reduction of total

cholesterol and increase of HDL-cholesterol. The blood glucose, triglyceride, LDL-cholesterol together with body weight of these rats positively decreased by supplementation of medium and high dosage extract. According to Abdul Kadir et al. (2015), prevention of overweight and obesity have come from high water content (85 – 90 %), high dietary fibre content (3.3 – 4.2 g/100 g) and low energy density of tamarillo. Increased total antioxidant status and significant lowered level of tumour necrosis factor-α (TNF-α) and interleukin (IL-6) activities were detected in rats treated with tamarillo-extract. This might be due to high content of polyphenols in tamarillo which has ability to neutralize reactive species. Thus, daily intake of tamarillo could help with weight control and prevention of obesity (Abdul Kadir et al., 2015).

According to epidemiological and clinical studies, many cancers may be prevented if a diet with 400 – 800 g of fruits and vegetables each day is consumed over many years. The phenolic profile from tamarillo extract has shown a high cytotoxic impact to prevent cancer in liver cell line (HepG2) and breast cancer cell line (MDA-MB-231) with IC_{50} values of 30 and 80 µg/mL, respectively (Mutalib et al., 2016; Mutalib et al., 2017). Phenolic acids, such as caffeic and vanillic acids, possess numerous biological activities and have correlation to the modulation of carcinogenesis. Gallic acid has capacity to inhibit oxidative damage and act against proliferation of several tumour cell lines (Mutalib et al., 2016). Together with antioxidant, anti-inflammatory and anti-allergenic capacities, flavonols (quercetin and kaempferol) also demonstrate other biological potentials including cardio-protective and vasodilatory effects as well as powerful anticancer ability on breast, gastric, leukaemia, liver and ovarian cancer cells (Joshi et al., 2011). More clinical trials or human intervention research should be further conducted to better understand the effect of bioactive compounds on human health and then validate the health benefits of various tamarillo cultivars. This is because the effect of bioactives on human cells and tissues identified by *in vitro* tests can not totally demonstrate the actual assessment of the *in vivo* effect.

It was found that the content of bioactive compounds in fruit by-products (peel/epicarp and seed) have been higher than that in edible tissue. Tamarillo peel and seed are richer source of bioactive compounds than the pulp. The possibility of using tamarillo by-products for food application have been investigated. Castro-Vargas, Benelli, Ferreira, and Parada-Alfonso (2013) reported on the effect of extracts from

tamarillo epicarp on minimization of lipid oxidation in cooked beef meat (CBM) during storage at 4°C. The extract gained by supercritical fluid extraction with CO_2/EtOH (50°C; 30 MPa and 2% of EtOH) exhibited the highest antioxidant capacity compared with other extracts (Castro-Vargas et al., 2013). This extraction conditions completely prevented lipid hydroperoxides (LHP) formation with unchanged values of LHP concentration in CBM sample post to storage at 4°C for 9 days. The extract also inhibited the formation of TBARS. This was because phenolic compounds in tamarillo extract had the ability to neutralize reactive oxygen species and free radicals which appeared during autoxidation. Also, these phenolic compounds were able to chelate ions to reduce the LHP formation (Castro-Vargas et al., 2013).

Application of tamarillo to milk gel has been made by Li, Scott, Hemar, Zhang, and Otter (2018b). They have purified protease, known as tamarillin – a serine protease, from tamarillo. It is a plant alkaline protease with optimal temperature of 60°C and pH of 7 to 11, with the maximum hydrolysis activity seen at pH 11. Li, Scott, Hemar, and Otter (2018a) has shown that tamarillin had wider caseinolytic activity on sodium caseinate than calf rennet. Hydrolysis of sodium caseinate with tamarillin for 24 hours had resulted in full degradation of all the caseins. Fifteen minutes of hydrolysis with tamarillin has resulted in appearance of the main peptide (14,290 Da) generated from κ-casein, indicating that this casein was the most susceptible to tamarillo protease action. High proteolytic activity towards α- and β-caseins was shown by tamarillin. α- and β-caseins have started to hydrolyse by tamarillo protease by 15 min and 1 hour, respectively while the rennet started hydrolysing both caseins around 4 hours.

Because of broader proteolytic activity of tamarillin, the milk gel made from tamarillin exhibited a faster increase in the elastic modulus (G′) at the early stage of gelation than the sample obtained by rennet (Li, Scott, Otter, Zhou, & Hemar, 2018c). Larger voids and more porosity were obtained in milk gel with tamarillin rather than with rennet due to high extent of casein hydrolysis (Li et al., 2018d). At pH between 6.5 and 6.7, the aggregation time of milk gels made from both rennet and tamarillin was the same. At pH below 6.5, the aggregation time of tamarillin-induced milk gel was higher than that of milk gels made from rennet. At 20°C, milk was coagulated by tamarillin extract in 2h, whereas milk was not coagulated by rennet within 3h. These results indicated that tamarillo protease can be applied in milk gelation, especially at low temperature

or high pH conditions (Li et al., 2018c). However, application of the enzymes (tamarillin) to other protein foods for protease action is relatively unexplored, hence further investigation of tamarillin should be implemented to achieve better utilisation of this protease. For example, actinidin, a protease from kiwifruit, had been applied into cooked beef brisket muscles to improve the rate of protein digestion under simulated gastric conditions (Zhu, Kaur, Staincliffe, & Boland, 2018).

The application of tamarillo to other food types (e.g., fortification) remain scarce when comparing with other fruits. For example, kiwifruit extract had been applied into gluten free-bread (Sun-Waterhouse et al., 2009) or kiwifruit-banana smoothies (Sun-Waterhouse & Zhou, 2010) and natural kiwifruit ice cream (Sun-Waterhouse et al., 2013a) had been made. As a result, developing food products with tamarillo-derived ingredients would be favourable in terms of increasing nutritional value while also making consumption easier and more accessible.

Table 2.7 Potential applications of tamarillo.

Used with permission (Diep et al., 2020d)

Beneficial effects	Application made to	Tamarillo source and sample	Observation	References
Health benefits				
Reduction of oxidative stress	Low-density lipoprotein (LDL)	Taiwan Extracted with ethanol and then partitioned in ethyl acetate and *n*-butanol	Phenolics in ethyl acetate and *n*-butanol fractions reduced copper-induced LDL oxidation.	Kou et al. (2009)
	Rat adrenal pheochromocytoma cell line, PC12 cells	Taiwan Extracted with ethanol and then partitioned in ethyl acetate and *n*-butanol	Phenolics in ethyl acetate fraction prevented neuronal PC12 cell death caused by oxidative stress.	Kou et al. (2009)
	HepG2 cells	Argentina Maceration, decoction and juice forms	HepG2 cell viability increased by 100%.	Ordóñez et al. (2009)
Anti-obesity	Male Sprague Dawley rats	Malaysia	Treated obese rats showed a significant increase in HDL-C and reduction in total cholesterol. Treated obese rats showed positive decrease in blood glucose, triglyceride, LDL-C and body weight. Positive improvement of superoxide dismutase (SOD) activity and glutathione peroxidase (GPx) activity, increase of total antioxidant status (TAS) and significant reduction of tumour necrosis factor-α (TNF-α) and interleukin (IL-6) activities.	Abdul Kadir et al. (2015)

Anticancer	Human tumour cell lines of liver (HepG2) and breast (MDA-MB-231)	Malaysia Extracted with 80% ethanol	Significant inhibition of proliferation of HepG2 and MDA-MB-231 cell lines was observed.	Mutalib et al. (2016)
	Human tumour cell lines of liver (HepG2) and breast (MDA-MB-231)	Malaysia Extracted with ethanol at room temperature for 24h. After drying at 50°C and then dissolving in dimethyl sulfoxide (DMSO), the extract was signified as crude ethanol extract. The rest fraction was partitioned in water, ethyl acetate and *n*-butanol for triplicate.	Proliferation and viability of HepG2 and MDA-MB-231 cell lines was inhibited by different extracts of tamarillo.	Mutalib et al. (2017)
Prebiotics	Bifidobacteria and lactobacilli. Predominant colonic bacteria	Malaysia Seed mucilage hydrocolloids extracted with 1% citric acid and water. Pulp mucilage hydrocolloids extracted with 20 mM HEPES buffer and 72% ethanol.	The growth of bifidobacteria and lactobacilli was stimulated; the proliferation of some pathogenic bacteria was inhibited. The hydrocolloids could act as fermentable substrates or prebiotics for the gut microbiota	Gannasin et al. (2015a)
Biological properties				
Antifungal activity	*Pycnosporus sanguineous*, *Ganoderma applanatum*, *Schizophyllum commune*, *Lenzytes elegans*, *Penicillium notatum*, *Phomopsis sojae* and *Fusarium mango*	Argentina Four μg of invertase inhibitory protein (IIP) per gram fresh weight was used.	The growth of all fungi was completely suppressed by the IIP (7.8 to 62.5 μg/ml)	Ordonez, Ordonez, Sayago, Moreno, and Isla (2006)
Antibacterial activity	*Xanthomonas campestris*, *Pseudomonas solanacearum*, *Pseudomonas corrugata*, *Pseudomonas syringae* and *Erwinia carotovora*	Inhibitory protein from Argentinean tamarillo with concentration of 4 μg of invertase inhibitory protein (IIP) per gram fresh weight.	The growth of all phytopatogenic bacteria was completely inhibited by the IIP with concentration required from 7.8 to 31.25 μg/ml.	Ordonez et al. (2006)

Food applications				
Lipid oxidation	Cooked beef meat (CBM)	Colombia (epicarp) Supercritical fluid extraction (SFE) and Soxhlet extraction (SE)	The extracts by SFE at 50°C, 30 MPa and with CO_2/2% of ethanol showed greater antioxidant activity than ter-buthyl hydroquinone (TBHQ) in inhibition against lipid oxidation of CBM.	Castro-Vargas et al. (2013)
Hydrolysis of casein	Milk casein	New Zealand Protease enzyme purification	Tamarillin hydrolyzed κ-casein and showed broader activity on α- and β-casein than calf rennet. Tamarillin could be used for milk gelation.	Li et al. (2018a), (2018b), (2018c), (2018d)
Functional ingredients	Bile acid and techno-functional properties	Malaysia Seed mucilage hydrocolloids extracted with 1% citric acid and water. Pulp mucilage hydrocolloids extracted with 20 mM HEPES buffer and 72% ethanol.	All tamarillo hydrocolloids could be used as food emulsifiers and bile acid binders. Pulp mucilage hydrocolloids could be potential for reduction of syneresis, prevention of staling and stabilization of high fat food. Seed mucilage hydrocolloids could be considered as foaming agents in foam-based food products.	Gannasin, Adzahan, Mustafa, and Muhammad (2016)

2.1.9 Conclusion and future perspective

Tamarillo fruit has been known for centuries, but the potential of this fruit has not been fully explored. Tamarillo can be recognized as a fruit with high nutritional adequacy score of 7.9 and 7.4 for gold and red varieties, respectively. Tamarillo contains high amount of dietary fibre, potassium, vitamin A, B_6, C and E as well as bioactive compounds including phenolics, carotenoids and anthocyanins. Tamarillo has a distinctive flavour (approximately 70 volatile compounds) and major contributors to the overall flavour of tamarillo should be identified through OAV value. In addition to its consumption as fresh fruit, tamarillo has numerous potentials for designing and developing fruit-based functional foods. However, this fruit is underutilized due to lack of nutritional knowledge and awareness of its value. Many of these reported values are outdated (more than 10 years ago) and relied on basic analytical instruments. From the biochemistry and pharmacology point of view, polyphenols are stated as the major bioactive component of tamarillo and may be the key target bioactive group to contribute various potential applications of this fruit. In particular, tamarillo peel is a rich source of bioactive compounds which also needs to be investigated further for health promoting effects. Several reports demonstrated that the phenolic content in tamarillo peel was higher than in the pulp. Although often discarded as waste, the peel may be used as a functional ingredient for further utilisation.

Comparing to other fruits, there is a lack of systematic study in tamarillo; and many of bioactives overlap in tamarillo, but application of these compounds remains unexplored. More novel extraction technologies should be developed and improved to better exploit natural compounds in tamarillo and to replace the traditional methods which are time-consuming and eco-unfriendly. A standardization of analytical method and expression of results as standard equivalents are considerable to achieve high quality of bioactive compounds as well as possibly compare different data from different studies. More research should be implemented to identify the complete profile of bioactive compounds in tamarillo and their relation to the antioxidant activities and/or other bioactive applications. Moreover, there is a lack of validation of bioactive compounds in health-related-area, hence further epidemiological and clinical studies are important to validate the health benefits and other applications of tamarillo. Therefore, there is a demand to conduct a systematic investigation about

these compositions and possible nutritional benefits of tamarillo sourced from New Zealand.

What is not known is how proximate, phytochemical and volatile contents in 'Amber' (yellow) and 'Mulligan' (purple-red) cultivars compares to 'Laird's Large' (red variety) known as the most commonly grown and consumed tamarillo cultivar, as well as how the antioxidant vitamin, polyphenol, carotenoid and chlorophyll pigments and volatile content of the peel compares to the pulp of the three main cultivars of tamarillo. It was hypothesized that the 'Amber' and 'Mulligan' cultivars as well as the peel of tamarillos could be considered as a dietary source of antioxidant vitamins and a source of phenolics, carotenoids pigments and volatiles.

The following sections aimed to evaluate the physicochemical compositions and nutrient contents, especially vitamins and bioactive compounds of tamarillo that may be beneficial for the human health. Characterization of tamarillo will make more available information to the export market, food companies and health and nutrition experts. This will promote New Zealand fruit to the world.

2.2 Materials and methods

2.2.1 Standards, chemicals, reagents

All chemicals and reagents used were AnalaR grade or greater. Acetone, acetonitrile (MeCN), dimethyl sulfoxide (DMSO), ethyl acetate, formic acid, isopropanol, methanol (MeOH), petroleum ether and sulphuric acid were from Thermo Fisher (Auckland, New Zealand). Copper sulphate, potassium sulphate, phenylmethylpyrazolone (PMP) and 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (accutag), Tetrabromophenyl porphyrin (TBP); metaphosphoric acid (MPA) Folin-Ciocalteu reagent; copper (II) chloride; neocuporine; Trolox (6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid); and 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) were purchased from either Thermo Fisher (Auckland, New Zealand) or Sigma-Aldrich (Sigma Aldrich Ltd, Auckland, New Zealand).

The analytical grade standards including sugars, amino acids standards (A9906 product), α-tocopherol, β-carotene and ascorbic acid were also purchased from Sigma-Aldrich (Auckland, New Zealand). Analytical standards of phenolics including *p*-

coumaric acid, caffeic acid, (±) catechin and (–) epicatechin were also obtained from Sigma-Aldrich (Auckland, New Zealand). Analytical standards of other phenolics (chlorogenic acid, ellagic acid, ferulic acid, gallic acid, kaempferol, rutin, kaempferol 3-rutinoside and isorhamnetin 3-rutinoside) and four anthocyanins (cyanidin 3-glucoside; cyanidin 3-rutinoside; delphinidin 3-rutinoside and pelargonidin 3-rutinoside) were purchased from Extrasynthese (Genay Cedex, France). Purite Fusion Milli-Q water purifying machine (Purite Limited, Thame, Oxon, UK) was used to produce Milli-Q water.

2.2.2 Preparation of tamarillo and summarization of methods

The commercially ripe, fresh fruits of tamarillo including yellow ('Amber'), red ('Laird's Large') and rich purple-red ('Mulligan') (Figure 2.7) used in this study were obtained from growers in the Northland region of New Zealand and kindly delivered by New Zealand Tamarillo Growers Association. From anthesis to commercial maturity, it takes between 21 and 24 weeks.

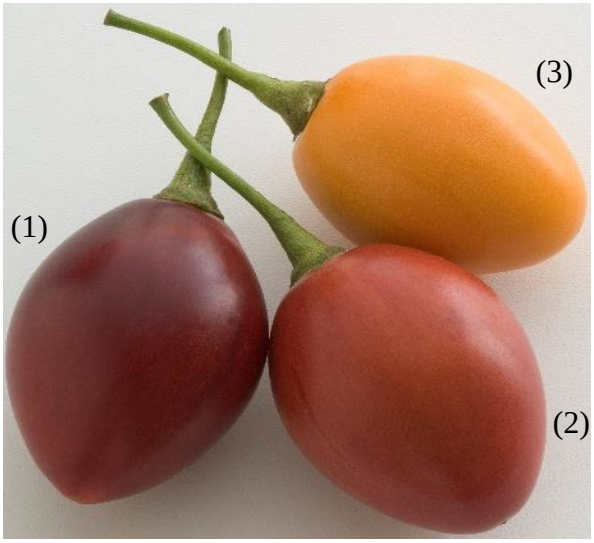

Figure 2.7 Three tamarillo cultivars sourced from New Zealand used in this study. (Reproduced with permission from New Zealand Tamarillo Growers Association) (1): 'Mulligan' cultivar (rich purple-red), (2): 'Laird's Large' cultivar (red), (3): 'Amber' cultivar (yellow)

Three tamarillo cultivars were randomly selected to obtain representative samples, measured physical parameters and then separated into peel and pulp. The fresh pulp of each type was used to identify chemical parameters such as pH, total soluble solids

(TSS), moisture and ash contents. Then, the peel and pulp of three tamarillo types were snap frozen by liquid nitrogen, lyophilized (Alpha 1-2 LD plus Freeze Dryer, Martin Christ, New Zealand), ground to powder, then stored at –20°C until further analysis. The freeze-dried pulp of tamarillo was used to investigate other chemical components (protein, fat, carbohydrate and dietary fibre) while identification of volatile compounds, bioactive compounds and antioxidant activity was implemented in both freeze-dried peel and pulp of three tamarillo cultivars. This stage of study and summarisation of methods for the analysis of the properties of tamarillo are schematically presented in Figure 2.8 and Table 2.8, respectively.

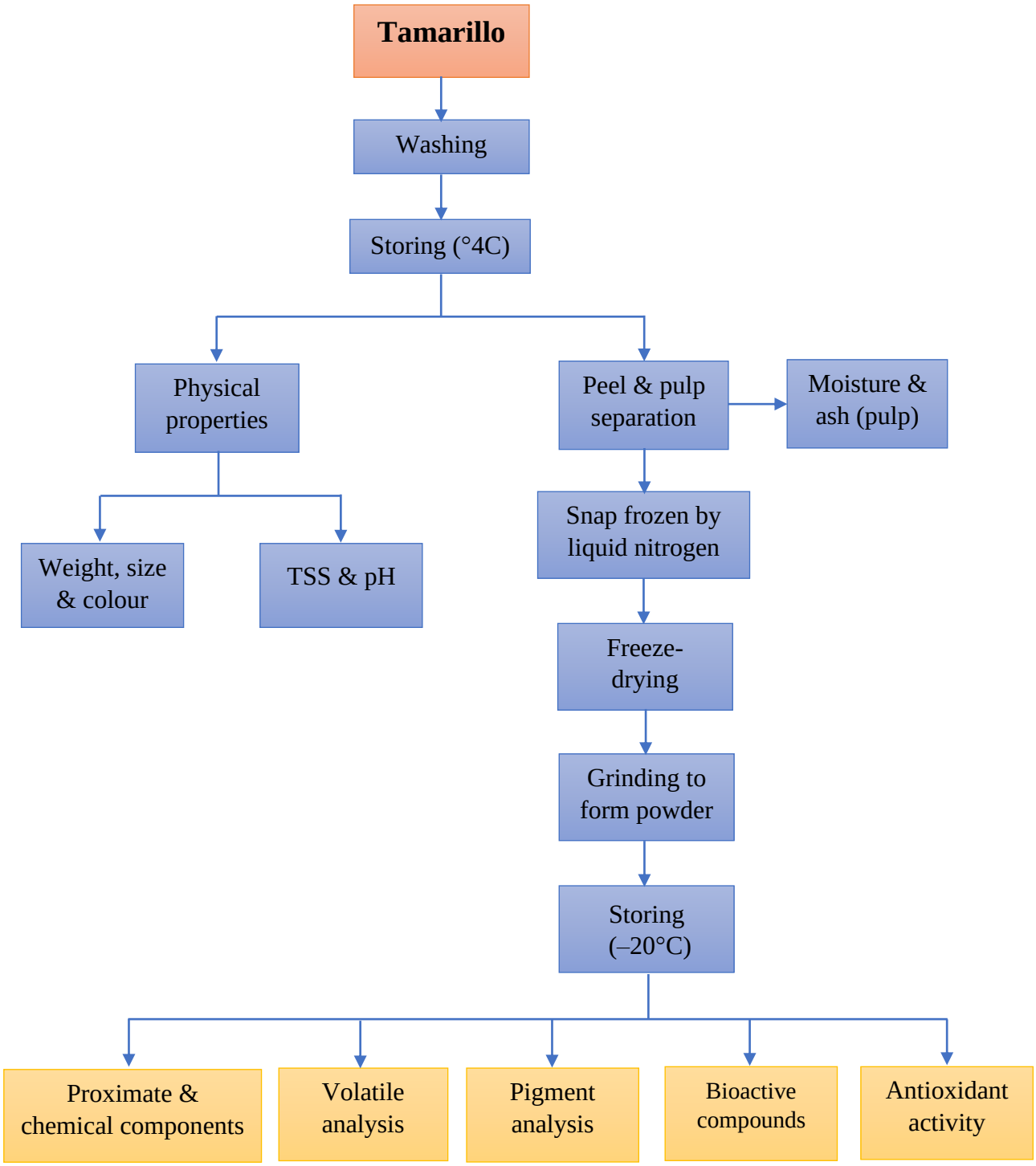

Figure 2.8 Schematic presentation of the analysis of the properties of physicochemical properties, bioactives and antioxidant activity of peel and pulp tamarillo fruits.

Table 2.8 Summary of methods for the analysis of the physicochemical properties, bioactives, antioxidant activity and volatiles of three tamarillo cultivars from New Zealand.

Constitutes	Fruit type	Methods	Extraction/derivatization solvent	Equipment/parameters
Physical properties				
- Weight	Whole fresh fruit	–	–	Analytical balance
- Size	Whole fresh fruit	–	–	Vernier
- Colour	Whole fresh fruit	–	–	Hunter Lab colour analyser
-TSS	Fresh pulp	–	–	Refractometer
- pH	Fresh pulp	–	–	pH meter
Proximate				
- Moisture	Fresh pulp	AOAC 950.46B	–	Drying oven/105°C for 8 hours
- Ash	Dried pulp	AOAC 920.153	–	Muffle furnace/550°C for 6 hours
- Protein	Freeze-dried pulp	AOAC 981.10 (Kjeldahl)	–	Heating block/420°C for 60 min & distillation systems
- Lipid	Freeze-dried pulp		Petroleum ether	
- Dietary fibre and carbohydrate	Freeze-dried pulp	AOAC 948.22 (Soxhlet)	–	–
		AOAC 996.11		Megazyme kit (K-ACHDF 08/16)
Volatiles	Freeze-dried peel and pulp	Chromatography		GC-MS couple with TD Phenomenex ZB-1701 column (30 m x 250 μm x 0.15 μm)
Organic acids	Freeze-dried peel and pulp	Derivatisation & chromatography	MCF (extraction and derivatisation)	GC-MS Phenomenex ZB-1701 column (35 m x 250 μm x 0.15 μm)
Reducing sugars	Freeze-dried peel and pulp	Derivatisation & chromatography	50% MeOH (extraction) PMP (derivatization)	LC-ESI-MS/MS Kinetex C18 column (150 x 2.1 mm, 1.7 μm)

Amino acids	Freeze-dried peel and pulp	Derivatisation & chromatography	50% MeOH (extraction) Accutag (derivatization)	LC-ESI-MS/MS Kinetex C18 column (150 x 2.1 mm, 1.7 μm)
Carotenoid and chlorophyll pigments	Freeze-dried peel and pulp	Chromatography	Acetone containing 1 mg/L TBP (extraction)	LC-ESI-MS/MS coupled with FLD and DAD XSelect C18 column (100 x 2.1 mm, 3.5 μm)
β-carotene and α-tocopherol	Freeze-dried peel and pulp	Chromatography	Acetone (extraction)	LC-APCI-MS/MS coupled with DAD XSelect C18 column (100 x 2.1 mm, 3.5 μm)
Ascorbic acid	Freeze-dried peel and pulp	Chromatography	3% MPA (extraction)	LC-ESI-MS/MS Poroshell 120 EC-C18 column (150 x 2.1 mm, 2.7 μm)
Phenolics	Freeze-dried peel and pulp	Chromatography	50% MeOH (extraction)	LC-ESI-MS/MS Cortecs C18 column (100 x 2.1 mm, 2.7 μm)
Anthocyanins	Freeze-dried peel and pulp	Chromatography	Formic acid, IPA and Milli-Q (extraction)	LC-ESI-MS/MS coupled with FLD, DAD XSelect C18 column (100 x 2.1 mm, 3.5 μm)
Total phenolic content	Freeze-dried peel and pulp	Spectroscopy (Folin-Ciocalteu)	50% MeOH and 70% acetone (extraction)	UV-spectrophotometer Absorbance measured at 765 nm
Antioxidant activity	Freeze-dried peel and pulp	Spectroscopy (CUPRAC)	50% MeOH and 70% acetone (extraction)	UV-spectrophotometer Absorbance measured at 450 nm
Antioxidant activity	Freeze-dried peel and pulp	Spectroscopy (FRAP)	50% MeOH and 70% acetone (extraction)	UV-spectrophotometer Absorbance measured at 593 nm

GC-MS: Gas chromatography-mass spectrometry; TD: Thermal desorption; MCF: Methyl chloroformate; MeOH: Methanol; LC-ESI-MS/MS: Liquid chromatography-electrospray ionization-mass spectrometry/mass spectrometry; TBP: Tetrabromophenyl porphyrin; PMP: Phenylmethylpyrazolone; LC-APCI-MS/MS: Liquid chromatography-atmospheric pressure chemical ionisation-mass spectrometry/mass spectrometry; MPA: Metaphosphoric acid; IPA: Isopropyl alcohol; CUPRAC: Cupric ion Reducing Antioxidant Capacity; FRAP: Ferric reducing antioxidant power

2.3 Analytical methods

All analysis methods presented here have been published and used with permission (Diep, Pook, & Yoo, 2020a; Diep, Pook, Rush, & Yoo, 2020b; Diep, Pook, & Yoo, 2020c; Diep et al., 2021)

2.3.1 Physical properties and proximate compositions

Thirty fruits of each variety were collected and the mass (± 0.1 mg) was identified using an analytical balance (Scaltec Company, Gottingen, Germany; model SPB31) with a maximum capacity of 210 g. The length and diameter of tamarillos were measured using a Vernier with a sensitivity of 0.01 mm. The colours were recorded using a Hunter Lab (45/0, Colorflex EZ) colour analyser based on CIE L*, a*, b* colour system. The L* value indicates the lightness of samples from black (0) to white (100), a* value indicates the green (– 60) to red (+ 60) characteristics and b* value indicates the blue (– 60) to yellow (+ 60) characteristics. Four randomly selected fruits of each tamarillo cultivar were peeled, and the pulp was homogenised to measure Total Soluble Solids (TSS) by Rudolph refractometer (J57 Automatic Refractometer, Rudolph Research Analytical, Hackettstown, USA). The homogenised tamarillo pulp (50 g) was mixed with 200 mL of Milli-Q water for pH measurement (HI 207, Hanna Instruments, USA).

The moisture content was determined by oven drying (Sanyo model MOV-112F, Sanyo Electric Co., Japan) at 105°C for 8 hours (AOAC 950.46B) (AOAC, 2005). For ash content, the dried samples were ashed in a muffle furnace at 550°C for 6 hours (AOAC 920.153) (AOAC, 2005). Kjeldahl method with nitrogen-protein conversion factor of 6.25 was used to estimate total protein content (AOAC 981.10) (AOAC, 2005). Lipid content was estimated by using Soxhlet extraction (AOAC 948.22) (AOAC, 2005). Megazyme Available Carbohydrates and Dietary Fibre assay kit (K-ACHDF 08/16) was used to assess the available carbohydrate and dietary fibre (AOAC 996.11) (AOAC, 2005).

2.3.2 Identification of chemical compositions in both peel and pulp of tamarillos

2.3.2.1 Reducing sugars

Freeze-dried powder of peel and pulp (50 ± 0.5 mg) was extracted with 1 mL of 20% acetonitrile (MeCN). The sample was vortexed for 30 s, incubated at 50°C for 60 min with vortexing every 10 min, and then centrifuged at 10,000 RCF at 4°C for 10 min. Then, the supernatant was used for derivatisation based on method of Dai et al. (2010) with some modifications. A 20 μL of extracts, standards or blank was mixed with 100 μL of PMP reagent (20 g/L) in Eppendorf tube to derivatise, followed by addition of 13 μL of NaOH (10 M) to alkalise the reaction. The mixture was vortexed and incubated at 70°C for 100 min, then cooled to room temperature before 100 μL of MilliQ was added into the solution. Ethyl acetate (500 μL) was used to remove excess PMP reagent for at least 3 times. Final derivatised solution (20 μL) was diluted with 980 μL of 1% formic acid, vortexed for 30 s and then kept at –20°C until analysis.

The LC-ESI-MS/MS consisting of an Agilent 1260 Infinity Quaternary LC System and an Agilent 6420 triple quadrupole mass spectrometer with multimode ionisation source (model G1978B) (Santa Clara, CA, USA) was used. The Kinetex C18 column (150 x 2.1 mm, 1.7 μm; Phenomenex, USA) was maintained at 50°C. The injection volume, flow rate and total run time were 1 μL, 0.2 mL/min and 20 min, respectively. The mobile phases: 0.1% formic acid in acetonitrile (A) and 0.6% formic acid in Milli-Q (B), with gradient elution: 0 – 10 min, 17% of A; 11 min, 80% of A; 12 – 20 min, 17% of A. The MS was run in the positive mode. The multimode inlet (MMI) source operation for electrospray ionisation (ESI) parameters was set as gas temperature of 300°C, gas flow of 6 L/min. Nebulizer and capillary voltage were 60 psi and to 2.2 kV, respectively. Chromatograms of standards are presented in Appendix B1 and summary of method validation are presented in Appendix B2.

2.3.2.2 Free amino acids (FAAs)

Freeze-dried tamarillo (14 – 15 mg) was extracted with 500 μL of 50% methanol in Eppendorf tube, followed by vortexing for 30 s and incubating for 60 min at room temperature with vortexing every 10 min. The samples were then centrifuged at 10,000 RCF for 10 min and 40 μL of the supernatant was mixed with 40 μL of methanol containing 10 mg/L of d4-alanine as internal standard-spiked methanol (ISSM). The

mixture was vortexed for 30 s and centrifuged at 10,000 RCF for 5 min at 4°C. The supernatant was used for derivatisation based on method Salazar, Armenta, and Shulaev (2012). A derivatisation reaction was prepared as: 70 μL of borate buffer (pH 8.8); 10 μL of samples, standards or blank; and 10 μL of accutag reagent. The vial was immediately capped, vortexed for 30s and then incubated at 55°C for 15 min. Then, 400 μL of 90% formic acid was added as neutralising solution, vortexed for 30s and kept at –20°C until analysis.

The LC-ESI-MS/MS equipped with Kinetex C18 column (150 x 2.1 mm, 1.7 μm; Phenomenex, USA) maintained at 25°C, was used to profile amino acids. Injection volume, flow rate and total run time were 3 μL, 0.225 mL/min and 33 min, respectively. Mobile phase gradient was set at: 0 – 8 min, 95% of A; 8 – 15 min, 90% of A; 15 – 16.5 min, 83% of A; 16.5 – 18.5 min, 20% of A; 18.5 – 33 min, 95% of A with 0.1% formic acid in Milli-Q (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B). The MS was run in the positive mode. The multimode inlet (MMI) source parameters were set for gas temperature and gas flow of 300°C and 6 L/min, respectively. Nebulizer and capillary voltage were set at 15 psi and to 4 kV, respectively. Chromatogram of standards and summary of method validation are presented in Appendix B3 and Appendix B4, respectively.

2.3.2.3 β-carotene and α-tocopherol

Dried, powdered samples (50 ± 0.5 mg) were extracted with 1 mL of acetone based on method of Gentili and Caretti (2011) The extract was vortexed for 30s and incubated in the dark for 30 min at 4°C with vortexing every 5 min, followed by centrifuging at 10,000 RCF for 10 min at 4°C. The supernatant was separated and stored at –20°C until analysis.

LC-APCI-MS/MS coupled with a diode array detector (DAD) and XSelect C18 column (100 x 2.1 mm, 3.5 μm; Waters, Ireland) was maintained at 50°C were used. The flow rate and the injection volume were 0.4 mL/min and 5 μL, respectively. Mobile phase gradient program was set up: 0 – 1 min, 5% of A; 1 – 9.2 min, 0% of A; 9.2 – 15 min, 5% of A, with A as 0.1% formic acid in Milli-Q and B as pure methanol. Double online detection was implemented in the DAD at 453 nm for β-carotene and 295 nm for both α-tocopherol and β-carotene. The MS was run in the positive mode.

The MMI source was operated at atmospheric pressure chemical ionization (APCI) parameters as follows: gas temperature as 300°C with a flow rate of 5 L/min. The capillary voltage and the nebuliser were set to 2 – 2.5 kV and at 50 psi, respectively. Vaporizer temperature was set at 250°C.

2.3.2.4 Ascorbic acid (vitamin C)

Freeze-dried tamarillo powder (50 ± 0.5 mg) were extracted with 1 mL of aqueous metaphosphoric acid (MPA) (3%) (Abushita, Hebshi, Daood, & Biacs, 1997). Samples were vortexed for 30s and incubated in the dark for 30 min at 4°C with vortexing every 5 min. The mixture was then centrifuged at 10,000 RCF for 10 min at 4°C and the supernatant was stored at –20°C until analysis.

The LC-ESI-MS/MS coupled with Poroshell 120 EC-C18 column (150 x 2.1 mm, 2.7 μm; Agilent, USA) and maintained at 22.6°C. A 0.1% formic acid in Milli-Q was mobile A and 0.1% formic acid in acetonitrile was mobile phase B. The LC gradient was kept constantly with 90% of A with the total time of 6.0 min. The injection volume and flow rate were 3.0 μL and 0.2 mL/min, respectively. Chromatograms of standards of α-tocopherol, β-carotene and ascorbic acid are presented in Appendix B5 and summary of method validation are presented in Appendix B6. The MS was run in the negative mode with the total time of 6.0 min. The MMI source operating at ESI parameters were as follows: the gas temperature of 300°C with a flow rate of 6 L/min. The capillary voltage and the nebulizer were set to 4 kV and at 15 psi, respectively.

2.3.2.5 Carotenoid and chlorophyll pigments

Dried and powdered tamarillo (50 ± 0.5 mg) were extracted with 1 mL of acetone containing 1 mg/L TBP as internal standard. The sample was vortexed for 30s and incubated in the dark for 5 min at 4°C, followed by centrifuged at 10,000 RCF at 4°C for 10 min. The supernatant was separated and kept at –20°C until analysis.

The LC-ESI-MS/MS coupled with diode-array detector (DAD), fluorescence detection (FLD) and a XSelect C18 column (100 x 2.1 mm, 3.5 μm; Waters, Ireland) were used. The injection volume and flow rate were 8 μL and 0.3 mL/min, respectively. The solvents used were: (A) 0.1% formic acid in Milli-Q; (B) 0.1% acetic acid in acetonitrile containing 10 mM NH_4; and (C) a mixture of 80% isopropanol, 20% acetonitrile and 0.1% acetic acid containing 10 mM NH_4. The mobile phases

gradient was: 0 – 0.5 min, 50% of A and 50% of B; 0.5 – 10 min, 30% of A and 70% of B; 10 – 20 min, 3% of A and 97% of B; 20 – 20.20 min, 3% of A and 47% of B; 20.20 – 21 min, 3% of A and 97% of B; 21 – 28 min, 50% of A and 50% of B. Double detection was implemented in a DAD between 300 and 640 nm. The MS was run in the positive ion mode. The MMI source operating at ESI were gas temperature of 300°C, gas flow of 5 L/min, vaporizer temperature of 200°C, nebulizer gas at 50 psi and capillary voltage of 2 – 2.5 kV.

2.3.2.6 Phenolic compounds

Freeze-dried tamarillo (50 ± 0.5 mg) was extracted with 1 mL of 50% methanol. The mixture was vortexed for 30s and incubated in the dark at 4°C for 30 min with vortexing every 5 min, then centrifuged at 10,000 RCF at 4°C for 10 min. The supernatant was collected and centrifuged again at 10,000 RCF at 4°C for 10 min. The extract was separated and then kept at –20°C until analysis.

The LC-ESI-MS/MS coupled with Cortecs C18 column (100 x 2.1 mm, 2.7 μm; Waters, Tauton, Ireland) was used. Flow rate and injection volume were 0.25 mL/min and 3 μL, respectively. Mobile phase A was 0.1% formic acid in Milli-Q and mobile phase B was 0.1% acetic acid in acetonitrile with gradient as: 0 – 12 min, 97% of A; 12 – 13.5 min, 75% of A; 13.5 – 15.5 min, 10% of A and 15.5 – 23 min, 97% of A. The MS was run in the negative mode with the total time of 23 min and the autosampler temperature was set at 4°C. The MMI source operated at ESI parameters of gas temperature of 325°C and gas flow of 6 L/min. Nebulizer and capillary voltage and were set at 60 psi and to 2 kV, respectively. The column temperature and vaporizer temperature were 25°C and 200°C, respectively.

2.3.2.7 Anthocyanin compounds

Freeze-dried tamarillo (50 ± 0.5 mg) was extracted with a mixture of 50 μL formic acid, 200 μL IPA and 400 μL Milli-Q. The sample was vortexed for 30s, sonicated in a water bath for 20 min at 50°C, then adding 1 mL toluene into the solution. The mixture was vortex-mixed again and centrifuged at 10,000 RCF for 10 min. The lower, aqueous phase (60 – 80 μL) was centrifuged at 10,000 RCF for 5 min. Then, 50 μL of the extract was diluted with 50 μL of Milli-Q, then stored at −20°C until further analysis.

LC-ESI-MS/MS coupled with FLD, DAD and XSelect C18 column (100 x 2.1 mm, 3.5 μm; Waters, Tauton, Ireland) were used. Injection volume, total run time and flow rate were 1 μL, 9 min and 0.4 mL/min and, respectively. The mobile phase A and B were 0.1 % formic acid in acetonitrile and 0.6% formic acid in Milli-Q, respectively. The elution gradient was set at 0 – 4 min, 18% of A; 4 – 9 min, 6% of A. Double detection was carried out in DAD at 520 nm as the preferred wavelength. The optimised MS program conditions operating at ESI were gas temperature of 300°C, gas flow of 6 L/min, vaporizer temperature of 300°C, nebulizer gas at 60 psi and capillary voltage of 2.2 – 2.5 kV. Chromatograms of phenolic and anthocyanins standards as well as summary of method validation are shown in Appendix B7 and Appendix B8, respectively.

2.3.3 Total phenolic content (TPC) and antioxidant activity

For total phenolic content (TPC) of tamarillo, Folin-Ciocalteu assay (Dorman, Koşar, Kahlos, Holm, & Hiltunen, 2003) was used with some modifications. Freeze-dried sale (100 ± 1) mg sample was extracted with 4 mL of 50% MeOH, then homogenised and left to rest for 60 min. The sample was centrifuged at 1,500 RCF for 15 min, and the supernatant was transferred into a 10 mL volumetric flask. Then, the residue was extracted for a second time with 4 mL of 70% acetone, and then homogenisation, resting and centrifugation as described above. The supernatant was transferred to volumetric flask containing the first extract, then Milli-Q was added to the volumetric flask to the 10 mL mark. Then, 1 mL from the volumetric flask was transferred to another volumetric flask and diluted to the 10 mL mark with Milli-Q. This was used as the extracted sample solution.

A 1.0 mL of extracted sample solution was mixed with 500 μL Folin-Ciocalteu reagent, and the mixture was kept at room temperature for 5 min. Then, 1.5 mL of 20% sodium carbonate (Na_2CO_3) solution was added, and the mixture was incubated for 120 min at room temperature in the dark. The extracted sample solution was transferred to a cuvette, and absorbance was measured at 765 nm against the blank using a UV-spectrophotometer (Ultrospec 7000, Cambridge, England). Results were presented as mg of gallic acid equivalent per 100 gram of dry weight (mg GAE/100 g DW).

Two different methods were used to determine the antioxidant activity, namely cupric ion reducing antioxidant capacity (CUPRAC) and ferric-reducing antioxidant power (FRAP) assays The CUPRAC assay was conducted according to Özyürek et al. (2011) with some modifications. Sample preparation was similar as procedure described in the TPC protocol. A 1 mL of sample extract solution was mixed with 1 mL of $CuCl_2$ (0.01 M); 1 mL of ammonium acetate (1.0 M, pH 7.0); 1 mL of Neocuporine (0.0075 M) in 96% ethanol and 0.1 mL of Milli-Q. The reaction was left for 5 min, then the absorbance was measured at 450 nm on a UV-spectrophotometer against MilliQ. The CUPRAC values were presented in μmol Trolox equivalent antioxidant capacity per gram of dry weight (μmol TEAC/g DW). A standard curve of gallic acid standard (2.5 – 200 mg/L) and Trolox standard (2.5 – 160 mg/L) are presented in Appendix B9.

2.3.4 Volatile profile of peel and pulp of tamarillos

2.3.4.1 Analysis of volatile compounds by TD-GC-MS

Freeze-dried sample (1000 – 1200 μg) was placed in a glass TDU. Gas Chromatography consisting of Agilent 6890B GC and 5977B MSD (Agilent Technologies, Santa Clara, CA, USA) equipped with a Gerstel Multipurpose Sampler, Thermal Desorption (TD) Unit and Cooled Inlet System (CIS) were used to extract and analyse the volatiles. A Phenomenex ZB-1701 column (Phenomenex NZ, New Zealand) measuring 30 m × 250 μm × 0.15 μm, with a 5 m guard, was used to separate polar and non-polar volatile compounds. Helium was used as carrier gas with a constant flow rate of 1.0 mL/min.

The autosampler spiked 2 μL of 2-chlorophenol (10 mg/L) into each sample immediately prior to TD. The TD unit was run in solvent venting mode for 1.0 min at 40°C, then switched to splitless extraction and the temperature was increased at 650°C/min to 150°C and held for 4 min. In solvent venting mode operation, the CIS trapped and cryofocused volatile compounds at –40°C with a helium flow of 80 mL/min. Then, the GC-MS started, and the CIS was operated in splitless mode with a fixed helium flow rate of 1.1 mL/min and a septum purge flow of 3 mL/min. Trapped volatile compounds were released from the CIS by ballistic heating at 11°C/s to 290°C and held for 4 min. Then, the helium flow to the split vent was set to 45 mL/min for

the rest of the run. The mass spectrometer scanned from 43 to 450 m/z at a rate of 5.5 scans per second.

Linear retention indices (LRIs) were calculated using the retention times of *n*-alkanes series (C_7–C_{30}). The peak areas of each feature in every sample were quantified relatively to that of the internal standard. The results for each target were blank subtracted and normalised to the sample mass.

2.3.4.2 Comparison of volatile component between fresh and dried samples

Volatile components of fresh and dried samples ('Laird's Large' pulp) was compared to assess whether loss of volatile compounds was considerable from the freeze-drying process using SPME-GC-MS. A 1 g of fresh fruit and 0.1 g of dried fruit were immediately placed in 10 mL headspace vial with addition of 2 μL of the internal standard (10 mg/L of 2-chlorophenol solution). The heads-space vial was heated at 50°C for 15 min using an incubator coupled with an agitator at a speed of 250 rpm. The SPME fibre was 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS), StableFlex fibre 24 ga, length of 2 cm (Supelco, Bellefonte, PA, USA). The GC-MS and Phenomenex ZB-1701 column described above were also used

To minimize the sugar artefact and oxidation effect in the fresh sample (90% of moisture content), another comparison test also carried out on 'Laird's Large' pulp sample using TD-GC-MS at 50°C. Also, the no solvent delay mode was set up to ensure the detection of volatile compounds with low boiling point. All other parameters were the same as the main experiment (Section 2.3.4.1). The fresh sample was stored at –20°C and placed in the GC-MS immediately before analysis to minimize enzymatic activity.

2.3.4.3 Odour threshold and relative odour activity value (OAV)

To identify the odour activity value (OAV) for tamarillo, odour threshold values from tomato (the same *Solanum* genus) (Wang et al., 2018a) and odour threshold values published by Leffingwell & Associates were used. The relative OAVs were calculated by dividing the relative concentrations of volatile compounds by their odour thresholds. the lowest value of the odour threshold was applied for the compounds having a range of odour thresholds (e.g. 5-hydroxymethylfurfural). Only the compounds with relative OAVs greater than 1 were acknowledged to contribute to the

tamarillo flavour. The odour description of these volatile compounds was retrieved from Acree and Arn (2004).

2.3.5 Statistical analysis

For each experiment, at least three independent measurements ($n \geq 3$) were used to calculate mean and standard deviation. One-way and two-way analysis of variance (ANOVA) and Fisher's (LSD) multiple comparison tests were applied to determine significant differences where appropriate. Pearson's correlation coefficient was applied to identify correlation Data analysis was implemented using SPSS 25.0 (IBM Corp., Armonk, New York, USA) and the statistical significance level was set at $p < 0.05$. Principal Component Analysis (PCA) were used to assess variability among samples, and the concentrations of the several analytes were visualised by heatmap and hierarchical clustering. Both PCA and heatmap were carried out using the MetaboAnalyst web interface (Chong, Yamamoto, & Xia, 2019).

2.4 Results and discussion

All results presented here have been published or partly published in peer-review journals and used with permission (Diep et al., 2020a; Diep et al., 2020b; Diep et al., 2020c; Diep et al., 2021)

2.4.1 Physical properties and proximate compositions of three New Zealand tamarillo cultivars

Significant difference in size, colour and proximate content was observed among three cultivars ($p < 0.05$) (Table 2.9). The 'Mulligan' was half weight of the 'Amber' and 'Laird's Large'. Comparing to the yellow and red cultivars, the mean diameter of the purple-red type was significantly lower by 22.7% and 26.6%, respectively. Meanwhile, the purple-red cultivar was significantly shorter by 17.6% and 21.1%, than that of the yellow and red types, respectively for the length. According to the BBCH (Biologische Bundesanstalt, Bundessortenamnt, Chemische Industrie) scale, all of the New Zealand tamarillos fell in the growth stage 8 and was classified as ripe (the weight of 30 to 160 g, diameter of 35 to 60 mm and length of 40 to 80 mm) (Acosta-Quezada et al., 2016). The colour parameters measured in L*, a* and b*

ranged from 27.77 to 61.75, 22.02 to 28.71 and 8.78 to 53.20, respectively (Table 2.9). The purple-red type showed the lowest value of L*, followed by red and yellow cultivars. It was logical to observe the 'Amber' type having the lowest a* and highest b* while these values were opposite for the 'Laird's Large' and 'Mulligan' cultivars. The purple-red type reached the ripeness stage of RS10 (RS1 to RS11: unripe to over-ripe). The red cultivar was at the RS9 ripeness stage (based on the L* and b* values) while based on a* value**,** this type was at the RS10 ripeness stage (Mwithiga, Mukolwe, Shitanda, & Karanja, 2007).

The pH range of 3.74 – 4.05 was observed for the New Zealand tamarillos and tamarillos from New Zealand showed higher pH range than that from Ecuador and Spain (Vasco et al., 2009). A significant difference ($p < 0.05$) in the pH was found, being lowest in the 'Amber' and the highest in the 'Mulligan'. The 'Mulligan' acidity was 7.6% and 6.9% lower than that of the 'Amber' and 'Laird's Large' varieties, respectively. This result indicated the 'Mulligan' and 'Amber' was the least and the most acidic cultivar, respectively. The 8.80 – 10.63°Brix range, known as total soluble solids (TSS), was observed in New Zealand tamarillos which differed from Ecuadorian purple-red and golden-yellow tamarillos with 11 to 12 and 10 to 11, respectively (Vasco et al., 2009). The sweetest and sourest cultivar was 'Laird's Large' and 'Mulligan', respectively. The 'Amber' and 'Mulligan' tamarillos showed 11.6% and 17.2% less of TSS than the 'Laird's Large', respectively.

Moisture was the main chemical component of the tamarillo pulp (Table 2.9) which could be correlated to the TSS where the higher the moisture content, the lower the TSS was found. The current results were relatively similar to moisture content for New Zealand gold (89%) and red (87.8%) tamarillo (Lister et al., 2005) and updated moisture content of yellow (86.3%) and red (86.1%) tamarillos (New Zealand Food Composition Database, 2019). The current results were consistent with the moisture content of 86 – 88% and 87 – 92% for yellow and purple-red tamarillos, respectively. (Schotsmans et al., 2011).

Ash content of all New Zealand tamarillos were in agreement with previous report as purple-red (0.69 – 1.26%) and gold (0.7 – 0.82%) cultivars (Schotsmans et al., 2011). The ash content of 0.8% in both yellow and red tamarillos was reported by New Zealand Food Composition Database (2019). The ash content of 'Mulligan' was significantly higher (by 16.2% and 33.3%) than that of the 'Laird's Large' and

‘Amber’, respectively. ‘Amber’ had similar ash content to golden-yellow tamarillo from Spain (0.7%) and Ecuador (0.8%), whereas ash contents in the red and purple-red varieties were higher than those from Spain (0.7%) and Ecuador (0.9%) (Vasco et al., 2009).

Significant difference of protein among three cultivars ($p < 0.05$) was observed, being lowest and highest in ‘Laird’s Large’ (1.15%) and ‘Amber’ (1.43%) cultivars, respectively (Table 2.9). The ‘Mulligan’ and ‘Amber’ tamarillos showed 8.7% and 19.6% more protein than ‘Laird’s Large’ variety, respectively. Result from the current study was slightly lower than findings of Lister et al. (2005) (1.9% and 1.8% for gold and red cultivars, respectively) and New Zealand Food Composition Database (2019) (1.9% and 2.0% for yellow and red varieties, respectively). This might be due to different analytical techniques: Kjeldahl method in the current research compared to combustion method by Lister et al. (2005). The protein contents found in this study were also lower than those from Ecuador and Spain (2.4 – 2.5% and 2.2% for golden-yellow and purple-red tamarillos, respectively) (Vasco et al., 2009).

Lipid contents in the New Zealand grown tamarillos from the current study were also lower (0.17 – 0.24%) than that from the previous reports (Lister et al., 2005; New Zealand Food Composition Database, 2019). Lipid contents of 0.2% in gold and 0.5% in red types have been reported by Lister et al. (2005), whereas an updated lipid value for red and yellow tamarillos was 0.4 and 0.5%, respectively (New Zealand Food Composition Database, 2019). Schotsmans et al. (2011) reported wide range of lipid content in tamarillo with 0.05 – 0.72% and 0.08 – 0.6% for yellow and purple-red varieties, respectively. The identification of lipid content might have been affected by the moisture content as dried samples were utilized for analysis.

The current available carbohydrate values were relatively similar to results reported by New Zealand Food Composition Database (2019); 3.5 and 3.4% for red and yellow tamarillos, respectively. The ‘Laird’s Large’ and ‘Amber’ showed significantly higher ($p < 0.05$) carbohydrate content by 28.7% and 12.1% to the ‘Mulligan’ cultivar, respectively. By calculating the sum of sugars and starch, Lister et al. (2005) reported the carbohydrate content of 4.6% and 4.3% for gold and red cultivars, respectively. Hence, the current values were lower, which might be due to the use of different analysis.

Approximately 3% of the dietary fibre in tamarillo has been found to be high in the current study, supporting previous findings in the literature. Insignificant differences in dietary fibre among the cultivars were found with 'Mulligan' and 'Amber' being the highest and lowest, respectively. Previous studies have reported dietary fibre value of 3.2% and 3.3% in New Zealand yellow and red tamarillos, respectively (New Zealand Food Composition Database, 2019). Using AOAC Prosky method, Lister et al. (2005) reported dietary fibre of 3.1 and % 3.6% for gold and red tamarillos, respectively. In comparison to several fruits such as peach (2.4%), banana (2.2%), mango (1.8%), orange (1.7%), grape (1.5%) and pineapple (1.1%) (Pérez-Jiménez & Saura-Calixto, 2017), tamarillo has a higher content of dietary fibre zthan those fruits. In addition to reducing blood cholesterol (Causey, Feirtag, Gallaher, Tungland, & Slavin, 2000) and blood sugar levels (Kaczmarczyk, Miller, & Freund, 2012), fruits with high amounts of dietary fibre may reduce the risk of coronary heart disease and cancer (Lattimer & Haub, 2010).

Table 2.9 Physical properties and proximate compositions of three New Zealand grown tamarillo cultivars.

Parameters	'Amber' (yellow)	'Laird's Large' (red)	'Mulligan' (purple-red)
Weight (g)	90.32 ± 14.12^{a}	107.52 ± 18.52^{b}	44.14 ± 3.64^{c}
Diameter (mm)	49.97 ± 3.26^{a}	52.63 ± 3.07^{b}	38.62 ± 1.76^{c}
Length (mm)	69.07 ± 3.49^{a}	72.10 ± 3.64^{b}	56.91 ± 2.75^{c}
Lightness (L*)	61.75 ± 2.33^{a}	30.64 ± 2.55^{b}	27.77 ± 1.52^{c}
Redness (a*)	22.02 ± 2.28^{a}	27.80 ± 3.82^{b}	28.71 ± 1.72^{c}
Yellowness (b*)	53.20 ± 2.24^{a}	11.98 ± 2.35^{b}	8.78 ± 1.23^{c}
pH	3.74 ± 0.02^{a}	3.77 ± 0.01^{b}	4.05 ± 0.01^{c}
TSS (°Brix)	9.40 ± 0.00^{a}	10.63 ± 0.06^{b}	8.80 ± 0.10^{c}
Moisture (%)	88.39 ± 0.36^{a}	88.14 ± 0.26^{a}	89.14 ± 0.40^{b}
Ash (%)	0.78 ± 0.03^{a}	$0.98 \pm 0.03^{a,b}$	1.17 ± 0.03^{b}
Protein (%)	1.43 ± 0.02^{a}	1.15 ± 0.04^{b}	1.26 ± 0.01^{c}
Lipid (%)	0.17 ± 0.01^{a}	0.29 ± 0.01^{b}	0.24 ± 0.01^{c}
Dietary fibre (%)	2.81 ± 0.13^{a}	2.99 ± 0.14^{a}	3.33 ± 0.18^{a}
Carbohydrate (%)	3.05 ± 0.18^{a}	3.76 ± 0.09^{b}	2.68 ± 0.01^{c}

TSS: Total soluble solids

* Data are presented as Mean ± SD (n ≥ 3). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row. Used with permission (Diep et al., 2020c)

2.4.2 Reducing sugars content

In tamarillo, the variety of sugar components play a key role in sweet taste and contribute as main precursor of aroma compounds. This study showed comprehensive sugar composition in tamarillo with usage of aqueous acetonitrile to enhance extractability of sugars. The poor extractability of sugars as well as strong bonds between cellulose and hemicellulose had caused difficulties in identification of mannose, xylose and ribose (Gannasin et al., 2015a). Previous studies had applied GC to analyse reducing sugars (Do Nascimento et al., 2013; Do Nascimento et al., 2016a; Lister et al., 2005), however, some disadvantages when using this technique have been proposed. Reducing sugars need to be converted into volatile derivatives with high thermal stability because these compounds are non-volatiles, hence this causes difficulty for accurate quantification of sugars. Rather than simultaneous quantification with other sugars, uronic acids were separately determined by using the *m*-hydroxybiphenyl method (Do Nascimento et al., 2013; Do Nascimento et al., 2016a). Hence, in the current study, PMP derivatization and LC-MS/MS were utilized to enhance the detection sensitivity and specificity for simultaneous quantification of complex reducing sugars and uronic acid contents with some advantages including causing no desialylation and isomerization, reacting PMP with reducing sugars under mild conditions and requiring no acid catalyst for reaction (Bai et al., 2015).

The concentrations of individual sugar, uronic acid and total reducing sugars (TRS) were significant differences ($p < 0.05$) among all samples (Table 2.10). Pulps showed higher TRS content than peels, approximately 2.4, 2.5 and 4.8times for ‘Mulligan’, ‘Laird’s Large’ and ‘Amber’, respectively. The TRS result was in agreement with the TSS results in which the highest and lowest TRS content was observed in ‘Laird’s Large’ and ‘Mulligan’ cultivars among all pulp samples. Two most dominant sugars in tamarillo were glucose and fructose, followed by galactose and mannose regardless of the cultivars and tissues. For these abundant sugars, the Laird’s Large showed the highest concentrations; while fructose showed higher concentrations than glucose in

all three cultivars. This result was similar to previous research reported by Acosta-Quezada et al. (2015) for five tamarillo groups. Prohens and Nuez (2001) had summarized the range of glucose and fructose concentrations in tamarillo from various sources with 0.5 – 1.0 g/100 g FW (3.2 – 6.4 g/100 g DW) and 0.7 – 1.2 g/100 g FW (4.48 – 7.69 g/100 g DW), respectively. Glucose content of both yellow and red tamarillos from New Zealand was 0.8 g/100 g FW (5.8 g/100g DW), whereas fructose content of 0.9 g/100 g FW (6.6 g/100g DW) has been recently reported in both yellow and red tamarillos (New Zealand Food Composition Database, 2019). The average concentrations of glucose and fructose in red and purple tamarillos were 8.9 – 9.0 g and 9.0 g/100 g DW, respectively (Acosta-Quezada et al., 2015). Glucose and fructose have been considered as positive molecules in terms of antioxidant ability (Hu, Sun, Pu, & Pan, 2016). High concentration of glucose accelerates the pentose phosphate routine, and then lead to a higher level of nicotinamide adenine dinucleotide phosphate (NADPH) which is related to protection against the toxicity of reactive oxygen species (ROS) (Cruz de Carvalho, 2008).

The next sugars in importance after glucose and fructose were galactose and mannose; with relatively similar concentrations of these sugars between the samples were observed (Table 2.10). Galactose has been an immediate precursor for ascorbic acid synthesis (Hu et al., 2016) as well as vital compound in energy delivery for human metabolism (Coelho, Berry, & Rubio-Gozalbo, 2015). Mannose showed a capacity to deal with urinary tract infections (UTIs) and bladder inflammation (Domenici et al., 2016) and decrease the growth of *in vitro* cancerous tumors (Gonzalez et al., 2018).

Besides, small amounts of arabinose, rhamnose, ribose and xylose (≤ 0.07 g/100 g DW) were observed in tamarillo cultivars with significant differences among all samples ($p < 0.05$) (Table 2.10). The existence of arabinose, galactose, xylose as well as uronic acid indicated the presence of pectin in tamarillo. Based on approach of Renard and Ginies (2009), the ratios of (arabinose + galactose) to rhamnose were 1:20, 1:49, 1:40, 1:107, 1:124 and 1:113 for ‘Amber’ peel, ‘Laird’s Large’ peel, ‘Mulligan’ peel, ‘Amber’ pulp, ‘Laird’s Large’ pulp and ‘Mulligan’ pulp, respectively. Hence, it could be concluded that pulp pectin had higher amounts of neutral side chains than peel pectin. Besides, ‘Laird’s Large’ showed higher amounts of neutral side chains of pectin than the ‘Amber’ and ‘Mulligan’ cultivars regardless of tissues. With a wide range of concentration, glucuronic acid has been recognized with capacity to bind

toxic molecules and improve their excretion by kidneys or intestines which showed benefits for human health (Jayabalan, Marimuthu, & Swaminathan, 2007).

PCA was performed to assess the correlation between the reducing sugars and uronic acid distribution in both peel and pulp of three tamarillo cultivars. The separation of various tamarillo samples based on the total reducing sugar profile was clearly visible (Figure 2.9A). Three New Zealand tamarillos were completely resolved by the tissue type on PC1 (describing 79.3% of the variance) and by the cultivar on PC2 (demonstrating another 11.1 % of the variance), respectively. Additionally, 6.7% of the variance was only explained by the third principal component (data not shown, 97.1% total variance explained) and thus, no further PCs were considered. Heatmap cluster analysis showed a clear separation between peel and pulp of tamarillo (Figure 2.9B). All peel samples were in the same cluster which were separated from pulp cluster.

Table 2.10 Reducing sugars contents in peel and pulp of three tamarillo cultivars from New Zealand.

Sugars / uronic acid	Concentration (g/100 g DW)					
	'Amber' peel	'Amber' pulp	'Laird's Large' peel	'Laird's Large' pulp	'Mulligan' peel	'Mulligan' pulp
Mannose	0.19 ± 0.03^{a}	0.95 ± 0.07^{b}	0.41 ± 0.03^{c}	1.1 ± 0.14^{d}	0.34 ± 0.02^{c}	1 ± 0.12^{bd}
Fructose	1.84 ± 0.51^{a}	9.19 ± 0.11^{b}	5.14 ± 0.29^{c}	13.15 ± 1.09^{d}	3.58 ± 0.23^{e}	8.43 ± 0.96^{b}
Xylose	0.02 ± 0^{a}	0.03 ± 0^{b}	0.03 ± 0^{b}	0.04 ± 0^{c}	0.03 ± 0^{d}	0.03 ± 0^{d}
Rhamnose	0.01 ± 0^{a}	0.01 ± 0^{b}	0.01 ± 0^{b}	0.01 ± 0^{c}	0.01 ± 0^{b}	0.01 ± 0^{c}
Galactose	0.16 ± 0.12^{a}	1.01 ± 0.07^{b}	0.44 ± 0.02^{c}	1.18 ± 0.15^{b}	0.37 ± 0.02^{c}	1.08 ± 0.13^{b}
Glucose	1.29 ± 0.35^{a}	6.33 ± 0.04^{b}	3.55 ± 0.2^{c}	9.05 ± 0.73^{d}	2.47 ± 0.15^{e}	5.82 ± 0.64^{b}
Ribose	0.02 ± 0.01^{a}	0.04 ± 0^{b}	0.03 ± 0^{a}	0.07 ± 0.01^{c}	0.02 ± 0^{a}	0.05 ± 0.01^{b}
Arabinose	0.04 ± 0.01^{ab}	0.06 ± 0^{d}	0.05 ± 0^{bc}	0.06 ± 0.01^{cd}	0.03 ± 0^{a}	0.05 ± 0.01^{cd}
Glucuronic acid	0.07 ± 0.02^{a}	0.04 ± 0^{b}	0.04 ± 0^{b}	0.04 ± 0.01^{b}	0.04 ± 0^{b}	0.06 ± 0.01^{a}
Total	3.64 ± 1.06^{a}	17.66 ± 0.3^{b}	9.69 ± 0.55^{c}	24.70 ± 2.12^{d}	6.91 ± 0.42^{e}	16.54 ± 1.88^{b}

* Data are expressed as Mean ± SD (n = 3). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row. Used with permission (Diep et al., 2020c). DW: Dry weight

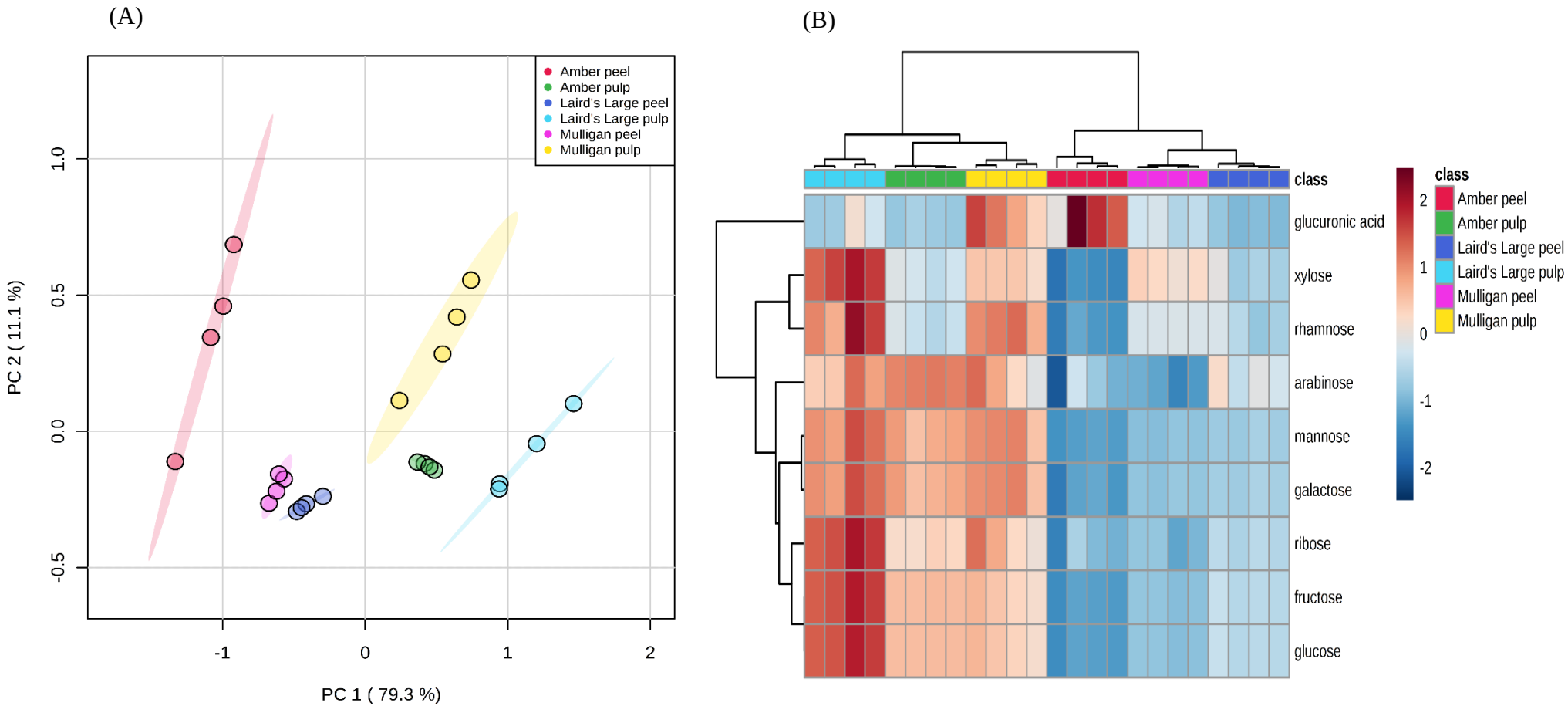

Figure 2.9 (A) Principal Component Analysis and (B) Heatmap of reducing sugars and uronic acid concentrations in both peel and pulp of three New Zealand tamarillo cultivars.

Used with permission (Diep et al., 2020c)

2.4.3 Free amino acid (FAA) content

Amino acid content have been considered an important parameter which can be used to determine the optimum time of ripeness and can influence quality of fruits (aroma, colour and taste as well as of fruit-derived products) (Silva et al., 2004). Building blocks of protein and polypeptides, maintenance of reproduction and immunity functions, and antioxidants have been significant roles of amino acids (Egydio, Santa Catarina, Floh, & dos Santos, 2013). Known as tree tomato, tamarillo would be expected to own high amount of GABA since this fruit has the same *Solanum* genus with tomato. The current study has applied the accutag derivatization method to overcome the issues (complete destruction of tryptophan and partial oxidization of methionine and cystine) from previous study of Gannasin et al. (2015a) who used acidic hydrolysis method.

Among 25 analysed amino acids, 22 compounds (9 essential and 13 non-essential amino acids) were detected in peel and pulp of the three tamarillo cultivars. Two essential amino acids (L-lysine and L-tryptophan) and 5 non-essential amino acids (L-ornithine, L-cystine, ethanolamine, taurine and amino-*n*-butyric acids in terms of α-, β- and γ- forms) are being reported for the first time in this study when comparing to 16 amino acids being detected in Malaysian tamarillo from study of Gannasin et al. (2015a). 'Amber' and 'Laird's Large' showed highest and the lowest of total free amino acids (TFAAs), respectively in both peel and pulp (Table 2.11). The FAA result was coherent with the protein result reported in section 2.4.1 with significantly different concentrations ($p < 0.05$) of amino acids among all samples (Table 2.11).

Glutamic acid was the most abundant amino acid in tamarillo regardless of the cultivar and tissue, which is in good agreement with Gannasin et al. (2015a). The amounts of glutamic acid, which carries umami taste and often used as a food additive and flavour enhancer in the form of monosodium glutamate (MSG) (Oruna-Concha, Methven, Blumenthal, Young, & Mottram, 2007), were higher in pulps than in peels (approximately 4 – 6 times). Tamarillo pulp showed relatively similar γ-Amino-n-butyric acid (GABA) content (approximately equal to 42.7 – 58.7 mg/100 g FW) to the average content in 18 tomato varieties with 50.3 – 66.8 mg/100 FW (Saito et al., 2008). Also, peel and pulp of tamarillo owned higher GABA content than peel and pulp of potato, the same *Solanum* genus, with 108 – 143 and 216 – 299 mg/100 g DW,

respectively and also higher than cherry tomato (306 mg/100 g DW) (Ramos-Ruiz, Poirot, & Flores-Mosquera, 2018). From findings of Ito, Ueno, and Kikuzaki (2017), daily intake of 10 – 20 mg of GABA has been enough to reduce blood pressure in human. Hence, long-term daily consumption of one tamarillo fruit, where about 53, 56 and 19 mg of GABA are present in 'Amber', 'Laird's Large' or 'Mulligan', may be sufficient to reduce blood pressure to a normal level.

Amino acid profiles of tamarillo were also dominated by L-aspartic acid as the second abundant compound in 'Amber' type and as the third one in 'Laird's Large' and 'Mulligan' regardless of the tissue types. L-proline, being recognized as the third abundant amino acid in 'Amber' peel, was presented in a higher quantity in pulp than in peel of 'Amber' and 'Mulligan' cultivars. Proline is an osmoprotectant and it is widely used in pharmaceutical, biotechnological industry (Chien-an, Phang, & Valle, 2008), which may suggest for alternative use of tamarillo. The potentials for health benefit of tamarillo can be contributed by the presence of 9 essential amnio acids. For example, valine is necessary for mental focus and emotional calm, whereas threonine and methionine are essential for healthy skin. Both leucine and isoleucine helps blood sugar regulation, and hormone production (Mohanty et al., 2014). Histidine accelerates creation of blood cells as well as enhances insulin resistance, reduces fat mass and inhibits inflammation and oxidative stress in obese women (Feng et al., 2013). The essential amino acid profile of tamarillo was dominated by L-histidine and then, L-lysine regardless of the tissues (Table 2.11). L-histidine and L-lysine showed higher contents in pulps than in peels by approximately 2 – 4 times and 1.6 – 2.4 times, respectively.

Amino acid profiles of tamarillo differed from cultivars and tissues were clearly visible through PCA (Figure 2.10A) which were perfectly resolved by tissue type on the PC1 (56.8% of the variance) and by cultivar on PC2 (24.8% of the variance). The third principal component explained 9.2% of the variance and therefore, as the samples were already resolved, no further PCs were considered. For PC1, all peel samples were located on the negative region while all of the pulp samples fell in the positive region. For PC2, pulp and peel of the 'Amber' cultivar were located on the positive region, pulp and peel of 'Laird's Large' and 'Mulligan' cultivars were in located on the negative area. All peel samples were in the same cluster which were clearly separated from pulp cluster which are shown in heatmap (Figure 2.10B).

Table 2.11 Free amnio acid (FAA) contents in the peel and pulp of three New Zealand grown tamarillo cultivars.

Amnio acids	Concentration (mg/100 g DW)					
	'Amber' peel	'Amber' pulp	'Laird's Large' peel	'Laird's Large' pulp	'Mulligan' peel	'Mulligan' pulp
L-Histidine	41.4 ± 3.8^{a}	164 ± 4.5^{b}	30.1 ± 2.8^{c}	76.0 ± 3.7^{d}	44.2 ± 1.5^{a}	80.2 ± 3.0^{e}
L-Threonine	8.6 ± 0.6^{a}	17.4 ± 0.4^{b}	10.0 ± 1.0^{c}	15.4 ± 0.7^{d}	11.9 ± 0.6^{e}	14.4 ± 0.6^{f}
L-Lysine	32.9 ± 3.7^{a}	77.3 ± 2.9^{b}	35.7 ± 3.5^{a}	67.1 ± 4.0^{c}	42.7 ± 2.7^{d}	69.0 ± 2.8^{c}
L-Valine	19.3 ± 1.7^{a}	25.8 ± 1.0^{b}	18.3 ± 1.3^{a}	22.4 ± 0.8^{c}	21.1 ± 0.4^{cd}	20.6 ± 0.9^{d}
L-Methionine	11.7 ± 1.1^{a}	21.0 ± 1.4^{b}	4.2 ± 0.5^{c}	7.6 ± 0.4^{d}	6.2 ± 0.3^{e}	9.9 ± 0.4^{f}
L-Leucine	20.7 ± 1.7^{a}	28.6 ± 1.2^{b}	17.0 ± 1.2^{c}	21.4 ± 0.9^{a}	24.4 ± 0.4^{d}	23.5 ± 0.9^{d}
L-Isoleucine	19.0 ± 2.1^{a}	28.3 ± 0.9^{b}	17.6 ± 1.2^{a}	24.6 ± 0.8^{c}	21.2 ± 0.3^{d}	26.2 ± 1.4^{e}
L-Phenylalanine	14.3 ± 0.9^{a}	24.4 ± 0.8^{b}	11.5 ± 0.9^{c}	16.2 ± 0.7^{d}	13.1 ± 0.8^{e}	17.1 ± 0.9^{d}
L-Tryptophan	8.6 ± 0.5^{a}	15.1 ± 0.3^{b}	8.1 ± 0.5^{a}	12.6 ± 0.6^{c}	10.7 ± 0.6^{d}	14.5 ± 0.9^{b}
TEFAAs	176 ± 16^{a}	402 ± 13.4^{b}	153 ± 12.9^{c}	263 ± 12.7^{d}	195 ± 7.4^{e}	275 ± 11.8^{f}
Hydroxy-L-Proline	2.6 ± 0.2^{a}	1.2 ± 0.1^{b}	2.3 ± 0.2^{c}	1.3 ± 0.1^{b}	2.3 ± 0.2^{d}	0.4 ± 0.03^{e}
L-Arginine	16.9 ± 2.9^{a}	50.2 ± 4.4^{b}	14.6 ± 1.0^{a}	30.1 ± 2.3^{c}	16.1 ± 1.1^{a}	29.8 ± 2.6^{c}
Ethanolamine	14.4 ± 0.9^{a}	20.0 ± 0.5^{b}	11.6 ± 0.9^{c}	21.1 ± 0.7^{d}	15.8 ± 0.5^{e}	23.1 ± 0.9^{f}
L-Serine	38.6 ± 3.6^{a}	54.0 ± 1.6^{b}	30.8 ± 2.7^{c}	42.0 ± 1.4^{d}	33.5 ± 0.6^{e}	37.9 ± 1.5^{a}
Glycine	3.2 ± 0.3^{a}	8.6 ± 0.3^{b}	2.8 ± 0.4^{c}	7.1 ± 0.2^{d}	5.1 ± 0.3^{e}	9.2 ± 0.4^{f}
Sarcosine	n.d	n.d	n.d	n.d	n.d	n.d
L-Aspartic acid	255 ± 18.1^{a}	574 ± 15.1^{b}	159 ± 10.4^{c}	309 ± 9.4^{d}	171 ± 4.8^{c}	314 ± 18.1^{d}
β-Alanine	9.0 ± 0.8^{a}	5.8 ± 0.2^{b}	1.1 ± 0.1^{ce}	1.5 ± 0.2^{cd}	1.9 ± 0.1^{d}	0.9 ± 0.1^{e}

Taurine	0.7 ± 0.1^{ab}	0.9 ± 0.2^{c}	0.6 ± 0.04^{a}	0.8 ± 0.1^{b}	0.7 ± 0.1^{ab}	0.7 ± 0.04^{ab}
L-Glutamic acid	707 ± 50.3^{a}	4032 ± 58.4^{b}	332 ± 17.1^{c}	2101 ± 59.4^{d}	540 ± 13.2^{e}	2284 ± 114^{f}
L-Alanine	54.7 ± 4.9^{a}	88.7 ± 1.1^{b}	35.2 ± 2.5^{c}	77.8 ± 2.3^{d}	83.6 ± 1.3^{e}	88.1 ± 4.3^{b}
γ-Aminobutyric acid	168 ± 14.5^{a}	489 ± 18.2^{b}	273 ± 25.4^{c}	433 ± 8.3^{d}	245 ± 5.9^{e}	355 ± 15.2^{f}
L-Proline	245 ± 18.5^{a}	286 ± 9.4^{b}	128 ± 7.0^{c}	121 ± 3.4^{c}	58.8 ± 2.1^{d}	47.3 ± 2.1^{e}
β-Amino-isobutyric acid	0.4 ± 0.04^{a}	0.2 ± 0.01^{b}	0.03 ± 0.02^{c}	0.02 ± 0.02^{cd}	0.04 ± 0.01^{c}	$< 0.004^{d}$
α-Aminobutyric acid	0.5 ± 0.1^{a}	0.6 ± 0.1^{b}	0.7 ± 0.1^{b}	1.1 ± 0.1^{c}	0.3 ± 0.02^{d}	0.3 ± 0.03^{d}
δ-Hydroxylysine	n.d	n.d	n.d	n.d	n.d	n.d
L-Ornithine	1.5 ± 0.3^{a}	3.0 ± 0.1^{a}	26.5 ± 3.4^{b}	21.3 ± 1.4^{c}	8.1 ± 0.6^{d}	3.2 ± 0.2^{a}
Cystathionine	n.d	n.d	n.d	n.d	n.d	n.d
L-Anserine	n.d	n.d	n.d	n.d	n.d	n.d
L-Cystine	0.1 ± 0.03^{a}	n.d	n.d	0.04 ± 0.03^{a}	0.01 ± 0.01^{b}	n.d
L-Tyrosine	60.1 ± 4.2^{a}	60.0 ± 1.7^{a}	22.4 ± 1.5^{b}	24.4 ± 1.2^{bc}	27.3 ± 1.7^{d}	26.2 ± 1.6^{cd}
L-Homocystine	n.d	n.d	n.d	n.d	n.d	n.d
TNEFAAs	*1577 ± 120^{a}*	*5675 ± 111^{b}*	*1040 ± 62.6^{c}*	*3192 ± 90.5^{d}*	*1209 ± 32.6^{e}*	*3220 ± 161^{d}*
TFAAs	*1753 ± 136^{a}*	*6077 ± 125^{b}*	*1192 ± 75.5^{c}*	*3455 ± 103^{d}*	*1405 ± 40.0^{e}*	*3495 ± 172^{d}*

n.d: not detected. Data are expressed as Mean ± SD (n ≥ 4). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row. DW: Dry weight; TEFAAs: total essential free amino acids; TNEFAAs: total non-essential free amino acids; TFAAs: total free amino acids Used with permission (Diep et al., 2020c)

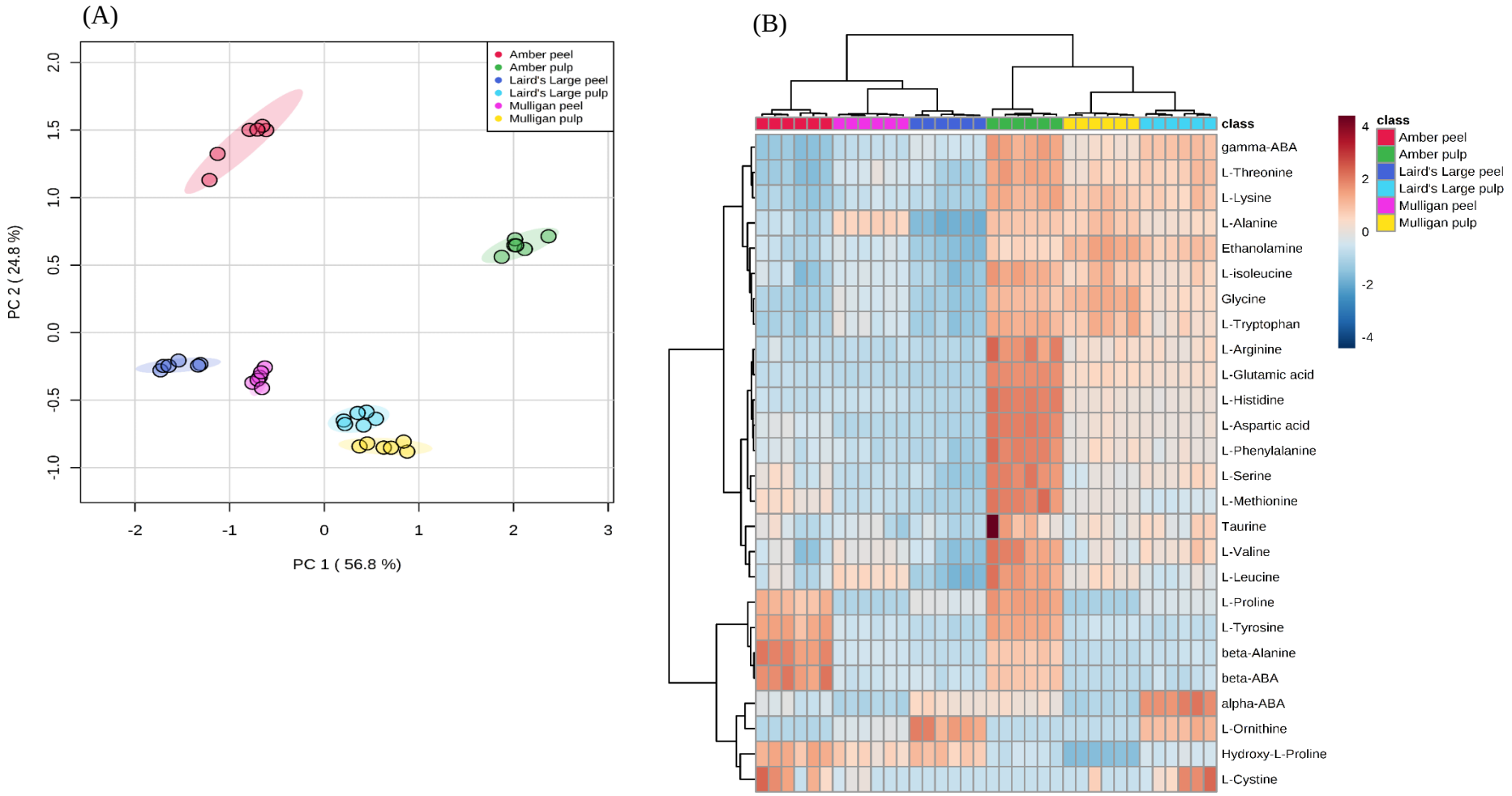

Figure 2.10 (A) Principal Component Analysis and (B) Heatmap of free amino acids concentration in both peel and pulp of three New Zealand tamarillo cultivars (ABA: Aminobutyric acid).

Used with permission (Diep et al., 2020c)

2.4.4 β-carotene and α-tocopherol contents

Beta-carotene has received a lot of attention as potential anti-cancer and anti-aging phytochemical. The results showed that pulp of the purple-red cultivar ('Mulligan') had more β-carotene than the yellow ('Amber') (Figure 2.11A) which was in agreement with previous work (2009) in Ecuador by Vasco et al. (2009). Similar to the current findings, Athar et al. (2003) recorded in 2003 that β-carotene contents were 0.600 and 0.763 mg/100 g FW in New Zealand red and yellow cultivars. 'Amber' cultivar had almost double the β-carotene content than that reported for yellow Ecuadorian tamarillos (0.46 mg/100 g FW) (Mertz et al., 2009). Meanwhile, 'Mulligan' variety showed higher β-carotene concentration than reddish-brown skin tamarillo from Malaysia with 4.80 mg/100 g DW which was equal to 0.7 mg/100 g FW (Mutalib et al., 2017). Also, tamarillo peel showed higher content of β-carotene than mango peel (3.16 mg/100g DW ~ 0.4 mg/100g FW) and pomegranate peel with and 2.47 mg/100g DW ~ 0.3 mg/100g FW) (Ghosh, Chatterjee, Chalkroborty, & Kundu, 2019).

Compared to other commonly consumed fruits, consumption of all three tamarillo cultivars would contribute meaningfully to dietary intake of β-carotene. The β-carotene quantities in one serve (120 g) (New Zealand Institute for Plant and Food Research) of the pulp of 'Amber', 'Laird's Large' and 'Mulligan' were 0.96, 0.48 and 1.08 mg, respectively which would supply 18, 9, and 20% of the RDI, respectively and classify 'Amber' and 'Mulligan' pulp as dietary sources of β-carotene (>10% RDI) (National Health and Medical Research Council, 2006) (Table 2.12). Administration of β-carotene at concentrations 0.4, 0.8 and 1.6 μM reduced lipid peroxidation formation by 28, 31 and 46%, respectively; and 1.6 μM of β-carotene significantly ($p < 0.05$) decreased DNA damage by 91% (Zhang & Omaye, 2001), which can all be achieved through one serve of 'Mulligan' tamarillo.

Alpha-tocopherol is recognized to have the highest biological activity among tocopherol (Raiola, Tenore, Barone, Frusciante, & Rigano, 2015). In contrast to the distribution of β-carotene, α-tocopherol was higher in the peel compared with the pulp of all three cultivars (1.2 – 1.7 times, $p < 0.05$) (Figures 2.11A and 2.11B). The current results are in the same magnitude as the previous New Zealand-based research of Athar et al. (2003) who reported in 2003 the α-tocopherol content of approximately

1.9 mg/100 g FW in both yellow and red tamarillos. The current results agreed more closely with results of Lister et al. (2005) who reported that content of tocopherols in gold and red tamarillos from New Zealand of 3.5 and 1.8 mg/100 g FW, respectively. Tamarillos from New Zealand should be considered as a good source of α-tocopherol compared to other fruits such as blackberries (1.4), kiwifruits (1.3), cranberries (1.2) and raspberries (0.9 mg/100g FW) as well as raw, peeled tomato (the same *Solanum* genus) with 0.6 mg/100g FW (Chun, Lee, Ye, Exler, & Eitenmiller, 2006).

'Laird's Large', 'Amber' and 'Mulligan' cultivars could all be considered as dietary sources (>10% AI) of α-tocopherol were 16, 18 and 23%, respectively which were highest AI compared to other common fruits in New Zealand (Table 2.12). The Recommended Daily Allowance (RDA) of α-tocopherol is 15 mg for adult men and women, which is equivalent to α-tocopherol from about 6.5 serves of 'Mulligan' tamarillo. Lipid peroxidation formation was significantly reduced by 15 and 16% and DNA damage was also reduced ($p < 0.05$) by 61 and 74% with administration of α-tocopherol at 10 and 20 μM, respectively (Zhang & Omaye, 2001) which can be retrieved from consumption of 1.9 to 3.8 serves of 'Mulligan' tamarillo.

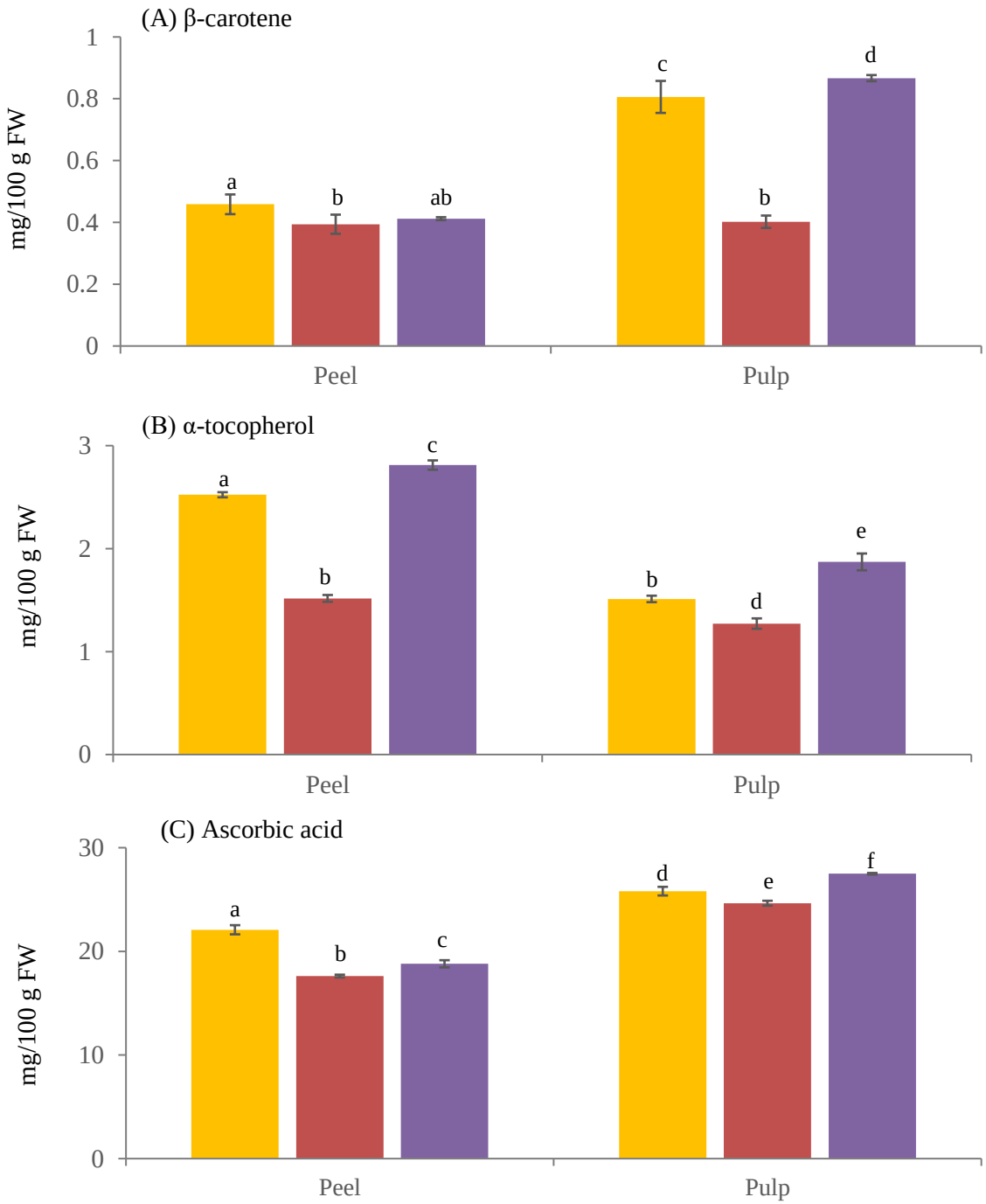

Figure 2.11 Concentrations of β-carotene (A), α-tocopherol (B), and ascorbic acid (C) in both peel and pulp of three New Zealand grown tamarillo cultivars. (■ 'Amber', ■ 'Laird's Large', ■ 'Mulligan'). Data are presented as mean (mg/100 g FW) and error bar (standard deviation) (n = 3). Different alphabets indicate statistical difference ($p < 0.05$). Used with permission (Diep et al., 2020b)

Table 2.12 Quantity (mg) and recommended dietary intake (RDI) of vitamins from three tamarillo cultivars and other commonly consumed fruits in New Zealand.

(New Zealand Institute for Plant and Food Research)

Fruits	Serving size (g)	β-carotene (mg/serve)	RDI of β-carotene	α-tocopherol (mg/serve)	AI of α-tocopherol	Ascorbic acid (mg/serve)	RDI of ascorbic acid
Tamarillos							
‘Amber’	120	0.96	**18%**	1.8	**18%**	31.2	**69%**
‘Laird’s Large’	120	0.48	9%	1.56	**16%**	30	**67%**
‘Mulligan’	120	1.08	**20%**	2.28	**23%**	33.6	**75%**
Avocado	85	0.041	1%	1.61	**16%**	6.2	**14%**
Blackcurrant	118	0.189	4%	0.94	9%	189	**420%**
Blueberry	157	0.012	0%	1.41	**14%**	6	**13%**
Boysenberry	133	0.4	7%	1.46	**15%**	12.1	**27%**
Feijoa	84	0.026	0%	0.15	2%	25.5	**57%**
Grapefruit	118	0	0%	–	–	47.2	**105%**
Kiwifruit	100	0.049	1%	1.7	**17%**	122	**271%**
Lemon	65	–	–	–	–	33.8	**75%**
Mandarin	150	0.478	9%	0.68	7%	31.5	**70%**
Passion fruit	18	0.002	0%	–	–	3.6	8%
Persimmon	83	0.058	1%	0	0%	42.2	**94%**
Raspberry	136	0	0%	0.48	5%	18.5	**41%**

Strawberry	175	0.01	0%	0.72	7%	79.8	**<u>177%</u>**

* Data was calculated based on the concentrations of the pulp samples. Used with permission (Diep et al., 2020b)

RDI: recommended dietary intake (National Health and Medical Research Council, 2006)

AI: Adequate Intake (used when an RDI cannot be determined)

1 µg retinol (vitamin A) equivalent = 6 µg all trans β carotene RDI = 900 retinol equivalents

Vitamin E: AI = 10 mg, UL = 300 mg

Vitamin C: Estimated average requirement (EAR) = 30 mg, RDI = 45 mg

– : not identified

Bolded numbers are dietary source (>10% RDI) and **<u>bolded and underlined</u>** numbers are good source (>25% RDI) (Food Standards Australia New Zealand, 2007)

2.4.5 Ascorbic acid content

All pulp samples of three tamarillo cultivars own higher ascorbic acid than the peel samples, approximately 15 – 30% with significant differences ($p < 0.05$) (Figure 2.11C). Vitamin concentrations in pulp of red and gold tamarillos from New Zealand was reported as 29.8 – 34.3 and 24.7 – 31.0 mg/100 g FW, respectively (Lister et al., 2005; New Zealand Food Composition Database, 2019). The current results are consistent with the ascorbic acid of 25 mg/100 g FW reported in literature (Ramirez & Kallarackal, 2019). The vitamin C concentrations in golden-yellow and purple-red tamarillos from Ecuador (17 and 16 mg/100 g FW, respectively) (Vasco et al., 2009) as well as in reddish-brown skin tamarillo from Malaysia (55.9 mg/100 g DW ~ 8 mg/100g FW) (Mutalib et al., 2017) were lower than that in New Zealand tamarillos from the current study. The vitamin C contents of three tamarillo cultivars were higher than that of blackberry, cherry, passionfruit, grape, persimmon and peach with 20, 20, 20, 10.8, 10 and 9.6 mg/100 g FW, respectively (Sivakumaran, Huffman, Sivakumaran, & Athar, 2015). The peel of three tamarillo cultivars showed higher ascorbic acid content than grapefruit, orange and lemon peels with 113.3, 110.4, and 58.59 mg/100 g DW, respectively which were equal to 11, 15 and 6 mg/100 g FW, respectively (Sir Elkhatim, Elagib, & Hassan, 2018)

One serve of 'Laird's Large', 'Amber' and 'Mulligan' tamarillos met 67, 69 and 75% of the RDI, respectively (Table 2.12) that were higher than avocado, blueberry, boysenberry, feijoa, passionfruit and raspberry; and approximately similar RDI to mandarin and lemon (known as rich vitamin C fruits) (National Health and Medical Research Council, 2006). The RDA for vitamin C is 75 and 90 mg for women and men, respectively (Moser & Chun, 2016) and this can be obtained from about 2.5 serves of 'Mulligan' tamarillos. Administration of 80 to 160 μM of ascorbic acid provided 34 and 61% protection against lipid peroxidation, respectively, and DNA damage was prevented by 95% at 160 μM of ascorbic acid ($p < 0.05$) (Zhang & Omaye, 2001). These effects could have been achieved by consumption of a single serve of tamarillo. The co-administration of vitamin A (mainly β-carotene), E (mainly α-tocopherol) and vitamin C can reduce the incidence and delay the progression of several cancers, such as colon, esophagus, mammary gland, skin and stomach (Barrita & Sánchez, 2013).

2.4.6 Carotenoid and chlorophyll pigments

These main carotenoids (β-carotene, β-cryptoxanthin, zeaxanthin, lutein and antheraxanthin) had been reported by De Rosso and Mercadante (2007) in Brazilian tree tomato; by Mertz et al. (2009) in Ecuadorian fruit; as well as by Giuffrida et al. (2018) in Colombian fruit. Violaxanthin had been explored in tamarillo from Brazil (De Rosso & Mercadante, 2007). However, this is the first time that astaxanthin; caricaxanthin; dinoxanthin; diatoxanthin; diadinoxanthin; flavoxanthin A; flavoxanthin B; fucoxanthin and siphonaxanthin were found in tamarillo. Among 21 analysed compounds, 20 carotenoid and chlorophyll pigments were detected in the peel and pulp of the three tamarillo cultivars except for chlorophyll D (Appendix B10). The major carotenoid pigment in all pulp samples was β-carotene which was consistent with results from Mertz et al. (2009). Meanwhile, β-cryptoxanthin was recognized as the most dominant carotenoid pigment in 'Mulligan' peel (31.82%) as well as second abundant compound in the rest samples (over 13%). carotenoid profile in tamarillo from Brazil was dominated by β-cryptoxanthin (45.3%), followed by β-carotene (26.1%) zeaxanthin (5.1%), and antheraxanthin (4.0%) (De Rosso & Mercadante, 2007). The difference might be due to the origin of fruits and methods of extraction.

The relative concentration of zeaxanthin in the peel was higher than in the pulp (approximately 1.3 – 1.8 times), except for the 'Mulligan' cultivar. Zeaxanthin comprised 8.63 – 25.11 and 7.87 – 13.47% in peels and pulps of total carotenoid and chlorophyll pigments (Appendix B11). Meanwhile, the concentration of lutein was higher in all pulps than in all peels, being the lowest and highest in 'Laird's Large' peel and 'Mulligan' pulp, respectively (Appendix B10). The percentage concentration (%) of lutein and antheraxanthin in all analyzed samples were over 5%. Phaeophytin was considered as a main chlorophyll pigment in 'Laird's Large' pulp with the relative percentage content of 6.46%, followed by the 'Mulligan' (4.21%) and then the 'Amber' (1.01%) (Appendix B11).

2.4.7 Phenolic content

For all of the cultivars, the total concentration of phenolic compounds detected in the pulp was slightly less than a half of the peel (Table 2.13). The total phenolics in peel

and pulp of Colombian tamarillo were 28.41 and 20.72 mg/100 g DW (Loizzo et al., 2019), which were lower than the current results.

Among 12 phenolics quantified in three tamarillo cultivars from New Zealand and six compounds had been previously found in tamarillo (Espin et al., 2016; Mutalib et al., 2016; Wrolstad & Heatherbell, 1974) and six other compounds (ellagic acid, rutin, catechin, epicatechin, kaempferol 3-rutinoside and isorhamnetin 3-rutinoside) were firstly explored in tamarillo. Concentrations of phenolics between different cultivars and tissues were significantly different ($p < 0.05$) (Table 2.13). The current result revealed chlorogenic acid as the most dominant phenolic compound regardless of the cultivars and tissues, that agrees with the findings of Espin et al. (2016) for tamarillos from Ecuador (25.04 – 42.73 and 50.33 mg/100 g DW) and New Zealand (163.62 mg/100 g DW) as well as of Loizzo et al. (2019) for both peel (25.38 mg/100 g DW) and pulp with seed (16.32 mg/100 g DW) of Colombian tamarillo. This phenolic acid has been recognized as the main soluble phenolic compound in other *Solanaceous* species (eggplant, potato and tomato) (Niggeweg, Michael, & Martin, 2004). The concentrations of this phenolic from previous studies (Espin et al., 2016; Loizzo et al., 2019) were much lower than the findings from the current project. Tamarillo pulp from New Zealand showed significantly higher chlorogenic acid concentration than tomato pulp (24.58 – 53.10 mg/100 g DW) (Gómez-Romero, Segura-Carretero, & Fernández-Gutiérrez, 2010). Anti-diabetic, anti-carcinogenic, anti-inflammatory and anti-obesity and antioxidant properties have been potential health benefits of this phenolic acid (Tajik, Tajik, Mack, & Enck, 2017), which would further enhcance the bioactive potential for tamarillo as a functional ingredient.

Rutin was recognised as the second most abundant polyphenol for ‘Amber’ peel and ‘Mulligan’ peel. Higher concentration of rutin was found in peel than in pulp (approximately 3.8, 7.5 and 9.6 times more for ‘Laird’s Large’, ‘Amber’ and ‘Mulligan’ varieties, respectively). Known as powerful antioxidant, rutin possess ability to strengthen the blood cell walls (Sun & Ho, 2005), with antitumor, antibacterial and antiviral (Calabro et al., 2005), antioxidant and anticarcinogenic properties (Yang, Guo, & Yuan, 2008). Kaempferol 3-rutinoside was considered as the second most dominant phenolic in pulp of all cultivars and in ‘Laird’s Large’ peel. For all three cultivars, concentration of kaempferol 3-rutinoside in pulps was higher than that in the peel (approximately 2.6 – 3.6 times) (Table 2.13). Recognized as a

powerful α-glucosidase inhibitor, this phenolic showed ability as anti-adipogenic property, which may act as an anti-obesity agent (Jang, Wang, Lee, Lee, & Lim, 2016). Also, isorhamnetin 3-rutinoside was detected in pulp of three tamarillo cultivars with trace amount.

For all tamarillo cultivars, caffeic acid showed approximately double concentration in peels rather than in pulps. In contrast, higher concentration in pulp than in peels was observed for *p*-coumaric acid. Caffeic acid (0.165 mg/100 g DW) and *p*-coumaric (0.041 mg/100 g DW) were observed in pulp of Malaysian tamarillo (Mutalib et al., 2016). In both peel and pulp of Colombian tamarillo, 0.02 mg/100 g DW of *p*-coumaric acid was reported by Loizzo et al. (2019). Most of current results was higher than these values from both previous projects. Pulp of 'Amber' and 'Laird's Large' showed extremely low concentration of ferulic acid, while all other samples contained this acid with trace amounts. Mutalib et al. (2016) also reported low concentration (0.005 mg/100 g DW) of ferulic acid in Malaysian tamarillo. Peel and pulp of Colombian tamarillo showed higher concentration of this acid with 0.87 and 0.76 mg/100 g DW, respectively (Loizzo et al., 2019). The higher content of hydroxycinnamic acids in red and purple-red New Zealand tamarillos compared with the yellow cultivar was consistent with the result reported for Ecuadorian red and yellow tree tomato (Mertz et al., 2009). Comprising over 85% and 55% of the total polyphenols in peel and pulp, respectively hydroxycinnamic acids group was recognized as one of the main polyphenolic classes in tamarillo, (Figure 2.12).

In all analysed samples, gallic acid and ellagic acid showed low concentrations with significant differences ($p < 0.05$) between cultivars. Concentration of gallic acid in Malaysian tamarillo was 0.302 mg/100 g DW) (Mutalib et al., 2016). Gallic acid has been shown as a protector against oxidative damage and chemo-preventive agent which led to death of several tumour cell lines (Mutalib et al., 2016). This is the first time ellagic acid was detected in tamarillo though trace amounts (approximately 0.1 mg/100 g DW) were observed (Table 2.13).

A very low concentration of kaempferol was identified in tamarillos from New Zealand, while tamarillo from Malaysia showed the kaempferol concentration of 0.05 mg/100 g DW (Mutalib et al., 2016), which was lower than the current result. This is the first time catechin and epicatechin were detected in tamarillos presenting 0.7 – 1.3% and 1.7 – 5.4% in peel and pulp of total phenolics, respectively (Figure 2.12).

Significantly higher concentration of catechin in both peel and pulp were found in 'Amber' rather than in two other cultivars. In contrast, the highest content of epicatechin in both peel and pulp was observed for 'Mulligan' cultivar (Table 2.13). Health benefits of catechin include neuroprotective properties, anti-obesity, anticancer and anti-inflammatory. Overall, it could be believed that bioactive values of tamarillo are compatible to other super fruits due to the presence of these phenolics.

Figure 2.13 showed shikimic acid pathway for synthesis of most phenolic compounds detected in tamarillo. According to Saltveit (2010), phenolic compounds in plants are generally synthesised from this pathway where carbohydrate precursors are converted into phenylalanine and then to *trans*-cinnamic acid, *p*-coumaric acid and dihydroflavonols. These compounds are further derived to form a variety of secondary phenolic compounds by adding hydroxyl groups and sugars and methylation. For instance, rutin, kaempferol 3-rutinoside and isorhamnetin 3-rutinoside were formed by adding sugar molecules to the flavonol skeletons.

Table 2.13 Phenolic and anthocyanin contents (mg/100 g DW) in peel and pulp of three tamarillo cultivars from New Zealand.

Bioactive compounds	Peel			Pulp			*p*-value*		
	'Amber'	'Laird's Large'	'Mulligan'	'Amber'	'Laird's Large'	'Mulligan'	C	T	C x T
Chlorogenic acid	225.85 ± 12.43^{ax}	231.18 ± 9.76^{bx}	278.03 ± 11.89^{cx}	54.67 ± 3.81^{ay}	66.35 ± 1.1^{by}	73.95 ± 1.98^{cy}	< 0.05	< 0.05	< 0.05
Caffeic acid	2.61 ± 0.18^{ax}	3.56 ± 0.52^{bx}	3.52 ± 0.85^{bx}	1.01 ± 0.03^{ay}	1.2 ± 0.06^{by}	1.32 ± 0.04^{by}	< 0.05	< 0.05	0.0328
p-coumaric acid	0.05 ± 0.01^{ax}	0.02 ± 0.01^{bx}	0.03 ± 0.01^{cx}	0.12 ± 0.01^{ay}	0.07 ± 0.01^{by}	0.08 ± 0.01^{cy}	< 0.05	< 0.05	< 0.05
Ferulic acid	0.03 ± 0.03^{ax}	0.01 ± 0.01^{bx}	0.04 ± 0.03^{cx}	$< 0.005^{ay}$	$< 0.005^{by}$	0.02 ± 0.02^{cy}	< 0.05	< 0.05	*0.1158*
Total hydroxycinnamic acids	228.53 ± 12.65^{ax}	234.77 ± 10.3^{bx}	281.63 ± 12.78^{cx}	55.80 ± 3.86^{ay}	67.62 ± 1.18^{by}	75.37 ± 2.04^{cy}	*< 0.05*	*< 0.05*	*< 0.05*
Gallic acid	0.8 ± 0^{ax}	0.79 ± 0^{bx}	0.8 ± 0.01^{bx}	0.79 ± 0^{ay}	0.93 ± 0.06^{by}	1 ± 0.2^{by}	< 0.05	< 0.05	< 0.05
Ellagic acid	0.12 ± 0.04^{x}	0.11 ± 0.03^{x}	0.1 ± 0.04^{x}	0.11 ± 0.02^{y}	0.09 ± 0.01^{y}	0.09 ± 0.02^{y}	*0.2304*	0.0483	*0.6023*
Total hydroxybenzoic acids	0.91 ± 0.05^{ax}	0.9 ± 0.03^{abx}	0.91 ± 0.05^{bx}	0.90 ± 0.03^{ay}	1.01 ± 0.07^{aby}	1.09 ± 0.22^{by}	*0.0227*	*< 0.05*	*0.0147*
Kaempferol	0.43 ± 0.01^{ax}	0.43 ± 0.01^{bx}	0.43 ± 0.01^{bx}	0.5 ± 0.04^{ay}	0.45 ± 0.01^{by}	0.45 ± 0.01^{by}	< 0.05	< 0.05	< 0.05
Total flavonols	0.43 ± 0.01^{ax}	0.43 ± 0.01^{bx}	0.43 ± 0.01^{bx}	0.5 ± 0.04^{ay}	0.45 ± 0.01^{by}	0.45 ± 0.01^{by}	*< 0.05*	*< 0.05*	*< 0.05*
Catechin	2.13 ± 0.83^{ax}	0.28 ± 0^{bx}	0.33 ± 0.02^{bx}	3.91 ± 0.68^{ay}	0.3 ± 0^{by}	0.32 ± 0^{by}	< 0.05	< 0.05	< 0.05
Epicatechin	1.36 ± 0.03^{a}	1.49 ± 0.12^{b}	2.6 ± 0.72^{c}	1.34 ± 0^{a}	1.73 ± 0.01^{b}	2.31 ± 0.02^{c}	< 0.05	*0.763*	*0.0545*
Total flavanols	3.49 ± 0.86^{ax}	1.78 ± 0.13^{bx}	2.94 ± 0.74^{cx}	5.25 ± 0.68^{ay}	2.03 ± 0.01^{by}	2.63 ± 0.02^{cy}	*< 0.05*	*< 0.05*	*< 0.05*
Rutin	24.33 ± 1.85^{ax}	3.71 ± 0.45^{bx}	12.68 ± 1.1^{cx}	3.23 ± 0.08^{ay}	0.97 ± 0.03^{by}	1.32 ± 0.03^{cy}	< 0.05	< 0.05	< 0.05
Kaempferol 3-rutinoside	8.32 ± 0.57^{ax}	19.22 ± 1.2^{bx}	12.45 ± 1.09^{cx}	30.72 ± 0.97^{ay}	50.04 ± 1.12^{by}	45.6 ± 1.41^{cy}	< 0.05	< 0.05	< 0.05
Isorhamnetin 3-rutinoside	0.59 ± 0.05^{ax}	0.96 ± 0.05^{bx}	0.9 ± 0.02^{cx}	0.16 ± 0.01^{ay}	0.13 ± 0^{by}	0.14 ± 0^{cy}	< 0.05	< 0.05	< 0.05
Total flavonol glycosides	33.25 ± 2.47^{ax}	23.89 ± 1.7^{bx}	26.03 ± 2.21^{bx}	34.11 ± 1.06^{ay}	51.14 ± 1.14^{by}	47.06 ± 1.44^{by}	*< 0.05*	*< 0.05*	*< 0.05*
Total phenolics	266.62 ± 16.04^{ax}	261.77 ± 12.16^{bx}	311.93 ± 15.80^{cx}	96.56 ± 5.67^{ay}	122.26 ± 2.42^{by}	126.60 ± 3.74^{cy}	*< 0.05*	*< 0.05*	*< 0.05*
Delphinidin 3-rutinoside	0.43 ± 0.02^{ax}	32.41 ± 2.98^{bx}	49.11 ± 2.23^{cx}	29.17 ± 2.47^{ay}	254.76 ± 6.33^{by}	273.36 ± 12.7^{cy}	< 0.05	< 0.05	< 0.05

Cyanidin 3-glucoside	n.d	0.33 ± 0.04^{a}	1.97 ± 0.14^{b}	n.d	n.d	n.d	< 0.05	–	–
Cyanidin 3-rutinoside	0.29 ± 0^{ax}	68.72 ± 4.33^{bx}	114.47 ± 5.97^{cx}	0.18 ± 0.02^{ay}	25.94 ± 1.99^{by}	30.67 ± 2.76^{cy}	< 0.05	< 0.05	< 0.05
Pelargonidin 3-rutinoside	0.52 ± 0.06^{ax}	54.36 ± 3.24^{bx}	93.63 ± 2.48^{cx}	0.35 ± 0.03^{ay}	200.66 ± 8.51^{by}	182.81 ± 11.17^{cy}	< 0.05	< 0.05	< 0.05
Total anthocyanins	*1.24 ± 0.08^{ax}*	*155.82 ± 10.58^{bx}*	*259.18 ± 10.81^{cx}*	*29.70 ± 2.52^{ay}*	*481.37 ± 16.83^{by}*	*486.84 ± 26.63^{cy}*	*< 0.05*	*< 0.05*	*< 0.05*

n.d: not detected; DW: Dry weight. Data are expressed as Mean ± SD (n = 4). Used with permission (Diep et al., 2020a)

* Statistical significance for cultivar (C), tissue (T) and the interaction of both types (C x T)

Means shown in $^{a, b, c}$ are significantly different at $p < 0.05$ between cultivars

Means shown in $^{x, y}$ are significantly different at $p < 0.05$ between tissues

SD values of less than 0.004 are presented as 0.

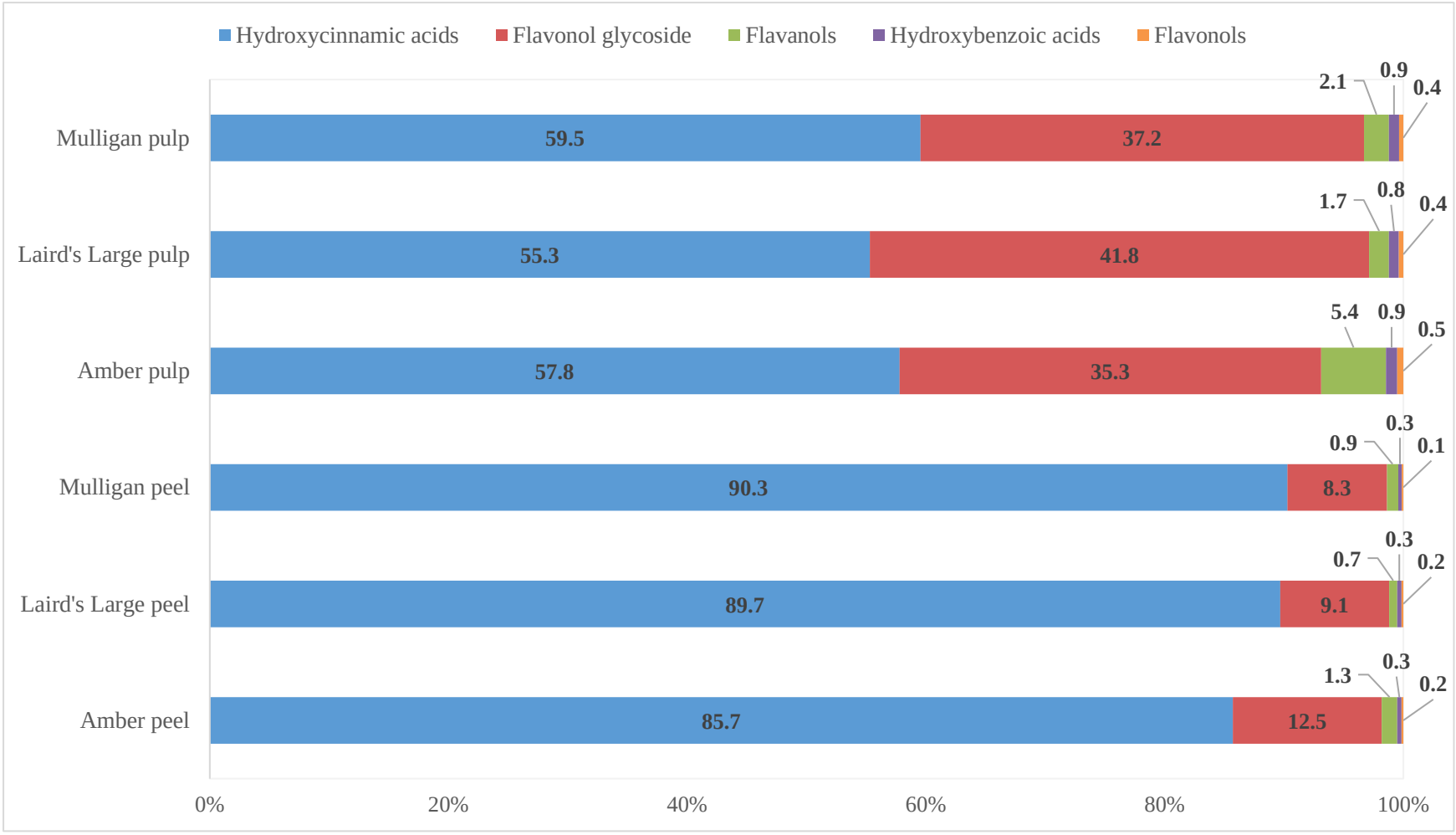

Figure 2.12 The percentage of five abundant phenolic classes separated by peel and pulp of three tamarillo cultivars from New Zealand. Used with permission (Diep et al., 2020a)

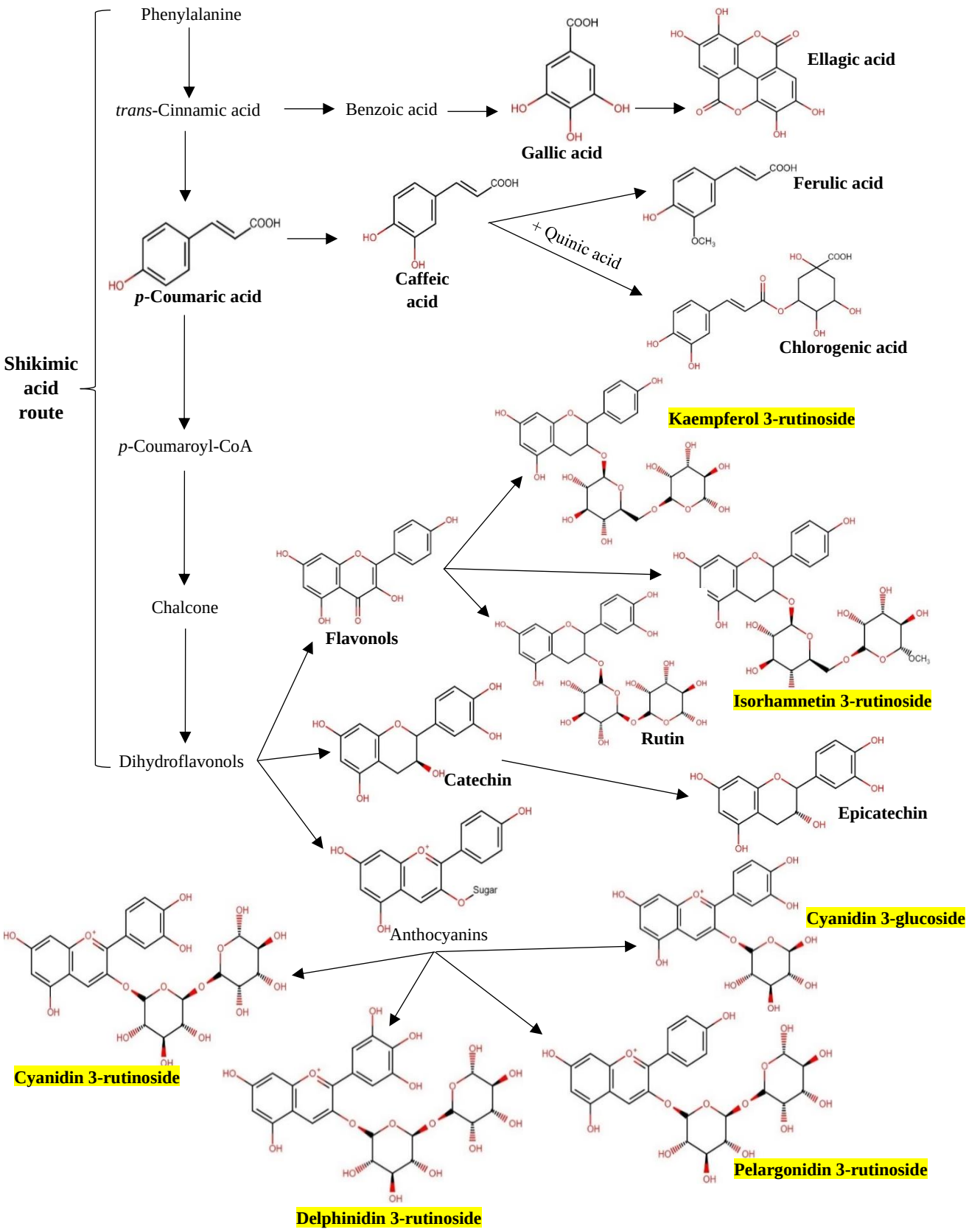

Figure 2.13 Outline of biosynthesis of phenolic and anthocyanin compounds from phenylalanine in tamarillo.

Used with permission (Diep et al., 2020a)

2.4.8 Anthocyanin content

In all cultivars, higher total anthocyanin concentration was found in pulps than in peels. For both peel and pulp, the red and purple-red cultivars had higher concentrations of each anthocyanin than yellow variety ('Amber') (Table 2.13). Concentration of total anthocyanins in tamarillo peel was evidently higher than the peels of other fruit with similar red colour such as the peels of red grape, red plum and red apple (27, 20 and 12 mg/100 g FW, respectively which were approximately equal to 138.75, 156.62 and 83.1 mg/100 g DW, respectively) (Kalt et al., 2019). Also, tamarillo pulp possessed higher total anthocyanins content than the pulp of strawberry, grape and cranberry (41.09, 28.09 and 19.30 mg/100 g FW, respectively which was approximately equal to 454.03, 144.35, 152.21 mg/100 g DW, respectively) (Corona, Tang, Vauzour, Rodriguez-Mateos, & Spencer, 2011).

Cyanidin 3-rutinoside and delphinidin 3-rutinoside have been reported as the main anthocyanin in peel (Wrolstad & Heatherbell, 1974) and pulp (Espin et al., 2016; Wrolstad & Heatherbell, 1974) of New Zealand purple cultivar, respectively. Similar results also have been explored in the current study with cyanidin 3-rutinoside and delphinidin 3-rutinoside as the most abundant anthocyanins in peels and pulps of both 'Laird's Large' and 'Mulligan' cultivars, respectively. The result from current study supports the previous findings that delphinidin-based anthocyanins are the dominant structure of *Solanaceous* species including pepper, tomato, eggplant and potato (Liu et al., 2018). These anthocyanins have shown capacity to inhibit thrombosis and reduce vascular inflammation (Watson & Schönlau, 2015) as well as to protect human skin against UV-B irradiance (Liu et al., 2018).

For red and purple-red cultivars, significantly higher concentrations of delphinidin 3-rutinoside and pelargonidin 3-rutinoside were found in pulps than in peels by 5.6 – 7.8 and 2.6 – 3.7 times, respectively. Conversely, pulps had a lower concentration of cyanidin 3-rutinoside than peels by 2.0 and 3.7 times for 'Laird's Large' and 'Mulligan' varieties, respectively (Table 2.13). Anthocyanins extracted from grape skin have been used to produce E163 (Khoo, Azlan, Tang, & Lim, 2017), therefore similar application might be applied for the peel of these tamarillos as food additives. The outcomes of current research also raise an attention to use anthocyanin as a natural colorant for replacement of synthetic food dye.

‘Amber’ cultivar, carrying yellow colour, possessed the least amounts of anthocyanins compared to purple and red tamarillos (‘Mulligan’ and ‘Laird’s Large’). The presence of anthocyanins has never been reported in New Zealand ‘Amber’ cultivar. The major anthocyanin in ‘Amber’ peel and pulp was pelargonidin 3-rutinoside and delphinidin 3-rutinoside, respectively (Table 2.13). Concentrations of cyanidin 3-rutinoside, delphinidin 3-rutinoside, pelargonidin 3-rutinoside in ‘Laird’s Large’ and ‘Mulligan’ tamarillo in this study were significantly higher than that reported in the red variety from Ecuador (Mertz et al., 2009), and in the purple cultivars from Ecuador and New Zealand (Espin et al., 2016). Also, the New Zealand ‘Laird’s Large’ and ‘Mulligan’ tamarillos had greater total anthocyanin content than Brazilian tamarillo (8.5 mg/100 g FW ~ 70.83 mg/100 g DW) (De Rosso & Mercadante, 2007), Ecuadorian tree tomato (102.35 – 165.1 mg/100 g DW (Espin et al., 2016; Mertz et al., 2009); and New Zealand purple cultivar from the previous study (168.88 mg/100 g DW) (Espin et al., 2016).

Difference in the phenolic and anthocyanin profiles of tamarillo between the current project and previous studies might be due to the cultivars and ripeness of fruit, post-harvest handling, storage period as well as extraction and analytical approaches for quantification. For example, acetone (Lister et al., 2005) and aqueous acetone containing 2% formic acid (Mertz et al., 2009) were used as extraction solvent for quantification of these compounds. A mixture of methanol and 0.1% formic acid as extraction solvent and 0.1% trifluoracetic acid and acetonitrile as mobile phases were used in study of Espin et al. (2016). Meanwhile, the current study used a combination of formic acid, Milli-Q, IPA, and toluene was used as extraction solvent with mobile phase of 0.1% formic acid in acetonitrile and 0.6% formic acid in Milli-Q. Water-based extracts were used in the current study since organic solvents such as methanol or ethanol can cause a toxicity issue for anthocyanin extraction (Khoo et al., 2017). Also, to enhance the stability of anthocyanins, lower pH of the solution was achieved by using pure formic acid. According to Khoo et al. (2017), the solubility of anthocyanins in water has been significantly improved by formation of flavylium cation at acidic condition. To improve the purity of the extracts, toluene was used to separate different layers while, as a good non-toxic alternative, IPA was used to preserve biological specimen such as anthocyanins.

2.4.9 Total phenolic content (TPC) and antioxidant activity

The TPC values in peels were almost two-fold compared to that in pulps and 'Mulligan' would be the most bioactive ($p < 0.05$), with relatively high TPC and strong antioxidant activities as identified by CUPRAC and FRAP assays (Table 2.14). New Zealand red and gold tamarillos showed 190.8 and 116.6 mg GAE/100 g FW (approximately equal to 1564 and 1060 mg/100 g DW) of TPC, respectively (Lister et al., 2005). Meanwhile, Ecuadorian golden-yellow and purple-red tamarillos showed the TPC 78 and 113 mg GAE/100 g FW (approximately equal to 557 and 1413 mg/100 g DW), respectively (Vasco et al., 2009).

It can be seen that TPs value calculated from all phenolic compounds identified by LC-MS/MS and TPC value determined by Folin-Ciocalteu method were slightly different. This difference might be because the LC-MS/MS technique identifies phenolic compounds containing glycosylated or ester linked groups (rutin, kaempferol 3-rutinoside or isorhamnetin 3-rutinoside), whereas the Folin-Ciocalteu technique only evaluates the TPC containing free phenolic groups. Also, the TPC were assessed for the entire extracts whereas the total concentration of phenolic compounds identified by LC-MS/MS results was only based on the determined compounds.

As shown in Table 2.14, among different cultivars and tissues, the antioxidant activity showed significant differences ($p < 0.05$) and a significant interaction between the cultivars and tissues was also found. The antioxidant activity determined by CUPRAC assay for pulps were lower than for peels (approximately 3.7, 2.8 and 2.6 times for 'Mulligan', 'Amber' and 'Laird's Large' cultivars, respectively). The same trend was observed when evaluated with the FRAP assay. Among three cultivars, peel showed higher antioxidant capacity than pulp. For both tissues, the strongest anti-radical efficacy was observed in 'Mulligan' type. The peels showed higher FRAP values than the pulps with 1.6 – 2.2 times. A previous study in 2016 reported the FRAP value for pulp of New Zealand purple tamarillo was 50 μmol TEAC/g DW (Espin et al., 2016), which was lower than the current result. Meanwhile, FRAP values for the pulp of Ecuadorian yellow and purple tamarillos were 10 – 17 and 15 μmol TEAC /g DW, respectively (Espin et al., 2016).

Peels exhibited higher TPC and stronger antioxidant activity than the pulps of tamarillo, that seemed generally common in fruits. High antioxidant activity of the

peel is associated with higher colour stability (Hurtado et al., 2009) which is helped by the existence of organic acids, phenolic acids, flavones, flavanones, flavonols, flavanols as well as anthocyanins (Eiro & Heinonen, 2002; Marković, Petranović, & Baranac, 2000). Extraction and purification of bioactive compounds, especially phenolics including anthocyanins, into a functional ingredient or additive may be applicable for food and pharmaceutical industries and to help with valorisation of by-product (peels).

Higher antioxidant activity being observed in New Zealand red tamarillo than in yellow cultivar was consistent with previous study of Lister et al. (2005) who applied ABTS assay. Similar phenomena was observed for Ecuadorian tamarillos with greater antioxidant capacity assessed by ORAC assay (Mertz et al., 2009) and DPPH assay (Vasco et al., 2008) in purple-red variety than yellow type. Several studies have illustrated that tamarillo showed higher antioxidant capacity than other fruits such as apple, orange, red grape (Mertz et al., 2009), kiwifruit, pineapple (Lister et al., 2005), tomato and cherry tomato (Noor Atiqah et al., 2014).

Table 2.14 Total phenolic content and antioxidant activity in peel and pulp of three tamarillo cultivars from New Zealand.

Tissues	Cultivars	TPC	CUPRAC	FRAP
Peel	'Amber'	1583.8 ± 40.09^{ax}	117.59 ± 9.35^{ax}	84.7 ± 11.13^{ax}
	'Laird's Large'	1673.28 ± 63.97^{bx}	136.68 ± 6.72^{bx}	102.55 ± 12.19^{bx}
	'Mulligan'	2225.06 ± 50.87^{cx}	265.29 ± 18.35^{cx}	161.74 ± 14.53^{cx}
Pulp	'Amber'	678.98 ± 19.09^{ay}	42.92 ± 8.73^{ay}	52.23 ± 6.7^{ay}
	'Laird's Large'	707.04 ± 30.65^{by}	52.42 ± 8.39^{by}	60.19 ± 5.21^{by}
	'Mulligan'	874.9 ± 30.48^{cy}	71.57 ± 7.81^{cy}	72.14 ± 9.41^{cy}
P value*	C	< 0.05	< 0.05	< 0.05
	T	< 0.05	< 0.05	< 0.05
	C x T	< 0.05	< 0.05	< 0.05

TPC: Total Phenolic Content; CUPRAC: Cupric ion Reducing Antioxidant Capacity; FRAP: Ferric reducing antioxidant power

* Statistical significance for cultivar (C), tissue (T) and the interaction of both types (C × T). Data are expressed as Mean ± SD (n = 4). Used with permission (Diep et al., 2020a)

Means shown in ^{a,b,c} are significantly different at $p < 0.05$ between cultivars.

Means shown in ^{x,y} are significantly different at $p < 0.05$ between tissues.

Units of TPC, CUPRAC and FRAP are mg GAE/100 g DW, μmol TEAC/g DW and μmol TEAC/g DW, respectively

Strong correlations between TPC and antioxidant activity as well as between two antioxidant activity assays were observed with all Pearson's Correlation Coefficient values of above 0.9 ($p < 0.01$) (Table 2.15). Previously, Acosta-Quezada et al. (2015) and then Mutalib et al. (2017) scrutinized the high correlation between TPC and antioxidant activity (evaluated by DPPH technique) of tamarillo with $r = 0.8607$ and $r = 0.998$, respectively. For purple-red and golden-yellow tamarillos, the Pearson's Correlation Coefficient values of 0.62 and 0.908 were observed for correlation of TPC – FRAP and DPPH – FRAP, respectively (Vasco et al., 2008). Based on the strong correlation between TPC and antioxidant activity (as determined by DPPH, CUPRAC, and FRAP assays), it can be concluded that polyphenols were the dominant antioxidant compounds in tamarillo regardless of the cultivars and tissues.

Table 2.15 Correlation between TPC, CUPRAC and FRAP values of three tamarillo cultivars from New Zealand.

		TPC	CUPRAC	FRAP
TPC	Pearson Correlation	1	.941**	.906**
	Sig. (2-tailed)		.000	.000
	N	24	24	24
CUPRAC	Pearson Correlation	.941**	1	.959**
	Sig. (2-tailed)	.000		.000
	N	24	24	24
FRAP	Pearson Correlation	.906**	.959**	1
	Sig. (2-tailed)	.000	.000	
	N	24	24	24

** Correlation is significant at the 0.01 level (2-tailed).

TPC: Total Phenolic Content; CUPRAC: Cupric ion Reducing Antioxidant Capacity; FRAP: Ferric reducing antioxidant power

Used with permission (Diep et al., 2020a)

According to Prior, Wu, and Schaich (2005), no one antioxidant activity assay truly reflect the total antioxidant capacity of a particular sample and the total antioxidant

capacity needs to reflect both lipophilic and hydrophilic capacity. There are several factors for selected test such as sensitivity, selectivity, reliability, reproducibility, usage of available reagents and instruments and wide variety measurement of antioxidant types including both lipophilic and hydrophilic antioxidants (Apak, Özyürek, Güçlü, & Çapanoğlu, 2016). However, a single specific assay can not meet these criteria. Therefore, to get better antioxidant activity profile of tamarillo, three assays (Folin, CUPRAC and FRAP) were used in the current study (section 2.4.9). This was because FRAP, CUPRAC, and Folin methods can be used for acidic (pH 3.6), neutral (pH 7.0), and alkaline (pH 10) media measurements, respectively (Apak et al., 2016). FRAP assay has been used for the assay of antioxidants in botanicals and reasonable screen for the ability to maintain redox status in cells or tissues (Prior et al., 2005). The Folin method is simple and can be useful in characterizing and standardizing botanical samples (Prior et al., 2005). For CUPRAC, the reagent is more stable and accessible than other chromogenic reagents (ABTS, DPPH). Also, the CUPRAC method can simultaneously measure hydrophilic (ascorbic acid) and lipophilic antioxidants (β-carotene and α-tocopherol) (Apak, Güclü, Özyürek, & Celik, 2008). Catechin, caffeic acid, epicatechin, gallic acid, rutin, and chlorogenic acid being detected in tamarillo showed the highest antioxidant capacities in the CUPRAC method (Apak et al., 2008). Hence, these methods are suitable for analysis of antioxidant activity of tamarillo.

2.4.10 Volatile profile

2.4.2.1 Volatile compounds in tamarillos separated by peel and pulp

All of volatiles identified in ‘Laird’s Large’ fresh pulp were detected in dried powder, except for 5 compounds that were completely absent (perhaps below the detection limit) in the fresh sample as shown in Appendix B12. From the freeze-drying process where water (88–90% in tamarillo) was removed and the rest of the constituents become more concentrated, higher amounts of volatiles would be expected. Comparative quantitative result of low boiling point volatiles between ‘Laird’s Large’ fresh pulp and freeze-dried powder using TD-GC-MS is presented in Appendix B13. Insignificant differences ($p > 0.05$) between volatiles from the fresh and lyophilised forms were observed. This result was in consistent with the previous results carried

out by using SPME. These results from both SPME and TDU techniques demonstrated that lyophilized tamarillo showed the characteristic volatiles of fresh tamarillo with some typical volatiles mentioned above. Therefore, lyophilized tamarillos still remaining unique flavour of fresh fruits should be used natural flavour enhancer or raw materials for other products such as juice, yoghurt, cake or an ingredient in pharmaceutical products.

A total of 121 features determined in peel and pulp of three tamarillos by TD-GC-MS and these were relatively quantified to the internal standard (Table 2.16). 'Amber' pulp had one less volatile compound than 'Amber' peel (119 compounds compared to 120). For 'Laird's Large', pulp had 117 compounds and peel had 115. By contrast, 'Mulligan' had 120 and 117 compounds in peel and pulp, respectively. The volatile compounds were further categorized to their chemical groups including 20 esters, 20 ketones, 19 fatty acids, 11 nitrogen compounds, 10 aldehydes, 10 furans, 8 alcohols, 8 benzenes, 6 hydrocarbons, 4 carboxylic acids and derivatives, 2 sulfur compounds, 2 terpenes and 1 pyran compound.

Ketones, in the form of pentanone and propanone, were the most dominating flavour volatiles found in all of the tamarillo samples, regardless of the cultivar and tissue types. The contents of ketone group in pulp were comparatively higher than that in peels, except for 'Laird's Large'. Among this class, 4-hydroxy-4-methyl-2-pentanone was the most abundant compound in all of the tamarillo peels, while for all the pulp samples 3,5-dihydroxy-2-methyl-4H-pyran-4-one or 5-hydroxymaltol was present in the highest amount (Table 2.16). In Colombian yellow tamarillo, 2479.2 μg/kg (~ 247.92 μg/100 g) fresh fruit of 4-hydroxy-4-methyl-2-pentanone had been reported (Garcia et al., 2016). Tamarillo cultivars sourced from New Zealand also contained maltol, which is known as a flavour enhancer and a flavouring agent and has a capacity to enhance oral bioavailability of gallium (Bernstein, Tanner, Godfrey, & Noll, 2000), as well as iron (Reffitt et al., 2000).

Esters, which contribute to fruity notes, were also found with twenty different compounds being higher relative content in peel (13.7–20.1%) than in pulp (9.7–11.7%) across all three cultivars. For all tamarillo samples, Phthalic acid, hept-4-yl isobutyl ester showed the highest relative concentration. The contribution of methyl butanoate, ethyl butanoate, methyl hexanoate, ethyl hexanoate and methyl octanoate

as crucial esters of tamarillo aroma has been previously reported as summarized in Table 2.16. Ethyl hexanoate has been reported in tamarillo from Columbia with concentration of < 100 μg/kg FW (~ 10 μg/100 g FW) (Torrado et al., 1995), whereas ethyl hexanoate and ethyl hexadecanoate have been found in tamarillo from Panama (Durant et al., 2013). Ethyl hexanoate is used in perfumes and fruit flavours, while ethyl hexadecanoate carries a wax-like aroma.

Ten aldehydes were identified in the New Zealand tamarillo cultivars. The aldehyde contents in pulp were significantly higher than in peels. Among this chemical group, nonanal was one of the abundant volatiles which was found in both peel and pulp of all three tamarillo cultivars. This compound was also detected in reddish-purple tamarillo from Panama with a relative amount of 9.0% (Durant et al., 2013) and possesses fungicidal effect towards *Penicillium cyclopium* (Zhang, Sun, Chen, Zeng, & Wang, 2017).

Synthesis of furan compounds has been associated with the sucrose content in fruit (Tylewicz, Inchingolo, & Rodriguez-Estrada, 2017). Ten furans were firstly identified in tamarillo by this study with furaneol being as abundant compound in both peel and pulp of 'Amber' and 'Mulligan' cultivars. This furan compound has been reported as one of significant volatiles in tomato, mango, raspberry, pineapple, strawberry and considered as a flavouring agent (Buttery, Takeoka, Naim, Rabinowitch, & Nam, 2001) that gives the caramel-like, strawberry-like, burnt-pineapple and sweet, floral flavours (Du & Qian, 2008). Meanwhile, the thermal breakdown induced by the TD process might cause the release of 5-hydroxymethylfurfural. This compound has been also found in tomato and utilized as an index of heat treatment and deterioration in some food products such as tomato paste, fruit juice and honey.

Flavour volatiles of tamarillos were dominated by eight alcohol compounds with higher content in peel than in pulp. Among these identified alcohols, 2,3-butanediol was the most abundant volatile in both peel and pulp for all of the three tamarillo cultivars from New Zealand. Compared to the findings by Torrado et al. (1995), the relative content of 2,3-butanediol in New Zealand tamarillos was significantly higher than the Columbian tamarillo (< 100 μg/kg ~ 10 μg/100 g). This compound has not been reported in tamarillos from Malaysia (Wong & Wong, 1997) and Panama (Durant et al., 2013). This compound gives rancid flavour, and it has been detected in yellow

tamarillo from Colombia, of 81.5 μg/kg (~ 8.15 μg/100 g) of fresh fruit (Garcia et al., 2016). Eugenol and α-terpineol, the main volatile compound in tamarillo from Colombia (Garcia et al., 2016; Torrado et al., 1995), Malaysia (Wong & Wong, 1997) and Panama (Durant et al., 2013), were also detected in the current project. Owing sweet and phenolic flavor (Wang, Li, Chen, Bao, & Yang, 2007), eugenol has been used in perfumes, flavorings, and essential oils (Li, Sun, & Zheng, 2004) whereas, with pleasant odor, α-terpineol has been usually used as an ingredient in perfumes, cosmetics, and flavors (Yao, Guo, Lu, & Jiang, 2005).

Tamarillo flavour was also contributed by other compounds classes including benzenes, hydrocarbons, nitrogen- and sulfur-containing compounds, pyrans, terpenes as well as carboxylic acids and derivatives (Table 2.16). Benzene-acetaldehyde was the most abundant benzene compound in tamarillo from New Zealand, except for 'Amber' and 'Mulligan' peels. All of the benzene and hydrocarbon compounds found in the current study have not been reported in tamarillo from previous studies. In addition, two sulphur-containing volatiles (dimethyl sulfone and 2,3-dihydro-thiophene) were detected for the first time with higher relative contents in peel than in pulp of all of the three cultivars. One pyran and two terpenes comprise about 1% of the total volatile profile, were determined in tamarillo for the first time. Farnesol (3,7,11-trimethyl-2,6,10-dodecatrien-1-ol) carrying sweet floral flavour was also detected, has been reported as a chemopreventative and anti-tumour agent (Joo & Jetten, 2010) and showed anti-bacterial activity (Kromidas, Perrier, Flanagan, Rivero, & Bonnet, 2006).

The current study reports a comprehensive diversity of volatiles in tamarillo compared to 46 volatile compounds found in Colombian red tamarillo (Torrado et al., 1995), 49 volatiles from Malaysian red tamarillo (Wong & Wong, 1997), 11 compounds from Colombian yellow tamarillo (Garcia et al., 2016), and 58 and 33 volatiles from Panama golden-yellow and reddish-purple tamarillo, respectively (Durant et al., 2013). This may be due to the use of TD-GC-MS MS which has been more superior than SPME. In contrast to SPME which was required the incubation time of 15 min at 50°C, no preheating was done before analysis by TD method in this study. The TD method did not alter the sample, and therefore remains authentic in retaining odoriferous properties of samples in extraction process. Solvent extraction followed by drying to concentrate flavour compounds has been used to study volatiles in tamarillo (Garcia

et al., 2016; Torrado et al., 1995; Wong & Wong, 1997). Du and Qian (2008) noted that solvent extraction (water, dichloromethane and/or a mixture of pentane-dichloromethane), which commonly used to analyse volatile in tamarillo, had a very poor elution power for some of the volatile compounds, including furaneol, which contributes significantly to the flavour of tamarillo. Ruan, Aalhus, Juárez, and Sabik (2015) mentioned that loss and degradation of analytes can be caused by liquid extraction. Hence, to partially solve this problem, the TD technique was used in this study. Advantages of using TD in volatile analysis has been reported including improving analytical sensitivity by enhanced desorption efficiency and sample dilution (Tholl et al., 2006), reducing manual sample preparation time, minimising impurities in organic solvent interfering with GC analysis (Ruan et al., 2015), detecting volatile and semi-volatile compounds in a diversity of sample types (Yang et al., 2018). TD may be superior in volatile analysis compared to traditionally used HS-SPME which has been mentioned by Kücklich et al. (2017). Only a total of 70 compounds were detected in Panama tamarillos from using HS-SPME with solvent extraction (Durant et al., 2013) or 73 volatiles detected in 'Laird's Large' pulp of the comparison experiment. Hence, the combination of TD-GC-MS could be considered as a simple, quick and promising analysis method to flavour analysis of tamarillo and be a good alternative to the traditional techniques.

From my knowledge, there are no reports on the compositions of tamarillo flavour sourced from New Zealand as well as no reports using Thermal Desorption Unit (TDU) to analyse volatile compounds of this fruit. Hence, it is necessary to evaluate the aroma profile of New Zealand tamarillo with advanced technique to ensure efficient and error-free operation. Comparison of volatile component between peel and pulp of tamarillo was firstly conducted herein. To our best knowledge, the volatile profile of the peel and there is still scare information about its contribution to total aroma of tamarillo. The current result showed that peels also consisted of as much as pulp of the major volatiles.

Table 2.16 Volatile compounds and their relative contents in peel and pulp of New Zealand grown tamarillo cultivars.

No	Compounds	RI	*m/z*	Relative concentration (μg/g DW)					
				'Amber' peel	'Amber' pulp	'Laird's Large' peel	'Laird's Large' pulp	'Mulligan' peel	'Mulligan' pulp
Alcohols									
1	R-(-)-1,2-Propanediol	975.9	45.1	340 ± 65a	996 ± 73b	287 ± 64ac	1062 ± 100b	158 ± 9c	344 ± 65a
2	2,3-Butanediol	996.6	45.1	1135 ± 1996a	1737 ± 1193ab	492 ± 602ab	1692 ± 1241ab	284 ± 356b	445 ± 551ab
3	Alpha-terpineol	1285.7	59.1	25 ± 3.1a	10 ± 3.4b	0.1 ± 0.1c	0.7 ± 0.6c	5.1 ± 0.4d	11 ± 2.2b
4	*p*-Mentha-1(7),2-dien-8-ol	1487.6	94	1.1 ± 0.1a	1.6 ± 0.3a	0.7 ± 0.1a	1 ± 0.2a	16 ± 3b	2.9 ± 0.2a
5	Eugenol	1536.1	164.1	134 ± 4.8a	24 ± 7.3bc	60 ± 63b	11 ± 5.4c	58 ± 5.3b	46 ± 3.9bc
6	2,4,7,9-Tetramethyl-5-decyn-4,7-diol	1571.0	151.1	31 ± 5.6a	29 ± 10ab	15 ± 2b	25 ± 7.7ab	28 ± 6.3ab	29 ± 9.2ab
7	trans-Isoeugenol	1614.9	164	94 ± 2.6a	6.3 ± 3.3b	40 ± 43cd	5.6 ± 3.2b	58 ± 3.8c	35 ± 3d
8	2,6-Dimethoxy-4-(2-propenyl)-phenol	1808.5	194	463 ± 28a	83 ± 24b	38 ± 41cd	9 ± 3.7d	79 ± 3.2b	52 ± 4.2bc
Aldehydes									
9	3-Furaldehyde	977.3	95	107 ± 8.9a	1592 ± 325b	484 ± 161c	4313 ± 1195d	141 ± 4.3a	1810 ± 175b
10	Methional	1036.4	104	23 ± 4.8a	187 ± 13b	15 ± 4.2a	118 ± 8.1c	14 ± 0.6a	129 ± 12c
11	Octanal	1078.4	55.1	0.3 ± 0.2a	n.d	n.d	37 ± 1.6b	1.4 ± 1.9a	14 ± 24a
12	5-Methyl-2-furancarboxaldehyde	1096.2	110.1	24 ± 3.9a	921 ± 101b	102 ± 26c	1913 ± 104d	19 ± 2.2a	739 ± 45e
13	Nonanal	1176.8	57	38 ± 10a	31 ± 12ab	19 ± 16bc	12 ± 3.4c	30 ± 3.5ab	11 ± 0.5c
14	1H-Pyrrole-2-carboxaldehyde	1209.7	94	20 ± 5ab	26 ± 8.9bc	11 ± 12a	30 ± 3.7bc	18 ± 3.3ab	33 ± 5.2c
15	(E)-2-Decenal	1364.9	70.1	4.8 ± 1.8a	2 ± 0.6b	2.3 ± 1.4ab	1.2 ± 0.1b	12.8 ± 0.3c	4.7 ± 2.6a
16	2-Undecenal	1472.5	70.1	0.7 ± 1.1a	0.1 ± 0.1a	n.d	0.8 ± 1.4a	7.3 ± 0.7b	0.3 ± 0.3a
17	4-Methyl-benzaldehyde	1479.4	120	12 ± 2.2a	29 ± 2b	4.7 ± 1c	7.5 ± 0.3d	5 ± 0.4c	14 ± 0.7b
18	2,4-Dihydroxy-6-methyl-benzaldehyde	1637.8	151	1.7 ± 1.3a	4.6 ± 1.4b	0.6 ± 0.6a	7.1 ± 0.8c	1.8 ± 1.4a	7.8 ± 2.1c
Benzenes									

19	4-Ethenyl-1,2-dimethyl-benzene	1138.6	132	13 ± 1.8ab	46 ± 3.7c	7.3 ± 2.9a	47 ± 2.6c	16 ± 0.6b	61 ± 4.6d
20	Benzeneacetaldehyde	1163.8	91.1	46 ± 7.1a	306 ± 23b	114 ± 33c	405 ± 11d	72 ± 2.8a	318 ± 25b
21	2-Acetoxy-5-hydroxyacetophenone	1429.6	137	7.3 ± 0.7a	22 ± 1.1b	8.6 ± 3.3a	21 ± 0.7b	4.8 ± 0.5c	23 ± 0.8b
22	3',5'-Dihydroxyacetophenone	1429.8	137.1	7.3 ± 0.7a	22 ± 1.1b	8.6 ± 3.3a	21 ± 0.8b	4.8 ± 0.5c	23 ± 0.8b
23	2',6'-Dihydroxy-3'-methylacetophenone	1514.1	151	4.2 ± 0.3ab	15 ± 0.2cd	5.6 ± 1.4a	16 ± 0.3c	3.6 ± 0.5b	14 ± 1.5d
24	1-Ethenyl-4-(2-methylpropyl)-benzene	1603.2	117	1.1 ± 0.3a	0.1 ± 0.1b	n.d	n.d	1 ± 0.2a	0.1 ± 0.1b
25	(E)-2,6-Dimethoxy-4-(prop-1-en-1-yl) phenol	1922.4	194	120 ± 12a	12 ± 6.6b	9.9 ± 7.5b	3.6 ± 1b	96 ± 9.4c	57 ± 3.9d
26	Diphenylacetylene	1956.5	178	13 ± 1.1a	4.7 ± 1.2b	2.7 ± 1.7c	3.7 ± 0.1bc	6.4 ± 0.5d	4.5 ± 0.5b
Carboxylic acids and derivatives									
27	Methylene-cyclopropane carboxylic acid	1028.7	98.1	32 ± 13a	28 ± 5.6a	5.9 ± 8.4a	81 ± 23b	17 ± 11a	29 ± 12a
28	Benzoic acid	1403.9	105	41 ± 2.4a	130 ± 11b	17 ± 5.5c	71 ± 0.9d	20 ± 3.3c	92 ± 6.4e
29	Valeric anhydride	1532.4	85	21 ± 5.8a	326 ± 22b	27 ± 4.8a	110 ± 162a	20 ± 3.3a	284 ± 7.8b
30	3-Amino-4-hydroxybenzoic acid	1678.7	153	84 ± 22a	106 ± 2.6b	22 ± 3.8c	41 ± 1d	14 ± 1.3c	63 ± 4e
Esters									
31	Hexanoic acid, ethyl ester	1055.4	88	7.3 ± 5.5ab	22 ± 2.3a	5.6 ± 2.4ab	12 ± 2.7ab	3.7 ± 1b	43 ± 20c
32	Butanoic acid, 3-hydroxy-, ethyl ester	1065.7	88.1	6 ± 3.9a	3 ± 3.1a	5.6 ± 2.4a	5.6 ± 8a	2.4 ± 1.3a	15 ± 24a
33	2-Propenoic acid, 2-methyl-, (tetrahydro-2-furanyl) methyl ester	1113.9	71.1	1.4 ± 2.5a	45 ± 10b	8.8 ± 2.8c	77 ± 7.6d	0.6 ± 1.1a	30 ± 9.5e
34	Propanoic acid, 2-methyl-, ethyl ester	1238.5	71.1	106 ± 17a	83 ± 33ab	33 ± 13cd	30 ± 3.3c	64 ± 3.3bd	66 ± 10bd
35	3-Furancarboxylic acid, methyl ester	1243.6	95	8.2 ± 5a	865 ± 32b	80 ± 21c	1585 ± 161d	5.4 ± 1.8a	912 ± 104b
36	Aspirin methyl ester	1289.4	120.1	4.7 ± 0.8a	1.2 ± 0.2b	1.7 ± 0.3b	1.2 ± 0.1b	1.1 ± 0.4b	5.1 ± 1a
37	1,2,3-Propanetriol, 1-acetate	1440.7	61	17 ± 4.1ab	31 ± 10.2a	18 ± 16ab	29 ± 2.9ab	16 ± 7.3b	27 ± 5.3ab

38	Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester	1505.3	71.1	13 ± 3a	9.5 ± 3.9a	8.9 ± 13a	10 ± 2.7a	10 ± 1.8a	6.1 ± 1.7a
39	Glycerol 1,2-diacetate	1527.6	43.1	12 ± 6.4ab	24 ± 6a	5.9 ± 3.4b	11 ± 13ab	9.5 ± 2.6ab	13 ± 12ab
40	Tributyl phosphate	1667.2	99.1	121 ± 27	116 ± 21	58 ± 5.3	89 ± 8.7	131 ± 31	127 ± 25
41	Carbamic acid, methylphenyl-, ethyl ester	1905.0	179	25 ± 4.4a	187 ± 5.7b	28 ± 4.8a	121 ± 6.2c	19 ± 1.3a	221 ± 23d
42	2-Ethylhexyl salicylate	1928.0	120	18 ± 1a	1.4 ± 0.2b	1.5 ± 0.7b	1.3 ± 0.1b	2.6 ± 0.2b	1.2 ± 0.2b
43	Homosalate	2020.9	138	29 ± 0.9a	0.1 ± 0.2b	n.d	0.1 ± 0b	0.5 ± 0.2b	n.d
44	Phthalic acid, hept-4-yl isobutyl ester	2058.3	149	869 ± 49a	728 ± 65abc	562 ± 238c	730 ± 13abc	821 ± 190ab	654 ± 88bc
45	Hexadecanoic acid, ethyl ester	2073.5	88	35 ± 4.4a	6.3 ± 0.6b	35 ± 29a	7.3 ± 1.4b	12 ± 1.2c	10 ± 1.1c
46	Benzoic acid, 2-benzoyl-, methyl ester	2193.4	163	3.2 ± 1.6a	4.2 ± 1.7a	2.5 ± 0.8a	2.5 ± 1.1a	3 ± 0.2a	2.6 ± 0.8a
47	Ethyl oleate	2259.7	55.1	39 ± 6.7a	2.3 ± 0.3b	4.2 ± 0.2b	1.5 ± 0.2b	14 ± 0.6c	1.5 ± 0.2b
48	Hexanedioic acid, bis(2-ethylhexyl) ester	2544.5	129	7.8 ± 4.1a	10 ± 4.8a	6 ± 2a	5.2 ± 2.2a	8.1 ± 4.1a	5.4 ± 2.2a
49	1,2-Cyclohexanedicarboxylic acid, dinonyl ester	2924.2	155	1.4 ± 0.9a	2.6 ± 1.7ab	3.1 ± 4.3ab	0.5 ± 0.5a	7 ± 5.6b	1.5 ± 0.9a
50	Phthalic acid, nonyl 4-octyl ester	2981.9	149	0.9 ± 1.4a	2.4 ± 0.2a	2.4 ± 2.4a	2.2 ± 2.1a	1.6 ± 2.7a	4.1 ± 4a
Fatty acids									
51	Propanoic acid	947.5	74.1	37 ± 7.4a	18 ± 8.6a	25 ± 7a	31 ± 10a	25 ± 20a	23 ± 19a
52	Butanoic acid	1003.8	60	67 ± 4.9a	0.2 ± 0.3b	5.7 ± 8.1bc	0.7 ± 1.2b	14 ± 9c	5.5 ± 9.5bc
53	Hexanoic acid	1166.3	45.1	117 ± 14abc	95 ± 39abc	80 ± 35a	128 ± 8.8c	86 ± 7.2ab	127 ± 20bc
54	Heptanoic acid	1253.7	60	25 ± 3.1ab	33 ± 0.7c	15 ± 7.2d	29 ± 0.5bc	21 ± 2.2ad	29 ± 3.2bc
55	2-Ethyl-hexoic acid	1281.6	88	19 ± 3.3a	30 ± 1.4b	16 ± 6.8a	27 ± 1.5b	14 ± 2.5a	26 ± 3.5b
56	Octanoic acid	1353.4	60	73 ± 10ab	103 ± 5c	48 ± 16d	87 ± 5.7be	69 ± 6.4a	90 ± 11ce
57	Nonanoic acid	1448.0	60	88 ± 18ab	134 ± 20c	63 ± 28a	98 ± 13ab	82 ± 6.1ab	117 ± 28bc
58	n-Decanoic acid	1546.7	73.1	54 ± 14abc	89 ± 19a	43 ± 18b	64 ± 11abc	53 ± 3.5bc	84 ± 36ac
59	Dodecanoic acid	1749.4	73.1	157 ± 28ab	214 ± 13bcd	119 ± 78a	261 ± 43d	164 ± 15abc	224 ± 14cd
60	Tetradecanoic acid	1932.9	73.1	n.d	n.d	n.d	n.d	7.5 ± 13	n.d

61	Myristoleic acid	1944.2	69	11 ± 2.7a	23 ± 7.1b	8.7 ± 1.6a	17 ± 6.9ab	7.7 ± 5.4a	12 ± 4.4a
62	Z-11-Tetradecenoic acid	1944.2	69.1	11 ± 2.7a	23 ± 7.1b	8.7 ± 1.6a	17 ± 6.9ab	7.7 ± 5.4a	12 ± 4.4a
63	Pentadecanoic acid	2052.0	73	32 ± 11ab	46 ± 6.8a	18 ± 0.6bc	36 ± 9.7ab	13 ± 9.4c	28 ± 13bc
64	Palmitoleic acid	2144.5	69.1	23 ± 12a	52 ± 11b	15 ± 0.7ac	28 ± 7.1a	7 ± 4.1c	20 ± 7.2ac
65	n-Hexadecanoic acid	2155.4	73	229 ± 75a	348 ± 32b	95 ± 37c	234 ± 50a	109 ± 56c	245 ± 69a
66	9-Octadecenoic acid	2341.8	83.1	5.6 ± 3.6a	14 ± 4.9b	1.9 ± 1.4a	9.9 ± 7.3ab	2.3 ± 0.3a	7.3 ± 5.3ab
67	(E)-9-Octadecenoic acid	2341.8	69	8 ± 1.1a	13 ± 4.6a	2 ± 1.5b	13 ± 7.7a	2.1 ± 1.1b	10 ± 5.1a
68	(Z,Z)-9,12-Octadecadienoic acid	2347.9	81.1	5.2 ± 0.4ab	14 ± 5.7ab	0.9 ± 0.7a	27 ± 27.2b	1.5 ± 0.4a	17 ± 10ab
69	Octadecanoic acid	2353.7	73	48 ± 22a	76 ± 5.3b	9.3 ± 6.6c	55 ± 8ab	24 ± 13c	60 ± 7.4ab
Furans									
70	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	1065.4	101.1	2 ± 1.5a	116 ± 30.2b	5.7 ± 0.3a	52 ± 15c	1.7 ± 0.4a	88 ± 5.8d
71	Dihydro-3-methylene-2(3H)-Furanone	1075.2	98.1	1 ± 1.7a	73 ± 16b	11 ± 10ac	48 ± 42bc	2.3 ± 0.4a	74 ± 6.9b
72	3-Furancarboxylic acid	1097.3	112	25 ± 5.5a	127 ± 21bc	28 ± 20ad	82 ± 14bd	19 ± 7.3a	145 ± 63c
73	Tetrahydro-3-furanmethanol	1113.4	71	1.4 ± 2.4a	26 ± 23b	8.6 ± 2.8ab	77 ± 7.6c	0.6 ± 1a	30 ± 9.5b
74	Furaneol	1239.5	128	255 ± 31a	849 ± 335b	164 ± 37a	411 ± 18c	160 ± 11a	731 ± 43b
75	1-(2-furanylmethyl)-1H-pyrrole	1298.9	81.1	4.5 ± 0.9a	12 ± 0.7b	6.5 ± 1.9c	7.1 ± 0.3c	3.5 ± 0a	10 ± 0.5d
76	5-Acetoxymethyl-2-furaldehyde	1483.1	126.1	1.3 ± 0.5a	6.2 ± 0.7ab	1.5 ± 0.2a	57 ± 4.5c	4.5 ± 4.4a	10 ± 2b
77	5-Hydroxymethylfurfural	1524.5	97	2.2 ± 0.3a	558 ± 51bc	115 ± 19b	5796 ± 371d	6.3 ± 2.1a	892 ± 137c
78	Dihydro-4-hydroxy-2(3H)-furanone	1556.1	44.1	20 ± 5.6ab	120 ± 9.1c	13 ± 4.8ab	34 ± 26a	8.1 ± 1.1b	84 ± 4.5d
79	2,3,5-Trimethyl-1H-indole	1692.1	158.1	15 ± 23a	3.3 ± 3.7a	4.9 ± 3.1a	2.7 ± 4.8a	22 ± 31a	11 ± 10a
Hydrocarbons									
80	1,1,5-Trimethyl-1,2-dihydronaphthalene	1412.9	157.1	5 ± 0.7a	2.6 ± 0.1b	4.3 ± 1.5a	2.9 ± 0.1b	8.3 ± 0.5c	2.5 ± 0.1b
81	Fluoranthene	2301.0	202	2 ± 0.2a	0.3 ± 0.4b	0.6 ± 0.3b	n.d	1.4 ± 0.1a	n.d
82	Tricosane	2307.3	57	10 ± 1.6a	2.3 ± 0.4b	10 ± 6.1ac	2.2 ± 0.7b	6.4 ± 0.4c	1.5 ± 0.4b
83	Hexadecanamide	2445.2	59.1	67 ± 10ab	37 ± 9.6c	26 ± 23c	47 ± 24bc	88 ± 6.4a	55 ± 17bc

84	(Z)-9-Octadecenamide	2636.4	59.1	38 ± 12ab	34 ± 16abc	14 ± 11c	21 ± 12bc	53 ± 2.1a	41 ± 13ab
85	Octadecanamide	2649.2	59.1	96 ± 14ab	56 ± 24ac	27 ± 31c	61 ± 38ac	131 ± 15b	81 ± 27a
Ketones									
86	4-Hydroxy-4-methyl-2-pentanone	992.4	59	1357 ± 364a	509 ± 544a	912 ± 1289a	617 ± 567a	980 ± 674a	n.d
87	1-(Acetyloxy)-2-propanone	1034.6	86.1	22 ± 2.5a	42 ± 16ab	22 ± 25a	29 ± 14a	15 ± 2.6a	66 ± 24b
88	4-Cyclopentene-1,3-dione	1046.0	96	10 ± 6.6a	122 ± 18b	27 ± 8.8a	142 ± 7.6b	6.4 ± 1.1a	141 ± 29b
89	1-(4-Methylphenyl)-1-pentanone	1062.3	119	13 ± 1.4a	11 ± 1.1a	5.1 ± 2b	12 ± 6.3ab	13 ± 3.4a	24 ± 8.6c
90	Butyrolactone	1118.9	86.1	62 ± 10a	303 ± 53b	42 ± 13a	154 ± 38c	60 ± 4.9a	229 ± 27d
91	2-Hydroxy-3-methyl-2-cyclopenten-1-one	1173.0	112	32 ± 4.9a	132 ± 2b	36 ± 15a	110 ± 8.9c	29 ± 4.3a	132 ± 10b
92	Acetophenone	1182.4	105	5.7 ± 0.3a	4.7 ± 0.7ab	1.3 ± 1.1c	2.5 ± 0.1cd	3.6 ± 0.6bd	7.6 ± 0.8e
93	Phorone	1189.4	123	5.3 ± 0.8a	5.6 ± 0.7a	2.5 ± 3.6a	3.4 ± 0.2a	4.4 ± 0.7a	5.5 ± 2.9a
94	1-Methyl-2,4-Imidazolidinedione	1239.2	114	120 ± 13a	271 ± 7.7b	112 ± 23a	234 ± 11c	104 ± 13a	339 ± 17d
95	Furyl hydroxymethyl ketone	1243.9	95	7.8 ± 5.1a	865 ± 32b	80 ± 21c	1585 ± 161d	4.9 ± 1.4a	912 ± 104b
96	Maltol	1263.0	126.1	143 ± 16ab	583 ± 17c	201 ± 53b	508 ± 25d	125 ± 12a	608 ± 63c
97	5-Acetyl-2-methylpyridine	1268.5	135.1	10 ± 1.1a	25 ± 1b	11 ± 2.8a	9.2 ± 0.5a	6.8 ± 0.4c	25 ± 1.3b
98	3,5-Dihydroxy-2-methyl-4H-pyran-4-one	1348.8	142	852 ± 67ab	2656 ± 2588bc	609 ± 216ab	2767 ± 392bc	293 ± 5.8a	3555 ± 113c
99	1-(2-Hydroxy-5-methylphenyl)-ethanone	1471.2	150.1	144 ± 28a	160 ± 12a	76 ± 25b	108 ± 3.4c	65 ± 1.2b	206 ± 13d
100	1,2-Dihydro-3H-1,2,4-triazol-3-one	1485.7	85.1	21 ± 5.8a	324 ± 18b	27 ± 4.8a	106 ± 166c	20 ± 3.3a	284 ± 7.8d
101	2-Imidazolidinone	1502.7	86.1	67 ± 7.7ab	245 ± 5.1c	42 ± 13ad	184 ± 40e	28 ± 4.2d	85 ± 2.1b
102	4,4,6-Trimethyltetrahydro-1,3-oxazin-2-one	1518.5	128	61 ± 11a	687 ± 39b	52 ± 10.1a	167 ± 4.3c	25 ± 1.6a	354 ± 23d
103	(S)-4-Ethyl-2-oxazolidone	1531.6	85.1	67 ± 7.7a	244 ± 5.1b	42 ± 13ad	160 ± 77c	28 ± 4.2d	85 ± 2.1a
104	1,3-Dioxol-2-one	1534.3	86.1	25 ± 3ab	118 ± 4.3d	32 ± 8.5ab	36 ± 16b	21 ± 1.6a	75 ± 2.1c
105	2,4,6-Tris(1,1-dimethylethyl)-4-methylcyclohexa-2,5-dien-1-one	1594.7	205.1	3.1 ± 0.1a	1.8 ± 0.5bc	1.5 ± 1.7bc	1.1 ± 0.3b	2.4 ± 0.6ac	0.8 ± 0.3b

Nitrogen compounds									
106	1H-Imidazole-4-methanol	1025.7	98.1	35 ± 11a	25 ± 6.1a	4.5 ± 6.4b	47 ± 43ab	9.1 ± 10b	30 ± 12a
107	N-Butyl-tert-butylamine	1149.5	114	21 ± 4.4a	86 ± 5.8b	17 ± 2.7a	60 ± 5.8d	8.7 ± 0.9c	85 ± 9.7e
108	3-Formyl-4,5-dimethyl-pyrrole	1162.2	123.1	20 ± 6.3a	3.8 ± 0.7b	n.d	n.d	n.d	1.3 ± 1.4b
109	5-Amino-2-methyl-2H-tetrazole	1273.5	71.1	17 ± 1.8a	63 ± 3.1b	12 ± 2.4a	47 ± 3.2c	14 ± 1.9a	61 ± 6.1b
110	2-Pyrrolidinone	1316.3	85.1	54 ± 63a	17 ± 29a	12 ± 1.9a	20 ± 18a	3.8 ± 1a	41 ± 71a
111	1-methyl-1H-pyrrole-2-carboxaldehyde	1343.2	109.1	17 ± 2.6a	196 ± 15b	20 ± 4.6a	95 ± 3.7c	10 ± 0.8e	104 ± 6c
112	1-Azabicyclo 3.1.0 hexane	1357.4	83.1	68 ± 14a	5.9 ± 3.3b	33 ± 45.4b	5.4 ± 6.2b	10 ± 14b	7.9 ± 2b
113	Succinimide	1417.2	99.1	17 ± 5.1a	34 ± 2.8b	7.7 ± 2.6c	24 ± 2.3d	11 ± 2ac	25 ± 4d
114	Caprolactam	1491.7	113.1	7.4 ± 0.8a	0.1 ± 0.1b	3.2 ± 3.2c	4.3 ± 0.4c	4.2 ± 0.6c	0.1 ± 0.1b
115	m-Aminophenylacetylene	1532.4	117	17 ± 5.6a	28 ± 4.2a	0.9 ± 1.3b	11 ± 4.7a	11 ± 4.1a	31 ± 29a
116	(Z)-13-Docosenamide	3033.2	59.1	493 ± 180a	1210 ± 229b	460 ± 100ac	150 ± 43c	243 ± 170ac	352 ± 112ac
Sulphur compounds									
117	Dimethyl sulfone	1175.8	79.1	9.3 ± 1.3a	30 ± 2.8b	5.2 ± 1.6c	15 ± 0.9d	5.8 ± 0.1c	17 ± 1.7d
118	2,3-Dihydro-thiophene	1502.8	86.1	67 ± 7.7ab	244 ± 5.1c	42 ± 13ad	182 ± 39e	28 ± 4.2d	85 ± 2.1b
Pyrans									
119	2,3-Dehydro-1,8-cineole	1028.6	109.1	17 ± 2.6a	27 ± 2.9b	8.2 ± 2.6c	12 ± 0.7ac	13 ± 2.5ac	40 ± 7.8d
Terpenes									
120	3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol	1853.7	69.1	75 ± 12a	5.6 ± 0.6b	12 ± 14b	4.8 ± 1.6b	2.1 ± 0.8b	5.7 ± 1.8b
121	Squalene	2854.7	69.1	36 ± 11a	26 ± 5.5a	33 ± 9.9a	24 ± 15ab	20 ± 3.4ab	9 ± 1.5b
Total relative contents by chemical class									
Alcohols				2224 ± 383	2886 ± 652	932 ± 178	2806 ± 655	686 ± 93	963 ± 172
Aldehydes				231 ± 32	2792 ± 542	639 ± 151	6438 ± 1419	251 ± 42	2763 ± 585
Benzenes				213 ± 40	427 ± 103	157 ± 38	518 ± 138	204 ± 37	499 ± 106
Carboxylic acids and derivatives				177 ± 27	589 ± 127	73 ± 9	303 ± 28	71 ± 3	468 ± 114
Esters				1326 ± 192	2144 ± 242	870 ± 124	2721 ± 377	1133 ± 182	2147 ± 241

Fatty acids	1010 ± 60	1324 ± 86	574 ± 35	1160 ± 74	708 ± 45	1136 ± 73
Furans	327 ± 79	1891 ± 284	359 ± 56	6566 ± 1810	228 ± 49	2074 ± 323
Hydrocarbon	219 ± 38	132 ± 23	82 ± 11	134 ± 26	288 ± 53	181 ± 34
Ketones	3028 ± 339	7308 ± 592	2333 ± 230	6937 ± 674	1835 ± 220	7134 ± 787
Nitrogen and sulphur compounds	842 ± 182	1942 ± 507	617 ± 162	659 ± 164	358 ± 87	841 ± 148
Terpenes and pyrans	128 ± 29	58 ± 12	54 ± 13	41 ± 10	35 ± 9	54 ± 19

n.d: not detected; RT: retention time; m/z: mass-to-charge ratio; DW: Dry weight

Data are presented as mean ± SD (n = 3) and listed in the order of group and then retention index. Different alphabets indicate statistical difference ($p < 0.05$) across each row.

Table 2.17 Relative content percentage (%) of volatile classes identified in the pulp and peel of three tamarillo cultivars.

The results are presented as mean ± SD

Chemical class	Relative content percentage (%)					
	'Amber' peel	'Amber' pulp	'Laird's Large' peel	'Laird's Large' pulp	'Mulligan' peel	'Mulligan' pulp
Alcohols	22.83 ± 2.2	13 ± 5.1	12.5 ± 8.9	9.6 ± 4.7	11.4 ± 6.1	5.3 ± 3.6
Aldehydes	2.4 ± 0.3	13.3 ± 3	10.2 ± 2.8	22.7 ± 3.6	4.4 ± 0.8	15.1 ± 1.1
Benzenes	2.2 ± 0.3	2 ± 0.4	2.5 ± 0.7	1.9 ± 0.2	3.6 ± 0.5	2.7 ± 0.2
Carboxylic acids and derivatives	1.8 ± 0.5	2.8 ± 0.7	1.2 ± 0.5	1.1 ± 0.7	1.2 ± 0.4	2.6 ± 0.1
Esters	13.7 ± 1.5	10.3 ± 2.3	14 ± 4.1	9.7 ± 1.1	20.1 ± 6.4	11.7 ± 1.5
Fatty acids	10.5 ± 2.8	6.4 ± 1.7	8.9 ± 1.9	4.1 ± 0.8	12.5 ± 4.2	6.2 ± 1.5
Furans	3.4 ± 0.6	9 ± 3	5.9 ± 2.1	23.4 ± 2.1	4.1 ± 1.5	11.3 ± 1.3
Hydrocarbons	2.3 ± 0.4	0.6 ± 0.2	1.1 ± 0.5	0.5 ± 0.3	5.1 ± 1.2	1 ± 0.3
Ketones	31.1 ± 4.7	32.9 ± 16.4	32.7 ± 20.5	24.5 ± 4.9	31.1 ± 11.2	39.1 ± 1.8
Nitrogen and sulphur compounds	8.6 ± 2.7	9.4 ± 3.1	10.1 ± 4.1	2.4 ± 0.6	6 ± 2.9	4.6 ± 1.4
Terpenes and pyrans	1.3 ± 0.2	0.3 ± 0	0.9 ± 0.7	0.2 ± 0.1	0.6 ± 0.2	0.3 ± 0.1

2.4.2.2 Principal component analysis (PCA)

To evaluate the correlation between the volatile distribution and the chemical groups of the volatile compounds in tamarillo pulp and peel from the three different cultivars, PCA was conducted (Figure 2.14). It can be seen that separation of different tamarillo samples based on the volatile profile was obviously visible. All tamarillo cultivars were absolutely resolved by the tissue on PC1 demonstrating 43.63% of the variance, and by the cultivar on PC2 explaining another 18.75% of the variance. The third PC component described another 7.96% of the variance (data not shown as 70% of the total variance explained) and therefore, no further PCs were considered. For PC1, the three cultivar pulps were located on the negative region, whereas all peel samples fell in the positive region. For PC2, pulp and peel of 'Laird's Large' cultivar fell in the positive region, Mulligan pulp and peel were in the middle and all 'Amber' samples were located on the negative area (Figure 2.14). This is the first time to classify and differentiate tamarillo samples according to their cultivars and tissues, by characterizing their volatile profile by TD-GC-MS and analysing the data by PCA. The TD-GC-MS, a superior analytical technique, has higher sensitivity for volatile compounds present in trace amount. Meanwhile, the PCA has successfully discriminated the different volatile compounds of the pulp and peel of the three tamarillo cultivars.

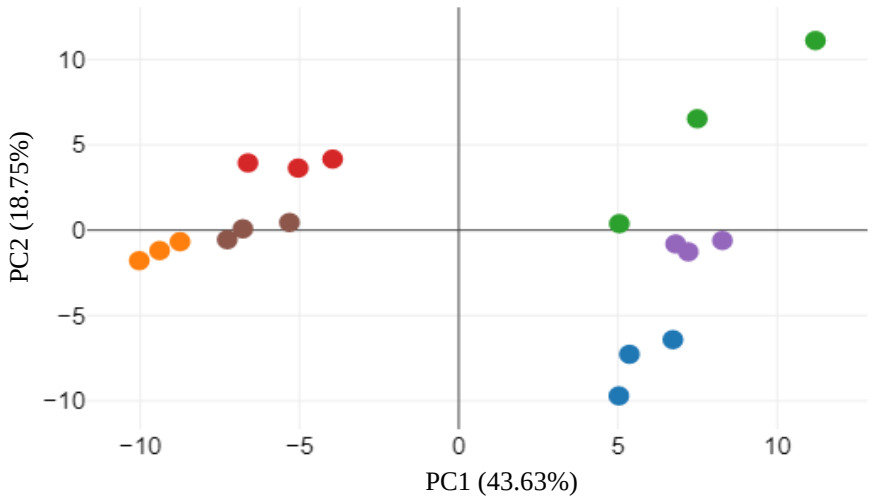

Figure 2.14 Score plots of PCA showing separations of 3 cultivars and 2 tissues of New Zealand tamarillo by all volatiles.

(● 'Amber' peel, ● 'Amber' pulp, ● 'Laird's Large' peel, ● 'Laird's Large' pulp, ● 'Mulligan' peel, ● 'Mulligan' pulp)

2.4.2.3 Odor threshold and relative odor activity value (OAV)

The OAV concept is an useful approach in aroma research although some limitations exist (Pino & Queris, 2011), it does help understand what a consumer will be able to sense. The OAV is calculated a ratio of concentration to odour threshold of the volatile, this value indicates contribution of each volatile to the typical flavour of any food product. If the OAV of a volatile compound is greater than 1, this compound considerably contributes to the overall fruit odour. The higher the OAV, the greater the possibility of a volatile compound to be sensed (Pino & Queris, 2011). A total of 36 compounds from 121 detected volatiles in three tamarillo cultivars were presented with odour detection threshold in water (ppb) and the relative OAVs due to lack of odour threshold data in the literature. In which, only 15 of these volatiles (5 aldehydes, 2 alcohols, 2 esters, 2 furan, 1 hydrocarbon, 1 ketone, 1 pyran and 1 terpene) were considered the most representative of lyophilized tamarillo flavour which presented beyond their odour threshold (Table 2.18).

Five aldehydes were found with relative OAVs higher than 1. In this class, methional had the highest relative OAVs in all of the analysed samples. It is described as potato and tomato flavour (Mayer et al., 2008), and tamarillo belongs to the family of these fruits, hence this compound being considered as the most representative of tamarillo flavour was reasonable. Nonanal, (E)-2-decenal and octanal compounds could be associated with fatty and green notes of tamarillo. Although showing higher relative OVAs in peel than in pulp, both nonanal and (E)-2-decenal also considerably contributed to the overall flavour of peel and pulp of three tamarillo cultivars. Octanal is recognized as an essential flavour contributor to the overall flavour of ‘Laird’s Large’ pulp (52.34) and ‘Mulligan’ pulp (19.59) as well as less important to flavour of ‘Mulligan’ peel (1.94). 3-furaldehyde having relatively low relative OAV (1.44) might also contribute to the fruit’s aroma character of ‘Laird’s Large’ pulp (Table 2.18).

Eugenol and 2,3-butanediol were major alcohols having relative contents higher than their odour detection thresholds. 2,3-butanediol which has been characterised by creamy and fruity notes greatly contributed to the overall flavour of tamarillo coming from the pulp rather than the peel. By contrast, eugenol, which carries a sweet odour, suggests the opposite location in the fruit. Two esters with relative OAVs > 1 were responsible for sweet and fruity characters of tamarillo. Hexanoic acid, ethyl ester or

ethyl hexanoate, a main volatile of tamarillo, have greater contribution to the overall flavour of all pulp rather than the peels. In contrast, propanoic acid, 2-methyl-, ethyl ester is likely to be more involved to the typical flavour of 'Amber' and 'Laird's Large' peels, while the relative OAVs of this compound determined for 'Mulligan' peel and 'Mulligan' pulp were quite similar.

Pulp and peel volatiles of all cultivars had caramel and fruity notes due to presence of furaneol, one of the most crucial aroma compounds in tomato (Du & Qian, 2008) and from our results, for tamarillo as well (Table 2.18). One hydrocarbon compound, 1,1,5-trimethyl-1,2-dihydronaphthalene, has great contribution to provide the typical flavour of tamarillo, in all of the samples with the relative OAV > 1. This compound is characterised by licoricey note and delicious wine odour. Ketones were the abundant volatiles in various tamarillo cultivars, however, only one ketone compound, 4-hydroxy-4-methyl-2-pentanone, has contribution towards the flavour of both the peel and pulp of tamarillo when evaluated using the relative OAVs, except for the 'Mulligan' pulp. This ketone compound has a minty note (Table 2.18).

Although the relative content of 2,3-dehydro-1,8-cineole and 3,7,11-trimethyl-2,6,10-dodecatrien-1-ol was low, it has shown favourable contribution to the flavour of tamarillo samples. The pyran compound (2,3-dehydro-1,8-cineole) was present in amounts higher than its odour threshold concentrations (relative OAV >1) for only 'Amber' and 'Mulligan' samples. This compound has minty and sweet characteristics. Only the 'Amber' peel sample had 3,7,11-trimethyl-2,6,10-dodecatrien-1-ol which exhibited the OAV > 1. This terpene is associated with sweet and floral flavour.

The fatty acids and the nitrogen-containing compounds may possibly contribute less to the perceived flavour of tamarillo, since they generally have high odour detection thresholds. Several determined volatile compounds having relative OAVs of less than 1 may probably have contributed to the fruity odour property of tamarillo in synergistical way. Therefore, quantitative analysis using dynamic headspace combined with GC-MS as well as sensory testing with trained panellists would provide a better understanding of how these volatile compounds contribute to the overall flavour of tamarillo.

Table 2.18 Odour threshold, relative odour activity value (OAV) and odour description of several volatiles in peel and pulp of New Zealand grown tamarillo cultivars.

No	Compounds	Odour threshold in water (ppb)[a]	Relative odour activity value (OAV)						Odour description[b]
			'Amber' peel	'Amber' pulp	'Laird's Large' peel	'Laird's Large' pulp	'Mulligan' peel	'Mulligan' pulp	
Alcohols									
1	2,3-Butanediol	30	37.84	57.89	16.39	56.41	9.46	14.82	Fruity, creamy
2	Alpha-terpineol	330-350	< 1	< 1	< 1	< 1	< 1	< 1	Floral, citrus, minty
3	Eugenol	6-30	22.33	3.98	9.94	1.78	9.74	7.58	Sweet
4	2,6-Dimethoxy-4-(2-propenyl)-phenol	1850	< 1	< 1	< 1	< 1	< 1	< 1	Sweet, spicy
Aldehydes									
5	3-Furaldehyde	3000	< 1	< 1	< 1	1.44	< 1	< 1	Almond
6	Methional	0.2	114.33	933.39	77.12	588.59	70.05	643.99	Tomato, potato
7	Octanal	0.7	< 1	< 1	< 1	52.34	1.94	19.59	Fatty, green
8	Nonanal	1	37.61	30.66	19.17	12.00	29.76	11.28	Fatty, green
9	(E)-2-Decenal	0.3-0.4	16.03	6.70	7.60	4.15	42.69	15.63	Fatty, green
Esters									
10	Hexanoic acid, ethyl ester	1	7.29	21.58	5.55	11.80	3.68	43.00	Sweet, fruity
11	Propanoic acid, 2-methyl-, ethyl ester	10	10.61	8.32	3.32	2.99	6.39	6.55	Sweet, fruity
12	Hexadecanoic acid, ethyl ester	2000	< 1	< 1	< 1	< 1	< 1	< 1	Fruity, creamy, waxy
Fatty acids									
13	Propanoic acid	20000	< 1	< 1	< 1	< 1	< 1	< 1	Dairy, soy
14	Butanoic acid	240	< 1	< 1	< 1	< 1	< 1	< 1	Fruity, dairy, cheesy
15	Hexanoic acid	3000	< 1	< 1	< 1	< 1	< 1	< 1	Fatty, cheesy

16	Heptanoic acid	3000	< 1	< 1	< 1	< 1	< 1	< 1	Fruity, cheesy, pineapple
17	Octanoic acid	3000	< 1	< 1	< 1	< 1	< 1	< 1	Fatty, cheesy
18	Nonanoic acid	3000	< 1	< 1	< 1	< 1	< 1	< 1	Fatty, green
19	n-Decanoic acid	10000	< 1	< 1	< 1	< 1	< 1	< 1	Fatty
20	Dodecanoic acid	10000	< 1	< 1	< 1	< 1	< 1	< 1	Fatty
21	Tetradecanoic acid	10000	< 1	< 1	< 1	< 1	< 1	< 1	Fatty
22	Myristoleic acid	10000	< 1	< 1	< 1	< 1	< 1	< 1	-
23	Z-11-Tetradecenoic acid	10000	< 1	< 1	< 1	< 1	< 1	< 1	-
24	n-Hexadecanoic acid	10000	< 1	< 1	< 1	< 1	< 1	< 1	Creamy
25	Octadecanoic acid	20000	< 1	< 1	< 1	< 1	< 1	< 1	Fatty
Furans									
26	Furaneol	60	4.25	14.15	2.74	6.86	2.67	12.19	Fruity, caramel
27	5-Hydroxymethylfurfural	3000-230000	< 1	< 1	< 1	1.93	< 1	< 1	Caramel, buttery
Hydrocarbons									
28	1,1,5-Trimethyl-1,2-dihydronaphthalene	2	2.48	1.28	2.16	1.43	4.13	1.26	Licoricey, delicious wine
Ketones									
29	4-Hydroxy-4-methyl-2-pentanone	280	4.85	1.82	3.26	2.20	3.50	< 1	Minty
30	2-Hydroxy-3-methyl-2-cyclopenten-1-one	1000	< 1	< 1	< 1	< 1	< 1	< 1	Sweet, fruity
31	Acetophenone	65	< 1	< 1	< 1	< 1	< 1	< 1	Sweet, flower
32	Maltol	35000	< 1	< 1	< 1	< 1	< 1	< 1	Sweet, fruity caramel
Nitrogen compounds									
33	N-Butyl-tert-butylamine	50000	< 1	< 1	< 1	< 1	< 1	< 1	-
34	Caprolactam	65	< 1	< 1	< 1	< 1	< 1	< 1	-
Pyrans									
35	2,3-Dehydro-1,8-cineole	12	1.44	2.21	< 1	< 1	1.07	3.31	Minty, sweet

Terpenes									
36	3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol (Farnesol)	20	3.75	< 1	< 1	< 1	< 1	< 1	Sweet, floral

2.5 Conclusion

New Zealand is the main producer of the fruit tamarillo, however, information of nutritional value and health benefits from New Zealand tamarillo cultivars are not fully investigated or understood. Current composition data of tamarillo do not reflect the large diversity of different cultivars available for consumers despite the clear importance of this fruit in diet. Hence, this part explored proximate components, antioxidant vitamins, bioactive compounds (phenolics including anthocyanins), antioxidant activity and volatile compounds in both endo- and mesocarp (pulp) and exocarp (peel) of three different tamarillo cultivars including 'Amber', 'Laird's Large' and 'Mulligan'. Measurements of physicochemical properties, nutrient contents and volatiles using standard (AOAC and spectrometry methods) and advanced techniques (metabolite derivatization, GC and LC-MS/MS) demonstrated significant differences between the cultivars as well as tissues. The current study showed comprehensive physicochemical and bioactive compositions in different tamarillo cultivars as well as tissues compared to previous studies. Tamarillo is nutrient-dense with nutrients and food-based bioactives identified were dietary fibre, β-carotene, α-tocopherol, vitamin C, phenolics including anthocyanins.

A comprehensive study to determine the physicochemical characteristics and nutrient and phytochemical content, especially bioactive compounds (polyphenols and related antioxidants) of tamarillo that may be beneficial for the human health is presented in this chapter. Some key findings are summarised as follows:

- High dietary fibre content of approximately 3% was determined in all tamarillo cultivars and nutritional adequacy score of tamarillos (7.9 and 7.4 for gold and red cultivars, respectively) was high compared to other fruit.
- Free amino acid profile of tamarillo was dominated by L-glutamic acid, GABA and L-aspartic acid. GABA concentration in tamarillos was high and relatively similar to tomato.
- Compared with standard serves of common New Zealand fruits, tamarillos showed the highest β-carotene (9-20% RDI/serve), high ascorbic acid (67-75% RDI/serve) and α-tocopherol (16-23% AI/serve) (FSANZ labelling) that could make a remarkable contribution to the daily intake of vitamins A, C and E.

- Chlorogenic acid was the most abundant polyphenol in tamarillo, whereas delphinidin 3-rutinoside was dominant compound in pulp of all tamarillo cultivars. Antioxidant activity of tamarillo revealed relatively high values and was strongly correlated with high content of total phenolic.
- This is the first study to analyze volatile compounds in tamarillo using TD-GC-MS, in which ketones, aldehydes and furans were dominant volatiles of pulp. Fifteen volatile compounds were present beyond their odour thresholds and methional was the most significant flavour contributor in tamarillo.

2.6 Summary and next questions

This chapter successfully employed LC-MS/MS and TD-GC-MS as advanced and sensitive analytical techniques to discriminate physicochemical compositions as well as phytochemical and volatile profiles of peel and pulp from three tamarillo cultivars. These techniques provided an in-depth identification and quantification of these compounds at low concentrations. The findings in this chapter demonstrated various amino acids, phenolics, anthocyanins, antioxidant vitamins and volatiles in different New Zealand tamarillos which fill the missing gaps from previous studies.

As hypothesized (Part I, chapter 2) it was confirmed that tamarillo is highly nutritious and contains many bioactive compounds with potential for well-being. Tamarillo grown in New Zealand has shown the potential to serve as a dietary source for antioxidant vitamins, and a source for polyphenols, carotenoids and pigments. Tamarillo skin (by-product) could also be used as a source of antioxidants, natural colorants and flavour enhancement to reduce food waste and improve sustainability of food production. The majority of tamarillo is eaten fresh, and the fruit is underutilized by the food industry. Although New Zealand produces a high amount of tamarillo, only a few tamarillo products are available in the market. The next part (Part II), therefore, proposes to develop yoghurt drink fortified with bioactive compounds from tamarillo. Further characterization of the fortified yoghurt will be performed to examine the nutritional properties and possible bioactivity and accessibility in the digestive system.

The questions to be answered in the next chapter is: What is the effect of fortification of yoghurt with dried tamarillo powder and the fermentation process on the physicochemical properties and nutrient and volatiles content of yoghurt?

PART II:
YOGHURT FORTIFIED WITH TAMARILLO

Chapter 3: Literature review of yoghurt production and fruit derived yoghurt

Globally yoghurt sales have increased markedly over the past few last decades from 982.6 million pounds in 1990 to about 4,742.1 million pounds in 2015(Aryana & Olson, 2017). Fortification, the addition of vitamins and minerals and reformulation, the addition or removal of ingredients, are important processes for improvement of the quality and quantity of nutrients and bioactive compounds in yoghurts. This would be one way of formulating a functional food and improving diet quality and healthiness of the diet.

Fruits are considered as rich sources of natural bioactive components including antioxidants such as polyphenols, carotenoids and vitamin C, E. Numerous studies have been carried out to supplement yoghurt with different fruit juices, pulp, seeds and pomace and to investigate changes of textural and rheological properties as well as antioxidant activity associated with the addition of fruit-derived bioactive compounds. Bio-accessibility and bioavailability of polyphenols and protein from yoghurt matrices is important, since only the compounds released after the gastrointestinal digestion are in a condition to exert their beneficial effects.

Therefore, this chapter summarises what is known already about the production of fortified yogurts (mainly through the addition of dietary fibres and bioactive compounds) as well as potential health benefits of fortified yoghurts. This chapter is organised as follows: Section 3.1 briefly summarizes the yoghurt production. Then, yoghurts fortified with fibre and with bioactive compounds from fruits are illustrated in Section 3.2 and 3.3, respectively

3.1 Production of yoghurt

Yoghurt, a fermented milk product can be formulated in a variety textures (smooth, liquid, set, frozen, powder), fat content (whole, low-fat or fat -free), flavours (natural, cereal, fruit, honey) and milk types (cow, goat, sheep) (Mckinley, 2005; Shah, 2003). The general process of yoghurt production is illustrated in Figure 3.1. Basically, the manufacture of yoghurt can be divided into three major stages: (1) preparatory

processing stage, (2) fermentation stage and (3) storage. Based on each stage, different types of yoghurt can be produced (Routray & Mishra, 2011; Shah, 2003). For example, there are two basic categories of yoghurt including: (1) standard culture yoghurt being produced from typical starter culture strains (*Lactobacillus delbrueckii. bulgaricus* and *Streptococcus thermophilus*) (Arena et al., 2015), and (2) bio-yoghurt being manufactured with probiotic strains (*Bifidobacterium* and *Lactobacillus acidophilus*) (Baltova & Dimitrov, 2014).

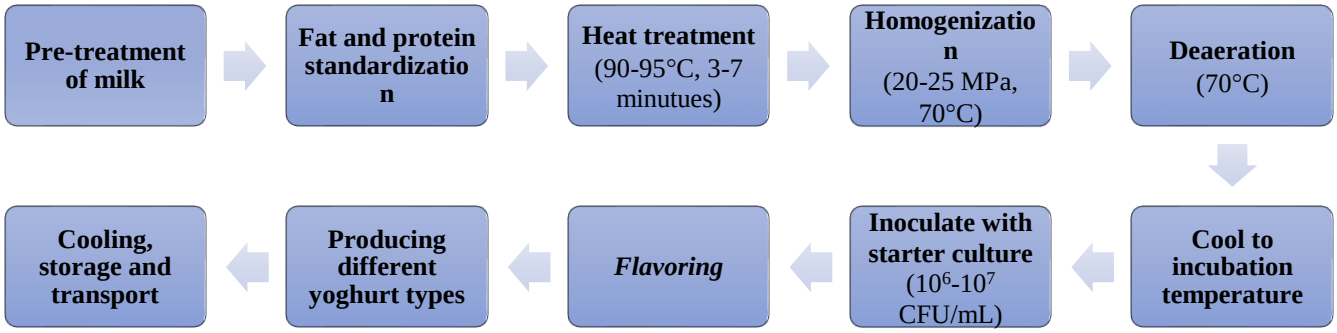

Figure 3.1 Schematic diagram of general yoghurt production process.

Yoghurt often owns a higher nutritional benefits (including protein bioavailability, calcium, biotin, folic acid and vitamin concentration) in comparison with milk (Walstra, Geurts, Walstra, & Wouters, 2005) (Table 3.1). In fact, yoghurt was recognized as the most frequently consumed vehicle of probiotics for consumers (Corrieu & Béal, 2016). Advantages of yoghurt when consumed as part of a healthy diet contribute towards weight management, reduction of cholesterol absorption as well as lower body fat mass and lower incidence of obesity from increased calcium intake (Martinez-Gonzalez et al., 2014; Zemel, Shi, Greer, Dirienzo, & Zemel, 2000). Functional components such as essential amino acids, medium chain fatty acids, whey protein, high concentration of calcium have been reported to have beneficial effects on the decrease of blood pressure, especially in elderly hypertensive patients (Aryana & Olson, 2017; Gahruie, Eskandari, Mesbahi, & Hanifpour, 2015). Yoghurt can be consumed by lactose intolerant customers without any detrimental influence since the fermentation process converts lactose to lactic acid (Gahruie et al., 2015). Besides, yoghurt also has protective capacity against pathogenic bacteria, viruses and intestinal infections, often leading to diarrhoea. The protective capacity comes from either the acidity of yoghurt or inhibitor molecules generated by fermentation and specific starter

cultures. Various strains of *Salmonella* are killed by the acidity of yoghurt, while *Lactobacillus* strains inhibit pathogens (*E. coli*, *Salmonella aureus* and *Listeria monocytogenes*) and prevent spoilage microorganisms (*Penicillium expansum*) from growing. Yoghurt consumption also has been reported to improve immune function (Corrieu & Béal, 2016) particularly of the gut through and increase in antibody and cytokine production, and natural killer cell and phagocytic activity. And these are associated with a lower incidence of cancers, tumour formation, allergic symptoms as well as gastrointestinal disorders such as bloating (Aryana & Olson, 2017; Mckinley, 2005). A recent study indicated that fermented milk product consisting of *L. rhamnosus* SD-11 have been able to enhance oral health through lowering *Streptococci spp.* in saliva (Rungsri et al., 2017).

Table 3.1 Comparison of chemical constituents of unflavoured whole-fat milk, whole-fat yoghurt, non-fat milk and non-fat yoghurt.

(Gahruie et al., 2015)

Constituents (/100g)	Whole-fat milk	Whole-fat yoghurt	Non-fat milk	Non-fat yoghurt
Protein (g)	3.3	3.8	3.5	4.4
Fat (g)	3.8	3.8	0.1	0.1
Potassium (mg)	157	157	150	187
Calcium (mg)	120	120	123	143
Phosphorus (mg)	92	92	97	109
Vitamin B_1 (μg)	30	60	40	50
Vitamin B_2 (μg)	170	270	170	250
Vitamin B_6 (μg)	60	100	60	90
Vitamin C (μg)	1	1	1	1
Biotin (μg)	1.9	2.6	1.9	2.9
Folic acid (μg)	6	18	5	17
Pantothenic acid (μg)	350	500	320	450
Lactic acid (g)	0	0.8	0	1.0

3.2 Yoghurt fortified with fibre

Yoghurt and other dairy products do not contain dietary fibre, an important constitute in the cell walls of fruit, vegetables, seeds and grains. Dietary fibre is defined as edible

carbohydrate polymers with three or more monomeric units that are resistant to the endogenous digestive enzymes and thus neither hydrolysed nor absorbed in the small intestine (Makki, Deehan, Walter, & Bäckhed, 2018). Hence, fortification of foods with extracted or synthesized non-digestible carbohydrates or their use in dietary supplements constitutes a strategy to increase fibre intake (Makki et al., 2018).

Value-added dairy products, particularly yoghurt, with the addition of ingredients with diverse dietary fibres would generate functional foods with health benefits and enhance their functionality (Gahruie et al., 2015). Numerous studies have reported on the health benefits of high-fibre products and these include inhibiting or reducing hypertension and obesity (Van Dam & Seidell, 2007), coronary heart disease (Pereira et al., 2004), type 2 diabetes (Anderson, Randles, Kendall, & Jenkins, 2004) and cancer (Bingham et al., 2003). According to Gahruie et al. (2015), the maximum amount of fibre to add for texture to be acceptable is 3%. For example, Fernández-Garía, McGregor, and Traylor (1998) confirmed the texture enhancement of unsweetened yoghurt through addition of 1.32% fibre from oat while fibre gained from by-products of asparagus manufacturing were fortified into yoghurt at the concentration of 1% (w/w) (Sanz, Salvador, Jimenez, & Fiszman, 2008). Hence, fibre fortified yoghurt should be further developed to improve human nutrition, gut health and dependent on the fibre type may help with mouthfeel to aid the process of swallowing. The contribution of one serving of yoghurt (150 g) to daily recommended dietary intake of 30 g would be small at 4.5g if 3%. However, it is the nature of the fibre that could be more important such as fructo-oligo saccharides (FOS also known as hydrocolloids) which are fermented in the gut to short chain fatty acids (Gannasin, Mustafa, Adzahan, & Muhammad, 2015b). According to Mobley, Slavin, and Hornick (2013), a good source of fibre must contain 10 – 19% of the daily value (25 gram per day) or 2.5 – 4.9 g of fibre per RACC (reference amount customarily consumed). Meanwhile, an excellent source must contain ≥ 20% of the daily value, or ≥ 5 g of fibre per RACC.

3.3 Yoghurt fortified with fruit bioactive compounds

Fruits are rich in bioactive compounds in which, phenolic substances have gained notable attention in recent years because of their antioxidant activity. Despite the

nutritional values and health benefits, fermented dairy products have not been recognized as the major source of phenolic substances and the concentration of phenolic compounds has been small (O'connell & Fox, 2001). Fortification with various fruit juices, pulp, seeds, powder, extracts, pomace and essential oil to enhance phenolic component as well as bioactivity of yoghurt have been made. Addition of essential oil from basil, peppermint or zataria in probiotic yoghurt has shown *in vitro* retardation of the growth of the bacteria *L. monocytogenes* and *E. coli* (Azizkhani & Tooryan, 2016). Yoghurt fortified with *Azadirachta indica* (neem) was recognized as a potentially functional dairy product for consumers with type-2 diabetes and hypertension. The fortified yoghurt had higher antioxidant activity than plain yoghurt over the storage period (28 days) and significantly inhibited α-amylase, α-glucosidase and angiotensin-1 converting enzymes activities which the authors postulated would have anti-diabetic and antihypertensive effects when consumed (Shori & Baba, 2013).

Phenolic compounds including anthocyanins, flavanols, flavonols and hydroxycinnamic acids from apple and blackcurrant berry could be added to drinking yoghurt during pre- and post-fermentation (Sun-Waterhouse, Zhou, & Wadhwa, 2012, 2013b). These studies report that the addition of polyphenols before fermentation resulted in drinking yoghurt with presence of phenolic acids, more polar polyphenols and higher total extractable polyphenol content compared to when added after fermentation. Chlorogenic acid, epicatechin, *p*-coumaric acid, phlorizin and phloretin derivative were retained in yoghurt enriched with apple polyphenol extract with pre and post addition (Sun-Waterhouse et al., 2012). In addition yoghurt enriched with blackcurrant polyphenols before or after the fermentation of yoghurt contained four typical anthocyanins (cyanidin 3-glucoside, cyanidin 3-rutinoside, delphinidin 3-glucoside and delphinidin 3-rutinoside) in which the concentration of cyanidin rutinoside was the highest (Sun-Waterhouse et al., 2013b).

Stirred yoghurt fortified with pomegranate peel extracts exhibited potentials in treatment of bronchitis, diarrhoea, fever, malaria, vaginitis and urinary tract infection (El-Said, Haggag, El-Din, Gad, & Farahat, 2014). No remarkable changes of appearance, flavour and texture have been observed when adding pomegranate peel extracts into stirred yoghurt, while the fortified yoghurt owned higher antioxidant activity than the plain sample. Sah, Vasiljevic, McKechnie, and Donkor (2016) supplemented pineapple by-product powder into yoghurt to investigate prebiotic,

antioxidant and antimutagenic capacities. The fortified yoghurt not only had higher cell count of *L. acidophilus*, *L. casei* and *Lactobacillus spp. Paracasei* strains but also demonstrated high antioxidant ability. The increase of phenolic component, antioxidant capacity and acidity as well as the reduction of pH, syneresis and fat in were achieved in grape skin added yoghurt (Marchiani et al., 2016).

Table 3.2 Fruit fortified yoghurts with bioactive compounds.

Fruits	Plant sources/extracts	Target bioactives	References
Apple	Pulp	Chlorogenic acid, epicatechin, p-coumaric acid, phlorizin	Sun-Waterhouse et al. (2012)
Blackcurrant	Pulp	Anthocyanins	Sun-Waterhouse et al. (2013b)
Grape	Pomace, skin, seed	Phenolic compounds, epicatechin	Chouchouli et al. (2013); (Marchiani et al., 2016; Mohamed, Zayan, & Shahein, 2014)
Strawberry	Pulp	Anthocyanins, phenolic compounds, catechin, kaempferol, quercetin-3-rutinoisde	Oliveira et al. (2015)
Pomegranate	Juice, peel	Phenols, flavonols, tannins, saponins, vitamin C	Ali (2016); (El-Said et al., 2014)

3.4 Summary and next questions

This chapter showed different nutritious components of fortified yoghurts and the beneficial impact of enriched yoghurts on preventing or treating disease. However, studies dealing with fortified dairy product with tamarillo food applications are still scarce. Therefore, there is reasonable potential for producing milk products from tamarillo such as yoghurts that provide opportunities for novel dairy products fortified with fruit-derived ingredients and health promoting effect from tamarillo.

From the literature, it is evident that the fibre content of New Zealand tamarillo meets the requirement of fortified-fibre yoghurt and phenolic compounds from this fruit can be supplemented into yoghurt during pre- and post-fermentation. Hence, it would be considerable to develop yoghurt with fortification of fibre and bioactive compounds from tamarillo and evaluate physicochemical properties, nutrients, bioactive compounds and volatile content of fortified yoghurts (Chapter 4). Also, changes of

amino acids, polyphenols and antioxidant activity in fortified yoghurts during *in vitro* digestion are explored in the Chapter 5.

Chapter 4: Effect of tamarillo fortification and fermentation process on physicochemical properties and nutrient and volatiles content of yoghurt

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Bright-red 'Laird's Large' tamarillo is a unique and under-utilized fruit which is a dietary source of carotenoids, vitamins C and E and dietary fibre. In this chapter, yoghurt samples enriched with tamarillo powder were produced in pre- and post-fermentation processes to achieve the potential health benefits. The effects of addition of freeze-dried tamarillo powder (5-15%) to milk and yoghurt starter either before (PRE) or after (POS) fermentation on physicochemical properties were examined. Using LC-MS and GG-MS, nutrient and volatile contents of tamarillo yoghurt were also examined. The addition of tamarillo prior to fermentation was associated with a more yellow color, higher concentrations of tocopherol compared to when tamarillo was added after fermentation. Higher elastic modulus, PUFAs, pro-vitamin A content and vitamin C retention were observed for POS than in PRE. All tamarillo yoghurts showed improvement in syneresis, lower lactose content and higher concentrations of antioxidant vitamins than the commercial premium-assorted fruits yoghurt from New Zealand Food Composition Data. Yoghurt fortified with tamarillo powder offers the potential for the development of a high-value nutrition product that could be a good source of vitamin C and a source of vitamin E and β-carotene and maintain the volatiles that give tamarillo its distinctive flavor.

This chapter is organised as follows: Section 4.1 briefly states the motivations and objectives of this study. Materials and methods are described in Section 4.2. The results and discussion are shown in Section 4.3. A brief summary is included in Section 4.4.

4.1 Introduction

Tamarillos (*Solanum betaceum* Cav.) are a source of polyphenols including anthocyanins (delphinidin 3-rutinoside, pelargonidin 3-rutinoside and cyanidin 3-rutinoside), hydroxy benzoic acids (gallic acid), hydroxycinnamic acids (chlorogenic acid, caffeic acid), flavonols (kaempferol), flavanols (catechin, epicatechin) and flavonol glycosides (rutin, kaempferol 3-rutinoside); fibre; carotenoids (β-carotene); potassium and vitamins C, E and B6 (Diep et al., 2020a; Diep et al., 2020b; Diep et al., 2020c). Many of these constituents are strong antioxidants which are associated with health benefits such as reducing lipid oxidation and reducing risk for certain cancers, cardiovascular disease and type 2 diabetes mellitus (Diep et al., 2020d; Schotsmans et al., 2011). Tamarillo fruit has the potential to be a functional food or a functional ingredient for food reformulation.

Recently, the formulation of functional fermented dairy products through the addition of fruit powder has become popular (Salehi, 2021). Yoghurt is already known to possess positive health benefits from the presence of probiotics (Aryana & Olson, 2017). Yoghurts also provide an ideal environment to carry bioactive phytochemicals to the human body. The bioactive compounds can be added prior to fermentation as an ingredient, or after fermentation as part of the usual practice of adding flavoring and coloring agents (Sun-Waterhouse et al., 2012). In our previous work, we have shown that freeze-dried tamarillo powder contains 10.6 mg/100 g α-tocopherol, 3.4 mg/100 g β-carotene and 162 mg/100 g vitamin C, demonstrating the unique properties of NZ grown 'Laird's Large' tamarillo (Diep et al., 2020b). In this study the effects of the addition of freeze-dried tamarillo powder (5-15%) to milk and yoghurt starter either before or after fermentation on physicochemical, rheological and textural properties and the nutrient, volatiles and fatty acid content of tamarillo yoghurt was examined. It was hypothesised that the addition of the powder before fermentation would enhance bioactivity but after fermentation would preserve the attractive red colour of the tamarillo.

4.2 Materials and methods

4.2.1 Fruit and yoghurt materials

Tamarillo ('Laird's Large' cultivar), standard milk (Anchor blue top) purchased from a local supermarket (Auckland, New Zealand) and starter culture, *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* (YoFlex® Express 1.1 powder) from CHR Hansen (Hoersholm, Denmark) were used as yoghurt ingredients.

Milk compositions were presented in Table 4.1. Tamarillo fruits were washed thoroughly with tap water, dried, and the skin was peeled. Pulp was freeze-dried (Alpha 1–2 LD plus Freeze Dryer, Martin Christ, New Zealand) and ground to powder.

All chemicals and reagents used were AnalaR grade or purer. A mixture of 37 fatty acid methyl esters (FAMEs) standards (Supelco 47,885-U), as well as analytical standards of reducing sugars, organic acids, α-tocopherol, β-carotene and ascorbic acid were purchased from Sigma-Aldrich (Auckland, New Zealand). Milli-Q water was produced by Purite Fusion Milli-Q water purifying machine (Purite Limited, Thame, Oxon, UK).

Table 4.1 Compositions of Anchor blue top milk used in this study.

Constitutes (per 100 mL)	Quantity
Protein	3.3 g
Fat – Total	3.4 g
– Saturated	2.3 g
Carbohydrate	4.8 g
– sugars	4.8 g
Sodium	40 mg
Calcium	117 mg
Vitamin A	43 μg
Vitamin B_2 (Riboflavin)	0.2 mg

4.2.2 Yoghurt preparation

Yoghurt samples were prepared using commercial yoghurt makers (Davis & Waddell, Steven, New Zealand). For the control yoghurt, starter culture and milk in the ratio of 0.1 : 100 (w/w), were placed in the yoghurt maker and set at 45°C for 8 hours and until the pH dropped below 5.0. The yoghurt was stored at 4°C overnight and then

homogenized at 4000 RPM (L4R Laboratory Mixer, Silverson, Waterside, England) for 2 min (Wang, Kristo, & LaPointe, 2020). Then, the yoghurt was divided into two parts. The first part was stored at 4°C for analyses of physical properties, some proximate compositions, rheology, texture and microstructure. The second part was snap frozen by liquid nitrogen, lyophilized and ground to powder. The freeze-dried yoghurt powder was stored in a freezer at –20°C for analyses of reducing sugars, organic acids and antioxidant vitamins.

Freeze-dried tamarillo pulp powder (5, 10 and 15%) was added into the yoghurt either before (PRE) or after (POS) the fermentation process. For PRE, tamarillo powder was added to the mixture of milk and starter culture at the start of yoghurt making process, prior to fermentation. For POS, tamarillo powder was added to yoghurt in the final homogenization step. Seven preparations in total were prepared.

A preliminary experiment was conducted to determine suitable concentration of fortification of yoghurt for appropriate texture and flavor, at concentrations of 1 to 20%. Difference in flavour to the control could not be detected when fortification was less than 5%. Fortification of above 15% was associated with an undesirably thick texture. Therefore, 5, 10 and 15% of fortification were chosen for further experiments.

4.2.3 Physicochemical properties analysis of yoghurt samples

Moisture, ash, protein, fat, carbohydrate and dietary fibre contents were determined according to the AOAC methods (AOAC, 2005). Total Soluble Solids (TSS) was identified by using Rudolph refractometer (J57 Automatic Refractometer, Rudolph Research Analytical, Hackettstown, USA). Color was recorded using a Hunter Lab (45/0, Colorflex EZ) color analyser, in L*, a* and b* values. The pH was measured with a digital pH meter (HI 207, Hanna Instruments, USA) equipped with a glass electrode without dilution of samples. Titratable acidity was determined based on AOAC method 942.15 (AOAC, 2005), with dilution in MilliQ water (in 1:4 (v/v) ratio), followed by titration against 0.1 M NaOH until the pH reached 8.1. The results are expressed as a percentage of lactic acid.

Syneresis index of yoghurt was determined according to Wang et al. (2020) with some modifications. Twenty grams of yoghurt sample was centrifuged (Eppendorf Centrifuge 5804R, Medi'Ray, Auckland, New Zealand) at 1500 RPM for 10 min at

4°C using an Eppendorf Centrifuges (ThermoFisher Scientific, Germany). The clear supernatant was poured off, weighed and recorded as syneresis (%) which was calculated as follows:

$$\text{Syneresis (\%)} = \frac{W_f}{W_i} \times 100$$

where W_i = weight of initial sample; W_f = weight of supernatant

4.2.4 Particle size distribution

The method of Wang et al. (2020) with some modifications was used to identify particle size distribution of yoghurt samples using static light scattering (Mastersizer 2000; Malvern Instruments, UK) with a refractive index of 1.33 for water as a dispersant. Samples were measured in triplicate after 2 h storage at 4°C. Sauter mean diameter D [3,2] known as surface area moment mean was achieved from the instrument software and calculated as:

$$D[3,2] = \frac{\Sigma d^3}{\Sigma d^2}$$

where d = diameter of the sphere with surface area or volume, respectively, equivalent to that of the particles being measured.

4.2.5 Rheological and textural analysis

Some modifications were made from Kristo, Miao, and Corredig (2011) for rheological measurements, using a rheometer (RST-SST, Brookfield Ametek, Middleboro, USA). The rheometer was calibrated following manufacturer's instructions before these measurements. For rheology, viscosity standard fluid (Brookfield Ametek) was used for calibration at 25°C. For texture, the calibration was carried out using Force with appropriate calibration weight (50 g) on the calibration platform. To determine viscosity profile (viscosity, consistency coefficient, flow behavior index), a concentric cylinder geometry (diameters of cup and bob were 28.92 and 26.66 mm, respectively) was used. A 20 mL of yoghurt sample was transferred into the cup, and a trap was used to prevent evaporation. Steady state viscosity measurements were performed at shear rates from 1 to 400 s^{-1}. To determine elastic modulus, a vane spindle (SSVANE) was used, and the measurement was conducted at

a speed of 0.5 rpm for 5 min. Data were acquired and processed with Rheo 3000 software.

A backward-extrusion test was conducted in yoghurt samples using TA-XT plus texture analyser (Texture Technologies Corp, New York, USA) according to Wang et al. (2020) with some modifications. The following parameters were applied: cylinder probe diameter of 50 mm, test speed of 1.0 mm/s, penetration distance of 25 mm and surface trigger force of 10 g. Firmness (N), consistency (N*s) and cohesiveness (N) were calculated from the exponent software. The test was carried out at ambient temperature with sample temperature of approximately 4 °C. The samples were taken directly from the refrigerator at 4°C and placed on the instrument stage for measurements.

4.2.6 Volatile analysis

A 1 – 2 g yoghurt sample was quickly introduced into 10 mL headspace vial. A 10 μL of internal standard (6.5 mg/L solution of dichlorobenzene in water) was added into the vial and vortexed for 30 s. GC-MS coupled with SPME fibre was used for volatile analysis according to our previous study (Diep et al., 2021). The total run time was 37 min.

4.2.7 Fatty acid analysis

Fatty acids were analysed according to Sun and Zhao (2014) with some modifications. A serial FAME standard solution was prepared in hexane and biphenyl solution (200 μg/mL: 10 mg biphenyl in 50 mL chloroform) was used as internal standard. Approximately 0.5 g of yoghurt sample was quickly introduced into centrifuge tube. Hexane 10 mL and 1 mL of sodium methoxide (5.4 M) in methanol solution were added into the vial. After strong vortex for 2 min and centrifuging at 3000 RPM for 10 min, 0.45 mL of the clear hexane layer was transferred to an autosampler vial and 50 μL of internal standard was added before injecting into GC-MS.

The GC-MS and DB- FATWAX-Ultra Inert (UI) column (Agilent Technologies, Australia), measuring 30 m × 250 μm × 0.25 μm, were used to analyse the fatty acids. Chromatographic conditions were as follows: the oven was held at 50°C for 2 min,

then raised to 200°C at a rate of 20°C/min and then raised to 240°C at a rate of 5°C/min held for 10 min. The equilibration time and total run time were 0.5 and 27.5 min, respectively. The inlet conditions were as follows: helium was the carrier gas. The mode of injection was split, and inlet temperature for the injection port was set to 250°C. The injection volume was 1 μL and the spilt ratio was 10:1. The MS was operated in the SIM mode with a source temperature of 250°C, a quadrupole temperature of 150°C, and transfer line temperature of 250°C. Concentrations of 35 fatty acids were quantified using Agilent MassHunter Quantitative software (Agilent Technologies, Australia). Peak areas were calculated for the reference ion for each target in each sample, standard and blank, and normalised to recovery of the internal standard. Standard curves were constructed using linear relationships between relative peak area and concentration. Quantification of fatty acids in tamarillo yoghurt was implemented using standard calibration curves fitted with at least six suitable concentrations. A coefficient of correlation (r^2) of > 0.99 was obtained for all fatty acids. Chromatograms of standard injections are shown in Appendix B14 and a summary of the method validation for all of the identified fatty acids is presented in Appendix B15.

The lipid indices of yoghurt including atherogenic index (AI), thrombogenic index (TI) and saturation index (SI) were calculated as follows (Ribeiro et al., 2021):

$$AI = (C12:0 + 4 * C14:0 + C16:0) / (\Sigma \text{ MUFAs} + \Sigma \text{ PUFAs})$$

$$TI = (C14:0 + C16:0 + C18:0) / [(0.5 * \Sigma \text{ MUFAs}) + (0.5 * \Sigma \text{ n-6}) + (3 * \Sigma \text{ n-3}) + (\Sigma \text{ n-3} / \Sigma \text{ n-6})]$$

$$SI = (C14:0 + C16:0 + C18:0) / (\Sigma \text{ MUFAs} + \Sigma \text{ PUFAs})$$

where Σ MUFAs is the sum of monounsaturated fatty acids and Σ PUFAs is the sum of polyunsaturated fatty acids.

4.2.8 Reducing sugars and organic acids analysis

Identification and quantification of reducing sugars were implemented according to our previous study (Diep et al., 2020c). Analytical standards of fructose, lactose, galactose, glucose, mannose, ribose, rhamnose, maltose, arabinose, xylose, fucose and glucuronic acid were used for calibration. Profile of organic acid was identified based

on Trigueros, Sayas-Barberá, Pérez-Álvarez, and Sendra (2012) with some modifications and then quantified by using LC-ESI-MS/MS. Briefly, freeze-dried samples (100 mg) were homogenized in 1 mL ultrapure water acidified with 0.1% formic acid and shaken vigorously for 30 s. The samples were incubated for 30 min at 4°C and then centrifuged for 10 min at 10,000 rpm at 4°C. The supernatants and the blank (0.1% formic acid) were injected into LC-MS/MS system. Separation was performed by using the Kinetex C18 column (100 x 2.1 mm, 1.7 μm; Phenomenex, USA). Mobile phase A was 0.1% formic acid in Milli-Q and mobile phase B was 0.1% formic acid in acetonitrile. LC gradient was set at: 0 – 3 min, 100% of A; 3 – 5 min, 80% of A; 5 – 10 min, 50% of A; 10 – 25 min, 100% of A. Flow rate and injection volume were 0.2 mL/min and 1 μL, respectively. The MS was run in the negative mode with the total time of 25 min. The MMI source operating at ESI parameters were gas temperature of 300°C, gas flow of 10 L/min. Nebulizer, capillary voltage, column temperature and vaporizer temperature were 40 psi, 4 kV, 25°C and 200°C, respectively. External standards used include malic acid, lactic acid, malonic acid, citric acid, succinic acid, fumaric acid, itaconic acid, vanillic acid, syringic acid and trans-cinnamic acid. Quantification of these analytes was undertaken using external pure standard calibration curves fitted with at least six suitable concentrations. A coefficient of correlation (r^2) of > 0.99 was obtained for all reducing sugars and organic acids.

4.2.9 Ascorbic acid, α-tocopherol and β-carotene

Identification and quantification of α-tocopherol, β-carotene and ascorbic acid were implemented according to our previous study (Diep et al., 2020b) without further modification.

4.2.10 Statistical analysis

Mean and standard deviation were calculated based on at least three independent measurements ($n \geq 3$) for each experiment. Correlation was determined by Pearson correlation coefficient, r. One-way analysis of variance (ANOVA) and Fisher's (LSD) multiple comparison tests were applied to identify whether significant differences

exist among different yoghurts. Data analysis was carried out using SPSS 25.0 (IBM Corp., Armonk, New York, USA) and the statistical significance level was set at $p < 0.05$. Principal Component Analysis (PCA) was used to assess variability among yoghurt samples, and the concentrations of various compositions were visualised by heatmap and hierarchical clustering. Both PCA and heatmap were carried out using the MetaboAnalyst web interface (Chong et al., 2019).

4.3 Results and discussion

4.3.1 Physical properties and proximate compositions of tamarillo yoghurts

For color, lightness (L*) decreased significantly ($p < 0.05$) by 1.1 – 2.1 times, while red/green (a*) increased as a higher dose of tamarillo powder was added. Fermentation process may have affected the color results from the absorption of water by dietary fibre and polyphenols in tamarillo. Variation in the a* and b* value was significant ($p < 0.05$) as the tamarillo powder has high redness compared to milk. POS showed more redness compared to the PRE of the same % fortification, e.g., by 3 times in POS5 compared to PRE5. This might be due to change in acidity of yoghurt during fermentation, causing decolouration of natural pigments, such as anthocyanins, to the yoghurt matrix (Ścibisz, Ziarno, & Mitek, 2019). Fermentation process might have favored the release of some pigments from tamarillo, mainly carotenoids, making the product more yellow. Therefore, most PRE samples showed higher b* value than the POS ones; e.g. 2.5 times higher in PRE5 compared to POS5.

Fortification, regardless of POS or PRE, substantially reduced syneresis. Addition of 10% and 15% tamarillo powder reduced the syneresis of yoghurt by approximately a half and one-third of that of the control, respectively (Table 4.2). Syneresis is the separation of liquid which occurs in weak gel-like structures such as yoghurt, and it is a visible defect having negative influence in consumer acceptability. It is mainly related to rearrangements of protein molecules (or the aggregation), caused by the difference of density between phases where whey proteins accumulate on the surface of yoghurt and expel the serum out of the food matrix (Wang et al., 2020). PRE possibly marginally lowered the syneresis through interactions of the added fibres and polyphenols from tamarillo with milk during fermentation which could form a stable gel structure. Syneresis was reduced with increased concentration of tamarillo (Table

4.2). A 5% increase in concentration was associated with a substantial 7% decrease in syneresis (16 to 9%). This phenomenon can be explained by insoluble fibre (cell wall in tamarillo) absorbing water through porous fibrillar structures (Harris & Smith, 2006), and enhancing the water holding capacity of the yoghurt. In addition, soluble fibre, such as pectin, creates linkages with both the aqueous phase and protein in the gel to increase the gel strength. Pectin can further stabilise casein aggregates through electrostatic and steric stabilisation that may lead to formation of casein-pectin complexes (Liu, Nakamura, & Corredig, 2006). Tamarillo contains a high amount of methoxyl pectin, which may form gel in an acidic environment incorporating the free serum and reducing syneresis (Do Nascimento, Simas-Tosin, Iacomini, Gorin, & Cordeiro, 2016b).

For both PRE or POS addition of tamarillo, TSS, ash, protein, carbohydrate and dietary fibre contents were increased proportionally with the level of fortification as shown in Table 4.2. In both of PRE and POS of 15% fortification, increases in TSS were 25–28%, ash 22–24%, protein 42–46% and carbohydrate 71–73% were observed. Fortification reduced fat content (by 3–11%) and syneresis by 63–68%, when compared to the control. Dietary fibre is often absent in yoghurt and dairy products. Addition of dietary fibre by fortification with tamarillo is able to change solubility, viscosity, hydration properties, oil-binding capacity and antioxidant activity (Elleuch et al., 2011), and enhance the viability of probiotic bacteria and their colonization ability (Do Espírito Santo et al., 2012b). POS10, POS15, PRE10 and PRE15 showed higher dietary fibre content (0.7, 1.1, 0.7, 1.0%) compared to the 0.4% reported for premium-assorted fruit yoghurt found in New Zealand Food Composition Database (NZ-FCD) (Sivakumaran, Huffman, & Sivakumaran, 2017). A standard serve of yoghurt is 150g and daily recommended dietary intake for an adult is 30g.

Higher TSS, ash, and protein were found in POS (2.5 – 4.0%, 2.9 – 4.9% and 6.7 – 9.9%, respectively) than PRE but fibre content was not different. PRE samples showed lower Brix value than the POS of the same % fortification as sugars in tamarillo were converted into acid during the fermentation process. This has caused higher acidity (lower pH) in PRE samples. Because the gel structure of yoghurt is mainly formed by denaturation of milk proteins (κ-casein and whey protein) at low pH, controlling the acid formation during fermentation is very important. The control samples showed the highest pH value and the lowest acidity (> 3 – 10% and < 13 – 32%, respectively)

compared to the tamarillo fortified yoghurts. Presence of acids, such as ascorbic acid, citric acid and malic acid, in tamarillo powder has contributed towards further acidification (Do Espírito Santo, Perego, Converti, & Oliveira, 2012a). Similar effect was also reported in yoghurt fortified with passion fruit peel powder (Do Espírito Santo et al., 2012a).

Table 4.2 Physical properties and proximate compositions of yoghurt samples.

Parameters	Tamarillo powder (%/DW)	Control	POS5	POS10	POS15	PRE5	PRE10	PRE15
Lightness (L*)	-	76.53 ± 0.87^{a}	52.98 ± 0.26^{b}	40.07 ± 0.36^{c}	35.23 ± 0.24^{d}	69.8 ± 0.93^{e}	50.05 ± 0.62^{f}	39.92 ± 0.12^{c}
Redness (a*)	-	-0.33 ± 0.10^{a}	29.13 ± 0.23^{b}	31.12 ± 0.25^{c}	32.58 ± 0.39^{d}	9.53 ± 0.40^{e}	24.13 ± 0.28^{f}	28.05 ± 0.19^{g}
Yellowness (b*)	-	11.63 ± 0.43^{a}	8.13 ± 0.29^{b}	9.82 ± 0.10^{c}	10.05 ± 0.15^{c}	20.07 ± 0.56^{d}	12.37 ± 0.23^{e}	9.78 ± 0.13^{c}
Syneresis (%)	-	30.19 ± 0.90^{a}	22.88 ± 1.84^{b}	18.21 ± 1.62^{c}	11.18 ± 1.19^{d}	20.65 ± 1.48^{b}	15.78 ± 1.07^{e}	9.59 ± 1.25^{d}
pH	-	4.47 ± 0.03^{a}	4.03 ± 0.01^{b}	4.01 ± 0.02^{c}	3.93 ± 0.01^{d}	4.35 ± 0.00^{e}	4.29 ± 0.02^{f}	4.06 ± 0.01^{g}
Acidity (%)	-	0.91 ± 0.02^{a}	1.18 ± 0.04^{b}	1.26 ± 0.03^{c}	1.33 ± 0.03^{d}	1.05 ± 0.02^{e}	1.21 ± 0.03^{b}	1.3 ± 0.01^{d}
TSS (°Brix)	-	7.32 ± 0.19^{a}	9.05 ± 0.14^{b}	9.57 ± 0.08^{c}	10.12 ± 0.13^{d}	8.81 ± 0.06^{e}	9.33 ± 0.06^{f}	9.71 ± 0.07^{c}
Moisture (%)	-	89.72 ± 0.02^{a}	85.11 ± 0.05^{b}	81.92 ± 0.08^{c}	78.78 ± 0.01^{d}	85.79 ± 0.16^{e}	81.94 ± 0.11^{c}	79.2 ± 0.13^{f}
Ash (%)	8.26	0.53 ± 0.10^{a}	0.61 ± 0.09^{a}	0.67 ± 0.13^{a}	0.7 ± 0.08^{a}	0.58 ± 0.11^{a}	0.64 ± 0.17^{a}	0.68 ± 0.12^{a}
Protein (%)	9.69	1.84 ± 0.08^{a}	2.43 ± 0.11^{b}	3.06 ± 0.05^{c}	3.41 ± 0.08^{d}	2.19 ± 0.10^{e}	2.76 ± 0.07^{f}	3.18 ± 0.09^{c}
Fat (%)	2.36	2.93 ± 0.18^{a}	1.54 ± 0.15^{b}	1.92 ± 0.34^{bc}	2.59 ± 0.22^{ad}	1.72 ± 0.25^{b}	2.28 ± 0.27^{cd}	2.85 ± 0.21^{a}
Carbohydrate (%)	31.7	2.37 ± 0.16^{a}	5.05 ± 0.41^{b}	6.5 ± 0.40^{c}	8.06 ± 0.78^{d}	4.81 ± 0.24^{b}	8.15 ± 0.33^{d}	8.89 ± 0.68^{d}
Dietary fibre (%)	15.2	0.00 ± 0.00	0.36 ± 0.09^{a}	0.72 ± 0.10^{b}	1.05 ± 0.05^{c}	0.31 ± 0.06^{a}	0.68 ± 0.10^{b}	0.96 ± 0.05^{c}

* Data are presented as Mean ± SD ($n \geq 3$). Different alphabet superscripts indicate statistical difference ($p < 0.05$) across each row. DW: dry weight. POS5, POS10 and POS15: 5, 10, 15% of tamarillo powder was added post fermentation, respectively. PRE5, PRE10 and PRE15: 5, 10, 15% of tamarillo powder was added to milk and starter culture prior to fermentation, respectively. TSS: total soluble solids. Used with permission (Diep, Yoo, & Rush, 2022)

4.3.2 Particle size distribution

The particle size distribution and parameters of yoghurt samples are shown in Figure 4.1 and Table 4.3, respectively. A monomodal distribution was observed in control sample, whereas yoghurts fortified with tamarillo powder showed a bimodal distribution. Addition of tamarillo powder after fermentation significantly increased the average particle size as well as Sauter mean diameter D [3,2], whereas the opposite phenomenon was observed for adding tamarillo before fermentation process when comparing to the control. According to Wang et al. (2020), Sauter mean diameter has been more sensitive to changes in fine particles which have a greater influence of the surface area. Hence, this parameter can provide useful information in a bimodal distribution. The increased size of fines with adding tamarillo powder after fermentation might indicate a possible interaction between tamarillo components and casein gel particles forming entities or complexes of larger particle size. Meanwhile, as already observed with D [3,2] decreased with addition of tamarillo before fermentation, which might be a result of reduced water availability and space to expand for tamarillo powder particles in the presence of higher amount of protein and other milk solids.

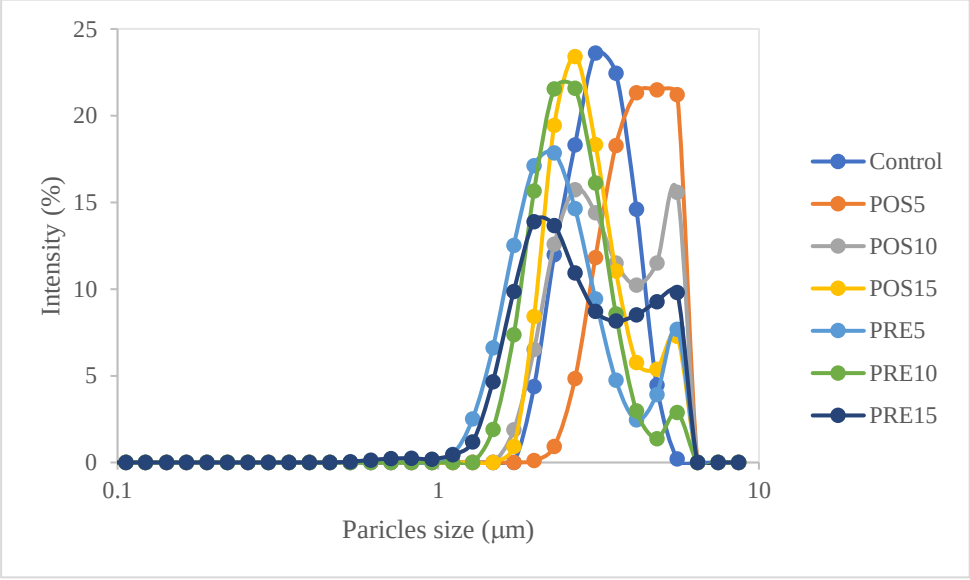

Figure 4.1 Particle size distribution of control yoghurt and yoghurts containing tamarillo powder produced from pre and post fermentation processes. POS5, POS10 and POS15: 5, 10, 15% of tamarillo powder was added post fermentation, respectively. PRE5, PRE10 and PRE15: 5, 10, 15% of tamarillo powder was added to milk and starter culture prior to fermentation, respectively

Table 4.3 Particle size distribution of control yoghurt and yoghurts containing tamarillo powder produced from pre and post fermentation processes.

Yoghurt samples	Z-average (d.μm)	PDI*	D [3,2]
Control	3.829 ± 0.458^{ab}	0.212 ± 0.068	3.917
POS5	4.9 ± 0.992^{c}	0.188 ± 0.06	5.228
POS10	4.512 ± 0.907^{bc}	0.232 ± 0.033	4.791
POS15	4.821 ± 0.866^{c}	0.245 ± 0.036	5.078
PRE5	3.089 ± 0.536^{a}	0.255 ± 0.094	3.251
PRE10	3.257 ± 0.216^{a}	0.242 ± 0.06	3.279
PRE15	3.627 ± 0.395^{a}	0.248 ± 0.053	3.701

* PDI: Polydispersity Index. Data are presented as Mean ± SD ($n \geq 3$). Different alphabet superscripts indicate statistical difference ($p < 0.05$) across each column. POS5, POS10 and POS15: 5, 10, 15% of tamarillo powder was added post fermentation, respectively. PRE5, PRE10 and PRE15: 5, 10, 15% of tamarillo powder was added to milk and starter culture prior to fermentation, respectively

4.3.3 Rheological analysis

Control yoghurt had a negligible viscosity constant, and 5% PRE and POS were also low. Both 10% and 15% were substantially more viscous. The increase between 5% and 10% was ~7x. The difference between 10% and 15% was 6x for PRE and 5x for POS (Table 4.4). The Oswald-de Waele power law model was used to model the flow behavior of the fortified yoghurt. For all yoghurt samples, the correlation coefficient for the model fit was above 0.96 (data not shown). The flow behavior index (n) of all samples were smaller than 1, hence yoghurt of all treatments in this study can be considered as non-Newtonian fluid with shear-thinning behavior. Similar results had been observed in yoghurt fortified with apple pomace (Wang et al., 2020) and orange pomace (Acharjee, Afrin, & Sit, 2020). According to Cui, Lu, Tan, Wang, and Li (2014), the pseudoplastic behaviour of yoghurt was due to breakage of bonds between the protein aggregates as a consequence of shear stress. The increase of tamarillo powder concentration led to the increase of consistency coefficient index and the decrease of flow behavior index. Whereas the fermentation process did not significantly affect these two values. The water holding capacity of tamarillo powder might have attributed to the increase of consistency coefficient value.

Elastic modulus describes the resistance of a material to deformation, and it relates to the solid-like characteristic that arises from the internal network structure. As shown in Table 4.4, the increase of tamarillo ratio resulted in higher elastic modulus of fortified yoghurt samples with significant difference ($p < 0.05$ for trend). The higher elastic modulus of 15% tamarillo yoghurt was indicative of a structure that is tending more towards solid than liquid. PRE resulted in a slightly lower elastic modulus compared to the POS. The type of interaction occurring between the fibres and polyphenols of tamarillo (mainly) and milk protein could contribute to this parameter. According to Chetachukwu, Thongraung, and Yupanqui (2018), this interaction can either be segregative or associative, depending on three different phenomena (co-solubility, complexation, or incompatibility). The hydration of fibres may also enhance the protein–protein interaction resulting in the increased gelation of the yoghurt structure (complexation). Apple, bamboo inulin and wheat, as source of dietary fibre had been used to improve rheological properties of yoghurt (Staffolo, Bertola, & Martino, 2004). The range of these interactions is governed by directed hydrogen bonding interactions that extend further than the electrostatics dependent screening length. Also, it is possible that the already weak protein structure of milk may have been disrupted. This is because some heat labile milk protein molecule may have undergone denaturation during fermentation, thereby limiting their surfactant capability at the oil–water interface of the emulsion.

Table 4.4 Rheological properties of control yoghurt and yoghurts containing tamarillo powder produced from pre and post fermentation processes.

Yoghurt samples	Consistency coefficient (K, Pa.s)	Flow behaviours index (n)	Viscosity at 350 s^{-1} (Pa.s)	Elastic modulus (Pa)
Control	< 0.005	0.88 ± 0.12	0.01 ± 0^{a}	N/A
POS5	0.30 ± 0.03	0.68 ± 0.02	0.05 ± 0^{b}	0.16 ± 0.003^{a}
POS10	2.22 ± 0.07	0.57 ± 0.01	0.18 ± 0.01^{c}	0.568 ± 0.005^{b}
POS15	13.1 ± 0.37	0.40 ± 0	0.40 ± 0.01^{d}	1.048 ± 0.029^{c}
PRE5	0.28 ± 0.02	0.65 ± 0.01	0.04 ± 0^{b}	0.123 ± 0^{d}
PRE10	2.25 ± 0.07	0.52 ± 0	0.14 ± 0.01^{e}	0.45 ± 0.005^{e}
PRE15	10.54 ± 0.82	0.42 ± 0.01	0.35 ± 0.01^{f}	0.929 ± 0.023^{f}

* N/A: not applicable. Data are presented as Mean ± SD (n ≥ 3). Different alphabet superscripts indicate statistical difference ($p < 0.05$) across each column. POS5, POS10 and POS15: 5, 10, 15% of tamarillo powder was added post fermentation, respectively. PRE5, PRE10 and PRE15: 5, 10, 15% of tamarillo powder was added to milk and starter culture prior to fermentation, respectively. Used with permission (Diep et al., 2022)

4.3.4 Textural analysis

According to Walia, Mishra, and Kumar (2013), the texture parameters are the key indicators for evaluating the physical and sensory quality of dairy products. These parameters depended not only by the arrangement of molecules in the gel network of products but also other factors such as manufacturing processes, protein and fat composition, other added ingredients, and particularly the concentration of properties of fibres. Addition of tamarillo powder has significantly ($p < 0.05$) improved the firmness, consistency, and cohesiveness of yoghurt as the dose of tamarillo powder increased regardless the fermentation process (Table 4.5). These may be explained by the phenolics including anthocyanin compounds present in tamarillo, which have hydroxyl (−OH) groups with a strong affinity for proline-rich proteins like casein (Yuksel, Avci, & Erdem, 2010). Similar findings with pomegranate extract were observed by Pan, Liu, Luo, and Luo (2019).

Fortification at 15% and PRE doubled the firmness and consistency when compared to the control (Table 4.5). According to Paseephol, Small, and Sherkat (2008), firmness of yoghurt depends on the total solid content of the milk mixture. Several factors affect the firmness of fermented milk such as the type of protein, the interaction between the ingredients used and the composition of the starter culture (Oliveira, Sodini, Remeuf, & Corrieu, 2001). By adding in dry tamarillo powder, total solids content also increased, and this may explain the higher firmness compared to the control. At 5 and 10% fortification, both POS and PRE samples showed similar firmness. However, for 15% fortification, PRE showed a higher increase in firmness of about 17% than the POS. The time of interaction (8 hours for fermentation) allows more binding sites between milk protein and tamarillo components (mainly amino acid side chains and polyphenol aromatic rings) to be produced and could explain the firmer texture. *L. delbrueckii* ssp. *bulgaricus* may also have contributed to this increase via exopolysaccharides, as shown in other study (Shihata & Shah, 2002).

According to Yildiz and Ozcan (2019), high consistency refers to a viscous product with high density. POS5 and PRE5 showed no change in consistency from the control. With 10 and 15% fortification, significantly higher consistency ($p < 0.05$) was observed when compared to the control (1.3 – 1.4 and 1.5 – 2.0 times, respectively). POS10 and PRE10 were similar in consistency, but by contrast, at 15% fortification, PRE produced higher consistency than POS. This may be explained by formation of complexes between fibre and polyphenols from tamarillo with the protein aggregates as part of the structural network formation as observed by others (Pan et al., 2019).

According to Peng, Serra, Horne, and Lucey (2009), the higher cohesiveness values with the addition of protein allows breakage of large number of casein–casein and protein linkages during stress application to reform after the stress was released. Cohesiveness is directly related to internal strength of the material structure. All of the fortified samples showed significantly higher cohesiveness than the control ($p < 0.05$) (Table 4.5). There was no clear pattern of differences in consistency within and between POS and PRE samples for different levels of fortification. The increase of cohesiveness in fortified samples may be attributed to the increase in viscosity from addition of tamarillo powder particles (both soluble and insoluble compounds), reinforcing the internal gel structure. It is likely that the rehydrated tamarillo powder absorbed the separated whey and also trapped casein clusters generated from shearing,

which may have strengthened the loose and opened protein structure to improve the consistency.

Table 4.5 Textural parameters of control yoghurt and yoghurt fortified with tamarillo powder from pre- and post-fermentation processes.

Yoghurt samples	Firmness (N)	Consistency (N.sec)	Cohesiveness (N)
Control	1.053 ± 0.004^{a}	15.797 ± 0.04^{a}	-0.003 ± 0.002^{a}
POS5	1.145 ± 0.007^{b}	16.607 ± 0.049^{a}	-0.136 ± 0.006^{b}
POS10	1.604 ± 0.022^{c}	21.915 ± 0.459^{b}	-0.341 ± 0.004^{c}
POS15	1.828 ± 0.08^{d}	24.189 ± 0.868^{c}	-0.496 ± 0.015^{d}
PRE5	1.133 ± 0.016^{b}	16.194 ± 0.102^{a}	-0.138 ± 0.014^{b}
PRE10	1.554 ± 0.023^{c}	21.189 ± 0.369^{b}	-0.28 ± 0.021^{e}
PRE15	2.211 ± 0.043^{e}	30.825 ± 1.065^{d}	-0.731 ± 0.048^{f}

* Data are presented as Mean ± SD ($n \geq 3$). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each column. POS5, POS10 and POS15: 5, 10, 15% of tamarillo powder was added post fermentation, respectively. PRE5, PRE10 and PRE15: 5, 10, 15% of tamarillo powder was added to milk and starter culture prior to fermentation, respectively. Used with permission (Diep et al., 2022)

4.3.5 Volatile profile

A total of 85 and 107 volatile compounds were identified in the control and fortified yoghurt samples, respectively. These were further classified into 22 alcohols, 19 esters, 15 acids, 13 benzenes, 9 aldehydes, 9 ketones, 5 hydrocarbons, 4 furans, 3 nitrogen compounds, 3 sulphur compounds, 2 terpenes and 2 pyrans in Table 4.6. Ketones were the most abundant in the control, POS5, POS10 and PRE5 and esters dominated POS15, PRE10 and PRE15 (Table 4.7). Among ketones, acetone with sweet and fruity aroma dominated in PRE samples, owing to the fermentation process. Fortification with tamarillo has significantly ($P < 0.05$) influenced the presence of acetone as well. Acetoin which has a creamy, slightly sweet and butter-like flavor (Routray & Mishra, 2011) was abundant (10.84 – 28.62 μg/g FW ~ 1084 – 2862 μg/100 g FW) in the control and POS samples. Acetoin is a common flavor compound

in cultured dairy products which is converted from diacetyl by diacetyl reductase (Cheng, 2010), reducing the harsh flavor (Chen et al., 2017).

Addition of tamarillo significantly increased ($p < 0.05$) methyl hexanoate and ethyl hexanoate in yoghurt sample (36 – 80 times) compared to the control. These esters have mainly originated from tamarillo (Durant et al., 2013; Torrado et al., 1995; Wong & Wong, 1997) and can minimize the sharpness and bitterness imparted by fatty acids and amines in yoghurt (Cheng, 2010). The concentration of ethyl hexanoate in fortified yoghurts was significantly lower than the original amount present in tamarillo fruit (Diep et al., 2021). This may be due to the matrix effect of yoghurt where proteins and exopolysaccharides as well as physicochemical interactions between components like pectin and sucrose have affected the release of volatile compounds (Routray & Mishra, 2011). The concentration of volatile compound in the headspace decreased when the matrix changed from water (i.e. tamarillo fruit) to yoghurt.

Acidity, perceived as sourness, is crucial for flavour acceptance of yoghurt (Cheng, 2010). Carboxylic acid was the second dominant group in the volatile profile of most yoghurt samples except for POS15 (Table 4.7). Hexanoic acid and butanoic acids were the two most abundant carboxylic acids in all samples. The highest concentrations of these acids were found in the control (18.3 and 13.3 μg/g FW ~ 1830 and 1330 μg/100 g FW), and POS had 1.3-1.7 times higher concentrations compared to PRE of the same level of fortification. Acetic acid and octanoic acid were also present at substantial concentrations, which could contribute to vinegar/acid notes and fruity notes, respectively (Cheng, 2010).

Acetaldehyde, 3-methyl-butanal and 2-Hexenal (E) were dominant aldehydes in tamarillo yoghurts which carry fruity aroma. Acetaldehyde is formed from breakdown of threonine by threonine aldolase, which is present in both *Lb. bulgaricus* and *S. thermophilus* (Routray & Mishra, 2011). While the concentration of acetaldehyde was 1.04 μg/g FW (~ 104 μg/100 g FW) in the control, fortification dropped its concentration as low as 0.12 μg/g FW (~ 12 μg/100 g FW), with higher concentration seen in POS in general. Similarly, lowered acetaldehyde concentration was observed in other studies with fruit fortification in yoghurt (Routray & Mishra, 2011). Addition of tamarillo resulted in extra volatile compounds that are normally not found in

yoghurt, and these include benzenes, hydrocarbons, nitrogen and sulphur compounds, terpenes and pyrans.

The effects of fortification were further analysed by principal component analysis (PCA) and presented as PCA plot and heatmap in Figure 4.2 and 4.3, respectively. The separation of control and tamarillo yoghurts based on the volatile profile was clearly visible. Principal component (PC) 1 and PC 2 explained 89% and 7.7% of the variance, respectively and therefore, no further PCs were considered. For PC1, the control sample was located on the positive region while all of POS were around the zero line and all of PRE fell in the negative region. For PC2, the control was located on the positive region, all POS were located on the negative area. For PRE, PRE5 and PRE10 were in the positive area but PRE15 fell in the negative region. The PC2 completely resolved fortified yoghurt samples based on the tamarillo concentration regardless of fermentation processes. The 5% samples were on the far-right side, the 15% samples were located on the left side while the 10% samples fell in between the 5% and 15% samples. A heatmap was generated to visualise the relative abundance of volatiles in each yoghurt group. Overall, clear differences were observed between control, PRE and POS yoghurt samples.

Table 4.6 Volatile compounds in control and tamarillo fortified yoghurts produced from pre and post fermentation processes.

No.	Compounds	RI	Relative Concentration (μg/g FW)						
			Control	POS5	POS10	POS15	PRE5	PRE10	PRE15
Acids									
1	Propanoic acid, anhydride	1162.0	0.01 ± 0a	0.19 ± 0.01bc	0.2 ± 0.02b	0.16 ± 0.01c	0.24 ± 0.01d	0.37 ± 0.04e	0.36 ± 0.01e
2	Acetic acid	1448.5	3.81 ± 0.2a	2.34 ± 0.07b	2.14 ± 0.13bc	2.06 ± 0.23c	0.88 ± 0.05d	0.88 ± 0.11d	0.83 ± 0.03d
3	Propanoic acid	1538.9	0.06 ± 0a	0.03 ± 0b	0.03 ± 0b	0.03 ± 0b	0.02 ± 0c	0.02 ± 0d	0.02 ± 0cd
4	Butanoic acid	1627.3	13.33 ± 0.64a	5.75 ± 0.17b	5.38 ± 0.06b	5.22 ± 0.05b	3.29 ± 0.14c	3.37 ± 0.13cd	3.91 ± 0.47d
5	3-Methyl-Butanoic acid	1671.7	0.16 ± 0ab	0.18 ± 0.01c	0.17 ± 0bc	0.17 ± 0.01bc	0.11 ± 0.01e	0.15 ± 0.02ad	0.15 ± 0d
6	Pentanoic acid	1740.0	0.2 ± 0.01a	0.07 ± 0b	0.07 ± 0b	0.07 ± 0b	0.05 ± 0c	0.06 ± 0.01c	0.06 ± 0c
7	3-Methyl-2-Butenoic acid	1797.5	0.01 ± 0a	0.01 ± 0b	0.02 ± 0c	0.02 ± 0d	0.01 ± 0a	0.01 ± 0e	0.01 ± 0f
8	3-Methyl-3-Butenoic acid	1797.5	n.d	0.01 ± 0a	0.06 ± 0b	0.03 ± 0c	< 0.005d	0.01 ± 0e	0.02 ± 0f
9	Hexanoic acid	1845.9	18.33 ± 0.77a	8.17 ± 0.25b	7.39 ± 0.08bc	7.21 ± 0.27c	4.71 ± 0.26d	5.12 ± 0.19d	5.33 ± 0.89d
10	Heptanoic acid	1956.0	0.22 ± 0a	0.07 ± 0b	0.06 ± 0bc	0.06 ± 0.01bc	0.06 ± 0.01bc	0.05 ± 0.02c	0.05 ± 0c
11	(E)-2-Hexenoic acid	1972.6	< 0.005a	0.06 ± 0b	0.06 ± 0.01bc	0.03 ± 0d	0.05 ± 0.01b	0.05 ± 0.01b	0.07 ± 0c
12	Octanoic acid	2098.2	4.92 ± 0.35a	1.62 ± 0.09b	1.24 ± 0.05c	1.24 ± 0.09c	0.66 ± 0.11d	0.7 ± 0.2d	0.82 ± 0.02d
13	Nonanoic acid	2386.1	0.08 ± 0.03a	0.05 ± 0b	0.03 ± 0.01bc	0.01 ± 0c	0.04 ± 0.01b	0.03 ± 0bc	0.01 ± 0c
14	n-Decanoic acid	2658.3	0.52 ± 0.06a	0.14 ± 0.01b	0.1 ± 0.01c	0.12 ± 0.01bc	0.05 ± 0.01d	0.05 ± 0.02d	0.08 ± 0.01e
15	Benzoic acid	3105.6	3.07 ± 0.59a	2.01 ± 0.31b	1.76 ± 0.15b	1.58 ± 0.11b	0.14 ± 0.04c	0.12 ± 0.03c	0.07 ± 0.01c
Alcohols									
16	Ethanol	913.5	0.01 ± 0.01a	0.82 ± 0.03b	1.72 ± 0.16c	0.7 ± 0.06b	0.44 ± 0.09d	1.01 ± 0.1e	1.07 ± 0.05e
17	1-Butanol	1149.3	0.06 ± 0a	0.06 ± 0ab	0.05 ± 0b	0.05 ± 0.01b	0.04 ± 0c	0.04 ± 0.01c	0.04 ± 0c
18	3-Methyl-1-Butanol	1210.7	0.1 ± 0.01a	0.11 ± 0.01a	0.12 ± 0.01a	0.07 ± 0.01a	0.28 ± 0.1b	0.1 ± 0.02a	0.25 ± 0.05b
19	3-Methyl-3-Buten-1-ol	1251.3	0.09 ± 0a	0.33 ± 0.01b	0.44 ± 0.03c	0.22 ± 0.02d	0.18 ± 0.04d	0.38 ± 0.05e	0.49 ± 0.01f

20	1-Pentanol	1253.4	1.23 ± 0.1a	0.08 ± 0b	0.07 ± 0b	0.07 ± 0b	0.1 ± 0.01b	0.05 ± 0.01b	0.08 ± 0b
21	Prenol	1323.8	0.22 ± 0.06a	0.14 ± 0b	0.23 ± 0.01a	0.13 ± 0.01b	0.06 ± 0.01c	0.1 ± 0.01bc	0.12 ± 0.01b
22	3-Pentanol	1344.1	5.03 ± 0.27a	2.24 ± 0.06b	2.14 ± 0.06b	2.48 ± 0.09c	0.01 ± 0d	< 0.005d	< 0.005d
23	1-Hexanol	1356.0	0.2 ± 0.01ab	0.19 ± 0.01a	0.35 ± 0.01c	0.28 ± 0.02bc	1.67 ± 0.1d	0.59 ± 0.06e	0.9 ± 0.03f
24	(E)-3-Hexen-1-ol	1366.1	< 0.005a	0.01 ± 0b	0.02 ± 0c	0.02 ± 0d	0.04 ± 0e	0.02 ± 0f	0.02 ± 0df
25	(Z)-3-Hexen-1-ol	1386.7	0.01 ± 0a	0.27 ± 0.01b	0.84 ± 0.03c	0.42 ± 0.03d	0.21 ± 0.01e	0.37 ± 0.04f	0.44 ± 0.01d
26	(Z)-2-Hexen-1-ol	1408.0	< 0.005a	0.01 ± 0a	0.01 ± 0a	0.01 ± 0a	0.1 ± 0.01b	0.03 ± 0c	0.09 ± 0.02b
27	1-Heptanol	1458.2	0.16 ± 0.01a	0.06 ± 0b	0.06 ± 0b	0.06 ± 0.01b	0.02 ± 0c	0.01 ± 0d	0.02 ± 0cd
28	2,6-Dimethyl-4-Heptanol	1474.2	0.04 ± 0a	0.02 ± 0b	0.02 ± 0b	0.02 ± 0c	< 0.005d	n.d	n.d
29	2,3-Butanediol	1542.0	0.12 ± 0.02a	0.09 ± 0b	0.09 ± 0.01b	0.27 ± 0c	0.06 ± 0.01d	0.09 ± 0.02b	0.13 ± 0.01a
30	1-Octanol	1560.6	0.03 ± 0a	0.01 ± 0b	0.01 ± 0b	0.01 ± 0b	0.01 ± 0c	0.01 ± 0c	0.01 ± 0c
31	Terpinen-4-ol	1606.7	n.d	0.01 ± 0a	0.02 ± 0b	0.01 ± 0a	0.01 ± 0a	0.01 ± 0a	0.02 ± 0b
32	1-Nonanol	1662.9	0.04 ± 0a	0.01 ± 0b	0.01 ± 0b	0.01 ± 0b	0.01 ± 0c	0.01 ± 0c	0.01 ± 0c
33	Alpha-Terpineol	1701.3	< 0.005a	0.04 ± 0b	0.05 ± 0c	0.07 ± 0d	0.04 ± 0b	0.08 ± 0.01e	0.1 ± 0f
34	p-Mentha-1,5-dien-8-ol	1729.5	n.d	0.04 ± 0a	0.07 ± 0b	0.18 ± 0.02c	0.02 ± 0d	0.09 ± 0.01b	0.16 ± 0e
35	p-Mentha-1(7),2-dien-8-ol	1785.4	n.d	0.06 ± 0a	0.11 ± 0b	0.29 ± 0.03c	0.04 ± 0d	0.12 ± 0.02b	0.22 ± 0.01e
36	Phenylethyl Alcohol	1914.8	0.02 ± 0a	0.02 ± 0a	0.01 ± 0a	0.01 ± 0a	0.06 ± 0.01b	0.02 ± 0a	0.05 ± 0.02b
37	[R-(R*,R*)]-1,2-diphenyl-1,2-Ethanediol	2169.4	< 0.005a	0.01 ± 0b	0.01 ± 0b	0.01 ± 0d	0.01 ± 0b	0.01 ± 0c	0.01 ± 0c
Aldehydes									
38	Acetaldehyde	629.8	1.04 ± 0.09a	0.12 ± 0.04a	0.55 ± 0.05b	0.79 ± 0.11c	0.22 ± 0.02d	0.16 ± 0.01d	0.13 ± 0.01d
39	Butanal	823.6	0.03 ± 0a	0.01 ± 0b	0.01 ± 0b	0.01 ± 0c	0.01 ± 0bc	0.01 ± 0d	0.01 ± 0d
40	2-Methyl-Butanal	876.3	0.04 ± 0a	0.11 ± 0.02a	0.22 ± 0.02b	0.29 ± 0.04b	0.24 ± 0.03b	0.89 ± 0.13c	0.99 ± 0.07c
41	3-Methyl- Butanal	883.1	0.04 ± 0a	0.14 ± 0ab	0.35 ± 0.04bc	0.47 ± 0.03c	0.53 ± 0.07c	2.29 ± 0.33d	2.39 ± 0.05d
42	Hexanal	1069.9	0.09 ± 0.01a	0.17 ± 0.01b	0.11 ± 0.01a	0.05 ± 0.01c	0.11 ± 0a	0.56 ± 0.05d	0.62 ± 0.02e

43	3-Methyl-2-Butenal	1195.3	0.24 ± 0.02a	0.27 ± 0.01b	0.33 ± 0.02c	0.48 ± 0.02d	0.09 ± 0.01e	0.16 ± 0.02f	0.15 ± 0f
44	(E)-2-Hexenal	1214.4	0.01 ± 0a	1.08 ± 0.09b	0.78 ± 0.03c	0.3 ± 0.05d	0.54 ± 0.05e	1.3 ± 0.21f	1.33 ± 0.09f
45	Nonanal	1392.5	0.03 ± 0.01a	0.01 ± 0b	0.01 ± 0bc	0.01 ± 0bc	0.01 ± 0b	0.01 ± 0b	0.02 ± 0c
46	(E,E)-2,4-Hexadienal	1396.1	0.01 ± 0a	0.04 ± 0b	0.03 ± 0c	0.01 ± 0d	0.02 ± 0e	0.04 ± 0b	0.05 ± 0f
Benzenes									
47	Toluene	1025.3	0.44 ± 0.09ab	0.41 ± 0.05a	0.66 ± 0.01bc	1.3 ± 0.18d	0.27 ± 0.07a	0.7 ± 0.25c	1.3 ± 0.09d
48	1,3-Dimethyl-Benzene	1126.4	0.03 ± 0.01a	0.02 ± 0b	0.03 ± 0a	0.03 ± 0.01a	< 0.005c	0.01 ± 0b	0.03 ± 0a
49	1-Methyl-3-(1-methylethyl)-Benzene	1265.2	0.01 ± 0a	0.04 ± 0bc	0.06 ± 0.01c	0.11 ± 0.03d	0.03 ± 0.01ab	0.05 ± 0.01c	0.1 ± 0.01d
50	1,2,3-Trimethyl-Benzene	1276.1	0.02 ± 0a	0.01 ± 0b	0.02 ± 0a	0.02 ± 0a	0.01 ± 0c	0.01 ± 0b	0.02 ± 0a
51	1-Methyl-3-(1-methylethenyl)-Benzene	1436.1	0.01 ± 0a	0.01 ± 0ab	0.02 ± 0b	0.04 ± 0.01c	0.01 ± 0a	0.02 ± 0.01b	0.03 ± 0c
52	1-Methyl-4-(1-methylethenyl)-Benzene	1436.1	0.01 ± 0ab	0.02 ± 0c	0.02 ± 0c	0.03 ± 0.01d	0.01 ± 0a	0.02 ± 0bc	0.03 ± 0d
53	Benzaldehyde	1523.2	0.08 ± 0ab	0.08 ± 0ab	0.1 ± 0.01bc	0.12 ± 0c	0.07 ± 0.01a	0.16 ± 0.03d	0.22 ± 0.01e
54	Benzonitrile	1606.2	0.03 ± 0a	0.01 ± 0b	0.01 ± 0b	0.01 ± 0b	0.01 ± 0c	0.01 ± 0c	0.01 ± 0c
55	Benzeneacetaldehyde	1641.3	0.03 ± 0.01a	0.15 ± 0.02b	0.29 ± 0.02c	0.27 ± 0c	0.29 ± 0.02c	1.3 ± 0.19d	1.53 ± 0.05e
56	3-Ethyl-Benzaldehyde	1710.3	0.01 ± 0a	< 0.005b	< 0.005b	< 0.005b	< 0.005b	< 0.005b	< 0.005b
57	Methoxy-phenyl-Oxime	1756.6	3.05 ± 0.56a	1.08 ± 0.14b	0.96 ± 0.1b	0.79 ± 0.13b	0.91 ± 0.34b	0.79 ± 0.18b	0.68 ± 0.12b
58	Meso-Hydrobenzoin	1878.3	< 0.005ab	0.01 ± 0a	0.01 ± 0c	0.01 ± 0d	< 0.005b	0.01 ± 0e	0.01 ± 0d
59	2-Methyl-Phenol	2001.9	< 0.005a	0.03 ± 0b	0.04 ± 0c	0.06 ± 0d	0.01 ± 0e	0.03 ± 0f	0.04 ± 0c
Esters									
60	Butanoic acid, methyl ester	965.7	0.01 ± 0a	1.42 ± 0.05b	2.25 ± 0.43c	4.74 ± 0.56d	0.95 ± 0.03b	2.19 ± 0.22c	2.13 ± 0.07c
61	Butanoic acid, ethyl ester	1024.9	n.d	0.13 ± 0a	0.49 ± 0.07b	0.77 ± 0.02c	0.51 ± 0.02b	1.1 ± 0.11d	1.35 ± 0.06e
62	Isopropyl butyrate	1029.9	0.01 ± 0a	0.23 ± 0.01b	0.18 ± 0.03c	0.05 ± 0d	0.14 ± 0.01e	0.33 ± 0.04f	0.43 ± 0.01g
63	3-Butenoic acid, 3-methyl-, methyl ester	1110.8	n.d	0.03 ± 0a	0.05 ± 0.01b	0.2 ± 0.01c	0.01 ± 0.01d	0.04 ± 0be	0.05 ± 0b
64	2-Butenoic acid, 3-methyl-, methyl ester	1161.3	n.d	0.12 ± 0.01ab	0.41 ± 0.06c	1.3 ± 0.06d	0.08 ± 0a	0.18 ± 0.02be	0.22 ± 0.01e

65	Hexanoic acid, methyl ester	1181.7	0.17 ± 0.08a	8.17 ± 0.42b	11.22 ± 2.5c	12.74 ± 0.82cd	6.19 ± 0.44b	10.84 ± 1.68c	13.69 ± 1.01d
66	3-Methyl-3-buten-1-ol, acetate	1190.8	0.05 ± 0.01a	1.69 ± 0.04b	1 ± 0.04c	0.62 ± 0.07d	0.62 ± 0.08d	1.3 ± 0.15e	1.65 ± 0.03b
67	Hexanoic acid, ethyl ester	1230.7	n.d	0.07 ± 0.01a	0.19 ± 0.05b	0.2 ± 0.02b	0.22 ± 0.01b	0.4 ± 0.06c	0.74 ± 0.05d
68	4-Hexenoic acid, methyl ester	1256.6	n.d	0.01 ± 0a	0.01 ± 0b	0.01 ± 0c	0.01 ± 0c	0.01 ± 0d	0.02 ± 0e
69	Acetic acid, methyl ester	1299.5	0.07 ± 0.01a	0.03 ± 0b	0.03 ± 0cd	0.03 ± 0bc	0.03 ± 0de	0.02 ± 0e	0.02 ± 0f
70	Butanoic acid, 4-pentenyl ester	1339.8	0.01 ± 0a	0.18 ± 0.01b	0.26 ± 0.04c	0.58 ± 0.02d	0.15 ± 0.01b	0.29 ± 0.05c	0.52 ± 0.03e
71	Octanoic acid, methyl ester	1389.5	n.d	0.02 ± 0a	0.03 ± 0b	0.04 ± 0c	0.02 ± 0a	0.03 ± 0.01b	0.06 ± 0d
72	Ethylene glycol di-n-butyrate	1515.1	n.d	0.02 ± 0a	0.08 ± 0b	0.03 ± 0c	0.01 ± 0d	0.03 ± 0c	0.04 ± 0e
73	Butanedioic acid, diethyl ester	1567.6	n.d	0.01 ± 0a	0.03 ± 0b	0.01 ± 0a	0.01 ± 0c	0.02 ± 0d	0.02 ± 0b
74	Hexanoic acid, 2-hydroxy-, methyl ester	1580.4	n.d	0.03 ± 0a	0.05 ± 0b	0.08 ± 0c	0.03 ± 0a	0.06 ± 0.01d	0.09 ± 0e
75	Benzoic acid, methyl ester	1624.2	0.03 ± 0.01a	0.1 ± 0.01b	0.14 ± 0.01c	0.24 ± 0.01d	0.07 ± 0.01e	0.15 ± 0.02c	0.22 ± 0f
76	Hexanoic acid, 4-oxo-, methyl ester	1643.3	n.d	0.07 ± 0a	0.12 ± 0b	0.09 ± 0c	0.04 ± 0d	0.11 ± 0.02b	0.17 ± 0.01e
77	Propanoic acid, 2-methyl-, ethyl ester	1692.8	n.d	0.04 ± 0a	0.13 ± 0b	0.12 ± 0c	0.02 ± 0d	0.06 ± 0.01e	0.09 ± 0f
78	Methyl salicylate	1778.8	0.02 ± 0.01a	0.05 ± 0b	0.07 ± 0c	0.07 ± 0.01c	0.03 ± 0a	0.05 ± 0.01b	0.06 ± 0c
Furans									
79	2-Pentyl-Furan	1225.0	0.01 ± 0a	0.02 ± 0ab	0.02 ± 0ab	0.1 ± 0.02c	0.07 ± 0d	0.02 ± 0.01ab	0.03 ± 0.01b
80	Furfural	1461.6	0.02 ± 0a	0.01 ± 0b	0.01 ± 0b	0.02 ± 0b	0.01 ± 0b	0.03 ± 0b	0.03 ± 0b
81	2-Acetyl-5-methylfuran	1663.2	n.d	0.01 ± 0a	0.01 ± 0b	0.01 ± 0b	< 0.005c	0.01 ± 0a	0.01 ± 0b
82	2-Vinylfuran	2006.0	0.03 ± 0a	0.02 ± 0b	0.01 ± 0c	0.01 ± 0c	0.01 ± 0d	0.01 ± 0c	0.01 ± 0c
Hydrocarbons									
83	3-Methylenecyclohexene	927.1	n.d	0.05 ± 0ab	0.11 ± 0c	0.22 ± 0.04d	0.03 ± 0a	0.07 ± 0.01b	0.12 ± 0c
84	4-methyl-1-(1-methylethyl)- Bicyclo [3.1.0] hex-2-ene	1088.5	<0.005a	0.01 ± 0a	0.01 ± 0.01a	0.01 ± 0.01a	n.d	0.01 ± 0.01a	0.01 ± 0.01a
85	2,2-Dimethylpropanoic anhydride	1376.5	n.d	0.02 ± 0a	0.02 ± 0b	< 0.005c	0.01 ± 0d	0.03 ± 0e	0.04 ± 0f

86	5-Ethyldecane	1600.1	0.05 ± 0a	0.02 ± 0b	0.02 ± 0b	0.02 ± 0b	0.01 ± 0c	0.01 ± 0c	0.01 ± 0c
87	2,6-Dimethyl-2-trans-6-octadiene	2154.3	n.d	0.02 ± 0a	0.02 ± 0b	0.03 ± 0c	0.01 ± 0d	0.02 ± 0b	0.03 ± 0c
Ketones									
88	Acetone	726.0	5.93 ± 0.29a	3.28 ± 0.08b	3.22 ± 0.27b	2.23 ± 0.21c	2.4 ± 0.23cd	2.67 ± 0.22d	1.69 ± 0.05e
89	2-Butanone	857.6	3.7 ± 0.23a	1.21 ± 0.06b	1.17 ± 0.08b	0.77 ± 0.08c	0.88 ± 0.05c	0.9 ± 0.12c	0.52 ± 0.05d
90	2,3-Butanedione	953.9	7.97 ± 0.58a	3.71 ± 0.22b	3.25 ± 0.19c	3.56 ± 0.12bc	0.66 ± 0.05d	0.08 ± 0.01e	0.06 ± 0.01e
91	2,3-Pentanedione	1046.7	1.34 ± 0.11a	0.57 ± 0.01b	0.48 ± 0.03c	0.93 ± 0.06d	0.09 ± 0.01e	0.01 ± 0e	0.01 ± 0e
92	2-Heptanone	1177.4	0.08 ± 0.01a	0.03 ± 0b	0.04 ± 0c	0.03 ± 0.01bc	0.01 ± 0d	0.02 ± 0d	0.01 ± 0d
93	Acetoin	1286.8	28.62 ± 1.43a	14.12 ± 0.32b	12.7 ± 0.42c	10.84 ± 0.3d	0.9 ± 0.04e	0.03 ± 0e	0.03 ± 0e
94	6-Methyl-5-Hepten-2-one	1337.0	0.01 ± 0a	0.01 ± 0b	0.02 ± 0c	0.01 ± 0d	0.01 ± 0b	0.02 ± 0e	0.03 ± 0f
95	2-Hydroxy-3-pentanone	1360.1	8.49 ± 0.43a	3.83 ± 0.11bc	3.67 ± 0.11b	4.22 ± 0.12c	5.65 ± 0.34d	2.01 ± 0.21e	3.04 ± 0.11f
96	3-(hydroxymethyl)-2-Nonanone	1389.1	0.19 ± 0.04a	0.06 ± 0b	0.06 ± 0b	0.07 ± 0.01b	0.03 ± 0c	0.03 ± 0c	0.03 ± 0c
Nitrogen compounds									
97	2-nitro-Propane	1117.3	0.06 ± 0.01a	0.06 ± 0ab	0.05 ± 0.01ab	0.03 ± 0.01cd	0.03 ± 0c	0.04 ± 0.02bd	0.03 ± 0c
98	Bromochloronitromethane	1293.4	0.01 ± 0a	< 0.005b	< 0.005b	< 0.005b	< 0.005b	< 0.005b	< 0.005b
99	4-Cyanocyclohexene	1566.5	0.01 ± 0a	0.01 ± 0a	0.02 ± 0b	0.02 ± 0b	0.01 ± 0c	0.01 ± 0d	0.02 ± 0b
Pyrans									
100	Tetrahydro-2H-Pyran-2-methanol	990.6	n.d	0.05 ± 0a	0.1 ± 0b	0.04 ± 0.01a	0.03 ± 0c	0.07 ± 0.01d	0.09 ± 0e
101	Tetrahydro-6-pentyl- 2H-Pyran-2-one	2470.0	0.24 ± 0.02a	0.08 ± 0b	0.07 ± 0bc	0.06 ± 0bc	0.04 ± 0.01d	0.06 ± 0.01cd	0.05 ± 0cd
Sulphur compounds									
102	Ethanethiol	659.4	0.07 ± 0a	0.14 ± 0.01b	0.24 ± 0.02c	0.23 ± 0.01c	0.17 ± 0.01d	0.42 ± 0.03e	0.39 ± 0.01f
103	Dihydro-2-methyl-3(2H)-Thiophenone	1530.2	0.4 ± 0.03a	0.09 ± 0b	0.07 ± 0c	0.06 ± 0c	n.d	n.d	n.d
104	Dimethyl sulfone	1899.3	0.35 ± 0.08a	0.21 ± 0.03b	0.12 ± 0.01c	0.13 ± 0.01c	0.14 ± 0.03c	0.08 ± 0.02c	0.09 ± 0.01c

Terpenes									
105	Limonene	1186.6	0.01 ± 0a	0.03 ± 0.01b	0.06 ± 0c	0.06 ± 0c	0.03 ± 0b	0.04 ± 0.01b	0.07 ± 0.01d
106	Eucalyptol	1204.2	n.d	0.1 ± 0a	0.2 ± 0b	0.09 ± 0.01ac	0.08 ± 0.01c	0.15 ± 0.02d	0.24 ± 0e
107	γ-Terpinene	1240.0	n.d	< 0.005a	< 0.005b	< 0.005bc	< 0.005d	< 0.005a	< 0.005c

* n.d.: not detected; RI: retention index; *m/z*: mass-to-charge ratio; FW: fresh weight. Data are presented as Mean ± SD (n = 3) and listed in the order of group and then retention index as determined using the homologous series of *n*-alkanes and found in library. Different alphabets indicate statistical difference ($p < 0.05$) across each row. POS5, POS10 and POS15: 5, 10, 15% of tamarillo powder was added post fermentation, respectively. PRE5, PRE10 and PRE15: 5, 10, 15% of tamarillo powder was added to milk and starter culture prior to fermentation, respectively. Used with permission (Diep et al., 2022)

Table 4.7 Relative content percentage (%) of volatile classes identified in yoghurt samples.

Chemical class	**Relative content percentage (%)**						
	Control	**POS5**	**POS10**	**POS15**	**PRE5**	**PRE10**	**PRE15**
Acids	38.76	29.44	25.89	13.68	27.45	23.54	21.88
Alcohols	6.39	6.58	8.93	4.09	9.08	6.73	7.85
Aldehydes	1.33	4.20	3.31	1.83	4.71	11.61	10.56
Benzenes	3.23	2.66	3.07	2.12	4.31	6.66	7.42
Esters	0.32	17.66	23.14	60.23	24.33	36.78	39.87
Furans	0.05	0.09	0.07	0.11	0.24	0.15	0.15
Hydrocarbons	0.04	0.17	0.25	0.21	0.16	0.30	0.39
Ketones	48.88	38.11	34.06	17.18	28.30	12.36	10.06
Nitrogen and sulphur compounds	0.78	0.73	0.69	0.36	0.93	1.18	0.98
Terpenes and pyrans	0.22	0.37	0.60	0.19	0.48	0.69	0.84

POS5, POS10 and POS15: 5, 10, 15% of tamarillo powder was added post fermentation, respectively. PRE5, PRE10 and PRE15: 5, 10, 15% of tamarillo powder was added to milk and starter culture prior to fermentation, respectively

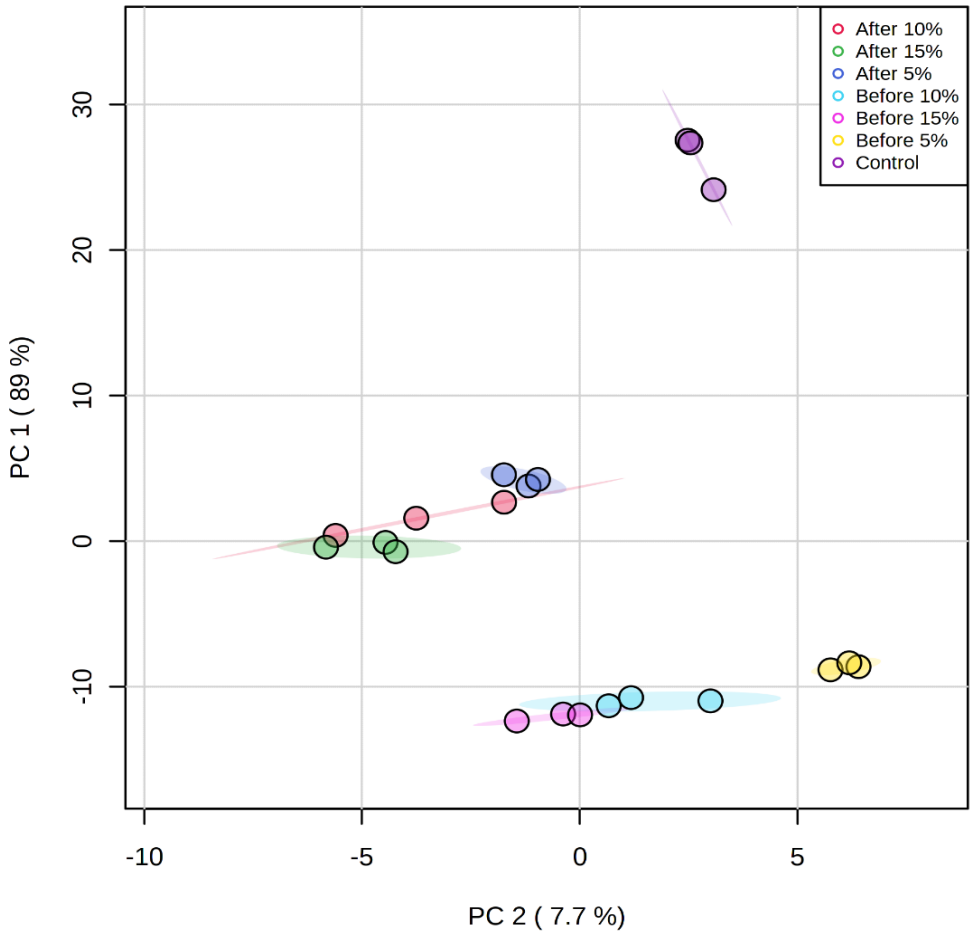

Figure 4.2 Score plot from principal component analysis of relative concentration of volatiles for control yoghurt and tamarillo yoghurts produced from pre and post fermentation processes.

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Figure 4.3 Heatmap of the relative concentration of volatiles quantified in control and tamarillo yoghurts produced from pre and post fermentation processes. The vertical bar (3 to −3) from deep red to deep blue shows high to low concentrations of each volatile in different groups of yoghurt samples. Used with permission (Diep et al., 2022)

4.3.6 Fatty acids profile

Addition of tamarillo significantly ($p < 0.05$) increased UFAs content and caused a subsequent decrease in SFAs to below 70% for both PRE and POS (Table 4.8). In all yoghurt samples, palmitic acid (C16:0) was the most abundant SFA as reported before in several other studies (Marand, Amjadi, Marand, Roufegarinejad, & Jafari, 2020; Ribeiro et al., 2021; Van Nieuwenhove et al., 2019), followed by myristic acid (C14:0) and then stearic acid (C18:0) (Table 4.8). Addition of tamarillo increased the PUFAs by 3.2 – 3.8, 5.3 – 6.2 and 6.5 times for 5, 10 and 15%, respectively, when compared to the control for both PRE and POS. Oleic acid (C18:1) and linoleic acid (C18:2) dominated monounsaturated fatty acid (MUFA) and PUFA profiles, respectively. Compared to the control, increase in oleic acid was observed in POS but a decrease was observed in PRE. The increase in PUFAs observed in fortified yoghurt may be caused by the presence of PUFAs (account for 72.05%) (Achicanoy, Benavides, & Martinez-Correa, 2018) in the mucilage of tamarillo pulp used in the study. A synergistic interaction between fruit fibre and the probiotic strain may also have contributed to the increase as well (Do Espírito Santo et al., 2012b). PUFAs in PRE was lower than that of the POS which may be explained by continuous oxidization of PUFAs to hydroperoxides, and subsequently aldehydes (Cheng, 2010). This finding also supports the elevated concentration of aldehydes in PRE compared to the POS in Table 4.7. POS5, POS10, POS15 and PRE15 showed similar or even higher C18:2 ω-6 content than assorted fruits yoghurt (66 mg/100 g) reported in New Zealand Food Composition Database (Sivakumaran et al., 2017). Total amounts of ω-3 and ω-6 increased significantly ($p < 0.05$) when comparing with the control, by 1.4 – 1.5 and 2.0 – 2.6 times higher in linolenic acid content for PRE and POS than the control, respectively. For linoleic acid, the increase was 5 – 8.5 and 7.9 – 13.7 times for PRE and POS compared to the control, respectively.

Atherogenic (AI), thrombogenic (TI) and saturation indices (SI) were calculated as a measure of nutritional quality indices (Do Espírito Santo et al., 2012b; Marand et al., 2020). The AI indicates the relationship between the sum of the main SFA and sum of MUFAs plus PUFAs. The SFAs (C12:0, C14:0 and C16:0) are considered pro-atherogenic (favoring the adhesion of lipids to cells of the immunological and circulatory system). The MUFAs and PUFAs are considered antiatherogenic (inhibiting the aggregation of plaque and diminishing the levels of esterified fatty acid,

cholesterol, and phospholipids, thereby preventing the appearance of micro-coronary and macro-coronary diseases) (Ribeiro et al., 2021). This value shows correlation of the risk of atherosclerosis, i.e., the increase of the level of blood cholesterol with the increase of the SFAs or the decrease of the ΣMUFAs, and ΣPUFAs. The TI value presents tendency to form clots in the blood vessels, which is defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenicity acids (MUFAs, ω-6 PUFAs and ω-3 PUFAs). According to Ribeiro et al. (2021), the influence of the different FAs ingested on coronary heart disease have been measured through these two values. The lower the AI and TI values, the greater the protective potential for coronary artery disease. In this study, tamarillo yoghurt exhibited lower values of AI and TI than the control sample. Addition of 5, 10 and 15% tamarillo powder resulted in reduction of AI by 23, 35 – 38 and 42%, respectively regardless of the fermentation process. Also, the TI values decreased in 79 – 87, 61 – 73 and 52 – 59% for adding 5, 10 and 15% tamarillo powder into yoghurt, respectively. The saturation index (SI) is another good indicator of the nutritional value of dietary fat, indicating the relationship between the sum of SFAs (pro-thrombogenic) and UFAs (anti-thrombogenic), and food with lower SI would be considered healthier foods (Ribeiro et al., 2021). Yoghurt fortified with tamarillo presented lower SI than the control (Table 4.8). Regardless of fermentation process, addition of 5, 10 and 15% tamarillo powder reduced the SI by 24 – 25, 36 – 39 and 42 – 44%, respectively. The higher the tamarillo fortification, the lower the AI, TI and SI values were, which could be used as an innovative strategy to increase the health appeal of high-fat yoghurts.

The PCA plot and heatmap were used to determine the effects of the addition of tamarillo powder as shown in Figure 4.4A and 4.4B, respectively. The control and tamarillo yoghurt samples were almost perfectly resolved on PC1, which explained 79.3% of the variance in the data, and on PC2, which explained another 13.7% of the variance. Therefore, no further PCs were considered. PC1 mostly resolved the difference tamarillo yoghurts between PRE and POS. For PC1, all of PRE fell in the negative region whereas most of POS was located on the positive region except for two samples of POS15. Two samples of control yoghurt fell in in the negative region and the other two was located on the opposite region. For PC2, the control, PRE5 and POS5 were located on the positive region. PRE10, POS10, PRE15 and POS15 were in the negative area except for one sample of PRE10. The PC2 also completely

resolved the samples based on the tamarillo concentration regardless of fermentation processes. Heatmap cluster analysis of these 37 fatty acids showed a clear separation between control and fortified yoghurts. Also, separation among tamarillo yoghurts based on the centration tamarillo added was observed. The PRE5 and POS5 were more closely related to control than the others. On the other side, PRE10 and PRE15 were in the same cluster which is separated from the POS10 and POS15 cluster.

Table 4.8 Fatty acid profiles and lipid indices of control and tamarillo fortified yoghurts produced from pre and post fermentation.

Fatty acids	Formula	Concentration (mg/100 g FW)						
		Control	**POS5**	**POS10**	**POS15**	**PRE5**	**PRE10**	**PRE15**
Saturated fatty acids (SFAs)								
Butyric acid	C4:0	35.98 ± 8.79^{ab}	40.07 ± 7.7^{a}	34.36 ± 3.45^{ab}	34.84 ± 10.08^{ab}	31.21 ± 2.69^{bc}	25.67 ± 2.27^{c}	23.81 ± 4.55^{c}
Hexanoic acid	C6:0	19.52 ± 4.35^{ac}	23.3 ± 1.41^{b}	22.02 ± 3.38^{ab}	22.5 ± 5.28^{ab}	18.82 ± 1.42^{ac}	16.09 ± 1.26^{cd}	14.61 ± 2.56^{d}
Octanoic acid	C8:0	11.87 ± 2.89^{ac}	15.17 ± 1.31^{b}	15.54 ± 2.59^{b}	13.99 ± 2.72^{ab}	12.38 ± 0.9^{ac}	10.2 ± 0.82^{cd}	9.49 ± 1.47^{d}
Decanoic acid	C10:0	25.33 ± 6.88^{a}	32.94 ± 2.28^{b}	34.09 ± 4.72^{b}	27.29 ± 4.86^{a}	26.53 ± 2.07^{a}	20.46 ± 1.07^{c}	19.35 ± 2.65^{c}
Undecanoic acid	C11:0	0.44 ± 0.12^{a}	0.56 ± 0.02^{b}	0.57 ± 0.07^{b}	0.44 ± 0.08^{a}	0.46 ± 0.04^{a}	0.34 ± 0.01^{c}	0.33 ± 0.04^{c}
Dodecanoic acid	C12:0	48.34 ± 12.74^{a}	64.97 ± 1.1^{b}	64.91 ± 6.57^{b}	48.06 ± 8.7^{a}	49.46 ± 4.18^{a}	36.55 ± 1.26^{c}	34.85 ± 4^{c}
Tridecanoic acid	C13:0	1.03 ± 0.18^{a}	1.2 ± 0.05^{b}	1.17 ± 0.07^{b}	0.95 ± 0.12^{a}	1.01 ± 0.07^{a}	0.83 ± 0.02^{c}	0.81 ± 0.05^{c}
Myristic acid	C14:0	98.12 ± 23.3^{a}	125.67 ± 11.27^{b}	119.26 ± 9.82^{b}	87.64 ± 16.87^{a}	96.04 ± 8.18^{a}	70.72 ± 3.83^{c}	66.85 ± 6.82^{c}
Pentadecanoic acid	C15:0	8.67 ± 1.92^{a}	10.75 ± 1.26^{b}	10.08 ± 0.78^{b}	7.35 ± 1.41^{ac}	8.35 ± 0.8^{a}	6.17 ± 0.43^{cd}	5.93 ± 0.58^{d}
Palmitic acid	C16:0	196.93 ± 40.37^{a}	248.27 ± 31.76^{b}	234.36 ± 17.21^{b}	172.12 ± 32.19^{ac}	184.79 ± 16.26^{a}	142.49 ± 11.18^{cd}	134.79 ± 16.31^{d}
Heptadecanoic acid	C17:0	4.28 ± 0.82^{ab}	5.25 ± 0.84^{c}	4.89 ± 0.36^{bc}	3.5 ± 0.61^{de}	3.96 ± 0.38^{ad}	3.06 ± 0.26^{e}	2.92 ± 0.35^{e}
Stearic acid	C18:0	81.77 ± 17.01^{a}	103.65 ± 17.18^{b}	96.08 ± 8.4^{b}	65.35 ± 12.58^{cd}	74.28 ± 8.01^{ac}	55.61 ± 5.59^{d}	52.9 ± 8.2^{d}
Arachidic acid	C20:0	0.85 ± 0.15^{ab}	1.18 ± 0.18^{c}	1.27 ± 0.13^{c}	0.9 ± 0.17^{a}	0.84 ± 0.09^{ab}	0.74 ± 0.05^{b}	0.79 ± 0.12^{ab}
Heneicosanoic acid	C21:0	0.2 ± 0.03^{a}	0.23 ± 0.04^{b}	0.24 ± 0.02^{b}	0.16 ± 0.03^{c}	0.16 ± 0.02^{c}	0.14 ± 0.02^{c}	0.13 ± 0.02^{c}
Behenic acid	C22:0	0.3 ± 0.06^{a}	0.57 ± 0.09^{b}	0.72 ± 0.09^{c}	0.56 ± 0.12^{b}	0.4 ± 0.04^{d}	0.4 ± 0.04^{d}	0.45 ± 0.09^{d}
Tricosanoic acid	C23:0	0.19 ± 0.03^{a}	0.26 ± 0.04^{bc}	0.29 ± 0.05^{b}	0.23 ± 0.03^{c}	0.19 ± 0.02^{a}	0.19 ± 0.03^{a}	0.19 ± 0.02^{a}
Lignoceric acid	C24:0	1.13 ± 0.06^{a}	1.48 ± 0.08^{bc}	1.75 ± 0.11^{d}	1.61 ± 0.16^{c}	1.3 ± 0.06^{e}	1.33 ± 0.16^{e}	1.43 ± 0.1^{be}
Monounsaturated fatty acids (MUFAs)								
Myristoleic acid	C14:1	7.43 ± 1.87^{a}	9.09 ± 0.48^{b}	9.02 ± 0.87^{b}	6.77 ± 1.23^{a}	7.33 ± 0.64^{a}	5.44 ± 0.22^{c}	5.16 ± 0.53^{c}
cis-10-Pentadecenoic acid	C15:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Palmitoleic acid	C16:1	13.33 ± 3.27^{a}	17.19 ± 2.01^{b}	17.03 ± 1.32^{b}	12.29 ± 2.48^{a}	12.87 ± 1.13^{a}	9.33 ± 0.71^{c}	9.1 ± 1.11^{c}
cis-10-Heptadecenoic acid	C17:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Elaidic, Oleic	C18:1 c+t	143.63 ± 31.91^{ab}	199.55 ± 29.6^{c}	202.5 ± 14.25^{c}	156.91 ± 24.19^{a}	157.16 ± 12.89^{a}	122.84 ± 10.51^{b}	121.52 ± 17.39^{b}
cis-11-Eicosenoic acid	C20:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Erucic acid	C22:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
cis-15-Tetracosenoic acid	C24:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Polyunsaturated fatty acids (PUFAs)								
Linoleaidic, Linoleic	C18:2 c+t	7.7 ± 1.72^{a}	60.85 ± 9.65^{bc}	105.72 ± 8.39^{d}	86.02 ± 18.7^{e}	38.25 ± 2.83^{f}	53.72 ± 4.68^{b}	65.5 ± 9.68^{c}
γ-Linolenic acid	C18:3 n-6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Linolenic acid	C18:3 n-3	4.44 ± 1.01^{a}	9 ± 0.84^{b}	11.74 ± 1.16^{c}	9.6 ± 2.2^{b}	6.27 ± 0.46^{d}	6.34 ± 0.31^{d}	6.63 ± 1.02^{d}
cis-11,14-Eicosadienoic acid	C20:2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
cis-8,11,14-Eicosatrienoic acid	C20:3 n-6	0.25 ± 0.09^{a}	0.34 ± 0.07^{b}	0.32 ± 0.05^{b}	0.19 ± 0.05^{ac}	0.22 ± 0.03^{a}	0.14 ± 0.03^{cd}	0.11 ± 0.03^{d}
Arachidonic acid	C20:4	0.63 ± 0.17^{bc}	0.76 ± 0.08^{a}	0.74 ± 0.1^{ab}	0.47 ± 0.1^{de}	0.52 ± 0.06^{cd}	0.38 ± 0.03^{ef}	0.34 ± 0.07^{f}
11,14,17-Eicosatrienoic acid	C20:3 n-3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
5,8,11,14,17-Eicosapentaenoic acid	C20:5	0.82 ± 0.19^{ab}	0.91 ± 0.09^{a}	0.89 ± 0.08^{a}	0.65 ± 0.13^{cd}	0.72 ± 0.07^{bc}	0.54 ± 0.04^{de}	0.51 ± 0.08^{e}
cis-13,16-Docasadienoic acid	C22:2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4,7,10,13,16,19-Docosahexaenoic acid	C22:6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total SFAs		*534.9 ± 119.7*	*675.5 ± 76.6*	*641.6 ± 57.8*	*487.5 ± 96*	*510.2 ± 45.2*	*391 ± 28.3*	*369.6 ± 47.9*
Total MUFAs		*164.4 ± 37*	*225.8 ± 32.1*	*228.5 ± 16.4*	*176 ± 27.9*	*177.4 ± 14.7*	*137.6 ± 11.4*	*135.8 ± 19*
Total PUFAs		*13.8 ± 3.2*	*71.9 ± 10.7*	*119.4 ± 9.8*	*96.9 ± 21.2*	*46 ± 3.5*	*61.1 ± 5.1*	*73.1 ± 10.9*
% SFAs		*75.01*	*69.41*	*64.84*	*64.11*	*69.55*	*66.30*	*63.89*
% MUFAs		*23.05*	*23.20*	*23.10*	*23.14*	*24.18*	*23.33*	*23.47*
% PUFAs		*1.94*	*7.38*	*12.07*	*12.75*	*6.27*	*10.36*	*12.63*
Atherogenic index (AI)		*3.58*	*2.74*	*2.23*	*2.09*	*2.77*	*2.32*	*2.09*

Thrombogenic index (TI)	*3.31*	*2.87*	*2.42*	*1.95*	*2.60*	*2.04*	*1.74*
Saturation index (SI)	*2.11*	*1.60*	*1.29*	*1.19*	*1.59*	*1.35*	*1.22*

* n.d: not detected; FW: fresh weight. Data are presented as Mean ± SD (n ≥ 3) and listed in the order of group and then number of carbons. Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row. POS5, POS10 and POS15: 5, 10, 15% of tamarillo powder was added post fermentation, respectively. PRE5, PRE10 and PRE15: 5, 10, 15% of tamarillo powder was added to milk and starter culture prior to fermentation, respectively. Used with permission (Diep et al., 2022)

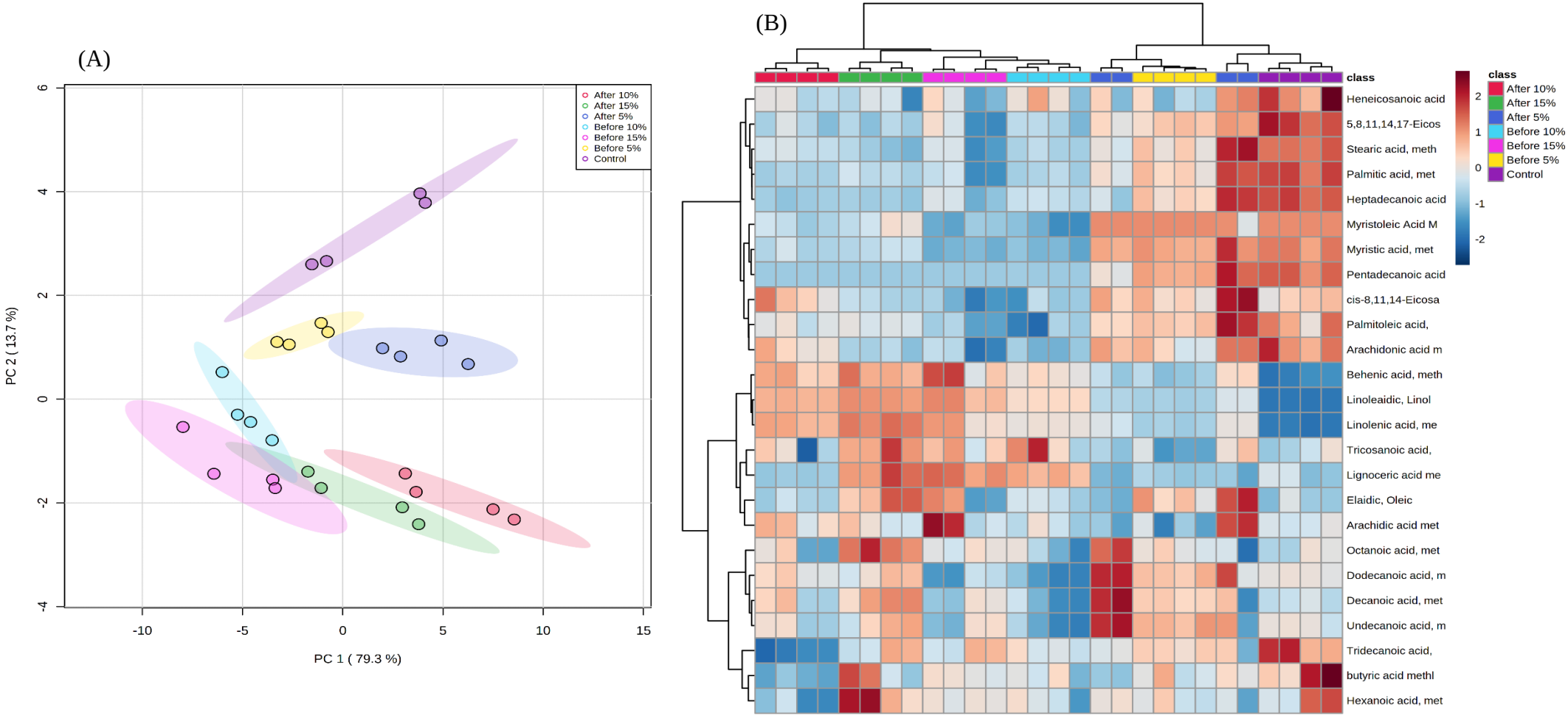

Figure 4.4 Score plot (A) and heatmap (B) diagrams from principal component analysis of fatty acids for control yoghurt and tamarillo yoghurts produced from two fermentation processes.

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4.3.7 Reducing sugar and organic acid profile of yoghurts

Lactose and maltose were the dominant sugars in all samples (Table 4.9) and the addition of tamarillo significantly increased ($p < 0.05$) the concentration of these sugars. Also, a higher concentration of lactose was found in tamarillo yoghurt produced from PRE than POS by 1.3 – 1.5 times. The concentration of lactose in cow milk with standard fat is 4.5 g/100 g (Sivakumaran et al., 2017). During fermentation, the content of lactose in milk significantly decreases and converts to lactic acid (Rybicka & Gliszczyńska-Świgło, 2021). This was observed in our results with lactose range of 1019 – 2277 mg/100 g, which was less than approximately 50% comparing to premium-assorted fruits yoghurt (4100 mg/100 g) from NZ-FCD (Sivakumaran et al., 2017).

As expected, with fruit added to the yoghurt the amount of fructose and glucose in fortified yoghurt ($p < 0.05$) was higher by 15 – 50 and 3 – 6 times, respectively, compared to the control (Gao, Wu, Wang, Xu, & Du, 2012; Temiz, Tarakci, Karadeniz, & Bak, 2012). Glucose content in all tamarillo yoghurts (214 – 495 mg/100 g) was lower than the fructose. Concentrations of fructose and glucose in all tamarillo yoghurt were lower than strawberry, peach, blueberry, raspberry and forest fruit yoghurts which ranged from 1200 – 4000 and 490 – 3890 mg/100 g, respectively Rybicka and Gliszczyńska-Świgło (2021). Hence, low concentration of fructose and glucose, and relatively low ratio of fructose to glucose (1.7:1 – 2.5:1) in the fortified tamarillo yoghurt may be considerable for developing functional food. Comparing galactose content, approximately 8 times of increase from PRE to POS was observed. The cultures used in yoghurt fermentation (*L. bulgaricus* and *S. thermophilus*) utilize galactose moiety of lactose rather than glucose the moiety. The concentrations of other sugars (ribose, rhamnose, mannose, arabinose, xylose, fucose) as well as glucuronic acid increased when yoghurt was fortified with tamarillo powder.

Organic acids are important indicators of bacterial metabolic activity in yoghurt, and they are considered as natural preservatives. Organic acids also contribute to the taste and flavor of the product together with other volatile and semi-volatile compounds (Trigueros, Pérez-Alvarez, Viuda-Martos, & Sendra, 2011). All organic acids detected were significantly ($p < 0.05$) affected by the type of yoghurt (Table 4.10). Lactic acid was the dominant organic acid in all samples, followed by citric acid with significantly ($p < 0.05$) higher concentrations found in the fortified than the control yoghurt ($p <$

0.05). Citric acid is the predominant organic acid in milk as a product of bovine metabolism and it promotes refreshing taste. This acid is known to be utilized during the fermentation process (Da Costa, Da Silva Frasao, Da Costa Lima, Rodrigues, & Junior, 2016).

Malic acid possesses antimicrobial property, where prevention of the growth of *Listeria monocytogenes*, *Salmonella gaminara*, and *Escherichia coli* O157 has been reported (Raybaudi-Massilia, Mosqueda-Melgar, & Martín-Belloso, 2009). The high concentration of malic as well as citric acids in tamarillo yoghurt resulted from the use of tamarillo fruit which is predominated by those two acids (Etienne, Génard, Lobit, Mbeguié-A-Mbéguié, & Bugaud, 2013). The concentration of malic acid increased with the increase of tamarillo concentration added. Similar phenomenon was also observed for cinnamic acid. Fumaric acid was only detected in yoghurt produced from POS15. This acid was found effective in the prevention of cardiovascular diseases (Delgado, Ramalhosa, Pereira, & Casal, 2018). Itaconic and syringic acids were only detected in tamarillo enriched yoghurt. The itaconic concentration was not much difference between PRE and POS but increasing the fortification to 10 and 15% has increased its content by 2.7 – 2.9 and 3.3 – 3.6 times, respectively. Itaconic acid has been shown as a potential bacteriocide and largely encountered as a component of the animalian immune response (Naujoks et al., 2016). Concentration of syringic acid in fortified yoghurts varied from 1.2 to 2.74 mg/100 g. POS showed slightly higher syringic acid concentration than the PRE. This acid has been reported with a wide range of therapeutic applications in prevention of diabetes, cancer, cerebral ischemia as well as it possesses antioxidant, antimicrobial and anti-inflammatory. The strong antioxidant activity of syringic acid may confer its beneficial effects for human health (Srinivasulu, Ramgopal, Ramanjaneyulu, Anuradha, & Kumar, 2018).

Table 4.9 Reducing sugar profiles of control and tamarillo fortified yoghurts produced from pre and post fermentation processes.

Reducing sugars	**Concentration (mg/100 g FW)**						
	Control	**POS5**	**POS10**	**POS15**	**PRE5**	**PRE10**	**PRE15**
Fructose	22.5 ± 3.08^{a}	482 ± 120^{b}	1066 ± 318^{cd}	1161 ± 269^{d}	365 ± 49.1^{b}	909 ± 346^{cd}	895 ± 156^{c}
Lactose	1019 ± 86.4^{a}	1483 ± 45.6^{b}	1376 ± 91.4^{c}	1263 ± 50.4^{d}	2277 ± 31.2^{e}	1938 ± 121^{f}	1702 ± 25.4^{g}
Galactose	283 ± 41.8^{a}	387 ± 16.5^{b}	372 ± 31.9^{b}	318 ± 9.93^{c}	46.8 ± 3.6^{d}	44.5 ± 4.98^{d}	36.4 ± 3.72^{d}
Glucose	79.2 ± 11.5^{a}	248 ± 11.1^{b}	427 ± 30.8^{c}	491 ± 26.9^{d}	$214 \pm 9.99e$	385 ± 30.3^{f}	495 ± 22.7^{d}
Mannose	1.72 ± 0.05^{a}	3.49 ± 0.15^{b}	4.21 ± 0.38^{c}	4.54 ± 0.28^{c}	4.66 ± 0.18^{c}	5.79 ± 0.53^{d}	6.12 ± 0.76^{d}
Ribose	0.17 ± 0.01^{a}	0.52 ± 0.08^{b}	0.57 ± 0.06^{b}	0.81 ± 0.13^{c}	1.94 ± 0.3^{d}	1.8 ± 0.4^{de}	1.64 ± 0.27^{e}
Rhamnose	n.d	$< 0.005^{ab}$	0.01 ± 0.01^{ab}	0.05 ± 0.03^{cd}	0.02 ± 0.02^{abc}	0.03 ± 0.04^{bc}	0.07 ± 0.04^{d}
Maltose	739 ± 55.4^{a}	1117 ± 131^{b}	1070 ± 69^{b}	1051 ± 73.3^{b}	1643 ± 161^{c}	1428 ± 163^{d}	1340 ± 105^{d}
Glucuronic acid	0.75 ± 0.05^{a}	1.2 ± 0.08^{b}	1.25 ± 0.16^{b}	1.23 ± 0.17^{b}	1.46 ± 0.2^{c}	1.31 ± 0.27^{bc}	1.31 ± 0.23^{bc}
Arabinose	0.07 ± 0.03^{a}	0.27 ± 0.04^{b}	0.33 ± 0.06^{b}	0.61 ± 0.09^{c}	0.42 ± 0.05^{d}	0.47 ± 0.1^{de}	0.51 ± 0.1^{e}
Xylose	0.04 ± 0.04^{a}	1.95 ± 0.29^{b}	3.46 ± 0.54^{c}	3.91 ± 0.59^{cd}	1.83 ± 0.31^{b}	2.73 ± 0.32^{e}	3.98 ± 0.53^{d}
Fucose	0.24 ± 0.03^{a}	0.31 ± 0.05^{b}	0.31 ± 0.07^{ab}	0.39 ± 0.06^{c}	0.34 ± 0.05^{bc}	0.34 ± 0.06^{bc}	0.32 ± 0.05^{b}
Total	*2145 ± 198*	*3725 ± 325*	*4321 ± 543*	*4296 ± 431*	*4558 ± 256*	*4717 ± 668*	*4483 ± 315*

* n.d: not detected; FW: fresh weight. Data are presented as Mean ± SD (n = 3) and listed in the order of retention time. Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row. POS5, POS10 and POS15: 5, 10, 15% of tamarillo powder was added post fermentation, respectively. PRE5, PRE10 and PRE15: 5, 10, 15% of tamarillo powder was added to milk and starter culture prior to fermentation, respectively. Used with permission (Diep et al., 2022)

Table 4.10 Organic acid profiles of control and tamarillo fortified yoghurts produced from pre and post fermentation processes.

Organic acids	**Concentration (mg/100 g FW)**						
	Control	**POS5**	**POS10**	**POS15**	**PRE5**	**PRE10**	**PRE15**
Malic acid	0.57 ± 0.09^{a}	20.7 ± 0.57^{b}	22.5 ± 0.18^{c}	43.4 ± 0.13^{d}	19.6 ± 0.33^{b}	34.1 ± 0.72^{e}	44.7 ± 1.09^{f}
Lactic acid	674 ± 26.5^{a}	920 ± 20.3^{b}	1479 ± 124^{c}	1683 ± 62.1^{d}	846 ± 5.91^{b}	1428 ± 20.6^{c}	1833 ± 37.5^{e}
Malonic acid	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Citric acid	116 ± 13.2^{a}	838 ± 12.5^{b}	887 ± 27.6^{c}	931 ± 62.9^{c}	751 ± 16.9^{d}	911 ± 32.6^{c}	885 ± 23.5^{c}
Succinic acid	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Fumaric acid	n.d	n.d	n.d	n.d	n.d	n.d	0.72 ± 0.05^{a}
Itaconic acid	n.d	0.28 ± 0.09^{a}	0.81 ± 0.1^{b}	0.91 ± 0.13^{bc}	0.31 ± 0.03^{a}	0.83 ± 0.11^{b}	1.14 ± 0.1^{c}
Vanilic acid	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Syringic acid	n.d	1.9 ± 0.02^{a}	2.28 ± 0.02^{b}	2.74 ± 0.07^{c}	1.2 ± 1.04^{d}	2.27 ± 0.02^{b}	2.63 ± 0.04^{c}
Cinnamic acid	0.43 ± 0.01^{a}	0.6 ± 0.05^{b}	0.76 ± 0.06^{c}	0.9 ± 0.06^{d}	0.66 ± 0.08^{b}	0.71 ± 0.02^{c}	0.84 ± 0.05^{d}
Total	*791 ± 39.8*	*1782 ± 33.5*	*2392 ± 152*	*2662 ± 125*	*1619 ± 24.3*	*2377 ± 54.1*	*2768 ± 62.4*

* n.d: not detected; FW: fresh weight. Data are presented as Mean ± SD (n = 3) and listed in the order of retention time. Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row. POS5, POS10 and POS15: 5, 10, 15% of tamarillo powder was added post fermentation, respectively. PRE5, PRE10 and PRE15: 5, 10, 15% of tamarillo powder was added to milk and starter culture prior to fermentation, respectively. Used with permission (Diep et al., 2022)

4.3.8 Content of α-tocopherol, β-carotene and ascorbic acid in yoghurts

Adding tamarillo significantly increased α-tocopherol (vitamin E), β-carotene (pro vitamin A) and ascorbic acid (vitamin C) in yoghurt (Table 4.11). The highest α-tocopherol concentration was found in PRE15. This trend is a clear indication that tamarillo fortification in yoghurt caused a significant increase ($p < 0.05$) approximately 5 – 11 times in vitamin E concentration in yoghurt regardless of the fermentation process. PRE10 and PRE15 possessed higher α-tocopherol content than those counterparts from POS. According to Sarkar, Salauddin, Hazra, and Chakraborty (2020), freeze-drying which reduced the water content had caused an increase in solid content thus increased the vitamin E concentration. Therefore, using tamarillo powder to fortify yoghurt should be more efficient than using fresh fruit or fruit juice. All of tamarillo yoghurt showed higher α-tocopherol content than premium-assorted fruits yoghurt (0.16 mg/100 g) from NZ-FCD (Sivakumaran et al., 2017) although only POS15, PRE10 and PRE15 samples were considered as a source of α-tocopherol. Tocopherols scavenge peroxide radicals by forming tocopheroxyl radical and at higher temperature through reacting with other antioxidants, this tocopheroxyl radical may be added back to tocopherol form (Sarkar et al., 2020). This could explain the higher tocopherol in PRE than the POS. Vitamin E has been reported as an impressive antioxidant compound that can prevent the lipid peroxidation, efficiently eliminate the free radicals, and plays a key role in prevention of several chronic diseases (Sarkar et al., 2020).

A significant amount of β-carotene was present in all types of yoghurt. Fortification significantly ($p < 0.05$) increased the β-carotene content by approximately 5 – 11 times (Table 4.11). Higher β-carotene content was observed in POS than in PRE. This might be because β-carotene has been degraded due to the isomerization process which took place during fermentation. Only POS15 sample could be considered as a dietary source of β-carotene (Table 4.11), however, other tamarillo yoghurt showed higher β-carotene content than premium-assorted fruits yoghurt (0.07 mg/100 g) from NZ-FCD (Sivakumaran et al., 2017). The high content of β-carotene as well as α-tocopherol in tamarillo yoghurt compared to premium-assorted fruits have resulted from the use of tamarillo fruit which was rich in these antioxidant vitamins. Similar to the α-tocopherol, the increase in β-carotene content of freeze-dried fruit compared to fresh samples had been reported (Gao et al., 2012) which proves that fortification with

tamarillo has potential. β-Carotene has been reported to own pro-oxidant and antioxidant activity against lipid peroxidation (Sarkar et al., 2020) which may affect the shelf life of yoghurt.

Tamarillo is a rich source of vitamin C or ascorbic acid (Diep et al., 2020b) whereas in milk, ascorbic acid is present in low amount, 1.65 – 2.75 mg/100 g. Tamarillo possesses 9 to 15-fold higher vitamin C content than that of milk. Thus, tamarillo fortification increased the ascorbic acid content of yoghurt significantly ($p < 0.05$) (Table 4.11). The highest ascorbic acid content resulted in POS15. Direct addition of tamarillo powder without any further thermal treatment enabled higher retention of vitamin C in fortified yoghurt in POS compared to the PRE. All tamarillo yoghurt showed higher vitamin C content than the premium-assorted fruits yoghurt (2.6 mg/100 g FW) as reported in NZ-FCD (Sivakumaran et al., 2017). Most tamarillo yoghurts were a good source of vitamin C (Table 4.11) except for the PRE5 and met higher % of RDI than premium-assorted fruits yoghurt (10%) (Sivakumaran et al., 2017) (National Health and Medical Research Council, 2006). The concentration of these three compounds in fortified yoghurt was lower than those present in raw fruit (α-tocopherol of 0.53 mg/100 g per 5% fortification), (β-carotene of 0.17 mg/100 g per 5% fortification), and (Vitamin C of 8.12 mg/100 g per 5% fortification). Further interaction between the components in yoghurt and matrix effect may have influenced the concentration of these analytes during extraction and analysis. Vitamin C is the most thermo-sensitive bioactive compound in plants and susceptible to degrade from high temperature, moisture and oxygen concentration (Sarkar et al., 2020). This can explain the lower concentration of ascorbic acid in PRE compared to the POS. Under aerobic condition, ascorbic acid firstly converts into dehydro-ascorbic acid via a reversible reaction pathway and then further hydrolysis and oxidation took place in an irreversible way (Sarkar et al., 2020). Vitamin C has played a crucial role in human physiology and body metabolism. This compound can act as a strong antioxidant agent that can prevent lipid peroxidation and as a strong free radical scavenging agent that can remove different types of the harmful free radical present in the human body (Sarkar et al., 2020).

Table 4.11 Concentrations and % RDI/AI of α-tocopherol, β-carotene and ascorbic acid in yoghurt samples.

Yoghurt samples	α-tocopherol (mg/100g FW)	%AI of α-tocopherol	β-carotene (mg/100g FW)	%RDI of β-carotene	Ascorbic acid (mg/100g FW)	%RDI of ascorbic acid
Control	0.09 ± 0^{a}	1%	0.03 ± 0^{a}	1%	0.57 ± 0.01^{a}	2%
POS5	0.46 ± 0.02^{b}	7%	0.17 ± 0.03^{b}	5%	7.77 ± 0.33^{b}	**26%**
POS10	0.62 ± 0.01^{c}	9%	0.26 ± 0.01^{c}	7%	11.57 ± 0.25^{c}	**39%**
POS15	0.89 ± 0.02^{d}	**13%**	0.35 ± 0.01^{d}	**10%**	15.85 ± 0.35^{d}	**53%**
PRE5	0.41 ± 0.01^{e}	6%	0.15 ± 0.02^{b}	4%	2.49 ± 0.02^{e}	8%
PRE10	0.72 ± 0.01^{f}	**11%**	0.24 ± 0.01^{e}	7%	6.27 ± 0.17^{f}	21%
PRE15	0.97 ± 0.04^{g}	**15%**	0.34 ± 0.03^{d}	9%	7.77 ± 0.05^{b}	**26%**

* Data are presented as Mean ± SD (n ≥ 3). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each column. FW: fresh weight. RDI: recommended dietary intake (FSANZ labelling). AI: Adequate Intake (used when an RDI cannot be determined) 1 μg retinol (vitamin A) equivalent = 6 μg all trans β carotene RDI = 900 retinol equivalents. Vitamin E: AI = 10 mg, UL = 300 mg. Vitamin C: Estimated average requirement (EAR) = 30 mg, RDI = 45 mg. – : not identified. **Bolded** numbers are dietary source (>10% RDI) and **bolded and underlined** numbers are good source (>25% RDI) (Diep et al., 2020b). The RDI and AI were calculated based on mg/serve in which serving size was 150g. Used with permission (Diep et al., 2022)

4.4 Conclusion

Addition of tamarillo in powder form both before and after fermentation increased the acidity, fibre, protein and lactic acid contents of yoghurts as well as maintaining the unique volatile compounds in the fruit. The addition of tamarillo resulted in a firmer yoghurt of lower deformability and higher elastic behavior and viscosity. The addition of powder after fermentation preserved the red color of the tamarillo as well as retained the vitamin C content. Yoghurt fortified with tamarillo powder offers the potential for the development of a value-added product that could be a good source of vitamin C and a source of vitamin E and β-carotene and maintain the volatiles that give tamarillo its distinctive flavor. Next steps include the determination of the organoleptic properties and consumer acceptability of tamarillo yoghurt.

4.5 Summary and next steps

This experimental investigation showed that tamarillo powder could be successfully employed as a functional ingredient to fortify yoghurt. This novel dairy product was formulated by enriching yoghurt with fruit powder either pre- or post the fermentation process. Tamarillo powder incorporation gave additional and essential healthy properties to yoghurt such as enhancing fibre content, improving the quality of the fatty acid profile, increasing the content of monounsaturated and polyunsaturated fatty acids as well as antioxidant vitamins. Yoghurt with added tamarillo fruit is a new product that can be manufactured and successfully marketed due to these properties.

The bio-accessibility of polyphenols from the food matrix is unknown and depends on the effects of the digestive process. In order to exert their beneficial effects on the small and large intestine the bioactive polyphenols and anthocyanins need to be released and not degraded during oral and gastric digestion. Hence, the next chapter will evaluate the influence of *in vitro* gastrointestinal digestion of tamarillo yoghurts on the digestibility of amino acids the stability and bio-accessibility of phenolic and anthocyanins compounds as well as on the antioxidant activity.

Chapter 5: Effect of *in vitro* gastrointestinal digestion on amino acids, polyphenols, and antioxidant capacity of tamarillo yoghurts

This chapter is reproduced from published article: "Effect of *In Vitro* Gastrointestinal Digestion on Amino Acids, Polyphenols and Antioxidant Capacity of Tamarillo Yoghurts" published in International Journal of Molecular Sciences with permission.

'Laird's Large' tamarillo powder is high in protein (10%), essential amino acids, γ-aminobutyric acid (GABA) and polyphenols (0.6% phenolics plus anthocyanins) and fibre (25%). This chapter aimed to investigate, using a standardized static *in vitro* digestion model, the stability of amino acids and antioxidant capacity of polyphenols in yoghurt fortified with 5, 10 and 15% tamarillo powder either before (PRE) or after (POS) fermentation. Compared to plain yoghurt, the fruit polyphenols (rutinosides and glycosides) were retained and substantial increases in FEAAs (free essential amino acids), total phenolic content (TPC) and antioxidant activity were observed particularly at the end of intestinal phase of digestion. Together with SDS-PAGE results, peptides and proteins in tamarillo yoghurts were more easily digested and therefore may be better absorbed in the small intestine compared to the control. TPC and antioxidant activity of fortified yoghurts increased significantly after *in vitro* digestion. Relatively high bioaccessibility of chlorogenic acid and kaempferol-3-rutinoside in digested PRE samples was observed. The results suggest that the yoghurt matrix might protect some compounds from degradation, increasing bioaccessibility and in the small intestine allow increased absorption and utilization possible. Fortification would deliver intact polyphenols and fibre to the large intestine and improve gut health. Further research of acceptability, shelf life, and then trials for health effects should be implemented.

This chapter firstly introduces the motivations and objectives of this study (Section 5.1) followed by the experimental work, which is described in Section 5.2. The amino acid, polyphenols and antioxidant activity profiles in tamarillo yoghurts before and after fermentation are described in Section 5.3. A brief summary is included in Section 5.4.

5.1 Introduction

The development of new food products with a potentially positive effect on health using traditional fruits, is generally desirable since there is an increasing interest among consumers to look for safe, healthy, sustainable and natural foods (Aschemann-Witzel, Gantriis, Fraga, & Perez-Cueto, 2021). Consumption of fruits is associated with health benefits which are usually related to their vitamin, mineral or specific antioxidant compounds, in particular, polyphenols which possess strong antioxidant activity and are associated with protective effects against chronic diseases including type 2 diabetes mellitus, cardiovascular diseases and cancer (Dreher, 2018).

The previous chapter (Chapter 2) has shown that the protein content of New Zealand-grown 'Laird's Large' cultivar of tamarillo is 1.2% fresh weight (FW) (88.1% moisture content) which is equivalent to 10% dry weight (DW) (Diep et al., 2020c) (Appendix B17). In addition, tamarillo powder has a high fibre content (25%), essential for bowel health. Twenty-two amino acids (9 essential and 13 non-essential were detected (Diep et al., 2020c). Tamarillo showed a high concentration of γ-aminobutyric acid (GABA) (433 mg/100 g DW) which is similar to tomato, the same *Solanum* genus (Diep et al., 2020c). In addition, tamarillo fruit contains a spectrum of polyphenol components including not only the blue-red coloured anthocyanins; delphinidin 3-rutinoside (255 mg/100 g DW) and pelargonidin 3-rutinoside (201 mg/100 g DW), but also the colourless phenolics, particularly chlorogenic acid (66.3 mg/100 g DW) and kaempferol 3-rutinoside (50.0 mg/100 g DW) contributing to high antioxidant activity (52.4 and 60.2 μmol TEAC/g DW ~ 5240 - 6020 μmol TEAC/100 g DW determined by CUPRAC and FRAP assays, respectively) (Diep et al., 2020a). It has been reported that tamarillo polyphenols have shown promising effects including reduction of oxidative stress (Kou et al., 2009), anti-obesity (Abdul Kadir et al., 2015), anticancer (Mutalib et al., 2016; Mutalib et al., 2017), anti-microbial activity (Ordonez et al., 2006) as well as protection against lipid oxidation (Castro-Vargas et al., 2013).

Among the principal issues concerning the beneficial effects of amino acids and polyphenols, their bioavailability and metabolic pathway must be considered. The bioavailability of a dietary compound is dependent on its release from the food matrix (referred to as bioaccessibility) based on particle size and form (Bohn, 2014), its digestive stability, and the efficiency of its transepithelial passage. In particular,

bioavailability differs greatly from one polyphenol to another, and can depend on the dietary source (Oliveira & Pintado, 2015). Research concerning the bio-accessibility of polyphenols from food matrices is important, since only the compounds released from the food matrix are potentially bio-accessible, and after the gastrointestinal digestion, are in a condition to exert their beneficial effects (Oliveira & Pintado, 2015).

In the food matrix, proteins are known to reversibly and irreversibly conjugate with polyphenols in different ways dependent on characteristics of the proteins, the polyphenols, the food matrix and the stage of digestion (Quan, Benjakul, Sae-leaw, Balange, & Maqsood, 2019). These characteristics include solubility, pH, thermal stability, non-covalent bonding and enzymatic reactions (Quan et al., 2019). Protein-polyphenol complexes may improve the stability of food emulsions on the shelf but also be a mechanism to prevent degradation of the polyphenol and target delivery to the intestinal tract.

Yoghurt is the most popular fermented dairy product (Aryana & Olson, 2017) and is highly appreciated for its nutritional value and good digestibility. The health benefits of yoghurt have been recognized due to the presence of bioactive peptides and probiotics (Helal & Tagliazucchi, 2018). However, plain yoghurt is not considered a source of polyphenols and therefore traditional foods such as spices, fruits, seed or extract had been used to enhance the polyphenol content of yoghurt. Many studies have attempted to investigate the effect of plant extracts in order to improve the quality of yoghurt such as increasing antioxidant activity and the viability of lactic acid bacteria during refrigerated storage (Anuyahong, Chusak, & Adisakwattana, 2020). The yoghurt matrix is an excellent delivery vehicle for plant-derived polyphenol compounds. The low pH of yoghurt increases the stability of phenolic compounds during storage (Helal & Tagliazucchi, 2018), whereas the presence of proteins or large peptides and fats maintain the integrity of polyphenols during digestion, increasing their bioaccessibility (Tagliazucchi, Helal, Verzelloni, & Conte, 2012).

The main aim of this chapter was to fortify yoghurt using tamarillo powder and to evaluate how amino acid, polyphenol concentrations and the antioxidant activity are affected during digestion. For this purpose, six tamarillo yoghurts (produced from pre- and post-fermentation processes at three different concentrations of fortification) and a control sample were examined for the successive effects of *in vitro* digestion at oral,

gastric and intestinal phases on amino acid, polyphenol concentrations and antioxidant activity.

5.2 Materials and methods

5.2.1 Materials

Yoghurt ingredients were standard milk (Anchor™ blue top, 3.3% protein and 3.4% fat) from Fonterra, New Zealand and starter culture, *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* (YoFlex® Express 1.1 powder) from CHR Hansen (Hoersholm, Denmark). Freeze dried tamarillo ('Laird's Large' cultivar) powder was used for fortification.

All chemicals and reagents used were AnalaR grade or purer. The analytical grade standards of amino acids standards (A9906 product), phenolics and anthocyanins were obtained from Sigma-Aldrich (Auckland, New Zealand) or Extrasynthese (Genay Cedex, France). All chemicals and gels for SDS-PAGE experiment including LDS Sample Buffer (4x), MES Running Buffer, Coomassive Blue R-250, Bis-Tris Protein Gels and Prestained standard were obtained from Thermo Fisher (Auckland, New Zealand). All chemicals for total phenolic content (TPC) and antioxidant activity were purchased from Sigma-Aldrich (Auckland, New Zealand). Milli-Q water was produced by a Purite Fusion Milli-Q water purifying machine (Purite Limited, Thame, Oxon, UK).

5.2.2 Preparation of tamarillo yoghurt and tamarillo water extract

Yoghurt preparation and experimental strategy are summarized in Figure 5.1. Yoghurt samples were prepared using commercial yoghurt makers (Davis & Waddell, Steven, New Zealand). For the control yoghurt, starter culture and milk in the ratio of 0.1 : 100 (w/w), were placed in the yoghurt maker and set at 45°C for 8 hours and until the pH dropped below 5.0. The yoghurt was stored at 4°C overnight and then homogenized at 4000 RPM (L4R Laboratory Mixer, Silverson, Waterside, England) for 2 min (Wang et al., 2020).

Freeze-dried tamarillo pulp powder (5, 10 and 15%) was added into the yoghurt either before (PRE) or after (POS) the fermentation process. For PRE, prior to fermentation, tamarillo powder was added to the mixture of milk and starter culture at the start of

the yoghurt making process. For POS, tamarillo powder was added to the yoghurt after fermentation in the final homogenization step. Counting the control yoghurt without tamarillo, seven different formulations in total were prepared for digestion. Calculated total protein content of each mixture was 3.3% for control, 3.6% for 5%, 4.0% for 10% and 4.3% for 15% as each 5% of tamarillo powder contributed 0.5% of tamarillo protein (Diep et al., 2020c). The polyphenol compounds in tamarillo powder have been identified in Chapter 2, and these were *p*-coumaric acid, caffeic acid, catechin, epicatechin, chlorogenic acid, ellagic acid, ferulic acid, gallic acid, kaempferol, rutin, kaempferol 3-rutinoside, isorhamnetin 3-rutinoside, cyanidin 3-rutinoside, delphinidin 3-rutinoside and pelargonidin 3-rutinoside (Diep et al., 2020a) with the concentration of main polyphenols has been described in Appendix B17.

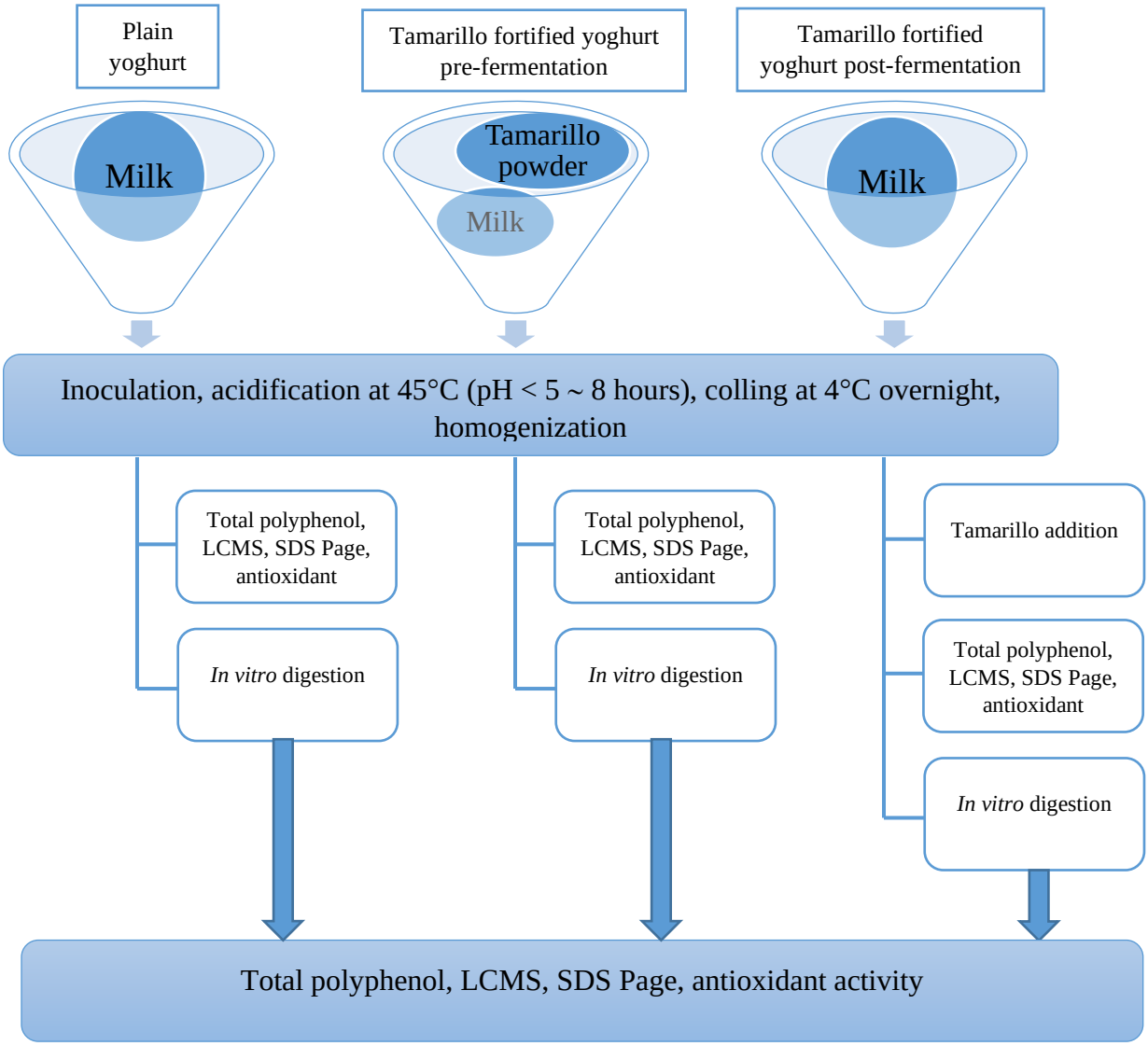


Figure 5.1 Experimental strategy for the preparation and characterization of tamarillo-fortified yoghurt produced from two fermentation processes.

5.2.3 *In vitro* digestion of yoghurt

A static *in vitro* enzymatic digestion method of Zhang, Yoo, Gathercole, Reis, and Farouk (2018) was used to examine digestibility of yoghurt samples. A consecutive three-stage digestion (oral, gastric and intestinal phases) was simulated in a single bioreactor in a shaking water bath at 37 °C at 100 RPM (Model G76, New Brunswick Scientific Co., Inc, U.S.A) to test 5 mL of each yoghurt sample for about 5 hours. Simulated Salivary Fluid (SSF), Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) and a multi-enzyme system composed of salivary α-amylase (from Aspergillus oryzae, A9857-5MU, Sigma-Aldrich), pepsin (from porcine gastric mucosa, P-7000, Sigma-Aldrich), pancreatin (from porcine pancreas 3X U.S.P., MP Biomedicals, LLC) and bile (Bile extract porcine, Sigma) were used to simulate the conditions for oral, gastric and intestinal phases, for 5 min, 120 min and 180 min, respectively. The simulated digestion fluids were prepared with electrolyte stock solutions, enzymes, and MilliQ according to Minekus et al. (2014). The stock solutions of digestion fluids were prepared with MilliQ water as given in Table 5.1.

For the oral phase (OP), a 5 mL of drinking yoghurt was mixed with 3.5 mL of SSF solution, 25 μL of 0.3 M $CaCl_2$ and 975 μL of MilliQ. The mixture was adjusted to pH 7.0, followed by pre-warming to 37°C. Addition of 0.5 mL salivary α-amylase was implemented, and the mixture was incubated for 5 min. For the gastric phase (GP), the mixture from oral phase was added with 7.5 mL of SGF solution, 5 μL of 0.3 M $CaCl_2$, 695 μL of MilliQ and 0.2 mL of 1 M HCl. The mixture was adjusted to pH 3.0, followed by pre-warming to 37°C. Then, 1.6 mL of pepsin was added, and the mixture was incubated for 120 min. For the intestinal phase (IP), the mixture from gastric phase was added with 11 mL of SIF solution, 40 μL of 0.3 M $CaCl_2$, 1.31 mL of MilliQ and 0.15 mL of 1 M HCl. The mixture was adjusted to pH 7.0, followed by pre-warming to 37°C. Then, 5 mL of pancreatin, 2.5 mL of bile, 780 μL of lipase were added, and the mixture was incubated for 180 min.

The sample (2 mL) was collected before digestion, after oral (5 min), gastric (120 min) and intestinal (180 min) phases. A total of 305 minutes and 4 sampling time points,

for each set of 7 formulations (28 samples) were achieved. Each sample was transferred into a centrifuge tube and snap-frozen in liquid nitrogen to stop further digestion. When the tube was defrosted at room temperature it was centrifuged at 2000 RPM for 10 min. The supernatant was transferred into another microcentrifuge tube and centrifuged again at 10000 RPM for 10 min. The supernatant was stored at –20°C until further analysis.

Table 5.1 Preparation of stock solutions of simulated digestion fluids.

Salt (stock solutions)	Stock solution added to prepare 450 mL of SSF (mL)	Stock solution added to prepare 450 mL of SGF (mL)	Stock solution added to prepare 450 mL of SIF (mL)
KCl (0.5 M)	15.1	6.9	6.8
KH_2PO_4 (0.5 M)	3.7	0.9	0.8
$NaHCO_3$ (1 M)	6.8	12.5	42.5
NaCl (2 M)	-	11.8	9.6
$MgCl_2(H_2O)_6$ (0.15 M)	0.5	0.4	1.1
$NH_4(CO_3)_2$ (0.5 M)	0.06	0.5	-

SSF: Simulated Salivary Fluid; SGF: Simulated Gastric Fluid; SIF: Simulated Intestinal Fluid

5.2.4 Analysis of soluble proteins using Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Protein profiles of control and tamarillo yoghurt samples, before and at the end of three phases of digestion were determined by the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method with 4 gels (before, oral, gastric and intestinal), each with 10 lanes (Figure 5.2). A 10 µL aliquot of pre-stained protein standard (SeeBlue® Thermo Fisher Scientific) with molecular weights in the range 3–198 kDa was applied to lanes 1, 5 and 10 of each gel.

For the yoghurt samples, 20 µL of each sample: PRE15, PRE10, PRE5, POS15, POS10, POS5 and control (n = 7 for each time point) were each mixed with 10 µL of LDS Sample Buffer (4X) and 10 µL of MilliQ water in an Eppendorf tube. After vortexing for 30 s, the mixture was incubated at 70 °C for 10 min. Then, 20 µL of each sample was carefully added into gel wells. Running buffer was prepared by mixing 20 mL of MES Running Buffer with 380 mL of MilliQ water. The electrophoresis was

performed by using a constant voltage (Bio-Rad, PowerPac™ Basic, California, USA) set at 150 V at room temperature for 40 min. At the completion of electrophoresis, the gel was removed from the cassette and placed in a vessel containing Coomassie gel stain which was prepared by mixing 1.0 g Coomassie Brilliant Blue with 450 mL of 95% methanol, 100 mL of glacial acetic acid and 450 mL of MilliQ water. After staining for 30 min, the gel stain solution was discarded, a gel destain solution containing 100 mL of 95% methanol, 100 mL of glacial acetic acid and 800 mL of MilliQ water were added. The gel was soaked overnight with the destaining solution. During this time, the destain solution was changed for three times. The gel was visually assessed when placed on a flat white surface under natural light according to the method agreed by international consensus (Liu, 2018).

5.2.5 Determination and quantification of free amino acids (FAAs)

Identification and quantification of free amino acids were implemented according to our previous study (Diep et al., 2020c) without further modification. The LC-ESI-MS/MS equipped with Kinetex C18 column (150 x 2.1 mm, 1.7 μm; Phenomenex, USA) and two Agilent MassHunter software (Qualitative Analysis and Quantitative Analysis for QQQ) (Santa Clara, CA, USA) were used to identify, qualify and quantify FAAs. Quantification of each FAA was carried out using standard calibration curves with a coefficient of correlation > 0.99.

5.2.6 Determination and quantification of phenolics including anthocyanins

Identification and quantification of phenolics including anthocyanins were implemented according to our previous study (Diep et al., 2020a) without further modification. Analytical standards of phenolics (gallic acid, ellagic acid, ferulic acid, chlorogenic acid, rutin, kaempferol, kaempferol 3-rutinoside and isorhamnetin 3-rutinoside) and four anthocyanins (cyanidin 3-glucoside, cyanidin 3-rutinoside, delphinidin 3-rutinoside and pelargonidin 3-rutinoside) were used for calibration. The LC-ESI-MS/MS was also used to identify polyphenols in yoghurts.

5.2.7 Total phenolic content (TPC) and antioxidant activity of yoghurt

The total phenolic content (TPC) of extracts and digests at each stage was determined using Folin-Ciocalteau assay as described in our previous study (Diep et al., 2020a). Two different methods were used to determine the antioxidant activity, namely cupric ion reducing antioxidant capacity (CUPRAC) and ferric-reducing antioxidant power (FRAP) assays (Diep et al., 2020a). Results of TPC and antioxidant activity were presented as mg gallic acid equivalent per 100 gram of yoghurt (mg GAE/100 g yoghurt) and mg Trolox equivalent antioxidant capacity per 100 g of yoghurt (mg TEAC/100 g yoghurt), respectively.

5.2.8 Statistical Analysis

Statistical analysis was carried out using SPSS 25.0 software (IBM Corp., Armonk, New York, USA). All experiments were carried out in triplicate, and data are reported as mean ± standard deviation. The differences among mean values of concentrations of polyphenols or antioxidant activity and that obtained in the different steps of the digestion were determined by one-way analysis of variance (ANOVA) with Fisher's (LSD) post hoc test. Statistical significance was set at $p < 0.05$.

5.3 Results and discussion

5.3.1 Protein fractions by mass

At a molecular level, marked differences in the protein fractions in control and tamarillo yoghurts before and after oral, gastric and intestinal phases of digestion (Figure 5.2) were observed. Prior to digestion, all yoghurts including the control (Figure 5.2A) had lower molecular weight (MW) whey proteins (α-lactalbumin and β-lactoglobulin) present. The caseins band was only observed in yoghurts fortified with 10% and 15% tamarillo powder. The intensity of β-lactoglobulin, the predominant component of whey protein, was similar for all yoghurt samples indicating that this compound was not degraded with or without addition of tamarillo powder. Meanwhile, α-lactalbumin showed higher intensity for control and the tamarillo yoghurts produced from the post-fermentation process rather than yoghurts fortified in pre-fermentation. These changes most probably were due to proteolysis induced by

tamarillo protease during fermentation (Li et al., 2018c). Hence, fermentation degraded α-lactalbumin but not the major whey proteins. This is similar to the changes in the protein profile observed 24 hours after a strawberry preparation was added to fermented yoghurt (Oliveira et al., 2015).

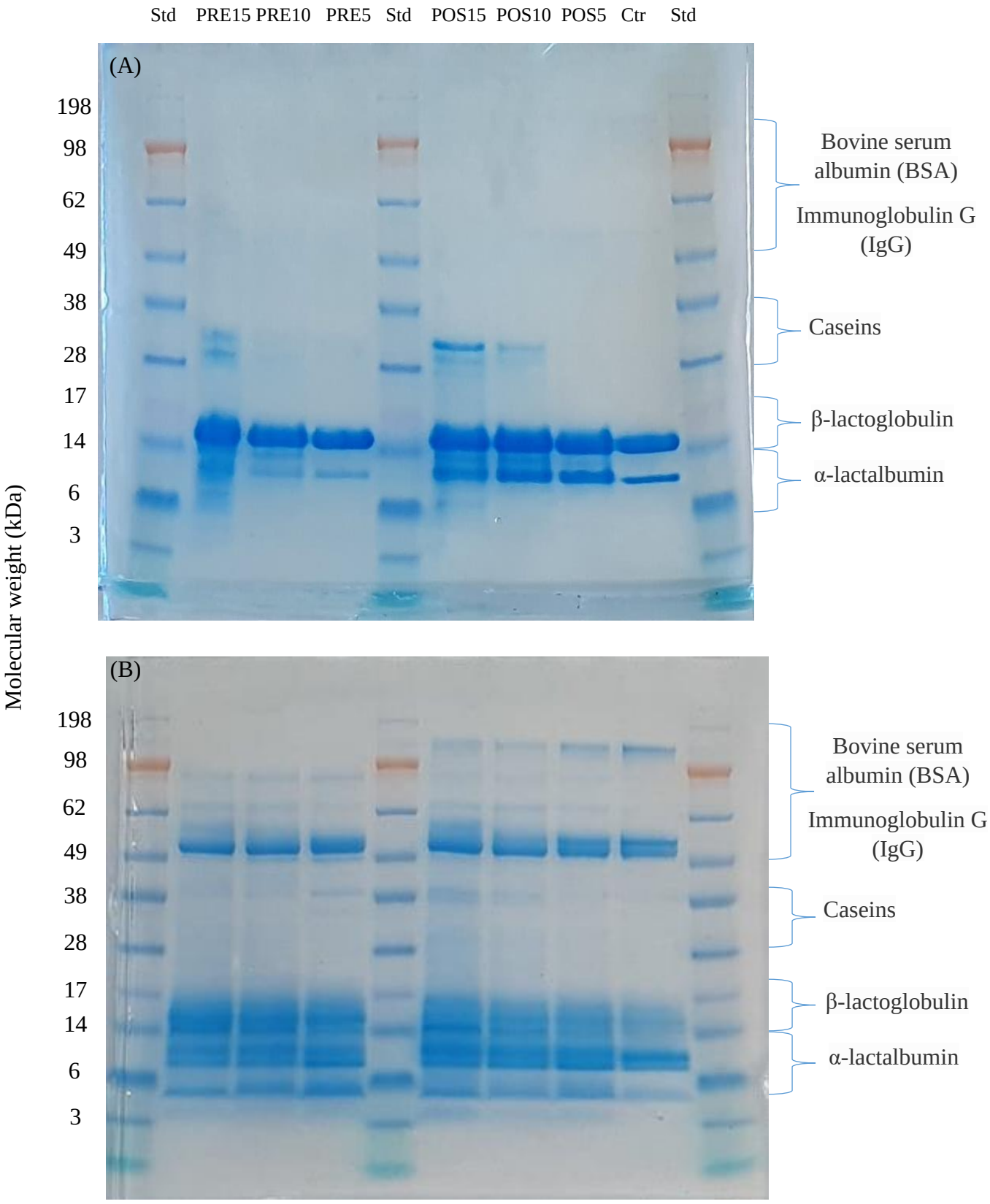
The proteins fractions in the yoghurt after the oral phase of digestion (Figure 5.2B) differed from the undigested yoghurts. Bands in the low molecular mass region (α-lactalbumin and β-lactoglobulin) were observed after the oral phase, corresponding to peptides produced by hydrolysis. A higher intensity of caseins was detected in tamarillo yoghurts with higher concentration of fortification (10 and 15%) compared to the 5% tamarillo yoghurts or control sample. In the high molecular mass region (> 98 kDa), the control and tamarillo yoghurts produced from post-fermentation showed higher intensity bands than the pre-fermentation fortified yoghurts, confirming for more extensive proteolysis in PRE samples as shown in undigested samples.

Gastric digestion (Figure 5.2C) resulted in further reduction of higher molecular weight proteins (> 38 kDa) and an increase in lower molecular weight protein and/or peptides than the β-lactoglobulin. Whey protein was hydrolyzed by simulated gastric acid and pepsin during the 120 minutes of gastric digestion. Meanwhile, β-lactoglobulin was not significantly different ($p > 0.05$) among the yoghurts produced from either pre- or post-fermentation process. The band of β-lactoglobulin was thicker for the control yoghurt than fortified samples, and some undefined bands appeared for tamarillo yoghurts but not for the control sample. For all yoghurt samples, only one casein band which was very thick, appeared after the gastric phase in contrast to several thin bands appearing in the oral phase. It was proposed that this band could represent gastric curd associated with α-casein with a molecular weight of 38 kDa (Wang, Ye, Lin, Han, & Singh, 2018b). Others have shown that pasteurization of the milk used in yoghurt slows the rate of hydrolysis of protein in the gastric phase (Rinaldi, Gauthier, Britten, & Turgeon, 2014) but we could not find any literature reporting the effect of thickening of yoghurt with fruit powder on the nature of the curd and protein digestion in the stomach. Faint bands still appeared in high molecular weight region (BSA and IgG with MW of 66.5 and 150 kDa, respectively)) for yoghurt fortified with high concentration of tamarillo powder (10 and 15%). This suggests different kinetics of protein release among yoghurt matrices thickened with higher concentrations of tamarillo powder and low concentration or without tamarillo

powder. In the previous study, the addition of tamarillo to yoghurt reduces syneresis and increases stiffness of the yoghurt matrix (Diep et al., 2022).

Following the 180 minutes of intestinal digestion, the patterns were similar for all yoghurt samples (Figure 5.2D). No bands were observed at the bottom of the gel, suggesting that proteins and large peptides (> 7 kDa) were further hydrolyzed into small peptides (< 7 kDa) and amino acids and therefore not detected. Lorieau et al. (2018) stated that the proteolysis to amino acids and peptides only begins with the addition of the pancreatin enzymes at the beginning of the intestinal phase. And this observation could be related to the appearance of several bands of smaller proteins below the α-lactalbumin band and peptides around 3 kDa. At the end of the intestinal phase, soluble caseins were totally degraded in all yoghurt samples, which was in line other studies (Mandalari et al., 2009; Rinaldi et al., 2014).

The current results are in agreement with Sousa, Portmann, Dubois, Recio, and Egger (2020). These authors observed that in whey protein isolate, serum albumin (64 kDa) hydrolysis occurred at the beginning of the gastric phase and was no longer present at the end of this phase. Meanwhile, β-lactoglobulin was resistant to pepsin in the gastric phase and immediately hydrolyzed at the beginning of the intestinal phase. The rate of protein digestion has been attributed to three factors: (i) hydrolysis by hydrochloric acid and pepsin; (ii) dilution of the digesta by the incoming gastric fluid during digestion; (iii) the size and denaturation of protein particles (Ye, Cui, Carpenter, Prosser, & Singh, 2019). The reduction in concentration of β-lactoglobulin was probably due to dilution by the gastric fluid as the reduction of β-lactoglobulin was close to or slower than the dilution of protein. However, the reduction in α-lactalbumin may have resulted from both dilution and pepsin hydrolysis, especially at long digestion times (beyond 160 min), when the pH was lower than pH 4.


Std
PRE15
PRE10
PRE5
Std
POS15
POS10
POS5
Ctr
Std
(A)
198
98
62
49
38
28
17
14
6
3
Molecular weight (kDa)
Bovine serum albumin (BSA)
Immunoglobulin G (IgG)
Caseins
β-lactoglobulin
α-lactalbumin
(B)
198
98
62
49
38
28
17
14
6
3
Bovine serum albumin (BSA)
Immunoglobulin G (IgG)
Caseins
β-lactoglobulin
α-lactalbumin

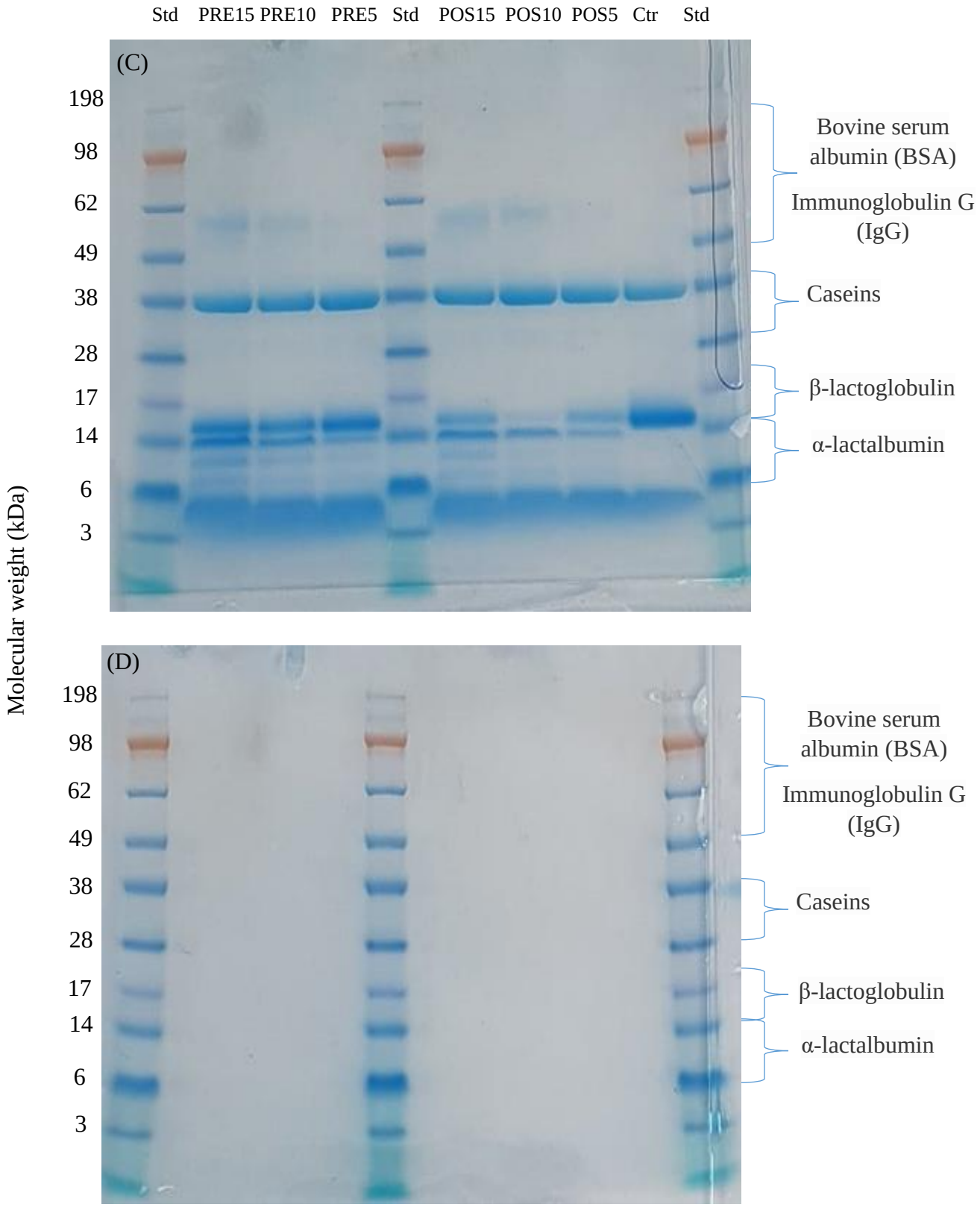


Figure 5.2 Representative SDS-PAGE images of proteins profile of control and tamarillo yoghurts during the gastrointestinal digestion simulation.

(A): undigested sample, (B) oral phase, (C): gastric phase, (D): intestinal phase. Numbers on y axis represent molecular weight in kDaltons (kDa). Lanes from left to right: standard, PRE15, PRE10, PRE5, standard, POS15, POS10, POS5, control and

standard, respectively. POS5, POS10 and POS15: 5, 10, 15% of tamarillo powder was added post fermentation, respectively. PRE5, PRE10 and PRE15: 5, 10, 15% of tamarillo powder was added to milk and starter culture prior to fermentation, respectively. Ctr: Control, Std: Standard.

5.3.2 Effect of *in vitro* digestion on FAAs profiles of tamarillo yoghurts

Milk protein is a complete protein containing all essential amino acids that are required for human growth and development. Consumption of extra plant proteins could further help to increase the intake of essential amino acids (Hertzler, Lieblein-Boff, Weiler, & Allgeier, 2020), which was observed from this study with higher essential free amino acid (EFAA) in tamarillo fortified yoghurt than the control (Table 5.2). Tamarillo powder which contains 80% or more protein by weight, make it possible to consume 10–20 g or more of plant-based protein in one serving of a ready-to-drink shake or powder mix (Hertzler et al., 2020).

The EFAA profile of PRE and POS yoghurts were dominated by isoleucine and histidine, respectively. The addition of tamarillo powder to the yoghurts pre and post fermentation (but before any digestion) was associated with substantial and dose-related increases in total free essential (up to 72x) and non-essential amino acids (up to 106x) (Table 5.2) in the yoghurt suggesting proteolytic activity of the tamarillo powder during and after fermentation. The total EFAAs of PRE was higher than for POS fermentation techniques. This might be due to the breakdown of protein during the fermentation process that led to increase in EFAA content in PRE samples.

Table 5.2 Concentrations (mg/100 g yoghurt) of total free amino acids in control and tamarillo fortified yoghurts in undigested and after intestinal *in vitro* digestion.

Yoghurt Samples	Undigested			After Intestinal Digestion		
	TEFAAs	TNEFAAs	TFAAs	TEFAAs	TNEFAAs	TFAAs
Control	1.56 ± 0.31^{a}	5.42 ± 0.81^{a}	6.98 ± 1.11^{a}	722 ± 129^{a}	599 ± 123^{a}	1321 ± 251^{a}
POS5	12.3 ± 0.48^{b}	238 ± 18.2^{b}	251 ± 18.7^{b}	778 ± 43.6^{b}	918 ± 50.7^{b}	1695 ± 94.4^{b}
POS10	20.0 ± 0.58^{c}	404 ± 4.37^{c}	424 ± 4.95^{c}	651 ± 91.6^{c}	1046 ± 128^{c}	1697 ± 220^{b}
POS15	35.2 ± 1.76^{d}	576 ± 44.6^{d}	611 ± 46.4^{d}	691 ± 87.7^{ac}	1437 ± 169	2129 ± 256^{c}
PRE5	77.4 ± 2.43^{e}	250 ± 8.01^{e}	327 ± 10.4^{e}	1009 ± 190^{d}	1282 ± 226^{e}	2290 ± 416^{c}
PRE10	105 ± 5.75^{f}	433 ± 15.5^{f}	538 ± 21.2^{f}	886 ± 146^{e}	1550 ± 255^{d}	2436 ± 401^{d}

PRE15	112 ± 9.96^{g}	538 ± 31.9^{d}	650 ± 42.0^{d}	905 ± 128de	1819 ± 233^{f}	2724 ± 361^{e}

* Data are expressed as Mean ± SD (n = 3). Different alphabet superscripts indicate statistical difference ($p < 0.05$) across each column. TEFAAs: total essential free amino acids, TNEFAAs: total non-essential free amino acids, TFAAs: total free amino acids. POS5, POS10 and POS15: 5%, 10%, 15% of tamarillo powder was added post fermentation, respectively. PRE5, PRE10 and PRE15: 5%, 10%, 15% of tamarillo powder was added to milk and starter culture prior to fermentation, respectively.

After the intestinal digestion phase, the effect of the addition of tamarillo powder and the increase in total free amino acids (TFAAs) was clear (Table 5.2). Before digestion, the lowest concentrations of TFAAs were seen in the control and the highest in the 15% tamarillo yoghurt. After intestinal digestion, the TFAAs of control increased 200x and the TFAAs in the 15% POS yoghurt increased 4x. The difference between the control and POS15 samples was possibly due to proteolysis induced by tamarillo protease which has been observed in a previous study (Li et al., 2018c). Our previous study (Diep et al., 2022) has also shown the benefits of fortified yoghurt with tamarillo powder including not only maintaining the distinctive flavor-associated volatiles from the fruit but also increasing the total acidity, fibre, protein and lactic acid contents of yoghurts. We have shown that yoghurt fortified with tamarillo powder could be a dietary source of α-tocopherol and β-carotene as well as a good dietary source of vitamin C (Diep et al., 2022).

There is concern that some fruit yoghurts may exhibit shorter shelf life and unfavorable flavor characteristics arising from the proteolysis with the addition of fruit. On the other hand it is important to enhance the digestibility of protein and take the FAAs present in the ileum into consideration (Wolfe, Rutherfurd, Kim, & Moughan, 2016). In the tamarillo yoghurts compared with control, the increased availability after intestinal digestion of EFAAs including L-glutamic acid, γ-aminobutyric acid (GABA) and L-aspartic acid, is of importance (Table 5.3). In response to the low bioavailability of GABA (produced from L-glutamic acid) in dairy products, researchers have used proteolytic co-culture of yoghurts using starters and the enzyme trypsin (Abd El-Fattah, Sakr, El-Dieb, & Elkashef, 2018).

The concentration of each FAA increased from oral to intestinal *in vitro* digestion, which led to a gradual increase in TFAAs, TEFAAs and TNEFAAs (Table 5.2) for the same route. After intestinal digestion the TFAAs and TNEFAAs in fortified yoghurts

with 15% tamarillo power added were approximately double and 2.4 – 3 times than control sample, respectively. The profiles of EFAA and TFAA of tamarillo yoghurts after intestinal digestion produced from pre and post fermentation process were quite similar (Table 5.3). In the control yoghurt, the TFAAs concentration increased to about 11 and 3 times by the end of intestinal phase compared to the oral and gastric phases, respectively. The TFAAs concentration increased to approximately 2.5 – 4.6 and 2 times by the end of intestinal phase compared to the oral and gastric phases, respectively, for tamarillo yoghurts (Table 5.2). This result is in agreement with the SDS-PAGE result where no protein fragments were detected in intestinal phase indicating potential absorption of amino acids within 5 h. Therefore, it can be assumed that peptides and proteins in tamarillo yoghurts have been completely digested and free amino acids could be absorbed in this time gastrointestinal tract. It is in coherent with the high digestibility of milk proteins, which are the most degradable proteins among food proteins with an ileal digestibility of 95% and 97% for caseins and whey proteins, respectively (Barbé et al., 2014).

The current finding indicated that the main role of gastric pepsin was to break down large proteins into smaller fragments which would be ready for the more complete digestion by pancreatic enzymes and the concomitant release of FAAs. Pancreatic enzymes contribute about 2 – 4 times more than pepsin to proteolysis during human gastrointestinal digestion (Zhang, Yoo, Realini, Staincliffe, & Farouk, 2021) similar to the magnitude observed in the current study. Several FAAs (L-alanine, L-glycine, L-isoleucine, L-leucine, and L-valine) could be produced as a result of further digestion by pancreatic endo- and exopeptidases (Zhang et al., 2021) which may explain the significant increase of these FAAs in yoghurts after pancreatic digestion.

For the oral phase, concentration of FAAs was significantly different among different yoghurt samples ($p < 0.05$). The control yoghurt showed the highest release rate of TFAAs, TEFAAs and TNEFAAs with 16, 51 and 6.6 times compared to undigested samples. The TEFAAs increased 3.1 – 7.4 and 1.3 – 2.7 times for fortified yoghurt produced from post- and pre-fermentation process, respectively. The TFAAs and TNEFAAs of both POS and PRE samples just increased 1.2 – 2.3 times compared to undigested samples. At the oral phase, L-glutamic acid was the highest FAA in all fortified yoghurts whereas, L-Lysine was the highest FAA in control sample after oral phase. There was a dose-dependent relationship between the amount of EFAAs and

concentration of tamarillo powder added. Higher amount of most FAAs as well as TFAAs, TEFAAs and TNEFAAs (approximately 1.4 – 2.8 times) were observed in yoghurts after the gastric phase compared to the oral phase. This was due to the shorter reaction time of oral phase (5 mins) while the gastric phase took 2 hours. After the intestinal phase, the key amino acids, L-glutamic acid, L-aspartic acid and GABA, in all fortified yoghurts was higher than control (Table 5.4). L-isoleucine was observed as the highest concentration in control sample. Since GABA is a non-protein amino acid, i.e. not a substrate for the digestive enzymes used in the study, relatively similar concentrations in fortified yoghurts after digestion were observed (Table 5.4).

Bioavailability and concentrations of amino acids in the intestine has a potential effect on protein metabolism at the splanchnic and peripheral levels. For example, leucine is known to stimulate the muscle protein synthesis (Barbé et al., 2014) and the concentration of this AA increased 6 – 8.5 and 28 – 95 times after intestinal digestion compared to undigested samples for PRE and POS, respectively (Table 5.3). Therefore, ingestion of tamarillo yoghurts may help to induce muscle protein synthesis. The present study thus contributes to the importance of food matrix design for the control of nutrients and phytochemicals delivery.

Quantification of FAAs has been affected by chemical reactions, oxidation or hydrolysis. For example, due to oxidation, methionine sulfone and cysteic acid have been quantified rather than methionine and cysteine, respectively. Accurate measurement of oxidation rate was difficult. Partial degradation (5%–10%) during hydrolysis have been reported for serine and threonine (Wolfe et al., 2016). As observed by (Wolfe et al., 2016), a decrease in uptake of phenylalanine and tryptophan was caused by increase in branched-chain amino acids, due to competition for the same transporter. As amino acids undergo structural changes during digestion, their availability for protein synthesis may also be affected. For example, methionine could be oxidized, and the oxidized derivatives may be either poorly utilized or not nutritionally available. The determination of both the amount and the profile of EFAAs absorbed from a protein was related to determination of the individual values of digestibility in the current study, following Wolfe et al (Wolfe et al., 2016).

Table 5.3 Concentrations (mg/100 g yoghurt) of free amino acids in control and tamarillo fortified yoghurts before *in vitro* digestion.

Free amino acids/phases	Undigested						
	Control	POS5	POS10	POS1	PRE5	PRE10	PRE15
L-Histidine	0.21 ± 0.11^{a}	5.90 ± 0.14^{b}	10.75 ± 0.37^{c}	18.1 ± 1.18^{a}	5.36 ± 0.15^{b}	10.75 ± 0.63^{c}	13.9 ± 1.82^{e}
L-Threonine	0.13 ± 0.03^{a}	0.67 ± 0.06^{b}	0.89 ± 0.01^{c}	1.90 ± 0.03^{d}	3.53 ± 0.14^{e}	5.21 ± 0.16^{f}	5.50 ± 0.35^{f}
L-Lysine	0.28 ± 0.01^{a}	2.50 ± 0.10^{b}	4.18 ± 0.02^{c}	7.26 ± 0.24^{d}	6.92 ± 0.23^{d}	10.6 ± 0.71^{e}	10.9 ± 0.90^{e}
L-Valine	0.10 ± 0.02^{a}	0.57 ± 0.04^{b}	0.85 ± 0.04^{c}	1.36 ± 0.04^{d}	10.2 ± 0.34^{e}	12.3 ± 0.55^{f}	11.3 ± 0.82^{ef}
L-Methionine	0.09 ± 0.00^{a}	0.14 ± 0.00^{b}	0.20 ± 0.01^{c}	0.33 ± 0.05^{d}	1.42 ± 0.12^{e}	2.00 ± 0.28^{f}	2.56 ± 0.52^{f}
L-Leucine	0.06 ± 0.01^{a}	0.48 ± 0.03^{b}	0.62 ± 0.03^{c}	1.47 ± 0.05^{d}	7.31 ± 0.21^{e}	8.99 ± 0.69^{f}	9.50 ± 0.41^{g}
L-isoleucine	0.18 ± 0.02^{a}	0.86 ± 0.04^{b}	1.13 ± 0.05^{c}	2.25 ± 0.05^{d}	24.3 ± 0.74^{e}	30.7 ± 2.37^{f}	33.0 ± 1.25^{f}
L-Phenylalanine	0.36 ± 0.07^{a}	0.73 ± 0.04^{b}	0.83 ± 0.03^{c}	1.42 ± 0.07^{d}	13.3 ± 0.35^{e}	18.0 ± 2.13^{f}	18.4 ± 0.92^{f}
L-Tryptophan	0.15 ± 0.03^{a}	0.39 ± 0.03^{b}	0.51 ± 0.02^{c}	1.15 ± 0.06^{d}	5.00 ± 0.14^{e}	6.85 ± 0.80^{f}	7.15 ± 0.40^{g}
TEFAAs	$\boldsymbol{1.56 \pm 0.31^{a}}$	$\boldsymbol{12.3 \pm 0.48^{b}}$	$\boldsymbol{20.0 \pm 0.58^{c}}$	$\boldsymbol{35.2 \pm 1.76^{d}}$	$\boldsymbol{77.4 \pm 2.43^{e}}$	$\boldsymbol{105 \pm 5.75^{f}}$	$\boldsymbol{112 \pm 9.96^{g}}$
Hydroxy-L-Proline	0.02 ± 0.00^{a}	0.14 ± 0.01^{b}	0.22 ± 0.00^{c}	0.21 ± 0.01^{c}	0.15 ± 0.01^{b}	0.25 ± 0.01^{d}	0.33 ± 0.05^{e}
L-Carnosine	0.06 ± 0.03^{a}	0.06 ± 0.02^{a}	0.07 ± 0.02^{a}	0.29 ± 0.14^{b}	0.11 ± 0.02^{c}	0.11 ± 0.01^{c}	0.20 ± 0.11^{b}
L-Arginine	0.41 ± 0.05^{a}	1.74 ± 0.02^{b}	2.71 ± 0.12^{c}	4.09 ± 0.26^{d}	6.09 ± 0.10^{e}	7.23 ± 0.53^{f}	7.48 ± 0.67^{f}
Ethanolamine	0.41 ± 0.05^{a}	1.64 ± 0.10^{b}	1.74 ± 0.06^{b}	3.31 ± 0.08^{c}	2.29 ± 0.08^{d}	2.78 ± 0.09^{e}	2.81 ± 0.20^{e}
L-Serine	0.10 ± 0.07^{a}	1.50 ± 0.10^{b}	2.98 ± 0.07^{c}	4.13 ± 0.11^{d}	3.88 ± 0.11^{e}	7.17 ± 0.27^{f}	7.49 ± 0.44^{f}
Glycine	0.04 ± 0.04^{a}	0.14 ± 0.02^{b}	0.18 ± 0.00^{c}	0.55 ± 0.05^{d}	1.80 ± 0.05^{e}	3.16 ± 0.11^{f}	3.26 ± 0.34^{f}
Sarcosine	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{b}	0.01 ± 0.00^{a}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{b}
L-Aspartic acid	0.16 ± 0.01^{a}	20.1 ± 1.51^{b}	37.3 ± 0.41^{c}	50.0 ± 2.37^{d}	19.5 ± 0.43^{b}	37.9 ± 1.49^{c}	47.97 ± 1.92^{e}
Taurine	0.36 ± 0.06^{a}	0.28 ± 0.02^{b}	0.26 ± 0.00^{c}	0.26 ± 0.02^{bc}	0.24 ± 0.01^{e}	0.22 ± 0.01^{ef}	0.21 ± 0.04^{f}

β-Alanine	< 0.005^{a}	0.32 ± 0.03^{b}	0.58 ± 0.00^{c}	0.61 ± 0.03^{c}	0.30 ± 0.02^{b}	0.57 ± 0.03^{c}	0.79 ± 0.04^{d}
L-Glutamic acid	0.81 ± 0.04^{a}	171 ± 13.5^{b}	285 ± 2.65^{c}	409 ± 37.6^{d}	160 ± 5.49^{e}	280 ± 7.88^{c}	358 ± 20.6^{f}
L-Citrulline	0.13 ± 0.05^{a}	0.17 ± 0.01^{b}	0.22 ± 0.01^{c}	0.28 ± 0.03^{d}	0.19 ± 0.01^{b}	0.23 ± 0.01^{c}	0.26 ± 0.01^{d}
L-Alanine	0.48 ± 0.04^{a}	3.22 ± 0.20^{b}	5.23 ± 0.08^{c}	10.7 ± 0.08^{d}	5.83 ± 0.15^{e}	9.48 ± 0.41^{f}	10.7 ± 0.48^{d}
γ-Aminobutyric acid	0.03 ± 0.00^{a}	29.2 ± 2.05^{b}	54.8 ± 0.39^{c}	71.5 ± 2.82^{d}	26.2 ± 0.80^{b}	48.6 ± 2.52^{e}	61.5 ± 2.96^{f}
L-Proline	1.64 ± 0.25^{a}	7.10 ± 0.41^{b}	10.1 ± 0.37^{c}	17.5 ± 0.67^{d}	11.0 ± 0.38^{e}	17.4 ± 0.74^{d}	19.5 ± 2.01^{f}
β-Amino-isobutyric acid	0.01 ± 0.00^{a}	0.03 ± 0.01^{b}	0.04 ± 0.00^{c}	0.04 ± 0.01^{c}	0.03 ± 0.00^{b}	0.04 ± 0.00^{c}	0.05 ± 0.00^{d}
α-Aminobutyric acid	0.01 ± 0.00^{a}	0.02 ± 0.01^{a}	0.06 ± 0.01^{b}	0.07 ± 0.00^{b}	0.04 ± 0.00^{c}	0.05 ± 0.01^{c}	0.07 ± 0.00^{b}
δ-Hydroxylysine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
L-Ornithine	0.04 ± 0.00^{a}	0.26 ± 0.02^{b}	0.89 ± 0.02^{c}	0.94 ± 0.02^{d}	0.25 ± 0.01^{b}	0.48 ± 0.04^{e}	0.62 ± 0.03^{f}
Cystathionine	0.02 ± 0.00^{a}	0.10 ± 0.02^{b}	0.10 ± 0.06^{b}	0.10 ± 0.02^{b}	0.04 ± 0.03^{c}	0.04 ± 0.00^{c}	0.06 ± 0.00^{d}
L-Cystine	n.d.	n.d.	< 0.005^{a}	< 0.005^{a}	n.d.	< 0.005^{a}	0.01 ± 0.00^{b}
L-Anserine	0.05 ± 0.00^{a}	0.07 ± 0.00^{b}	0.11 ± 0.02^{c}	0.12 ± 0.03^{c}	0.13 ± 0.02^{d}	0.25 ± 0.11^{e}	0.30 ± 0.16^{f}
L-Tyrosine	0.62 ± 0.12^{a}	1.18 ± 0.09^{b}	1.89 ± 0.08^{c}	2.73 ± 0.12^{d}	11.6 ± 0.29^{e}	16.9 ± 0.92^{f}	16.3 ± 2.02^{f}
L-Homocystine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
TNEFAAs	***5.42 ± 0.81^{a}***	***238 ± 18.2^{b}***	***404 ± 4.37^{c}***	***576 ± 44.6^{d}***	***250 ± 8.01^{e}***	***433 ± 15.5^{f}***	***538 ± 31.9^{d}***
TFAAs	***6.98 ± 1.11^{a}***	***251 ± 18.7^{b}***	***424 ± 4.95^{c}***	***611 ± 46.4^{d}***	***327 ± 10.4^{e}***	***538 ± 21.2^{f}***	***650 ± 42^{d}***

* n.d.: not detected. Data are expressed as Mean ± SD (n = 3). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row. TEFAAs: total essential free amino acids, TNEFAAs: total non-essential free amino acids, TFAAs: total free amino acids. POS5, POS10 and POS15: 5, 10, 15% of tamarillo powder was added post fermentation, respectively. PRE5, PRE10 and PRE15: 5, 10, 15% of tamarillo powder was added to milk and starter culture prior to fermentation, respectively

Table 5.4 Concentrations (mg/100 g yoghurt) of free amino acids in control and tamarillo fortified yoghurts after each step of *in vitro* digestion.

Free amino acids/phases	Oral phase						
	Control	POS5	POS10	POS1	PRE5	PRE10	PRE15
L-Histidine	4.41 ± 1.62^{a}	7.72 ± 2.39^{b}	12.7 ± 1.11^{c}	15.7 ± 2.14^{d}	18.0 ± 3.54^{e}	17.3 ± 4.95^{e}	15.6 ± 2.48^{d}
L-Threonine	6.58 ± 0.44^{a}	4.21 ± 1.77^{b}	4.06 ± 0.93^{b}	4.96 ± 1.90^{b}	10.8 ± 5.18^{c}	7.68 ± 2.49^{ac}	7.10 ± 0.96^{a}
L-Lysine	20.3 ± 2.97^{a}	22.0 ± 8.22^{b}	27.6 ± 6.15^{c}	27.8 ± 3.69^{c}	44.6 ± 17.0^{d}	34.6 ± 11.5^{e}	35.7 ± 2.65^{e}
L-Valine	4.52 ± 0.34^{a}	6.47 ± 1.98^{b}	8.05 ± 1.53^{c}	8.05 ± 1.65^{c}	18.8 ± 5.37^{d}	16.3 ± 5.05^{e}	16.0 ± 1.95^{e}
L-Methionine	4.47 ± 1.45^{a}	4.18 ± 2.92^{a}	4.47 ± 1.52^{a}	3.85 ± 0.69^{b}	10.1 ± 5.97^{c}	5.72 ± 2.13^{d}	6.18 ± 1.40^{d}
L-Leucine	4.14 ± 0.31^{a}	5.76 ± 1.62^{b}	7.88 ± 1.55^{c}	8.29 ± 1.78^{d}	13.4 ± 3.92^{e}	13.2 ± 3.82^{e}	13.6 ± 1.83^{e}
L-isoleucine	19.4 ± 2.12^{a}	18.0 ± 5.96^{b}	20.2 ± 4.78^{ab}	19.5 ± 3.6^{a}	45.6 ± 18.1^{c}	38.4 ± 12.7^{d}	37.5 ± 4.60^{d}
L-Phenylalanine	13.7 ± 0.69^{a}	18.8 ± 6.09^{cb}	20.6 ± 5.89^{b}	17.2 ± 3.42^{cb}	40.4 ± 19.9^{d}	29.1 ± 9.60^{e}	26.4 ± 4.26^{e}
L-Tryptophan	2.99 ± 0.32^{a}	3.79 ± 1.34^{b}	4.13 ± 1.20^{c}	3.56 ± 0.42^{b}	10.44 ± 5.41^{d}	7.92 ± 2.44^{de}	6.70 ± 1.12^{e}
TEFAAs	***80.6 ± 10.3^{a}***	***90.9 ± 32.3^{b}***	***110 ± 24.7^{c}***	***109 ± 19.3^{c}***	***212 ± 84.3^{d}***	***170 ± 54.7^{e}***	***165 ± 21.3^{e}***
Hydroxy-L-Proline	0.07 ± 0.00^{a}	0.36 ± 0.02^{b}	0.54 ± 0.04^{c}	0.60 ± 0.07^{c}	0.25 ± 0.02^{d}	0.41 ± 0.01^{e}	0.56 ± 0.03^{f}
L-Carnosine	n.d	n.d	n.d	n.d	n.d	n.d	n.d
L-Arginine	1.69 ± 1.24^{a}	5.34 ± 1.74^{b}	12.7 ± 3.80^{c}	9.96 ± 3.68^{d}	18.3 ± 7.58^{e}	10.4 ± 4.78^{d}	12.9 ± 1.30^{c}
Ethanolamine	1.95 ± 0.21^{a}	3.02 ± 0.67^{b}	3.71 ± 0.35^{b}	4.77 ± 0.31^{c}	4.67 ± 1.48^{c}	4.42 ± 1.11^{e}	4.70 ± 0.48^{c}
L-Serine	5.63 ± 0.63^{a}	6.19 ± 2.09^{b}	6.49 ± 1.82^{b}	7.59 ± 1.65^{c}	15.3 ± 5.76^{d}	14.8 ± 4.33^{d}	10.5 ± 1.28^{e}
Glycine	1.28 ± 0.14^{a}	1.89 ± 0.36^{b}	1.98 ± 0.46^{b}	2.32 ± 0.28^{c}	5.78 ± 2.82^{d}	7.08 ± 3.69^{e}	4.59 ± 0.62^{d}
Sarcosine	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.01^{a}
L-Aspartic acid	1.21 ± 0.21^{a}	20.5 ± 3.95^{b}	40.7 ± 3.42^{c}	49.5 ± 3.51^{d}	34.5 ± 13.8^{e}	43.4 ± 13.4^{c}	52.8 ± 5.02^{d}
Taurine	1.76 ± 0.11^{a}	1.39 ± 0.01^{b}	1.11 ± 0.09^{c}	1.20 ± 0.13^{c}	1.57 ± 0.10^{d}	1.48 ± 0.08^{d}	1.31 ± 0.06^{b}
β-Alanine	0.10 ± 0.01^{a}	1.18 ± 0.03^{b}	1.94 ± 0.08^{c}	1.35 ± 0.17^{d}	0.54 ± 0.02^{e}	0.95 ± 0.09^{b}	1.25 ± 0.07^{bd}

L-Glutamic acid	4.77 ± 0.27^{a}	180 ± 39.0^{b}	370 ± 19.8^{c}	557 ± 45.2^{d}	376 ± 152^{c}	462 ± 139^{e}	572 ± 62.1^{d}
L-Citrulline	0.26 ± 0.02^{a}	0.32 ± 0.08^{b}	0.39 ± 0.06^{b}	0.54 ± 0.16^{c}	0.33 ± 0.02^{b}	0.49 ± 0.05^{bc}	0.42 ± 0.16^{bc}
L-Alanine	5.18 ± 0.60^{a}	5.87 ± 1.47^{b}	8.91 ± 1.88^{c}	11.8 ± 1.68^{d}	13.0 ± 6.13^{de}	14.1 ± 3.93^{e}	14.8 ± 1.31^{e}
γ-Aminobutyric acid	0.16 ± 0.02^{a}	31.0 ± 6.41^{b}	59.1 ± 6.92^{c}	62.3 ± 2.20^{d}	38.2 ± 13.7^{b}	49.8 ± 14.2^{c}	60.5 ± 5.75^{d}
L-Proline	3.23 ± 0.14^{a}	9.50 ± 2.39^{b}	16.7 ± 3.10^{c}	20.0 ± 2.67^{d}	16.6 ± 5.30^{c}	19.6 ± 4.16^{d}	22.6 ± 3.14^{d}
β-Amino-isobutryic acid	0.02 ± 0.00^{a}	0.06 ± 0.01^{b}	0.08 ± 0.03^{c}	0.08 ± 0.01^{c}	0.04 ± 0.02^{d}	0.06 ± 0.03^{b}	0.05 ± 0.01^{b}
α-Aminobutyric acid	0.12 ± 0.00^{a}	0.17 ± 0.02^{b}	0.24 ± 0.01^{c}	0.29 ± 0.02^{d}	0.24 ± 0.03^{c}	0.25 ± 0.03^{cd}	0.28 ± 0.01^{d}
δ-Hydroxylysine	0.02 ± 0.00^{a}	0.42 ± 0.08^{b}	1.04 ± 0.16^{c}	1.22 ± 0.11^{d}	0.18 ± 0.01^{e}	0.81 ± 0.08^{f}	0.80 ± 0.02^{f}
L-Ornithine	0.34 ± 0.01^{a}	1.69 ± 0.10^{b}	2.68 ± 0.12^{c}	2.08 ± 0.18^{d}	0.97 ± 0.04^{e}	1.53 ± 0.16^{b}	1.96 ± 0.08^{d}
Cystathionine	0.17 ± 0.01^{a}	0.10 ± 0.01^{b}	0.08 ± 0.02^{c}	0.13 ± 0.03^{b}	0.06 ± 0.01^{d}	0.08 ± 0.01^{c}	0.06 ± 0.02^{d}
L-Cystine	0.19 ± 0.04^{a}	0.14 ± 0.10^{b}	0.14 ± 0.07^{b}	0.28 ± 0.02^{c}	0.22 ± 0.02^{d}	0.22 ± 0.03^{d}	0.22 ± 0.04^{d}
L-Anserine	0.22 ± 0.10^{a}	0.14 ± 0.10^{b}	0.10 ± 0.11^{b}	0.22 ± 0.08^{a}	0.13 ± 0.04^{b}	0.22 ± 0.09^{a}	0.16 ± 0.09^{b}
L-Tyrosine	7.43 ± 0.08^{a}	8.87 ± 2.97^{a}	11.1 ± 3.68^{b}	9.82 ± 2.39^{ab}	22.8 ± 11.7^{c}	19.2 ± 5.72^{cd}	18.1 ± 3.06^{d}
L-Homocystine	0.09 ± 0.06^{a}	0.24 ± 0.06^{b}	0.35 ± 0.11^{c}	0.36 ± 0.10^{c}	0.35 ± 0.05^{c}	0.39 ± 0.10^{d}	0.30 ± 0.06^{bc}
TNEFAAs	***35.9 ± 3.9^{a}***	***278 ± 61.7^{b}***	***543 ± 41.4^{c}***	***741 ± 69.3^{d}***	***549 ± 221^{c}***	***652 ± 195^{e}***	***781 ± 84.7^{d}***
TFAAs	***117 ± 14.2^{a}***	***369 ± 94.0^{b}***	***653 ± 66.1^{c}***	***849 ± 88.6^{d}***	***762 ± 305^{e}***	***822 ± 250^{d}***	***945 ± 106^{f}***

* n.d.: not detected. Data are expressed as Mean ± SD (n = 3). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row. TEFAAs: total essential free amino acids, TNEFAAs: total non-essential free amino acids, TFAAs: total free amino acids. POS5, POS10 and POS15: 5, 10, 15% of tamarillo powder was added post fermentation, respectively. PRE5, PRE10 and PRE15: 5, 10, 15% of tamarillo powder was added to milk and starter culture prior to fermentation, respectively

Table 5.4 Concentrations (mg/100 g yoghurt) of free amino acids in control and tamarillo fortified yoghurts after each step of *in vitro* digestion (Cont.).

Free amino acids/phases	Gastric phase						
	Control	POS5	POS10	POS1	PRE5	PRE10	PRE15
L-Histidine	4.35 ± 1.53^{a}	11.8 ± 5.17^{b}	11.3 ± 3.35^{b}	17.5 ± 3.22^{c}	11.6 ± 3.07^{b}	13.7 ± 0.47^{d}	16.9 ± 4.82^{c}
L-Threonine	13.1 ± 1.01^{a}	17.2 ± 2.97^{b}	13.4 ± 3.46^{a}	14.1 ± 1.96^{a}	19.6 ± 1.35^{c}	18.4 ± 1.82^{bc}	19.7 ± 3.16^{c}
L-Lysine	16.1 ± 1.60^{a}	21.6 ± 5.29^{b}	18.3 ± 3.54^{ab}	22.0 ± 3.12^{b}	27.5 ± 2.40^{c}	29.2 ± 1.20^{c}	35.1 ± 4.43^{d}
L-Valine	16.5 ± 1.13^{a}	22.3 ± 4.26^{b}	17.5 ± 5.34^{a}	17.7 ± 2.12^{a}	28.4 ± 1.98^{c}	27.6 ± 1.97^{c}	30.5 ± 5.01^{d}
L-Methionine	8.56 ± 3.45^{a}	11.9 ± 2.53^{b}	9.53 ± 5.93^{ab}	8.27 ± 0.34^{a}	11.0 ± 2.47^{b}	12.9 ± 2.81^{c}	11.2 ± 5.22^{b}
L-Leucine	11.2 ± 0.77^{a}	15.0 ± 2.78^{b}	12.2 ± 2.55^{ab}	12.9 ± 1.29^{ab}	18.5 ± 0.70^{c}	19.6 ± 1.74^{c}	22.1 ± 2.51^{d}
L-isoleucine	58.2 ± 2.92^{a}	72.6 ± 11.7^{b}	56.3 ± 15.8^{a}	54.6 ± 4.69^{a}	84.0 ± 6.68^{cd}	80.5 ± 5.75^{c}	88.7 ± 16.7^{d}
L-Phenylalanine	51.4 ± 5.86^{a}	66.6 ± 13.6^{b}	51.2 ± 11.0^{a}	48.7 ± 4.18^{c}	69.0 ± 3.45^{c}	62.5 ± 5.53^{b}	63.5 ± 13.1^{b}
L-Tryptophan	15.3 ± 1.79^{a}	18.8 ± 3.83^{b}	13.4 ± 4.93^{c}	12.3 ± 1.09^{c}	19.4 ± 0.57^{b}	16.8 ± 1.27^{a}	16.4 ± 4.07^{a}
TEFAAs	***195 ± 20.1**^{a}*	***258 ± 52.1**^{b}*	***203 ± 55.9**^{c}*	***208 ± 22.0**^{c}*	***289 ± 22.7**^{d}*	***281 ± 22.6**^{d}*	***304 ± 59.0**^{e}*
Hydroxy-L-Proline	0.33 ± 0.03^{a}	0.59 ± 0.05^{b}	1.03 ± 0.21^{c}	0.89 ± 0.11^{d}	0.51 ± 0.08^{b}	0.85 ± 0.10^{d}	0.98 ± 0.24^{cd}
L-Carnosine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
L-Arginine	12.6 ± 7.75^{a}	3.91 ± 1.37^{b}	2.55 ± 1.63^{c}	6.19 ± 1.35^{d}	6.07 ± 2.72^{d}	8.91 ± 1.24^{e}	13.9 ± 7.89^{a}
Ethanolamine	2.90 ± 0.06^{a}	4.34 ± 0.84^{b}	4.29 ± 0.55^{b}	6.07 ± 0.57^{c}	4.90 ± 0.34^{b}	5.63 ± 0.60^{bc}	6.55 ± 0.65^{d}
L-Serine	14.7 ± 2.69^{a}	20.7 ± 4.48^{b}	16.8 ± 4.41^{c}	16.7 ± 3.25^{c}	21.3 ± 1.81^{b}	22.7 ± 1.50^{d}	23.9 ± 2.58^{d}
Glycine	13.3 ± 0.88^{a}	15.8 ± 2.36^{b}	12.9 ± 3.35^{a}	12.6 ± 1.22^{a}	17.2 ± 0.28^{c}	17.1 ± 1.09^{c}	17.2 ± 1.77^{c}
Sarcosine	0.01 ± 0.00^{a}	0.04 ± 0.01^{b}	0.14 ± 0.02^{c}	0.10 ± 0.08^{c}	0.12 ± 0.00^{c}	0.04 ± 0.06^{b}	0.01 ± 0.00^{a}
L-Aspartic acid	25.7 ± 2.38^{a}	58.7 ± 10.1^{b}	61.9 ± 14.3^{b}	73.7 ± 8.51^{c}	56.9 ± 4.00^{b}	70.1 ± 4.95^{c}	91.4 ± 13.8^{d}
Taurine	10.0 ± 0.76^{a}	8.26 ± 0.62^{b}	9.04 ± 1.10^{c}	9.16 ± 0.52^{ac}	9.37 ± 0.57^{ac}	9.75 ± 1.03^{a}	9.49 ± 1.21^{ac}

β-Alanine	0.29 ± 0.03^{a}	1.22 ± 0.03^{b}	2.38 ± 0.15^{c}	1.64 ± 0.04^{b}	0.74 ± 0.07^{d}	1.27 ± 0.10^{b}	1.67 ± 0.17^{b}
L-Glutamic acid	45.7 ± 2.11^{a}	300 ± 41.4^{b}	410 ± 128^{c}	650 ± 64.1^{d}	372 ± 33.6^{c}	552 ± 41.0^{e}	771 ± 98.9^{f}
L-Citrulline	0.79 ± 0.11^{a}	0.68 ± 0.09^{b}	0.89 ± 0.16^{c}	0.98 ± 0.03^{d}	0.88 ± 0.05^{c}	1.05 ± 0.08^{d}	0.70 ± 0.55^{b}
L-Alanine	20.1 ± 2.09^{a}	27.8 ± 4.34^{b}	23.4 ± 6.70^{c}	26.7 ± 3.23^{bc}	30.0 ± 1.07^{b}	30.5 ± 1.65^{b}	35.6 ± 4.62^{d}
γ-Aminobutyric acid	0.56 ± 0.13^{a}	42.3 ± 4.78^{b}	60.4 ± 11.5^{c}	62.5 ± 9.30^{c}	31.1 ± 3.01^{d}	52.7 ± 1.81^{e}	74.5 ± 11.3^{f}
L-Proline	6.82 ± 0.18^{a}	13.2 ± 1.99^{b}	14.4 ± 4.77^{b}	19.1 ± 1.45^{c}	14.9 ± 1.35^{b}	20.9 ± 1.61^{c}	26.2 ± 3.84^{d}
β-Amino-isobutyric acid	0.05 ± 0.01^{a}	0.08 ± 0.02^{b}	0.10 ± 0.02^{c}	0.08 ± 0.02^{b}	0.11 ± 0.02^{c}	0.16 ± 0.02^{d}	0.11 ± 0.03^{c}
α-Aminobutyric acid	0.13 ± 0.02^{a}	0.16 ± 0.01^{b}	0.27 ± 0.02^{c}	0.19 ± 0.05^{b}	0.21 ± 0.01^{b}	0.29 ± 0.01^{c}	0.19 ± 0.15^{b}
δ-Hydroxylysine	0.03 ± 0.01^{a}	0.05 ± 0.02^{b}	0.07 ± 0.02^{c}	0.08 ± 0.04^{c}	0.05 ± 0.01^{b}	0.06 ± 0.00^{b}	0.08 ± 0.00^{c}
L-Ornithine	3.51 ± 0.26^{a}	4.51 ± 0.18^{b}	6.25 ± 0.25^{c}	5.64 ± 0.26^{d}	4.60 ± 0.09^{b}	4.88 ± 0.35^{b}	5.44 ± 0.28^{d}
Cystathionine	0.28 ± 0.06^{a}	0.22 ± 0.04^{b}	0.23 ± 0.02^{b}	0.22 ± 0.07^{b}	0.22 ± 0.05^{b}	0.22 ± 0.04^{b}	0.30 ± 0.02^{a}
L-Cystine	7.71 ± 0.60^{a}	6.67 ± 0.54^{b}	7.69 ± 0.57^{a}	7.64 ± 0.13^{a}	7.39 ± 0.14^{c}	7.20 ± 0.35^{c}	7.53 ± 0.42^{a}
L-Anserine	6.82 ± 0.29^{a}	6.17 ± 0.25^{b}	8.04 ± 0.57^{c}	7.29 ± 0.74^{d}	7.29 ± 0.41^{d}	6.59 ± 0.97^{a}	7.00 ± 0.71^{c}
L-Tyrosine	29.8 ± 2.70^{a}	39.1 ± 8.29^{b}	30.4 ± 9.95^{a}	27.5 ± 2.61^{c}	41.3 ± 1.92^{d}	38.6 ± 3.04^{b}	39.5 ± 8.11^{bd}
L-Homocystine	0.28 ± 0.07^{a}	0.23 ± 0.04^{b}	0.23 ± 0.02^{b}	0.30 ± 0.07^{a}	0.29 ± 0.07^{a}	0.36 ± 0.11^{c}	0.26 ± 0.07^{ab}
TNEFAAs	***202 ± 23.2^{a}***	***555 ± 83.6^{b}***	***673 ± 188^{c}***	***935 ± 97.8^{d}***	***627 ± 51.7^{c}***	***852 ± 61.8^{d}***	***1133 ± 157^{e}***
TFAAs	***397 ± 43.3^{a}***	***813 ± 136^{b}***	***876 ± 244^{b}***	***1143 ± 120^{c}***	***916 ± 74.4^{d}***	***1134 ± 84.3^{c}***	***1437 ± 216^{e}***

* n.d.: not detected. Data are expressed as Mean ± SD (n = 3). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row. TEFAAs: total essential free amino acids, TNEFAAs: total non-essential free amino acids, TFAAs: total free amino acids. POS5, POS10 and POS15: 5, 10, 15% of tamarillo powder was added post fermentation, respectively. PRE5, PRE10 and PRE15: 5, 10, 15% of tamarillo powder was added to milk and starter culture prior to fermentation, respectively

Table 5.4 Concentrations (mg/100 g yoghurt) of free amino acids in control and tamarillo fortified yoghurts after each step of *in vitro* digestion (Cont.).

Free amino acids/phases	Intestinal phase						
	Control	POS5	POS10	POS1	PRE5	PRE10	PRE15
L-Histidine	29.3 ± 7.75^{a}	30.1 ± 6.95^{a}	28.8 ± 10.7^{a}	37.1 ± 12.41^{b}	44.3 ± 12.7^{c}	38.5 ± 6.98^{b}	42.9 ± 6.72^{c}
L-Threonine	32.4 ± 6.11^{ab}	34.8 ± 1.49^{a}	29.6 ± 5.37^{b}	32.0 ± 3.87^{ab}	52.6 ± 13.8^{c}	43.0 ± 6.95^{d}	43.8 ± 6.34^{d}
L-Lysine	177 ± 27.7^{a}	194 ± 10.1^{b}	164 ± 13.2^{a}	179 ± 18.8^{a}	236 ± 30.3^{cd}	229 ± 40.6^{c}	241 ± 40.9^{d}
L-Valine	51.6 ± 11.0^{ab}	55.5 ± 1.44^{a}	46.2 ± 8.32^{b}	48.2 ± 4.53^{b}	80.6 ± 17.8^{c}	69.9 ± 12.0^{d}	70.9 ± 9.61^{d}
L-Methionine	13.7 ± 5.40^{a}	13.0 ± 1.18^{a}	10.4 ± 1.85^{b}	17.4 ± 3.81^{c}	31.2 ± 19.1^{d}	12.2 ± 2.26^{a}	13.7 ± 4.36^{a}
L-Leucine	43.0 ± 8.13^{a}	45.6 ± 2.62^{a}	37.9 ± 6.34^{b}	40.8 ± 3.98^{ab}	61.8 ± 14.2^{c}	54.7 ± 8.86^{d}	57.0 ± 7.22^{cd}
L-isoleucine	191 ± 34.7^{a}	200 ± 12.1^{a}	163 ± 21.2^{b}	169 ± 20.2^{b}	251 ± 36.0^{c}	221 ± 33.4^{ac}	227 ± 31.0^{ac}
L-Phenylalanine	133 ± 19.1^{a}	148 ± 4.58^{b}	126 ± 16.6^{a}	125 ± 13.6^{a}	179 ± 29.3^{c}	162 ± 25.4^{c}	157 ± 16.3^{b}
L-Tryptophan	51.0 ± 8.74^{ab}	55.5 ± 3.22^{a}	45.2 ± 8.14^{b}	43.8 ± 6.51^{b}	73.0 ± 17.3^{c}	55.7 ± 9.70^{a}	53.1 ± 5.44^{a}
TEFAAs	***722 ± 129^{a}***	***778 ± 43.6^{b}***	***651 ± 91.6^{c}***	***691 ± 87.7^{ac}***	***1009 ± 190^{d}***	***886 ± 146^{e}***	***905 ± 128de***
Hydroxy-L-Proline	0.50 ± 0.04^{a}	0.91 ± 0.05^{b}	1.60 ± 0.21^{c}	1.10 ± 0.08^{d}	0.70 ± 0.05^{e}	1.15 ± 0.03^{d}	1.25 ± 0.17^{f}
L-Carnosine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
L-Arginine	109 ± 44.3^{a}	101 ± 27.3^{a}	81.7 ± 20.4^{b}	91.3 ± 25.3^{ab}	168 ± 37.8^{c}	155 ± 29.8^{c}	111 ± 25.1^{a}
Ethanolamine	5.94 ± 0.33^{a}	6.93 ± 0.31^{b}	7.11 ± 0.48^{b}	8.98 ± 0.06^{c}	8.21 ± 0.74^{c}	9.76 ± 1.21^{d}	10.0 ± 0.32^{d}
L-Serine	31.5 ± 7.18^{a}	32.9 ± 3.75^{a}	26.6 ± 4.76^{b}	28.6 ± 2.75^{b}	63.1 ± 19.9^{c}	47.1 ± 8.85^{d}	42.8 ± 5.83^{d}
Glycine	74.9 ± 10.5^{a}	81.0 ± 1.32^{b}	69.6 ± 8.21^{c}	69.8 ± 7.71^{c}	101 ± 17.04^{d}	90.4 ± 15.3^{e}	86.0 ± 8.64^{be}
Sarcosine	0.08 ± 0.06^{a}	0.11 ± 0.08^{ab}	0.07 ± 0.01^{a}	0.13 ± 0.04^{b}	0.13 ± 0.01^{b}	0.15 ± 0.02^{c}	0.10 ± 0.07^{ab}
L-Aspartic acid	34.0 ± 6.57^{a}	59.8 ± 1.90^{b}	68.5 ± 7.15^{c}	93.3 ± 9.95^{d}	78.6 ± 16.0^{e}	97.8 ± 18.7^{d}	117 ± 19.3^{f}
Taurine	23.1 ± 0.23^{a}	23.6 ± 0.64^{a}	27.9 ± 3.77^{b}	21.8 ± 1.38^{c}	22.1 ± 1.49^{c}	25.2 ± 0.76^{ab}	22.8 ± 1.57^{ac}

β-Alanine	0.82 ± 0.02^{a}	1.91 ± 0.09^{b}	2.09 ± 0.17^{c}	3.62 ± 0.35 ^{d}	1.26 ± 0.05^{e}	1.96 ± 0.12^{b}	2.26 ± 0.19^{c}
L-Glutamic acid	75.3 ± 13.3^{a}	310 ± 11.1^{b}	452 ± 33.6^{c}	806 ± 91.03^{d}	470 ± 69.1^{c}	750 ± 119^{d}	1027 ± 123^{e}
L-Citrulline	0.25 ± 0.07^{a}	0.38 ± 0.17^{b}	0.47 ± 0.01^{c}	1.04 ± 0.15^{d}	0.56 ± 0.11^{c}	1.17 ± 0.07^{d}	1.30 ± 0.03^{e}
L-Alanine	43.0 ± 8.16^{a}	47.9 ± 0.85^{b}	42.4 ± 6.69^{a}	47.5 ± 4.42^{b}	70.6 ± 14.9^{c}	60.6 ± 9.94^{c}	66.0 ± 9.14^{c}
γ-Aminobutyric acid	0.49 ± 0.40^{a}	39.0 ± 3.19^{b}	61.6 ± 3.21^{c}	73.8 ± 8.32^{d}	34.3 ± 3.36^{b}	66.1 ± 10.17^{c}	90.6 ± 13.9^{e}
L-Proline	14.4 ± 3.30^{a}	13.8 ± 0.43^{a}	20.2 ± 0.59^{b}	27.9 ± 1.21^{c}	29.4 ± 6.44^{c}	35.1 ± 5.77^{d}	40.3 ± 3.93^{d}
β-Amino-isobutyric acid	0.19 ± 0.02^{a}	0.18 ± 0.03^{a}	0.25 ± 0.06^{b}	0.22 ± 0.03^{ab}	0.20 ± 0.03^{ab}	0.23 ± 0.01^{ab}	0.17 ± 0.07^{b}
α-Aminobutyric acid	1.08 ± 0.21^{a}	1.17 ± 0.21^{a}	1.47 ± 0.21^{b}	1.06 ± 0.24^{a}	1.26 ± 0.04^{ab}	1.26 ± 0.10^{ab}	0.99 ± 0.09^{a}
δ-Hydroxylysine	0.10 ± 0.01^{ab}	0.08 ± 0.01^{a}	0.13 ± 0.04^{b}	0.13 ± 0.02^{b}	0.12 ± 0.01^{b}	0.11 ± 0.05^{ab}	0.10 ± 0.03^{ab}
L-Ornithine	4.32 ± 0.13^{a}	5.53 ± 0.40^{b}	8.56 ± 0.80^{c}	5.81 ± 0.29^{b}	5.43 ± 0.28^{b}	6.32 ± 1.10^{d}	6.54 ± 0.65^{d}
Cystathionine	0.77 ± 0.07^{a}	0.62 ± 0.02^{b}	0.76 ± 0.07^{a}	0.67 ± 0.05^{b}	0.83 ± 0.05^{c}	0.82 ± 0.14^{c}	0.66 ± 0.18^{b}
L-Cystine	16.6 ± 0.77^{a}	16.4 ± 1.68^{a}	20.4 ± 3.92^{b}	15.0 ± 1.83^{c}	16.4 ± 0.92^{a}	17.5 ± 2.92^{ab}	15.9 ± 1.80^{ac}
L-Anserine	17.2 ± 1.99^{ab}	16.0 ± 3.58^{b}	21.4 ± 4.40^{c}	15.5 ± 0.78^{b}	16.3 ± 1.37^{b}	17.8 ± 2.63^{a}	17.4 ± 1.18^{a}
L-Tyrosine	146 ± 24.7^{a}	158 ± 4.09^{b}	132 ± 17.8^{c}	124 ± 12.74^{c}	193 ± 35.5^{d}	166 ± 27.9^{b}	158 ± 17.3^{b}
L-Homocystine	0.06 ± 0.01^{a}	0.14 ± 0.03^{b}	0.31 ± 0.03^{c}	0.23 ± 0.04^{d}	0.11 ± 0.08^{b}	0.19 ± 0.06^{b}	0.20 ± 0.02^{b}
TNEFAAs	***599 ± 123^{a}***	***918 ± 50.7^{b}***	***1046 ± 128^{c}***	***1437 ± 169^{d}***	***1282 ± 226^{e}***	***1550± 255^{d}***	***1819 ± 233^{f}***
TFAAs	***1321 ± 251^{a}***	***1695 ± 94.4^{b}***	***1697 ± 220^{b}***	***2129 ± 256^{c}***	***2290 ± 416^{c}***	***2436 ± 401^{d}***	***2724 ± 361^{e}***

* n.d.: not detected. Data are expressed as Mean ± SD (n = 3). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row. TEFAAs: total essential free amino acids, TNEFAAs: total non-essential free amino acids, TFAAs: total free amino acids. POS5, POS10 and POS15: 5, 10, 15% of tamarillo powder was added post fermentation, respectively. PRE5, PRE10 and PRE15: 5, 10, 15% of tamarillo powder was added to milk and starter culture prior to fermentation, respectively

5.3.3 Effect of *in vitro* digestion on TPC and antioxidant acidity of tamarillo yoghurts

Fortified yoghurts using pre- or post-fermentation approach had higher TPC values (2.5 – 5 times) and antioxidant activity (82 – 95%) than the control yoghurt ($p < 0.05$) (Figure 5.3A), suggesting that the added polyphenols from tamarillo had been retained. Higher concentration of tamarillo fortification led to a higher TPC as well as antioxidant activity of yoghurts. The undigested PRE samples showed higher TPC value than POS with 37%, 10% and 2% with addition of 5%, 10% and 15% tamarillo added, respectively. The polyphenols from tamarillo added in pre-fermentation might be released from protein-phenol conjugates and become more extractable (Sun-Waterhouse et al., 2013b). This might be because of the catabolic action of enzymes in the tamarillo (Li et al., 2018c) and also produced during fermentation (Salar, Purewal, & Bhatti, 2016). Additionally, the activities of β-glucosidase would be able to hydrolyze conjugated phenolics to release free phenolics (Salar, Certik, & Brezova, 2012). According to Adebo and Gabriela Medina-Meza (2020), increased extractability of polyphenols might be due to structural breakdown of cell walls after fermentation. The breakdown of the cell wall also led to the release of various bioactive compounds which cause increase TPC. The TPC of control was most likely related to the presence of milk compounds that are not polyphenols such as low molecular weight antioxidants (<900 Da) such as organic acids, fatty acids, vitamins and minerals as well as free amino acids, peptides and proteins that interfere with the Folin–Ciocalteu reagent (Helal & Tagliazucchi, 2018). Similar results had been observed for yoghurt fortified cinnamon powder (Helal & Tagliazucchi, 2018) and *Rhus coriaria* leaf powder (Simonetti, Perna, Grassi, & Gambacorta, 2021). The antioxidant activity of control is mainly due to the formation of bioactive peptides with radical scavenging activity because of the proteolytic activity of *Lactobacilli* in the starter culture. The POS5 showed higher CUPRAC and FRAP values than the PRE5, while with 10% and 15% tamarillo added, relatively similar antioxidant activity between POS and PRE samples were observed.

At neutral pH (oral and intestinal phases), the PRE samples showed higher TPC than POS ones (Figure 5.3B and D); whereas at acidic pH (gastric phase), similar TPC value between PRE and POS was observed for 5% and 15% tamarillo added (Figure 5.3C). According to Simonetti et al. (2021), at yoghurt pH (4.5), the binding affinity between polyphenols and milk proteins is enhanced which may lead to a decrease in

the digestibility and absorption of nutrients and bioactives from yoghurt. However, during *in vitro* digestion, hydrolytic enzymes and pH changes lead to hydrolysis of proteins and/or peptides, resulting in the release of polyphenols that were bound, and therefore this would increase their bioaccessibility. Pepsin at acidic pH (gastric phase) would digest majority of the proteins to polypeptides thus interrupting the protein–polyphenol interaction and resulting in the release of more free polyphenols into the digestive fluid; whereas, pancreatin at neutral pH (intestinal phase) would complete the hydrolysis to smaller peptides and some amino acids (Simonetti et al., 2021). In line with this, the TPC after *in vitro* gastric digestion represented about 84% and 75–87% of the TPC released after complete *in vitro* digestion for control and fortified yoghurts, respectively. A similar trend was observed in yoghurt fortified with strawberry and peach (Oliveira & Pintado, 2015), cinnamon (Helal & Tagliazucchi, 2018) and *Rhus coriaria* leaf powder (Simonetti et al., 2021). After pancreatic digestion, the TPC of fortified yoghurts shows an increase in phenolic content compared to the undigested yoghurt of about 6–10 times. According to Simonetti et al. (2021), Folin–Ciocalteu method detects the free polyphenols from tamarillo, the endogenous milk phenols derived from animal feed, and nonphenolic compounds from milk such as free amino acids and peptides that interfere with the Folin–Ciocalteu reagent. However, this method is not able to detect the polyphenols fraction that remains linked to milk components such as protein, lipids, and carbohydrates. Additionally, the accuracy of this assay can be affected by several compounds including ascorbic acid, dehydroascorbic acid (DHA), and reducing sugars (glucose and fructose) (Sánchez-Rangel, Benavides, Heredia, Cisneros-Zevallos, & Jacobo-Velázquez, 2013). Ascorbic acid and dehydroascorbic acid (both enediols) rapidly react with polyphosphotungstate under acidic pH of the reagent, showing a blue color right after mixing the extract with the reagent. Under the alkali conditions of the assay, enediol reductones from reducing sugars are formed and react with the reagent. Therefore, the results for TPC determinations are skewed (Sánchez-Rangel et al., 2013). To get a better antioxidant activity profile of yoghurts, three assays (Folin, CUPRAC and FRAP) were used in the current study. This was because FRAP, CUPRAC, and Folin methods can be used for acidic (pH 3.6), neutral (pH 7.0), and alkaline (pH 10) media, respectively (Apak et al., 2016).

The difference between TPC identified by the Folin–Ciocalteu method mgGAE% (Figure 5.3) and concentration of polyphenols determined by LC-MS/MS mg% (Tables 5.5 and 5.6) had been observed in our previous study (Diep et al., 2020a). The Folin–Ciocalteu colorimetric method is a measure of the reduction capacity of phenolic compounds, but free amino acid, fatty acids, organic acid, and vitamins (A, C and E) may interfere. Whereas the LC-MS/MS directly quantified specific polyphenol compounds including glycosylated or ester-linked groups. Additionally, possible explanations for the ambiguous relationship between TPC and polyphenol concentration is that synergy in a mixture makes TPC not only dependent on polyphenol concentration but also on the structure and interactions among polyphenols (Adebo & Gabriela Medina-Meza, 2020). Hence, directly linking TPC in food and responsible components might be somewhat difficult, as methods of extraction, identification, and/or quantification of TPC and individual polyphenol vary, which lead to difficulty in drawing comparisons and, subsequently, extrapolating conclusions (Adebo & Gabriela Medina-Meza, 2020).

The antioxidant activity of yoghurts (CUPRAC and FRAP values) was associated with polyphenols content and as expected, after each phase of *in vitro* digestion, it increased significantly ($p < 0.05$) (Figure 5.3B, C and D). Yoghurts fortified with strawberry and peach (Oliveira & Pintado, 2015), cinnamon (Helal & Tagliazucchi, 2018) and *Rhus coriaria* leaf powder (Simonetti et al., 2021) have shown a similar trend. Antioxidant activity in polyphenol fortified dairy products increased during digestion as a result of peptic and pancreatic enzyme activity, breaking down the protein structures. These enzymes promoted the release of both polyphenols bound in protein – polyphenol complex and bioactive peptides and amino acids bound in milk protein sequences (Rashidinejad, Birch, & Everett, 2016). After the gastric phase, CUPRAC and FRAP values of fortified yoghurts corresponded to an increase of over 72 and 67% compared to the same values after intestinal phase, respectively, except for FRAP value of POS5 with 45%. It can be concluded that the acidic pH together with enzymes action led to both higher polyphenol extractability from protein – polyphenol complexes and release of bioactive peptides. After the intestinal phase, the CUPRAC and FRAP values of fortified yoghurts increased about 2.4 – 3.5 and 1.4 – 3.1 times, respectively, compared to the undigested samples. The same antioxidant activity trend was observed by others in yoghurts fortified with strawberry and peach (ORAC assay) (Oliveira &

Pintado, 2015) as well as *Rhus coriaria* leaf powder (ABTS and FRAP assays) (Simonetti et al., 2021).

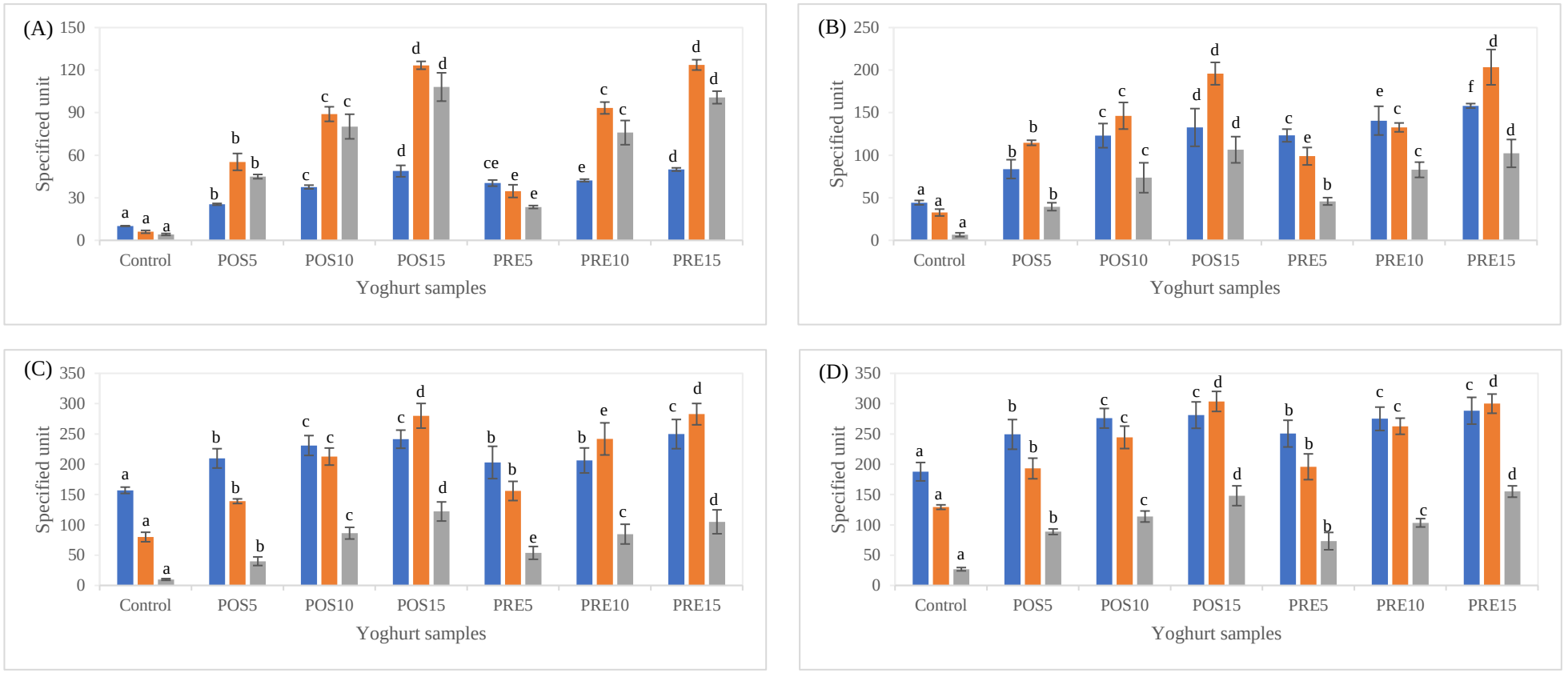


Figure 5.3 Total phenolic content and antioxidant activity of yoghurts fortified with tamarillo powder before digestion (A), after oral (B), gastric (C) and intestinal (D) phases of *in vitro* digestion.

TPC (■) (mg/100 g yoghurt) CUPRAC (■) (mg TEAC/100 g yoghurt) and FRAP (■) (mg TEAC/100 g yoghurt). Data are presented as mean and error bar (standard deviation) (n = 3). Different alphabets indicate statistical difference ($p < 0.05$) for each assay. POS5, POS10, and POS15: 5, 10, and 15% tamarillo powder was added post-fermentation, respectively. PRE5, PRE10, and PRE15: 5, 10, and 15% tamarillo powder was added to milk and starter culture prior to fermentation, respectively.

5.3.4 Effect of *in vitro* digestion on polyphenol profile of tamarillo yoghurts

In the fortified yoghurts prepared using the pre- or post-fermentation approach, all polyphenol compounds from tamarillo fruit (Diep et al., 2020a) were detected (Table 5.5), thus, the yoghurt matrix has helped retain these individual polyphenols during fermentation. Compared to the estimated contributions of tamarillo powder to nutrient and phytochemical content of fortified yoghurt (Appendix B17), the actual concentration of main polyphenols (Table 5.5) as well as TPC, CUPRAC and FRAP values (Figure 5.3A) in all undigested tamarillo yoghurt samples were lower or relatively similar except for chlorogenic acid (in POS5) and delphinidin 3-rutinoside (in POS5 and PRE5) with higher concentrations were overserved. The increase in tamarillo powder added led to higher concentration of polyphenols in fortified yoghurts ($p < 0.05$). As expected, chlorogenic acid, kaempferol 3-rutinoside, delphinidin 3-rutinoside and pelargonidin 3-rutinoside (which account for over 90% of the total polyphenol content in yoghurts) showed high concentrations of all the identified polyphenol compounds in the fortified yoghurts. Chlorogenic acid showed relatively similar concentrations between POS and PRE samples for all fortified yoghurts. For kaempferol 3-rutinoside and pelargonidin 3-rutinoside, POS5 and POS15 showed higher concentration than the PRE5 and PRE15, whereas POS10 and PRE10 have had the same concentration of these polyphenols. Similar concentration of delphinidin 3-rutinoside was observed for POS and PRE samples with 5% and 10% tamarillo added, while PRE15 showed a lower content of this compound than the POS15. This could be explained by the yields of polyphenols identified by LC-MS, which were related to the extractability of polyphenols from the food matrix and the stability of these polyphenols during food processing (Sun-Waterhouse et al., 2013b). Polyphenol extractability might also be influenced by chemical and physical effects such as the gel structure of yoghurt, binding to amphipathic yoghurt peptides or complexation with proteins and polysaccharides (Sun-Waterhouse et al., 2013b). The high concentration of chlorogenic acid in both POS and PRE samples suggested that chlorogenic acid was not metabolized by the starter cultures or was not degraded into other molecules, regardless of addition approach. Similar trend had been observed in yoghurt fortified with apple polyphenols by Sun-Waterhouse et al. (2012). These authors concluded that the fermentation process showed less impact on chlorogenic acid than other polyphenols. Acidity of yoghurt induced acid hydrolysis of

polyphenols, hence low concentration of flavonol compound (kaempferol) in yoghurts has been reported for fortified yoghurts (Sun-Waterhouse et al., 2012).

As shown in Table 5.5 and Table 5.6, different behaviours of identified polyphenols before and after *in vitro* digestion were observed. Major polyphenols (chlorogenic acid, kaempferol 3-rutinoside, delphinidin 3-rutinoside and pelargonidin 3-rutinoside) in fortified yoghurts showed high concentration at the end of each digestion phase compared to other polyphenol compounds ($p < 0.05$). After *in vitro* digestion, chlorogenic acid represented about 44 – 54% and 42 – 71% of this compound concentration in undigested POS and PRE samples, respectively, indicating relatively high bioaccessibilty of this polyphenol in tamarillo yoghurt. A chlorogenic acid level of 61% after *in vitro* digestion compared to undigested sample had been observed in peach yoghurt (Oliveira & Pintado, 2015), owing to chlorogenic acid forming stable milk casein complexes under simulated gastrointestinal conditions (Simonetti et al., 2021). During *in vitro* digestion, the oxidation and polymerization could cause the degradation of chlorogenic acid to form quinone (Tagliazucchi et al., 2012). For PRE samples, the percentage ratio between kaempferol 3-rutinoside content in post-pancreatic digested and undigested samples was over 65%, while the number for POS sample was 31–50%. This might be due to kaempferol 3-rutinoside being broken down into smaller forms that would be more extractable, when added prior to fermentation.

A similar phenomenon was observed for caffeic acid with over 75% and 20 – 43% for PRE and POS samples, respectively. For all fortified yoghurts, the percentage ratio of catechin and epicatechin in digested and undigested samples was below 20% and above 75%, respectively. This may be explained by epicatechin having lower interactions with α- and β-casein than catechin, in terms of lower binding constant value and number of polyphenols bound for casein molecule. This would result in a less protection of epicatechin and therefore, a greater susceptibility to the enzymes action, which can be detected with LC-MS analysis (Hasni et al., 2011). Also, lower release of catechin during digestion is due to a ring structure in the molecule that facilitates dissolution in milk fat globule membrane (Sirk, Brown, Friedman, & Sum, 2009). After the pancreatic digestion, rutin showed a percentage ratio of 51 – 63% and 45 – 83% between digested and undigested for POS and PRE samples, respectively (Table 5.5 and Table 5.6). The percentage ratio of rutin in yoghurt fortified with strawberry, peach and *Rhus coriaria* leaf powder was 60, 67 and 52%, respectively

(Oliveira & Pintado, 2015; Simonetti et al., 2021). According to Simonetti et al. (2021), rutin, with low dissolution rate and bioavailability, forms complexes only with the bovine serum albumin and the β-lactoglobulin, while the free rutin can undergo several chemical and enzymatic degradation steps in the gastrointestinal environment.

The percentage ratio for delphinidin 3-rutinoside content between post-pancreatic digested and undigested samples was 23 – 38% and 6 – 14% for PRE and POS samples, respectively (Table 5.5 and Table 5.6). For pelargonidin 3-rutinoside, the ratio was 16 – 34% and 18 – 21% for PRE and POS samples, respectively. The transition from the acidic gastric to the mild alkaline intestinal environment caused a decrease in the amount of bio-accessible anthocyanins (Oliveira & Pintado, 2015). High instability of anthocyanins at neutral or slightly basic pH due to the formation of the colourless chalcone pseudo-base has resulted in the destruction of an anthocyanin chromophore, as reported by (Bermúdez-Soto, Tomás-Barberán, & García-Conesa, 2007; Durmus, Capanoglu, & Kilic-Akyilmaz, 2021; McDougall, Fyffe, Dobson, & Stewart, 2005a; McDougall, Dobson, Smith, Blake, & Stewart, 2005b). Anthocyanins from strawberry have been reported as highly stable under the acidic conditions of the stomach while they have been degraded under the alkaline conditions of the intestine (Oliveira & Pintado, 2015). The same finding has been observed in this study.

Strengths of the current study are that this is the first study to fortify yoghurt with tamarillo powder, either pre-fermentation or post-fermentation and effect of *in vitro* digestion on amino acids, polyphenols and antioxidant activity was examined. This study highlights after simulated gastro-pancreatic digestion the high concentrations and bioaccessibility of diverse free amino acids, free polyphenols, and antioxidant activity, in yoghurt fortified with tamarillo powder. Our results have demonstrated that tamarillo pulp powder can be used to fortify yoghurt with essential amino acids, GABA and polyphenols. The results suggest that the yoghurt matrix allowed the protection of some compounds from degradation increasing bioaccessibility and making absorption and utilization possible. In fortified yoghurts, polyphenol compounds released from the yoghurt were stable in the digestive environment, thus would be able to exert their biological effects on the gastrointestinal system, which is more important than the content of these compounds in the corresponding undigested food. For example, chlorogenic acid in milk casein complex form has shown stability under simulated gastrointestinal conditions (Dupas, Marsset Baglieri, Ordonaud,

Tomé, & Maillard, 2006). Additionally, high stability of chlorogenic acid and caffeic acid under digestive conditions has been reported (Olthof, Hollman, & Katan, 2001). However, to obtain a better understanding on polyphenols bioaccessibility from tamarillo-fortified yoghurt, further *in vivo* studies evaluating both the action of digestive enzymes and the action of microbiota metabolism should be performed. Additionally, the changes of polyphenols in freeze-dried tamarillo pulp only (as a control) during *in vitro* digestion should be implemented to highlight the possible protective effects operated by yoghurt on polyphenols.

Table 5.5 Concentrations (mg/100 g yoghurt) of individual polyphenols in yoghurts fortified with cubosome containing tamarillo extract before *in vitro* digestion.

Polyphenols/phases	Undigested					
	POS5	POS10	POS15	PRE5	PRE10	PRE15
Phenolics						
Gallic Acid	0.044 ± 0.000^{a}	0.045 ± 0.000^{a}	0.049 ± 0.001^{b}	0.044 ± 0.000^{a}	0.048 ± 0.001^{b}	0.049 ± 0.001^{b}
Catechin	0.119 ± 0.085^{a}	0.165 ± 0.070^{b}	0.219 ± 0.104^{c}	0.035 ± 0.014^{d}	0.113 ± 0.097^{a}	0.208 ± 0.142^{c}
Caffeic acid	0.021 ± 0.001^{a}	0.031 ± 0.003^{b}	0.050 ± 0.005^{c}	0.024 ± 0.002^{a}	0.059 ± 0.014^{d}	0.060 ± 0.006^{d}
Chlorogenic acid	4.387 ± 0.274^{a}	7.005 ± 1.290^{b}	10.31 ± 0.458^{c}	4.072 ± 0.031^{a}	6.052 ± 0.379^{b}	9.502 ± 0.393^{c}
Epicatechin	0.695 ± 0.198^{a}	1.339 ± 0.391^{b}	1.670 ± 0.470^{c}	0.837 ± 0.083^{d}	0.886 ± 0.147^{d}	1.245 ± 0.185^{b}
p-coumaric acid	0.017 ± 0.000^{a}	0.028 ± 0.001^{b}	0.048 ± 0.002^{c}	0.026 ± 0.000^{b}	0.029 ± 0.001^{b}	0.040 ± 0.003^{c}
Ferulic acid	0.003 ± 0.000^{a}	0.005 ± 0.001^{b}	0.009 ± 0.002^{c}	0.028 ± 0.001^{d}	0.033 ± 0.001^{e}	0.037 ± 0.004^{e}
Rutin	0.011 ± 0.000^{a}	0.023 ± 0.005^{b}	0.035 ± 0.009^{c}	0.011 ± 0.000^{a}	0.022 ± 0.002^{b}	0.033 ± 0.004^{c}
Ellagic Acid	0.004 ± 0.000^{a}	0.003 ± 0.001^{a}	0.009 ± 0.001^{b}	0.002 ± 0.000^{c}	0.002 ± 0.000^{c}	0.005 ± 0.000^{a}
Kaempferol 3-rutinoside	2.702 ± 0.075^{a}	4.208 ± 0.102^{b}	8.218 ± 0.707^{c}	2.206 ± 0.010^{a}	4.290 ± 0.054^{b}	6.022 ± 0.140^{d}
Isorhamnetin 3-rutinoside	0.003 ± 0.000^{a}	0.005 ± 0.001^{b}	0.008 ± 0.001^{c}	0.003 ± 0.001^{a}	0.005 ± 0.001^{b}	0.007 ± 0.001^{c}
Kaempferol	0.010 ± 0.001^{a}	0.011 ± 0.001^{a}	0.016 ± 0.001^{b}	0.026 ± 0.003^{c}	0.028 ± 0.002^{c}	0.028 ± 0.001^{c}
Anthocyanins						
Delphinidin 3-rutinoside	20.59 ± 1.648^{a}	23.96 ± 5.714^{b}	38.22 ± 2.137^{c}	19.62 ± 0.149^{a}	21.37 ± 1.807^{ab}	31.66 ± 1.650^{d}
Cyanidin 3-rutinoside	0.503 ± 0.066^{a}	0.685 ± 0.122^{b}	1.267 ± 0.111^{c}	0.539 ± 0.025^{a}	0.626 ± 0.080^{b}	0.947 ± 0.096^{d}
Pelargonidin 3-rutinoside	7.733 ± 0.494^{a}	11.12 ± 1.484^{b}	23.56 ± 1.063^{c}	5.100 ± 0.218^{d}	11.72 ± 0.702^{b}	16.36 ± 0.685^{e}

* Data are expressed as Mean ± SD (n = 3). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row. No polyphenols were detected in the control yoghurt. POS5, POS10, and POS15: 5, 10, and 15% tamarillo powder was added post-fermentation, respectively. PRE5, PRE10, and PRE15: 5, 10, and 15% tamarillo powder was added to milk and starter culture prior to fermentation, respectively.

Table 5.6 Concentrations (mg/100 g yoghurt) of individual polyphenols in yoghurts fortified with cubosome containing tamarillo extract after each step of *in vitro* digestion.

Polyphenols/phases	Oral					
	POS5	POS10	POS15	PRE5	PRE10	PRE15
Phenolics						
Gallic Acid	0.009 ± 0.000^{a}	0.009 ± 0.001^{ab}	0.010 ± 0.000^{b}	0.009 ± 0.000^{a}	0.009 ± 0.000^{a}	0.010 ± 0.002^{b}
Catechin	0.011 ± 0.007^{a}	0.163 ± 0.008^{b}	0.187 ± 0.038^{c}	0.005 ± 0.000^{a}	0.013 ± 0.004^{ad}	0.019 ± 0.006^{d}
Caffeic acid	0.006 ± 0.001^{a}	0.014 ± 0.005^{b}	0.014 ± 0.006^{b}	0.005 ± 0.001^{a}	0.009 ± 0.003^{b}	0.028 ± 0.006^{c}
Chlorogenic acid	0.712 ± 0.033^{a}	0.718 ± 0.011^{a}	1.159 ± 0.059^{b}	0.568 ± 0.012^{c}	0.649 ± 0.010^{d}	1.011 ± 0.022^{b}
Epicatechin	0.381 ± 0.033^{a}	0.415 ± 0.072^{b}	0.453 ± 0.036^{b}	0.301 ± 0.042^{c}	0.396 ± 0.016^{a}	0.404 ± 0.046^{b}
p-coumaric acid	0.008 ± 0.001^{a}	0.008 ± 0.005^{ab}	0.010 ± 0.004^{b}	0.004 ± 0.001^{c}	0.006 ± 0.004^{ac}	0.007 ± 0.001^{ac}
Ferulic acid	0.005 ± 0.002^{a}	0.008 ± 0.005^{ab}	0.009 ± 0.003^{b}	0.008 ± 0.004^{ab}	0.012 ± 0.004^{c}	0.015 ± 0.005^{c}
Rutin	0.015 ± 0.003^{a}	0.016 ± 0.006^{a}	0.018 ± 0.004^{b}	0.010 ± 0.002^{c}	0.012 ± 0.000^{c}	0.021 ± 0.003^{d}
Ellagic Acid	0.016 ± 0.002^{a}	0.021 ± 0.003^{b}	0.024 ± 0.004^{bd}	0.014 ± 0.001^{c}	0.015 ± 0.003^{ac}	0.027 ± 0.007^{d}
Kaempferol 3-rutinoside	2.407 ± 0.402^{a}	3.276 ± 0.234^{b}	4.561 ± 0.434^{c}	2.812 ± 0.344^{a}	3.532 ± 0.184^{b}	4.222 ± 0.146^{c}
Isorhamnetin 3-rutinoside	0.002 ± 0.001^{a}	0.002 ± 0.000^{a}	0.002 ± 0.000^{a}	0.002 ± 0.001^{b}	0.002 ± 0.001^{b}	0.003 ± 0.000^{b}
Kaempferol	0.007 ± 0.001^{a}	0.007 ± 0.002^{a}	0.009 ± 0.003^{b}	0.006 ± 0.001^{a}	0.008 ± 0.001^{ab}	0.010 ± 0.003^{b}
Anthocyanins						
Delphinidin 3-rutinoside	1.785 ± 0.005^{a}	1.934 ± 0.003^{b}	5.899 ± 0.002^{c}	1.892 ± 0.004^{a}	4.042 ± 0.003^{d}	4.371 ± 0.003^{e}
Cyanidin 3-rutinoside	0.143 ± 0.002^{a}	0.146 ± 0.023^{a}	0.147 ± 0.012^{ab}	0.145 ± 0.050^{a}	0.147 ± 0.027^{ab}	0.151 ± 0.033^{b}
Pelargonidin 3-rutinoside	0.573 ± 0.059^{a}	0.962 ± 0.039^{b}	2.132 ± 0.236^{c}	0.627 ± 0.126^{a}	1.026 ± 0.041^{b}	1.902 ± 0.209^{c}

* Data are expressed as Mean ± SD (n = 3). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row. No polyphenols were detected in the control yoghurt. POS5, POS10, and POS15: 5, 10, and 15% tamarillo powder was added post-fermentation, respectively. PRE5, PRE10, and PRE15: 5, 10, and 15% tamarillo powder was added to milk and starter culture prior to fermentation, respectively.

Table 5.6 Concentrations (mg/100 g yoghurt) of individual polyphenols in yoghurts fortified with cubosome containing tamarillo extract after each step of *in vitro* digestion (Cont.)

Polyphenols/phases	Gastric					
	POS5	POS10	POS15	PRE5	PRE10	PRE15
Phenolics						
Gallic Acid	0.024 ± 0.002^{a}	0.027 ± 0.002^{b}	0.028 ± 0.001^{b}	0.025 ± 0.001^{ab}	0.024 ± 0.000^{a}	0.025 ± 0.001^{ab}
Catechin	0.008 ± 0.001^{a}	0.015 ± 0.005^{b}	0.040 ± 0.010^{c}	0.012 ± 0.003^{b}	0.013 ± 0.004^{b}	0.028 ± 0.010^{d}
Caffeic acid	0.019 ± 0.002^{a}	0.025 ± 0.004^{b}	0.031 ± 0.002^{c}	0.024 ± 0.003^{b}	0.027 ± 0.003^{b}	0.034 ± 0.006^{c}
Chlorogenic acid	3.043 ± 0.130^{a}	3.194 ± 0.152^{b}	5.931 ± 0.291^{c}	2.937 ± 0.133^{a}	3.480 ± 0.142^{d}	5.635 ± 0.218^{c}
Epicatechin	0.427 ± 0.025^{a}	0.500 ± 0.014^{b}	0.500 ± 0.003^{b}	0.428 ± 0.024^{a}	0.508 ± 0.074^{b}	0.535 ± 0.080^{c}
p-coumaric acid	0.005 ± 0.001^{a}	0.006 ± 0.002^{ab}	0.007 ± 0.002^{b}	0.005 ± 0.001^{a}	0.006 ± 0.002^{ab}	0.006 ± 0.001^{ab}
Ferulic acid	0.003 ± 0.001^{a}	0.004 ± 0.001^{ab}	0.005 ± 0.001^{b}	0.006 ± 0.001^{bc}	0.008 ± 0.004^{c}	0.008 ± 0.003^{c}
Rutin	0.022 ± 0.005^{a}	0.040 ± 0.010^{b}	0.046 ± 0.008^{b}	0.019 ± 0.004^{a}	0.030 ± 0.005^{c}	0.040 ± 0.002^{b}
Ellagic acid	0.026 ± 0.003^{a}	0.029 ± 0.001^{ab}	0.035 ± 0.008^{b}	0.031 ± 0.007^{b}	0.037 ± 0.010^{b}	0.069 ± 0.019^{c}
Kaempferol 3-rutinoside	1.560 ± 0.227^{a}	3.365 ± 0.361^{b}	6.305 ± 0.488^{c}	2.665 ± 0.347^{d}	4.458 ± 0.475^{e}	5.748 ± 0.261^{f}
Isorhamnetin 3-rutinoside	0.002 ± 0.000^{a}	0.005 ± 0.002^{b}	0.007 ± 0.001^{c}	0.003 ± 0.000^{a}	0.003 ± 0.001^{ab}	0.003 ± 0.001^{ab}
Kaempferol	0.009 ± 0.001^{a}	0.010 ± 0.001^{ab}	0.011 ± 0.002^{b}	0.011 ± 0.001^{b}	0.012 ± 0.001^{c}	0.012 ± 0.001^{c}
Anthocyanins						
Delphinidin 3-rutinoside	1.912 ± 0.040^{a}	3.312 ± 0.092^{b}	7.617 ± 0.035^{c}	7.683 ± 0.058^{c}	8.861 ± 0.022^{d}	9.300 ± 0.107^{e}
Cyanidin 3-rutinoside	0.352 ± 0.042^{a}	0.491 ± 0.050^{b}	0.489 ± 0.042^{b}	0.352 ± 0.025^{a}	0.420 ± 0.011^{c}	0.471 ± 0.055^{bc}
Pelargonidin 3-rutinoside	1.722 ± 0.143^{a}	3.200 ± 0.208^{b}	5.835 ± 0.393^{c}	2.388 ± 0.170^{d}	3.720 ± 0.139^{e}	4.678 ± 0.085^{f}

* Data are expressed as Mean ± SD (n = 3). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row. No polyphenols were detected in the control yoghurt. POS5, POS10, and POS15: 5, 10, and 15% tamarillo powder was added post-fermentation, respectively. PRE5, PRE10, and PRE15: 5, 10, and 15% tamarillo powder was added to milk and starter culture prior to fermentation, respectively.

Table 5.6 Concentrations (mg/100 g yoghurt) of individual polyphenols in yoghurts fortified with cubosome containing tamarillo extract after each step of *in vitro* digestion (Cont.)

Polyphenols/phases	Intestinal					
	POS5	POS10	POS15	PRE5	PRE10	PRE15
Phenolics						
Gallic Acid	0.002 ± 0.001^{a}	0.002 ± 0.001^{a}	0.003 ± 0.002^{b}	0.003 ± 0.001^{b}	0.008 ± 0.004^{c}	0.009 ± 0.002^{c}
Catechin	0.014 ± 0.006^{a}	0.033 ± 0.010^{b}	0.036 ± 0.006^{b}	0.005 ± 0.000^{c}	0.022 ± 0.008^{ad}	0.026 ± 0.002^{d}
Caffeic acid	0.004 ± 0.001^{a}	0.008 ± 0.003^{b}	0.021 ± 0.012^{c}	0.019 ± 0.003^{c}	0.045 ± 0.015^{d}	0.048 ± 0.013^{d}
Chlorogenic acid	1.926 ± 0.034^{a}	3.767 ± 0.198^{b}	4.553 ± 0.260^{c}	1.724 ± 0.045^{d}	4.507 ± 0.264^{c}	5.825 ± 0.207^{e}
Epicatechin	0.679 ± 0.015^{a}	0.803 ± 0.062^{b}	0.871 ± 0.185^{c}	0.793 ± 0.018^{b}	0.834 ± 0.025^{bc}	0.835 ± 0.088^{c}
p-coumaric acid	0.011 ± 0.006^{a}	0.012 ± 0.006^{b}	0.012 ± 0.003^{a}	0.010 ± 0.005^{a}	0.011 ± 0.005^{a}	0.012 ± 0.008^{b}
Ferulic acid	0.005 ± 0.002^{a}	0.005 ± 0.001^{a}	0.008 ± 0.001^{b}	0.009 ± 0.003^{b}	0.014 ± 0.003^{c}	0.014 ± 0.004^{c}
Rutin	0.006 ± 0.002^{a}	0.015 ± 0.005^{b}	0.018 ± 0.005^{c}	0.005 ± 0.001^{a}	0.019 ± 0.006^{c}	0.020 ± 0.004^{c}
Ellagic acid	0.065 ± 0.011^{a}	0.073 ± 0.011^{b}	0.076 ± 0.019^{b}	0.059 ± 0.009^{a}	0.064 ± 0.006^{a}	0.092 ± 0.009^{c}
Kaempferol 3-rutinoside	0.825 ± 0.092^{a}	1.880 ± 0.227^{b}	4.137 ± 0.380^{c}	1.501 ± 0.249^{b}	3.647 ± 0.525^{d}	4.155 ± 0.272^{c}
Isorhamnetin 3-rutinoside	0.005 ± 0.003^{a}	0.006 ± 0.002^{ab}	0.007 ± 0.003^{b}	0.003 ± 0.001^{a}	0.004 ± 0.001^{a}	0.004 ± 0.001^{a}
Kaempferol	0.018 ± 0.001^{a}	0.018 ± 0.002^{a}	0.023 ± 0.005^{b}	0.018 ± 0.001^{a}	0.022 ± 0.004^{b}	0.024 ± 0.001^{b}
Anthocyanins						
Delphinidin 3-rutinoside	1.147 ± 0.010^{a}	2.802 ± 0.064^{b}	5.527 ± 0.004^{c}	4.560 ± 0.008^{d}	8.159 ± 0.090^{e}	9.033 ± 0.016^{f}
Cyanidin 3-rutinoside	0.639 ± 0.024^{a}	0.641 ± 0.051^{b}	0.672 ± 0.052^{c}	0.653 ± 0.210^{b}	0.667 ± 0.181^{c}	0.687 ± 0.192^{c}
Pelargonidin 3-rutinoside	1.601 ± 0.019^{a}	2.072 ± 0.059^{b}	4.147 ± 0.092^{c}	1.740 ± 0.067^{a}	2.557 ± 0.045^{d}	2.617 ± 0.009^{d}

* Data are expressed as Mean ± SD (n = 3). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row. No polyphenols were detected in the control yoghurt. POS5, POS10, and POS15: 5, 10, and 15% tamarillo powder was added post-fermentation, respectively. PRE5, PRE10, and PRE15: 5, 10, and 15% tamarillo powder was added to milk and starter culture prior to fermentation, respectively.

5.4 Conclusion

Tamarillo powder was successfully employed either before or after fermentation to produce yoghurts fortified with tamarillo and these were highly nutritious containing essential amino acids, GABA and polyphenols. The availability of amino acids at the end of intestinal digestion was increased by adding tamarillo powder. Protein profiling showed that 10–15% addition of tamarillo powder could improve both proteolytic activity and protein content. At the end of digestion, soluble caseins were totally hydrolyzed in all yoghurt samples. For undigested samples, addition of tamarillo powder led to a dose-dependent increase in FAA content, especially the precursor of GABA as well as the TFAAs, TEFAAs and TNEFAAs. PRE samples showed higher amount of TFAAs (6–26%), TEFAAs (3–6 times) and TNEFAAs (4.6–13%) than the POS ones. The highest content of FAAs and no molecular band from SDS-PAGE after intestinal phase indicated that protein hydrolysis was complete, and these would be absorbable in the small intestine.

For either of the fermentation approaches used, the four major tamarillo polyphenols were significantly retained in the final yoghurt products with high TPC and antioxidant activity in undigested samples. With 10% and 15% of tamarillo added, relatively similar antioxidant activity between POS and PRE samples were observed. The antioxidant capacity of fortified yoghurts from both fermentation processes increased under the influence of intestinal digestion, due to chemical changes of the polyphenols and presence of bioactive amino acids or peptides. The percentage ratio of main polyphenols in digested and undigested yoghurt samples was high indicating high bioaccessibility of these bioactive compounds. The results suggested that yoghurt matrix allowed the protection of some polyphenols from hydrolysis, having become bioaccessible and making absorption and utilization possible. Further research of acceptability, shelf life, and then trials for health effects should be implemented. Additionally, the polyphenol-protein interaction needs further investigation to get a better understanding of effect on content of free polyphenols, antioxidant capacity and bioavailability of polyphenols in yoghurt matrix.

5.5 Summary and next steps

The bioactive compounds in tamarillo may be influenced by negative gastrointestinal digestion interactions which result in low bioavailability and loss of some beneficial properties during digestion. Polyphenols are the main bioactive compound in tamarillo. If polyphenols could be encapsulated protection from enzymatic digestion may be possible. Hence, in the next chapter, (Chapter 6) what is known about targeted delivery vehicles and in particular a novel encapsulation technique (cubosomes) is explored prior to inform experimentally encapsulating tamarillo polyphenols before addition to a yoghurt matrix.

Chapter 6: Literature review of novel encapsulation technique (cubosome) to protect bioactive compounds

There are a number of challenges in developing functional foods, especially with respect to the direct utilization of certain bioactive compounds. This is because bioactive compounds can show issues such as instability or reaction with other food matrix ingredients. Encapsulation has thus emerged as a potential approach to deal with these problems and, also to provide controlled or targeted delivery or release when consumed. In recent years there has been interest in using lyotropic liquid crystals (LLCs), particularly cubosomes for delivery of food extracts and drug applications (Mezzenga et al., 2019). The particles show, *in vitro*, the potential of efficient, controlled and target selective release of bioactive compounds (Mezzenga et al., 2019).

This chapter aims to summarize the potential of LLCs or cubosomes to protect and release bioactive compounds to ensure their bioavailability and applications of cubosome techniques in food is discussed. This chapter is organised as follows: Section 6.1 briefly introduce liquid crystal particles and cubosome. Type and components of cubosome particle is summarized in Section 6.2. A brief production and application are shown in Section 6.3 and 6.4, respectively. Section 6.5 presents the summary and next question for the next chapter.

6.1 Characteristics of lyotropic liquid crystals and cubosome encapsulation

Liquid crystals are often classified as materials which exist in a mesomorphic state between liquid and crystalline solid. Therefore, liquid crystals can show the mechanical stability of solids as well as flow like liquids (Omray, 2013; Tadwee, Shahi, Ramteke, & Syed, 2012). General properties of liquid crystals include birefringence (the double refraction of light), sensitivity to temperature resulting in colour changes, optical activity in twisted nematic phases as well as response to magnetic and electric fields. In general, liquid crystals are classified into two types: lyotropic liquid crystals (LLCs) and thermotropic liquid crystals (TLCs). The formation of lyotropic liquid crystals is often created by adding a liquid (an organic polar solvent or water while the formation of thermotropic crystals is the results of

cooling an isotropic melt or heating a crystalline solid. In comparison to TLC, LLC are more popular for food and pharmaceutical applications (Omray, 2013).

Lamellar, hexagonal and cubic phases are three main types of LLCs based on their structures. The lamellar phase is a linear arrangement of lipid bilayers whereas the hexagonal phases are cylindrical structures that form a hexagonal lattice. Cubic phase is as the cubic packing of spherical micelles, creating a dense cubic lattice (Kim et al., 2015). By dispersing lamellar, hexagonal and cubic phases into the aqueous phase in the presence of suitable stabilizers, the nano-structured liquid crystals (100 – 1000 nm size range) can be generated including liposomes, hexosomes and cubosomes, respectively (Bhosale, Osmani, Harkare, & Ghodake, 2013; Spicer, 2014). Because of the co-existence of hydrophilic, hydrophobic and amphiphilic molecules in the structure, cubosomes have shown many advantages rather than other lipid-based systems. In cubosomes, hydrophobic actives are located within the lipid bilayers, the hydrophilic components are placed in the aqueous channels or around the polar head of the lipid, and amphiphilic molecules can be separated partially at the lipid-water interface (Kwon & Kim, 2014; Liu et al., 2013; Peng, Zhou, & Han; Tu et al., 2014). Cubosomes have achieved considerable interest in numerous applications in recent years because bioavailability of poorly soluble compounds is enhanced and there is the ability to encapsulate diverse active molecules (Lancelot, Sierra, & Serrano, 2014; Nanjwade, Hundekar, Kamble, & Srichana, 2014; Sagalowicz & Leser, 2010).

6.2 Category and components of cubosomes

The formation of cubosome particles has replied on the combination of lipid, stabiliser and loaded proteins through self-assembly to create bicontinuous cubic phases (Q_{II}). Under excessive water conditions, three types of cubosome based on the structures will be formed: (i) gyroid surface (Ia3d, Q230, G-surface); (ii) diamond surface (Pn3m, Q224, D-surface); (iii) primitive surface (Im3m, Q229, P-surface) (Rizwan & Boyd, 2015) (Figure 6.1).

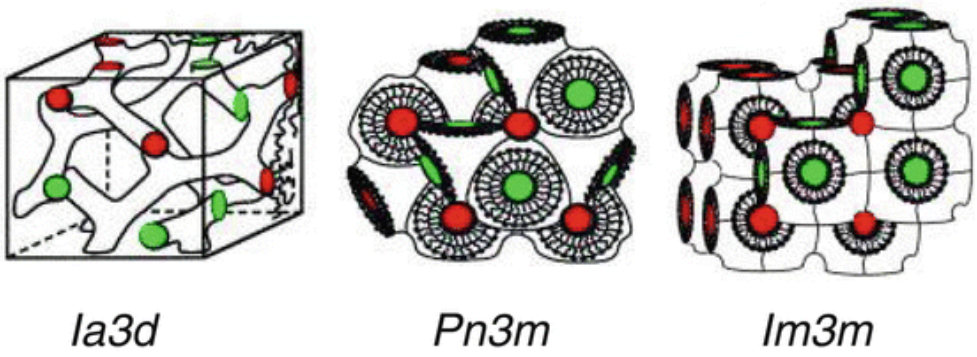


Figure 6.1 Schematic representations of three bicontinuous cubic phase types.

(Rizwan & Boyd, 2015)

Various lipids have been used to produce cubosomes, in which two most commonly used are phytantriol (PHYT) and monoolein or glyceryl monooleate (GMO). Because there are hydrocarbon chains in the tail and hydroxyl groups in the head region, GMO has both hydrophobic and hydrophilic properties at the same time. Moreover, monoolein is classified as Generally Recognized As Safe (GRAS) and is mainly used as an emulsifier in the food industry. According to Karami and Hamidi (2016), this lipid as a biodegradable and biocompatible material, is favored for pharmaceutical applications due to high purity. Meanwhile, PHYT is considered as an alternative for monoolein in cubosomal production. This has been because fatty acid-based GMO is susceptible to esterase-catalyzed hydrolysis, by contrast the higher structural stability of PHYT could be generated by phytanyl backbone (Boyd, Whittaker, Khoo, & Davey, 2006). Karami and Hamidi (2016) stated that although molecular structures of GMO and PHYT are different, both chemicals exhibit similar behavior of phase transition as temperature and water concentration increase.

A stabilizing or dispersing agent is crucial to cover the outer surface of cubosome particles in order to prevent aggregation as well as supply colloidal stability (Barriga, Holme, & Stevens, 2019; Karami & Hamidi, 2016). Also, the agent should contribute to the lipid–water assembly without disrupting the cubic liquid crystallinity of the structure (Rizwan & Boyd, 2015). Tri-block copolymers including Synperonic F-108 (PEO_{127}-PPO_{48}-PEO_{127}) and Pluronic F127 (PEO_{99}–PPO_{67}–PEO_{99}) (being considered as gold standard) are the most-applied stabilizers in cubosomal production. The PPO portion gives the hydrophilic property and the PEO portion is responsible for the hydrophobic character (Rizwan & Boyd, 2015). According to Karami and Hamidi (2016), the PPO chains are located within the bilayer structure or at the surface of the

cubosomes, whereas the PEO portions are correlated with the water phase. Also, polyethylene glycol and β-casein have been displayed as a suitable stabilizer for cubosome formation in terms of enhancing uptake in cellular systems and reducing cytotoxicity (Barriga et al., 2019; Zhai et al., 2011). Currently used in drug delivery applications, Tween 80 (polysorbate 80, polyoxyethylene sorbitan monooleate) has been recommended as stabilizer due to some advantages including incorporating with biologically active diglycerides and enhancing drug delivery across the blood-brain barrier (Barriga et al., 2019).

6.3 Production of cubosomes

The dispersion of bulk cubic phase into aqueous phase can use popular methods such as homogenization (Deshpande et al., 2014), sonication (Demurtas et al., 2015), shearing (Salentinig, Yaghmur, Guillot, & Glatter, 2008), incorporation with hydrotropes (Spicer, Hayden, Lynch, Ofori-Boateng, & Burns, 2001) and mechanical stirring (Barauskas, Misiunas, Gunnarsson, Tiberg, & Johnsson, 2006). The 'top-down' and 'bottom-up' methods, two common approaches for production of cubosomse are illustrated in Figure 6.2.

The 'top-down' method is carried out in two steps: (i) formation of the viscous bulk cubic phase by mixing lipid(s) with stabilizer(s) to circumvent aggregation and (ii) dispersing bulk cubic phase into aqueous medium by high energy of homogenization or sonication (Spicer, 2005). Cubosomes formed from this method are reproducible and stable without addition of solvents. Therefore, re-investigation of phase behavior is unnecessary and the cellular toxicity of solvents is not a concern (Barriga et al., 2019). The drawbacks of this method are the need for a high energy supply, the need to heat the mixture, damage to amphiphilic particles or encapsulated drugs, and vesicle production as a by-products (Barriga et al., 2019; Lancelot et al., 2014). For improvement of monodispersity and reduction of the vesicles number, shear equipment coupled with a Couette cell should be used in the dispersion of the bulk cubic phase. This technique will minimize the heating energy input and allow heat cycling (Barauskas, Johnsson, Joabsson, & Tiberg, 2005; Barauskas et al., 2006).

Spicer et al. (2001) developed the liquid precursor or solvent dilution method for cubosome production that was known as the 'bottom-up' method. The vital feature of

this dilution method is the utilization of a hydrotrope which possessing capacity to dissolve water-insoluble lipids into liquid precursors (Bhosale et al., 2013). The main advantage of the 'bottom-up' method is the lower polydispersity of cubosomes and hence the number of vesicles formed is reduced (Spicer et al., 2001). According to Rizwan et al. (2011), there are some shortcomings of this method including cellular toxicity (caused by residual solvent) and phase behavior transformation for biological applications. The authors also stated that several solvents (polyglycerol ester or propylene glycol) could be applied to optimize 'bottom-up' method for delivery of protein vaccines.

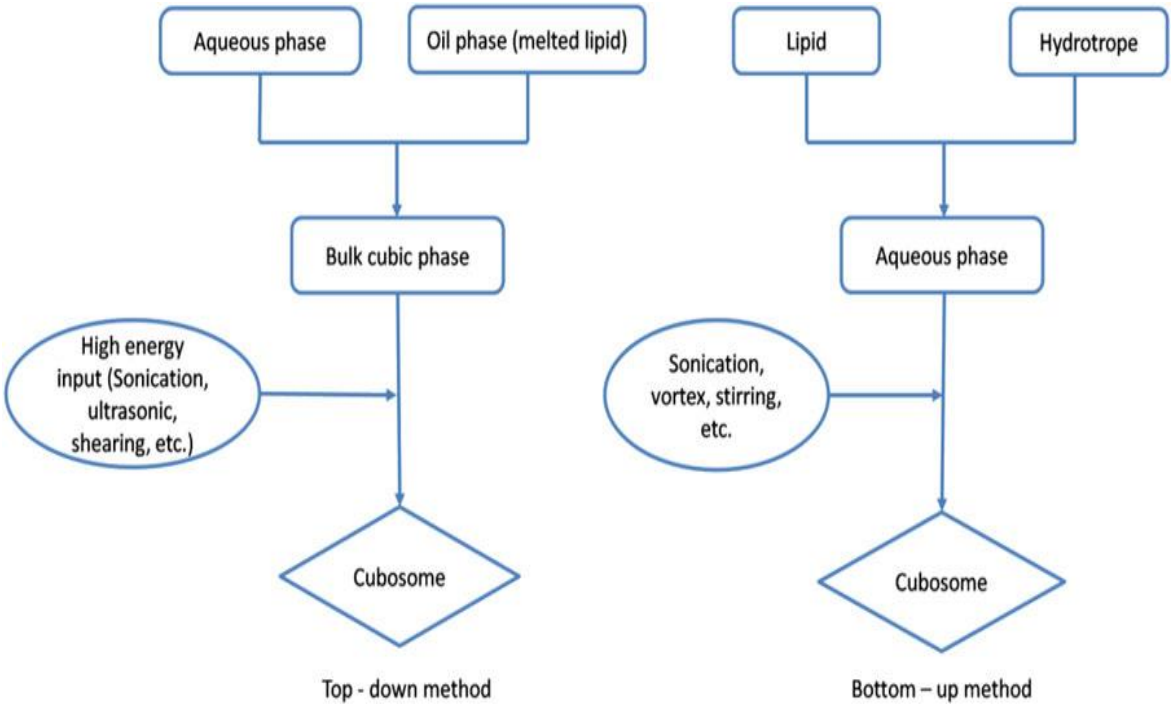


Figure 6.2 Two common techniques to produce cubosome.

(Huynh Mai, Thanh Diep, Le, & Nguyen, 2020). Used with permission

6.4 Application of cubosomes

Due to abilities of enhancement of bio-adhesion and biocompatibility, solubilization of hydrophobic, amphiphilic and hydrophilic compounds at the same time, as well as the protection of substances from physical and enzymatic degradation, cubosomes are considered as an ideal encapsulation material in general. Besides, lyotropic liquid crystals such as cubosomes are classified as non-toxic materials. Hence, they are considered as potential tools to control release and enhance the bioavailability of

bioactives in food products. For example, cubosomes can be used to protect phytochemicals and aromas from chemical degradation (e.g., oxidation) and can be designed to release at the right place and during the required period (e.g., burst or sustained release) (Mezzenga et al., 2019). Previous studies reported that a cubic phase resulted in a distinct release rate of aroma compounds when compared to a water-in-oil emulsion (Vauthey et al., 2000a; Vauthey et al., 2000b). Another study showed that self-assembled structures (inverse micellar cubic phase or inverse hexagonal phase) have delayed the release of caffeine (Martiel et al., 2015). According to Mezzenga et al. (2019), sustained release of caffeine may be useful to have alertness longer than the 3 or 4 h that corresponds to immediate absorption. Besides, cubosomes can play a role as micro-reactor controlling the interactions between food components during processing. Sagalowicz, Leser, Watzke, and Michel (2006) informed that cubosomes increased the efficiency of Maillard reaction and acted as a reactor for the production of a flavor compound, 2-furfurylthiol (FFT). Bicontinuous cubic phase showed higher productivity of FFT about seven times than the aqueous phase.

Bicontinuous cubic phase had been used to solubilize vitamin E and increase the shelf-life of tocopherol-acetylated form under pathophysiological conditions had been observed (Nagy et al., 2014). This result suggests a potential ability of the cubic phase to enhance vitamin bioavailability. In recent years, there has been a growing interest in the development of LLCs (especially cubosomes) as nanocarriers for delivering natural polyphenols including poorly water-soluble compounds. According to Yaghmur (2019), bioavailability and therapy improvements of natural polyphenols are important outcomes in the design of food nanostructured colloids to deliver these compounds. For example, to overcome the low aqueous solubility of quercetin, a natural polyphenol compound with anticancer and antioxidant activities, monoolein-cubosomes have been used as nanocarriers to deliver this compound (Cortesi et al., 2017). The encapsulation efficiency of quercetin-loaded cubosomes was over 84% and incorporation of quercetin into cubosomes enhanced the solubility of quercetin in water up to 20 times (Cortesi et al., 2017). Additionally, the attractiveness of the use of cubosomes to enhance the solubilization and bioavailability of curcumin, a natural anticancer herbal compound, has been reported. Puglia et al. (2013) found that encapsulation efficiency of curcumin-loaded cubosomes was above 82%. Cubosomes could control curcumin diffusion through the skin and had ability to improve the anti-

inflammatory/antioxidant effects of this polyphenol, which confirmed the protective effect of encapsulation on its chemical stability (Puglia et al., 2013). Both piperine and curcumin were successfully encapsulated in the interior of the cubosome particles which considerably enhanced the oral bioavailability of curcumin (Tu et al., 2014). Another study has shown that cubosomes increase the stability and antibacterial activity of curcumin (Archana, Vijayasri, Madhurim, & Kumar, 2015) (Table 6.1).

Table 6.1 Using cubosomes as nanocarriers for delivering bioactive compounds and their effects on food applications.

Compounds	**Effects**	**References**
2-furfurylthiol	Increased productivity	Sagalowicz et al. (2006)
Caffeine	Delayed caffeine release	Martiel et al. (2015)
Vitamin E	Increased solubility Longer shelf-life	Nagy et al. (2014)
Quercetin	Increased aqueous solubility	Cortesi et al. (2017)
Curcumin	Increased stability Control of curcumin diffusion	Puglia et al. (2013)
Curcumin	Improved bioavailability	Tu et al. (2014)
Curcumin	Increased antibacterial activity	Archana et al. (2015)

In the pharmaceutical applications, cubosomes have shown ability to cope with many problems in the oral delivery of different substances including poor absorption, poor aqueous solubility and molecular size. GMO-based cubosomes have shown enhancement of oral intake of insulin (Chung, Kim, Um, Kwon, & Jeong, 2002). According to Jin et al. (2013), compared to the free 20(S)-protopanaxadiol (PPD), PPD-loaded cubosome in oral formulation could enhance the permeability by 53%. Avachat and Parpani (2015) observed higher the C_{max} and AUC values of efavirenz (EFV)-loaded cubosome in the oral formulation than those of free drug with 1.93 and 1.48 times, respectively after 12h *in vitro* release. For targeting the brain parenchyma, Elnaggar, Etman, Abdelmonsif, and Abdallah (2015) orally applied the Tween-modified GMO cubosomes loaded with piperine and the results from *in vivo* experiment showed significant enhancement of the piperine cognitive effect. Rizwan et al. (2011) reported ovalbumin loaded by phytantriol based cubosomes and observed the sustained-release profiles of the ovalbumin. In addition, the immune properties of immunostimulants could be potentiated by PHYT-based cubosomes loaded with

polysaccharides through intensifying antigen transport into lymph nodes and enhancing the immune response (Liu et al., 2016).

The most comprehensive research of the cubosome applications is for cancer therapy. Cancer drugs such as Paclitaxel (Aleandri, Bandera, Mezzenga, & Landau, 2015), Quercetin (Murgia et al., 2013), Sorafenib (Thapa et al., 2015), Irinotecan (Ali, Noguchi, Iwao, Oka, & Itai, 2016), 5-Fluorouracil (Astolfi et al., 2017; Nasr, Ghorab, & Abdelazem, 2015) and Doxorubicin (Nazaruk, Majkowska-Pilip, & Bilewicz, 2017) had been encapsulated by cubosome particles. An increased uptake of 5-fluorouracil released from cubosomes into the livers of rats demonstrated that cubosome particle delivery might improve the efficiency of low 5-fluorouracil doses (Nasr et al., 2015). Due to the extensive water channel networks, cubosomes could carry both water-soluble drugs and poorly water-soluble ones (Lancelot et al., 2014). The efficiency of Paclitaxel (a poorly water-soluble medicine) in cancer therapy could be enhanced by coating with cubosomes (Aleandri et al., 2015). On the other hand, Irinotecan (a water-soluble drug) with stability at pH of 3.5, could maintain active form at neutral pH if being encapsulated by cubosomes (Ali et al., 2016).

6.5 Summary and next questions

Encapsulation using lyotropic liquid crystals and in particular cubosomes has emerged as a potential approach to add improve the bioavailability of bioactives extracted from functional foods, thus extending their benefits to a wider population. Cubosomes have been shown to help control the behaviour and release of bioactives and therapies for cancer. This knowledge could be applied to formulate a functional food such as yoghurt fortified with tamarillo extract. In the next experimental chapter (Chapter 7) the is asked: Does the encapsulation of tamarillo extract enhance the stability and bio-accessibility of bioactive compounds in tamarillo yoghurt and maintain texture compared to yoghurt fortified with unencapsulated tamarillo extract?.

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Chapter 7: Tamarillo polyphenols encapsulated-cubosome: formation, characterization, stability during digestion and application in yoghurt

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Tamarillo extract is a good source of phenolic and anthocyanin compounds which are well-known for beneficial antioxidant activity, but their bioactivity maybe lost during digestion. In this study, promising prospects of tamarillo polyphenols encapsulated in cubosome nanoparticles prepared via a top-down method was explored. The prepared nanocarriers were examined for their morphology, entrapment efficiency, particle size and stability during *in vitro* digestion as well as potential fortification of yoghurt. Tamarillo polyphenol-loaded cubosomes showed cubic shape with a mean particle size of 322.4 ± 7.27 nm and the entrapment efficiency for most polyphenols was over 50%. The encapsulated polyphenols showed high stability during the gastric phase of *in vitro* digestion and were almost completely, but slowly released in the intestinal phase. Addition of encapsulated tamarillo polyphenols to yoghurt (5, 10 and 15 wt% through pre- and post-fermentation) improved the physicochemical and potential nutritional properties (polyphenols concentration, TPC) as well as antioxidant activity. The encapsulation of tamarillo polyphenols protected against pH changes and enzymatic digestion and facilitated a targeted delivery and slow release of the encapsulated compounds to the intestine. Overall, the cubosomal delivery system demonstrated the potential for encapsulation of polyphenols from tamarillo for value-added food product development with yoghurt as the vehicle.

This chapter is organised as follows: Section 7.1 briefly states the motivations and objectives of this study. Materials and methods are described in Section 7.2. The results and discussion are shown in Section 7.3. A brief summary is included in Section 7.4.

7.1 Introduction

Inverse bicontinuous liquid crystalline nanoparticles, termed cubosomes, have advantageous properties that may be suitable for the delivery of bioactive compounds to the small intestine. Amphiphilic lipids such as the monoglyceride monoolein can self-assemble in water to produce dispersions of cubosomes. The basic structure of a cubosome is a honeycomb-like structure with two non-intersecting internal aqueous channels separated by lipid bilayers. The internal hydrophilic (aqueous) areas are separated by lipid bilayers that are twisted into a tightly packed three-dimensional honeycomb structure which has a high internal surface area to volume. Within this structure, encapsulation of diverse hydrophilic, hydrophobic and amphiphilic compounds of small to large molecular weights, such as proteins, peptides, amino acids, and nucleic acids is possible (Mezzenga et al., 2019). Within cubosomes, hydrophobic molecules can be located within the lipid bilayers, hydrophilic components in the aqueous channels or around the polar head of the lipid, and amphiphilic molecules can be located at the lipid-water interface. This structure generally maintains the efficacy; stability of actives (vitamins and proteins) without adverse effects on the recipient (Barriga et al., 2019). Both polar and non-polar compounds can be solubilized in the water channel and the bilayers, respectively, therefore both can be loaded into these particles. According to Meikle et al. (2021), cubosomes are relatively non-toxic, stable over a broad range of biologically relevant environmental conditions, and can be formulated using a wide array of lipids and stabilizers. Their size, surface charge, and bilayer structure can be tuned through adjustments in their composition. Moreover, previous studies have demonstrated the ability of cubosomes to deliver drugs to both the eukaryotic and prokaryotic cells (Boge et al., 2019a; Kwon, Hong, & Kim, 2012; Luo et al., 2015; Saber, Al-Mahallawi, Nassar, Stork, & Shouman, 2018; Zabara et al., 2019) found in the human digestive system. Cubosome encapsulation has been used to deliver quercetin *in vitro* (Cortesi et al., 2017) and curcumin to the skin (Puglia et al., 2013) and demonstrated improved solubility and availability with an entrapment of 84% and 82%, respectively. Improved anti-inflammatory/antioxidant effects and controlled diffusion of encapsulated curcumin through the skin were observed (Puglia et al., 2013). Cubosome encapsulated has also enhanced the stability and antibacterial activity of curcumin (Archana et al., 2015). Another study had reported successful encapsulation

of both piperine and curcumin in the interior of the cubosome particles (Tu et al., 2014).

Tamarillo (*Solanum betaceum* Cav.), a common fruit of New Zealand, is a good source of polyphenols compounds including anthocyanins, hydroxy benzoic acids, hydroxycinnamic acids, flavonols, flavanols and flavonol glycosides. The main polyphenols in the dried pulp of 'Laird's Large' tamarillo cultivar were identified in our previous study (Diep et al., 2020a) as delphinidin 3-rutinoside (255 mg/100g), pelargonidin 3-rutinoside (201 mg/100g DW), chlorogenic acid (66 mg/100g), kaempferol 3-rutinoside (50 mg/100g) and cyanidin 3-rutinoside (26 mg/100g). These polyphenols are strong antioxidants possessing many potential health benefits such as preventing lipid oxidation and are associated with reduced risk for certain cancers, cardiovascular diseases and type 2 diabetes mellitus (Cory, Passarelli, Szeto, Tamez, & Mattei, 2018). Tamarillo fruit therefore has the potential to be an ingredient in functional food products (Diep et al., 2020d). Evaluation of the stability of polyphenol compounds is important as these compounds are often degraded by oxidation during digestion, resulting in reduced biological activity (Martínez-Ballesta et al., 2018). For example, anthocyanins, present in high amounts in the nutrient-dense tamarillo, are oxidized into quinones, reducing the biological power of these molecules during digestion (Martínez-Ballesta et al., 2018). To overcome this obstacle, new generation - functional foods often use encapsulation technologies to protect polyphenols from degradation as well as maintaining their bioavailability (Martínez-Ballesta et al., 2018).

This chapter aimed to investigate the morphology, entrapment efficiency, and particle size of cubosome-encapsulated tamarillo extract (CUBTAM) and test the stability and antioxidant activity and release of the CUBTAM before and after *in vitro* digestion. *In vitro* digestion of yoghurt fortified with CUBTAM was similarly investigated for the stability and release of polyphenol compounds.

7.2 Materials and methods

7.2.1 Materials

The yoghurt ingredients included standard milk (Anchor™ blue top, Fonterra) from a local supermarket (Auckland, New Zealand) and starter culture containing

Lactobacillus delbrueckii subsp. bulgaricus and *Streptococcus thermophilus* (YoFlex® Express 1.1 powder) from CHR Hansen (Hoersholm, Denmark).

Dimodan® MO 90/D (monoolein) was kindly provided by Danisco (Auckland, New Zealand). Pluronic F127 (PEO_{99}–PPO_{67}–PEO_{99}) was purchased from Sigma-Aldrich (Auckland, New Zealand). The analytical grade standards of phenolics including anthocyanins were obtained from Sigma-Aldrich (Auckland, New Zealand) or Extrasynthese (Genay Cedex, France). Purite Fusion Milli-Q water purifying machine (Purite Limited, Thame, Oxon, UK) was used to produce Milli-Q water. All chemicals and reagents used were AnalaR grade or purer.

7.2.2 Tamarillo extract (EXT) preparation and identification of polyphenol components

Fresh fruits of 'Laird's Large' (red) tamarillo cultivar were collected from growers in the Northland region of New Zealand with commercial maturity of 21 – 24 weeks from anthesis. The fruits were cleaned, peeled, then the pulp was packed in polyethylene bags, sealed and frozen at −18°C and defrosted (15 min) immediately before use. The extraction process was based on the method of Piovesana and Noreña (2018) with some modifications. Aqueous citric acid (2 % w/v) was used for extraction in the ratio of 1:5 (tamarillo : aqueous citric acid, w/w) and homogenised in a blender, and agitated (1000 rpm) at 55°C in a water bath (1000 rpm) (Heidoplph, LABOROTA) for one hour after the addition of (20 μL/100 mL) pectin lyase (Novozym 33095), to improve the extraction of the bioactive compounds. Parafilm and tightly sealed lids of container and centrifuge tube were used to prevent entry of air and oxidation of polyphenols. The mixture was centrifuged at 10,000 RPM for 10 min and the supernatant (tamarillo extract) was separated and stored at –20°C until being injected into LC-MS for identification of polyphenol component as reported in our previous study (Diep et al., 2020a).

7.2.3 Preparation of cubosome and cubosome containing tamarillo extract

The lyotropic liquid cubic phase was prepared and dispersed into cubosomes (CUB) as previously described in previous studies (Boge et al., 2019b; Park & Kim, 2019;

Seo, Kang, Ha, & Kim, 2013). Briefly, molten monoolein (50°C) was mixed with MilliQ water at a mass ratio of 60:40 to form the cubic phase. The cubic phase gel was equilibrated at room temperature for at least 48 hours. Then, 25 mg of cubic phase gel was added to 5 mL of Pluronic F127 solution (2 % (w/v)) and sonicated using a probe sonicator (BEM-150A, Bueno Biotech Ltd, Nanjing, China) (50% amplitude, pulsing 5 s on, 5 s off, for 7 min total run time) to obtain cubosomes (CUB). In parallel, the water was replaced by the tamarillo extract with a mass ratio of 60:40 (monoolein : tamarillo extract) to produce tamarillo polyphenol loaded-cubosomes (CUBTAM).

7.2.4 Polarized light microscopy (PLM) and scanning electron microscopy (SEM)

Microstructure and morphology of CUB and CUBTAM particles were observed using a polarized light microscope (DM750, Leica, Germany) equipped with a digital camera (ICC50HD, Leica, Germany). Dried CUB and CUBTAM were coated by using Platinum (Pt) using a sputtering technique with a Sputter Coater (Hitachi E-1045, Japan) for 60 s at room temperature. The particle morphology was then observed by a scanning electron microscope (Hitachi SU-70 Schottky, Japan) at 25 mA and 10 kV (Wei et al., 2019).

7.2.5 Dynamic light scattering (DLS)

Each sample of the CUB and CUBTAM diluted 20X in MilliQ was measured in triplicate using disposable plastic cuvettes at 25°C Particle size and its distribution were determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, U.K.) The mean *z*-average diameter and polydispersity indices (PDI) were obtained from cumulative analysis using the Malvern software version 7.13. (Boge et al., 2019b).

7.2.6 Determination of the entrapment efficiency (EE)

The sample preparation procedure was applied according to Zhou et al. (2015) with minor modifications. Briefly, 20 μL of CUB or CUBTAM were transferred into a 1 mL Eppendorf microcentrifuge tube and made up to volume with methanol. Then, the sample solutions were centrifuged at 5,000 RPM for 5 min and the supernatant was

separated and transferred to an amber 1.8 mL glass vial, then injected into the LC-MS system.

The phenolic and anthocyanin compounds were analysed by using LC-MS according to our previous study (Diep et al., 2020a) without further modification. Quantification of individual polyphenol was implemented using standard calibration curves fitted with at least six suitable concentrations to obtain a coefficient of correlation > 0.99. According to Patil, Pawara, Gudewar, and Tekade (2019), the entrapment efficiency (EE %) was calculated as follows:

$$EE\% = \frac{A - B}{A} \times 100$$

where A is the polyphenol concentration added into cubosome and B is the polyphenol concentration present in the supernatant

7.2.7 Yoghurt fermentation and fortification with tamarillo polyphenol loaded-cubosome

CUBTAM suspensions from section 7.2.3 were snap frozen by liquid nitrogen, and then lyophilized for 36 h (Alpha 1-2 LD plus Freeze Dryer, Martin Christ, New Zealand). The tamarillo bioactive loaded-cubosome (CUBTAM) powder was stored at –20°C until use.

Kitchen yoghurt makers (Davis & Waddell, Stevens, New Zealand) were purchased to produce the yoghurt. For the control yoghurt, starter culture and milk in the ratio of 0.1 : 100 (w/w), were placed in the yoghurt maker. Incubation was implemented at 45°C for 8 hours until the pH of below 5.0 was obtained. The yoghurt was stored at 4°C overnight and then homogenized at 4000 RPM using laboratory mixer (Silverson, Waterside, England) for 2 min (Wang et al., 2020). The yoghurt was stored at 4°C until further analyses within 24 h.

The CUBTAM powder with concentration of 5, 10 and 15% were fortified into the yoghurt either before (PRE) or after (POS) the fermentation process. For PRE, CUBTAM powder was added to the mixture of milk and starter culture at the start of yoghurt making process, prior to fermentation. For POS, CUBTAM was added to yoghurt in the final homogenization step.

7.2.8 Determination of physicochemical properties of fortified yoghurts

The pH was measured with a digital pH meter to one decimal place. Syneresis index of yoghurt was identified based on method of Wang et al. (2020). Some modifications were made from Kristo et al. (2011) for rheological measurements, using a rheometer (RST-SST, Brookfield Ametek, Middleboro, USA). The viscosity profile (viscosity, consistency coefficient, flow behavior index), was determined with a concentric cylinder with the diameters of cup and bob of 28.92 and 26.66 mm, respectively. Elastic modulus was determined with a vane spindle (SSVANE-) at a speed of 0.5 rpm for 5 min.

TA-XT plus texture analyser (Texture Technologies Corp, New York, USA) was used to perform textural analysis with a backward-extrusion test based on method of Wang et al. (2020) with some modifications. The parameters of test included cylinder probe diameter of 50 mm, test speed of 1.0 mm/s, penetration distance of 25 mm and surface trigger force of 10 g.

7.2.9 *In vitro* digestion

The EXT, CUB, CUBTAM and yoghurt samples fortified with CUBTAM were subjected to *in vitro* digestion to identify the bioavailability of bioactive contents using method of Zhang et al. (2018) without further modification. The sample (2 mL) was collected before digestion and after oral (5 min), gastric (120 min) and intestinal phases (180 min). The samples were snap frozen using liquid nitrogen to stop enzyme activity and centrifuged at 10,000 RPM at 4°C for 10 min. The supernatants were collected and stored at – 20°C before being injected into the LC-MS for phenolic and anthocyanins identification as well as total phenolic content (TPC) and antioxidant activity analysis (Diep et al., 2020a).

7.2.10 Total phenolic content and antioxidant activity

The TPC of extracts and digests at each stage of digestion was determined using Folin-Ciocalteu assay as described in our previous study (Diep et al., 2020a). Two different methods were used to determine the antioxidant activity, namely cupric ion reducing antioxidant capacity (CUPRAC) and ferric-reducing antioxidant power (FRAP) assays

which were mentioned in our previous study (Diep et al., 2020a). Results of TPC and antioxidant activity are presented as mg gallic acid equivalent per 100 gram of tamarillo or yoghurt (mg GAE/100 g) and μmol Trolox equivalent antioxidant capacity per 100 g of tamarillo or yoghurt (μmol TEAC/100 g), respectively.

7.2.11 Statistical analysis

Measurements of all the analytes were undertaken in triplicate, and the results are presented as mean ± standard deviation (SD). For comparison among different samples, one-way analysis of variance (ANOVA) was applied using SPSS 25.0 (IBM Corp., Armonk, NY, USA). Fisher's (LSD) multiple comparison tests were used to determine the magnitude of differences between means. A p value of < 0.05 was considered statistically significant.

7.3 Results and discussion

7.3.1 Characterization of cubosomal suspensions containing tamarillo extract (CUBTAM)

Adapting the temperature-composition phase diagram of a monoolein/water system (Caffrey & Cherezov, 2009), pure monoolein cubosomes (CUB) and tamarillo polyphenols loaded-cubosomes (CUBTAM) were prepared in a top–down approach. This method allows the formation of reproducible, stable cubosomes without adding solvents. Therefore, it is unnecessary to reinvestigate phase behaviour, and there are no solvent concerns for cellular toxicity (Barriga et al., 2019). The concentration of the surfactant F127 was chosen to be 2 wt% which yields stable cubosome dispersions (Flak et al., 2020). The CUB dispersion appeared homogenously milky white and CUBTAM appeared semi-opaque pink (picture not shown).

Figure 7.1 shows the PLM and SEM photos of CUB and CUBTAM while their particle size distribution (PSD) is summarized in Figure 7.2 and Table 7.1. The addition of tamarillo extract did not significantly affect the morphology of cubosome particles. The initial cubic periodicity can be clearly visualized for both samples using PLM and SEM. Because tamarillo extracts are mainly water-soluble compounds (phenolics including anthocyanins), they should be dispersed in the water channel of the

cubosome and should minimally affect the structure of the nanoparticles (Park & Kim, 2019).

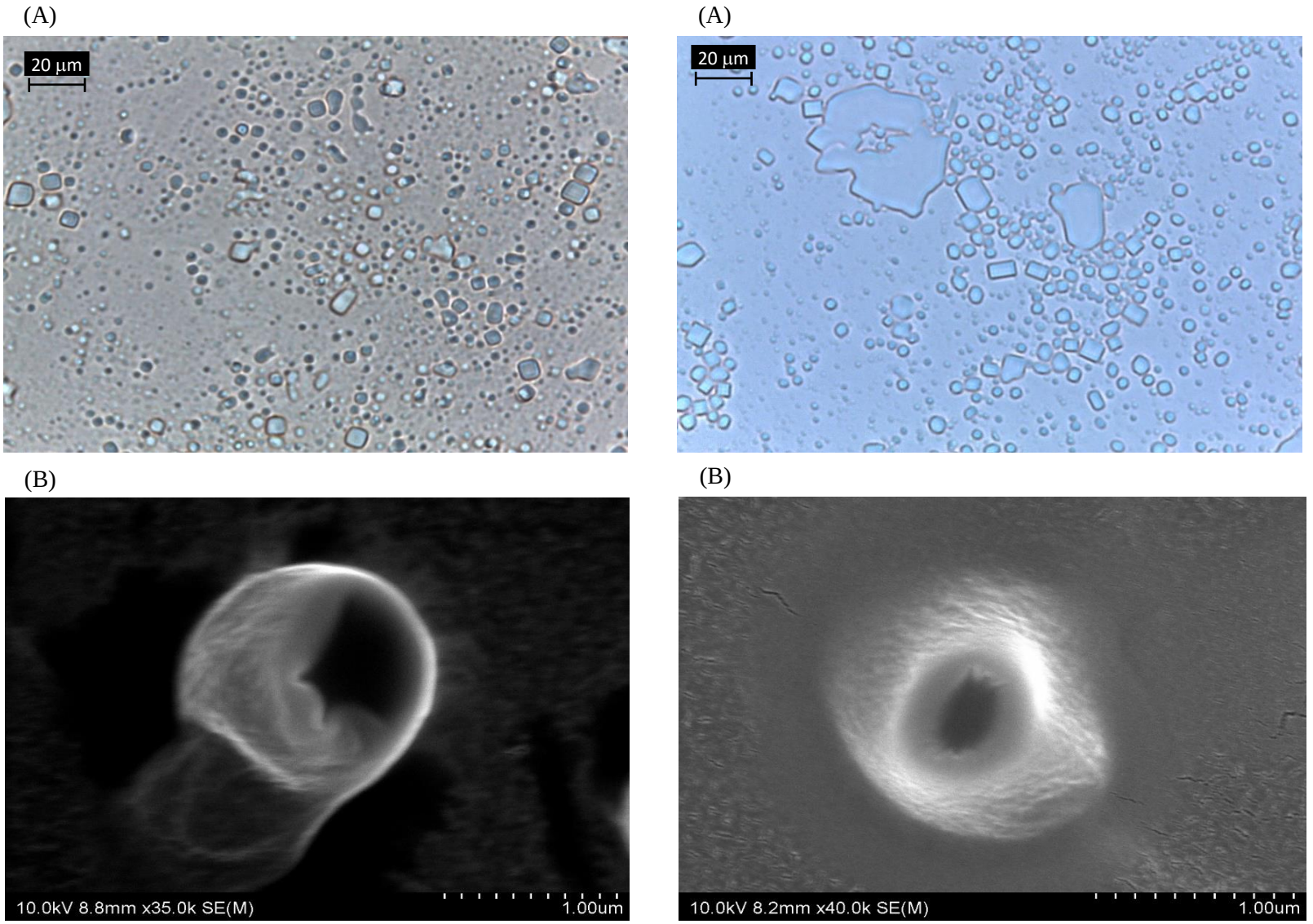

Figure 7.1 (A) PLM and (B) SEM micrographs of cubosome (left) and cubosome containing tamarillo extract (right).

For particle size distribution, CUBTAM had an unimodal curve and its polydispersity index (PDI) was quite small (below 0.3), as shown in Figure 7.2. The mean hydrodynamic diameter of liquid crystal particles increased significantly (from 270 to 327 nm) with the addition of the tamarillo extract. In general, this parameter depends on several factors such as the concentration of amphiphile (lipid and polymer), the presence of charged lipids, the ionic strength and the interactions between groups (Patil et al., 2019). For CUBTAM, the addition of hydrophilic groups contributed to increasing electrostatic interaction as well as the coalescence between colloidal particles resulting in a bigger average particle size. However, most of the cubic particles in CUBTAM were still limited to a sub-micron range (100-1000 nm). According to Danaei et al. (2018) the small particle size and the narrow size distribution (small PDI) creates a large surface area that benefits cellular uptake.

Table 7.1 Particle size and polydispersity index (PDI) of cubosome and cubosome containing tamarillo extract.

Parameters	Cubosomes	Cubosomes containing tamarillo extract
Z-average (nm)	270.9 ± 5.61	322.4 ± 7.27
PDI	0.237	0.272

* Data are presented as Mean ± SD (n ≥ 3). PDI: polydispersity index

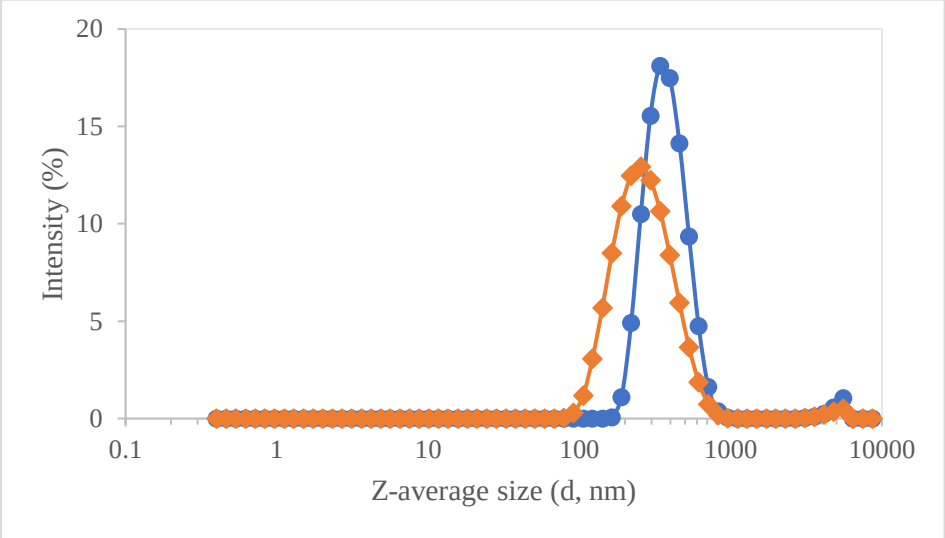

Figure 7.2 Size distribution of cubosome (◆) and cubosome containing tamarillo extract (●).

Entrapment efficiency (EE%) of bioactive compounds from CUBTAM ranged from 19.8 (catechin) to 87.7% (kaempferol 3-rutinoside) (Figure 7.3). Twelve of the fourteen tamarillo bioactive compounds had an EE of more than 50%. Also, it is noteworthy that we show high EE % (> 69%) for the major polyphenols in tamarillo (chlorogenic acid, kaempferol 3-rutinoside, delphinidin 3-rutinoside, cyanidin 3-rutinoside and pelargonidin 3-rutinoside). The high EE in the CUBTAM could be attributed to the fact that polyphenols in tamarillo extract are water-soluble compounds which embed in the water channels. The EE difference between polyphenols encapsulated by cubosome might also depend on the number of -OH groups in molecular structure. For example, the hydroxycinnamic acids chlorogenic acid, caffeic acid and ferulic acid (with > 2 -OH groups) showed higher EE than p-coumaric acid (which has only 1 -OH group). More -OH groups will more easily attach in the aqueous channel of cubosome particles. Also, different polyphenol classes showed different EE. For instance, the hydroxycinnamic acids (chlorogenic acid, caffeic acid and ferulic acid), hydroxybenzoic acid (gallic acid), flavonol glycosides (rutin, kaempferol 3-rutinoside, isorhamnetin 3-rutinoside) and anthocyanins (delphinidin 3-rutinoside, cyanidin 3-rutinoside, pelargonidin 3-rutinoside) showed higher EE than flavanols (catechin, epicatechin). According to Patil et al. (2019), the EE is dependent on particle size rather than the amount of poloxamer (Pluronic F127) used to stabilise the cubosome. The larger the nanoparticles, the higher entrapment efficiency for the polyphenols. This is because surface area to volume ratio of large particles is less than that of smaller particles and exposure to water of active compounds also decreased. Thus, the active compound loss due to diffusion also decreased in larger particles.

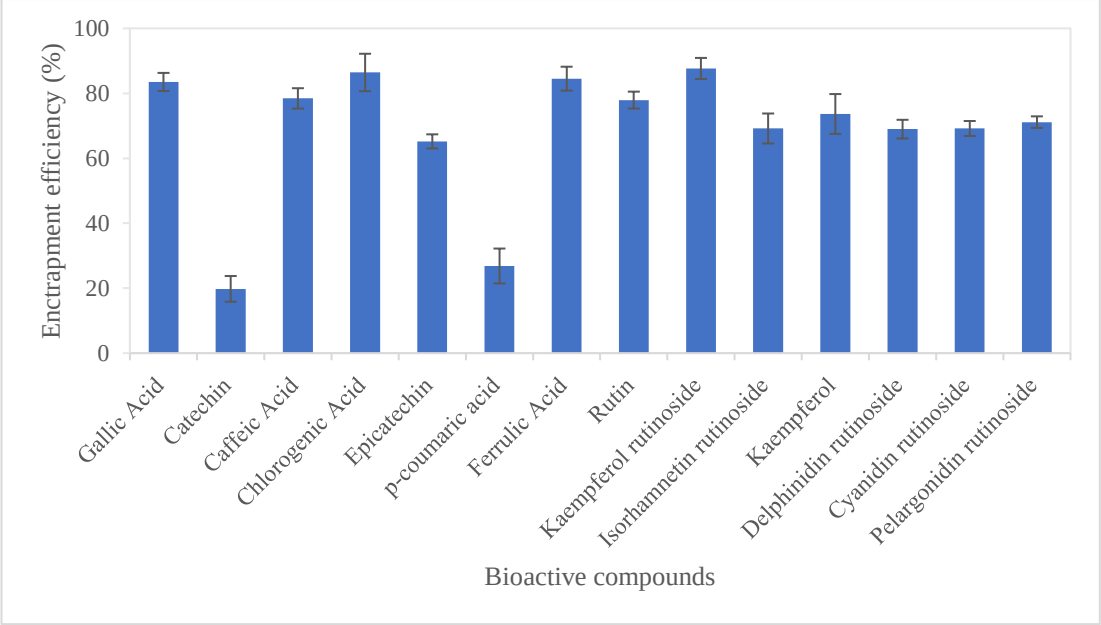

Figure 7.3 Entrapment efficiency of polyphenols from tamarillo extract using lyotropic liquid crystalline nanoparticles (cubosomes).
Data are presented as mean and error bar (standard deviation) (n = 3)

7.3.2 Total phenolic content (TPC) and antioxidant activities of encapsulated and non-encapsulated extracts during *in vitro* digestion

The impact of digestion on TPC and antioxidant activities of EXT and CUBTAM is shown in Figure 7.4. The TPC and antioxidant activities decreased significantly after digestion for both non-encapsulated and encapsulated in comparison to the undigested samples.

For both EXT and CUBTAM, there were significant differences ($p < 0.05$) between the amounts of TPC in the supernatant after each stage of digestion. Gastric digests recorded the highest TPC for EXT, while in the oral and intestinal phases, no significant differences were observed (Figure 7.4A). Low values of TPC in the supernatant of the oral digest (after 2 min of digestion) are related to the short time for diffusion and low solubility of polyphenols. The loss of polyphenols during digestion could be explained by physicochemical transformations, such as oxidation or the presence of yoghurt molecules (fats and proteins) in the digestion mixture. Also, the decrease of bioactive content could arise from precipitation of several compounds with proteins or enzymes in the digest (Ortega, Macià, Romero, Reguant, & Motilva, 2011).

However, for the CUBTAM, the release of polyphenols increased during the digestion and was greater in the intestinal phase than the gastric phase (Figure 7.4B) which was not seen for the non-encapsulated extract, demonstrating a protective effect of the cubosome encapsulation technique against digestive enzymes and pH changes during gastric digestion. Results obtained from LC-MS (Table 7.2) further support the protective effect of the cubosomes on polyphenols. Similar findings have been reported on release properties of encapsulated blueberry extract (Flores, Singh, Kerr, Phillips, & Kong, 2015) and carob pulp extract (Ydjedd et al., 2017) during simulated gastrointestinal digestion. Both studies showed that TPC in the supernatant increased throughout gastric to intestinal digestion. The materials used for lipid bilayer and stabilisation of cubosomes determines susceptibility of polyphenols to digestive enzymes as well as pH at each stage (Saura-Calixto, Serrano, & Goñi, 2007). The reduction in TPC of tamarillo extract during *in vitro* digestion might be associated to sensitivity of phenolic compounds to higher pH (> 6), since at that pH, monomers obtained by hydrolysis from larger molecules might be less stable (Pavan, Sancho, & Pastore, 2014). The increase in the TPC of CUBTAM could be related to the release of complexed bioactive compounds as a result of the digestive process (Da Silva Haas et al., 2019).

The antioxidant activity of tamarillo fruit phenolic extracts is mainly linked to their anthocyanins, chlorogenic acid and kaempferol 3-rutinoside compounds. However, due to the chemical transformations from different mechanisms, the antioxidant properties of these compounds might change during digestion. Thus, the influence of digestion on the antioxidant capacity of tamarillo pulp extracts in non-encapsulation and encapsulation form was assessed by using CUPRAC and FRAP assays (Figure 7.4).

All activities tested significantly ($p < 0.05$) decreased after digestion in comparison to the raw material, which coincide with the decrease in bioactive compounds, mainly polyphenols, after digestion (Table 7.2). There were substantial and significant differences in CUPRAC values between the non-encapsulated and encapsulated extracts ($p < 0.05$) throughout the process of *in vitro* digestion. Tamarillo extract had the highest CUPRAC value in the gastric phase, whereas for CUBTAM, the highest supernatant activity was noted in the intestinal medium. EXT and CUBTAM presented significant differences ($p < 0.05$) in FRAP values during the digestion process. The

highest FRAP value was observed in the oral phase for non-encapsulated extract, whereas for encapsulated samples, this activity increased with the progress of digestion with the highest FRAP activity of the supernatant was recorded at the end of the intestinal phase. In other studies, an increase in the FRAP with digestion was most pronounced at the intestinal phase for both encapsulated blueberry extract (Flores et al., 2015) and carob pulp extract (Ydjedd et al., 2017).

The difference in FRAP and CUPRAC activities, in the oral and intestinal phases, respectively, may not be due to the content of phenolics including anthocyanins, but rather to the diversity and characteristics of the polyphenols present. However, the highest activities (FRAP and CUPRAC) after the gastric phase might be due to the higher release of phenolics including anthocyanins content and the quenching and reducing properties of the acidic medium of the sample. The effect of the pH could also be different among various polyphenols. At neutral pH, some polyphenols have exhibited pro-oxidant activities, whereas at lower pH, others have demonstrated antioxidant activities (Ydjedd et al., 2017). Also, the difference might be related to *in vitro* digestion conditions used and/or change of polyphenol availability related to the release of matrix associated compounds (Ferreyra, Torres-Palazzolo, Bottini, Camargo, & Fontana, 2021). In fact, free polyphenols have shown higher antioxidant activity than iron-phenol chelates. Together with the enzymatic action, the pH influence within the gastrointestinal digestion and the presence of compounds that were not analysed in this study (e.g. peptides or complex polyphenols) enhance antioxidant activity (Da Silva Haas et al., 2019). The increase in antioxidant power of the supernatant between the acidic gastric phase and alkaline intestinal phase environments, as seen in this study, can be partially explained by the deprotonation of the hydroxyl groups on the aromatic rings of the polyphenols (Ferreyra et al., 2021).

Under the intestinal conditions, the decrease in antioxidant activity (CUPRAC and FRAP) for the non-encapsulated extract would be related to the lower TPC alongside transformation of some polyphenols into conformations related to the neutral pH (Figure 7.4A). Meanwhile, the highest antioxidant activities for CUBTAM supernatant, in the intestinal phase (Figure 7.4B) could be explained by their release from the microcapsules as they are degraded in the neutral pH. The weak activities recorded in oral and gastric phases of digestion might be due to a small amount of

polyphenol release from the microcapsule surface and/or via the penetration of salivary and gastric fluids into the microcapsules through their surface pores.

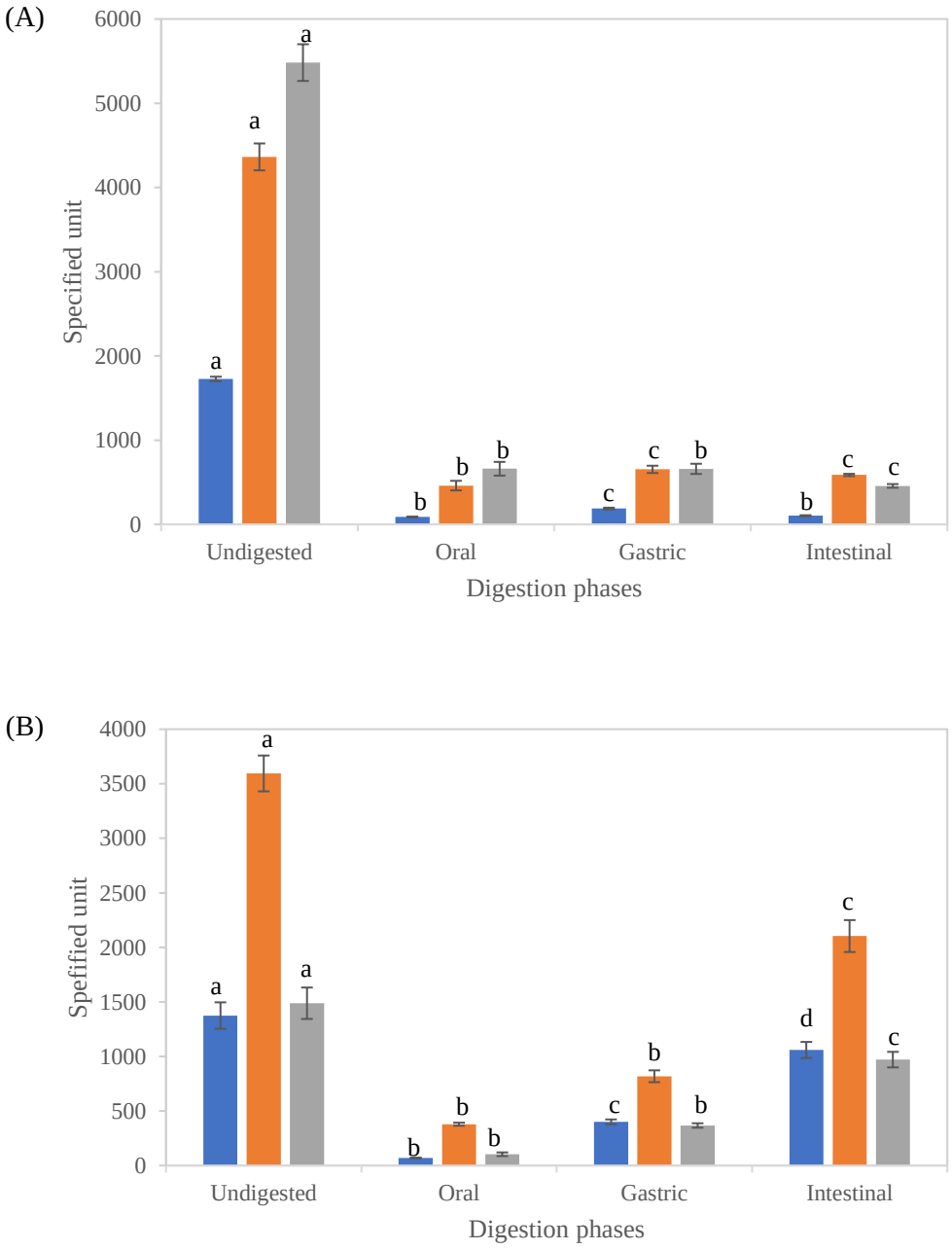

Figure 7.4 Changes in the total phenolic content and antioxidant activities of tamarillo extract (A) and tamarillo polyphenol-loaded cubosome (B) before and after *in vitro* digestion.

The unit of TPC (■), CUPRAC (■) and FRAP (■) were mg GAE /100 g tamarillo, µmol TEAC /100 g tamarillo and µmol TEAC /100 g tamarillo, respectively. Data are presented as mean and error bar (standard deviation) (n = 3). Different alphabets indicate statistical difference ($p < 0.05$) for each assay

7.3.3 *In vitro* release of tamarillo bioactive from cubosomes during digestion

In order to evaluate the stability of individual polyphenol compounds during digestion, a total of fourteen compounds were evaluated by LC-MS (Table 7.2). The CUB was also analysed as a control. The results showed that 11 phenolic compounds and 3 anthocyanins were released from the microcapsules after the digestion process, demonstrating that these phytochemical compounds were well encapsulated by the cubosomes.

The phenolics presented different behaviours during the simulated digestion (Table 7.2). Analysis of phenolics released from EXT during digestion showed a significant instability for the major phenolic acids (gallic acid, chlorogenic acid and *p*-coumaric acid), other phenolics (epicatechin, rutin and kaempferol 3-rutinoside) as well as all anthocyanins after oral and gastric phases. For gallic acid, the concentrations in oral phase remained stable and only a significant ($p < 0.05$) increase was observed in the gastric phase (46.49%) when compared to the initial undigested EXT. Then, the concentration of this acid dropped down to 20.4%. Tagliazucchi, Verzelloni, Bertolini, and Conte (2010) reported the degradation (43%) of pure gallic acid after gastrointestinal digestion, while the total degradation for gallic acid from grape extract and carob pulp extract had been explored by Jara-Palacios, Gonçalves, Hernanz, Heredia, and Romano (2018) and Ydjedd et al. (2017), respectively. Meanwhile, caffeic acid showed insignificant changes during the digestion (24.13% at the oral phase, 31.42% after the gastric phase and 21.94% at the end of the intestinal phase). According to Wojtunik-Kulesza et al. (2020), the remaining percentage of caffeic acid decreased to 75% and 78% after oral and gastric phases, respectively. Some studies have reported that the gastric phase has increased the bioaccessibility of some phenolic acids, while during the intestinal phase, their concentrations could be decreased. This behaviour has been closely related to the stability and structural changes that each type of polyphenolic acid undergoes (Tagliazucchi et al., 2010). Due to its low molecular weight, gallic acid has been better absorbed in humans compared to other phenolic acids, which makes it highly bioaccessible (Ferreyra et al., 2021). For chlorogenic acid, the highest concentration was detected in the gastric phase, then the concentration of this compound reduced by 63% in the intestinal phase (Table 7.2). According to Tagliazucchi et al. (2012), the degradation of chlorogenic acid during

gastro-pancreatic digestion might be due to the oxidation and polymerization to form quinone in an alkaline environment. Significant reductions of free phenolic acids (gallic, chlorogenic, caffeic, *p*-coumaric acids) during *in vitro* digestion have been reported in previous studies (Celep, Charehsaz, Akyüz, Acar, & Yesilada, 2015; Kamiloglu et al., 2017; Sengul, Surek, & Nilufer-Erdil, 2014). These decreases in phenolic acids could be related to changes in pH and the presence of bile salts in the intestinal phase, which may lead to the formation of precipitates (Kamiloglu et al., 2017), which may explain the reductions observed at the end the intestinal phase of this study.

The concentrations of kaempferol 3-rutinoside in EXT remained stable after the oral and gastric phases but decreased significantly ($p < 0.05$) at the end of the intestinal phase (Table 7.2). A similar trend for kaempferol 3-rutinoside during *in vitro* digestion of the Cactus Cladodes plant had been observed (De Santiago, Pereira-Caro, Moreno-Rojas, Cid, & De Pena, 2018). Hydrolysis of glycoside flavonoids starts in the mouth by means of β-glycosidase action but the degree of hydrolysis depends on the types of sugars present in the flavonoid compounds. For example, polyphenol compounds with more hydrophobic properties often interact more strongly with proteins (Wojtunik-Kulesza et al., 2020). Degradation of polyphenols with high molecular weights (such as kaempferol 3-rutinoside) may be related to their strong affinities with human salivary proline- and histidine-rich proteins to form non-covalent and covalent associations (Wojtunik-Kulesza et al., 2020).

All of the anthocyanins, especially delphinidin 3-rutinoside and pelargonidin 3-rutinoside, showed the same releasing behavior during *in vitro* digestion (Table 7.2). For these main anthocyanins in tamarillo extract, significantly ($p < 0.05$) a higher proportion of anthocyanins (43 to 76%) was released after the intestinal phase when compared to the undigested samples. The instability of anthocyanins at neutral or slightly basic pH has been observed for polyphenols from grape and chokeberry (Bermúdez-Soto et al., 2007; Tagliazucchi et al., 2010). The instability can be explained by the formation of a colourless chalcone pseudo-base, resulting in the destruction of the anthocyanin chromophore (McDougall et al., 2005a). The current results support these previous findings, suggesting that anthocyanins are stable in the acidic conditions of the gastric phase but are degraded in the alkaline/neutral conditions of the intestinal phase. The reduction of anthocyanins may also be related

to the fact that in aqueous solution in response to changes in pH, anthocyanins undergo structural rearrangements, change colour, may form complexes with proteins in food and digestate and be degraded to phenolic acids (Kamiloglu et al., 2017).

The quantity of individual bioactive compounds from the CUBTAM at the end of each digestive phase varied by compound (Table 7.2). Catechin, epicatechin, isorhamnetin 3-rutinoside and all anthocyanins (delphinidin 3-rutinoside, cyanidin 3-rutinoside and pelargonidin 3-rutinoside) were released after the gastric phase in acidic medium; gallic acid, caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid, rutin, kaempferol and kaempferol 3-rutinoside were released into the neutral medium after oral and intestinal phases. It is worth noting that the percentage of free polyphenols was lower in CUBTAM (encapsulated) than in EXT (non-encapsulated) one and remained fairly constant along different *in vitro* digestion phases. These results were also expected, because the initial amount of polyphenols in the encapsulated sample was lower owing to the encapsulated efficiency (over 50%). According to Ydjedd et al. (2017), the properties of encapsulating material play a significant role in enhancing the entrapment efficiency and controlled release of the core compounds. They reported a slow release of some phenolics (gallic acid, p-coumaric acid and kaempferol) from the microcapsules and a period of more than 3 h in the intestinal phase (neutral medium) has been necessary for complete release of these compounds, when the encapsulating material was completely degraded (Ydjedd et al., 2017).

The present study is the first to report the proportion of cubosome encapsulated polyphenols released after each phase of *in vitro* digestion, demonstrating the potential of cubosomes to protect bioactive compounds in their matrix. Similarly, reduction of the degradation in cubosome encapsulated bioactive antimicrobial peptide has been reported, showing resistance towards the enzymatic degradation (Boge et al., 2019b). Cubosomes have a high viscosity which hinders the diffusion of polyphenols into the release medium and slows the entry of water which sustains the slow release profile (Zhang et al., 2020). The rate of release controlled by the structure also depends both on the partition coefficient and on the diffusion of the drug through the hydrocarbon tail region (Martiel et al., 2015).

Table 7.2 LC−MS/MS polyphenol profiles of tamarillo pulp extract and tamarillo polyphenol loaded-cubosome after each step of *in vitro* simulated gastrointestinal digestion*.

Bioactive/phases	**Tamarillo extract**			**Tamarillo polyphenol-loaded cubosome**		
	Oral	**Gastric**	**Intestinal**	**Oral**	**Gastric**	**Intestinal**
Phenolics						
Gallic Acid	3.86 ± 0.84^{a}	46.49 ± 6.02^{b}	20.4 ± 1.86^{c}	4.51 ± 0.79^{a}	8.57 ± 0.85^{a}	52.92 ± 11.65^{d}
Catechin	24.51 ± 5.01^{a}	27.18 ± 7.93^{a}	25.39 ± 6.09^{a}	6.37 ± 0.52^{b}	23.53 ± 4.09^{a}	16.31 ± 0.39
Caffeic Acid	24.13 ± 4.55^{a}	31.42 ± 3.90^{b}	21.94 ± 7.06^{a}	81.21 ± 15.17^{c}	2.73 ± 0.91^{d}	8.03 ± 0.35^{e}
Chlorogenic Acid	9.40 ± 1.24^{a}	67.7 ± 12.28^{b}	4.82 ± 1.12^{c}	4.99 ± 0.44^{c}	9.33 ± 0.43^{a}	28.76 ± 4.02^{d}
Epicatechin	24.19 ± 6.22^{a}	55.15 ± 10.33^{b}	13.91 ± 1.10^{c}	9.42 ± 0.49^{d}	16.56 ± 2.62^{c}	16.31 ± 3.66^{ac}
p-coumaric acid	38.82 ± 8.36^{a}	39.39 ± 6.29^{a}	4.14 ± 0.36^{b}	31.12 ± 1.16^{c}	16.16 ± 0.97^{d}	23.2 ± 4.20^{e}
Ferulic Acid	5.55 ± 0.98^{a}	13.37 ± 2.62^{b}	17.79 ± 2.64^{b}	54.38 ± 8.75^{c}	3.42 ± 0.98^{a}	32.31 ± 7.54^{d}
Rutin	30.96 ± 6.09^{a}	38.72 ± 7.40^{a}	22.99 ± 5.96^{b}	16.46 ± 1.39^{bc}	14.69 ± 2.69^{c}	24.02 ± 5.79^{b}
Kaempferol 3-rutinoside	32.07 ± 8.67^{a}	37.61 ± 5.87^{a}	15.53 ± 2.00^{b}	21.00 ± 3.76^{c}	21.47 ± 4.49^{c}	29.06 ± 4.72^{ac}
Isorhamnetin 3-rutinoside	8.21 ± 1.04^{a}	10.29 ± 1.08^{b}	9.81 ± 1.08^{ab}	3.76 ± 0.52^{c}	10.23 ± 2.20^{b}	9.43 ± 1.89^{ab}
Kaempferol	43.84 ± 12.10^{a}	20.35 ± 5.01^{b}	5.83 ± 0.70^{c}	29.59 ± 4.51^{b}	20.22 ± 5.21^{b}	33.06 ± 3.25^{d}
Anthocyanins						
Delphinidin 3-rutinoside	10.11 ± 0.76^{a}	24.61 ± 5.97^{b}	7.87 ± 0.40^{c}	5.30 ± 0.32^{d}	10.28 ± 1.16^{a}	19.41 ± 1.14^{b}
Cyanidin 3-rutinoside	14.26 ± 1.26^{a}	35.94 ± 6.17^{b}	4.31 ± 0.73^{c}	8.68 ± 0.55^{d}	17.05 ± 6.90^{a}	25.18 ± 0.09^{e}
Pelargonidin 3-rutinoside	20.61 ± 1.49^{a}	48.74 ± 5.26^{b}	6.16 ± 0.31^{c}	10.31 ± 1.46^{d}	14.62 ± 2.80^{d}	27.83 ± 1.36^{e}

* Results are expressed as % of starting material. Data are expressed as Mean ± SD (n = 3). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row

7.3.4 Physicochemical properties of yoghurt fortified with CUBTAM

The addition of 5, 10 and 15% CUBTAM to yoghurt was associated with a small but statistically significant fall in pH and reduced syneresis (Table 7.3). This can be explained by the use of the freeze-drying treatment to prepare the powder for the cubosome, which would result in an increase in total dry solids, which in turn would increase the water holding capacity, reduce porosity and reduce the syneresis.

Viscosity of yoghurt increased with the increase of the concentration of CUBTAM, showing significant ($p < 0.05$) differences across the yoghurt samples (Table 7.3). Within the same % CUBTAM fortification, there was no significant ($p > 0.05$) difference in viscosity between En–PRE and En–POS. Based on the Oswald-de Waele power law model, yoghurts fortified with CUBTAM made from both fermentation processes can be considered as non-Newtonian fluids with shear-thinning behaviour due to the flow behaviour index (n) below 1. The breakage of bonds between the protein aggregates as a consequence of shear stress led to the pseudoplastic behaviour of the yoghurt samples (Cui et al., 2014). The consistency index (K) and flow behaviour index (n) of yoghurts were not significantly influenced by the fermentation process; whereas the increase of encapsulated powder concentration led to the increase of K and decrease of n values. The increase of K value might be attributed to the water holding capacity, caused by the addition of powder.

The elastic modulus of all yoghurts was very low, indicating the same relatively weak structure regardless of whether with or without CUBTAM (Table 7.3).

Table 7.3 Physicochemical properties of yoghurts fortified with tamarillo bioactive-loaded cubosome.

Parameters/samples	Control	En–POS5	En–POS10	En–POS15	En–PRE5	En–PRE10	En–PRE15
pH	4.35 ± 0.03^{a}	4.27 ± 0.02^{b}	4.14 ± 0.01^{c}	4.09 ± 0.02^{d}	4.30 ± 0.00^{a}	4.18 ± 0.01^{c}	4.10 ± 0.02^{cd}
Synergies (%)	29.30 ± 0.91^{a}	28.82 ± 1.48^{a}	27.12 ± 1.53^{ab}	26.58 ± 1.91^{b}	28.65 ± 1.36^{a}	27.87 ± 1.70^{b}	26.94 ± 1.52^{b}
Textural parameters							
Firmness (N)	1.027 ± 0.005^{a}	1.031 ± 0.004^{a}	1.034 ± 0.002^{ab}	1.042 ± 0.008^{b}	1.033 ± 0.004^{a}	1.035 ± 0.003^{ab}	1.045 ± 0.006^{b}
Consistency (N.sec)	16.079 ± 0.004^{a}	16.117 ± 0.029^{a}	16.215 ± 0.021^{b}	17.189 ± 0.068^{c}	16.094 ± 0.102^{a}	16.229 ± 0.039^{b}	17.215 ± 0.065^{d}
Cohesiveness (N)	-0.005 ± 0.003^{a}	-0.007 ± 0.002^{a}	-0.011 ± 0.004^{ab}	-0.014 ± 0.005^{b}	-0.008 ± 0.003^{a}	-0.010 ± 0.001^{ab}	-0.016 ± 0.007^{b}
Rheological parameters							
Viscosity constant (K, Pa.s)	$< 0.005^{a}$	0.009 ± 0.003^{b}	0.010 ± 0.003^{b}	0.014 ± 0.007^{b}	0.008 ± 0.020^{b}	0.012 ± 0.005^{b}	0.015 ± 0.008^{b}
Power Law exponent (n)	0.781 ± 0.012^{a}	0.729 ± 0.022^{a}	0.658 ± 0.010^{b}	0.604 ± 0.018^{c}	0.735 ± 0.014^{a}	0.678 ± 0.020^{b}	0.642 ± 0.015^{b}
Viscosity at 350 s^{-1} (Pa.s)	0.026 ± 0.000^{a}	0.030 ± 0.000^{b}	0.033 ± 0.001^{c}	0.041 ± 0.001^{d}	0.032 ± 0.000^{b}	0.037 ± 0.002^{cd}	0.045 ± 0.003^{d}
Elastic modulus (Pa)	N/A	0.003 ± 0.000^{a}	0.005 ± 0.000^{b}	0.005 ± 0.001^{b}	0.003 ± 0.000^{a}	0.004 ± 0.001^{b}	0.006 ± 0.002^{b}

* N/A: not applicable. Data are expressed as Mean ± SD (n = 3). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row. En–POS5, En–POS10, En–POS15: addition of 5, 10, 15% of CUBTAM post to fermentation process, respectively. En–PRE5, En–PRE10 and En–PRE15: addition of 5, 10, 15% of CUBTAM prior to fermentation process, respectively.

7.3.5 Total phenol content, antioxidant activity and release of polyphenol compounds in yoghurt fortified with CUBTAM during digestion

In a yoghurt matrix, catalase and super oxidase enzymes, casein as well as lactic acid bacteria which have antioxidant properties are present (Tavakoli, Hosseini, Jafari, & Katouzian, 2018). Without digestion, as expected addition of CUBTAM led to a dose-dependent increase in TPC and total antioxidant capacity; i.e.. as expected a higher level of fortification led to a higher TPC as well as antioxidant activity ($p < 0.05$). Also, fortification in En–PRE resulted in higher TPC and antioxidant activity than in En–POS at the same concentration (Figure 7.5A).

After the oral phase, TPC and total antioxidant capacity determined using CUPRAC and FRAP assays in both POS and PRE samples at all fortified concentrations reduced by 7.6 – 8.9%, 12.9 – 15.5% and 7.8 – 9.4%, respectively compared to undigested samples (Figure 7.5B). After the gastric phase, significant increases in TPC and total antioxidant capacity were obtained compared to those of the oral phase (up to 3-, 2- and 3-fold, respectively), ($p < 0.05$) (Figure 7.5C). After simulated intestinal digestion further significant increases in TPC (64 – 71%) were observed ($p < 0.05$). Also, total antioxidant activity resulted in an additional 39 – 50% and 63 – 70% increase for CUPRAC and FRAP, respectively (Figure 7.5D). These results were in line with the measures of TPC and antioxidant activity of encapsulated tamarillo extracts (CUBTAM) (Figure 7.4) as well as polyphenol concentration determined by LC-MS (Table 7.2), in which the TPC and antioxidant activity increased greatly during the gastric and intestinal phases of *in vitro* digestion. Previous research showed that the antioxidant activity of the yoghurt samples containing encapsulated phenolics was increased due to the controlled release of the phenolic components from the encapsulation network (Tavakoli et al., 2018). Some researchers have considered the Folin-Ciocalteau assay as an antioxidant capacity test since this assay not only measures phenolic compounds but also the total reducing capacity of a sample, and hence (Kamiloglu et al., 2017). This may explain the difference in polyphenols content measured by LC-MS and by the colorimetric tests. Considering the pH conditions of the total antioxidant capacity assays performed in this study, the CUPRAC assay (pH 7.0) could be more appropriate for evaluating total antioxidant activity after the oral and intestinal phases, while the FRAP assay (pH 3.6) could be more suitable to assess total antioxidant activity after the gastric phase.

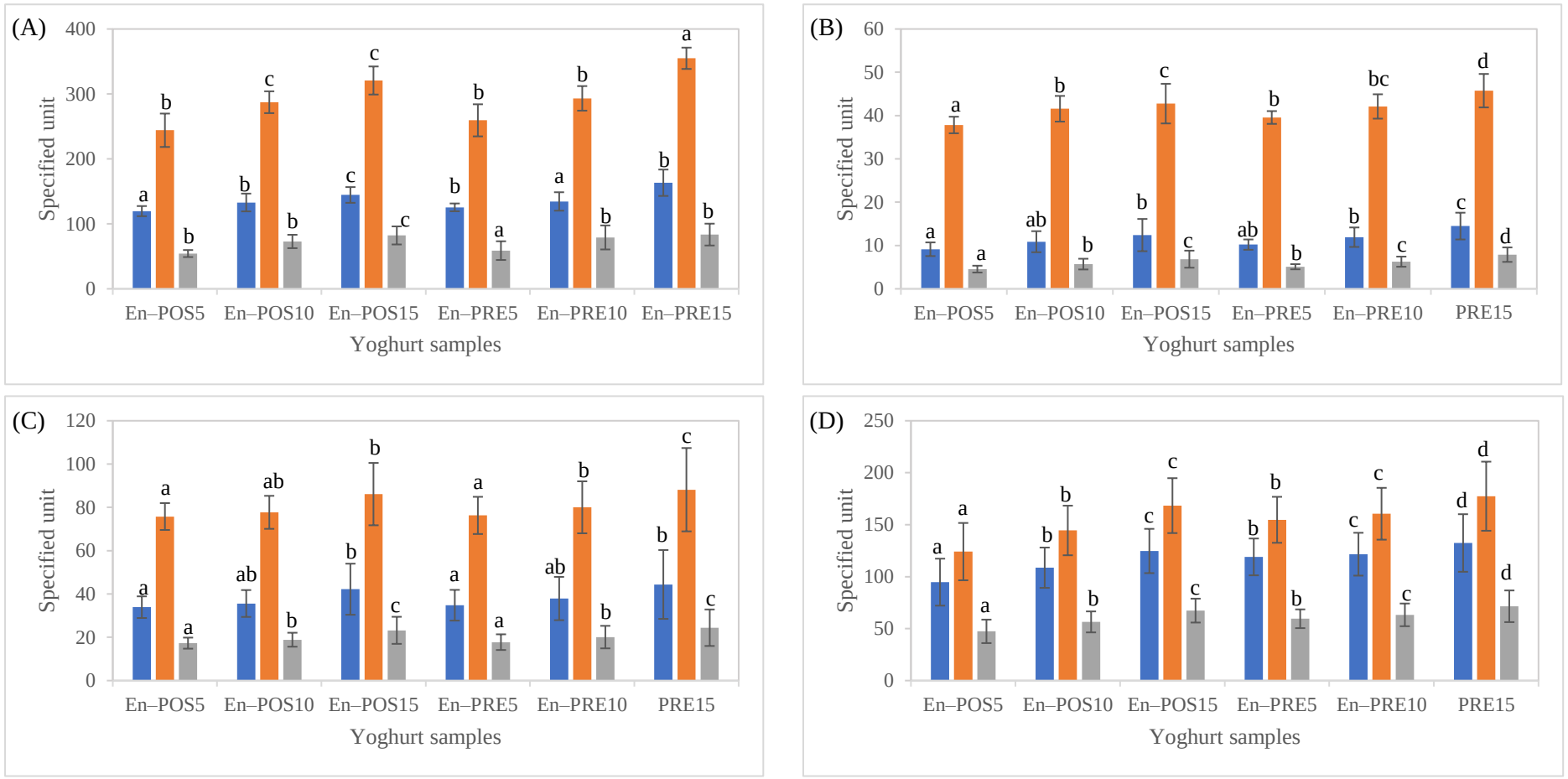

Figure 7.5 Total phenolic content and antioxidant activity of yoghurts fortified with cubosome containing tamarillo extract before digestion (A), after oral (B), gastric (C) and intestinal (D) phases of *in vitro* digestion.

The unit of TPC (■), CUPRAC (■) and FRAP (■) were mg GAE /100 g yoghurt, μmol TEAC/100 g yoghurt and μmol TEAC/100 g yoghurt, respectively. Data are presented as mean and error bar (standard deviation) (n = 3). Different alphabets indicate statistical difference ($p < 0.05$) for each assay

The data in Table 7.4 is described as percentage of each digestion phase in the supernatant compared to the content of the undigested sample. The TPC and antioxidant activities measured by CUPRAC and FRAP between CUBTAM and yoghurt fortified with CUBTAM were relatively similar at each phase of the digestion simulation (Table 7.4), demonstrating that yoghurt was a suitable carrier of cubosome without significant interference. For the oral phase, the TPC released and antioxidant activities in CUBTAM fortified yoghurt were higher than that for the CUBTAM itself by 1.5 – 1.7, 1.2 – 1.5 and 1.1 – 1.4 times, respectively. For the gastric phase, the amount of TPC released for CUBTAM was slightly higher than the fortified yoghurts whereas opposite trend was observed for the antioxidant activities. For the intestinal phase, the TPC and FRAP of CUBTAM were lower than that for CUBTAM fortified yoghurts, whereas for the CUPRAC, the CUBTAM showed similar value to En–PRE5, which were both much higher compared to the rest of the samples. Overall, cubosomes containing tamarillo extract showed effective protection for polyphenols in the oral and gastric phases.

Table 7.4 Measures of total phenolic content and antioxidant activity of supernatant of cubosome encapsulated tamarillo and yoghurt fortified with encapsulated tamarillo during *in vitro* digestion.

Phases	**Samples**	**TPC**	**CUPRAC**	**FRAP**
Oral	CUBTAM	5.07	10.55	6.97
	En–POS5	7.64	15.50	8.38
	En–POS10	8.19	14.47	7.83
	En–POS15	8.59	13.34	8.33
	En–PRE5	8.14	15.25	8.67
	En–PRE10	8.86	14.36	7.93
	En–PRE15	8.87	12.89	9.45
Gastric	CUBTAM	29.13	22.77	24.70
	En–POS5	28.34	31.04	31.84
	En–POS10	26.79	27.05	25.88
	En–POS15	29.20	26.86	28.24
	En–PRE5	27.75	29.41	30.18
	En–PRE10	28.18	27.29	25.39
	En–PRE15	27.19	24.84	29.25

Intestinal	CUBTAM	77.03	58.53	65.27
	En–POS5	79.20	50.83	87.22
	En–POS10	81.72	50.27	77.47
	En–POS15	86.30	52.49	81.92
	En–PRE5	94.90	59.64	101.19
	En–PRE10	90.41	54.74	79.94
	En–PRE15	81.08	49.99	85.62

* Data was presented as % of the sample before digestion. TPC: Total Phenolic Content, CUPRAC: Cupric ion Reducing Antioxidant Capacity, FRAP: Ferric reducing antioxidant power. CUBTAM: tamarillo polyphenols loaded-cubosomes. En–POS5, En–POS10 and En–POS15: yoghurts with addition of 5, 10, 15% of CUBTAM post to fermentation process, respectively. En–PRE5, En–PRE10 and En–PRE15: yoghurts with addition of 5, 10, 15% of CUBTAM prior to fermentation process, respectively.

The differences in the LC-MS profiles between En–PRE and En–POS were significant ($p < 0.05$). Major polyphenols in tamarillo were also detected in CUBTAM fortified yoghurts (both from En–PRE and En–POS) before digestion (Table 7.5). Thus, the yoghurt matrix as well as encapsulation had helped to retain these individual polyphenols during processing. Encapsulation of bioactive compounds promoted lower loss of polyphenols under refrigerated conditions (De Moura et al., 2019). At the same concentration of CUBTAM yoghurt, addition of tamarillo polyphenol loaded-cubosomes prior to fermentation was associated with higher concentration of major polyphenols than addition post fermentation. The concentrations of chlorogenic acid, kaempferol 3-rutinoside and delphinidin 3-rutinoside (accounted for over 65% of the total polyphenol content in yoghurts) were higher for pre-fermentation versus post fermentation approach (Table 7.5). Delphinidin 3-rutinoside was the dominant anthocyanin in fortified yoghurts that was in agreement with the main anthocyanin in ‘Laird’s Large’ tamarillo pulp reported in our previous study (Diep et al., 2020a). The results showed that fermentation process appeared to have little impact on anthocyanins present in both yoghurts which might be due to the encapsulation of anthocyanins.

The yields of polyphenols were associated with the extractability of polyphenols from the original tamarillo extract. According to Sun-Waterhouse et al. (2012), during

fermentation, the yoghurt starter cultures could transform the polyphenols into other forms/types of compounds, e.g., via flavonoid glycosides hydrolysis or C-ring cleavage. Such a conversion may result in the deactivation of bioactive compounds or activation of previously inactive compounds, e.g., polyphenol glycosides are hydrolyzed into their aglycones of higher free radical scavenging ability, and procyanidins break down to flavan-3-ols or to smaller molecular phenolic acids. Acidity of yoghurt may have induced acid hydrolysis of polyphenols, hence this could explain an increased amount of hydroxycinnamates such as caffeic acid, chlorogenic acid, ferulic acid and p-coumaric acid in the fortified yoghurts. Sun-Waterhouse et al. (2013b) stated that the yields of hydroxycinnamic acids and flavonols detected might be dependent on the extractability of these polyphenols from different product matrices.

Despite the importance of the recovery in each digestion phase, bioactive compounds will need to be released from their food matrix and reach the intestine where they can be absorbed and be metabolised (Ribeiro et al., 2021). The release rate during *in vitro* digestion has been considered as an indicator to assess the effectiveness of compound carriers (Remanan & Zhu, 2021). In general, non-encapsulated phenolic compounds in drinking yoghurt were highly degraded after digestion (Altin, Gültekin-Özgüven, & Ozcelik, 2018) while microencapsulated formulation showed ability to preserve the antioxidant activity of extract in yoghurt when compared with the free form (De Moura et al., 2019; Martins et al., 2014). The amount of individual polyphenol in yoghurt samples was significantly different ($p < 0.05$) after each phase of *in vitro* digestion. The quantity of polyphenols released at each stage is dependent on the time at each phase, pH and the concentration of CUBTAM (Table 7.6). Most polyphenols components were detected in both En–PRE and En–POS in each digestion phase. The oral digestion lasts for a few minutes; the encapsulated polyphenol in yoghurts release from oral digestion was significantly lower than the gastric (post to 2h) and intestinal (post to 3h) simulated digests. This data is in line with the findings from Section 7.3.3 that encapsulating polyphenol in cubosome particles could effectively protect the bioactive compounds from the gastric enzymes and facilitate the utilization of polyphenols in human body. Studies on the metabolism of bioactive compounds in the humans have shown that bioactives are mainly metabolised by a large number of small and large intestinal bacteria, and the metabolites are absorbed into the human blood

(Aravind, Wichienchot, Tsao, Ramakrishnan, & Chakkaravarthi, 2021). Therefore, for the polyphenols to be absorbed by the human body or to be active in the microbiome of the small and large intestines, the polyphenols should be protected in encapsulated form until completely released in the intestinal tract. When the polyphenol capsules were present in the simulated oral phase, the concentration of all bioactive compounds was low, indicating good retention in the cubosomes. Also, compared to undigested samples, significantly ($p < 0.05$) lower amount of most polyphenols (percentage loss < 10%) were released during the oral phase (except for epicatechin, rutin and kaempferol 3-rutinoside). The findings provide evidence that cubosomes protect bioactive compounds from interaction with for example milk proteins and digestive enzymes, reducing the risk of polyphenol degradation.

To our knowledge, most of reports of the loads of cubosomes are for proteins or small molecules such as drugs while research about encapsulation of hydrophilic polyphenols, mainly presented in tamarillo, using the cubosome is limited. Effects of the digestion process on properties and stability of cubosome as well as application of cubosome in food are still scarce. Hence, a strength of this study is it is the first attempt to encapsulate polyphenols from tamarillo and has demonstrated the proof of principle that this technique can be used to fortify yoghurt with a fruit extract. Entrapment efficiency was greater than 50% and together with enhancement of antioxidant effects, stability and bioavailability of polyphenols *in vivo* and *in vitro* (Barriga et al., 2019), the results showed potential of cubosome to minimize degradation of polyphenols and contribute to controlled release of these and other bioactives during digestion. Application of encapsulated polyphenols into yoghurt did not significantly change texture and rheology of yoghurts when compared to the control, except for 15% fortification which higher texture and rheology values were observed. However, there are still challenges about applications of cubosomes, including a deeper understanding of the stabilizer and possible cytotoxicity. In the future interaction between yoghurt components (mainly protein), starter culture and encapsulated bioactives should be evaluated with longer intestinal digestion and possible effects on the microbiota explored. According to Wei et al. (2019), monoolein has been easily hydrolysed to free oleic acid due to the presence of pancreatic lipase and bile salt, therefore it can be assumed that cubosome particles (with 60% of monoolein in components) only be degraded in intestinal phase. However, degradation or stability of cubosome after each

phase of digestion should be evaluated to ensure the safety, effectiveness and acceptability to the consumer of this approach. It is a limitation that we did not validate the correct encapsulation of the tamarillo polyphenols in the cubosome. In future work, we could confirm the encapsulation with confocal Raman/FTIR microscopy.

Table 7.5 Concentrations (μg/g yoghurt) of individual polyphenols in yoghurts fortified with CUBTAM before *in vitro* digestion.

Polyphenols/phases	Undigested					
	En–POS5	En–POS10	En–POS15	En–PRE5	En–PRE10	En–PRE15
Phenolics						
Gallic Acid	0.011 ± 0.001^{a}	0.028 ± 0.002^{b}	0.068 ± 0.004^{c}	0.012 ± 0.001^{a}	0.050 ± 0.002^{d}	0.070 ± 0.001^{c}
Catechin	0.002 ± 0.001^{a}	0.004 ± 0.002^{b}	0.005 ± 0.002^{b}	0.004 ± 0.002^{b}	0.004 ± 0.003^{b}	0.004 ± 0.003^{b}
Caffeic acid	0.174 ± 0.032^{a}	0.336 ± 0.091^{b}	0.761 ± 0.168^{c}	0.163 ± 0.008^{a}	0.482 ± 0.078^{b}	0.700 ± 0.027^{c}
Chlorogenic acid	0.848 ± 0.017^{a}	1.696 ± 0.055^{b}	3.392 ± 0.138^{c}	1.275 ± 0.056^{b}	2.646 ± 0.055^{d}	3.821 ± 0.097^{c}
Epicatechin	0.004 ± 0.002^{a}	0.011 ± 0.007^{b}	0.014 ± 0.012^{c}	0.002 ± 0.000^{a}	0.003 ± 0.003^{a}	0.003 ± 0.001^{a}
p-coumaric acid	0.030 ± 0.010^{a}	0.064 ± 0.020^{b}	0.107 ± 0.025^{c}	0.050 ± 0.014^{b}	0.061 ± 0.030^{b}	0.086 ± 0.006^{d}
Ferulic acid	0.037 ± 0.003^{a}	0.106 ± 0.012^{b}	0.132 ± 0.015^{c}	0.053 ± 0.008^{d}	0.162 ± 0.014^{e}	0.159 ± 0.037^{e}
Rutin	0.047 ± 0.014^{a}	0.051 ± 0.023^{a}	0.060 ± 0.031^{a}	0.019 ± 0.007^{b}	0.029 ± 0.015^{b}	0.039 ± 0.012^{c}
Kaempferol 3-rutinoside	0.333 ± 0.062^{a}	0.547 ± 0.009^{b}	0.847 ± 0.023^{c}	0.345 ± 0.018^{a}	0.644 ± 0.017^{b}	0.860 ± 0.032^{c}
Isorhamnetin 3-rutinoside	0.103 ± 0.049^{a}	0.136 ± 0.003^{b}	0.136 ± 0.003^{b}	0.134 ± 0.000^{ab}	0.137 ± 0.004^{b}	0.135 ± 0.001^{c}
Kaempferol	0.058 ± 0.016^{a}	0.236 ± 0.031^{b}	0.363 ± 0.026^{c}	0.216 ± 0.015^{b}	0.348 ± 0.058^{c}	0.597 ± 0.016^{d}
Anthocyanins						
Delphinidin 3-rutinoside	0.171 ± 0.008^{a}	0.249 ± 0.004^{b}	0.419 ± 0.010^{c}	0.156 ± 0.005^{a}	0.378 ± 0.019^{bc}	0.443 ± 0.057^{c}
Cyanidin 3-rutinoside	0.023 ± 0.014^{a}	0.041 ± 0.010^{b}	0.061 ± 0.031^{c}	0.025 ± 0.011^{a}	0.073 ± 0.040^{c}	0.094 ± 0.040^{d}
Pelargonidin 3-rutinoside	0.067 ± 0.019^{a}	0.100 ± 0.035^{b}	0.228 ± 0.120^{c}	0.136 ± 0.060^{b}	0.164 ± 0.082^{bc}	0.283 ± 0.161^{c}

* Data are expressed as Mean ± SD (n = 3). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row. No polyphenols were detected in the control yoghurt. En–POS5, En–POS10, En–POS15: addition of 5, 10, 15% of CUBTAM post to fermentation process, respectively. En–PRE5, En–PRE10 and En–PRE15: addition of 5, 10, 15% of CUBTAM prior to fermentation process, respectively.

Table 7.6 Concentrations (μg/g yoghurt) of individual polyphenols in yoghurts fortified with CUBTAM after each step of *in vitro* digestion.

Polyphenols/phases	Oral					
	En–POS5	En–POS10	En–POS15	En–PRE5	En–PRE10	En–PRE15
Phenolics						
Gallic Acid	0.001 ± 0.000^{a}	0.001 ± 0.000^{a}	0.001 ± 0.001^{a}	0.001 ± 0.000^{a}	$< 0.0005^{a}$	$< 0.0005^{a}$
Catechin	0.001 ± 0.000^{a}	0.001 ± 0.001^{a}	0.001 ± 0.000^{a}	$< 0.0005^{a}$	0.001 ± 0.002^{a}	0.001 ± 0.001^{a}
Caffeic acid	0.008 ± 0.004^{a}	0.012 ± 0.003^{b}	0.052 ± 0.011^{c}	0.009 ± 0.005^{a}	0.018 ± 0.004^{b}	0.020 ± 0.004^{b}
Chlorogenic acid	0.006 ± 0.002^{a}	0.005 ± 0.002^{a}	0.014 ± 0.010^{b}	0.005 ± 0.001^{a}	0.006 ± 0.004^{a}	0.010 ± 0.001^{c}
Epicatechin	0.001 ± 0.000^{a}	0.001 ± 0.000^{a}	0.001 ± 0.001^{a}	0.001 ± 0.000^{a}	0.001 ± 0.001^{a}	0.001 ± 0.000^{a}
p-coumaric acid	0.012 ± 0.005^{a}	0.010 ± 0.002^{a}	0.009 ± 0.006^{a}	0.015 ± 0.006^{b}	0.024 ± 0.011^{bc}	0.037 ± 0.013^{c}
Ferulic acid	0.004 ± 0.003^{a}	0.005 ± 0.001^{a}	0.006 ± 0.002^{a}	0.004 ± 0.001^{a}	0.005 ± 0.002^{a}	0.004 ± 0.002^{a}
Rutin	0.007 ± 0.003^{a}	0.012 ± 0.005^{ab}	0.014 ± 0.005^{b}	0.007 ± 0.003^{a}	0.009 ± 0.005^{a}	0.013 ± 0.007^{ab}
Kaempferol 3-rutinoside	0.101 ± 0.021^{a}	0.217 ± 0.008^{b}	0.349 ± 0.036^{c}	0.124 ± 0.018^{a}	0.250 ± 0.012^{b}	0.354 ± 0.043^{c}
Isorhamnetin 3-rutinoside	0.030 ± 0.001^{a}	0.030 ± 0.000^{a}	0.030 ± 0.001^{a}	0.029 ± 0.000^{a}	0.030 ± 0.001^{a}	0.030 ± 0.000^{a}
Kaempferol	0.001 ± 0.001^{a}	0.002 ± 0.001^{ab}	0.003 ± 0.001^{b}	0.002 ± 0.001^{ab}	0.004 ± 0.003^{b}	0.003 ± 0.001^{b}
Anthocyanins						
Delphinidin 3-rutinoside	n.d	n.d	n.d	n.d	n.d	n.d
Cyanidin3- rutinoside	n.d	n.d	n.d	n.d	n.d	n.d
Pelargonidin 3-rutinoside	n.d	n.d	n.d	n.d	n.d	n.d

* n.d: not detected. Data are expressed as Mean ± SD (n = 3). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row. No polyphenols were detected in the control yoghurt. En–POS5, En–POS10, En–POS15: addition of 5, 10, 15% of CUBTAM post to fermentation process, respectively. En–PRE5, En–PRE10 and En–PRE15: addition of 5, 10, 15% of CUBTAM prior to fermentation process, respectively.

Table 7.6 Concentrations (μg/g yoghurt) of individual polyphenols in yoghurts fortified with CUBTAM after each step of *in vitro* digestion (Cont.)

Polyphenols/phases	Gastric					
	En–POS5	En–POS10	En–POS15	En–PRE5	En–PRE10	En–PRE15
Phenolics						
Gallic Acid	0.006 ± 0.001^{a}	0.011 ± 0.001^{b}	0.021 ± 0.001^{c}	0.002 ± 0.001^{d}	0.018 ± 0.001^{c}	0.023 ± 0.002^{c}
Catechin	0.002 ± 0.002^{a}	0.002 ± 0.000^{a}	0.001 ± 0.001^{a}	0.001 ± 0.000^{a}	0.002 ± 0.001^{a}	0.002 ± 0.001^{a}
Caffeic acid	0.040 ± 0.002^{a}	0.119 ± 0.003^{b}	0.191 ± 0.010^{c}	0.015 ± 0.001^{d}	0.112 ± 0.004^{b}	0.147 ± 0.007^{e}
Chlorogenic acid	0.234 ± 0.088^{a}	0.256 ± 0.116^{a}	0.425 ± 0.171^{b}	0.113 ± 0.004^{c}	0.129 ± 0.028^{c}	0.161 ± 0.048^{ac}
Epicatechin	0.001 ± 0.001^{a}	0.004 ± 0.001^{b}	0.008 ± 0.003^{c}	0.002 ± 0.001^{a}	0.001 ± 0.000^{a}	0.002 ± 0.001^{ab}
p-coumaric acid	0.022 ± 0.010^{a}	0.051 ± 0.020^{b}	0.049 ± 0.021^{b}	0.009 ± 0.005^{c}	0.009 ± 0.006^{c}	0.012 ± 0.006^{c}
Ferulic acid	0.018 ± 0.004^{a}	0.038 ± 0.013^{b}	0.043 ± 0.022^{bc}	0.034 ± 0.015^{b}	0.059 ± 0.013^{c}	0.106 ± 0.014^{d}
Rutin	0.013 ± 0.005^{a}	0.014 ± 0.004^{a}	0.019 ± 0.009^{b}	0.013 ± 0.007^{a}	0.011 ± 0.006^{a}	0.015 ± 0.006^{a}
Kaempferol 3-rutinoside	0.243 ± 0.012^{a}	0.454 ± 0.174^{b}	0.529 ± 0.204^{bc}	0.292 ± 0.039^{a}	0.517 ± 0.096^{b}	0.637 ± 0.026^{c}
Isorhamnetin 3-rutinoside	0.060 ± 0.000^{a}	0.065 ± 0.000^{a}	0.065 ± 0.001^{a}	0.061 ± 0.001^{a}	0.065 ± 0.000^{a}	0.064 ± 0.001^{a}
Kaempferol	0.018 ± 0.002^{a}	0.032 ± 0.020^{b}	0.100^{c} ± 0.010^{c}	0.032 ± 0.006^{b}	0.044 ± 0.020^{d}	0.035 ± 0.014^{bd}
Anthocyanins						
Delphinidin 3-rutinoside	0.085 ± 0.018^{a}	0.114 ± 0.018^{b}	0.251 ± 0.003^{c}	0.068 ± 0.038^{a}	0.152 ± 0.066^{b}	0.251 ± 0.008^{c}
Cyanidin 3-rutinoside	n.d	0.001 ± 0.000^{a}	0.009 ± 0.003^{b}	n.d	0.022 ± 0.013^{c}	0.060 ± 0.035^{d}
Pelargonidin 3-rutinoside	0.014 ± 0.002^{a}	0.022 ± 0.001^{b}	0.070 ± 0.007^{c}	0.041 ± 0.005^{d}	0.097 ± 0.001^{e}	0.110 ± 0.006^{f}

* n.d: not detected. Data are expressed as Mean ± SD (n = 3). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row. No polyphenols were detected in the control yoghurt. En–POS5, En–POS10, En–POS15: addition of 5, 10, 15% of CUBTAM post to fermentation process, respectively. En–PRE5, En–PRE10 and En–PRE15: addition of 5, 10, 15% of CUBTAM prior to fermentation process, respectively.

Table 7.6 Concentrations (μg/g yoghurt) of individual polyphenols in yoghurts fortified with CUBTAM after each step of *in vitro* digestion (Cont.)

Polyphenols/phases	Intestinal					
	En–POS5	En–POS10	En–POS15	En–PRE5	En–PRE10	En–PRE15
Phenolics						
Gallic Acid	0.004 ± 0.002^{a}	0.016 ± 0.001^{b}	0.025 ± 0.001^{c}	0.002 ± 0.001^{a}	0.014 ± 0.004^{b}	0.021 ± 0.005^{c}
Catechin	0.004 ± 0.003^{a}	0.003 ± 0.001^{ab}	0.004 ± 0.003^{a}	0.002 ± 0.001^{b}	0.002 ± 0.000^{b}	0.002 ± 0.001^{b}
Caffeic acid	0.042 ± 0.003^{a}	0.266 ± 0.066^{b}	0.494 ± 0.052^{c}	0.055 ± 0.011^{a}	0.268 ± 0.088^{b}	0.458 ± 0.043^{c}
Chlorogenic acid	0.342 ± 0.078^{a}	0.531 ± 0.111^{b}	0.694 ± 0.102^{c}	0.280 ± 0.029^{a}	0.560 ± 0.032^{b}	0.667 ± 0.189^{c}
Epicatechin	0.003 ± 0.001^{a}	0.002 ± 0.002^{a}	0.004 ± 0.002^{b}	0.003 ± 0.001^{a}	0.003 ± 0.002^{a}	0.003 ± 0.003^{b}
p-coumaric acid	0.042 ± 0.016^{a}	0.044 ± 0.010^{a}	0.063 ± 0.009^{b}	0.022 ± 0.011^{c}	0.034 ± 0.013^{bc}	0.044 ± 0.012^{a}
Ferulic acid	0.025 ± 0.011^{a}	0.031 ± 0.014^{ab}	0.043 ± 0.014^{b}	0.014 ± 0.006^{c}	0.053 ± 0.017^{b}	0.095 ± 0.018^{d}
Rutin	0.019 ± 0.008^{a}	0.014 ± 0.007^{a}	0.026 ± 0.010^{b}	0.018 ± 0.008^{a}	0.014 ± 0.005^{a}	0.042 ± 0.011^{c}
Kaempferol 3-rutinoside	0.174 ± 0.016^{a}	0.463 ± 0.021^{b}	0.725 ± 0.033^{cd}	0.267 ± 0.020^{a}	0.600 ± 0.128^{c}	0.755 ± 0.136^{d}
Isorhamnetin 3-rutinoside	0.031 ± 0.001^{a}	0.037 ± 0.000^{b}	0.038 ± 0.001^{b}	0.032 ± 0.000^{a}	0.037 ± 0.000^{b}	0.037 ± 0.001^{b}
Kaempferol	0.013 ± 0.003^{a}	0.042 ± 0.007^{b}	0.065 ± 0.013^{c}	0.032 ± 0.003^{d}	0.061 ± 0.010^{c}	0.098 ± 0.012^{e}
Anthocyanins						
Delphinidin 3-rutinoside	0.030 ± 0.015^{a}	0.178 ± 0.038^{b}	0.249 ± 0.009^{c}	0.032 ± 0.006^{a}	0.099 ± 0.036^{d}	0.314 ± 0.099^{e}
Cyanidin 3-rutinoside	0.003 ± 0.001^{a}	0.006 ± 0.002^{b}	0.013 ± 0.001^{c}	$< 0.0005^{d}$	0.018 ± 0.001^{e}	0.016 ± 0.002^{e}
Pelargonidin 3-rutinoside	0.054 ± 0.031^{a}	0.088 ± 0.029^{b}	0.134 ± 0.054^{c}	0.057 ± 0.032^{a}	0.065 ± 0.039^{ab}	0.148 ± 0.045^{c}

* Data are expressed as Mean ± SD (n = 3). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row. No polyphenols were detected in the control yoghurt. En–POS5, En–POS10, En–POS15: addition of 5, 10, 15% of CUBTAM post to fermentation process, respectively. En–PRE5, En–PRE10 and En–PRE15: addition of 5, 10, 15% of CUBTAM prior to fermentation process, respectively.

7.4 Conclusion

This study demonstrated the proof-of-principle that tamarillo polyphenols could be effectively encapsulated by cubosome nanoparticles with relatively high loading efficiency and preservation of high antioxidant activity. Compared to the unencapsulated extract, cubosomal encapsulation provided a protective effect to the tamarillo polyphenols under simulated gastrointestinal conditions, exhibiting good free polyphenol concentrations at the end of the intestinal phase. A cubosomal system was employed for the delivery of tamarillo polyphenols via yoghurt and the addition of encapsulated bioactive improved the physicochemical and nutritional properties of yoghurt. The addition of CUBTAM at increasing concentrations successfully increased the concentration of polyphenols, TPC and antioxidant activity of yoghurts, with controlled stability during digestion suggesting that polyphenols with enhanced bioavailability could be delivered in a dose-controlled manner. This research informs application of cubosome encapsulation to fortification of food products, for example both water-soluble and lipid-soluble vitamins, carotenoids (β-carotene). However, although the components of cubosomes (monoolein and Pluronic F127) are listed as generally recognised as safe (GRAS) by the FDA and approved in principle, further investigations should be carried out before sensory testing or consumption by humans as a food.

Chapter 8: Discussion, major conclusions and future prospects

In New Zealand, tamarillo fruits are grown in relatively small quantities in New Zealand but as a fruit it has high nutrient density and bioactive phytochemical content. This thesis has explored the potential of the addition of dried tamarillo powder as a functional ingredient in yoghurt. The research questions asked were:

What are the physicochemical characteristics of New Zealand grown tamarillo and can a yoghurt fortified with freeze-dried tamarillo be developed that possesses the potential bioactive properties of fresh fruit after formulation and after *in vitro* digestion? Further characterization of the fortified yoghurt could be performed to examine the nutritional and other health benefits? Does the encapsulation of tamarillo powder enhance the stability and bio-accessibility after digestion of bioactive compounds in tamarillo yoghurt and maintenance of texture compared to yoghurt fortified with unencapsulated tamarillo powder?

The gap in knowledge addressed was to provide evidence for the potential of dried tamarillo powder as a functional ingredient in food product development as case study of yoghurt.

Key findings/New knowledge:

The key findings of this body of work are that tamarillo peel and pulp contain antioxidant vitamins, bioactive compounds and distinct volatiles (Chapter 2) and the freeze-dried tamarillo powder is a potential ingredient to fortify yoghurt with these components (Table 8.1.). This is because of 1. Nutrients and phytochemicals, 2. Physicochemical properties and 3. Volatile characteristics

Table 8.1 Summary of key findings and new knowledge of this thesis.

Chapter	Novel techniques applied/questions	Confirmed	New knowledge
2. Review & investigation of tamarillo (Contributed to 5 papers)	• PMP derivatization coupled with LC-MS for sugar analysis • Accutag derivatization coupled with LC-MS/MS for amino acid analysis • Water-based solution for anthocyanin extraction • TD to separate volatiles and measure with GC-MS	• Good source of protein, fibre, vitamin C, β-carotene, α-tocopherol and phenolics including anthocyanins • Differences between red and gold cultivars • High in protein (for a fruit) • High TPC and antioxidant activity • Strong correlation between TPC and antioxidant activity	• Nutritional adequacy score of 7.9 (gold) and 7.4 (red) • Compared compositions of 3 cultivars (red, purple red, yellow/gold) of tamarillo • Macro nutrients: total protein, carbohydrate, fibre • Profiled sugars, free amino acids including GABA, phenolics, anthocyanins, carotenoids, volatiles • Phytochemical activity antioxidants including flavanols, flavonol glycosides and volatiles • Differences in peel and pulp. Peel showed higher TPC and antioxidant activity than pulp • High RDI (β-carotene and vitamin C) and AI (α-tocopherol)
3. Review of yoghurt & fortification	N/A		
4. Physicochemical properties of tamarillo yoghurt (1 paper published)	Add tamarillo before or after fermentation		• Higher elastic modulus, PUFAs, pro-vitamin A content and vitamin C retention in POS than in PRE samples • More yellow color, higher tocopherol concentration in POS than in PRE • Better syneresis – yoghurt thickened with addition of tamarillo • Higher concentrations of antioxidant vitamins than the commercial premium-assorted fruits yoghurt
5. Digestion of tamarillo yoghurt (1 paper published)	Bioavailability after oral, gastric and intestinal digestion		• Polyphenols originated from fruits are retained • Peptides and proteins may be better digested and absorbed in the gastro-intestinal tract • high bioaccessibility of polyphenols

6. Review of novel encapsulation	N/A	
7. Encapsulation of polyphenols & application into yoghurts (1 paper published)	• Polyphenols extracted then encapsulated and applied into yoghurt • Polyphenol stability during digestion	• Proof of principle: Phytochemical activity of extract preserved • encapsulation of tamarillo polyphenols in against pH changes and enzymatic digestion and a slow release and targeted delivery

GC: Gas chromatography; LC: liquid chromatography; MS: mass spectrometry; TDU: Thermal desorption; TPC: total phenolic content; GABA: γ-Amino-n-butyric acid; PMP: Phenylmethylpyrazolone; Accutag: 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate; RDI: Recommended dietary intake; AI:Adequate Intake; PUFAs: polyunsaturated fatty acids; POS: tamarillo powder was added post-fermentation; PRE: tamarillo powder was added pre-fermentation; N/A: not applicable

Discussion:

1. Main components and bioactive compounds in tamarillos that may benefit human health:

The current study showed comprehensive physicochemical and bioactive compositions in different tamarillo cultivars as well as tissues compared to previous studies. This study showed comprehensive sugar composition in tamarillo (section 2.4.2) than previous studies which have mainly reported glucose, fructose and sucrose (Acosta-Quezada et al., 2015; Lister et al., 2005; Vasco et al., 2009). Known as tree tomato, tamarillo owned high amount of GABA since this fruit has the same *Solanum* genus with tomato. The current study has applied the accutag derivatization method to determine 25 amino acids in which 2 EFAAs and 5 NEFAAs in tamarillo were firstly reported herein (section 2.4.3).

The determination of carotenoid compounds in tamarillo was important to New Zealand to have a local source of foods with high carotenoid content. This was because 80% of participants having less than the optimal carotenoid concentrations for health have been recently reported (Rush, Amoah, Diep, & Jalili-Moghaddam, 2020). Tamarillo fruit could make a remarkable contribution to the daily intake of vitamins A, E and C with high AI and RDI values of α-tocopherol and β-carotene, respectively (FSANZ labelling) (section 2.4.4 and 2.4.5). The equivalent of one serving of tamarillo consumed each day would provide these vitamins in quantities that would improve nutrition and promote health. Novel food additives can be produced from extracts of tamarillo peel which can improve the functional qualities of food as well as reduce food waste.

Together with known phenolic compounds reported in previous studies, the current study has explored other six phenolics for the first time with high concentration of kaempferol 3-rutinoside. The concentration of various anthocyanins in different tamarillo New Zealand grown cultivars has not been reported, hence this study will fill the missing gap from previous studies (section 2.4.6, 2.4.7 and 2.4.8). Tamarillo can be potential for further utilisation in food and pharmaceutical industries due to presence of these bioactive compounds (phenolics including anthocyanins). Owing certain phenolic compounds or other components which have stronger reducing properties such as carotenoids, phenolics, anthocyanins and ascorbic acid; tamarillo

showed higher antioxidant capacity than other fruits (tomato and cherry tomato, apple, orange, red grape, kiwifruit, pineapple).

The current study reports a comprehensive diversity of volatiles in tamarillo (section 2.4.10) compared to previous studies. This is the first study comparison volatile component between peel and pulp of tamarillo has attempted to contribute to the knowledge of volatile properties of this fruit. There is still limited information about volatile profile of peel and its contribution to total flavour of tamarillo. Results showed that peels also contained significant amounts of volatiles, especially key compounds of tamarillo flavour, suggesting that this tissue also had a contribution to overall flavour quality. Although the peel is often discarded as by-product, it might be used as a flavour enhancer in lyophilized or preserved form.

2. Tamarillo fortify yoghurt to increase nutritional values and enhance physiochemical characteristics:

Yoghurt samples fortified with tamarillo powder were produced in pre- and post-fermentation processes to achieve the potential health benefits (Chapter 4 and Figure 8.1). The increase in protein, carbohydrate and fibre contents in the yoghurt was mainly contributed by the tamarillo powder. The addition of tamarillo powder significantly decreased pH, syneresis and increased the viscosity, firmness, consistency and cohesiveness of the fortified yoghurts which may be more appealing to the consumer. The addition of tamarillo powder significantly increased the UFA contents, especially for the observed PUFA concentrations with respect to the control. The use of tamarillo powder as a promising fortification source of omega-3 and omega-6 FAs as well as PUFA content.

A firmer yoghurt of lower deformability, higher elastic behavior and viscosity as well as higher antioxidant vitamins (α-tocopherol, β-carotene and ascorbic acid) were observed through addition of tamarillo resulted. The red color of the tamarillo was preserved, and the vitamin C content was retained in fortified yoghurts by the addition of powder after fermentation. The flavour characteristics of enriched yoghurts were preserved as the originated fruit. Results from the current study showed a potential for fortifying yoghurt with tamarillo, where improvements in flavor and nutritional properties were observed. Fortification with tamarillo may be applicable to other products where natural red color from anthocyanins will be favored or acceptable. Use

of tamarillo powder enhanced the fibre, volatile, fatty acid and vitamin compositions of yoghurt, and tamarillo-flavoured yoghurt may be offered for consumers as an alternative type of flavoured yoghurt.

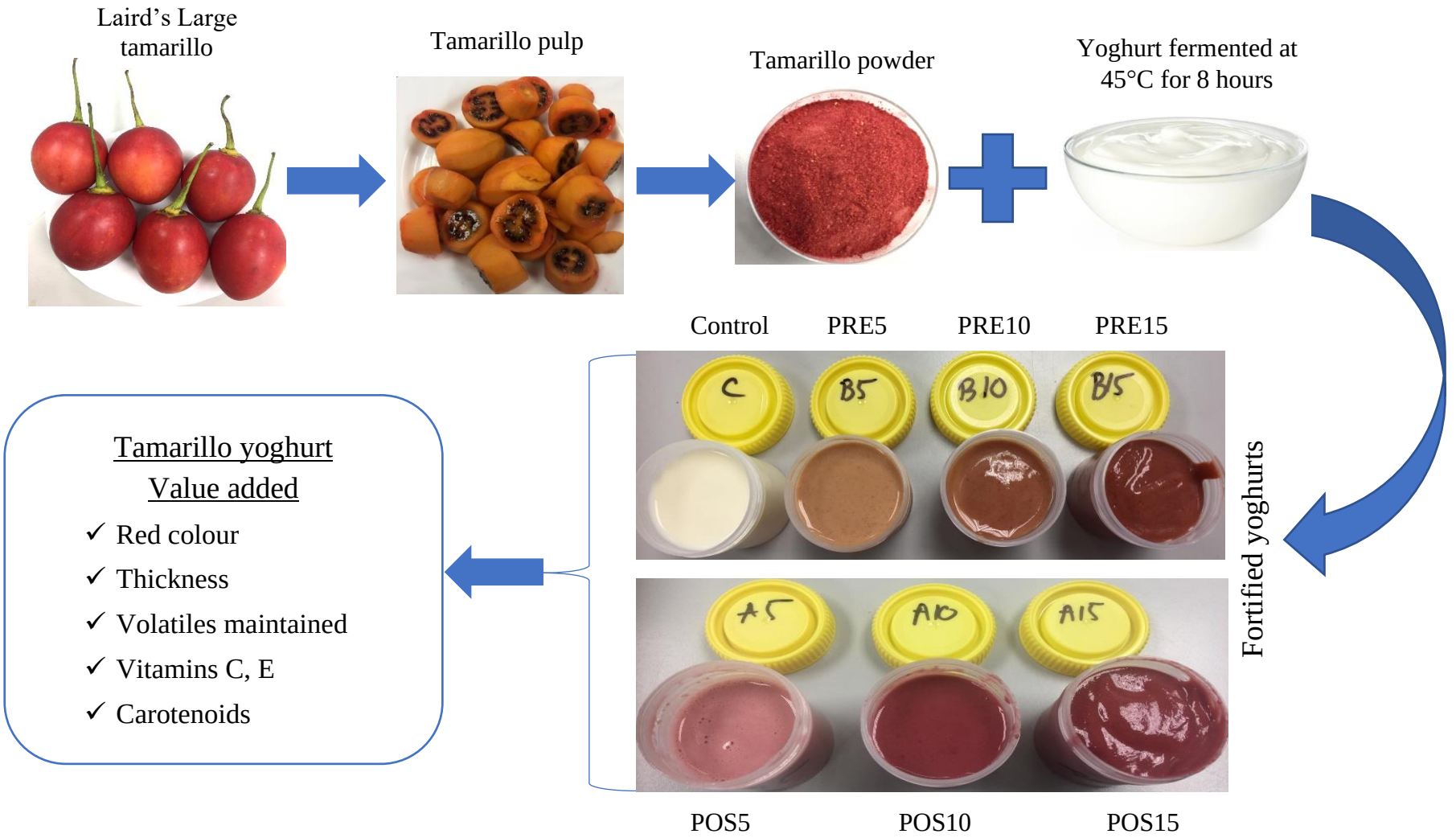

Figure 8.1 Schematic outline for fortifying tamarillo powder into yoghurt through pre- and post-fermentation processes and nutrient value added

Tamarillo powder was fortified into yoghurt as a functional ingredient and main bioactive compounds originated from fruits were retained (Chapter 5). The outline for effect of *in vitro* gastrointestinal digestion on amino acids and polyphenols, of tamarillo yoghurts was shown in Figure 8.2. Tamarillo pulp powder can be utilized as added essential amino acids, GABA and polyphenols in yoghurt. Tamarillo fortified yoghurt displayed higher essential amino acids; total phenolic content antioxidant activity compared to plain yoghurt. The tamarillo yoghurts produced from post-fermentation (POS) showed higher GABA concentration and lower TPC than the tamarillo yoghurts produced from pre-fermentation (PRE); while relative similar antioxidant activity between POS and PRE samples were observed for 10 and 15% tamarillo added. *In vitro* digestion of the tamarillo yoghurt resulted in the release of free amino acids (FAAs) indicating the main role of pepsin enzymes (gastric) was to break down large proteins into smaller fragments which would be ready for the more complete digestion by pancreatic enzymes (intestine). Relatively high bioaccessibilty of chlorogenic acid and kaempferol 3-rutinoside in PRE samples demonstrated that yoghurt matrix allowed the protection of some compounds from degradation, having become bio-accessible and making absorption and utilization possible. In fortified yoghurts, the evaluation of bioactive compounds released from the yoghurt and stable in the digestive environment, thus able to exert their biological effects on the gastrointestinal system, is more important than the content of these compounds in the corresponding food. These findings showed the influence of *in vitro* digestion on antioxidant capacity and polyphenols recovery in fortified yoghurts and may help in the design of dairy products with better functional quality.

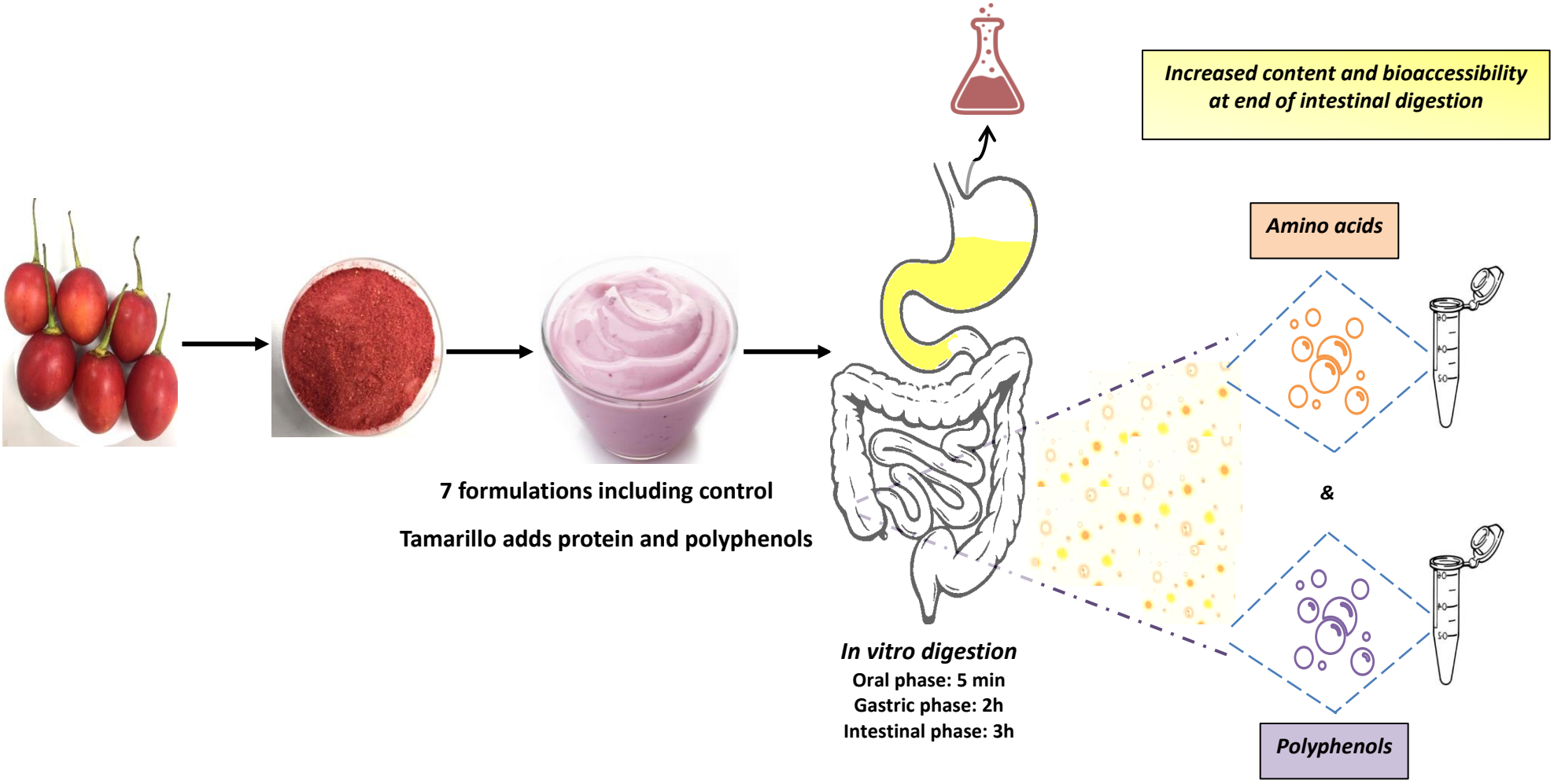

Figure 8.2 Schematic outline for effect of *in vitro* gastrointestinal digestion on amino acids, polyphenols, and antioxidant capacity of tamarillo yoghurts (graphics sourced from creative commons sources).

3. Encapsulation enhance stability and bio-accessibility of bioactive compounds in tamarillo yoghurt with an acceptance of texture:

Figure 8.3 showed diagram of encapsulating tamarillo bioactive using cubosome and application of tamarillo bioactive loaded-cubosomes into yoghurts. Tamarillo bioactives could be effectively encapsulated by cubosome nanoparticles with encapsulation efficiency over 50%. The characterization of the cubosome containing tamarillo extract suggested cubosomal structures as a successful technique for encapsulation. Compared to the unencapsulated extract, cubosomal encapsulation provided a protective effect on the tamarillo polyphenols under simulated gastrointestinal conditions, exhibiting good and sustained release characteristics. As a result, the nanoparticles containing tamarillo extract performed well in antioxidant tests, further suggesting that the cubosomal particle system can be used as carriers of bioactive compounds to enhance the health benefits of food products.

Cubosomal systems were employed for the delivery of tamarillo polyphenols via yoghurt and addition of encapsulated polyphenols improved the physicochemical and nutritional properties of yoghurt as well as successfully increased the concentration of polyphenols, TPC and antioxidant activity of yoghurts. It was observed that stability of polyphenols in yoghurt samples showed great results after *in vitro* digestion regarding total phenolics and antioxidant capacity. The encapsulation of tamarillo polyphenols showed a protective effect against pH changes and enzymatic activities along digestion, thereby promoting a controlled release and targeted delivery of the encapsulated compound, which contributed to an increase in its bioaccessibility in the yoghurt. In addition, each individual bioactive compound of yoghurts in cubosomal system showed the good stability after *in vitro* digestion. Cubosomes can, therefore, be considered an excellent carrier of active ingredients, and cubosome-based encapsulation has potential in the development of functional yoghurts.

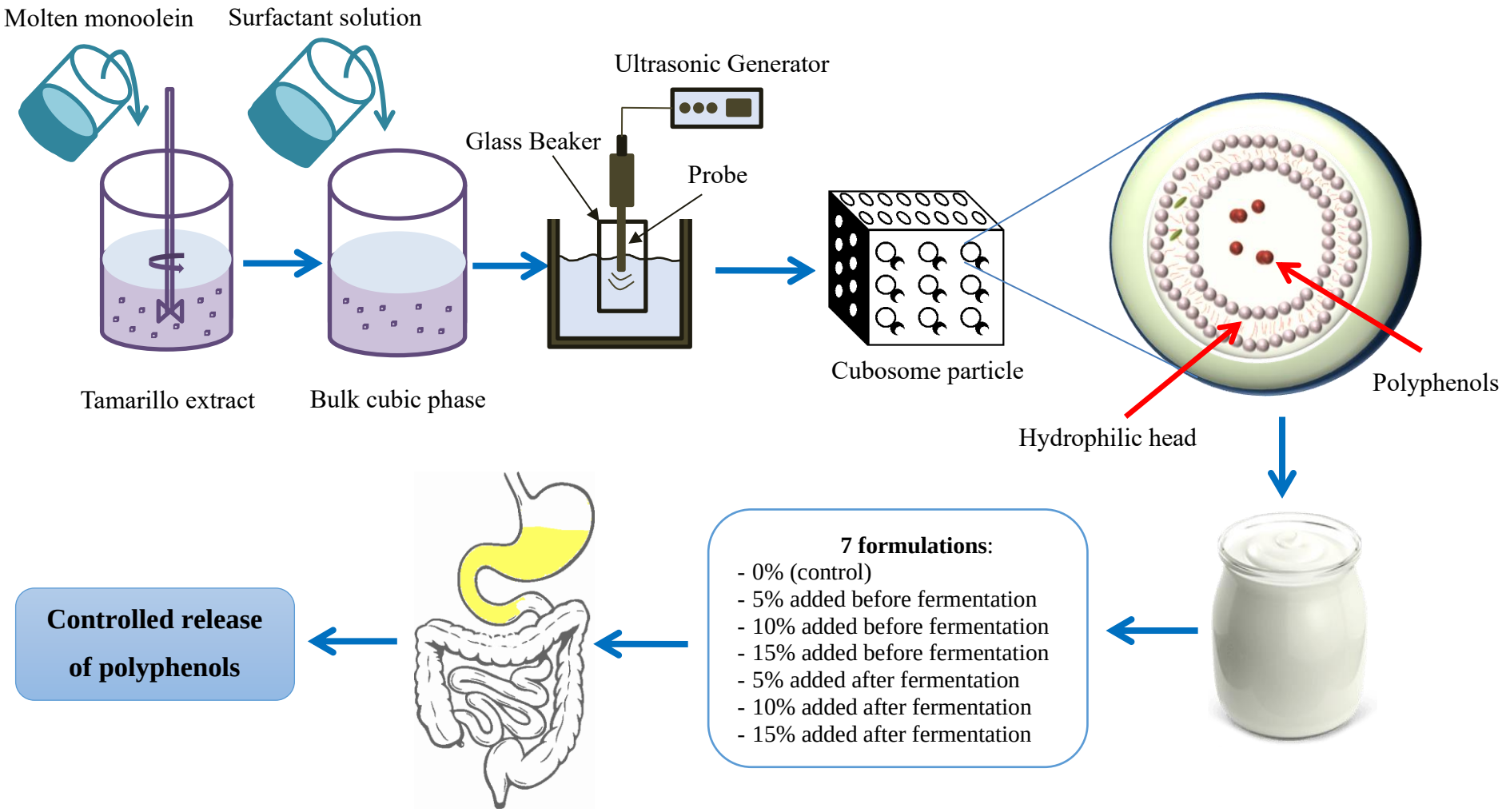

Figure 8.3 Schematic outline for encapsulating tamarillo bioactive using cubosome and application in yoghurt (graphics sourced from creative commons sources).

Practical implication and limitations

Over the last decade there has been a rapid increase in nutrition-related non-communicable disease, hence a lot of studies from different sectors to add the goodness of fruits into popular consumer foods such as yoghurt has been implemented. This thesis has significantly contributed to the current knowledge of nutrient contents, especially bioactive compounds (polyphenols and related antioxidants) of tamarillo that may be beneficial for the human health as well as potential for producing yoghurts from tamarillo. Also, fruit by-products (peels) have a high concentration of phytochemicals and may also have antioxidant properties. This makes the extraction of these compounds important and attractive for application as food ingredients, pharmaceuticals and cosmetic formulations. These data may be useful for the design and interpretation of intervention studies investigating the health effects of polyphenols from tamarillo. Besides, the TDU technique could be utilized as a rapid, reliable and non-destructive tool in quality control for other foods. Additionally, the potential of cubosomes to protect bioactive compounds in their matrix has been demonstrated. These results should be useful for the design and development of effective delivery system for polyphenols and several nutrients with different solubilities.

There are limitations of this project. The effect of post-harvest time and storage conditions on physicochemical properties and nutrient compositions has not been measured to get better understanding the difference about these components between three cultivars. Analysis for minerals (copper, manganese, magnesium, calcium, iron, and especially potassium) and other vitamins (B3, B6, B12, D and folate) in tamarillo may give more information about the nutrient density of this nutritious fruit as well as updated nutrient adequacy score. Testing of the human odour threshold with panellists has not been implemented to advance the sensorial characterization of the complex composition, flavour and odour of tamarillo pulp and peel. For tamarillo yoghurt, a lack of sensory tests also caused insufficient understanding on how yoghurt flavour and texture would be perceived by the addition of fruit. *In vivo* studies evaluating both the action of digestive enzymes and the action of microbiota metabolism should be performed. Although the nutritional properties of enriched yoghurt were not monitored in this study, it is suggested that these properties be taken into consideration

in determining the form of encapsulation to be added to a food product. Engagement with the tamarillo industry was limited.

Finally, this work is limited in that there were no *in vivo* studies to measure bioavailability or effects of consumption of tamarillo fruit or the yoghurt. This is an area which needs attention for any health claims to be possible.

Final conclusions and future work:

This research answers all the research questions and addresses all six objectives with the highlights of this research are listed as follows:

- A high percentage of dietary fibre (approximately 3%) was found in tamarillo and GABA content in this fruit is comparatively similar to that in tomato.
- Two essential amino acids, 5 non-essential amino acids and 6 phenolic compounds in tamarillo were newly reported.
- L-glutamic acid, β-carotene, chlorogenic acid and delphinidin 3-rutinoside dominated amino acid, carotenoid, phenolic and anthocyanins profiles of tamarillo, respectively.
- The presence of tamarillo in the diet could contribute substantially to the intake of vitamins A, C, and E.
- Tamarillo possessed higher antioxidant activity than kiwifruit and apple.
- First study to analyze volatile compounds in tamarillo with TD-GC-MS in which 85 volatile compounds were reported herein for the first time.
- Methional was the most significant flavour contributor in tamarillo.
- Tamarillo powder contributed a higher amount of dietary fibre and antioxidant vitamin to the yoghurt while retained the fruit flavour.
- Compared to plain yoghurts, tamarillo yoghurts being produced either before or after fermentation showed lower syneresis and higher viscosity, firmness, consistency and cohesiveness.
- The antioxidant vitamins present in tamarillo yoghurts (pro-vitamin A, vitamin C, and vitamin E) may possess health and nutritional benefits.

- Tamarillo pulp powder can be utilized as added essential amino acids, GABA and polyphenols in yoghurts.
- Tamarillo fortified yogurt displayed higher essential amino acids, total phenolic content and antioxidant activity compared to plain yoghurt.
- Protein hydrolysis in tamarillo yoghurts was complete and absorbed in the gastrointestinal tract.
- The encapsulation efficiency for most polyphenols in tamarillo was over 50% and encapsulated polyphenols showed high stability during the gastric phase of *in vitro* digestion and were almost completely released in the intestinal phase.
- Application of cubosomal tamarillo polyphenols in yoghurt improved the physicochemical and nutritional properties as well as antioxidant activity.
- During digestion, the encapsulation of cubosomal tamarillo polyphenols protected against pH shifts and enzymatic activity and facilitated the controlled release of the compound encapsulated.

This PhD project provided an overview picture of nutrient components, bioactives as well as volatile profile of New Zealand tamarillo as well as develop value-added yoghurt products using naturally occurring bioactive compounds (polyphenols and related antioxidants) from New Zealand grown tamarillo. Using natural sources in the development and formulation of yoghurt products will contribute to increase in research and industrial application of fruits. The findings of project also open new doors for further studies:

- Food product development may be advanced through the extraction of dietary fibre, soluble sugars, and amino acids from tamarillo.
- Interaction of these phenolics including anthocyanins with other food components should be explored. Changes in phenolics including anthocyanin profiles and their antioxidant activity of tamarillo from long term storage being influenced during food processing would be advantageous. Understanding of the effects of pH, temperature, processing mechanisms and storage conditions on maintaining the bioactive components of tamarillo will enhance application and utilisation of the fruit. Tamarillo has a relatively long fruit season, and they are relatively shelf stable

compared to other fruit (e.g. strawberry) which may be of advantageous in its application compared to other fruit.

- Determination of the total antioxidant activity and bioavailability of phytochemicals from tamarillo for their synergistic functional roles in *in vitro* cell lines, animal models and human clinical studies as well as bioavailability of phytochemicals in foods formulated and fortified with tamarillo extracts would be advantageous. Tamarillos can be preserved and the extracts can be fortified to boost nutritional value of processed food.
- To provide more information about the nutrient density of tamarillo yoghurt the minerals (iron, calcium, potassium) and other vitamin (B, D, K) in fortified yoghurt should be analysed. Sensory test including determination of the organoleptic properties and consumer acceptability of tamarillo should be implemented to understand how yoghurt flavor and texture would be perceived by the addition of fruit. The interaction between fibres and stater culture (viability, total colony counts) should be performed in the future.
- Further research of acceptability, shelf life then trials for health effects should be carried out. A better understanding of polyphenols bioaccessibility from tamarillo fortified yoghurt will require further *in vivo* studies related to evaluation of both the action of digestive enzymes and microbiota metabolism.
- Interaction between yoghurt components (mainly protein), starter culture and encapsulated polyphenols should be assessed with longer intestinal digestion and possible effects on the microbiota in the future. Also, the effect of pH change during *in vitro* digestion on cubosome stability should be further investigated

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APPENDICES

Appendix A. Papers published from this project and permission from journals for reproduction and usage in this thesis

Appendix A1. Paper of "Physiochemical properties and proximate composition of tamarillo fruits from New Zealand" published in Journal of Food Composition and Analysis

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Original Research Article

Physicochemical properties and proximate composition of tamarillo (*Solanum betaceum* Cav.) fruits from New Zealand

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Dietary fibre
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ABSTRACT

This study reports physical parameters, proximate compositions, reducing sugar and amino acid contents in Amber, Laird's Large and Mulligan tamarillos that were produced in New Zealand. Across all three cultivars, about 3 % of dietary fibre was present. Higher amounts of neutral side chains were observed in pectin from Laird's Large compared to other cultivars and in pectin from pulps compared to peels. Among 22 detected amino acids, 2 essential amino acids and 5 non-essential amino acids were reported herein for the first time. The total amino acids content in peel and pulp of tamarillos ranged from 1192 to 1753 and 3455–6077 mg $100\,g^{-1}$ dry weight, respectively. L-glutamic acid, γ-aminobutyric acid and L-aspartic acid dominated amino acid profile of tamarillo except for Amber peel. L-histidine and L-lysine dominated the essential amino acid profile of all tamarillo samples. Principal component analysis revealed a clear separation among soluble sugar and amino acid profiles of different cultivars and tissues of tamarillo.

1. Introduction

Tamarillo, also known as tree tomato (*Solanum betaceum Cav.*), is relatively low in carbohydrates, though it is a good source of dietary fibre; vitamins A, B_6, C and E; minerals including calcium, copper, iron, magnesium, manganese, phosphorus and zinc (Vasco et al., 2009). It possesses a variety of functional bioactives, such as phenolics, anthocyanins (Diep et al., 2020) and carotenoids, that are beneficial to human health (Skinner and Hunter, 2013). New Zealand is one of the main producers and exporters of tamarillo with a yield of approximately 450 tons per annum. The country possesses a cultivated area of some 10 ha with 40 growers (Aitken and Warrington, 2018). Three cultivars, which differentiate by colour and size, are called Mulligan (purple-red), Laird's Large (red) and Amber (yellow). The red cultivar contributes to approximately 80 % of the total tamarillos exported from New Zealand (Schotsmans et al., 2011).

Consumer acceptance of fruits is influenced by skin colour, firmness, size and weight. The skin of tamarillo is green at development stage and turns into a full yellow, orange or red colour during maturation stage and the firmness decreases (Schotsmans et al., 2011). In the first 25 weeks of development, total soluble solids (TSS) increase to 12°Brix. Breakdown of starch and accumulation of reducing sugars are seen as fruit matures (Ramírez and Kallarackal, 2019). However, starch accumulation is not associated with tamarillo growth nor TSS content in tamarillos, which is different from tomato, the fruit of the same *Solanum* genus. One of the vital quality and maturity features of fruits is sugar content, where glucose and fructose are the major components (Hu et al., 2016). Soluble sugars start to accumulate from ripening and these serve as substrates for respiratory reactions, which significantly influence the development of overall flavour and texture of tamarillo (Hu et al., 2016). Most of previous studies have focused on reporting on glucose, fructose and sucrose (Acosta-Quezada et al., 2015; Vasco et al., 2009), except for Gannasin et al. (2015) who had detected eight soluble sugars, except fructose, from different fractions of Malaysian tamarillo.

Amino acid content is a result of metabolic changes during growth and ripening of fruits, hence this parameter can be used to identify the optimum ripening time (Silva et al., 2004). Amino acids affect quality of fruits in terms of aroma, colour and taste. When heat treatment is involved in producing fruit-derived products such as juices and jams, amino acids play a greater role in influencing aroma, colour and taste through Maillard reaction (Silva et al., 2004). Evaluation of amino acid in fruits may help to identify falsification or adulteration in fruit-derived products, including wines, juice and jam (Silva et al., 2004). The presence of six essential amino acids (arginine, histidine, isoleucine,

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Appendix A2. Paper of "Quantification of Carotenoids, α-tocopherol and Ascorbic acid in New Zealand tamarillos" published in Journal of Foods

foods

MDPI

Article

Quantification of Carotenoids, α-Tocopherol, and Ascorbic Acid in Amber, Mulligan, and Laird's Large Cultivars of New Zealand Tamarillos (*Solanum betaceum* Cav.)

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Abstract: Amber (yellow), Laird's Large (red) and Mulligan (purple–red) cultivars of New Zealand tamarillo fruit were separated into pulp (endo- and mesocarp) and peel (exocarp), and analyzed by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) for carotenoids, α-tocopherol and ascorbic acid contents. Fresh Mulligan pulp had the highest content of β-carotene (0.9 mg/100 g), α-tocopherol (1.9 mg/100 g), and ascorbic acid (28 mg/100 g). Higher concentrations of β-carotene and ascorbic acid, and lower concentrations of α-tocopherol were detected in pulps compared with peels. Compared with standard serves of other fruit, tamarillo had the highest β-carotene (9–20% RDI (recommended dietary intake)/serve), high ascorbic acid (67–75% RDI/serve), and α-tocopherol (16–23% adequate intake/serve). All cultivars had diverse carotenoid profiles dominated by provitamin A carotenoids (β-carotene and β-cryptoxanthin) and xanthophyll carotenoids (lutein; zeaxanthin and antheraxanthin). Favorable growth conditions (high light intensity and low temperature) may explain the higher antioxidant vitamin content in New Zealand tamarillos compared to those from other countries. Tamarillo peels may be used as natural food coloring agent to reduce waste and deliver sustainable production.

Keywords: tamarillo; dietary antioxidants; β-carotene; ascorbic acid; α-tocopherol; carotenoids; provitamin A

1. Introduction

Antioxidant compounds in fruit and vegetables reduce oxidative stress and these in turn contribute towards treatment and prevention of cardiovascular diseases and cancers, as demonstrated by many of biochemical and epidemiological studies [1]. Carotenoids (β-carotene, β-cryptoxanthin, lutein, and zeaxanthin), α-tocopherol and vitamin C are some of the most significant antioxidants present in fruit and vegetables [1]. Carotenoids are classified into two subgroups depending on their structure: carotenes (containing carbon and hydrogen atoms) and xanthophylls (containing at least one oxygen molecule) [2]. Carotenoids possess ability to trap singlet oxygen and eliminate peroxyl radical, thereby known as strong antioxidants. They act as photoprotectors to protect membrane lipids against

Appendix A3. Paper of "Phenolic and Anthocyanin Compounds and Antioxidant activity of Tamarillo" published in Journal of Antioxidants

antioxidants

MDPI

Article

Phenolic and Anthocyanin Compounds and Antioxidant Activity of Tamarillo (*Solanum betaceum* Cav.)

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Abstract: This study examined phenolics and anthocyanins present in Amber, Laird's Large and Mulligan cultivars of tamarillo that were cultivated in Whangarei, Northland of New Zealand. Samples were further separated by their tissue types, peel and pulp. Using LC-MS/MS, twelve polyphenols were quantified and six (ellagic acid, rutin, catechin, epicatechin, kaempferol-3-rutinoside and isorhamnetin-3-rutinoside) were detected for the first time in tamarillo. Mulligan cultivar showed the highest amounts of phenolic and anthocyanin compounds and the highest antioxidant activity. Phenolic compounds were mostly synthesized from shikimic acid route, and chlorogenic acid dominated the profile regardless of cultivar and tissue types. Anthocyanin profile was dominated by delphinidin-3-rutinoside in pulp. Higher amounts of anthocyanins were detected in this study, which may be explained by favourable growth conditions (high light intensity and low temperature) for anthocyanin biosynthesis in New Zealand. Higher antioxidant activity and total phenolic content in peels than in pulps were found when assessed by Cupric Ion-Reducing Antioxidant Capacity (CUPRAC), Ferric Reducing Ability of Plasma (FRAP) and Folin–Ciocalteu assays, and a positive correlation ($r > 0.9$, $p \leq 0.01$) between the three assays was observed. Current findings endorse that tamarillo has a great bioactive potential to be developed further as a functional ingredient with considerable levels of antioxidant compounds and antioxidant activity.

Keywords: Tamarillo; phenolics; anthocyanins; antioxidant; chlorogenic acid; delphinidin-3-rutinoside

1. Introduction

Tamarillo (*Solanum betaceum* Cav.) is a fruit species of family *Solanaceae* genus *Solanum*, which is also known as tree tomato as its flesh closely resembles to that of the tomato [1]. As a subtropical fruit, tamarillo is mainly grown in warmer and sheltered areas of the North Island in New Zealand (Auckland and Hawkes Bay) [2]. New Zealand is one of the leading producers of tamarillo [3], with the main export markets including America, Australia, Hong Kong, Singapore and Japan. The ripe fruit turns to various colours (yellow, orange, red or purple) depending on the cultivars and exhibits a slightly bitter, sour and astringent taste with a unique aroma [4]. In New Zealand, tamarillo are available in yellow, red and purple-red cultivars, with the red being more popular and more common than the others. Tamarillo is considered as underutilized fruit due to its texture, strong flavour and some unidentified properties.

Phenolics (or polyphenols) and anthocyanins are secondary plant metabolites which are also known as antioxidants. Health-promoting effects arising from prolonged high intake of polyphenols have been reported extensively in the literature, where reduction in risks of certain cancers, cardiovascular

Appendix A4. Paper of "Effect of tamarillo fortification and fermentation process on physicochemical properties and nutrient and volatile content of yoghurt" published in Journal of Foods

foods

MDPI

Article

Effect of Tamarillo Fortification and Fermentation Process on Physicochemical Properties and Nutrient and Volatiles Content of Yoghurt

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Abstract: Bright-red Laird's Large tamarillo is a unique and under-utilised fruit that is a dietary source of carotenoids, vitamins C and E, and dietary fibre. The effects of the addition of freeze-dried tamarillo powder (5–15%) to milk and yoghurt starter either before (PRE) or after (POS) fermentation on physicochemical properties were examined. Using LC-MS and GG-MS, nutrient and volatile contents of tamarillo yoghurt were also examined. The addition of tamarillo prior to fermentation was associated with a more yellow colour and higher concentrations of tocopherol compared to when tamarillo was added after fermentation. Higher elastic modulus, PUFAs, pro-vitamin A content, and vitamin C retention were observed for POS than PRE. All tamarillo yoghurts showed improvement in syneresis, lower lactose content, and higher concentrations of antioxidant vitamins than the commercial premium-assorted fruits yoghurt from New Zealand Food Composition Data. Yoghurt fortified with tamarillo powder offers the potential for the development of a high-value nutritional product that could be a good source of vitamin C and a source of vitamin E and β-carotene, and maintain the volatiles that give tamarillo its distinctive flavour.

Keywords: tamarillo; yoghurt; fermentation; physicochemical properties; vitamins; volatiles

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1. Introduction

Tamarillos (*Solanum betaceum* Cav.) are a dietary source of polyphenols, including anthocyanins (delphinidin rutinoside, pelargonidin rutinoside, and cyanidin rutinoside), hydroxy benzoic acids (gallic acid), hydroxycinnamic acids (chlorogenic acid, caffeic acid), flavonols (kaempferol), flavanols (catechin, epicatechin), and flavonol glycosides (rutin, kaempferol-3-rutinoside); fibre; carotenoids (β-carotene); potassium; and vitamins C, E, and B6 [1–3]. Many of these constituents are strong antioxidants that are associated with health benefits such as reducing lipid oxidation and reducing risk for certain cancers, cardiovascular disease, and type 2 diabetes mellitus [4,5]. Tamarillo fruit has the potential to be a functional food or a functional ingredient for food reformulation.

Recently, the formulation of functional fermented dairy products through the addition of fruit powder has become popular [6]. Yoghurt is already known to possess positive health benefits from the presence of probiotics. It is a good source of proteins, calcium, potassium, phosphorus, and vitamins [7]. Compared to other dairy products, yoghurt is gaining more popularity due to the presence of probiotics, nutrients in higher digested form, gel-like structure, taste, and mouthfeel [8]. It also has protective capacity against pathogenic bacteria, viruses, and intestinal infections that often lead to diarrhoea. The protective capacity comes from either the acidity of yoghurt or the inhibitor molecules generated by

Appendix A5. Paper of "Effect of In Vitro Gastrointestinal Digestion on Amino Acids, Polyphenols and Antioxidant Capacity of Tamarillo Yoghurts" published in International Journal of Molecular Sciences

International Journal of *Molecular Sciences*

Article

Effect of In Vitro Gastrointestinal Digestion on Amino Acids, Polyphenols and Antioxidant Capacity of Tamarillo Yoghurts

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Abstract: Laird's Large tamarillo powder is high in protein (10%) essential amino acids (EAAs), gamma-aminobutyric acid (GABA) and polyphenols (0.6% phenolics plus anthocyanins) and fibre 25%. This study aimed to investigate, using a standardized static in vitro digestion model, the stability of amino acids and antioxidant capacity of polyphenols in yoghurt fortified with 5, 10 and 15% tamarillo powder either before (PRE) or after (POS) fermentation. Compared to plain yoghurt, the fruit polyphenols (rutinosides and glycosides) were retained and substantial increases in FEAAs (free essential amino acids), total phenolic content (TPC) and antioxidant activity were observed particularly at the end of intestinal phase of digestion. Together with SDS-PAGE results, peptides and proteins in tamarillo yoghurts were more easily digested and therefore may be better absorbed in the small intestine compared to the control. TPC and antioxidant activity of fortified yoghurts increased significantly after in vitro digestion. Relatively high bioaccessibilty of chlorogenic acid and kaempferol-3-rutinoside in digested PRE samples was observed. The results suggest that the yoghurt matrix might protect some compounds from degradation, increasing bioaccessibility and in the small intestine allow increased absorption and utilization possible. Fortification would deliver intact polyphenols and fibre to the large intestine and improve gut health. Further research of acceptability, shelf life, and then trials for health effects should be implemented.

Keywords: tamarillo; yoghurt; fermentation; in vitro digestion; amino acids; polyphenols; antioxidant activity

Citation: Diep, T.T.; Yoo, M.J.Y.; Rush, E. Effect of In Vitro Gastrointestinal Digestion on Amino Acids, Polyphenols and Antioxidant Capacity of Tamarillo Yoghurts. *Int. J. Mol. Sci.* 2022, 23, 2526. https://doi.org/10.3390/ijms23052526

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1. Introduction

The development of new food products with a potentially positive effect on health using traditional fruits, is generally desirable since there is an increasing interest among consumers to look for safe, healthy, sustainable and natural foods [1]. Consumption of fruits is associated with health benefits which are usually related to their vitamin, mineral or specific antioxidant compounds, in particular, polyphenols which possess strong antioxidant activity and are associated with protective effects against chronic diseases including type 2 diabetes mellitus, cardiovascular diseases and cancer [2].

We have previously shown that the protein content of New Zealand-grown Laird's Large cultivar of tamarillo is 1.2% fresh weight (FW) (88.1% moisture content) which is equivalent to 10% dry weight (DW) [3] (Supplementary Table S1). In addition, tamarillo powder has a high fibre content (25%), essential for bowel health. Twenty-two amino acids (9 essential and 13 non-essential were detected [3]. Tamarillo showed a high concentration of γ-aminobutyric acid (GABA) (433 mg/100 g DW) which is similar to tomato, the same *Solanum* genus [3]. In addition, tamarillo fruit contains a spectrum of polyphenol components including not only the blue-red coloured anthocyanins; delphinidin-3-rutinoside (254.76 mg/100 g DW) and pelargonidin-3-rutinoside (200.66 mg/100 g DW),

Appendix A6. Paper of "Tamarillo Polyphenols Encapsulated-Cubosome: Formation, Characterization, Stability during Digestion and Application in Yoghurt" published in Journal of Antioxidants

antioxidants

Article

Tamarillo Polyphenols Encapsulated-Cubosome: Formation, Characterization, Stability during Digestion and Application in Yoghurt

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Abstract: Tamarillo extract is a good source of phenolic and anthocyanin compounds which are well-known for beneficial antioxidant activity, but their bioactivity maybe lost during digestion. In this study, promising prospects of tamarillo polyphenols encapsulated in cubosome nanoparticles prepared via a top-down method were explored. The prepared nanocarriers were examined for their morphology, entrapment efficiency, particle size and stability during in vitro digestion as well as potential fortification of yoghurt. Tamarillo polyphenol-loaded cubosomes showed cubic shape with a mean particle size of 322.4 ± 7.27 nm and the entrapment efficiency for most polyphenols was over 50%. The encapsulated polyphenols showed high stability during the gastric phase of in vitro digestion and were almost completely, but slowly released in the intestinal phase. Addition of encapsulated tamarillo polyphenols to yoghurt (5, 10 and 15 wt% through pre- and post-fermentation) improved the physicochemical and potential nutritional properties (polyphenols concentration, TPC) as well as antioxidant activity. The encapsulation of tamarillo polyphenols protected against pH changes and enzymatic digestion and facilitated a targeted delivery and slow release of the encapsulated compounds to the intestine. Overall, the cubosomal delivery system demonstrated the potential for encapsulation of polyphenols from tamarillo for value-added food product development with yoghurt as the vehicle.

Keywords: tamarillo extract; yoghurts; cubosome; polyphenols; encapsulation; in vitro digestion

Citation: Diep, T.T.; Yoo, M.J.Y.; Rush, E. Tamarillo Polyphenols Encapsulated-Cubosome: Formation, Characterization, Stability during Digestion and Application in Yoghurt. *Antioxidants* 2022, *11*, 520. https://doi.org/10.3390/antiox11030520

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1. Introduction

Inverse bicontinuous liquid crystalline nanoparticles, termed cubosomes, have advantageous properties that may be suitable for the delivery of bioactive compounds to the small intestine. Amphiphilic lipids such as the monoglyceride monoolein can self-assemble in water to produce dispersions of cubosomes. The basic structure of a cubosome is a honeycomb-like structure with two non-intersecting internal aqueous channels separated by lipid bilayers. The internal hydrophilic (aqueous) areas are separated by lipid bilayers that are twisted into a tightly packed three-dimensional honeycomb structure that has a high internal surface area to volume. Within this structure, encapsulation of diverse hydrophilic, hydrophobic and amphiphilic compounds of small to large molecular weights, such as proteins, peptides, amino acids and nucleic acids, is possible [1]. Within cubosomes, hydrophobic molecules can be located within the lipid bilayers, hydrophilic components in the aqueous channels or around the polar head of the lipid, and amphiphilic molecules can be located at the lipid–water interface. This structure generally maintains the efficacy—stability of actives (vitamins and proteins) without adverse effects

Appendix A7. Paper of "Tamarillo: A review of Physicochemical and Bioactive properties and potential Applications" published in Journal of Food Review International

FOOD REVIEWS INTERNATIONAL
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Tamarillo (*Solanum betaceum* Cav.): A Review of Physicochemical and Bioactive Properties and Potential Applications

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ABSTRACT

Tamarillo (*Solanum betaceum* Cav.) is a sub-tropical fruit with unique flavour and colour, known to be highly nutritious. Tamarillo has nutritional adequacy score of 7.4 (red type) to 7.9 (gold type) and it is a rich source of vitamins A, B_6, C, dietary fibre and potassium. Phenolics, carotenoids and anthocyanins are considered as the main bioactive components, with 70 volatile compounds and organic acids that contribute to flavour. Potential health benefits include antioxidant, anti-obesity, anti-cancer and prebiotic properties. Antimicrobial and antifungal activities and proteolytic activity have also been demonstrated. This review summarizes chemical composition and bioactive properties of tamarillo from eight different countries (Argentina, Brazil, Colombia, Ecuador, New Zealand, Malaysia, Panama and Taiwan). Information on carbohydrates, dietary fibre, vitamins, minerals, volatiles, phenolic compounds, anthocyanins, total phenolic content and antioxidant activities of tamarillo are compared based on cultivars and geographical sources. Tamarillo possesses higher antioxidant activity than apples and kiwifruit. Applications of tamarillo as a functional ingredient for health, food, cosmetics and pharmaceutical products are also highlighted. Recently reported antimicrobial and antifungal properties make it even more attractive as a functional ingredient to enhance safety and shelf life of foods.

KEYWORDS

Tamarillo; antioxidant; antimicrobial activity; bioactive compounds; prebiotic; nutritional adequacy score; food safety

Introduction

Tamarillo, also known as tree tomato (*Solanum betacea* or *Cyphomandra betacea Cav.*), is categorized in the family *Solanaceae*, genus *Solanum* together with tomato, capsicum and eggplant (Fig. 1a).[1] The egg-shaped fruit has purple-red to golden-yellow skin and small seeds.[2] The exact origin of this fruit is unknown, but wild cultivars exist in South American countries including Bolivia, Chile, Ecuador and Peru.[3] In the late 19th century, the fruit was globally introduced to Oceania (Australia and New Zealand), South-East Asia (India, Malaysia, Thailand, Indonesia and Vietnam), Europe (Italy, Germany, Spain, Portugal, France and Netherlands) as well as Africa (South Africa, Uganda and Rwanda).[1,3-5] At present, only three countries, Australia, Colombia and New Zealand, commercially grow tamarillo.

In New Zealand, yellow and purple cultivars were developed by Hay & Sons in the late 1800's,[3] and purple-red type was developed by an Auckland nurseryman from South America around 1920.[5] In 1967, the New Zealand name 'tamarillo' was coined by combining 'tama', a Maori word with meaning of leadership, and 'rillo', a Spanish word. The new name 'tamarillo' was considered more marketable than its original name, tree tomato.[6] According to Schotsmans, et al.,[7] tamarillo successfully grew in New Zealand through improvements in species cultivation and storage conditions. As a sub-tropical

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Appendix A8. Paper of "Volatile components and preliminary antibacterial activity of tamarillo" published in Journal of Foods

foods

MDPI

Article

Volatile Components and Preliminary Antibacterial Activity of Tamarillo (*Solanum betaceum* Cav.)

Tung Thanh Diep [1,2], Michelle Ji Yeon Yoo [1,2,*], Chris Pook [3], Saeedeh Sadooghy-Saraby [1], Abhishek Gite [1] and Elaine Rush [2,4]

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Abstract: Tamarillo is a nutrient-dense fruit with a unique aroma from its volatile compounds (VCs). In this study, we aimed to compare the volatile profiles: (i) of fresh and freeze-dried tamarillo; (ii) detected using Thermal Desorption–Gas Chromatography–Mass Spectrometry (TD–GC–MS) and Solid-Phase MicroExtraction–Gas Chromatography-Mass Spectrometry (SPME–GC–MS); (iii) of freeze-dried pulp and peel of New Zealand grown tamarillo. The possible antibacterial activity of freeze-dried tamarillo extracts was also investigated. We show that freeze-drying maintained most of the VCs, with some being more concentrated with the loss of water. The most abundant VC in both fresh and freeze-dried tamarillo was hexanoic acid methyl ester for pulp (30% and 37%, respectively), and (E)-3-Hexen-1-ol for peel (36% and 29%, respectively). With the use of TD–GC–MS, 82 VCs were detected for the first time, when compared to SPME–GC–MS. Methional was the main contributor to the overall aroma in both peel (15.4 ± 4.2 µg/g DW) and pulp (118 ± 8.1 µg/g DW). Compared to water as the control, tamarillo extracts prepared by water and methanol extraction showed significant antibacterial activity against *E. coli*, *P. aeruginosa*, and *S. aureus* with zone of inhibition of at least 13.5 mm. These results suggest that freeze-dried tamarillo has a potential for use as a natural preservative to enhance aroma and shelf life of food products.

Keywords: freeze-dried tamarillo; TD–GC–MS; volatiles; antimicrobial activity

Citation: Diep, T.T.; Yoo, M.J.Y.; Pook, C.; Sadooghy-Saraby, S.; Gite, A.; Rush, E. Volatile Components and Preliminary Antibacterial Activity of Tamarillo (*Solanum betaceum* Cav.). *Foods* 2021, *10*, 2212. https://doi.org/10.3390/foods10092212

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1. Introduction

Tamarillo (*Solanum betaceum* Cav.) is a good source of phenolics and anthocyanins with high antioxidant activity (52.42–60.19 µmol TEAC/g DW) [1]. It is cultivated in New Zealand, Ecuador, Brazil, and Colombia, and it is mostly consumed as fresh fruit [1]. Laird's Large, also known as red type, is the most common cultivar. Tamarillo pulp contains high amounts of dietary fiber, vitamins A, B_6, C, and E, and minerals (Ca, K, Cu, Fe, Mg, Mn, P, and Zn) [2–5]. Chlorogenic acid and kaempferol rutinoside are dominant phenolics in red tamarillo from New Zealand; high concentrations of delphinidin rutinoside and pelargonidin rutinoside were found in this variety [1]. Meanwhile, β-carotene and β-cryptoxanthin were identified as the most abundant carotenoids in tamarillo from Australia and Brazil [6] as well as New Zealand [3]. Potential health benefits including antioxidant, antiobesity, anticancer, and prebiotic properties of tamarillo fruit have been reported [5]. Recognized for a distinctive aroma, tamarillo pulp is mostly consumed in the fresh form and the peel is discarded. Aroma is one of the important parameters for the quality of fruit and fruit-derived products, with volatile compounds (VCs) determining the aroma

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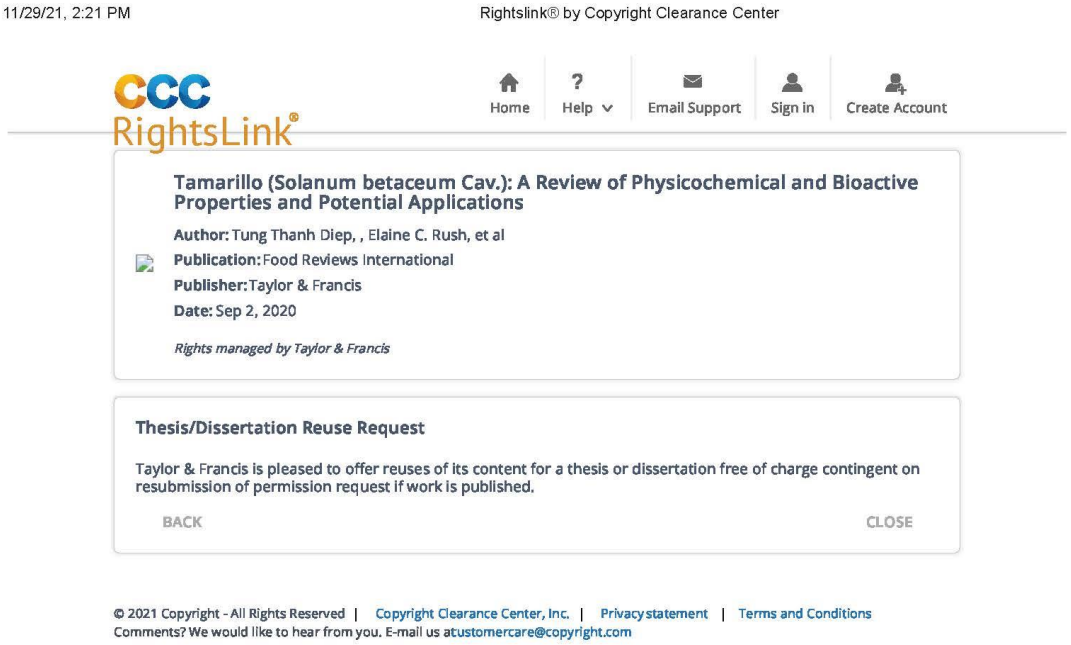

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Tamarillo (Solanum betaceum Cav.): A Review of Physicochemical and Bioactive Properties and Potential Applications

Author: Tung Thanh Diep, , Elaine C. Rush, et al
Publication: Food Reviews International
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Physicochemical properties and proximate composition of tamarillo (Solanum betaceum Cav.) fruits from New Zealand

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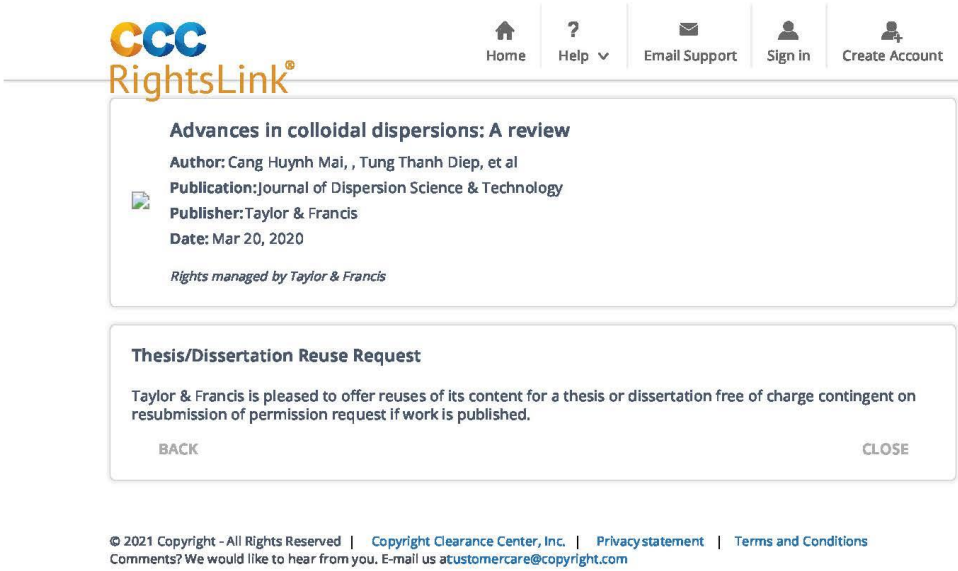
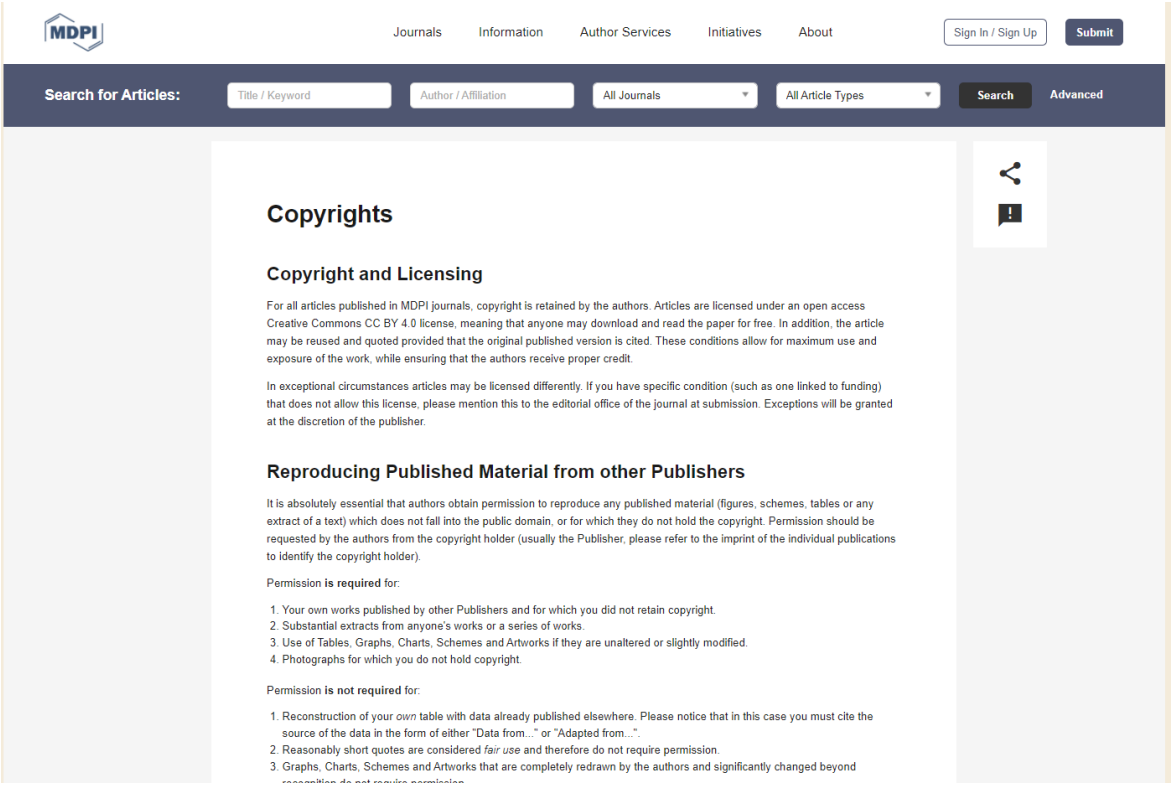
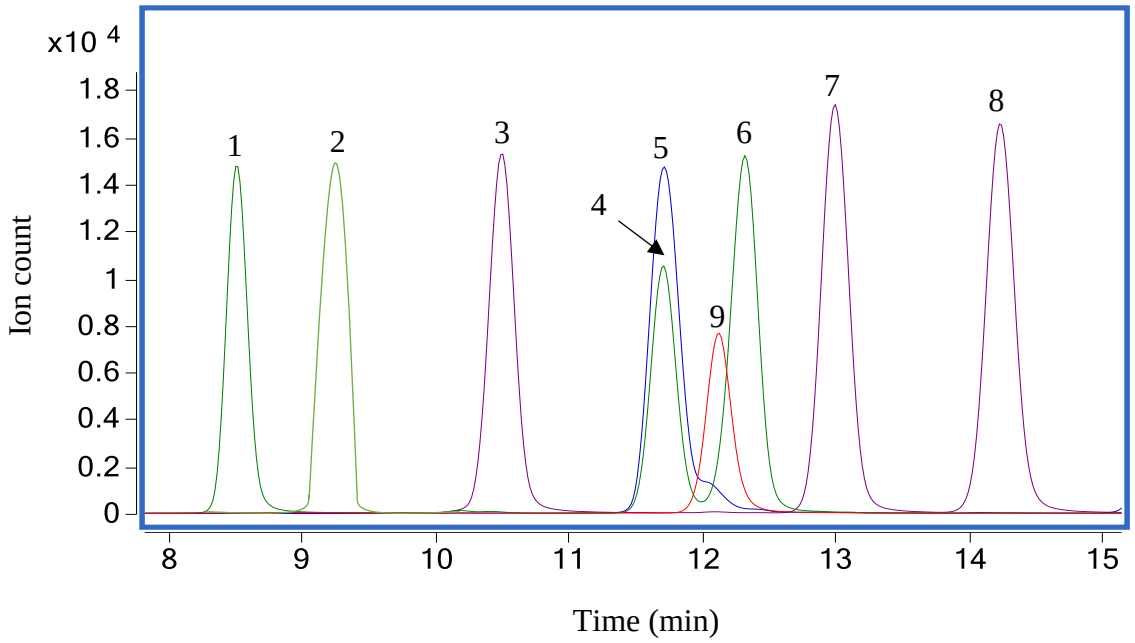




Appendix B. Chromatograms and method validation for analysis of different compounds in tamarillo and tamarillo yoghurts

Appendix B1. MRM chromatograms of 8 reducing sugars and one uronic acid standards were identified by using LC-MS



1: Mannose 2: Fructose 3: Xylose

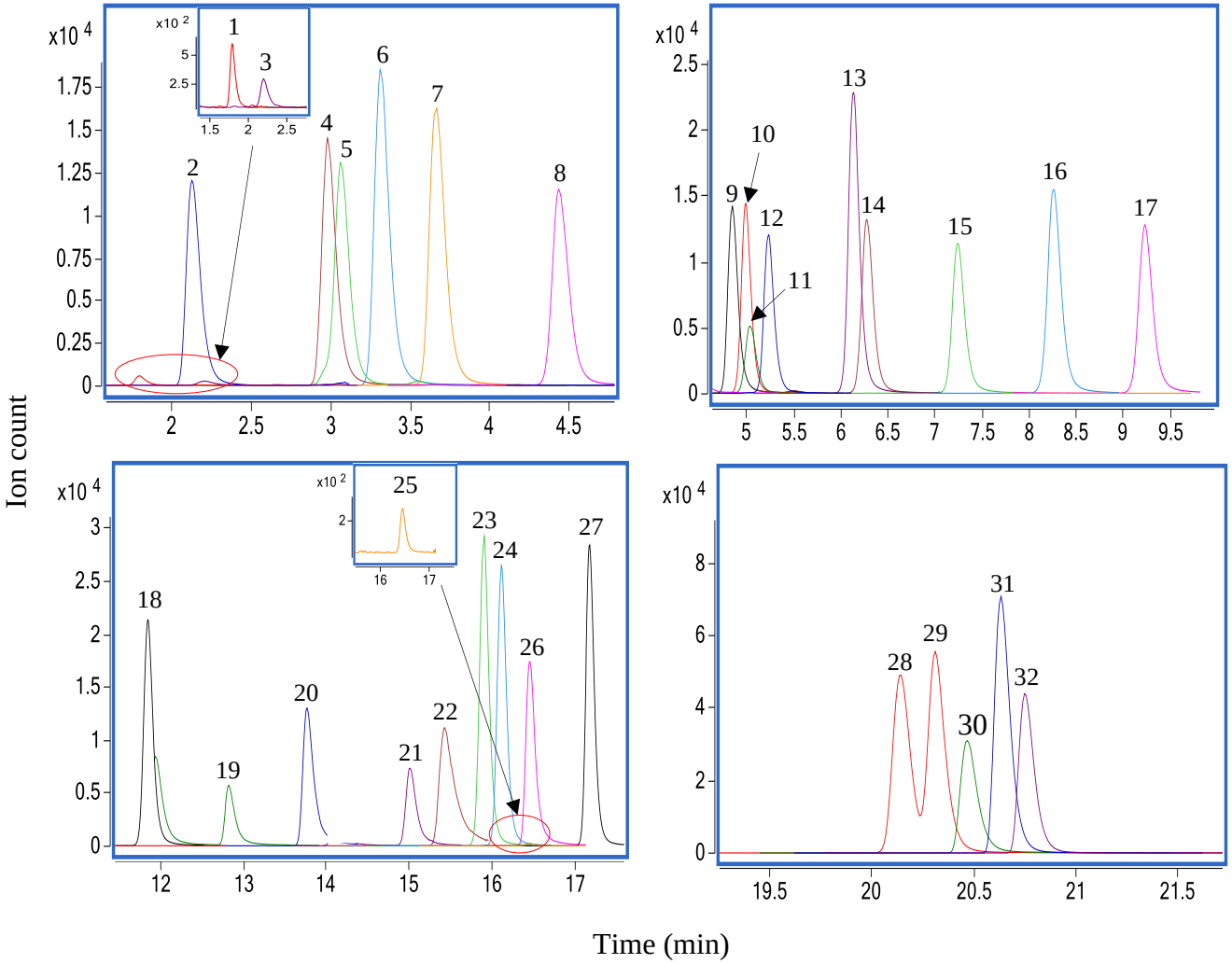
4: Galactose 5: Rhamnose 6: Glucose

7: Ribose 8: Arabinose 9: Glucuronic acid

Appendix B2. Method validation for quantification of reducing sugars and uronic acid. Compounds were listed in the order of retention time (RT)

Analytes	RT (min)	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy (V)	Regression equation	Linear fit correlation coefficient (R^2)	Calibration range (mg/L)	LOD (mg/L)	LOQ (mg/L)	Ion mode
Mannose	8.498	511.2	175.2	25	y = 171.05 x – 1428.80	0.9974	1.9531 – 1000	0.3365	1.0197	Positive
Fructose	9.400	511.2	175.2	25	y = 157.97 x – 1563.07	0.9958	1.9531 – 1000	0.4619	1.3998	Positive
Xylose	10.485	481.2	175.2	25	y = 248.24 x – 2393.50	0.9950	1.9531 – 500	0.1681	0.5093	Positive
Galactose	11.708	511.2	175.2	25	y = 147.99 x + 1021.39	0.9953	1.9531 – 1000	0.0614	0.1861	Positive
Rhamnose	11.712	495.2	175.2	21	y = 305.79 x – 1447.02	0.9963	1.9531 – 250	0.2770	0.8394	Positive
Glucuronic acid	12.125	525.2	175.2	25	y = 107.45 x – 66.57	0.9994	1.9531 – 1000	0.2541	0.7700	Positive
Glucose	12.316	511.2	175.2	25	y = 214.42 x – 1652.30	0.9971	1.9531 – 1000	0.2799	0.8480	Positive
Ribose	12.994	481.2	175.2	25	y = 263.04 x + 478.72	0.9953	1.9531 – 1000	0.2932	0.8885	Positive
Arabinose	14.225	481.2	175.2	25	y = 259.14 x + 146.71	0.9972	1.9531 – 1000	0.2318	0.7025	Positive

Appendix B3. MRM chromatograms of 31 amino acid standards and one internal standard were identified by using LC-MS



1: L-Histidine 2: Hydroxy-L-Proline 3: L-Arginine 4: Ethanolamine

5: L-Serine 6: Glycine; 7: Sarcosine 8: L-Aspartic acid

9: β-Alanine 10: L-Threonine 11: Taurine 12: L-Glutamic acid

13: D_4-Alanine 14: L-Alanine 15: γ-Aminobutyric acid

16: L-Proline 17: β-Amino-isobutryic acid

18: α-Aminobutyric acid 19: δ-Hydroxylysine 20: L-Ornithine

21: Cystathionine 22: L-Lysine 23: L-Valine 24: L-Methionine

25: L-Anserine 26: L-Cystine 27: L-Tyrosine 28: L-Leucine

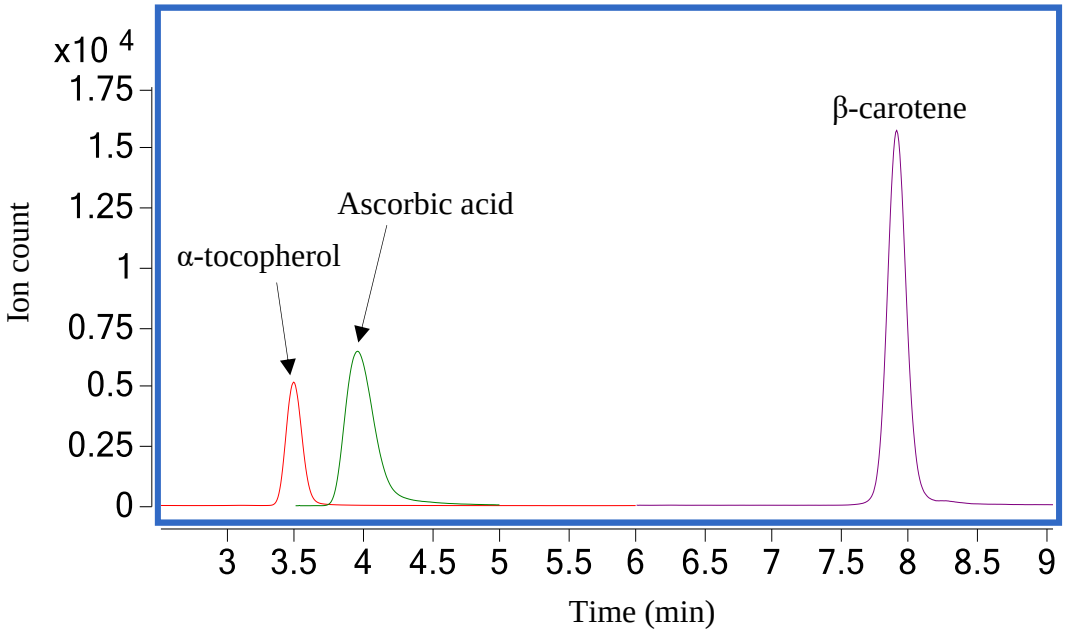
29: L-isoleucine 30: L-Homocystine 31: L-Phenylalanine 32: L-Tryptophan

Appendix B4. Method validation for quantification of free amino acids. Compounds were listed in the order of retention time (RT)

Analytes	RT (min)	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy (V)	Regression equation	Linear fit correlation coefficient (R^2)	Calibration range (μM)	LOD (μM)	LOQ (μM)	Ion mode
L-Histidine	1.806	326	171	20	$y = 0.0078\ x - 5.4*10^{-4}$	0.9956	3.125 – 100	0.2690	0.8151	Positive
Hydroxy-L-Proline	2.120	302	171	20	$y = 0.2119\ x + 3.96*10^{-4}$	0.9992	0.7813 – 100	0.0170	0.0516	Positive
L-Arginine	2.245	345	171	20	$y = 0.051\ x - 1.72*10^{-4}$	0.9984	1.5625 – 100	0.4056	1.2290	Positive
Ethanolamine	3.029	232	171	20	y = 0.2643 x + 0.0035	0.9968	0.7813 – 100	0.0186	0.0565	Positive
L-Serine	3.108	276	171	20	y = 0.25 x + 0.0081	0.9954	0.7813 – 100	0.0224	0.0680	Positive
Glycine	3.361	246	171	20	y = 0.3478 x + 0.0064	0.9992	0.7813 – 100	0.0119	0.0360	Positive
Sarcosine	3.709	260	171	20	y = 0.569 x + 0.0038	0.9964	0.7813 – 50	0.0248	0.0751	Positive
L-Aspartic acid	4.493	304	171	20	y = 0.2376 x + 0.0059	0.9974	0.7813 – 100	0.0212	0.0643	Positive
β-Alanine	4.700	260	171	20	y = 0.2945 x + 0.0076	0.9975	0.7813 – 100	0.0170	0.0516	Positive
L-Threonine	5.048	290	171	20	y = 0.2964 x + 0.0049	0.9976	0.7813 – 100	0.0224	0.0679	Positive
Taurine	5.079	296	171	20	y = 0.1188 x – 0.001	0.9994	0.7813 – 100	0.0163	0.0493	Positive
L-Glutamic acid	5.293	318	171	20	y = 0.2596 x + 0.0052	0.9983	0.7813 – 100	0.0179	0.0542	Positive
L-Alanine	6.070	260	171	20	y = 0.3232 x + 0.0018	0.9992	0.7813 – 100	0.0126	0.0382	Positive
γ-Amino-n-butyric acid	6.960	274	171	20	y = 0.3012 x + 0.0016	0.9985	0.7813 – 100	0.0144	0.0437	Positive
L-Proline	8.343	286	171	20	y = 0.4472 x – 0.0027	0.9997	0.7813 – 100	0.0064	0.0194	Positive
DL-β-Amino-isobutryic acid	9.337	274	171	20	$y = 0.3839\ x + 2.33*10^{-4}$	0.9994	0.7813 – 100	0.0095	0.0287	Positive
L-α-Amino-n-butyric acid	11.898	274	171	20	y = 0.4757 x + 0.0025	0.9999	0.7813 – 100	0.0073	0.0221	Positive
δ-Hydroxylysine	12.870	252	171	20	y = 0.1348 x + 0.0029	0.9979	0.7813 – 100	0.0368	0.1116	Positive
L-Ornithine	13.802	237	171	20	y = 0.3517 x + 0.0044	0.9996	0.7813 – 100	0.0109	0.0330	Positive
Cystathionine	15.000	282	171	20	y = 0.1893 x + 0.0022	0.9996	0.7813 – 100	0.0211	0.0639	Positive
L-Lysine	15.489	244	171	20	y = 0.4288 x + 0.0032	0.9991	0.7813 – 100	0.0097	0.0295	Positive

L-Valine	15.922	288	171	20	$y = 0.662 x + 0.0047$	0.9995	0.7813 – 100	0.0058	0.0177	Positive
L-Methionine	16.137	320	171	20	$y = 0.5912 x – 0.0042$	0.9997	0.7813 – 100	0.0052	0.0157	Positive
L-Anserine	16.468	411	171	20	$y = 0.0049 x + 5.27*10^{-5}$	0.9978	0.7813 – 100	0.9157	2.7748	Positive
L-Cystine	16.473	291	171	20	$y = 0.4095 x + 8.88*10^{-4}$	0.9980	0.7813 – 100	0.0104	0.0315	Positive
L-Tyrosine	17.185	352	171	20	$y = 0.5708 x – 0.0112$	0.9986	0.7813 – 100	0.0035	0.0107	Positive
L-Leucine	19.900	302	171	20	$y = 0.8063 x + 8.69*10^{-4}$	0.9998	0.7813 – 100	0.0041	0.0125	Positive
L-isoleucine	20.320	302	171	20	$y = 0.9159 x + 0.0149$	0.9993	0.7813 – 100	0.0046	0.0139	Positive
L-Homocystine	20.451	305	171	20	$y = 0.5203 x + 0.0039$	0.9987	0.7813 – 100	0.0082	0.0250	Positive
L-Phenylalanine	20.624	336	171	20	$y = 1.0483 x – 0.0189$	0.9981	0.7813 – 100	0.0016	0.0048	Positive
L-Tryptophan	20.739	375	171	20	$y = 0.6495 x – 0.0216$	0.9953	0.7813 – 100	0.0011	0.0034	Positive

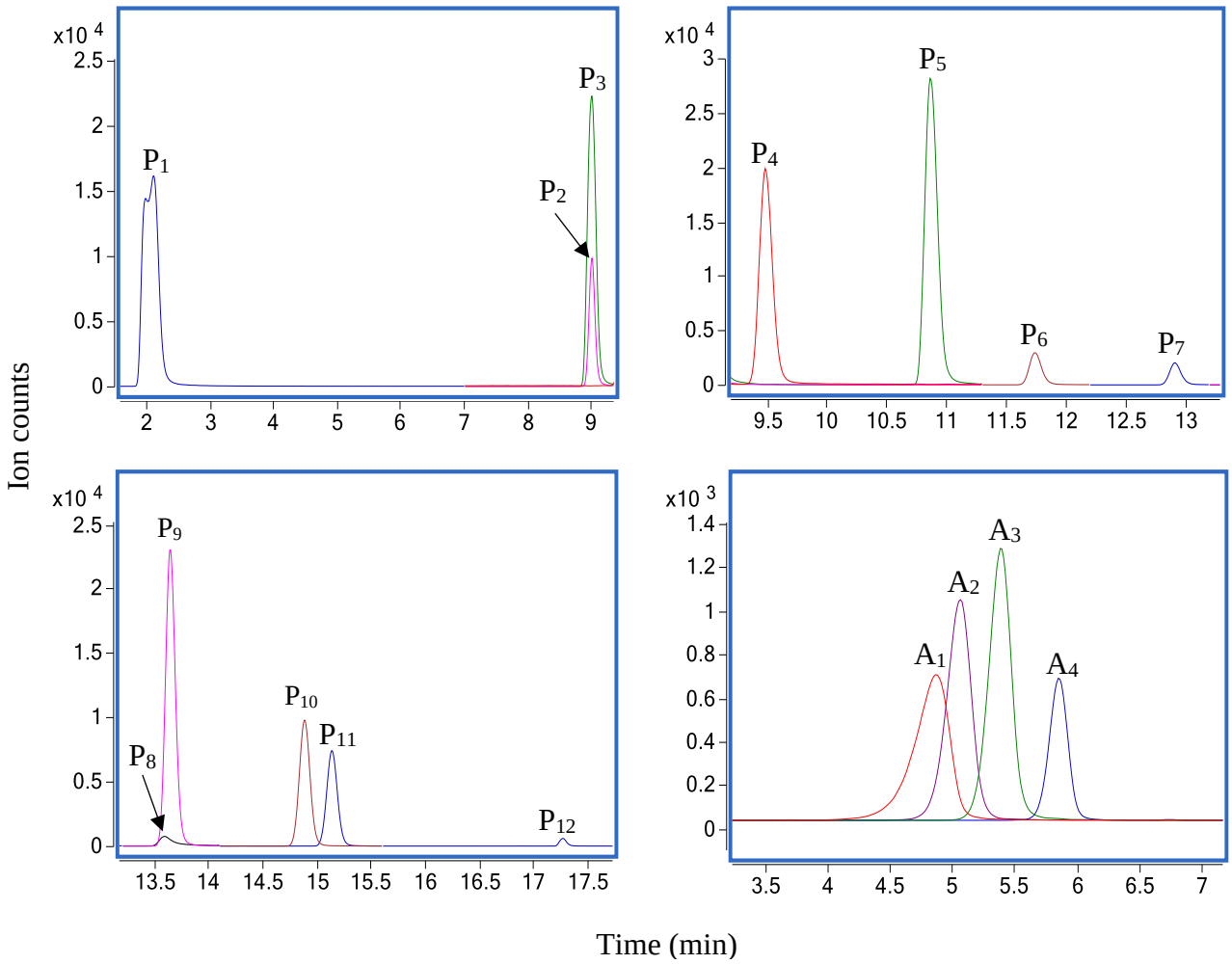
Appendix B5. MRM chromatograms of α-tocopherol, β-carotene and ascorbic acid standards were identified by using LC-MS



Appendix B6. Method validation for identification and quantification of α-tocopherol, β-carotene and ascorbic acid by using LC-MS

Parameters	α-tocopherol	β-carotene	Ascorbic acid
RT (min)	3.524	7.931	3.965
Precursor ion (*m/z*)	431.0	537.0	175.0
Product ion (*m/z*)	165.0	537.0	115.0
Collision energy (V)	32	1	8
Regression equation	y = 4122.59 x + 437.03	y = 13372 x – 1741.49	y = 1063.14 x – 506.73
Linear fit correlation coefficient (R^2)	0.9991	0.9998	0.9991
Calibration range (mg/L)	0.1563 – 20	0.1563 – 20	0.625 – 20
Limit of detection (µg/L)	0.1771	0.0258	0.8638
Limit of quantification (µg/L)	0.5366	0.0782	2.617
Ion mode	Positive	Positive	Negative

Appendix B7. MRM chromatograms of 12 phenolics and 4 anthocyanins standards were identified using LC-MS



P_1: gallic acid P_2: chlorogenic acid P_3: catechin P_4: caffeic acid

P_5: epicatechin P_6: *p*-coumaric acid P_7: ferulic acid P_8: ellagic acid

P_9: rutin P_{10}: kaempferol 3-rutinoside

P_{11}: isorhamnetin 3-rutinoside P_{12}: kaempferol

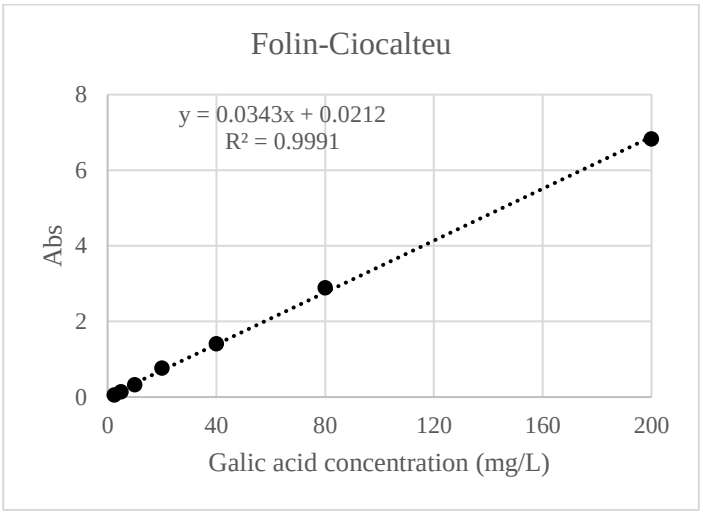
A_1: delphinidin 3-rutinoside A_2: cyanidin 3-glucoside

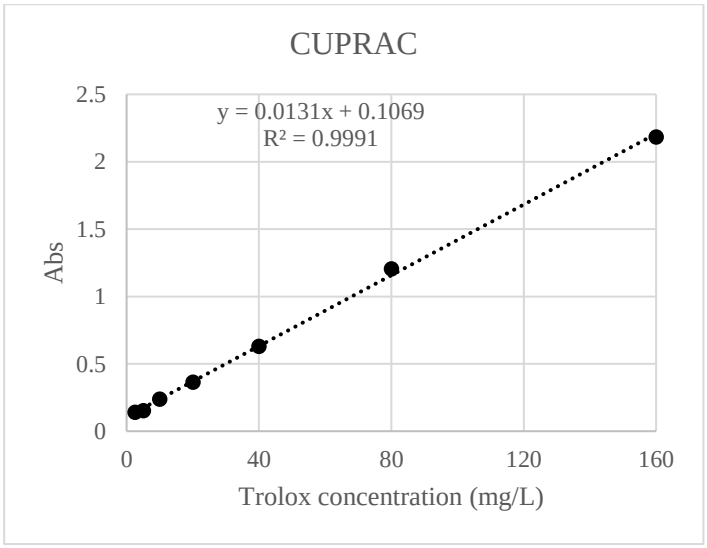
A_3: cyanidin 3-rutinoside A_4: pelargonidin 3-rutinoside

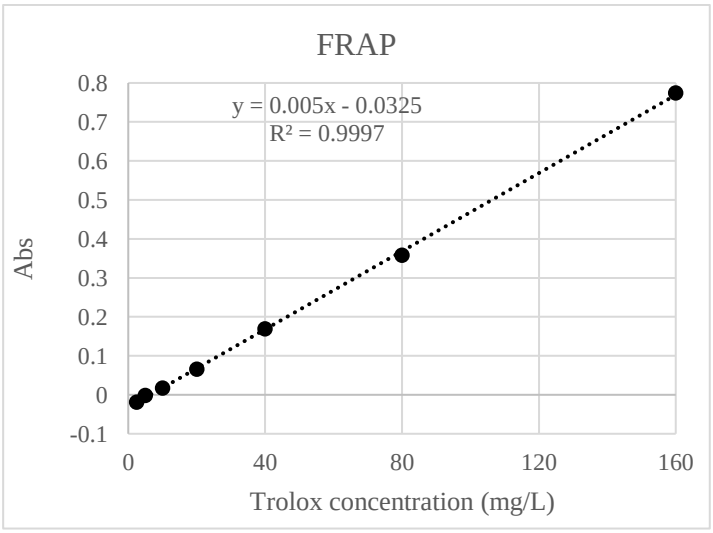
Appendix B8. Method validation for identification and quantification of phenolics including anthocyanins. Compounds were listed in the order of chemical group and then retention time (RT)

Analytes	RT (min)	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy (V)	Regression equation	Linear fit correlation coefficient (R^2)	Calibration range (mg/L)	LOD (μg/L)	LOQ (μg/L)	Ion mode
Polyphenols										
Gallic acid	2.089	169.0	125.0	10	y = 37448.95 x + 1792.43	0.9964	0.0488 – 6.25	0.2980	0.9030	Negative
Chlorogenic acid	8.908	353.0	191.0	13	y = 9149.52 x – 1284.97	0.9971	0.0977 – 6.25	0.1920	0.5817	Negative
Catechin	9.020	289.0	245.0	6	y = 7589.21 x – 1046.10	0.9985	0.4883 – 31.25	0.6137	1.8598	Negative
Caffeic acid	9.381	179.0	135.0	13	y = 14247.18 x – 5425.88	0.9994	0.0879 – 11.25	0.0419	0.1270	Negative
Epicatechin	10.870	289.0	245.0	8	y = 8305.81 x – 5475.92	0.9937	0.2441 – 15.625	0.7679	2.3270	Negative
p-coumaric acid	11.651	163.0	119.0	12	y = 2839.08 x + 114.77	0.9992	0.0586 – 7.5	0.0911	0.2761	Negative
Ferulic acid	12.827	193.0	134.0	12	y = 822.08 x + 37.54	0.9999	0.1074 – 13.75	0.1878	0.5692	Negative
Ellagic acid	13.557	301.0	145.0	36	y = 787.13 x – 24.83	0.9992	0.0732 – 9.375	1.7155	5.1984	Negative
Rutin	13.622	609.0	300.0	40	y = 23979.25 x – 1254.12	0.9992	0.0488 – 6.25	0.0092	0.0279	Negative
Kaempferol 3-rutinoside	14.852	593.0	285.0	30	y = 10917.23 x – 340.32	0.9984	0.0488 – 6.25	0.0287	0.0869	Negative
Isorhamnetin 3-rutinoside	15.096	623.0	315.0	28	y = 8272.36 x – 297.53	0.9991	0.0488 – 6.25	0.0408	0.1237	Negative
Kaempferol	17.228	285.0	239.0	24	y = 380.98 x – 80.54	0.9993	0.0586 – 7.5	0.8451	2.5610	Negative
Anthocyanins										
Delphinidin 3-rutinoside	4.890	609.1	300.1	33	y = 333.47x – 32.98	0.9998	0.0391 – 40.0	2.3044	6.9831	Negative
Cyanidin 3-glucoside	5.064	447.1	284.1	21	y = 364.07x + 39.51	0.9994	0.0391 – 40.0	3.8123	11.5523	Negative
Cyanidin 3-rutinoside	5.396	593.2	284.1	33	y = 409.22x + 2.45	0.9998	0.0391 – 40.0	1.8851	5.7124	Negative
Pelargonidin 3-rutinoside	5.848	577.2	269.1	25	y = 175.67x + 4.21	0.9999	0.0391 – 40.0	3.3400	10.1212	Negative

Appendix B9. Standard curves for quantification of total phenolic content and antioxidant activity







Appendix B10. Carotenoid and chlorophyll pigment compounds and their relative concentrations determined in the pulp and peel of three tamarillo cultivars by using LC-MS

Pigments	Relative concentration (mg/100 g FW)					
	'Amber' peel	'Amber' pulp	'Laird's Large' peel	'Laird's Large' pulp	'Mulligan' peel	'Mulligan' pulp
Provitamin A carotenoids						
β-Carotene	0.23 ± 0.07^{a}	0.72 ± 0.19^{b}	0.15 ± 0.02^{a}	0.36 ± 0.1^{a}	0.15 ± 0.03^{a}	0.89 ± 0.2^{b}
β-Cryptoxanthin	0.25 ± 0.08^{a}	0.24 ± 0.04^{a}	0.1 ± 0.01^{b}	0.12 ± 0.03^{b}	0.23 ± 0.05^{a}	0.22 ± 0.04^{a}
Xanthophyll carotenoids						
Astaxanthin	0.02 ± 0.01^{a}	$< 0.005^{b}$	0.01 ± 0^{b}	$< 0.005^{b}$	$< 0.005^{b}$	$< 0.005^{b}$
Violaxanthin	0.04 ± 0.02^{a}	0.02 ± 0.01^{ab}	0.04 ± 0.02^{a}	0.01 ± 0^{b}	0.04 ± 0.01^{a}	0.02 ± 0.01^{ab}
Diadinoxanthin	0.01 ± 0.01^{a}	0.01 ± 0^{ab}	0.01 ± 0.01^{ab}	$< 0.005^{b}$	0.01 ± 0.01^{ab}	0.01 ± 0^{ab}
Antheraxanthin	0.15 ± 0.03^{a}	0.09 ± 0.02^{bc}	0.09 ± 0.04^{b}	0.04 ± 0.01^{c}	0.07 ± 0.03^{bc}	0.11 ± 0.03^{ab}
Dinoxanthin	$< 0.005^{a}$	$< 0.005^{a}$	$< 0.005^{a}$	$< 0.005^{a}$	$< 0.005^{a}$	$< 0.005^{b}$
Lutein	0.13 ± 0.02^{abc}	0.17 ± 0.09^{bc}	0.06 ± 0.02^{a}	0.08 ± 0.03^{a}	0.09 ± 0.03^{ab}	0.2 ± 0.06^{c}
Flavoxanthin A	0.02 ± 0^{ab}	0.02 ± 0.01^{ab}	0.01 ± 0^{c}	0.02 ± 0.01^{a}	0.01 ± 0.01^{ac}	0.03 ± 0^{b}
Diatoxanthin	$< 0.005^{a}$	$< 0.005^{ab}$	n.d	n.d	n.d	$< 0.005^{b}$
Zeaxanthin	0.32 ± 0.06^{a}	0.24 ± 0.08^{ab}	0.11 ± 0.07^{cd}	0.06 ± 0.02^{d}	0.06 ± 0.01^{d}	0.2 ± 0.06^{bc}
Siphonaxanthin	0.01 ± 0.01^{a}	0.01 ± 0^{a}	0.01 ± 0.01^{a}	$< 0.005^{a}$	0.01 ± 0.01^{a}	$< 0.005^{a}$
Caricaxanthin	n.d	$< 0.005^{a}$	$< 0.005^{a}$	n.d	n.d	n.d
Flavoxanthin B	0.01 ± 0^{a}	0.01 ± 0.01^{ab}	$< 0.005^{a}$	0.01 ± 0.01^{ab}	0.01 ± 0^{b}	$< 0.005^{a}$
Fucoxanthin	n.d	$< 0.005^{a}$	n.d	$< 0.005^{a}$	n.d	n.d

Chlorophyll pigments						
Chlorophyll A	n.d	$< 0.005^{a}$	$< 0.005^{b}$	$< 0.005^{b}$	$< 0.005^{b}$	$< 0.005^{b}$
Chlorophyll C1	0.01 ± 0.01^{a}	0.02 ± 0.01^{a}	0.03 ± 0.02^{a}	0.01 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.02^{a}
Chlorophyll C2	0.06 ± 0.01^{a}	$< 0.005^{b}$	$< 0.005^{b}$	$< 0.005^{b}$	$< 0.005^{b}$	$< 0.005^{b}$
Chlorophyll C3	0.02 ± 0.01^{a}	$< 0.005^{b}$	$< 0.005^{b}$	$< 0.005^{b}$	n.d	$< 0.005^{b}$
Chlorophyll D	n.d	n.d	n.d	n.d	n.d	n.d
Phaeophytin	n.d	0.02 ± 0.01^{a}	n.d	0.05 ± 0.01^{a}	n.d	0.07 ± 0.05^{a}

n.d: not detected. Data results are presented as mean ± SD and listed in the order of bioactive groups, then retention time (RT). Different alphabets superscripts indicate statistical difference ($p < 0.05$) across each row.

Appendix B11. Carotenoid and chlorophyll pigment compounds and their relative percentage contents (%) determined in the pulp and peel of tamarillo cultivars.

Pigments	RT (min)	*m/z*	Relative percentage contents (%)					
			'Amber' peel	'Amber' pulp	'Laird's Large' peel	'Laird's Large' pulp	'Mulligan' peel	'Mulligan' pulp
Provitamin A carotenoids								
β-Carotene	22.7	536.4	17.68 ± 4.08	45.62 ± 10.45	22.72 ± 4.02	47.23 ± 7.71	21.32 ± 4.38	49.79 ± 9.64
β-Cryptoxanthin	20.3	522.4	18.73 ± 2.85	13.61 ± 2.76	16.36 ± 4.29	16.62 ± 5.29	31.82 ± 3.3	14.02 ± 1.88
Xanthophyll carotenoids								
Astaxanthin	6.4	596.4	1.75 ± 0.89	0.07 ± 0.06	0.96 ± 0.58	0.03 ± 0.05	0.49 ± 0.18	0.18 ± 0.16
Violaxanthin	9.7	600.4	3.01 ± 0.96	1.18 ± 0.56	6.79 ± 3.6	1.2 ± 0.65	6.25 ± 1.65	1.24 ± 1.01
Diadinoxanthin	11.4	583.4	1.06 ± 0.39	0.62 ± 0.08	1.66 ± 1.4	0.36 ± 0.22	1.44 ± 0.54	0.65 ± 0.11
Antheraxanthin	11.4	584.4	11.8 ± 1.1	4.95 ± 1.12	13.78 ± 5.22	5.41 ± 1.04	10.31 ± 4.69	6.67 ± 1.21
Dinoxanthin	12.5	642.4	0.02 ± 0.02	0.04 ± 0.03	0.03 ± 0.03	0.04 ± 0.03	0.03 ± 0.03	0.15 ± 0.13
Lutein	12.9	568.4	10.49 ± 0.74	9.76 ± 5.07	13.63 ± 6.64	8.41 ± 2.45	12.85 ± 4.55	12.58 ± 3.96
Flavoxanthin A	12.9	584.4	1.45 ± 0.64	1.07 ± 0.53	0.75 ± 0.57	2.36 ± 1.22	1.18 ± 0.79	1.82 ± 0.23
Diatoxanthin	13.0	566.4	0.27 ± 0.16	0.08 ± 0.06	n.d	n.d	n.d	0.04 ± 0.04
Zeaxanthin	13.0	568.4	25.11 ± 2.11	13.47 ± 5.1	16.43 ± 8.73	7.87 ± 2.02	8.63 ± 1.18	12.62 ± 3.97
Siphonaxanthin	17.1	600.4	1.1 ± 1	0.45 ± 0.07	1.64 ± 1.27	0.24 ± 0.2	0.81 ± 0.79	0.11 ± 0.07
Caricaxanthin	18.2	522.4	n.d	0.05 ± 0.04	0.11 ± 0.1	n.d	n.d	n.d
Flavoxanthin B	19.0	598.4	0.46 ± 0.06	0.55 ± 0.31	0.45 ± 0.28	1.32 ± 1.28	2.03 ± 0.36	0.16 ± 0.13

Fucoxanthin	21.1	658.4	n.d	0.03 ± 0.03	n.d	0.05 ± 0.04	n.d	n.d
Chlorophyll pigments								
Chlorophyll A	23.3	892.5	n.d	0.26 ± 0.16	0.08 ± 0.06	0.08 ± 0.07	0.13 ± 0.11	0.05 ± 0.01
Chlorophyll C1	18.5	610.2	0.84 ± 0.8	1.15 ± 0.65	4.5 ± 1.88	2.1 ± 1.83	2.62 ± 1	1.46 ± 1.18
Chlorophyll C2	8.1	608.2	4.49 ± 0.12	0.11 ± 0.06	0.11 ± 0.08	0.08 ± 0.07	0.1 ± 0.06	0.06 ± 0.05
Chlorophyll C3	8.0	652.2	1.74 ± 0.87	0.05 ± 0.04	0.02 ± 0.01	0.12 ± 0.09	n.d	0.04 ± 0.02
Chlorophyll D	21.6	894.5	n.d	n.d	n.d	n.d	n.d	n.d
Phaeophytin	23.8	896.8	n.d	1.01 ± 0.44	n.d	6.46 ± 1.74	n.d	4.21 ± 2.88

n.d: not detected

Appendix B12. Comparison of volatile compounds between fresh and freeze-dried pulp samples of 'Laird's Large' cultivar using SPME-GC-MS (data presented as percentage of relative concentration)

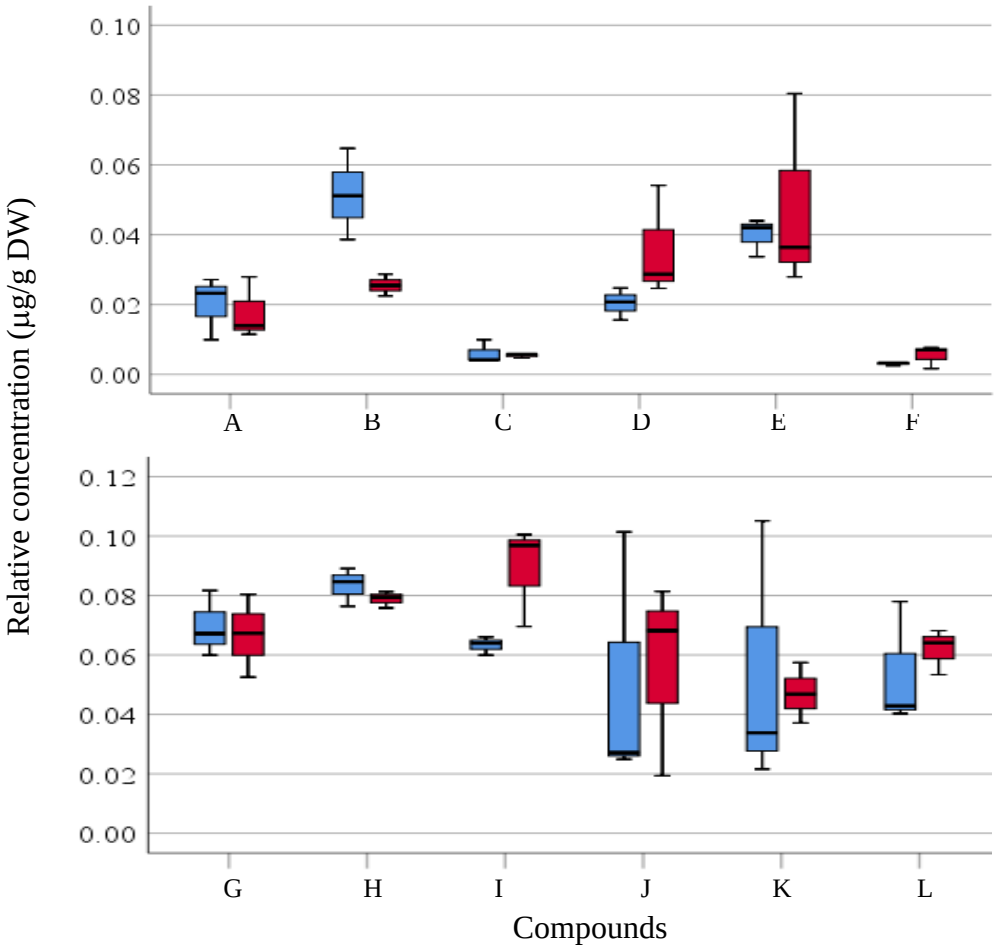
Used with permission (Diep et al., 2021)

No	Compounds	Fresh Pulp (%)	Dried Pulp (%)	% (dried – fresh)
1	3-methyl-Butanal	0.017	0.086	80.7
2	1-Methoxy-3-methyl-3-butene	0.898	0.137	- 84.7
3	Butanoic acid, methyl ester	10.146	8.470	- 16.5
4	1-methyl-1,4-Cyclohexadiene	1.145	0.260	- 77.3
5	Methyl isovalerate	0.241	0.154	- 36.1
6	3-methyl-3-Buten-1-ol	1.861	0.909	- 51.2
7	Butanoic acid, ethyl ester	0.018	0.548	96.7
8	3-Butenoic acid, 3-methyl-, methyl ester	1.614	0.476	- 70.5
9	1-Pentanol	0.026	0.122	78.9
10	Hexanal	1.443	0.949	34.2
11	3-Hexenal	0.522	0.323	- 38.2
12	Prenol	0.417	0.390	- 6.5
13	Isopropyl butyrate	0.263	0.127	- 51.8
14	2-Ethyl-tetrahydropyran	0.165	0.145	- 11.9
15	2-methyl-2-Butenal	0.557	0.729	23.6
16	2-Butenoic acid, 3-methyl-, methyl ester	7.818	2.624	66.4
17	(S)-(+)-1,2-Propanediol	n.d	1.194	
18	4-methyl-1-(1-methylethyl)- Bicyclo[3.1.0] hex-2-ene	0.110	0.109	- 1.7
19	1-Butanol, 2-methyl-, acetate	0.037	0.032	- 13.9
20	Alpha-Pinene	0.067	0.145	54.0
21	2,2-dimethyl- 3-Octene	0.091	0.093	2.0
22	3-Methyl-3-buten-1-ol, acetate	15.543	6.633	- 57.3
23	2-Hexenal	3.494	3.187	- 8.8
24	[R-(R*,R*)]-2,3-Butanediol	n.d	16.426	
25	(Z)- 3-Hexen-1-ol	1.802	1.610	- 10.6
26	[S-(R*,R*)]-2,3-Butanediol	n.d	0.668	
27	Propyl-Cyclopropane	3.926	0.675	- 82.8
28	Hexanoic acid, methyl ester	30.200	36.926	18.2
29	2-Buten-1-ol, 3-methyl-, acetate	1.860	0.745	- 60.0
30	2,3-Dehydro-1,8-cineole	0.596	2.225	73.2
31	Ether, 2-ethylhexyl tert-butyl	0.014	0.552	97.5
32	D-Limonene	0.025	0.101	75.6
33	Hexanoic acid, ethyl ester	0.025	0.505	95.0
34	3-Methyl-3-butenoic acid	0.017	0.647	97.4
35	o-Cymene	0.171	0.336	49.2
36	Eucalyptol	1.292	0.496	- 61.6
37	(Z)- 3-Hexen-1-ol, acetate	0.150	0.064	- 57.4
38	2-methyl-3-(1-methylethenyl)-, (1a,2a,3a)-Cyclohexanol	0.046	0.138	66.8

39	Pentanoic acid, 2-hydroxy-3-methyl-, methyl ester	0.030	0.163	81.5	
40	.+/-.-Tetrahydro-3-furanmethanol	n.d	0.714		
41	Butanoic acid, 4-pentenyl ester	10.474	2.641		- 74.8
42	Butanoic acid, 3-methylbut-2-enyl ester	0.239	0.062		- 74.2
43	1-methyl-2-octyl-Cyclopropane,	0.026	0.110	76.3	
44	Octanoic acid, 3-hydroxy-, methyl ester	0.008	0.104	92.3	
45	Butanedioic acid, methyl-, dimethyl ester	0.016	0.245	93.4	
46	Octanoic acid, methyl ester	0.284	0.204		- 28.2
47	Nonanal	0.051	0.265	80.9	
48	Linalool	0.024	0.010		- 60.2
49	Ethylene glycol di-n-butyrate	0.125	0.237	47.1	
50	Benzoic acid, hydrazide	0.417	0.499	16.5	
51	Hexanoic acid, 4-oxo-, methyl ester	0.124	1.863	93.3	
52	(Z)- Butanoic acid, 3-hexenyl ester	0.007	0.042	82.8	
53	5-ethyldihydro-2(3H)-Furanone	0.052	0.485	89.3	
54	2-Acetyl-5-methylfuran	0.014	0.172	91.7	
55	[R-(R*,R*)]-1,2-diphenyl-, 1,2-Ethanediol	0.031	0.070	56.1	
56	Terpinen-4-ol	0.063	0.017		- 73.8
57	4-methyl-1-Hexanol	0.251	0.086		- 65.7
58	(E,E)-2,6-Nonadienal	0.251	0.086		- 65.7
59	Butyric acid, 2-hydroxy-3-methyl-, methyl ester	0.005	0.219	97.9	
60	p-Mentha-1,5-dien-8-ol	0.062	0.269	76.9	
61	L-.alpha.-Terpineol	0.399	0.201		- 49.7
62	Methyl salicylate	0.071	0.012		- 82.7
63	p-Mentha-1,5-dien-8-ol	0.110	0.595	81.5	
64	1,3,3-trimethyl- 2-Oxabicyclo[2.2.2]octan-6-ol,	0.001	0.098	98.5	
65	Hexanoic acid, 4-pentenyl ester	0.068	0.025		- 63.6
66	1,3,3-trimethyl-2-Oxabicyclo[2.2.2]octan-6-one	0.026	0.042	37.8	
67	2,6,6-trimethyl- Bicyclo(3.1.1)heptane-2,3-diol	n.d	0.086		
68	Butanoic acid, 2,3-dihydroxypropyl ester	0.030	0.030	2.2	
69	(Z)- 2-methoxy-4-(1-propenyl)-Phenol	0.049	0.012		- 75.9
70	Methyleugenol	0.007	0.0002		- 97.4
71	2,6-Dimethyl-2-trans-6-octadiene	0.014	0.208	93.4	
72	l-Alanine, N-(2,3,4-trifluorobenzoyl)-, methyl ester	0.002	0.011	84.5	
73	Aristol-1(10)-en-9-yl isovalerate	0.029	0.122	76.5	

Appendix B13. Comparison of relative concentration of some compounds between fresh and dried pulp samples of 'Laird's Large' cultivar analysed by TD-GC-MS (■ fresh sample, ■ freeze-dried sample)

Used with permission (Diep et al., 2021)



A: 2,4-hexadien-1-ol

B: 2,4,7,9-tetramethyl-5-decyn-4,7-diol

C: 5-hydroxymethylfurfural

D: benzeneacetaldehyde

E: nonanal

F: *p*-mentha-1,5-dien-8-ol

G: 2,3-dehydro-1,8-cineole

H: acetophenone

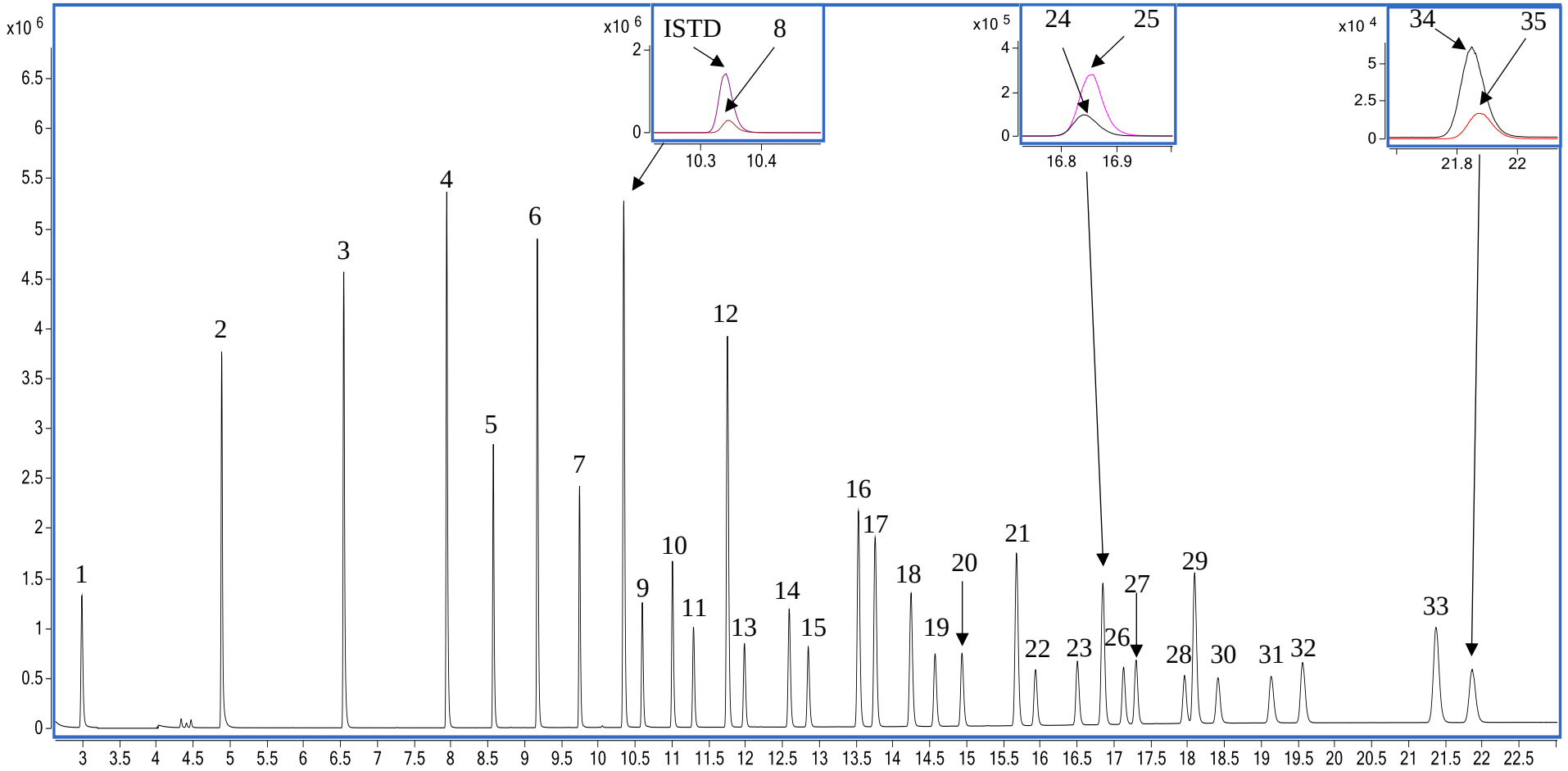
I: benzaldehyde

J: butanoic acid

K: octanal

L: propanoic acid

Appendix B14. MRM chromatograms of 35 fatty acids standards and biphenyl (internal standard) were identified using GC-MS. The compounds were numbered as Appendix 15



Appendix B15. Method validation for quantification of fatty acids standards in tamarillo yoghurt. Compounds were listed "in the order of retention time (RT)

No.	Analytes	RT (min)	SIM ion (*m/z*)*	Regression equation	Linear fit correlation coefficient (R^2)	Calibration range (mg/L)	LOD (μg/L)	LOQ (μg/L)
1	Butyric acid, methyl ester	2.983	74; 87	y = 0.3786 x + 0.0015	0.9996	0.4 – 80	0.0877	0.2658
2	Hexanoic acid, methyl ester	4.887	74.1; 87.1; 99	y = 0.6488 x + 0.003	0.9997	0.4 – 80	0.0731	0.2216
3	Octanoic acid, methyl ester	6.544	74.1; 87.1; 127	y = 0.7784 x + 0.0054	0.9995	0.4 – 80	0.0820	0.2484
4	Decanoic acid, methyl ester	7.94	74.1; 87.1; 186	y = 0.8442 x – 0.003	0.9991	0.4 – 40	0.1168	0.3538
5	Undecanoic acid, methyl ester	8.573	74; 87; 169	y = 0.8391 x – 0.0032	0.9976	0.2 – 20	0.0685	0.2076
6	Dodecanoic acid, methyl ester	9.171	74.1; 87.1; 214	y = 0.7975 x – 0.0049	0.9977	0.4 – 40	0.2422	0.7338
7	Tridecanoic acid, methyl ester	9.744	74.1; 87.1; 228	y = 0.7544 x – 0.0029	0.9963	0.2 – 20	0.1356	0.4109
8	Myristic acid, methyl ester	10.345	199; 143; 242	$y = 0.1308\ x – 6.3*10^{-4}$	0.9977	0.4 – 40	2.0674	6.2650
9	Myristoleic acid, methyl ester	10.596	55; 166; 208	$y = 0.2192\ x – 7.3*10^{-4}$	0.9968	0.2 – 20	0.5325	1.6136
10	Pentadecanoic acid, methyl ester	11.008	74; 87; 256	y = 0.6436 x – 0.0029	0.9962	0.2 – 20	0.2087	0.6325
11	cis-10-Pentadecenoic acid, methyl ester	11.29	55; 96; 222	$y = 0.2231\ x – 8.3*10^{-4}$	0.9969	0.2 – 20	0.5886	1.7836
12	Palmitic acid, methyl ester	11.753	74; 87; 270	y = 0.5999 x – 0.0073	0.9977	0.6 – 60	0.8514	2.5800
13	Palmitoleic acid, methyl ester	11.981	55; 194; 236	$y = 0.1626\ x – 4.8*10^{-4}$	0.9974	0.2 – 20	0.9011	2.7305
14	Heptadecanoic acid, methyl ester	12.588	74; 87; 284	y = 0.5526 x – 0.0025	0.9961	0.2 – 20	0.2574	0.7801
15	cis-10-Heptadecenoic acid, methyl ester	12.847	55; 208; 250	$y = 0.1703\ x – 6.2*10^{-4}$	0.9968	0.2 – 20	0.8877	2.6900
16	Stearic acid, methyl ester	13.53	74; 87; 298	y = 0.5327 x + 0.0011	0.9997	0.4 – 40	0.5280	1.6000
17	Elaidic, Oleic	13.759	264; 296; 222	$y = 0.0591\ x – 8.2*10^{-4}$	0.9963	0.6 – 60	8.1200	24.606
18	Linoleaidic, Linoleic	14.239	81; 95; 294	$y = 0.1912\ x + 1.2*10^{-4}$	0.9999	0.4 – 40	1.4913	4.5191

19	γ-Linolenic acid, methyl ester	14.569	79.1; 93.1; 292	$y = 0.1780\ x - 8.3*10^{-4}$	0.9967	0.2 – 20	0.8241	2.4972
20	Linolenic acid, methyl ester	14.935	79.1; 95.1; 292	$y = 0.2399\ x - 0.0012$	0.9965	0.2 – 20	0.6023	1.8251
21	Arachidic acid, methyl ester	15.672	74.1; 87.1; 326	$y = 0.4953\ x - 3.3*10^{-4}$	0.9996	0.4 – 40	0.5410	1.6385
22	cis-11-Eicosenoic acid, methyl ester	15.937	292; 97; 250	$y = 0.0592\ x + 3.7*10^{-5}$	0.9997	0.2 – 20	2.3276	7.0534
23	cis-11,14-Eicosadienoic acid, methyl ester	16.502	81.1; 95.1; 322	$y = 0.1968\ x - 6.0*10^{-5}$	0.9996	0.2 – 20	0.7027	2.1292
24	cis-8,11,14-Eicosatrienoic acid methyl ester	16.84	79.1; 80.1; 320	$y = 0.1740\ x + 1.8*10^{-4}$	0.9999	0.2 – 20	0.7828	2.3721
25	Heneicosanoic acid, methyl ester	16.851	74; 87; 340	$y = 0.4887\ x - 9.2*10^{-5}$	0.9995	0.2 – 20	0.2657	0.8051
26	Arachidonic acid, methyl ester	17.128	79; 91; 150	$y = 0.1711\ x + 9.0*10^{-5}$	0.9999	0.2 – 20	0.8057	2.4414
27	11,14,17-Eicosatrienoic acid methyl ester	17.297	79; 95.1; 320	$y = 0.2213\ x - 6.8*10^{-5}$	0.9998	0.2 – 20	0.6217	1.8840
28	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	17.958	79; 91	$y = 0.1974\ x - 2.6*10^{-4}$	0.9993	0.2 – 20	0.6962	2.1098
29	Behenic acid, methyl ester	18.099	354; 311; 143	$y = 0.0661\ x + 8.8*10^{-6}$	0.9995	0.4 – 40	4.1327	12.523
30	Erucic acid, methyl ester	18.412	320; 97; 278	$y = 0.0593\ x - 3.0*10^{-5}$	0.9993	0.2 – 20	2.3649	7.1664
31	cis-13,16-Docasadienoic acid, methyl ester	19.134	67; 81.1; 350	$y = 0.1774\ x - 2.3*10^{-4}$	0.9993	0.2 – 20	0.7270	2.2030
32	Tricosanoic acid, methyl ester	19.56	368; 325	$y = 0.0638\ x - 3.9*10^{-5}$	0.9993	0.2 – 20	2.0733	6.2827
33	Lignoceric acid, methyl ester	21.372	382; 74; 339	$y = 0.0596\ x - 6.3*10^{-4}$	0.9955	0.4 – 40	4.6593	14.119
34	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	21.851	79; 91; 119	$y = 0.1794\ x - 1.1*10^{-4}$	0.9997	0.2 – 20	0.7163	2.1707
35	cis-15-Tetracosenoic acid, methyl ester	21.877	348; 97; 380	$y = 0.0539\ x - 3.4*10^{-5}$	0.9993	0.2 – 20	2.4609	7.4573

*The first ion was used for quantification and the second (and third) ions were used for qualification

Appendix B16. Concentrations (μg/g FW) of individual bioactive compounds in tamarillo extract and cubosome containing tamarillo extract after each step of *in vitro* digestion.

Bioactive	Tamarillo extract				Tamarillo bioactive-loaded cubosome			
compounds	Undigested	Oral	Gastric	Intestinal	Undigested	Oral	Gastric	Intestinal
Phenolics								
Gallic Acid	0.41 ± 0.009	0.016 ± 0	0.191 ± 0.001	0.084 ± 0	0.068 ± 0.003	0.003 ± 0	0.006 ± 0	0.036 ± 0
Catechin	0.037 ± 0.004	0.009 ± 0	0.01 ± 0	0.009 ± 0	0.03 ± 0.014	0.002 ± 0	0.007 ± 0.001	0.005 ± 0
Caffeic acid	22.675 ± 1.471	5.471 ± 0.111	7.124 ± 0.205	4.975 ± 0.104	4.89 ± 0.48	3.971 ± 0.106	0.134 ± 0.004	0.392 ± 0.016
Chlorogenic acid	105.933 ± 5.436	9.953 ± 0.176	71.712 ± 0.994	5.106 ± 0.061	14.377 ± 0.891	0.717 ± 0.013	1.341 ± 0.04	4.135 ± 0.063
Epicatechin	0.1 ± 0.017	0.024 ± 0.001	0.055 ± 0.003	0.014 ± 0.001	0.035 ± 0.007	0.003 ± 0	0.006 ± 0	0.006 ± 0
p-coumaric acid	0.935 ± 0.085	0.363 ± 0.007	0.368 ± 0.005	0.039 ± 0	0.684 ± 0.655	0.213 ± 0.008	0.111 ± 0.006	0.159 ± 0.027
Ferulic acid	1.783 ± 0.051	0.099 ± 0.001	0.238 ± 0.001	0.317 ± 0.002	0.276 ± 0.112	0.15 ± 0.01	0.009 ± 0.001	0.089 ± 0.01
Rutin	0.882 ± 0.022	0.273 ± 0.002	0.342 ± 0.002	0.203 ± 0.001	0.195 ± 0.006	0.032 ± 0	0.029 ± 0	0.047 ± 0
Kaempferol 3-rutinoside	58.811 ± 1.473	18.858 ± 0.128	22.119 ± 0.087	9.135 ± 0.03	7.258 ± 0.213	1.524 ± 0.008	1.558 ± 0.01	2.109 ± 0.01
Isorhamnetin 3-rutinoside	0.123 ± 0.005	0.01 ± 0	0.013 ± 0	0.012 ± 0	0.038 ± 0.004	0.001 ± 0	0.004 ± 0	0.004 ± 0
Kaempferol	0.491 ± 0.003	0.215 ± 0	0.1 ± 0	0.029 ± 0	0.129 ± 0.022	0.038 ± 0.001	0.026 ± 0.001	0.043 ± 0.001
Anthocyanins								
Delphinidin 3-rutinoside	54.2 ± 2.426	5.481 ± 0.067	13.337 ± 0.145	4.265 ± 0.01	16.814 ± 1.833	0.892 ± 0.005	3.04 ± 0.076	1.581 ± 0.02
Cyanidin 3-rutinoside	2.481 ± 0.186	0.354 ± 0.006	0.892 ± 0.011	0.107 ± 0.001	0.765 ± 0.152	0.066 ± 0.001	0.207 ± 0.01	0.04 ± 0
Pelargonidin 3-rutinoside	24.392 ± 1.953	5.027 ± 0.029	11.889 ± 0.103	1.504 ± 0.006	7.041 ± 0.594	0.726 ± 0.021	1.029 ± 0.017	0.551 ± 0.008

* Data are expressed as Mean ± SD (n = 3). Different alphabets superscripts indicate statistical difference ($P < 0.05$) across each row

Appendix B17. Estimated contributions of 'Laird's Large' tamarillo and milk to nutrient and phytochemical content of fortified yoghurt

Nutrients	Tamarillo pulp (g%)	Tamarillo powder (g%)	Milk (g%)	5% yoghurt (g%)	10% yoghurt (g%)	15% yoghurt (g%)
Water	88.1	3	87.6	83.4	79.1	74.9
Protein	1.2	10.08	3.3	3.64	3.98	4.32
Lipid	0.29	2.44	3.1	3.07	3.03	3.00
Fibre	3	25.21	0	1.26	2.52	3.78
Carbohydrate	3.8	31.93	4.5	5.87	7.24	8.61
Phytochemicals		(mg%)	(mg%)	(mg%)	(mg%)	(mg%)
γ–Amino butyric acid (GABA)	n.a	433	n.a	21.7	43.3	65.0
Total Phenolics	n.a	122.26	n.a	6.1	12.2	18.3
Rutin	n.a	0.97	n.a	0.05	0.10	0.15
Kaempferol 3-rutinoside	n.a	50.04	n.a	2.5	5.0	7.5
Chlorogenic acid	n.a	66.35	n.a	3.3	6.6	10.0
Total anthocyanins	n.a	481.37	n.a	24.1	48.1	72.2
Delphinidin 3-rutinoside	n.a	254.76	n.a	12.7	25.5	38.2
Pelargonidin 3-rutinoside	n.a	200.66	n.a	10.0	20.1	30.1
Total phenolic content and antioxidant activity						
TPC (mg GAE%)	n.a	707.04	n.a	35.4	70.7	106.1
CUPRAC (mg TEAC%)	n.a	1312.02	n.a	65.6	131.2	196.8
FRAP (mg TEAC%)	n.a	1004.33	n.a	75.3	150.7	225.9

Appendix B18. Concentration of polyphenols added into cubosome (■) and concentration of polyphenols present in the supernatant (■)