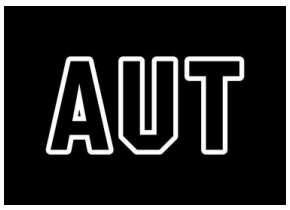

Exploring New Zealand Honeydew Honey, Yacon Concentrate, and their Novel Food and Health Applications

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Thesis Abstract

In order to understand the physical and functional properties of food products, it is essential to obtain physicochemical and sensory information. Without this information, it would be impossible to establish market opportunities, as manufacturers and researchers would be unable to predict consumer acceptance. In this thesis, physicochemical principles were used to understand the properties of New Zealand honeydew honey (NZHDH) and New Zealand yacon concentrate (NZYC). Subsequently, NZHDH and NZYC were incorporated into a novel food product (low alcohol beer), which was analysed using both physicochemical principles and sensory evaluations. Finally, alternative applications of NZHDH and NZYC were investigated, including their inhibitory effects on the formation of acrylamide, their anti-diabetic and anti-obesity properties, and their anti-microbial properties.

In Chapter 3, the physicochemical properties of NZHDH such as proximate composition, mineral, sugar, phenolic, and amino acid profiles, and antioxidant activity, were determined. Results indicated the major mineral in NZHDH was potassium, with significant amounts of phosphorus, magnesium, and sodium. The major sugars were fructose and glucose, while sugars unique to NZHDH such as palatinose, turanose, erlose, and melezitose were also detected, with erlose dominating over melezitose. The major phenolic compounds were pinocembrin, abscisic acid, and pinobanksin, with this being the first comprehensive phenolic profiling of NZHDH conducted to the author's knowledge. Proline was the major amino acid; L-aspartic acid, L-glutamic acid, L-alanine, and L-phenylalanine were the next most abundant, with this being the first profiling of the amino acid content of NZHDH to the author's knowledge. The antioxidant activity of NZHDH was comparable to literature values for Mānuka honey, considered to be the "gold standard" for antioxidant activity. Overall, NZHDH was comparable to other more well-described varieties of HDH in the literature, suggesting it would have similar health-promoting benefits.

In Chapter 4, the physicochemical properties of NZYC such as proximate composition, mineral, sugar, phenolic, amino acid, and organic acid profiles, antioxidant activity, and glycaemic index (GI), were determined. Results indicated that the major mineral in NZYC was potassium, with significant amounts of phosphorus, calcium, and magnesium also identified. Fructose was the major sugar; NZYC was also found to have high total content of fructooligosaccharides (FOS) and inulins (non-digestible prebiotic carbohydrates) which have been associated with numerous health benefits including gut health and weight loss, among

others. Chlorogenic acid and caffeic acid were the major phenolic compounds; L-arginine, L-glutamic acid, L-proline, L-aspartic acid, and asparagine were the major amino acids; citric acid was the major organic acid. The antioxidant activity of NZYC was found to be several times higher than Mānuka honey, and NZYC was determined to be a low-GI food. Overall, these results indicate that NZYC may have excellent health-promoting benefits, particularly in the areas of gut health, weight loss, and antioxidative effects.

In Chapter 5, five novel flavoured low alcohol beers (LABs) (and one control beer) incorporating NZHDH and/or NZYC were developed. The physicochemical properties of these LABs such as ethanol content, fructooligosaccharide and inulin presence, volatile profile, colour, and amino acid profile were determined. All six LABs had sufficiently low ethanol content to be classified as LABs in New Zealand. Beers that were flavoured with NZYC were found to contain fructooligosaccharides and/or inulins, while beers which were not flavoured with NZYC were not. The LABs could not be differentiated in terms of their volatile profile; however, crude NZHDH was found to differ from crude NZYC, particularly due to the presence of compounds associated with "earthy" flavours. Significant differences were not consistently identified across all LAB samples in terms of colour; however, samples containing NZYC tended to have darker colour than samples which did not. The LABs could not be differentiated in terms of their amino acid profiles. Sensory evaluation of the LABs was also conducted using unstructured line scales, just-about-right (JAR) scales, and check-all-that-apply (CATA) questions. Fifty-three participants completed the sensory evaluation (thirty-one male and twenty-two female). For unstructured line scales, the LAB samples could not be differentiated but were overall somewhat liked by the participants. For JAR, three attributes were evaluated and a high proportion of participants indicated that sweetness, hoppiness, and bitterness were too low in the six LAB samples. Twenty-six terms were used for CATA, and seven of these terms reached statistical significance between the LAB samples. Results from physicochemical analysis supported the incorporation of NZYC into a functional LAB beverage; however, both physicochemical and sensory results suggest that reformulation would be required to achieve greater differentiation between the samples and increased overall liking.

In Chapter 6, the potential health benefits of NZHDH and NZYC were investigated with respect to inhibition of the formation of acrylamide in the Maillard reaction, anti-diabetic and anti-obesity activity, and anti-bacterial activity. Results suggest that NZHDH, NZYC, and phenolic compounds found in NZHDH and

NZYC are unable to significantly reduce the formation of acrylamide at the tested concentrations. Phenolic extracts of NZHDH and NZYC were found to exhibit anti-diabetic activity, which was in agreement with literature concerning the major phenolic compounds identified in Chapters 3 and 4. Crude NZYC was found to exhibit anti-obesity activity, which was in agreement with literature concerning the major phenolic compounds identified in Chapter 4, while crude NZHDH exhibited negligible anti-obesity activity. Phenolic extracts of NZHDH and NZYC were unable to inhibit the growth of *Escherichia coli, Pseudomonas aeruginosa,* or *Saccharomyces cerevisiae*.

Overall, this thesis provides insightful information regarding (a) physicochemical information on NZHDH, NZYC, and novel LAB beverages formulated containing NZHDH and/or NZYC, (b) sensory information on the novel LAB beverages, and (c) some potential health benefits of NZHDH and NZYC. In terms of the practicality of the research, manufacturers and researchers now have basic physicochemical information about NZHDH and NZYC, some of which was not previously available in the literature, while NZYC has been highlighted due to its high FOS content and notable anti-obesity effects. Further experimentation should be considered such as reformulation of the LABs to improve functional properties, sample differentiation, and overall liking.

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Attestation of authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor used artificial intelligence tools or generative artificial intelligence tools (unless it is clearly stated, and referenced, along with the purpose of use), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

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Candidate contributions to co-authored papers

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Chen	**Acquisition of research data, analysis and interpretation**
Hamid	**Critical revising**
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Wong	**Acquisition of research data, analysis and interpretation**
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Chapters 1. Introduction

1.1 A brief background on honeydew honey and yacon concentrate

Honey, a natural plant product produced by bees, has been used for centuries for food, medicinal, and cosmetic applications. It can be broadly divided into two classes according to botanical origin; blossom honey (from the nectar of flowers), and honeydew honey (HDH; from honeydew excreted by plant-sucking insects) (Pita-Calvo & Váquez, 2017).

It is likely that honey was a significant food source for prehistoric ancestors of *Homo sapiens* as an energy-rich food source which required minimal mechanical breakdown; however, the earliest record of human harvesting of honey dates back 40,000 to 80,000 years ago (Allsop & Miller, 1996; Crittenden, 2011). Medicinal use of honey dates to the ancient Egyptians, Assyrians, Chinese, Greeks, and Romans (Abou El-Soud, 2012; Eteraf-Oskouei & Najafi, 2013; Kuropatnicki, Kłósek, & Kucharzewski, 2018). HDH has been found to have greater antibacterial and antioxidant properties than blossom honey, meaning it has greater potential for use in medicinal applications (Pita-Calvo & Vázquez, 2018). The advent of modern synthetic medicines saw a decline in medicinal applications of honey; however, recent rises in antibiotic resistance and renewed interest in natural remedies have led to a resurgence.

HDH is mainly composed of carbohydrates (especially glucose and fructose) and water, with small amounts of protein and ash, which is largely mineral in nature. Of especial interest are its bioactive compounds, such as phenolic compounds, amino acids, and organic acids. Phenolic compounds previously identified in New Zealand HDH (NZHDH) include 4-hydroxybenzoic acid, 4-hydroxy-cinnamic acid, benzoic acid, cinnamic acid, syringic acid, and vanillic acid (Weston, Brocklebank, & Lu, 2000). The amino acid profile of NZHDH is unknown, while literature suggests that the organic acid citric acid is typically more abundant in HDH than in blossom honey (Rahman, Hossain, & Barman, 2023; Seraglio et al., 2019).

In the modern food industry, honey is sold directly or used in various applications such as baking, cereals, confectionery, dairy, dressings and sauces, non-alcoholic beverages, mead, and spreads (Bellik & Iguerouada, 2014; Senn, Cantu, & Heymann, 2021; White, 1978). Novel food applications for HDH in the literature include kefir (Bielska et al., 2021), honey powder (Jedlińska et al., 2019; Tomczyk, Zagula,

Tarapatskyy, Kačániová, & Dżugan, 2020), fortified food (Némedi et al., 2021), flavoured mead (Chitarrini et al., 2020), and kombucha (Quach, 2020).

Yacon (*Smallanthus sonchifolius*) is an ancient Andean crop, native to the lower latitudes of South America (0 to 25°S). It is typically grown in subtropical and warm-temperate environments at temperatures from 18 to 25°C and altitudes between 600 and 3500 m (Douglas et al., 2005; Ojansivu, Ferreira, & Salminen, 2011). In the early 1980s, yacon was introduced to New Zealand and grown at latitudes from 37 to 45°S; the higher temperatures and longer seasons of the North Island were found to be favourable for yield (Douglas et al., 2005).

Yacon concentrate (YC; also known as yacon syrup) is a product that has arisen due to growing global interest in yacon. It is a sweet syrup produced by concentration of juice extracted from the roots of the yacon plant, and has physical and sensorial characteristics similar to that of honey or sugar cane syrup (Genta et al., 2009). YC typically has carbohydrate content between 65 and 70% by weight, though the carbohydrate profile varies widely depending on cultivar (Manrique, Párraga, & Hermann, 2005b). The fructooligosaccharide (FOS) content is of particular interest; FOS makes up between 40 and 70% of dry matter in yacon tubers by weight, higher than that of any other plant matter (Ojansivu et al., 2011). FOS are non-digestible prebiotic carbohydrates, associated with a number of potential health benefits including suppression of inflammation, reduced risk of colorectal cancer, improved gut health, decreased postprandial insulin response, and reduced body-mass index (BMI) (Genta et al., 2009; Sales et al., 2023; M. R. Yan, Welch, Rush, Xiang, & Wang, 2019). In terms of bioactive compounds, chlorogenic acid is the major phenolic compound identified in yacon tubers (Manrique et al., 2005b), while L-tryptophan is the major amino acid identified in YC (M. F. G. Silva et al., 2018).

In the literature, YC has been used as a sugar substitute in baked goods such as bread and sponge cake (K.-H. Kim & Lee, 2016; W.-M. Kim, Kim, Byun, & Lee, 2012), as a supplement in yoghurt (Mendes et al., 2019), and as an osmotic solution for pre-treatment in the drying of banana slices (Macedo, da Silva Araújo, Vimercati, Saraiva, & Teixeira, 2021). Various clinical studies have also been carried out, with varying degrees of success, to assess the potential health benefits of YC with respect to weight loss, satiety, and anti-diabetic effects (Adriano et al., 2019; Adriano et al., 2020; Dionísio et al., 2019; Sales et al., 2023; Törrönen et al., 2013).

1.2 Research motivation

The rationale of this research is to extend our knowledge about NZHDH and NZYC and to explore the potential food applications of these products. One potential application is the incorporation of NZHDH and NZYC in the production of a novel and functional low-alcohol beer. Additionally, as will be discussed in **Chapter 2**, NZHDH and NZYC contain phenolic compounds which have the potential to retard acrylamide formation in food and exert several health benefits including anti-obesity, anti-diabetes, and anti-bacterial effects. These will be explored in this thesis. As stated in **Section 2.2.5 Existing research and research gap**, information in the literature on the chemical composition and/or nutritional value of NZHDH and NZYC is limited. Therefore, research on NZHDH and NZYC should be explored as there is evidence that other varieties of HDH and YC are of growing global interest due to their purported health benefits.

This research aims to assist food scientists, food industries, and market researchers in developing an understanding of NZHDH and NZYC. In addition, the NAB and LAB market is rapidly expanding in New Zealand, though information on how to produce these beers using accessible methods is limited or disregarded in the literature. This research aims to develop and document a method of producing LAB that is accessible to homebrewers while also developing a functional LAB that is accessible to consumers. Documenting the sensory attributes of these LABs will aid new product development as it will provide researchers with information on what is liked or disliked in the LABs and how to improve overall liking. Furthermore, this research aims to investigate the potential health benefits of NZHDH and NZYC with respect to reduction of acrylamide in the Maillard reaction, anti-diabetes and anti-obesity effects, and anti-bacterial activity This PhD research will include three components: (a) physicochemical analysis of NZHDH, NZYC, and LAB, (b) sensory evaluation of LAB, and (c) quantitative analysis of acrylamide production, spectrophotometric assessment of anti-diabetes and anti-obesity activity, and disk diffusion assay for anti-bacterial activity, to answer the research objectives.

The novelty of this work will be (a) providing the most comprehensive physicochemical analyses of NZHDH and NZYC in the literature, both of which are under-utilised and under-researched materials, (b) developing a novel food-based application of NZHDH and NZYC as replacement for priming sugars during secondary fermentation of LABs, (c) providing the first instance in the literature of brewing of LABs using materials, methods, and equipment which are entirely accessible to home-brewers, and (d) an investigation

into the potential health applications of NZHDH and NZYC, including a particular focus on the anti-bacterial activity of their phenolic constituents.

1.3 Aims and objectives

Specific objectives of thesis research include:

- To produce comprehensive chemical analyses of NZHDH and NZYC (Chapters 3 and 4)
- To produce a comprehensive chemical analysis of functional LAB beverages (Chapter 5)
- To compare the flavour and sensory attributes of different LAB samples using chemical analysis and sensory evaluation (Chapter 5)
- To understand the consumer acceptance of the formulated LAB samples among New Zealand consumers (Chapter 5).
- To evaluate the acrylamide-reducing, anti-diabetes, anti-obesity, and anti-bacterial effects of NZYC, NZHDH, or their phenolic constituents (Chapter 6).

As chapters 3 and 4 are exploratory in nature, they do not have inherent hypotheses. However, the results from chapters 3 and 4 have been used to inform the key hypotheses of chapters 5 and 6, which are as follows:

For chapter 5, NZHDH and NZYC can be utilised for secondary fermentation to produce low-alcohol beers (ABV ≤ 1.15%). The ethanol content of NZHDH, NZYC, and control LABs will not be significantly different, while the addition of NZHDH and/or NZYC will result in significant differences in colour and volatile profile, and therefore in hedonic liking and descriptive analysis by panellists in sensory evaluation.

For chapter 6, the phenolic compounds present in NZHDH and NZYC will have an inhibitory effect on:

a) The formation of acrylamide in the Maillard reaction, relative to a model system,
b) The hydrolysis of *p*-nitrophenyl α-D-glycopyranoside by alpha-glucosidase, and

c) The growth of *Escherichia coli*, *Staphylococcus aureus,* and *Pseudomonas aeruginosa*, while

d) Crude NZYC will exhibit greater inhibitory effects on the hydrolysis of *p*-nitrophenyl acetate by pancreatic lipase than crude NZHDH.

The majority of the objectives will be embedded in different chapters of this thesis and will be answered according to the experiments conducted. Due to the structure of the thesis, experimental chapters will include different aspects and research objectives as illustrated in the bullet points above.

1.4 Overview of the thesis

This research thesis will consist of seven chapters, with four experimental chapters. The four experimental chapters (**Chapters 3, 4, 5 and 6**) will include an introduction of the experimental design and research gap, methods and materials used, results and discussion and also a summary. **Chapter 7** will conclude this research thesis with future implications and recommendations identified from this study.

Chapter 1. Introduction

A brief background on honeydew honey, yacon concentrate, and low alcohol beer, research rationale, research objectives and description of the thesis research.

Chapter 2. Literature Review

A history, general nutritional profile, bioactive profile, and food industry and research applications of honeydew honey, yacon concentrate, and low alcohol beer; a review of the production methods for low alcohol beer; a review on acrylamide and current methods of reduction, anti-diabetes and anti-obesity activity, and anti-bacterial activity.

Chapter 3. A comprehensive chemical analysis of New Zealand honeydew honey

Chapter 3 explores the chemical composition of NZHDH with respect to proximate composition, mineral, sugar, phenolic, and amino acid profile, and antioxidant activity. This chapter was published in *Food Research International*.

Chapter 4. A comprehensive chemical and nutritional analysis of New Zealand yacon concentrate

Chapter 4 explores the chemical compositional and nutritional value of NZYC with respect to proximate composition, mineral, sugar, phenolic, amino acid, and organic acid profiles, antioxidant activity, and glycaemic index. This chapter was published in *Foods*.

Chapter 5. Developing a novel flavoured low alcohol beer using New Zealand honeydew honey and yacon concentrate

Chapter 5 formulated six LABs (two containing NZHDH, two containing NZYC, one containing NZHDH and NZYC, and one control) which were produced using thermal distillation. Physicochemical analysis was conducted with respect to ethanol content, fructooligosaccharide and inulin content, volatile compounds, colour, and amino acids. Sensory evaluation was conducted using unstructured line scales, Just-About-Right scales, and Check-All-That-Apply questions. This chapter was published in the *Applied Food Research.*

Chapter 6. An investigation into potential health benefits of New Zealand honeydew honey and New Zealand yacon concentrate

Chapter 6 was conducted in three parts; (1) an investigation on the inhibitory effects of NZHDH and NZYC phenolics on the formation of acrylamide in the Maillard reaction, (2) an investigation into the anti-diabetes activity of NZHDH and NZYC phenolics and the anti-obesity activity of

NZHDH and NZYC, and (3) an investigation into the inhibitory effects of NZHDH and NZYC phenolics on *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. This chapter was not published.

Chapter 7. Overall conclusion and future research directions

Finally, this chapter will conclude the findings and research implications of this PhD research.

Chapters 2. Literature review

This chapter presents an overview of New Zealand honeydew honey (NZHDH), yacon concentrate (YC), low alcohol beer (LAB), and provides the background for potential applications of NZHDH and YC. This literature review aims to provide information regarding (1) the nutritional and bioactive profiles of NZHDH and YC and their applications in food industry and research, (2) the production, the nutritional, bioactive profiles, and the sensory evaluation of low alcohol beer, and (3) an overview of acrylamide, anti-diabetes, anti-obesity activity, anti-bacterial activity, and how these relate to NZHDH and YC.

2.1 Honeydew honey

2.1.1 A history of honeydew honey

Honey is a natural plant product produced by honeybees (*Apis mellifera*), among other species of bees, and has been used for centuries by humans in a variety of food, medicinal, and cosmetic applications (Farooqui & Farooqui, 2011; Seraglio et al., 2019). Honey can be broadly divided into two classes according to botanical origin; blossom honey (also known as floral honey), and honeydew honey (HDH) (Bentabol Manzanares, García, Galdón, Rodríguez, & Romero, 2011; Pita-Calvo & Váquez, 2017; Seraglio et al., 2019; Vasić et al., 2019). Blossom honey is produced by bees from the nectar of flowers such as acacia, lavender, orange blossom, thyme, heather, and myrtle; HDH is produced by bees from honeydew, which is secreted from the living parts of plants or excreted by plant-sucking insects such as aphids (Pita-Calvo & Váquez, 2017).

As honey is one of the most energy-dense natural food products, it was likely a significant food source for early hominins. During the Pliocene era (5.3 to 2.6 million before the common era (BCE)), Oldowan (chipped stone) tools emerged and grasslands spread across East Africa, meaning that *Homo* species would have been capable of – and likely did – harvest honey (Crittenden, 2011). This theory is supported by the evolution of larger, more metabolically expensive hominid brains 1.5 to 2 million years BCE, which coincided with a reduction in the size of molar teeth, indicative of a more energy-rich diet which required less mechanical breakdown (Crittenden, 2011). The first rock art depicting human harvesting of honey dates back to the Upper Palaeolithic era (40,000 to 8,000 years ago), with multiple examples found in Spain, India, Australia, and Southern Africa (Allsop & Miller, 1996; Crittenden, 2011). The large number of

rock paintings depicting honey indicates it was important to the Palaeolithic diet; however, no quantitative data is available, and the beehives and stick tools used to collect honey do not survive the archaeological record (Allsop & Miller, 1996; Crittenden, 2011). Modern hunter-gatherer diets do further support the importance of honey to the Palaeolithic diet; honey makes up approximately 15% of the diet of the Hazda foragers of Tanzania, and during the "honey season" (July-August) it provides up to 80% of the energy in the diet of the Mbuti pygmies of the Congo (Allsop & Miller, 1996; Crittenden, 2011).

HDH has been found to have greater antibacterial and antioxidant properties than blossom honey, meaning it has greater potential for use in medicinal applications (Pita-Calvo & Vázquez, 2018). Honey has been used in traditional medicines dating back to the ancient Egyptians, Assyrians, Chinese, Greeks, and Romans (Abou El-Soud, 2012; Eteraf-Oskouei & Najafi, 2013; Kuropatnicki et al., 2018). Babylonian tablets contain recipes for honey-based medicines, and the Ebers papyrus (dated to around 1550 BCE) includes details on 147 medicines for external applications containing honey (Kuropatnicki et al., 2018). Hippocrates of Kos, the 'Father of Modern Medicine', claimed that honey was able to "cause heat, clean sores and ulcers, soften hard ulcers of the lips, and heal carbuncles and running sores" (Kuropatnicki et al., 2018). While the use of honey in traditional and folk medicine persisted throughout the centuries, advancements in chemistry and biochemistry gave rise to modern synthetic medicines which led to a decline in the medicinal use of honey until recently (Kuropatnicki et al., 2018). However, increasing antibiotic resistance and renewed interest in natural remedies have seen a resurgence in the therapeutic use of honey. Studies in the literature have investigated the uses of honey in treating wounds, gastrointestinal tract diseases, fungal infections, ophthalmological conditions, and diabetes, among other medical conditions (Eteraf-Oskouei & Najafi, 2013).

Archaeological records dating back to predynastic Egypt (around 4500 BCE), a Sumerian tablet from around 3000 BCE, and the Ebers papyrus all detail the use of honey in cosmetic applications; honey was also important in the embalming process in ancient Egypt. (Burlando & Cornara, 2013). In ancient times, honey was often used as a binder in pastes or as the vehicle for creams and lotions, while Medieval records show it was used as a skin moisturiser, hair dye, lip softener, and face mask (Burlando & Cornara, 2013). The use of honey in cosmetic applications has continued to the present day, with honey included in the International Nomenclature of Cosmetic Ingredients (Burlando & Cornara, 2013). According to Martinotti, Bonsignore,

and Ranzato (2023), anecdotal data on the therapeutic and cosmetic effects of honeydew honey remains scant, especially in comparison to blossom honey; however, one study did develop a shampoo containing honeydew honey that was effective as a treatment for mild to moderate scalp psoriasis (Mariano, De Padova, Lorenzi, & Cameli, 2018).

2.1.2 General nutritional profile of honeydew honey

HDH is mainly composed of carbohydrates and moisture, with small amounts of protein and ash (Chua & Adnan, 2014). The major sugars present in honey are reducing sugars (glucose and fructose), which make up between 59.9 and 79.7% of HDH by weight (Seraglio et al., 2019). Other sugars which may be present in HDH include erlose, isomaltose, maltose, melezitose, nigerose, raffinose, sucrose, trehalose, turanose, and xylose (Astwood, Lee, & Manley-Harris, 1998; Bentabol Manzanares et al., 2011; Mateo & Bosch-Reig, 1997; Primorac et al., 2009; Rybak-Chmielewska, 2007; Tomczyk et al., 2020; Victorita et al., 2008). Erlose and melezitose are of particular interest concerning HDH, as these sugars are produced by transferase enzymes in the gut of the plant-sucking insect, and are thus considered to be markers of HDH (Astwood et al., 1998; Pita-Calvo & Váquez, 2017; Sanz, Gonzalez, de Lorenzo, Sanz, & Martínez-Castro, 2005). According to Astwood et al. (1998), either erlose or melezitose will dominate in HDH depending on which insect feeds on the tree sap. Erlose dominates in NZHDH produced from nectar exuded by *Ultracoelostoma assimile*; conversely, when feeding on spruce trees the aphid species *Cinara piceae* and *C. pilcornis* produce nectar which contains high proportions of melezitose (Shaaban, Seeburger, Schroeder, & Lohaus, 2020). Palatinose and turanose are of particular interest as these sugars have previously been reported in NZHDH (Astwood et al., 1998). They are also of general interest due to their reported health effects. Palatinose has been shown to positively affect exercise performance, glycaemic control, and cognitive performance in animal experiments and clinical trials. Meanwhile, recent studies have demonstrated that turanose exerts anti-inflammatory and adipogenesis suppression effects in vitro (Tian, Deng, Zhang, & Mu, 2019).

According to Seraglio et al. (2019), the moisture content of HDH falls within the range of 10.5 to 20.5% depending on botanical and geographical origin. While HDH typically has higher ash content than blossom honey, which is largely mineral in nature, the value is usually less than 1.2% (Karabagias, Karabournioti, Karabagias, & Badeka, 2020; Seraglio et al., 2019; Vasić et al., 2019). Potassium is the major

mineral present in HDH, with values ranging from 307 to 6610 mg per kg honey; other significant minerals include calcium, magnesium, phosphorus, and sodium (Bergamo, Tischer Seraglio, Gonzaga, Fett, & Costa, 2018; González-Miret, Terrab, Hernanz, Fernández-Recamales, & Heredia, 2005; Madejczyk & Baralkiewicz, 2008; Vanhanen, Emmertz, & Savage, 2011).

The protein content of honey typically falls below 2%, with the protein content of various HDH types identified in the literature falling between 0.03 and 1.16 g/100g (Quirantes-Piné et al., 2024; Seraglio et al., 2019). As the protein content of HDH is low, and the extraction and characterisation of proteins is challenging, relatively little data is available in the literature on the identities of the various proteins present in HDH (Seraglio et al., 2019); however, it is known that the protein content of HDH is approximately double that of blossom honey, and relative ratios of proteins of various masses can be used for the characterisation of HDH and blossom honey (Quirantes-Piné et al., 2024). One study found that the major protein bands in Brazilian HDH, Brazilian blossom honey, and German blossom honey correspond to 55 kDa and 70 kDa, molecular masses which are consistent with those of major royal jelly proteins (MRJPs) (B. Silva et al., 2021). Royal jelly is an acidic secretion of young worker honey bees which is fed to queen larvae and is also added to honey by honey bees during production and ripening (Mureşan et al., 2022). MJRPs have been linked with a variety of health benefits in humans, including anti-inflammatory, immuno-stimulatory, antimicrobial, anti-tumour, hypocholesterolaemic, hypotensive, cell proliferation, anti-ageing, antioxidant, wound healing, immuno-modulatory, and neuroprotective (Mureşan et al., 2022).

2.1.3 Bioactive profile of honeydew honey

HDH is known to contain a variety of bioactive compounds; these include phenolic compounds, amino acids, proteins, and organic acids, among others (Seraglio et al., 2019). Bioactive compounds are those which exert various health benefits arising from their antioxidant and anti-inflammatory properties (Sorrenti, Burò, Consoli, & Vanella, 2023).

Phenolic compounds are important in honey as phenolic compounds have antioxidative and anti-free radical effects (Nešović et al., 2020; Vasić et al., 2019). These compounds may impart bitter, astringent, and floral flavours (Silici, Sarioglu, & Karaman, 2013). A variety of phenolic compounds have been identified in European HDH in the literature, including acacetin, apigenin, caffeic acid, catechin, chlorogenic acid, chrysin, coumaric acid, epicatechin, ferulic acid, gallic acid, hesperidin, kaempferol, luteolin, naringenin, *p*-

coumaric acid, *p*-hydroxybenzoic acid, pinocembrin, protocatechuic acid, quercetin, rutin, sinapic acid, taxifolin, and vanillic acid (Nešović et al., 2020; Silici et al., 2013; C. F. Silva et al., 2020). Only one previous study investigated the phenolic composition of NZHDH, which qualitatively identified 4-hydroxybenzoic acid, 4-hydroxy-cinnamic acid, benzoic acid, cinnamic acid, syringic acid, and vanillic acid (Weston et al., 2000).

The concentration of amino acids may be strongly correlated to the radical scavenging activity of honey, even more so than the concentration of polyphenolic compounds (Pérez, Iglesias, Pueyo, González, and de Lorenzo (2007). Amino acids are also important contributors to flavour, and precursors for aroma compounds (Kivrak, 2015). A variety of free amino acids identified in European HDH include aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, threonine, arginine, β-alanine, α-alanine, γ-aminobutyric acid, tyrosine, valine, tryptophan, phenylalanine, isoleucine, leucine, ornithine, lysine, proline, *trans*-4-hydroxy proline, and methionine (Iglesias, de Lorenzo, Polo, Martín-Álvarez, & Pueyo, 2004; Kivrak, 2015; Pérez et al., 2007).

Organics acids make up less than 0.5% of honey, but they contribute significantly to its colour, aroma, taste, pH, acidity, and contribute somewhat to electrical conductivity (M. T. Sancho, Mato, Huidobro, Fernández-Muiño, & Pascual-Maté, 2013). They are produced as a result of aerobic and anaerobic fermentation; for example, gluconic acid is produced by the action of bee glucose-oxidase on nectar glucose and by the metabolic activity of *Gluconobacter* bacteria in the bee gut (Rahman et al., 2023). Formic acid and levulinic acid arise from the hydration of 5-hydroxymethylfurfural, while lactic acid and acetic acid both arise from fermentation in the digestive system of the bee (Seraglio et al., 2019). Gluconic acid is the major organic acid in all kinds of honey, with at least 30 different non-aromatic organic acids identified in the literature (Rahman et al., 2023). Citric acid can be used to discriminate between HDH and blossom honeys, as it is present in higher concentrations in HDH than in blossom honey (Rahman et al., 2023; Seraglio et al., 2019).

2.1.4 Food industry and research applications of honeydew honey

In the food industry honey can be sold directly as a table sweetener or spread, or used in various applications such as baking, cereals, confectionery, dairy, dressings and sauces, non-alcoholic beverages, and spreads (Bellik & Iguerouada, 2014; White, 1978). Honey provides a number of advantages when used in these applications, including exertion of antimicrobial activity, improvement of colour and flavour,

contribution to browning reactions, lowering of freezing point, and action as a binding agent among others (Bellik & Iguerouada, 2014). It also has the advantage of being perceived as natural and healthy, and imparting "old-fashioned goodness" (White, 1978). Honey is also fermented in the food industry to produce mead, an ancient alcoholic beverage which has experienced a recent resurgence in the American market (Senn et al., 2021). According to Senn et al. (2021), in 2017 a new meadery emerged every three days in the United States, while the global rate was one every seven days.

In the literature, there has been a growing number of research articles using HDH for novel food applications. HDH has been added to kefir (a fermented milk beverage), increasing its water-holding capacity, reducing water activity, and increasing viscosity (Bielska et al., 2021). Honey powder is another food product of interest as an alternative sweetener, and is advantageous compared to liquid honey as it overcomes the issues of viscosity, density, and spontaneous crystallisation, and is easier to store and transport (Jedlińska et al., 2019). HDH powder exhibits greater antioxidant activity and possesses higher mineral content than other varieties of honey powder (Tomczyk et al., 2020), and has better recovery and flowability, lower hygroscopicity, and higher glass transition temperature than rapeseed honey powder (Jedlińska et al., 2019). Jedlińska et al. (2019) were able to optimise their process to obtain HDH powder containing 80% honey solids with recovery above 90%; typically honey powder contains no more than 60% honey solids. Némedi et al. (2021) developed a novel HDH-based (84%) functional food fortified with pumpkin (10%), sea buckthorn (5%), and inulin (1%), and conducted a 13-week, double-blind, placebo-controlled human clinical trial to establish the impact of the product on human health. It was determined that consuming the product did not increase blood sugar or any significant changes in cholesterol levels (Némedi et al., 2021).

Chitarrini et al. (2020) produced mead using HDH and blossom honey, flavoured with or without blackcurrant. HDH meads had greater total phenolic content; however, the more traditional blossom honey mead was favoured by the sensory panel (44 nontrained panellists). HDH meads were richer in carboxylic acids, esters, and short-chain fatty acids, which are associated with negative sensory terms such as "rancid", "cheesy", and "fatty" aroma (Chitarrini et al., 2020). Quach (2020) developed kombucha using HDH or sucrose as a substrate for fermentation and found that the organic acids from HDH lead to greater titratable

acidity and therefore sourness, while the amino acids from HDH were metabolised by bacteria in the tricarboxylic acid cycle, increasing the rate of fermentation.

2.2 Yacon concentrate

2.2.1 A history of yacon and yacon concentrate

Yacon (*Smallanthus sonchifolius*) is an ancient Andean crop grown from Colombia to North-western Argentina; depictions dating to 500-1200 A.D. have been found in an archaeological deposit of Nazca, while the first written record is dated to 1615 A.D. (Douglas et al., 2005; Ojansivu et al., 2011). It is native to the lower latitudes of South America (0-25°S), and grows in subtropical and warm temperate environments at altitudes between 600 and 3500 m (Douglas et al., 2005). Optimum growth occurs between 18 and 25°C, while temperatures below 10 to 12°C can be detrimental to growth (Douglas et al., 2005). The roots of the yacon plant are said to have a sweet taste and crisp texture and can be consumed raw, boiled, or baked, or they can be juiced; the young stems of the yacon plant can also be consumed similar to celery (Ojansivu et al., 2011; R. Pedreschi, Campos, Noratto, Chirinos, & Cisneros-Zevallos, 2003). Traditionally, yacon has also been used medicinally for skin rejuvenation and treatment of intestinal, hepatic, and renal disorders (Ojansivu et al., 2011).

In the early 1980s, yacon was imported into New Zealand as a novel vegetable; from New Zealand, it was introduced to Japan, and from Japan to Korea and Brazil (Douglas et al., 2005; Ojansivu et al., 2011). An unpublished study conducted in New Zealand planted yacon in four sites at latitudes from 37 to 45°S. The two South Island sites (Lincoln and Mosgiel) had very low yields compared to the North Island sites of Pukekohe and Hamilton. This can be attributed to the climatic differences between the North and South Islands, wherein the higher temperatures and longer season on the North Island match more closely to the Andean conditions (Douglas et al., 2005).

In recent years, there has been growing global interest in yacon due to its potential as a functional food, which can be related to its unique profile of bioactive and prebiotic compounds, including antioxidants and phenolic compounds; these will be discussed in greater detail in **section 2.2.3** (Douglas et al., 2005; Ojansivu et al., 2011; M. R. Yan et al., 2019). One product that has recently arisen in the market as a result

of growing interest in yacon is yacon syrup or yacon concentrate (YC); it is associated with physical and sensorial characteristics similar to that of honey or sugar cane syrup (Genta et al., 2009).

According to Manrique, Párraga, and Hermann (2005a), YC is produced by extracting juice from the roots of the yacon plant and concentrating to around 72° Brix. The roots are first washed and disinfected, and then peeled, which removes 18 to 20% of the initial mass (Manrique et al., 2005a). Yacon peel contains compounds which promote oxidation of the juice, as well as resins and other compounds which impart a bitter and mildly spicy flavour (Manrique et al., 2005a). The roots are then juiced. During the peeling and juicing steps some cell membranes are ruptured, releasing polyphenol oxidase enzymes which catalyse the oxidation of phenols; the products of this reaction bind with sulfhydryl or amino groups of proteins and reducing sugars, yielding melanins which change the colour of the juice from orange to oily dark green (Manrique et al., 2005a). To prevent this from occurring, antioxidants are often added to the juice. During the juicing stage, a further 18 to 20% of the initial mass is lost as insoluble solid waste.

After juicing, the juice is filtered to remove insoluble solids (3 to 5% of the initial mass) and is then concentrated to 50 to 60° Brix, yielding a pre-syrup which is then filtered again to remove foam and crystallised sugar (3 to 5% of the initial mass) (Manrique et al., 2005a). The pre-syrup is then concentrated to 68 to 70° Brix, filtered, and bottled. As it cools, water continues to evaporate and so the final concentration increases to about 72° Brix.

2.2.2 General nutritional profile of yacon concentrate

In terms of proximate composition, carbohydrates make up 65-70% of YC by weight, while water accounts for around 25%, protein for 1-2%, and fat for around 0.1% (Manrique et al., 2005b). The only significant micronutrient in YC is potassium, which accounts for around 1% of the total weight (Manrique et al., 2005b). The carbohydrate profile of YC can vary widely between cultivars; for instance, YC produced from cultivar CLLUNC118 contains 10.9% fructooligosaccharides (FOS), 15.5% free glucose, 25.4% free fructose, and 12.2% free sucrose, while YC produced from cultivar AMM5163 contains 47.6% FOS, 2.6% free glucose, 7.9% free fructose, and 20.0% free sucrose (Manrique et al., 2005b). To the extent of the researcher's knowledge, no information is available in the literature on the identity of proteins or lipids present in YC or in yacon tubers.

2.2.3 Bioactive profile of yacon concentrate

The FOS content of YC is of particular interest; FOS makes up between 40 and 70% of dry matter in yacon tubers by weight, which is higher than that of any other plant matter (Ojansivu et al., 2011; M. R. Yan et al., 2019). FOS are short-chain oligosaccharide fructans with a degree of polymerisation (DP) lower than nine, classed as non-digestible prebiotic carbohydrates. They are non-digestible as amylase enzymes are unable to hydrolyse β-(2,1) bonds; similarly, salivary and internal digestive enzymes are unable to hydrolyse FOS (Genta et al., 2009; M. R. Yan et al., 2019). Thus, FOS have a low calorific value for humans and pass undigested through to the colon. In the colon they are fermented by anaerobic bacteria, forming short-chain fatty acids (SCFA). Animal studies suggest that the formation of SCFA can increase local immune response and reduce colon pH, suppressing inflammation and reducing the risk of colorectal cancer (M. R. Yan et al., 2019). Furthermore, consumption of FOS supports the growth of beneficial gut bacteria (particularly those of the genus *Bifidus* and *Lactobacillus*), which may aid in the easing of constipation and reduction of lipid and glucose in the blood (Genta et al., 2009; M. R. Yan et al., 2019). Conversely, in the few clinical studies carried out on yacon, only bloating and flatulence have been reported as adverse effects attributable to FOS content, and only in levels of daily intake above 0.14 g FOS/kg body weight; the only significant concern related to consumption of yacon is one reported case of anaphylaxis (Genta et al., 2009). Yacon roots may also contain inulin; like FOS, inulins are fructan-type oligosaccharides – however, they can have DP of up to sixty. Inulins are known to have similar health-promoting effects to those of FOS (M. R. Yan et al., 2019).

Phenolic compounds are secondary metabolites in plants which are of interest primarily due to their antioxidative properties, as well as their anti-inflammatory and anti-carcinogenic properties (Khajehei, Merkt, Claupein, & Graeff-Hoenninger, 2018; M. R. Yan et al., 2019). Yacon tubers, from which YC is derived, contain a notably high level of phenolic compounds – approximately 200 mg per 100 g fresh weight (Manrique et al., 2005b). Chlorogenic acid has been identified as a major phenolic compound present in yacon tubers (Manrique et al., 2005b); other phenolic compounds identified include quercetin, ferulic acid, quinic acid, 3,5-dicaffeoylquinic acid, caffeic acid, and three ester derivatives of caffeic acid (Manrique et al., 2005b; Ojansivu et al., 2011; Takenaka et al., 2003). Amino acids, similar to phenolic compounds, are of interest primarily due to their antioxidative properties. The major amino acid identified in YC in the literature is L-tryptophan; glutamine, arginine, alanine, threonine, and valine have also been identified as

present in YC (M. F. G. Silva et al., 2018; X. Yan et al., 1999). The organic acids formic acid, fumaric acid, citric acid, and malic acid have also been identified as being present in YC (M. F. G. Silva et al., 2018).

2.2.4 Food industry and research applications of yacon and yacon concentrate

As well as syrups and concentrates, the food industry produces many other products which contain or are derived from yacon. These include juices, confectionaries, marmalades, tea leaves, chip-like snacks, sweet pastries, flour, fermented vegetables, and vinegars; additionally, yacon may be used a source of ethanol (Ojansivu et al., 2011). In New Zealand, yacon is considered to be a "non-traditional" but "not novel" food as it has a history of safe use in other countries and there are no concerns regarding its composition (Food Standards Australia New Zealand, 2024).

In the literature, YC has been used as a substitute for sugar in baked goods such as bread and sponge cake. It was found that the substitution of sugar with YC improved bread quality with regard to crust and crumb colour, taste, flavour, sweetness, moistness, and residual mouth feel, leading to increased consumer acceptance (W.-M. Kim et al., 2012). However, increasing the degree of substitution of sugar with YC in sponge cake increased moistness, decreased cake height, volume, and springiness, and decreased overall acceptance (K.-H. Kim & Lee, 2016). Another study carried out by Mendes et al. (2019) supplemented yoghurt with YC and/or cashew apple extract, and found that yoghurt supplemented with just YC had the highest overall acceptance, liking of appearance and texture, and purchase intention.

Osmotic dehydration is a pre-treatment method for drying where the product is immersed in an osmotic solution and water is removed due to osmotic pressure, while the solute is incorporated into the product (Macedo et al., 2021). Typical osmotic solutions include sucrose, glucose, fructose, and sodium chloride; Macedo et al. (2021) used yacon syrup as an osmotic solution for the pre-treatment of banana slices and found that osmotically dehydrating the banana in a solution of 45°Brix before drying at 60°C optimally decreased water activity, increased hardness, and increased yacon solids gain, including FOS.

Various studies in the literature have assessed the potential health benefits of supplementing diet with yacon syrup. One such study, a double-blind randomised crossover clinical trial, found that supplementing a standard breakfast with 40 grams of yacon syrup (including 14 grams of FOS) had no effect on the concentrations of appetite biomarkers postprandial ghrelin or glucagon-like peptide-1, or on hunger,

desire to eat, satiety, or fullness sensation (Adriano et al., 2020). Similarly, a randomised, double-blind, placebo-controlled pilot trial which investigated the effects of consuming 40 g of yacon syrup (8.74 g FOS) per day for two weeks found no effects on blood lipid, glucose, or metabolic endotoxemia (Dionísio et al., 2019).

Postprandial insulin is produced for the maintenance or improvement of postprandial glucose metabolism, and a lower postprandial insulin requirement may be beneficial for short and long-term health, including the prevention of type-2 diabetes (Törrönen et al., 2013). A double-blind, crossover, randomised clinical trial carried out by Sales et al. (2023) determined that the profile of gut microbiota before the trial did affect the postprandial insulin response to FOS. There was a positive association between the abundance of the *Actinobacteria* phylum and *Bifidobacteriales* order in the group that responded (i.e. experienced decreased postprandial insulin response) to the intervention (a 40 g dose of yacon syrup, 14 g of which was FOS) (Sales et al., 2023). Sales et al. (2023) also reported that consumption of yacon syrup over 120 days (with at least a week washout period between interventions) led to significant decreases in waist circumference and BMI. Similarly, a randomised, crossover, double-blind clinical trial carried out by Adriano et al. (2019) reported that yacon syrup had a reducing effect on postprandial glucose and insulin levels, and had no effect on postprandial triglyceride levels.

2.3 Low alcohol beer

2.3.1 A history of low alcohol beer

The definition of ‘beer’ has developed over the course of recorded human history, making it somewhat challenging to provide a definitive account of the history of beer brewing and consumption. Historically, drinks brewed from fruits, sugar cane, honey, and cereals have all been referred to as ‘beer’, though nowadays some would be considered as ‘wine’ or another variety of distilled alcoholic beverages (Poelmans & Swinnen, 2011). A more narrow definition of beer would classify it as “the hopped drink obtained from liquefied starch after fermentation with specific strains of *Saccharomyces* yeast”; however, this definition would omit the origins of brewing technology and beer culture (Meussdoerffer, 2009). The basic requirements for humans to be considered capable of ‘brewing’ are as follows: (a) the availability of suitable grains, (b) a controllable source of energy (such as a fireplace), and (c) appropriate brewing vessels (such as pottery or metal kettles); according to Meussdoerffer (2009) these technologies were not widely

accessible earlier than 5000 BC. However, the oldest evidence for the production of fermented beverages comes from China, and dates as far back as 7000 BC (Meussdoerffer, 2009; Poelmans & Swinnen, 2011), while one the oldest known recipes for 'beer' was found on a clay tablet in Mesopotamia, dating back to 6000 BC (Poelmans & Swinnen, 2011).

Non-alcoholic beer (NAB) and low alcohol beer (LAB) are a much more recent addition to the market and can be traced back to the Prohibition era in the United States (Muller et al., 2019). From 1920 to 1933 the production of beer was outlawed in America, and so breweries began to produce dyes, malt extracts, and a novel malt-based drink called "near beer", which contained less than 0.5% alcohol (Muller et al., 2019). Almost simultaneously, the First World War caused a reduced supply of cereals across Europe, leading to the development of beers with lower alcohol content (Muller et al., 2019). Although the first patents for NAB and LABs date back to the 1890's, demand for these products only reached significant levels towards the end of the 20th century when laws in America and Europe limited the intake of alcohol for drivers (Muller et al., 2019). Other drivers for the growing demand for non-alcoholic and low alcohol alternatives to traditionally alcoholic beverages include health and lifestyle changes and changing gender roles (Anderson, 2023; Liguori et al., 2018; Myles et al., 2020).

According to Anderson (2023), sales of LAB and NAB across 10 focus markets (Australia, Brazil, Canada, France, Germany, Japan, South Africa, Spain, the United Kingdom, and the United States) were in excess of US$11 billion in 2022 and were forecasted to increase by 33% by 2026, representing by far the largest growth of any segment of the alcohol beverage market. From 2019 to 2023, consumption of low-alcohol beer in New Zealand (defined as ABV $\leq$ 1.15%) increased by 750% and represented 13% of the entire beer market, which was valued at $3.3 billion in September 2022 (Enerva, 2023). However, according to the World Health Organisation (2023), the availability of information on the production, consumption, and potential health impacts of LAB and NAB is limited; furthermore, the labelling and marketing of these beers are weakly regulated and vary widely between countries.

2.3.2 Production of low alcohol beer

There are two major classes of production processes for NAB and LAB; physical processes (which may be further categorised as either thermal treatments or membrane separations), and biological processes.

2.3.2.1 Physical processes – thermal treatments

Thermal treatments are conducted post-fermentation to either completely or partially remove alcohol; the most common examples of these include falling film evaporation, thin layer evaporation, and continuous vacuum rectification. All three of these methods yield a low alcohol or alcohol-free beer concentrate which requires the addition of water and carbon dioxide (Montanari et al., 2009).

Falling film evaporation is the cheapest thermal method as falling film evaporators contain no moving parts and are easiest to clean (Brányik et al., 2012; Montanari et al., 2009). In falling film evaporation, beer is pre-heated in a vacuum (3.5 to 20 kPa) to evaporation temperature (30 to 60°C) and flows downward through the column by the action of gravity and co-current vapour flow (Brányik et al., 2012; Salanță et al., 2020). The process only takes a few seconds, and as such the colour, the concentration of hop-bittering substances, and the pH of the beer are not substantially affected; however, the concentration of volatile compounds is significantly decreased (Montanari et al., 2009). A multi-stage falling film evaporator may also be used to further save energy by utilising the alcohol-containing vapour from the first and second evaporators as the heating steam for the second and third evaporators; however, this requires relatively high temperature in the first evaporator, resulting in a significant thermal impact on the final product (Brányik et al., 2012; Montanari et al., 2009).

Thin layer evaporation similarly operates under vacuum at low temperatures (35 to 60°C). Beer enters the evaporator and is distributed onto the underside of a hollow rotating cone; centrifugal force immediately spreads the beer over the entire surface in a layer approximately 0.1 mm thick (Brányik et al., 2012; Montanari et al., 2009; Salanță et al., 2020). The beer passes over the entire heating surface in less than one second and is then collected, resulting in minimal thermal impact; however, there is a risk of oxygen penetrating the seals of the moving parts and thus being introduced into the beer (Brányik et al., 2012; Montanari et al., 2009).

In continuous vacuum rectification, beer is filtered, pre-heated, and degassed under vacuum, resulting in the removal of volatile compounds (Brányik et al., 2012; Montanari et al., 2009; Salanță et al., 2020). The beer is then passed through a vacuum column where alcohol is removed between 42 and 48°C; the liberated volatile compounds are then re-incorporated into the beer by spraying the degassed CO_2 with dealcoholised beer or water, which is then added back into the dealcoholised beer (Brányik et al., 2012;

Montanari et al., 2009; Salanță et al., 2020). If the liberated volatile compounds are not re-incorporated into the dealcoholised beer then the volatile profile is dominated by 2-phenyl ethanol, a higher alcohol with floral odour (Brányik et al., 2012).

2.3.2.2 Physical processes – membrane separation

Membrane separation processes include reverse osmosis, osmotic distillation, dialysis, and pervaporation; compared to thermal methods they utilise lower operational temperatures and require lower energy consumption, thus reducing operational costs; however, they still require the purchase of specialised brewing equipment (Salanță et al., 2020).

In reverse osmosis, filtered beer flows tangentially to a semi-permeable membrane where pressure differential results in the removal of ethanol and water from the beer (Montanari et al., 2009). The process occurs over three stages: (a) concentration, where a permeate consisting of alcohol, water, carbon dioxide, and volatile compounds is removed from the beer, increasing the apparent alcohol content; (b) diafiltration, where sterile, demineralised, and deaerated water is added to the beer to reach the desired alcohol content, and (c) the make-up phase, where the volume of the dealcoholised beer is further increased with diafiltration water to the original volume (Brányik et al., 2012; Montanari et al., 2009; Salanță et al., 2020). The beer must then be carbonated following reverse osmosis, and the overall method is not economically feasible for beer with ABV lower than 0.45% (Montanari et al., 2009; Salanță et al., 2020). Osmotic distillation utilises a semi-permeable membrane at low pressure and temperature, and is more energy-efficient than reverse osmosis; however, it results in a greater loss of volatile compounds and so requires additional investment in a recovery unit (Salanță et al., 2020).

In dialysis, substances are exchanged between different liquids on either side of a semi-permeable membrane via diffusion, with the rate of exchange determined by concentration gradient, and the degree of exchange determined by contact time (Montanari et al., 2009). Counter-current flow is utilised to maintain a high concentration gradient and so optimise the diffusion of alcohol. For dealcoholisation, the process is usually carried out at low temperature to minimise the thermal impact (1 to 6°C), and under pressure at least equal to the saturation pressure of carbon dioxide to prevent or minimise loss of CO_2 (Brányik et al., 2012). Disadvantageously, low-molecular-weight components present in beer also diffuse through the membrane, resulting in almost total loss of esters and higher alcohols, and removal of around 50% of short-chain fatty

acids, though this can be regulated by controlling the flow rate or enriching the dialysate with beer volatiles (Brányik et al., 2012; Montanari et al., 2009).

Pervaporation utilises selective semipermeable membranes to remove alcohol at low temperatures by diffusion in its gas phase. By controlling parameters such as temperature and the nature of the membrane, pervaporation can also extract and concentrate volatile compounds and reincorporate them into the dealcoholised beer (Salanță et al., 2020).

2.3.2.3 Biological processes

While physical processes focus on removing alcohol after fermentation, biological processes focus on minimising the production of alcohol during fermentation. Biological processes can be subdivided into methods that utilise traditional brewery equipment (changed mashing, arrested or limited fermentation, cold contact, and specialised yeast), and methods that require specialised equipment such as continuous limited fermentation (Brányik et al., 2012; Salanță et al., 2020). Traditional breweries, for obvious reasons, tend to favour a combination of methods which only utilise traditional brewery equipment.

Mashing utilises a series of physical, chemical, and enzymatic processes to completely break down starch into fermentable sugars and soluble dextrins (Brányik et al., 2012). The goal of changed mashing is to modify the mashing process to obtain a wort with a lower concentration of fermentable sugars. This can be achieved in several ways, and often multiple of these strategies are used in tandem: (a) inactivation of β-amylase (which produces the fermentable sugar maltose) by mashing at temperatures higher than 75°C; (b) cold water malt extraction, which obtains wort with maximum malt flavour while limiting the extraction of fermentable sugars; (c) modifying the grain bill by using maize or rice to reduce the fermentable extract / unfermentable extract ratio; (d) re-mashing of spent grains to produce a second extract with limited fermentable sugars; and (e) using varieties of barley with β-amylase deficiency or varied β-amylase thermostability (Brányik et al., 2012; Montanari et al., 2009; Salanță et al., 2020). However, modifying the mash process and generating higher unfermentable sugars in the wort makes the final product unpleasantly sweet, imparts a wort-like taste, and increases the risk of microbial contamination (Salanță et al., 2020). Hence multiple corrective measures are required, which can include vigorous wort boiling to lower aldehyde levels, wort acidification, limited fermentation, and colour and bitterness adjustments (Brányik et al., 2012; Salanță et al., 2020).

Arrested fermentation (also known as stopped fermentation) aims to keep ethanol content low by removing yeast after a partial fermentation (filtration or centrifugation), or by temperature inactivation (cooling to 0°C or pasteurisation); limited fermentation aims to keep ethanol content low by manipulating brewing conditions to restrict yeast metabolism, typically by use of the 'cold contact process' (Brányik et al., 2012; Montanari et al., 2009; Salanță et al., 2020). For arrested fermentation, the fermentation is typically carried out at low temperatures (about 2-3°C) for 150-200 hours; higher temperatures may be used, but this requires shorter and more tightly controlled fermentation times (Brányik et al., 2012; Montanari et al., 2009). Strategies must also be used during arrested fermentation to mitigate 'worty' taste and optimise the concentration of desired beer volatiles; these can include brewing at higher temperatures, using top fermentation yeast rather than bottom fermentation yeast, or reducing oxygen concentration in the wort (Brányik et al., 2012; Montanari et al., 2009).

The cold contact process can be carried out at higher temperatures (15-20°C) for a short time period (0.5-8 hours), but lower temperatures (0-5°C) and longer periods (up to 24 hours) are favoured (Montanari et al., 2009). Under these conditions, negligible ethanol is produced, while there is moderate production of esters and fusel alcohols, and moderate reduction of carbonyls (Brányik et al., 2012; Montanari et al., 2009). When free mass (i.e., not immobilised) yeast is used, the wort must be stripped at low temperature in an atmosphere of carbon dioxide or nitrogen to remove sulfur compounds which are typically eliminated by yeast during normal fermentation (Montanari et al., 2009).

As an alternative to free mass yeast, immobilised yeast may be used for continuous limited fermentation. This is advantageous as it means that fermentation time remains limited while the yeast may be used for a long time, although it does require additional materials such as carriers for cellular attachment of yeast, a porous matrix for cellular entrapment, or microporous membrane filters or microcapsules for cellular containment; alternatively, yeast cells may self-aggregate via flocculation – a reversible, asexual, and calcium-dependent process. (Montanari et al., 2009). The immobilisation of yeast cells can cause changes in yeast metabolism; rapid yeast growth promotes the anabolic formation of amino acid precursors, while under or over-aeration accelerates or inhibits ester formation (Brányik, Vicente, Dostálek, & Teixeira, 2008). Furthermore, the metabolism of yeast cells grown in continuous culture shifts over time as a result of aging

and spontaneous genetic mutation (Brányik et al., 2008), meaning that the sensory attributes of beers produced by this method are difficult to control (Brányik et al., 2012).

Specialised yeasts may also be utilised to produce low-alcohol beers. For instance, *Saccharomycodes ludwigii* is a yeast that is capable of fermenting glucose, sucrose, and fructose but not maltose, as it lacks invertase and maltase enzymes (Montanari et al., 2009). Similarly, certain strains of *Saccharomyces* yeast are unable to utilise maltose (Brányik et al., 2012). As approximately 75% of fermentable sugar in malt worts is maltose, the resultant beers will typically contain less than 0.5% ABV, or only require minor dilutions (Brányik et al., 2012). *Zygosaccharomyces rouxii* is a yeast which consumes ethanol under aerobic conditions; however, this requires oxygenation of the beer and has adverse effects on sensory properties (Salanță et al., 2020). A potential future direction is genetic engineering of yeast to obtain a strain with desired metabolic properties; however, attempts to date have failed to make decisive breakthroughs and, at present, consumer acceptance of genetically modified yeast is low (Brányik et al., 2012).

2.3.3 General nutritional profile of low alcohol beer

According to Bamforth (2005), 'regular' beer contains between 2.8 and 5.6 grams of carbohydrates per 100 mL, while LAB contains around 1.6 grams per 100 mL. However, according to Statista (2024), selected NABs in Australia in 2020 had carbohydrate content ranging from 2.7 to 7 grams per 100 mL. Generally speaking, LABs have lower carbohydrate content than full-strength beers; however, their carbohydrate profile is higher in starch and oligosaccharides such as maltose and maltotriose, meaning they have relatively high glycaemic index values of around 80 (Lamiquiz-Moneo et al., 2022). According to I. M. P. L. V. O. Ferreira (2009), NABs from Brazil, Portugal, and Spain contained 0.25 ± 0.01 g/100 mL fructose, 0.56 g ± 0.15 g/100 mL glucose, 4.41 ± 1.21 g/100 mL maltose, 1.09 ± 0.29 g/100 mL maltotriose, and 0.24 ± 0.10 g/100 mL maltotetraose.

According to analysis of 31 NABs from Czech producers, the protein content of NAB ranges from 0.09 to 0.31 g/100 mL (Olšovská, Štěrba, Vrzal, & Čejka, 2019), while LABs produced by da Silva, Borges, Crestani, Dognini, and de Jesus (2024) had protein content from 0.09 to 0.12 g/100 mL. Most proteins present in beer originate from malt and are modified by proteolytic and other chemical reactions that occur during the brewing process (Nogueira, Martins, Ferreira, & Trugo, 2004). These barley malt proteins include albumins, globulins, prolamines, and glutelins; the latter three are particularly susceptible to chemical

modification during brewing (Gänz, Becher, Drusch, & Titze, 2022). Conversely, albumins such as protein Z and lipid transfer proteins LTP1 and LTP2 are relatively unchanged throughout the brewing process and play an important role in the quality of beer foam and beer turbidity (Gänz et al., 2022).

According to Brányik et al. (2012), beer is known to contain no fat or cholesterol; however, data released by the United States Department of Agriculture (USDA) claims that "malt beverages, including non-alcoholic beer" contain 0.12 g of fat per 100 g (Kozłowski, Dziedziński, Stachowiak, & Kobus-Cisowska, 2021). This may be due to the fact that malt barley does contain some fat (less than 3.4% w/w) (Piornos et al., 2023). During normal fermentation, the presence of lipids contributes to the formation of Strecker aldehydes (Piornos et al., 2023); however, this occurs under high temperatures in the presence of oxygen. As discussed earlier in this review, not all methods of producing LAB or NAB operate under these conditions, so it may be that some malt barley fat persists through low and no-alcohol brewing. Other sources of fats in beer include the metabolism of *Saccharomyces cerevisiae*, which produces glycerol, fatty acids, and lipids during fermentation, as well as oils released by hops.

Information on the total ash content of LAB is not available in the literature to the extent of the author's knowledge; however, it is known that ash in food is comprised of minerals and other inorganic matter. The general mineral profile of beer, according to Bamforth (2002), is presented below in **Table 2.1**.

Table 2.1 General mineral profile of beer (Bamforth, 2002).

Mineral	Range in beer (per litre)
Calcium (mg)	40–140
Phosphorus (mg)	90–400
Magnesium (mg)	60–200
Potassium (mg)	330–1000
Sodium (mg)	40–230
Iron (mg)	0.1–0.5
Zinc (mg)	0.01–1.48
Selenium (μg)	<0.4–7.2

One study carried out by Alcázar, Pablos, Martín, and González (2002) determined the mineral content of 18 LABs "obtained from local stores" in Spain. A summary of these results is presented below in **Table 2.2**. The concentrations of calcium, phosphorus, and zinc in LABs identified by Alcázar et al. (2002) fell entirely within the general range for beer as described by Bamforth (2002). Some LABs had

concentrations of magnesium, potassium, sodium, and/or iron lower than the general range; however, other LABs did fall within the general range. According to Alcázar et al. (2002), potassium and phosphorus are the major minerals in beer and originate from the raw materials (hops, barley, and other cereals), as well as yeast; magnesium, calcium, and sodium – also present in relatively high concentrations – likewise originate from the raw materials, including water. Hops are a source of trace elements, especially iron, as well as zinc and manganese (Alcázar et al., 2002).

Table 2.2 Summarised mineral profile of LABs purchased from local Spanish stores (Alcázar et al., 2002)

Mineral	Range in LABs (mg per litre)
Zinc	0.012–0.975
Phosphorus	108.61–297.27
Boron	0.115–0.447
Manganese	0.031–0.128
Iron	0.060–0.378
Magnesium	43.10–96.67
Aluminium	0.04–0.63
Strontium	0.127–0.740
Calcium	29.07–107.89
Barium	0.014–0.055
Sodium	8.36–82.06
Potassium	200.2–555.1

2.3.4 Bioactive and volatile profile of low alcohol beer

The phenolic compounds present in beer originate from the raw materials (hops, cereals, and malts) (Mellor, Hanna-Khalil, & Carson, 2020; Moura-Nunes et al., 2016). Typically, LAB and NABs have lower phenolic content than regular beers; this is due to differences in fermentation and yeast strains used, as well as losses that arise from dealcoholisation procedures (Bartolomé et al., 2000). The type and concentration of phenolic compounds in beer influence a number of sensory characteristics, including taste, aroma, colour, and colloidal and foam stability (Ambra et al., 2021); the lower phenolic content of LAB and NABs is likely the cause for their reportedly weaker aroma (Bartolomé et al., 2000).

Bartolomé et al. (2000) analysed twelve phenolic compounds in standard and alcohol-free beers, and found that the concentration of tyrosol, caffeic acid, *p*-coumaric acid, ferulic acid, and vanillic acid were significantly lower in alcohol-free beers than in standard beers. Tyrosol was the most abundant phenolic compound in both standard and alcohol-free beers; tyrosol is an intermediate product of yeast metabolism of

tyrosine, so limited fermentation or use of alternate yeasts may account for the lower tyrosol content in alcohol-free beer (Bartolomé et al., 2000). The hydroxycinnamic acids (caffeic acid, *p*-coumaric acid, and ferulic acid) and vanillic acid (a benzoic acid) are largely derived from malt, so the differences between standard and alcohol-free beers may be attributed to losses caused by dealcoholisation (Bartolomé et al., 2000).

Amino acids in beer mostly originate from the grains used in the brewing process. During malting, protease enzymes hydrolyse storage proteins into polypeptides, peptides, and amino acids; amino acids play an important role in yeast reproduction and fermentation (Fontana & Buiatti, 2009). Wort amino acids can be categorised into four different groups based on the rate of metabolic uptake by yeasts (see **Table 2.3**). Amino acids are taken up to supply nitrogen to the yeast cell, which produces a range of compounds including proteins, newly synthesised amino acids, α-keto acids, aldehydes, higher alcohols, and carboxylic acids, among others (Fontana & Buiatti, 2009). Fermentation temperature affects the rate of amino acid metabolism; at higher temperatures, amino acid decrease is more significant while the rate of yeast growth is higher. For instance, after fermentation at 15°C, 90% of amino acids are metabolised except for glycine and proline.

Table 2.3 Classification of wort amino acids according to their consumption rate by yeast (Fontana & Buiatti, 2009)

Group A – Fast absorption	**Group B – Intermediate absorption**	**Group C – Slow absorption**	**Group D – Little or no absorption**
Glutamic acid	Valine	Glycine	Proline
Aspartic acid	Methionine	Phenylalanine	
Asparagine	Leucine	Tyrosine	
Glutamine	Isoleucine	Tryptophan	
Serine	Histidine	Alanine	
Threonine			
Lysine			
Arginine			

In terms of volatiles, the most important compounds in beer fall into the following categories: aldehydes, higher alcohols, esters, vicinal diketones, sulfur compounds, and hop-derived compounds (Blanco et al., 2016; Piornos et al., 2023; Riu-Aumatell et al., 2014).

Aldehydes are mainly formed during wort production (mashing and boiling) and are additionally formed by yeast metabolism during fermentation (Piornos et al., 2023; Riu-Aumatell et al., 2014). Pathways for aldehyde formation during mashing and boiling include the Maillard reaction and Strecker degradation (Piornos et al., 2023). During normal fermentation aldehydes are subsequently converted to alcohols and esters; LABs, particularly those brewed by biological methods, have higher concentrations of aldehydes as process conditions are not conducive to the formation of alcohols and esters (Piornos et al., 2023). These aldehydes, of which 3-methylthiopropionaldehyde is prominent, contribute to the unpleasant 'malty' or 'worty' characteristics of LAB and NABs (Blanco et al., 2016; Piornos et al., 2023; Riu-Aumatell et al., 2014).

Higher alcohols, such as 3-methylbutanol, 2-methylbutanol, 2-phenylethanol, and 2-methylpropanol, contribute to fruity flavours in normal beers (Piornos et al., 2023); however, they may also contribute to alcoholic aroma, rough flavours or harshness, or phenolic-like flavours (Blanco et al., 2016). The immediate precursors to higher alcohols are 2-oxo acids, which are synthesised by yeast during fermentation via both catabolic and anabolic pathways (Blanco et al., 2016). In the catabolic pathway 2-oxo acids are formed by the transamination of amino acids while in the anabolic pathway they are derived from carbohydrate metabolism; 2-oxo acids are then decarboxylated to form aldehydes and subsequently reduced to form higher alcohols (Blanco et al., 2016). Fermentation parameters determine the level of higher alcohol production; nutrient availability (amino acids, oxygen, lipids, and zinc), increased temperature, and increased agitation are conducive to yeast growth and therefore higher alcohol production (Blanco et al., 2016). Conversely, low temperature or high carbon dioxide pressure restrict yeast growth and thus higher alcohol production.

Like higher alcohols, esters also contribute to fruity flavours in normal beers (Blanco et al., 2016; Piornos et al., 2023). Esters often have low flavour thresholds and a major impact on overall flavour; however, for many LABs or NABs, esters are only introduced to the final beer when flavours are added at the end of production. This is because biological methods usually prevent the formation of esters, while physical methods remove esters along with ethanol (Piornos et al., 2023). However, for LAB or NABs brewed with alternative strains of yeast, it is common to have one or more esters present in high concentration and therefore have an unbalanced or overpowered aroma (Piornos et al., 2023). Esters are formed by an enzyme-catalysed reaction (mostly alcohol acyltransferase) between acetyl-CoA and higher

alcohols, both of which are products of yeast metabolism (Blanco et al., 2016; Piornos et al., 2023). As well as enzyme and substrate availability, factors that influence ester production include temperature, carbon dioxide concentration or pressure, oxygen level, pH, and amino acid concentration (Blanco et al., 2016). There is a relationship between the concentrations of amino acids, higher alcohols, and esters present in beer which determines whether the overall flavour will be more alcoholic or fruity; in lagers, the ideal alcohol-to-ester ratio falls between 4:1 and 4.7:1 (Blanco et al., 2016). For example, the amino acid valine is converted to the higher alcohol isobutanol which is converted to the ester isobutyl acetate; similarly, leucine is converted to 3-methylbutanol which is converted to isoamyl acetate, while phenylalanine is converted to 2-phenylethanol which is converted to phenylethyl acetate (Blanco et al., 2016).

Vicinal diketones, sulfur compounds, and hop-derived compounds, though often present in normal and LAB or NABs, are not usually key aroma compounds and have secondary roles in overall flavour (Piornos et al., 2023). Vicinal diketones are byproducts of the synthesis of some amino acids; the most significant of this class of compounds are 2,3-butanedione and 2,3-pentanedione which provide buttery or "unmatured" aroma in fresh beers, and a "defective" aroma after long storage periods (Blanco et al., 2016; Piornos et al., 2023). Sulfur compounds have a low perception threshold and characteristic aroma; major sulfur compounds include dimethyl sulfide (sweetcorn aroma) and 3-methyl-2-butene-1-thiol (skunky aroma) (Piornos et al., 2023). S-methylmethionine forms in the embryo of barley during germination; during brewing, it thermally decomposes to form dimethyl sulfide or breaks down into dimethyl sulfoxide which is reduced to dimethyl sulfide by yeast. 3-methyl-2-butene-1-diol is formed by the degradation of iso-α-acids present in hops, in the presence of light (Piornos et al., 2023). Hops contribute a large variety of volatile compounds to both regular and LAB or NABs, most of which are terpenes and sesquiterpenes (Piornos et al., 2023; Riu-Aumatell et al., 2014). The essential oil of hops, present in the lupulin glands of the female hop flower, may contain more than 1000 compounds; additionally, yeast metabolism can convert these hop-derived compounds to other compounds such as the conversion of geraniol to β-citronellol (Riu-Aumatell et al., 2014).

2.3.5 Sensory evaluation of low alcohol beer

This section of the literature review will be divided into two parts, the first part is a brief overview of sensory evaluation, and the second part is about the sensory research conducted on LAB that is published in the literature.

2.3.5.1 An overview of sensory evaluation

One commonly accepted definition of sensory evaluation is "a scientific method used to evoke, measure, analyse, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste, and hearing" (Stone, Bleibaum, & Thomas, 2020). The purpose of sensory evaluation is to accurately measure human responses to food while minimising potentially biasing effects of brand identity and other information influences, thereby isolating and providing useful information on the sensory properties themselves (Lawless & Heymann, 2010). To achieve this, the four activities laid out in the definition of sensory evaluation (evocation, measurement, analysis, and interpretation) must be carefully considered.

In order to evoke useful human responses, testing conditions must be controlled and designed to account for human behaviour. For example, panellists are often placed in individual booths so that the responses they give are their own and not influenced by the judgments of those around them (Lawless & Heymann, 2010). Samples should be labelled with random numbers to avoid judgements based on labels, and presentation order should be randomised between panellists to control for the sequential effects of seeing one product after another (Lawless & Heymann, 2010). Other factors such as sample temperature, volume, and tasting intervals should also be controlled to minimise variation and improve precision (Lawless & Heymann, 2010).

In order to utilise descriptive and inferential statistics, responses to stimuli during sensory evaluation must be accurately measured (Stone et al., 2020). The test objective, panellist qualifications, and product characteristics necessarily influence, and so must precede, the method and selection of scale (Stone et al., 2020). A wide range of methods and scales have been developed for sensory evaluation, and those used will differ between assessing, for instance, the proportion of panellists who can discriminate small changes in products and the proportion of panellists who express a preference for one product over another. To obtain meaningful results of measurements from sensory evaluation, proper analysis of the data is required. This

requires the usage of appropriate statistical techniques, as well as good experimental design (Lawless & Heymann, 2010). Finally, the results must be appropriately interpreted. Conclusions must be drawn based on the data, analyses, and results, accounting for the method used, limitations of the experiment, and the background and contextual framework of the study (Lawless & Heymann, 2010).

Experiments and tests utilised in sensory evaluation can be broadly divided into three categories: difference (or discrimination), descriptive, and affective. Difference and descriptive testing are analytical techniques, while affective testing is hedonic. Difference testing is the simplest of the three categories, in that the aim is merely to determine whether any perceptible difference exists between two types of product (Lawless & Heymann, 2010). Examples of difference tests include paired comparison, duo-trio, triangle, A-not-A, and the two-alternative forced choice (Stone et al., 2020). Typically, difference tests are carried out with 25-40 panellists who have been screened for their ability to detect common product differences and who are familiar with the test procedures (Lawless & Heymann, 2010). Analysis of data is typically based on the statistics of frequencies and proportions of panellists who correctly or incorrectly identify product differences or lack thereof.

Descriptive tests are the most complex category of sensory tests, and also the most useful, providing word descriptors (or attributes) of food products as well as qualitative or quantitative data on attribute intensity (Lawless & Heymann, 2010; Stone et al., 2020). This allows for the comparison of product similarities and differences, the grouping of attributes which best correlate with differences in preference, and the relation of attribute differences with specific ingredients or process variables (Stone et al., 2020). Descriptive analysis methods which provide qualitative data include the flavour profile method and product experts; methods which provide quantitative data include the texture profile method, quantitative descriptive analysis (QDA), spectrum analysis, free-choice profiling, and diagnostic descriptive analysis (Stone et al., 2020). Affective tests are used in sensory evaluation to quantify the degree of liking or disliking of a product (Lawless & Heymann, 2010). The two most common affective tests are the paired comparison test and the 9-point hedonic scale (Stone et al., 2020).

2.3.5.2 Sensory research on low alcohol beer

From available research on the sensory evaluation of LAB and NABs, the majority of the studies conducted used QDA (a subcategory of descriptive testing) where panellists were asked to rate the intensity

of various sensory attributes on a scale, typically from 0-9 or 1-10 or similar. These studies mainly produced LAB or NAB using alternative strains of yeast (Bellut, Michel, Hutzler, et al., 2019; Bellut, Michel, Zarnkow, et al., 2019; Bellut et al., 2018; Callejo et al., 2019; Dziedziński, Stachowiak, Kobus-Cisowska, Faria, & Ferreira, 2023; Johansson et al., 2021; Krogerus, Eerikäinen, Aisala, & Gibson, 2022; Methner et al., 2022; Pater, Januszek, & Satora, 2024; Pater, Satora, & Januszek, 2024; Simões et al., 2023), including a comparison of the flavour stability of LAB with and without oxygen in the headspace (Sileoni, Maranghi, De Francesco, Perretti, & Marconi, 2023) and the use of alternative yeasts coupled with brewer's spent grains (Canonico, Agarbati, Comitini, & Ciani, 2023). Other methods of producing LAB or NAB which were evaluated by QDA included osmotic distillation (De Francesco et al., 2021; De Francesco, Sileoni, Marconi, & Perretti, 2015; Loredana et al., 2018), low-temperature fermentation (Petelkov et al., 2021), or were commercially obtained (Krebs, Müller, Becker, & Gastl, 2019; Lafontaine et al., 2020; Paixão, Tavares Filho, & Bolini, 2020; D. Sancho, Blanco, Andrés-Iglesias, & Caballero, 2021).

2.3.5.2.1 Sensory research on low alcohol beer – selected studies utilising alternative yeasts and analysed by QDA

According to Simões et al. (2023), their LABs produced with alternative yeasts tended to contain volatile compounds at concentrations lower than traditional beer thresholds. Two of their yeast strains (*Saccharomyces cerevisiae* var. *Chevalieri* and an engineered strain of *S. cerevisiae*) produced LABs with significantly intense estery/fruity characteristics, while six other strains had poor estery/fruity characteristics. LAB samples tended to be rated most intense in the attributes of 'cereal-like', 'malty', and 'worty' (Simões et al., 2023). According to Pater, Januszek, et al. (2024), LABs produced with *S. cerevisiae* var. *Chevalieri* without the addition of hops were characterised as having either woody and roasted or woody and chemical aroma, depending on the mash profile used.

Sileoni et al. (2023) reported that attributes 'fruity/estery', 'alcoholic/solvent', 'fruity/citrusy', and 'hop' were low for all LAB samples across all storage periods, due to low levels of hops and the presence of esters and higher alcohols at concentrations far below perception thresholds. All LABs had high 'DMS/cooked vegetable' and 'worty' aromas and flavours, likely due to the high concentration of methional. Although 2-methylbutanal and 3-methylbutanal were present below reported threshold levels, the perception thresholds in LABs are lower than in regular beer due to the absence of masking compounds (Sileoni et al.,

2023). As such, these aldehydes may be responsible for the reported ‘malty’ aroma and ‘bread’ and ‘almond’ taste of LABs. Across 120 days of storage, the presence of oxygen in beer headspace was responsible for the worsening of beer taste and aroma (Sileoni et al., 2023).

LABs produced by Methner et al. (2022) were fermented using two different strains of *Saccharomycopsis fibuligera*, at either 20°C or 28°C. Regardless of temperature, the LABs were found to have pronounced plum- and berry-like flavours, as well as dried fruit flavours. However, flavour expression tended to be higher in LABs fermented at 20°C, while slight wort flavours were detected in the LABs fermented at 28°C (Methner et al., 2022).

Krogerus et al. (2022) produced LABs using *Candida sojae* and *Trigonopsis cantarellii*, isolated from the natural microbiota of a brewery. These LABs contained esters and higher alcohols at concentrations mostly below detection limits. Aldehyde levels, associated with off-flavours, were lowest in *T. cantarellii* beer, while *C. sojae* beer contained higher levels of diacetyl and dimethyl sulfide, associated with ‘buttery’ and ‘cooked corn’ aromas. Compared to reference beers, across all attributes assessed, the LABs produced with alternative yeast strains were only significantly different concerning sweetness; both alternative LABs were significantly sweeter than the reference beers (Krogerus et al., 2022).

Johansson et al. (2021) produced LAB using two yeast strains isolated from sourdough culture: *Kazachstania servazzii* and *Pichia fermentans*. *P. fermentans* was deemed to be suitable for low-alcohol wheat beers due to its production of 4-vinylguaiacol (spice/clove aroma), while *K. servazzii* showed potential for lager-style beer due to its clean flavour profile and tolerance for low temperature. *P. fermentans* LAB had a banana and melon taste, which could not be correlated with any volatile compounds, while *K. servazii* LAB had pear and apple flavours, associated with 3-methylbutyl acetate and 2-phenylethyl acetate. Both *P. fermentas* and *K. servazzii* LABs had predominant dimethyl sulfide (DMS), cereal, and sweet taste.

Bellut et al. (2018) produced LAB using six strains of non-*Saccharomyces* yeasts isolated from kombucha. *S. cerevisiae* beer was strongly associated with a fruitier aroma and acidic/sour taste, while the non-*Saccharomyces* LABs were strongly associated with a sweeter taste and wort-like aroma. In descriptive analysis, all non-*Saccharomyces* LABs were given attributes such as “wort-like”, “bread-like”, and “honey-

like" by the panellists, while attributes such as "cereal-like", "black tea", "caramel", "slightly grassy", "fruity", and "white wine" were given to specific strains.

These results show that vast differences in the aroma and flavour profile of LAB and NAB are generated by utilisation of alternative yeasts in LAB and NAB brewing. However, utilisation of alternative yeasts typically results in decreased formation of volatiles, and aroma and flavour profiles which score highly in descriptors not desirable in beers, for instance 'worty', 'vegetable', 'butter', and 'dimethyl sulfide'.

2.3.5.2.2 Sensory research on low alcohol beer – selected studies utilising osmotic distillation, low-temperature fermentation or commercially obtained beers and analysed by QDA

According to Loredana et al. (2018), compared to an original beer, LABs produced by osmotic distillation had decreased body and "alcohol/solvent" taste and aroma. Supplementing the LAB with hop extract and pectin improved the body and provided 'hop' and 'fruity-citrus' notes comparable to the original beer, but led to a perceived decrease in sweetness and in 'cereal', 'caramel', 'bitter', and 'linger' notes relative to the un-supplemented LAB (Loredana et al., 2018). The original beer was associated with higher levels of higher alcohols and esters, while the LAB beers were largely associated with aldehydes and ketones.

De Francesco et al. (2021) investigated the effects of osmotic dealcoholisation on four varieties of commercial beer. For lager beer, osmotic dealcoholisation led to decreased aroma intensity with respect to attributes such as "fruity-estery", "alcoholic-solvent", "fruity-citrus", "hop", "malty", "DMS", and "other sulfur", and increased intensity for "worty". In terms of taste intensity, osmotic dealcoholisation led to decreased "linger", "body", "astringent", "alcoholic/solvent", and "fruity-estery", and increased "sour". For bitter beer, osmotic dealcoholisation led to decreased aroma intensity to "fruity-estery", "alcoholic/solvent", and "hop", decreased taste intensity concerning "fruity/estery", "alcoholic/solvent", "body", and "linger", and increased taste intensity with respect to "sour", "sweet", and "worty" (De Francesco et al., 2021).

For milk stout, aroma intensity decreased with respect to "alcoholic/solvent" and "hop", while taste intensity decreased with respect to "fruity/estery", "alcoholic/solvent", "sweet", "body", and "linger", and increased with respect to "worty" and "sour" (De Francesco et al., 2021). For stout, aroma intensity decreased with respect to "fruity/estery", "alcoholic/solvent", "malty", "burnt", "other sulfur", and "sweet",

while taste intensity decreased with respect to “fruity-estery” and “alcoholic/solvent”, and increased with respect to “worty” and “sour” (De Francesco et al., 2021).

Similarly, De Francesco et al. (2015) found that osmotic dealcoholisation of beer fermented with Belgian ale yeast led to decreases in “body”, “linger” and “sweetness”, attributed to loss of ethanol and higher alcohols, as well as a significant decrease in “estery”, due to the loss of esters.

Petelkov et al. (2021) found that limited or low-temperature fermentation at 10°C leads to the formation of esters, higher alcohols, and vicinal diketones at concentrations two to four times lower than conventional beers brewed with the same strain of yeast, and the formation of aldehydes at a concentration approximately 20 times lower than conventional beers. At lower temperatures (7°C and 5°C), the formation of the major metabolites was even more restricted. As an additional strategy to further limit ethanol formation, Petelkov et al. (2021) attempted fermentation with lower concentrations of yeast at both 10 and 7°C. Results from sensory analysis showed that the LAB brewed with the regular concentration of yeast at 10°C was the most preferred sample, particularly with respect to flower notes, fruity notes, and feeling of alcohol. LABs brewed with lower concentrations of yeast were described as less carbonated than other samples, while the LAB brewed with lower concentration of yeast at 5°C scored notably lower than all other samples in terms of fruity notes and aroma intensity.

Paixão et al. (2020) assessed six commercial Pilsen beers (three alcoholic and three non-alcoholic) and found that, unsurprisingly, the alcoholic beers scored higher for the descriptors ‘alcohol flavour’ and ‘alcohol aroma’. Partial least squares regression analysis revealed that these descriptors contributed positively to panellist acceptance of beers, with alcoholic aroma most positively contributing to acceptance.

These results show that the feeling and aroma of alcohol play an important role in consumer preference, an obvious challenge when producing LAB or NAB. Additionally, flavours which are associated with beer may be negatively correlated with consumer preference in LAB and NAB, while flavours not typically associated with beer such as ‘flowery’ or ‘fruity’ may be positively correlated. This could be due to differences in volatile profiles between regular beer (higher in higher alcohols and esters) and LAB or NAB (aldehydes and ketones); certain ‘beer-like’ volatiles may be present in LAB or NAB at relatively higher concentrations than in regular beer, leading to unbalanced or under-developed flavour and aroma profiles.

This idea has been discussed previously, wherein LAB and NAB beers are often associated with ‘worty’ and other off flavours.

2.3.5.2.3 Sensory research on low alcohol beer – selected studies analysed by affective testing methods

Affective testing was also used in the literature, where overall liking, as well as liking of appearance, aroma, flavour, taste, and/or mouthfeel were rated on hedonic scales. These studies included LAB produced by interrupted fermentation (De Fusco et al., 2019), cold mash process (da Silva et al., 2024; Dalberto et al., 2021), or were commercially obtained (Moss, Barker, & McSweeney, 2022; Ramsey et al., 2018).

De Fusco et al. (2019) developed a low-alcohol isotonic beer and compared it to two commercially available NABs. There were no significant differences between the three samples with respect to appearance, aroma, taste, global impression, or purchase intent, with all samples liked ‘slightly’ to ‘moderately’. da Silva et al. (2024) developed an international pale lager style LAB which scored twenty-seven out of fifty following the beer judge certification program, indicating a beer which was ‘good’ but ‘missed the mark on style and/or minor flaws’. Appearance scored extremely well; however, mouthfeel was not favourably evaluated; this was likely due to the LAB having a specific gravity of 1.005, lower than the recommended range of 1.008 to 1.012 for an international pale lager (da Silva et al., 2024). da Silva et al. (2024) also noted that there was a pronounced bitterness in the beer, with an unbalanced ratio between bitterness and original gravity.

Dalberto et al. (2021) produced LAB by modifying mash temperature, sifting the malt to remove some starch content, and varying the amount of Pilsen malt used to provide less fermentable sugars. Two LABs; one made using cold mash and 200 g/L sifted Pilsen malt and another made using cold mash and 200 g/L non-sifted Pilsen malt, compared favourably to a standard alcoholic beer. However, it should be noted that these LABs were formulated according to Brazilian legislations, which allow up to 2.5% alcohol in a LAB.

From the results from a focus group, Moss et al. (2022) determined that those most likely to consume NABs are those who are avoiding alcohol or want the flavour of beer without its intoxicating effects. The focus group also identified NAB consumers who are interested in a healthy lifestyle and that

NABs are consumed in social settings, but not in restaurants. From sensory analysis, Moss et al. (2022) found that NAB versions of lager, stout, and pale ale were less liked than regular alcoholic varieties, and were associated with wateriness and bland flavour.

Ramsey et al. (2018) found no significant differences between beer with 0%, 0.5%, 2.8%, and 5% ethanol in terms of overall liking; however, cluster analysis identified three distinct clusters of consumers. Cluster 1 liked the 5% beer significantly more than all other samples. Cluster 2 showed no significant difference in liking of beer samples, with all samples 'liked slightly' or 'liked moderately'. Cluster 3 liked the 5% beer significantly less than all other samples but disliked all beer samples. However, it should be noted that sample size was too small to make meaningful inferences on the different clusters.

These results show that LABs and NABs are often deficient in terms of mouthfeel and body and that the removal of ethanol can lead to imbalances in flavour, particularly in terms of sweetness and bitterness. Results also suggest that consumers may be clustered in terms of liking LAB and NAB; however, increased consumer participation would be required to draw conclusions on cluster membership in terms of beer consumption patterns, gender, age, types of beer consumed, and familiarity with beer styles.

2.3.5.2.4 Sensory research on low alcohol beer – selected studies analysed by the Check-All-That-Apply method

The Check-All-That-Apply (CATA) method was also used in the literature, a descriptive test where panellists are asked to check (or tick) all attributes from a predetermined list of sensory descriptors that they believe apply to the sample they are evaluating (Krebs et al., 2019; Lafontaine et al., 2020; Moss et al., 2022; Ramsey et al., 2018).

Krebs et al. (2019) spiked commercial LAB samples with various classes of polymers and assessed the effect of these compounds (maltodextrin, β-glucan, and isomaltulose) on mouthfeel by means of the CATA test. LAB spiked with isomaltulose was described as 'soft' and 'mouth-coating', while all spiked LABs were more 'smooth' than the unspiked control. β-glucan-spiked LAB was classed as 'slimy' by 45% of panellists, while dextrin-spiked LAB was classed as 'slimy' by about 28% of panellists and isomaltulose-spiked LAB and the control were classed as 'slimy' by less than 10% of panellists (Krebs et al., 2019). As discussed above, LAB and NAB often have deficient mouthfeel, so polymers may be added to enhance

palate fullness or mouthfeel. These results show that the overall molar masses and molar mass distributions of polymers have divergent effects on specific descriptors for mouthfeel impressions, allowing for targeted improvements of specific sensory attributes of LAB and/or NABs.

Lafontaine et al. (2020) assessed 42 commercial non-alcoholic brands, and found that the descriptors 'malty', 'cheerios', 'grape nuts', 'dried yeast', and 'skunk' were positively correlated with beer similarity, while the descriptors 'overall aroma intensity', 'citrus', 'lemon', 'orange', 'tropical', 'stone fruit', and 'cola' were negatively correlated with beer similarity. These negatively correlated variables, along with 'floral', were significantly positively correlated with consumer aroma liking, indicating that panellists from Northern California were least satisfied with NABs which were perceived to be most beer-like. Volatile compounds negatively correlated to beer likeness were heptanal, octanal, nonanal, decanal, trans,trans-2,4-decadienal, α-pinene, limonene, trans-linalool oxide, and α-terpineol; these were positively correlated with citrusy, tropical, stone fruit or floral aromas and were additionally hypothesised to mask or suppress cheerio, grape nut, and dried yeast aromas which panellists were not satisfied with. These results further support the developed theme in the literature where descriptors not typically associated with beers are liked in LAB or NAB by panellists, while descriptors associated with beers are not liked. As discussed, this is due to variations in the formation of volatile compounds during LAB or NAB brewing, wherein the formation of higher alcohols and esters is limited while the formation of aldehydes and ketones is promoted.

Moss et al. (2022) assessed stout, lager, pale ale, and their NAB counterparts by means of the CATA test and analysed the results using correspondence analysis (CA). The resultant CA map dimensions explained 83.05% of the experimental variability; 63.98% on dimension one, and 19.07% on dimension two. Dimension one separated the samples by level of carbonation and appearance; the stouts and NAB lager were correlated with 'clear' and 'low carbonation', while the pale ales and regular lager were correlated with 'cloudy' and 'high carbonation'. Dimension two separated the samples by taste; the lagers and regular pale ale were correlated with 'sweet', while the stouts and the NAB pale ale were correlated with 'sour' and 'bitter'. Penalty lift analysis was also used to determine the effect of CATA terms on overall liking; 'sweet' and 'aromatic' had meaningful positive impact on overall liking, while 'watery', 'thin', 'bland', and 'bitter' had meaningful negative impact. The results show that panellists were able to distinguish between the regular and NAB variations of the lagers (separated on dimension 1) and the pale ales (separated on

dimension 2) and confirm that mouthfeel and flavour play important roles in overall liking of beer, including NAB.

Ramsey et al. (2018) utilised temporal CATA (TCATA) to assess beers with various concentrations of ethanol (0, 0.5, 2.8, and 5%). The average proportions of citations for 'sweet', 'fullness of body', and 'alcohol warming' were significantly higher for 5% ethanol than all other samples; additionally, 2.8% ethanol was cited as 'sweet' significantly more than 0% ethanol. Panellists could be separated into three distinct clusters. Cluster one enjoyed the attributes 'tingly' and 'fullness/body', while 'sweetness' and 'bitterness' were negative drivers of liking. Cluster two enjoyed 'malty' and 'sweet' attributes, and disliked 'astringent' and 'tingly' as ethanol concentration increased. Cluster three liked 'sour' and 'tingly' sensations, and disliked 'bitterness' as ethanol concentration increased. TCATA analysis also revealed that some attributes were cited more frequently earlier in the evaluation time, and others later. Tingly appeared early in the evaluation for all beer samples, while malty flavour, bitterness, and hoppy flavour appeared after swallowing. Results showed that the time in tasting in which consumers judge their overall liking of beer varies depending on the consumer and on the ethanol content of the beer. Overall liking of higher alcohol beers was determined more rapidly compared to lower alcohol beers. These results show the importance of ethanol in beer, as it affects the perception of taste and mouthfeel, and highlight which attributes should be targeted by manufacturers when developing new low-alcohol products.

2.4 Potential applications for honeydew honey and yacon concentrate

2.4.1 Using honeydew honey and yacon concentrate to prevent the formation of acrylamide

In this section, a brief background on acrylamide will be discussed, along with its major formation pathways in food and strategies presented in the literature to mitigate its formation.

2.4.1.1 A brief historical background on acrylamide

Acrylamide is an industrially important substituted olefinic monomer with the structure CH_2=CHCONH$_2$, and has been available commercially since the 1950s (Rice, 2005). It can be readily polymerised to form polyacrylamides which have various uses in industry, including strengthening aids for paper manufacturing, flocculants during water treatment, soil conditioning agents, and grouting agents for dam, tunnel, and sewer construction (Mills, Mottram, & Wedzicha, 2008). The discovery of acrylamide in foods occurred because of contamination from the construction of a railway tunnel in Sweden. An

acrylamide polymer was used as a sealant, which leaked into a nearby stream and contaminated local ground and water systems. Dead fish were found downstream of the tunnel, as well as herds of paralysed cows, while tunnel workers reported neurotoxic symptoms (Mills et al., 2008). Researchers discovered that the fish, cows, and tunnel workers had elevated levels of acrylamide-hemoglobin adducts; however, they also discovered that the background levels of these adducts in unexposed people were higher than the background levels in unexposed wild animals and cattle. This led to the theory that acrylamide exposure in humans was caused from another source, i.e. cooked food (Mills et al., 2008).

This hypothesis was tested by feeding rats a fried animal standard diet for one or two months, and these rats showed a significant increase in the level of the acrylamide-hemoglobin adduct (Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2000). It was also demonstrated that (a) acrylamide was formed during the heating of the feed, (b) the levels of acrylamide in the feed could be related to the levels of the acrylamide-hemoglobin adduct, and (c) the levels of adduct in the experimental rats were of a magnitude similar to background levels in non-smoking humans (Tareke et al., 2000). These results supported the hypothesis that cooked food was a source of exposure to acrylamide for humans.

Studies on mice and rats have demonstrated that, when taken orally, acrylamide is readily bioavailable and absorbed by the gastrointestinal tract (Doerge, Young, McDaniel, Twaddle, & Churchwell, 2005a, 2005b). From there, it is distributed throughout the body, including to foetuses, and has been identified in human breast milk (Food and Agricultural Organization of the United States & World Health Organisation, 2005). Once ingested, acrylamide may be rapidly metabolised to form glycidamide (Calleman, Bergmark, & Costa, 1990). According to Mills et al. (2008), glycidamide is thought to be responsible for the genotixic effects of acrylamide; both acrylamide and glycidamide are electrophilic and are so able to form adducts on DNA and protein.

According to the Joint FAO/WHO Expert Committee on Food Additives (2006), single oral doses of acrylamide only produce acute toxic effects when greater than 100 mg/kg of body weight, while the reported median lethal dose is greater than 150 mg/kg of body weight. However, repeated exposure at lower doses has been shown to cause degenerative peripheral nerve changes in rats, for instance at doses of 50 mg/kg of body weight for 11 days and of 21 mg/kg body weight for 40 days (Joint FAO/WHO Expert Committee on Food Additives, 2006). Prolonged dietary intake of acrylamide has been demonstrated to cause degeneration of

nerve terminals in areas of the brain responsible for learning, memory, and other cognitive functions. The estimated no observed adverse effects level (NOEL) for morphological changes in nerves in rats was 0.2 mg/kg body weight per day, while the NOEL for reproductive and developmental effects in rats was 2 mg/kg body weight per day. As well as the aforementioned neurotoxic, reproductive, and developmental effects, acrylamide has been clearly shown *in vitro* to be a direct-acting clastogen in mammalian cells (i.e. can cause damage to chromosomes) and has been clearly shown *in vivo* to be mutagenic (i.e. can cause genetic mutations) (European Commission: Joint Research Centre, 2002). Furthermore, acrylamide has been shown to induce tumours in rats that have been dosed with acrylamide via drinking water (Friedman, Dulak, & Stedham, 1995; Johnson et al., 1986).

According to Mills et al. (2008), evidence suggests that acrylamide primarily acts on the nervous system in humans. Up to 2008, occupational exposure to acrylamide had not been linked to overall cancer mortality; studies in the literature were limited and failed to account for the confounding effects of tobacco smoking or dietary intake. Based on their NOEL levels in rats for morphological changes in nerves (0.2 mg/kg of body weight per day) and for reproductive and developmental effects (2 mg/kg of body weight per day), the Joint FAO/WHO Expert Committee on Food Additives (2006) calculated margins of exposure (MOEs) from 50 to 200 and 500 to 2000 respectively. The MOE is a ratio between the NOEL in animal studies and the estimated human exposure level or dose; at these levels, it was concluded that adverse effects were unlikely in humans but morphological changes in nerves could not be ruled out for high-intake individuals (Joint FAO/WHO Expert Committee on Food Additives, 2006).

When considering carcinogenic effects, however, the MOE is a ratio between the benchmark dose lower limit (BMDL) and the estimated human exposure level or dose. The BMDL for the induction of mammary tumours in rats was 0.3 mg/kg of body weight, and so the MOE for induction of tumours was calculated to be between 75 and 300 (Joint FAO/WHO Expert Committee on Food Additives, 2006). For genotoxic and carcinogenic compounds, these values were considered to be low and indicative of a potential human health concern.

A number of epidemiological studies have been conducted to investigate associations between dietary intake of acrylamide and the incidence of cancer in humans. Studies have failed to show associations between acrylamide consumption and colorectal cancer (Mucci, Adami, & Wolk, 2006), bowel, kidney, and

bladder cancer (Mucci, Dickman, Steineck, Adami, & Augustsson, 2003), renal cell cancer (Mucci, Lindblad, Steineck, & Adami, 2004), breast cancer (Larsson, Akesson, & Wolk, 2009), or oral cavity and pharynx, oesophagus, larynx, large bowel, breast, or ovarian cancer (Pelucchi et al., 2003). However, one study did observe a significantly increased risk of endometrial cancer among high acrylamide consumers, and a non-significantly increased risk of ovarian cancer overall with a significantly increased risk of serous tumours (Wilson, Mucci, Rosner, & Willett, 2010). A separate study, when accounting for smoking behaviour, observed a positive association between acrylamide-haemoglobin levels and oestrogen receptor with breast cancer. An estimated incidence rate ratio of 2.7 per 10-fold increase in acrylamide-haemoglobin levels were observed (Olesen et al., 2008). According to Hogervorst and Schouten (2022) the literature remains inconclusive, with the strongest potential association between dietary acrylamide and cancer being for sex hormone-driven cancer in women.

In the European Union, Commission Regulation (EU) 2017/2158 is currently in effect, which was created to raise the level of protection of human health and provide mitigation strategies for acrylamide levels in food (Koszucka, Nowak, Nowak, & Motyl, 2020; Pietropaoli, Pantalone, Cichelli, & d'Alessandro, 2022). This regulation provided benchmark levels for acrylamide in a range of food products, for instance ready-to-eat French fries (500 µg/kg), potato crisps (750 µg/kg), wheat-based bread (50 µg/kg), and roast coffee (400 µg/kg), among others. In 2019, the list of foods to be monitored was updated by Commission Recommendation (EU) 2019/1888 (Pietropaoli et al., 2022).

Conversely, in the United States there are no legally binding obligations on companies to meet benchmark levels of acrylamide in food. Instead, the United States Food and Drug Department (US FDA) has provided non-binding recommendations on how to minimise the levels of acrylamide in certain foods (Quesada-Valverde, Artavia, Granados-Chinchilla, & Cortés-Herrera, 2022). However, in the state of California under Proposition 65, food businesses are required to put warning labels on their food if it causes exposure to more than 0.2 µg of acrylamide per day, and there is a maximum allowable dose level of 140 µg acrylamide per day (Quesada-Valverde et al., 2022). An updated no significant risk level (NSRL) of 1.1 µg of acrylamide per day has been proposed based on more recent data (B. Wang, Guerrette, Whittaker, & Ator, 2020); however, the NSRL set by Proposition 65 remains at 0.2 µg. As of October 2024, there are no set benchmark levels for acrylamide for food sold in Australia or New Zealand, though Food Standards

Australia New Zealand (FSANZ) does state the importance of reducing acrylamide in food to as low as reasonably possible (Food Standards Australia New Zealand, 2023b).

2.4.1.2 Formation of acrylamide in food

In food, there are two main pathways through which acrylamide may be formed. The first and most significant is the Maillard reaction in the presence of free amino acids (mainly asparagine), at temperatures higher than 120°C (Maan et al., 2022; F. Pedreschi, Mariotti, & Granby, 2014; Y. Xu et al., 2014). In the Maillard reaction, asparagine is converted to acrylamide through thermal decarboxylation and deamination, which requires the presence of a carbonyl group such as that of a reducing sugar (Y. Xu et al., 2014); according to Maan et al. (2022), the reactions involved are most efficient for short-chain sugars which form cyclic hemiacetals and are thus exposed to nucleophilic attack by alpha-amino acids. Model studies have shown that α-hydroxy carbonyls are more effective than di-carbonyls, and that fructose (which contains two α-hydroxy carbonyl groups) increases the formation of acrylamide approximately two-fold compared to other reducing sugars such as glucose (Y. Xu et al., 2014).

The first stage of acrylamide formation in the Maillard reaction is the reaction of the reducing sugar with acrylamide, resulting in the formation of a Schiff base via *N*-glycosyl conjugation (F. Pedreschi et al., 2014). After dehydration at high temperatures, the Schiff base is decarboxylated, and then either decomposes directly to form acrylamide, or hydrolyses to form aminopropionamide which can be converted to acrylamide by elimination of an ammonia group (Maan et al., 2022; F. Pedreschi et al., 2014).

The second main but less significant pathway through which acrylamide is formed in food is the acrolein pathway. Acrylic acid and its precursor, acrolein, are primarily generated when oils are heated above their smoke point; at these temperatures, glycerol is degraded to form acrolein (Maan et al., 2022). Acrylic acid and acrolein may also be derived from small molecules such as acetaldehyde or formaldehyde or generated via protein metabolism or organic acid decarboxylation (Y. Xu et al., 2014). The carbonyl group of acrylic acid is available for reaction with the amino group of free amino acids such as asparagine, resulting in the subsequent formation of acrylamide (Maan et al., 2022). It should be noted that the carbonyl group of reducing sugars are more reactive with asparagine, and so the formation of acrylamide via the acrolein pathway is marginal compared to the Maillard reaction (Y. Xu et al., 2014).

2.4.1.3 Mitigation of acrylamide in the literature

There are a number of factors that influence the formation of acrylamide; these include temperature and time, pH, water activity, the food matrix, availability of asparagine, and storage conditions (Isleroglu et al., 2012; Keramat, LeBail, Prost, & Jafari, 2011; Sadd, Hamlet, & Liang, 2008). The water content of the food matrix plays an important role in acrylamide formation; in dry systems, the formation of acrylamide has been observed to decrease when temperature increases above a certain point, while in other food systems, researchers have found that acrylamide content increases as temperature increases (Bråthen & Knutsen, 2005). This has been attributed to water evaporation, which decreases the effective temperature of the food system. According to De Vleeschouwer, Van der Plancken, Van Loey, and Hendrickx (2007), acrylamide formation increases exponentially with water activity (a_w), with a maximum value in the range of 0.6 – 0.8; above this value, acrylamide formation declines. It should also be noted that prolonged heating has been found to decrease the acrylamide content in various food systems, especially at higher temperatures, indicating that acrylamide reacts further or is eliminated through evaporation (Bråthen & Knutsen, 2005). One possible mechanism through which acrylamide may be eliminated is by reaction with amino acids; addition of amino acids or protein-rich ingredients has been shown to reduce acrylamide formation, while protein-rich foodstuffs typically have relatively low acrylamide levels (Bråthen & Knutsen, 2005).

Decreasing pH (i.e. increasing acidity) significantly reduces the formation of acrylamide. In a model system, reducing pH from 8 to 4 led to a ten-fold reduction in the reaction rate at 160°C (De Vleeschouwer, Van der Plancken, Van Loey, & Hendrickx, 2006). Another study reported higher formation of acrylamide in a buffered system (pH 6.0) than in a non-buffered system (pH 4.6) (Y. Wang et al., 2019). However, changing the pH, as well as heating temperature and/or time, will result in changes to the Maillard reaction and thus the colour and aroma of the food product (De Vleeschouwer et al., 2006). As such, any strategies designed to reduce acrylamide formation must also consider colour and aroma formation in the final product.

A range of methods have been proposed to reduce the formation of acrylamide in baked goods; however, many have only been tested in model systems (Sadd et al., 2008). These mitigation strategies tend to focus on reducing or diluting precursors to the Maillard reaction such as free asparagine, for instance by the addition of enzymes, yeasts, or amino acids, by binding asparagine with a complexing agent, removing

accelerants such as ammonium salts, lowering the pH, or minimising heating temperature and time (Sadd et al., 2008).

Another method through which the formation of acrylamide may be reduced is by the addition of phenolic compounds; however, the literature is inconclusive on this. One study investigated the effects of pure phenolic compounds (cinnamic, gallic, ferulic, coumaric and caffeic acids, catechin and epicatechin) on the formation of acrylamide in a model system, and found that the compounds investigated had no mitigation effects (Bassama, Brat, Bohuon, Boulanger, & Günata, 2010). However, another study investigated the effects of the flavonoid naringenin on acrylamide formation in a model system and found that naringenin did inhibit acrylamide formation by forming adducts with asparagine degradation products (Cheng et al., 2009). Studies have also shown that polyphenolic extracts from certain natural sources such as grape pomace, mint, cumin seeds, or star anise have greater inhibitory effects than pure phenolic compounds (C. Xu et al., 2015; Zhu, Cai, Ke, & Corke, 2009). Zhu et al. (2009) did note that some plant extracts (*Ilex cornuta Lindl. et Paxt*.) and pure phenolic compounds (ferulic acid and hesperetin) appeared to increase acrylamide formation in a model system.

C. Xu et al. (2015) found that one mechanism through which phenolic extracts inhibit acrylamide formation may be the trapping of sugar fragments and other intermediates at high temperature, but stated further research was needed. Zhu et al. (2009) stated that, for plant extracts, the different types and contents of phenolic compounds, as well as the chemical structures of these compounds, resulted in different effects on acrylamide formation depending on synergistic or antagonistic interactions.

As stated in sections **2.1.3** and **2.2.3**, a variety of phenolic compounds have previously been identified in European HDH, NZHDH, and fresh yacon tubers. Some of these compounds, such as *p*-coumaric acid, gallic acid, quercetin, chlorogenic acid, catechin, hesperidin and naringenin were found by Zhu et al. (2009) to have inhibitory effects on the formation of acrylamide in a model system.

2.4.2 Potential anti-diabetes and anti-obesity activity

In this section, a brief background on the anti-diabetes and anti-obesity assays will be discussed, along with compounds in food which are attributed to anti-diabetes or anti-obesity activity.

2.4.2.1 Anti-diabetes assay

In recent years, there has been a concerning rise in diagnoses of diabetes, to the point where it can be considered an epidemic (Benalla, Bellahcen, & Bnouham, 2010). Type 1 diabetes is an autoimmune disease where pancreatic beta-cells which produce insulin are destroyed, causing inulin deficiency which leads to chronic hyperglycaemia (high blood sugar), while type 2 diabetes (which makes up 90-95% of all diabetes cases) is a group of metabolic diseases similarly characterised by chronic hyperglycaemia, caused by a combination of insulin resistance and impaired insulin secretion (Benalla et al., 2010; Dirir, Daou, Yousef, & Yousef, 2022; Jaber, 2023). Hyperglycaemia is the major cause of diabetes complications which include heart disease, nerve, kidney, and eye damage, skin and mouth conditions, Alzheimer's disease, and depression (Dirir et al., 2022); type 1 diabetes is typically treated by insulin injection, while type 2 diabetes is treated with drugs which hinder glucose absorption, suppress hepatic gluconeogenesis, and inhibit the reabsorption of glucose by the kidneys. Alpha-glucosidase inhibitors are a common treatment for type 2 diabetes as they reversibly and competitively bind with alpha-glucosidase, delaying the digestion of carbohydrates and so reducing blood glucose levels (Bhatia, Singh, Arora, & Arora, 2019; Dej-Adisai, Rais, Wattanapiromsakul, & Pitakbut, 2021). Alpha-amylase inhibitors are another common treatment; alpha-amylase is responsible for the cleavage of α-1,4-glycosidic bonds in polysaccharides, breaking them down to smaller oligosaccharides which are subsequently degraded by alpha-glucosidase (Trinh, Staerk, & Jäger, 2016).

In the alpha-glucosidase assay, anti-diabetes activity is measured by absorbance change upon cleavage of the substrate *p*-nitrophenol α-d-glycopyranoside (PNPG) to form glucose and *p*-nitrophenol, which absorbs strongly at 405 nm (Schmidt, Lauridsen, Dragsted, Nielsen, & Staerk, 2012). Successful inhibition of alpha-glucosidase (i.e. anti-diabetes activity) will result in reduced or eliminated cleavage of PNPG and therefore reduced or eliminated absorbance-change at 405 nm. Similarly, the alpha-amylase assay measures anti-diabetes activity by absorbance-change upon cleavage of substrates such as 2-chloro-4-nitrophenyl-α-maltotrioside (CNP-G3) or *p*-nitrophenyl-α-d-maltoheptaoside (PNP-G7) to form chromophores such as 2-chloro-4-nitrophenolate or *p*-nitrophenolate respectively (Okutan, Kongstad, Jäger, & Staerk, 2014).

2.4.2.2 Analysis of anti-diabetes activity in the literature

Although alpha-glucosidase and alpha-amylase inhibitors (which include acarbose, miglitol, and voglibose) are currently used in the treatment of type-2 diabetes, they are also known to have adverse side effects. These include diarrhoea, abdominal pain, and flatulence, which are caused by the fermentation of undigested carbohydrates by gut bacteria resulting in increased intestinal gas production (Dej-Adisai et al., 2021; Dirir et al., 2022). In recent years, there has been growing interest in the use of natural products in a variety of therapeutic treatments, including for type 2 diabetes. Natural products have several advantages; they have been used for centuries in traditional medicines around the world and so are trusted by consumers, can have decreased toxicity and severity of side effects, and can be lower in cost than conventional drugs (Bhatia et al., 2019; Dej-Adisai et al., 2021; Dirir et al., 2022).

Tadera, Minami, Takamatsu, and Matsuoka (2006) evaluated the yeast and rat small intestine alpha-glucosidase and the porcine alpha-amylase inhibitory activity of six groups of flavonoids (flavone, flavonol, flavanone, isoflavone, flavan-3-ol, and anthocyanidin). Anthocyanidins, isoflavones, and flavonols, as well as the flavan-3-ol epigallocatechin gallate, were potent inhibitors for yeast alpha-glucosidase, with the structure of the A, B, and C rings closely related to the inhibitory activity. For A and C rings, hydroxylation at the 3 and 5 points enhanced inhibitory activity, as did linkage of the B ring at the 3 position; saturation of the 2,3-double bond in the C ring decreased inhibitory activity (Tadera et al., 2006). Many flavonoids weakly inhibited rat small intestine alpha-glucosidase; hydroxylation at the 3 position of flavone and hydroxyl substitution on the B ring enhanced inhibitory activity (Tadera et al., 2006). The flavonoids myricetin and quercetin and the flavone luteolin were potent inhibitors of porcine alpha-amylase; linkage of the B ring at the 3 position, the 2,3-double bond, and hydroxylation at the 5 position of flavonols or isoflavones enhanced inhibitory activity. However, unlike for the alpha-glucosidases, hydroxylation at the 3 point was unfavourable for inhibitory activity (Tadera et al., 2006).

Another enzyme of interest in the treatment of diabetes is aldose reductase, which participates in the polyol pathway and catalyses the conversion of glucose to sorbitol; accumulation of intracellular sorbitol contributes to clinical complications arising from diabetes such as cataracts and neurological diseases (Wu, Luo, & Xu, 2015). According to Wu et al. (2015), ethanolic extracts of lychee, blueberry, and plum exhibit

strong alpha-glucosidase inhibitory activity, while lemon peel, eggplant, and bitter gourd extracts exhibited the highest aldose reductase inhibitory activity.

Cassia alata is a shrub native to South America which has also been introduced in many pantropical countries in Southeast Asia, Africa, and North America. It is one of the most popular natural antidiabetic remedies in Africa, the Caribbean, and India; Varghese, Bose, and Habtemariam (2013) analysed active fractions of methanolic extracts from *C. alata* leaves to determine mechanisms of antidiabetic action. The fractions which mostly contained kaempferol and kaempferol 3-*O*-gentiobioside displayed the greatest inhibitory effects on alpha-glucosidase. The fraction which mainly contained kaempferol exerted greater inhibitory effects than isolated kaempferol, suggesting that minor constituents of the kaempferol fraction exerted synergistic inhibitory effects or exerted particularly potent inhibitory effects themselves (Varghese et al., 2013).

Mohamed Sham Shihabudeen, Hansi Priscilla, and Thirumurugan (2011) demonstrated that cinnamon bark extract was able to competitively and reversibly inhibit α-glucosidase enzymes in rats, suppressing maltose- and sucrose-induced postprandial blood glucose spikes. Preliminary LC-MS work suggested that the presence of flavonoid glycosides may have contributed to the inhibitory effects of the extract.

The phenolic compounds found in honey are unique and specific to the type of honey, and determine characteristics such as flavour, colour, and functional properties (Peláez-Acero et al., 2022). Previous studies have shown that phenolic honey extracts have the ability to inhibit alpha-glucosidase, with IC_{50} values ranging from 55 to 153 µg/mL (Ali, Bakar, Majid, Muhammad, & Lim, 2020; Zaidi et al., 2019). Inhibition of alpha-glucosidase can be achieved by the binding of hydrogen to the active site of the enzyme (Zaidi et al., 2019), and so the hydroxyl group of phenolic compounds such as those found in NZHDH and NZYC may facilitate inhibition of alpha-glucosidase.

2.4.2.3 Anti-obesity assay

In recent years, there has been a rapid global rise in obesity. Obesity causes abnormal physiological metabolism, adversely affecting physiological, psychological, and social well-being (T.-T. Liu, Liu, Chen, & Shi, 2020). Particularly, obesity is known to be a significant risk factor for conditions such as heart disease,

hypertension, hyperlipidaemia, diabetes, and cancer (T.-T. Liu et al., 2020). Pancreatic lipase is an enzyme responsible for breaking fat down into glycerol and fatty acids, which are then absorbed by the body and used for metabolism (T.-T. Liu et al., 2020). Pancreatic lipase inhibitors are therefore used as drug treatments for obesity as they limit the level of lipids entering the blood. Similarly to the alpha-glucosidase assay, anti-obesity activity may be measured by absorbance change upon cleavage of substrates such as *p*-nitrophenylpalmitate to form chromophores which absorb strongly at specific wavelengths (Slanc et al., 2009).

2.4.2.4 Analysis of anti-obesity activity in the literature

Orlistat, also sold as Xenical, is one example of a pancreatic lipase inhibitor approved by the United States Food and Drug Administration (FDA) which is used for long-term treatment of obesity. Orlistat works by covalently bonding to serine in the active site of gastrointestinal and pancreatic lipase (Seyedan, Alshawsh, Alshagga, Koosha, & Mohamed, 2015). Cetilistat is another example of a pancreatic lipase inhibitor, approved for use by the Pharmaceuticals Medical Devices Agency of Japan (PMDA) (A. Kumar & Chauhan, 2021). Both orlistat and cetilistat have been associated with gastrointestinal side effects such as flatulence, liquid stool, diarrhoea, oily spotting, incontinence, and abdominal cramping; as such, novel and naturally derived inhibitors are of interest for the treatment of obesity (A. Kumar & Chauhan, 2021; Seyedan et al., 2015).

Cardullo, Muccilli, Pulvirenti, and Tringali (2021) investigated the inhibitory effects of the isoflavones daidzein, genistein, and formononetin, as well as a small library of their semisynthetic derivatives. Of the natural isoflavones, genistein exhibited the greatest inhibitory effect on pancreatic lipase, while hydroxylation and bromination of the isoflavones to yield semisynthetic derivatives increased potency (Cardullo et al., 2021). It is worth noting that orlistat had a much lower IC_{50} value (0.6 ± 0.1 μM) than any of the natural isoflavones or their semisynthetic derivatives, with the lowest IC_{50} value being 60 ± 5 μM.

L. Zhang et al. (2021) developed a novel method to screen pancreatic lipase inhibitors, guided by the interactions between orlistat and pancreatic lipase. They virtually screened 20 natural compounds, of which 13 met the criteria. These 13 molecules were tested *in vitro* and four (curcumin, sinensetin, quercetin, and nobiletin) exerted similar inhibitory activity to orlistat. Curcumin and sinensetin, the two with the lowest IC_{50} values, were then tested *in vivo* using diet-induced obese mice. Mice treated with orlistat gained the least fat;

curcumin and sinensetin also suppressed the accumulation of fat and did not cause adverse effects in the body, with curcumin being more effective than sinensetin (L. Zhang et al., 2021).

Qin et al. (2022) investigated the inhibitory effects of over 100 herbal medicines on human pancreatic lipase (hPL). Most displayed weak anti-hPL activity, with vine tea (*Ampelopsis grossedentata*) extract (AGE) displaying the most potent anti-hPL effect. Two of the major flavonoid constituents of AGE (myricetin and quercetin) displayed strong hPL inhibition in subsequent testing, with IC_{50} values around 1.5 μM. Their mechanism of inhibition was determined to be non-competitive, most likely as allosteric regulators which bind to a region on hPL distinct to the active site and so induce a conformational change, reducing hPL activity (Qin et al., 2022).

These results from the literature show that phenolic compounds, in particular flavonoids, may be excellent candidates for natural alternatives to synthetic drugs currently used in the treatment of obesity. As discussed in **sections 2.1.3** and **2.2.3**, both NZHDH and yacon are sources of a variety of phenolic compounds including quercetin, which was highlighted by Qin et al. (2022) as an inhibitor of hPL.

2.4.3 Anti-bacterial activity

2.4.3.1 Anti-bacterial activity of honeydew honey

There are several properties of honey that may contribute to its antibacterial activity. The first is osmolarity; as honey is a saturated or super-saturated solution of sugars, it has low water activity (typically around 0.6) and so exerts an osmotic or water-withdrawing effect on various species of bacteria (Molan, 1992). However, the antibacterial activity of honey is attributable to more than just osmolarity. Studies have shown that dilute solutions of honey, or honey that have been dialysed to remove sugar, still exhibit antibacterial activity; similarly, ‘artificial honey’ (solutions of sugars at the same proportionality of honey) have been shown to exhibit antibacterial activity lower than natural honey (Molan, 1992).

It has been theorised that the acidity of honey may play a role in its antibacterial activity. Honey is an acidic medium, with pH typically between 3.2 and 4.5. Although studies tend to show that the acidity of honey does not contribute to antibacterial activity, these experiments often use heavily diluted honey and/or growth medium that neutralises the pH of honey (Molan, 1992). Many species of wound-infecting bacteria have minimum pH values for growth above or around the pH range for honey; these include *E. coli* (4.3),

Salmonella species (4.0), *Pseudomonas aeruginosa* (4.4), and *Streptococcus pyogenes* (4.5). If used in direct topical applications, then the acidity of honey may well exhibit some antibacterial effect (Molan, 1992).

Hydrogen peroxide has also been proposed as a potential explanation for the antibacterial effects of honey. Glucose oxidase, an enzyme present in honey which converts β-d-glucose to gluconolactone and hydrogen peroxide, becomes more effective when honey is diluted; studies have shown that the antibacterial activity of diluted honey is decreased when enzymes that destroy hydrogen peroxide (such as catalase or peroxidase) are added, supporting the theory that hydrogen peroxide contributes to the antibacterial effects of honey (Molan, 1992). Hydrogen peroxide is not antibacterial; however, the catalytic action of metal ions in bacterial cells generates damaging hydroxyl free radicals, and this effect is enhanced by the presence of ascorbic acid (vitamin C).

Non-peroxide compounds in honey which have been associated with antibacterial activity include pinocembrin, lysozyme, syringic acid, methyl syringate, 3,4,5-trimethoxybenzoic acid, and 2-hydroxy-3-phenylpropionic acid, among others (Molan, 1992). However, studies tend to show that the contribution of these compounds to antibacterial activity is insignificant, and when significance is shown, it is typically due to the fact that the extracted antibacterial compounds have been concentrated to levels far greater than that which they naturally occur at in honey (Molan, 1992). Bogdanov (1997) fractionated ten different honey samples and found that the acidic fraction exerted the greatest non-peroxide antibacterial activity (44% on average), followed by the basic fraction (24%) and the nonpolar/non-volatile fraction (21%), with the volatile fraction exerting the lowest antibacterial activity (11%). Interestingly, almost all the non-peroxide antibacterial activity of Manuka honey (90%) was found in the acidic fraction.

By use of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays, Zivkovic, Sunarić, Stanković, Mihajilov-Krstev, and Spasić (2019) determined that Serbian HDH had very strong activity against *Streptococcus pneumoniae, Enterococcus faecalis, Pseudomonas aeruginosa, Escherichia coli,* and *Acinetobacter baumanii*. For all honey samples analysed by Zivkovic et al. (2019), there was a strong correlation between antibacterial activity and the colour intensity, total phenolic content, total flavonoid content, and antioxidant activity, with HDH, oregano honey, and forest honey exerting the greatest antibacterial activity.

2.4.3.2 Anti-bacterial activity of yacon

Lin, Hasegawa, and Kodama (2003) extracted six sesquiterpenoid lactones from yacon leaves; four of which were previously identified in the literature (sonchifolin, uvedalin, enhydrin, and fluctuanin). Two were newly identified; 8-*β*-tigloyloxymelampolid-14-oic acid methyl ester (compound 1) and 8*β*-methacryloyloxymelampolid-14-oic acid methyl ester (compound 2). Lin et al. (2003) assessed the antimicrobial activity of these compounds against the bacteria *Bacillus subtilis* and the fungus *Pyricularia oryzae*. Against *B. subtilis*, fluctuanin was the most effective, while compound two was more effective than compound one. Against *P. oryzae*, compound two and sonchifolin exhibited significantly greater antifungal effects than all other compounds. The structure of the various compounds was found to be determinative of their antibacterial activity. The three most effective compounds had an acetoxy group at the C-9 position, while the presence of an epoxide group at C-4/C-5 meant that uvedalin was more effective than enhydrin (Lin et al., 2003). Compound 1, an isomer of sonchifolin with a tigloyl group instead of an angeloyl group, showed greater antibacterial activity against *B. subtilis* than sonchifolin (Lin et al., 2003).

Joung et al. (2010) obtained a methanolic extract of yacon leaves and subsequently fractionated the extract using *n*-hexane, ethyl acetate, *n*-butanol, and water. The fractions were assessed for their antibacterial activity against six strains of methicillin-resistant *Staphylococcus aureus* and one standard methicillin-susceptible *S. aureus* strain. In the absence of light, no antibacterial activity was observed; however, under 4000 lux, the *n*-hexane fraction had an MIC of 15.6 µg/mL. Furthermore, pre-illumination of the *n*-hexane fraction increased its effectiveness against all seven strains (Joung et al., 2010). However, it was not determined what compounds present in the extract were modified in the presence of light, nor was the antibacterial mechanism of these modified compounds.

2.4.4 Existing research and research gap

According to Ministry for Primary Industries (2020), in the year ending June 2020 “comb honey and honeydew honey” represented 3% (321 tonnes) of New Zealand’s honey export volume, and 1% (NZ $2 million) of New Zealand’s honey export value. In the same year, Mānuka honey represented 76% (7854 tonnes) of export volume and 88% (NZ$375 million) of export volume (Ministry for Primary Industries, 2020). In the year ending June 2020, beekeepers were paid NZ$2.50 – NZ$4.00 per kilogram for bulk honey classified as “dark, including honeydew”; this increased to NZ$4.00 – NZ$12.00 in the year ending June

2023 (Ministry for Primary Industries, 2023). Over the same period of time, the price beekeepers were paid per kilogram for bulk Mānuka honey increased from NZ$4.50 – NZ$130.00 to $NZ6.00 – NZ$160.00; the range in price was influenced by ratings on industry grading systems such as UMF® and MGO™, as well as other market requirements (Ministry for Primary Industries, 2023). Data on the export volume and export value of NZHDH was not readily available after 2020. These statistics show that, compared to Mānuka honey, NZHDH is an underutilised and undervalued resource. Prior to this work, a comprehensive chemical profiling of NZHDH had not been carried out; in particular, to the extent of the researcher's knowledge, no information on the amino acid profile of NZHDH had been previously published in the literature, while several phenolic compounds were profiled in NZHDH for the first time. Similarly, yacon has only recently been introduced to New Zealand, with NZYC being an even more novel introduction to the market. Prior to this work, literature on the chemical composition and nutritional value of NZYC was limited or scant.

A vast quantity of research exists concerning sensory evaluation of NAB and LAB – see **Chapter 2.3.5.2** for more detail. Sensory evaluation on NAB and LAB brewed with alternative yeasts showed that alternative yeasts generate unique aroma and flavour profiles, although volatile content is lower than normal beer, and these profiles tend to score highly in undesirable descriptors such as 'worty', 'vegetable, 'butter', and 'dimethyl sulfide'. Sensory evaluation on NAB and LAB prepared by osmotic distillation or low-temperature fermentation, or commercially obtained, showed that the feeling and aroma of alcohol plays an important role in consumer preference. Additionally, potentially due to differences in volatile profile, flavours typically associated with beer were negatively correlated with consumer preference, while flavours not typically associated with beer were positively correlated. Affective testing of various kinds of NAB and LAB showed that NAB and LAB are often deficient in mouthfeel and body, and that removal of ethanol can lead to imbalances in sweetness and bitterness. Finally, testing by the Check-All-That-Apply method showed that (a) polymers can be selected based on molar masses to improve the mouthfeel of NAB and LAB, (b) panellists tend to like descriptors typically not associated with beers in NAB and LAB while disliking typical beer descriptors, and (c) mouthfeel and flavour play an important role in overall liking of beer. However, although industrial methods to produce LAB and NAB are well-described in the literature, research on the production of LAB or NAB in a home-brew context is non-existent. Most industrial processes are inaccessible to home-brewers due to the need for specialised equipment or inputs only available to industry

(e.g. specialised yeasts). The most accessible method to home brewers – thermal distillation – is disregarded in the literature, as the removal of alcohol under higher temperatures has been found to have significant adverse effects on beer taste (Brányik et al., 2012).

As discussed in **Chapter 2.2.4**, YC has been assessed in clinical studies with respect to its potential health benefits, particularly treatment of obesity and diabetes. However, to the extent of the researcher's knowledge, this work has not included assessment of NZYC.

3 A comprehensive chemical analysis of New Zealand honeydew honey

3.1 Prelude

Chapter 3, *A comprehensive chemical analysis of New Zealand honeydew honey,* examines the chemical profile of honeydew honey (HDH) in scientific literature and compares it to experimental data generated for New Zealand honeydew honey (NZHDH). Although honey has long been used in a variety of applications, including food, medicines, and cosmetics, and demand for HDH is growing around the world, NZHDH remains an underutilised and undervalued resource, especially compared to Mānuka honey. Furthermore, information on the chemical profile of NZHDH in the literature remains scant. Therefore, it is important to develop a comprehensive chemical profile of NZHDH to allow researchers, manufacturers, and the general public to better understand the potential health benefits of NZHDH.

This chapter fills the knowledge gap in the literature. The chemical composition of NZHDH has been determined concerning its proximate composition, mineral profile, sugar profile, phenolic profile, antioxidant activity, and amino acid profile. Results of interest have been compared to those published in the scientific literature for Mānuka honey, and NZHDH compares favourably, particularly about antioxidant activity. New Zealand Mānuka honey has been described in the literature as the "gold standard" for antioxidant activity, The antioxidant activity of NZHDH according to the ferric-reducing antioxidant power (FRAP) assay (47.42 ± 2.09 – 53.77 ± 1.38 mg Trolox equivalents per 100 grams) is higher than that described for Mānuka honey in multiple studies. However, other studies have also found Mānuka honey to have higher FRAP activity than that determined for NZHDH in the present study. Nevertheless, the potential health benefits of NZHDH have been highlighted by this research and would be of interest to researchers, manufacturers, and the general public, potentially increasing the market potential of NZHDH.

3.2 Introduction

Honey is a natural plant product produced by honeybees (*Apis mellifera*) and used by humans in a variety of food, medicinal, and cosmetic applications (Farooqui & Farooqui, 2011; Seraglio et al., 2019). Honey can be broadly divided into two classes according to botanical origin; blossom (or floral) honey, and honeydew honey (HDH) (Bentabol Manzanares et al., 2011; Pita-Calvo & Váquez, 2017; Seraglio et al.,

2019; Vasić et al., 2019). HDH is produced from nectar exuded from insects such as aphids. For instance, sources of HDH in New Zealand include the giant willow aphid *Tuberolachnus salignus*, which mainly feeds on willow trees, and the scale insects *Ultracoelostoma assimile* and *U. brittini*, which feed on most species of the Southern beech (*Nothofagus* spp.) found in the South Island (Astwood et al., 1998; Swears & Manley-Harris, 2021). It is estimated that honeydew is available as a resource in New Zealand in a land area of around 1,000,000 hectares, on the eastern side from Mount Somers northwards, and on the western side from Greymouth northwards (Beggs, 2001). According to K. Zhou, general manager of Streamland Honey Group Ltd., (personal communication, March 7, 2022), the Nelson beech forest is the source of all commercial honeydew in New Zealand, and one of the leading suppliers of NZHDH supplies NZHDH of the *U. assimile* type. NZHDH produced by the giant willow aphid rapidly crystallises, is low in sweetness, and has an unacceptable taste which can be attributed to salicylic and malic acids; therefore it is usually discarded by beekeepers and is not of commercial interest (Swears & Manley-Harris, 2021). It can therefore be understood that most commercial NZHDH is produced from nectar exuded by *U. assimile* in the Nelson beech forest.

According to Ministry for Primary Industries (2020), in the year ending June 2020 "comb honey and honeydew honey" represented 3% (321 tonnes) of New Zealand's honey export volume, and 1% (NZ $2 million) of New Zealand's honey export value. In the same year, Mānuka honey represented 76% (7854 tonnes) of export volume and 88% (NZ$375 million) of export volume (Ministry for Primary Industries, 2020). In the year ending June 2020, beekeepers were paid NZ$2.50 – NZ$4.00 per kilogram for bulk honey classified as "dark, including honeydew"; this increased to NZ$4.00 – NZ$12.00 in the year ending June 2023 (Ministry for Primary Industries, 2023). Over the same period of time, the price beekeepers were paid per kilogram for bulk Mānuka honey increased from NZ$4.50 – NZ$130.00 to $NZ6.00 – NZ$160.00; the range in price was influenced by ratings on industry grading systems such as UMF® and MGO™, as well as other market requirements (Ministry for Primary Industries, 2023). Data on the export volume and export value of NZHDH was not readily available after 2020. These statistics show that, compared to Mānuka honey, NZHDH is an underutilised and undervalued resource.

HDH is characterised by stronger flavour and darker colour, as well as distinct amino acid, polyphenol, and sugar profiles, in comparison to blossom honey (Pita-Calvo & Váquez, 2017; Seraglio et al., 2019; Vasić et al., 2019). HDH is of interest to both the food industry and consumers due to its potential anti-

microbial, anti-inflammatory, and antioxidant properties (Seraglio et al., 2019). Although in recent years there has been a growth in demand for HDH, particularly in central Europe (Karabagias et al., 2020; Nešović et al., 2020; Vasić et al., 2019), the information on New Zealand honeydew honey (NZHDH) present in literature remains scant, with the majority of research focussed on European HDH.

HDH is mainly composed of carbohydrates and moisture, with small amounts of protein and ash (Chua & Adnan, 2014). The major sugars present in honey are reducing monosaccharides (glucose and fructose), which make up between 59.9 and 79.7% of HDH by weight (Seraglio et al., 2019). Other sugars which may be present in HDH include erlose, isomaltose, maltose, melezitose, nigerose, raffinose, sucrose, trehalose, turanose, and xylose (Astwood et al., 1998; Bentabol Manzanares et al., 2011; Mateo & Bosch-Reig, 1997; Primorac et al., 2009; Rybak-Chmielewska, 2007; Tomczyk et al., 2020; Victorita et al., 2008). Erlose and melezitose are of particular interest with respect to HDH, as these sugars are produced by transferase enzymes in the gut of the plant-sucking insect, and are thus considered to be markers of HDH (Astwood et al., 1998; Pita-Calvo & Váquez, 2017; Sanz et al., 2005). According to Astwood et al. (1998), either erlose or melezitose will dominate in HDH depending on which insect feeds on the tree sap. Erlose dominates in NZHDH produced from nectar exuded by *Ultracoelostoma assimile*. Palatinose and turanose are of particular interest as these sugars have previously been reported in NZHDH (Astwood et al., 1998), They are also of general interest due to their reported health effects. Palatinose has been shown to positively affect exercise performance, glycemic control, and cognitive performance in animal experiments and clinical trials. Meanwhile, recent studies have demonstrated that turanose exerts anti-inflammatory and adipogenesis suppression effects in vitro (Tian et al., 2019).

According to Seraglio et al. (2019), the moisture content of HDH falls within the range of 10.5 to 20.5% depending on botanical and geographical origin. While HDH typically has higher ash content than blossom honey, which is largely mineral in nature, the value is usually less than 1.2% (Karabagias et al., 2020; Seraglio et al., 2019; Vasić et al., 2019). Potassium is the major mineral present in HDH, with values ranging from 307 to 6610 mg per kg honey; other significant minerals include calcium, magnesium, phosphorus, and sodium (Bergamo et al., 2018; González-Miret et al., 2005; Madejczyk & Baralkiewicz, 2008; Vanhanen et al., 2011).

Phenolic compounds are important in honey as phenolic compounds have antioxidative and anti-free radical effects (Nešović et al., 2020; Vasić et al., 2019). These compounds may impart bitter, astringent, and floral flavours (Silici et al., 2013). A variety of phenolic compounds have been identified in European HDH in the literature, including acacetin, apigenin, caffeic acid, catechin, chlorogenic acid, chrysin, coumaric acid, epicatechin, ferulic acid, gallic acid, hesperidin, kaempferol, luteolin, naringenin, p-coumaric acid, p-hydroxybenzoic acid, pinocembrin, protocatechuic acid, quercetin, rutin, sinapic acid, taxifolin, and vanillic acid (Nešović et al., 2020; Silici et al., 2013; C. F. Silva et al., 2020). Only one previous study investigated the phenolic composition of NZHDH, which qualitatively identified 4-hydroxybenzoic acid, 4-hydroxy-cinnamic acid, benzoic acid, cinnamic acid, syringic acid, and vanillic acid (Weston et al., 2000).

The concentration of amino acids may be strongly correlated to the radical scavenging activity of honey, even more so than the concentration of polyphenolic compounds (Pérez et al. (2007). Amino acids are also important contributors to flavour, and precursors for aroma compounds (Kivrak, 2015). A variety of free amino acids identified in European HDH include aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, threonine, arginine, β-alanine, α-alanine, γ-aminobutyric acid, tyrosine, valine, tryptophan, phenylalanine, isoleucine, leucine, ornithine, lysine, proline, trans-4-hydroxy proline, and methionine (Iglesias et al., 2004; Kivrak, 2015; Pérez et al., 2007).

The aim of this research was to provide a comprehensive chemical analysis of NZHDH with respect to proximate analysis, mineral content, sugar profile, phenolic profile, antioxidant activity, and amino acid profile. This was done in order to compare NZHDH to other varieties of HDH better described in the literature, and further demonstrate its potential health-promoting benefits.

3.3 Materials and methods

3.3.1 Chemicals and reagents

All reagents used in this study are ≥99% in purity, unless otherwise stated. The NZHDH used in this study was produced from nectar exuded by the scale insect *Ultracoelostoma assimile*, which feeds on the Southern beech (*Nothofagus* spp.), and was sourced from Streamland Honey Group Ltd, New Zealand. NZHDH samples were collected from three different production batches over a period of one year. Ultrapure water (UPW) was produced using a Purite Select Fusion water deionisation unit (Suez Water Technologies

& Solutions, USA). D(+)-melezitose monohydrate was sourced from Acros Organics, USA. Ammonium heptamolybdate (81-83% as MoO_3), glacial acetic acid, and sodium carbonate were sourced from Ajax FineChem, Australia. D-Turanose (98%) and palatinose hydrate (98+%) was sourced from Alfa Aesar, USA. 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate was sourced from Apollo Scientific, UK. p-Hydroxybenzoic acid and sucrose (purity not stated) were sourced from BDH Laboratory Supplies, UK. Potassium sulphate was sourced from ECP Ltd, New Zealand. Kaempferol was sourced from Extrasynthese, France. Methanol, ethanol, acetonitrile, ammonium acetate, d-glucose anhydrous, chloroform, and formic acid were sourced from Fisher Scientific, UK. Monopotassium phosphate (purity not stated) was sourced from InterChem Agencies, New Zealand. Gallic acid was sourced from LobaChemie, India. Copper sulphate pentahydrate was sourced from Merck KGaA, Germany. Xylitol was sourced from Nirvana Organics, Australia. D(-)-Fructose was sourced from Panreac, Spain. Strata C18-E (500 mg/3 mL) SPE cartridges were sourced from Phenomenex, USA. Sakuranetin (≥98.0%) and pinocembrin (≥98.0%) were sourced from PhytoLab, Germany. Sodium tetraborate decahydrate (borax) was sourced from PureScience, New Zealand. Iron (III) chloride was sourced from Scharlau Chemie, Spain. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, 97%), 2,4,6-tripyridyl-S-triazine (TPTZ), Amino Acid Standard A9906, neocuporine (≥98%), ellagic acid (≥95%), abscisic acid (≥98%), luteolin (≥98%), quercetin (≥95%), pinobanksin (≥95%), chrysin (97%), acacetin (≥97%), Multielement Standard Solution 6, N-methyl-N-(trimethylsilyl) trifluoroacetamide (≥98.5%), Folin & Ciocalteu's phenol reagent, L-alanine-2,3,3,3-d_4, methoxyamine hydrochloride, erlose (≥94%), pyridine, and dry acetonitrile were sourced from Sigma-Aldrich, USA. Sodium acetate trihydrate was sourced from Thermo Fisher Scientific, New Zealand. Sulphuric acid (95-98%), hydrochloric acid (36%), nitric acid (70%), mannitol, boric acid, and sodium hydroxide (97%) were sourced from Univar, USA. Copper (II) chloride dihydrate was sourced from VWR Chemicals, USA.

3.3.2 Proximate analysis

Protein content of NZHDH was determined by the Kjeldahl method (Bicsak & Collaborators, 1993). Honey sample (0.50 g) was mixed with copper sulphate (0.50 g) and potassium sulphate (7.00 g) in a digestion vessel, to which 10 mL of concentrated sulphuric acid was added. Samples were digested for 60 minutes at 420°C using a DK 20 heating digester (Velp Scientifica, Italy) equipped with a JP reticulating

water aspirator (Velp Scientifica, Italy). After digestion, samples were cooled to room temperature. Samples were then distilled with 80 mL 35% NaOH, 80 mL 4% boric acid, and 100 mL distilled water using 100% steam power, and titrated with 0.01 M hydrochloric acid using a VAP 450 distillation unit (Gerhardt, Germany) coupled with a Titroline easy titrator (SI Analytics, Germany).

Moisture content was determined by refractive index, using the method and calculation described by Sesta (2008). Honey was homogenised and allowed to thermally equilibrate before measurement of refractive index at 25°C using a J57 automatic refractometer (Rudolph Research, USA). Water content was then calculated according to **Equation 3.1**, where RI = refractive index. As the calculation is written for refractive index as measured at 20°C, a correction factor of an additional 0.00023% was applied for every degree above this temperature.

$$W(\%) = \frac{[-0.2681 - \log(RI - 1)]}{0.002243} \qquad \textbf{Eq. 3.1}$$

Ash content was determined by mass difference after heating honey sample in a Perfect Fire HDTP-56-55 furnace (Canadian Instrumentation Company, Canada) at 550°C for 6 hours according to Thiex, Novotny, and Crawford (2012). Carbohydrate content was determined by mass difference once protein, moisture, and ash content were determined.

3.3.3 Mineral profile

The mineral profile of NZHDH was determined by microwave plasma atomic emission spectrometry (MP-AES). Sample was prepared for analysis by digesting either 0.1 or 1.0 g honey in 10 mL 70% nitric acid (HNO_3), and making up to 25 mL with 2% HNO_3, or by digesting 1.0 g honey in 5 mL 70% HNO_3, and making it up to 10 mL with 2% HNO_3. This was done to obtain three different concentrations of digested honey sample. All digestions were carried out in Multiwave Go microwave digestion system (Anton-Paar, Austria), with ramp time of 10 minutes, hold time of 40 minutes, and digestion temperature of 170°C. Standards were prepared by diluting Multielement Standard Solution 6 in UPW to appropriate concentrations, ranging from 0.005 to 9.000 ppm. Standards and samples were analysed using the Agilent 4200 MP-AES system (Agilent Technologies, US).

3.3.4 Sugar profile

3.3.4.1 Determination of disaccharides and trisaccharides by trimethylsilyl (TMS) derivatisation

The concentrations of selected disaccharides and trisaccharides (turanose, palatinose, erlose, and melezitose) in NZHDH was determined by TMS derivatisation using gas chromatography – mass spectrometry (GC-MS), according to a modified version of the procedure described by Zarate et al. (2016). Honey (3.0 g) was made up to 10 mL in UPW, and sugar standards were prepared at appropriate concentrations (five concentration levels per sugar). These levels were 4-20 mg/mL (turanose), 2-10 mg/mL (palatinose), 0.5 to 2.5 mg/mL (erlose), and 0.1 to 0.5 mg/mL (melezitose). For the sample, 20 µL of honey solution was transferred into a 1.5 mL amber glass GC vial. For the sugar standards, 20 µL of each standard solution was combined in the same 1.5 mL amber glass GC vial at each concentration level, resulting in five standard mixes. The internal standard, xylitol (50 µL, 0.2 mg/mL) and methanol (60µL) was added to each GC vial, which were then vortexed to mix. All samples and standards were then frozen in liquid nitrogen to prevent losses and dried overnight using an Advantage Pro Freeze Dryer (SP VirTis, USA). After freeze-drying, 80 µL of methoxyamine hydrochloride (2 mg/100 mL pyridine) was added to each vial, which were then vortexed and incubated for 90 minutes at 30°C. After incubation, 80 µL of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) was added to each vial, which were then incubated for a further 30 minutes at 37°C. After this second incubation, 100 µL from each vial were transferred to a separate 1.5 mL amber glass GC vial containing a 0.1 mL glass insert.

Using an Agilent 7683B Series injector, derivatised samples were injected into an Agilent 6890N Network GC system coupled to an Agilent 5973 Network Mass Selective Detector with a quadrupole mass selective detector (Electron Ionisation) operated at 70 eV. The column used for the analysis of TMS-derivatised samples was an Agilent 122-5532 UI DB – 5MS UI, 30 m x 250 µm (internal diameter) x 0.25 µm (film thickness). The MS was operated in scan mode, where scanning started after 4.5 minutes (mass range 40 to 650 atomic mass units (a.m.u.) at 2 scans/s. The GC-MS parameters were as follows:

The oven temperature was initially held at 70°C for 5 minutes. Temperature was then raised at a rate of 12°C/minute to 220°C. Temperature was held for 1 minute, then raised with a gradient of 1.5°C/minute to 265°C. Again, temperature was held for 1 minute, and was then raised at a rate of 10°C/minute to 280°C. Temperature was held for 1 minute, then raised at a rate of 1°C/minute to 290°C. Finally, temperature was

held for 8 minutes. The flow through the column was held constant at 1.3 mL helium/minute. The injection volume was 1 µL, and the split ratio was 25:1. The inlet temperature was 230°C, the source temperature was 250°C, and the quadrupole temperature was 150°C. The GC column was equilibrated for 0.50 minutes prior to each injection.

3.3.4.2 Determination of monosaccharides by high performance liquid chromatography (HPLC) with evaporating light scattering detector (ELSD)

The concentrations of fructose, glucose, sucrose, and maltose present in NZHDH were determined by HPLC/ELSD. Eight standard mix solutions of these sugars (100 to 1500 µg/mL) were prepared in UPW. 1 mL of each standard mix was transferred into 2 mL amber glass GC vials and spiked with 20 µL of 0.05 g/mL xylitol as internal standard.

Honey (0.15 g) was dissolved in 50 mL of UPW. 3 mL of this solution was spiked with 60 µL of 0.051 g/mL mannitol as internal standard. The sample was washed three times with 3 mL chloroform to remove all non-polar organic compounds. 1.2 mL of the remaining aqueous layer was then centrifuged at 4,466 xG for 5 minutes, and 1 mL was transferred into a 2 mL GC vial for HPLC/ELSD analysis.

HPLC/ELSD analysis was carried out using a Shimadzu LC-10AT liquid chromatogram coupled to an Agilent 385-ELSD. Samples were injected with a Shimadzu SIL-10A auto injector. The column was a Luna Omega 3µm Sugar 100Å 250 x 4.6mm (Phenomenex 00G-4775-E0) with a SecurityGuard Cartridge (Phenomenex AJ0-4495). The HPLC parameters were as follows: the mobile phase was acetonitrile:UPW (80:20, v/v), isocratic elution, pump flow rate of 0.5 mL/minute, analysis time of 75 minutes, injection volume of 10 µL, sample loop volume of 50 µL, temperature of 25°C. The ELSD parameters were as follows: evaporator temperature was 80°C, nebuliser temperature was 50°C, and the inert gas flow rate (N_2) was 1.20 standard litres per minute (SLM).

3.3.5 Phenolic profile

3.3.5.1 Total phenolic content

The total phenolic content of NZHDH was determined by the Folin-Ciocalteu (FC) assay (Permal, Leong Chang, Seale, Hamid, & Kam, 2020). Gallic acid standards ranging from 2.5 to 200 mg/L were

prepared by dissolving gallic acid in UPW. Honey sample was prepared by dissolving 1 g of honey in 10 mL of UPW. 500 µL of FC phenol reagent was added to 1 mL of sample, standard, or blank (UPW) solution and held at room temperature for 5 minutes. Then, 1.5 mL of 20% sodium carbonate solution was added and the reaction mixture was incubated for 2 hours at room temperature in the dark. Absorbance of the standard and sample solutions were then measured at 765 nm against the blank.

3.3.5.2 Quantitative phenolic profile by liquid chromatography – mass spectrometry (LC-MS)

The phenolic profile of NZHDH was determined according to the procedure described by Gašić et al. (2014). "Honey samples (5g) were mixed with 5 mL of UPW, adjusted to pH 2.0 with 0.1% hydrochloric acid and homogenised in an ultrasonic bath for 30 min at room temperature. The samples were then filtered through filter paper to remove solid particles. An SPE cartridge was conditioned by washing with 3 mL of acetonitrile and 9 mL of ultrapure water. The filtrate was passed through cartridge, which was then washed with 6 mL of acidified water to remove all sugars and other polar constituents of honey. The adsorbed compounds were eluted with acetonitrile (1.5 mL)." The extracts were centrifuged at 4,466 xG for 5 minutes, and the supernatant was used for analysis by LC-MS.

A 20 mg/L stock solution of a mixture of phenolic compounds (p-hydroxybenzoic acid, ellagic acid, abscisic acid, luteolin, quercetin, sakuranetin, pinobanksin, kaempferol, pinocembrin, chrysin, and acacetin) was prepared in methanol, and diluted with methanol to yield standards at concentrations ranging from 0 to 5 mg/L. Stock and standard solutions were stored in the dark at 4°C.

LC-MS analyses were conducted using an Agilent 1260 Infinity Quaternary LC System (Santa Clara, CA 95051 USA). The LC-MS system consisted of the following components: 1260 quaternary pump (model number: G1311B), 1260 infinity ALS sampler (model number: G1329B), 1260 infinity TCC column component (model number: G1316A), 1260 infinity diode array detector (DAD) (model number: G4212B), connected to a 6420 triple quadrupole LC/MS system with electrospray ionisation (ESI) source (model number: G1948B). The column used was a Kinetex Evo C18 (2.1 x 150 mm, 1.7 µm) (Phenomenex, USA).

The MS ionisation source conditions were as follows: capillary voltage of 4 kV, drying gas temperature of 300°C, drying gas flow of 10 L/min, nebulizer pressure of 40 psi. The negative and positive ionisation modes were performed with multiple reaction monitoring (MRM) for quantitative analysis. The

LC-MS conditions were as follows: Mobile phase A was 0.1% formic acid in UPW. Mobile phase B was 0.1% formic acid in acetonitrile. The flow rate was 0.3 mL/min.

The gradient program was as follows: The initial percentage of mobile phase B (B%) was 10% and held for 1 minute. B% was then raised at a rate of 3% per minute to 25%. B% was then raised at a rate of 5% per minute to 40% and held for 7 minutes. B% was then raised at a rate of 10% per minute to 80% and held for 3 minutes. B% was then lowered at a rate of 70% per minute to 10%, and the run was complete.

3.3.6 Ferric reducing antioxidant power (FRAP), cupric ion reducing antioxidant capacity (CUPRAC), and phosphomolybdenum assays

6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) standards were prepared within the concentration range of 5 to 160 mg/L in 75% ethanol. NZHDH sample was prepared by making 1.0 g of NZHDH up to 10 mL with UPW.

The antioxidant activity of NZHDH was determined by the FRAP assay according to the procedure described by Permal et al. (2020). To make a 300 mM acetate buffer, 1.55 g of sodium acetate trihydrate was added to 8 mL glacial acetic acid, adjusted to pH 3.6, and made up to 500 mL with UPW. A 10 mM solution of 2,4,6-tripyridyl-S-triazine (TPTZ) was prepared by making 0.3123 g of TPTZ up to 100 mL with 40 mM hydrochloric acid. A 20 mM solution of $FeCl_3$ was prepared by making 1.3515 g of $FeCl_3$ up to 250 mL with UPW. To prepare the FRAP reagent, 1 mL of TPTZ solution was added to 1 mL of $FeCl_3$ solution and 10 mL of the acetate buffer. The mixture was then heated to 36°C. To determine ferric-reducing antioxidant power, 100 µL of sample, standard, or blank (UPW) was added to 900 µL of UPW and 2 mL of FRAP reagent. The reaction mixtures were inverted to mix and left to rest for 4 minutes before measuring absorbance at 593 nm.

The antioxidant activity of NZHDH was determined by the CUPRAC assay according to the procedure described by Permal et al. (2020). A 10 mM $CuCl_2$ solution was prepared by dissolving 0.4262 g $CuCl_2.2H_2O$ in UPW and making up to 250 mL. A 1.0 M ammonium acetate buffer was prepared by dissolving 19.27 g NH_2Ac in UPW, making up to 250 mL, and adjusting the pH to 7.0. A 7.5 mM solution of neocuporine (Nc) was prepared by dissolving 0.039 g of Nc in 96% ethanol and making up to 25 mL. To determine cupric ion reducing capacity, 1 mL of standard, sample, or blank (UPW) was added to 1 mL each

of $CuCl_2$ solution, NH_2Ac buffer, and Nc solution and 0.1 mL of UPW. The reaction mixtures were left for five minutes before reading absorbance at 450 nm.

The antioxidant activity of NZHDH was determined by the phosphomolybdenum assay according to the procedure described by Permal et al. (2020). A 1.1 M solution of monopotassium phosphate was prepared by weighing out 1.36 g and making it up to 100 mL with UPW. A 1.1 M solution of ammonium heptamolybdate was prepared by weighing out 12.36 g and making it up to 100 mL with UPW. To determine antioxidant activity by reduction of phosphate-molybdenum (VI) to phosphate-molybdenum (V), 1 mL of sample, standard, or blank (UPW) was mixed with 2.8 mL monopotassium phosphate solution, 6 mL of 1 M sulphuric acid, 0.4 mL of ammonium heptamolybdate solution, and 0.8 mL of UPW. The reaction mixtures were heated at 90°C for 2 hours and then rapidly cooled in an ice bath. Absorbance was then measured at 700 nm.

3.3.7 Amino acid profile

The amino acid profile of NZHDH was determined by the AccQTag (6-aminoquimolyl-N-hydroxysuccinimidyl carbamate) derivatisation method and analysed using liquid chromatography-mass spectrometry (LC-MS), according to the method described by Salazar, Armenta, and Shulaev (2012). The AccQTag reagent was prepared by warming up 2.8 mg 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate in 1 mL of dry acetonitrile to dissolve. Borate buffer was prepared by warming and sonicating 7.63 g of sodium tetraborate decahydrate in 90 mL of UPW and 10 mL acetonitrile and adjusting the pH to 8.8. A neutralising solution was prepared by mixing 10 mL of formic acid with 90 mL of UPW. Internal standard-spiked methanol (ISSM) was prepared by dissolving 1 mg of d4-alanine in 100 mL of methanol.

Standards were prepared ranging from 200 µM to 0.781 µM by diluting amino acid standard A9906 in UPW. 50 µL of ISSM was added to 50 µL of each standard. Honey sample was prepared by dissolving approximately 0.3 g HDH in 10 mL UPW, then adding 40 µL of this solution or 40 µL UPW (blank) to 40 µL of ISSM and vortexing at 4,466 xG for 5 min. 10 µL of sample, standard, or blank was added to 70 µL borate buffer in a microcentrifuge tube, followed by 10 µL of the AccQTag reagent. The tubes were vortexed immediately, capped, and incubated at 55°C for 15 minutes. Neutralising solution (400 µL) was then added to each tube, and the solutions were transferred to LC-MS vials for analysis.

LC-MS analyses were conducted using an Agilent 1260 Infinity Quaternary LC System (Santa Clara, CA 95051 USA). The LC-MS system consisted of the following components: 1260 quaternary pump (model number: G1311B), 1260 infinity ALS sampler (model number: G1329B), 1260 infinity TCC column component (model number: G1316A), 1260 infinity diode array detector (DAD) (model number: G4212B), connected to a 6420 triple quadrupole LC/MS system with electrospray ionisation (ESI) source (model number: G1948B). The column used was a Kinetex Evo C18 (2.1 x 150 mm, 1.7 µm) (Phenomenex, USA).

The MS ionisation source conditions were as follows: capillary voltage of 4 kV, drying gas temperature of 300°C, drying gas flow rate of 10 L/min, and nebuliser pressure of 30 psi. The positive ion mode was performed with multiple reaction monitoring (MRM) for quantitative analysis. The LC-MS conditions were as follows: Mobile phase A was 0.1% formic acid in UPW. Mobile phase B was 0.1% formic acid in acetonitrile. The flow rate was 0.225 mL/min, and the column temperature was 25°C.

The gradient program was as follows: The initial percentage of mobile phase B (B%) was 5% and held for 3 minutes. B% was then raised at a rate of 1% per minute to 10% and was held for 2 minutes. B% was then raised at a rate of 1.2% per minute to 17%, then raised at a rate of 42% per minute to 80% and held for 0.5 minutes. B% was then lowered at a rate of 50% per minute to 5% and the run was complete.

3.3.8 Statistical analysis

Statistical analysis was conducted using Microsoft Excel version 2104 and R Studio version 1.1.463. Proximate composition, sugar profile, phenolic profile, antioxidant activity and amino acid measurements were done in triplicates from different batches of NZHDH and the results were reported as mean values ± standard deviation as calculated in Excel and analysed by one-way analysis of variance (ANOVA) using R Studio. Post hoc analysis was conducted in R studio using Tukey's honestly significant difference (HSD) test with a 95% family-wise confidence interval. Mineral profile measurements were done in triplicates from a single batch of NZHDH, and results were reported as mean values ± standard deviation as calculated in Excel.

3.4 Results and discussion

3.4.1 Proximate analysis

The protein content of NZHDH, determined by the Kjeldahl method, was 0.47 ± 0.04 g/100g. The moisture content, determined by refractive index, was 15.90 ± 0.20 g/100g. The ash content, determined by mass difference after incineration at 550°C, was 0.28 ± 0.08 g/100g, and the carbohydrate content, determined by mass difference, was 83.35 ± 0.22 g/100g. These results are consistent with the reviewed literature for HDH, wherein ash and protein are minor components, and moisture content is reported to be between 10.5 and 20.5 g/100g (Chua & Adnan, 2014; Seraglio et al., 2019).

3.4.2 Mineral profile

Fourteen minerals were quantified in NZHDH by MP-AES, and the results are presented in **Table 3.1**. Potassium was the most abundant mineral (238.6 ± 10.5 mg/100g), followed by phosphorus (16.41 ± 0.16), magnesium (5.911 ± 0.044), and sodium (4.697 ± 0.247). These findings are consistent with the literature for HDH, in that potassium is by far the most abundant and that phosphorus, magnesium, and sodium are amongst the next most abundant (Vanhanen et al., 2011). The total evaluated mineral content was 269.8 ± 10.55 mg/100g, which is comparable with the ash content (0.28 ± 0.08 g/100g), and thus consistent with the claim that the ash of HDH is mostly mineral in nature (Seraglio et al., 2019).

Table 3.1 Mineral profile of New Zealand honeydew honey (NZHDH) (n=3).

Mineral	mg/100g NZHDH
Potassium	238.6 ± 10.54
Phosphorus	16.41 ± 0.156
Magnesium	5.911 ± 0.044
Sodium	4.697 ± 0.049
Calcium	2.279 ± 0.106
Iron	0.649 ± 0.050
Zinc	0.366 ± 0.066
Aluminium	0.260 ± 0.042
Titanium	0.197 ± 0.009
Boron	0.156 ± 0.007
Copper	0.121 ± 0.015
Antimony	0.088 ± 0.007
Nickel	0.049 ± 0.006
Lead	0.046 ± 0.004
Total	**269.8 ± 10.55**

3.4.3 Sugar profile

The sugar profile of NZHDH is presented in **Table 3.2**. The combination of fructose and glucose ranged from 55.239 to 56.575 g/100g, which is close to the range reported for European HDH (59.9 to 79.7 g/100g), and above the lower legal limit of 45 g/100g for the combination of these two sugars in HDH (Codex Alimentarius Commission, 2001). Sucrose was not detected, and no significant differences were identified in the concentration of maltose between the three batches of NZHDH, which was approximately 6 g/100g (see Table 2).

Erlose and melezitose were quantified as these sugars are produced by transferase enzymes in the gut of the plant-sucking insect and thus considered to be markers of HDH (Astwood et al., 1998; Pita-Calvo & Váquez, 2017; Sanz et al., 2005). Significant differences were identified in the concentrations of erlose between the three batches of NZHDH ($p = 0.006$); batch 1 was found to have significantly higher erlose content than batch 2 and batch 3, which were not significantly different to one another ($p > 0.05$). Erlose concentration in NZHDH was much higher than melezitose, which is consistent with the results reported by Astwood et al. (1998) for NZHDH produced from nectar exuded by *Ultracoelostoma assimile*.

The sum of sugars profiled is much lower than the total sugar content as determined by difference (83.35 ± 0.22 g/100g). Some of this difference can be attributed to the fact that the sugar profile is not comprehensive, with some unknown oligosaccharides not accounted for, as well as many of the disaccharides and trisaccharides identified in the literature review that were not quantified. In particular, three other peaks were observed in the HPLC-ELSD chromatogram **(Figure 3.1)**, which were neither glucose, fructose, sucrose, or maltose. These three unknown peaks may have included any of palatinose, turanose, melezitose, or erlose; however, owing to the price and quantity of standards available, only TMS derivatisation was used to quantify these saccharides. TMS derivatisation was chosen for these lower-concentration sugars as derivatisation is able to improve peak shape and resolution, among other factors (Krull & Strong, 2000).

Table 3.2 Sugar profile of New Zealand honeydew honey (NZHDH) (n=9).

Sugar	g/100g NZHDH Batch 1	g/100g NZHDH Batch 2	g/100g NZHDH Batch 3
Glucose*	20.288 ± 0.154[a]	19.417 ± 0.415[b]	19.821 ± 0.134[ab]
Fructose	36.287 ± 0.388	35.822 ± 0.655	36.470 ± 0.460

Sucrose	n.d.	n.d.	n.d.
Maltose	5.914 ± 0.398	5.843 ± 0.386	6.192 ± 0.213
Palatinose	0.869 ± 0.117	0.815 ± 0.083	0.851 ± 0.053
Turanose	2.727 ± 0.115	2.493 ± 0.416	2.892 ± 0.252
Melezitose	0.094 ± 0.005	0.091 ± 0.015	0.113 ± 0.014
Erlose**	0.430 ± 0.025[a]	0.336 ± 0.029[ab]	0.331 ± 0.026[b]

* Significant differences existed between batches at the .05 probability level. Values with different superscript letters are significantly different.

** Significant differences existed between batches at the .01 probability level. Values with different superscript letters are significantly different.

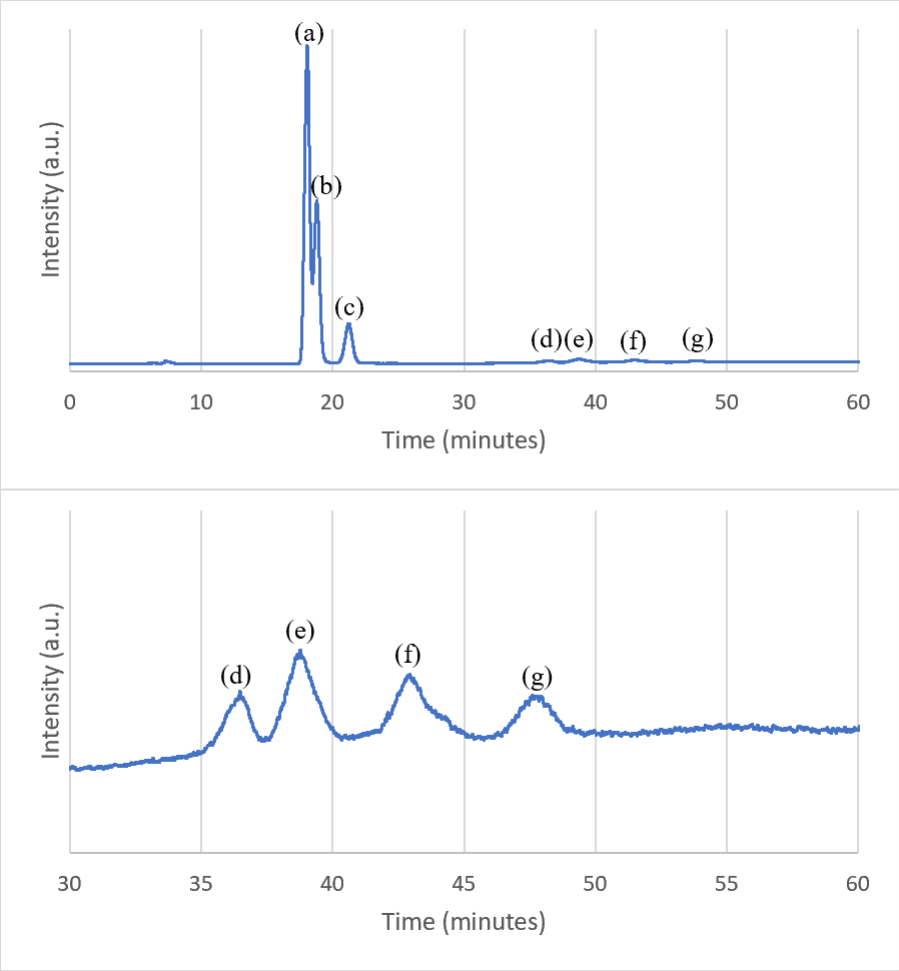

Figure 3.1 HPLC-ELSD chromatogram of time (minutes) vs intensity (arbitrary units (a.u.)) for New Zealand honeydew honey (n=3). Peak (a) = xylitol; (b) = fructose; (c) = glucose; (e) = maltose; (d), (f), and (g) = unknown.

3.4.4 Phenolic profile

The total phenolic content (TPC) of NZHDH, determined by the FC assay, was 62.30 ± 6.261 mg gallic acid equivalents (GAE) per 100 g. No significant differences were identified between the three batches of NZHDH. A study on honey from Burkina Faso found that the TPC of two HDH samples were 113.05 ±

1.10 mg GAE/100 g (harvested July 2003, sourced from an apiarist) and 114.75 ± 1.30 mg GAE/100g (harvested December 2002, sourced from the Fada Beekeeping Cooperative) (Meda, Lamien, Romito, Millogo, & Nacoulma, 2005). A separate study on Montenegro honey found that the mean TPC of eight HDH samples was 72.884 ± 9.106 mg GAE/100 g (Nešović et al., 2020). These results from the literature are comparable to the TPC of NZHDH in the current study.

The phenolic profile of NZHDH is presented in **Table 3.3**. Significant differences between batches were observed for p-hydroxybenzoic acid, ellagic acid, abscisic acid, pinobanksin, kaempferol, luteolin, chrysin, and pinocembrin. The most abundant compounds were pinocembrin, abscisic acid, and pinobanksin. However, batch 1 had much lower abscisic acid content (0.9624 ± 0.0091 mg/kg) than batches 2 and 3 (2.3231 ± 0.0370 and 2.2991 ± 0.0728 mg/kg respectively). Ellagic acid, p-hydroxybenzoic acid, kaempferol, luteolin, chrysin, and quercetin were all present at concentrations greater than 0.1 mg/kg, while sakuranetin and acacetin were present at concentrations less than 0.1 mg/kg.

While no study in the literature has quantified sakuranetin in HDH, all other compounds **(Table 3.3)** have been quantified in concentrations comparable, in terms of order of magnitude, to those previously reported in the literature for various sources of HDH (Bertoncelj, Polak, Kropf, Korošec, & Golob, 2011; Daher & Gülaçar, 2008; Halouzka, Tarkowski, & Zeljković, 2016; Haroun, Poyrazoglu, Konar, & Artik, 2012; Kováčik, Grúz, Biba, & Hedbavny, 2016; Nešović et al., 2020; Seraglio et al., 2016; Vasić et al., 2019).

Although sakuranetin has not previously been quantified in HDH, the sakuranetin content of NZHDH as identified in this research is very low. Sakuranetin has been shown to have anticancer, antimicrobial, antiprotozoal, antiviral, and anti-inflammatory effects (Stompor, 2020). However, the IC_{50} (half maximal inhibitory concentration) and MIC (minimum inhibitory concentration) values for these effects are much higher than the concentration of sakuranetin in NZHDH (Stompor, 2020). Sakuranetin has previously been quantified in floral honey varieties at similarly low concentrations; for instance, 0.0316 ± 0.0258 mg/kg in Mānuka honey, 0.0170 ± 0.0150 mg/kg in buckwheat honey, and 0.00649 ± 0.00262 mg/kg in acacia honey (Shen et al., 2019).

Only one study analysed phenolic compounds in NZHDH, qualitatively identifying p-hydroxybenzoic acid, p-coumaric acid, benzoic acid, cinnamic acid, pinobanksin, pinocembrin, chrysin, and galangin (Weston et al., 2000). Of these compounds, p-hydroxybenzoic acid, pinobanksin, pinocembrin, and chrysin were common in this research. However, only p-hydroxybenzoic acid was quantified by Weston et al. (2000) at 4.4 µg/100 g (0.044 mg/kg), which is notably lower than the results from this study. Factors that can affect the phenolic profile of honey include the botanical source, geographical and entomological origin, and climatic conditions (Martinello & Mutinelli, 2021; Weston et al., 2000).

Table 3.3 Phenolic profile of New Zealand honeydew honey (NZHDH) (n=9).

Phenolic compound	mg/kg NZHDH Batch 1	mg/kg NZHDH Batch 2	mg/kg NZHDH Batch 3
Pinocembrin**	2.649 ± 0.029^{a}	2.422 ± 0.049^{b}	2.325 ± 0.048^{b}
Abscisic acid**	0.962 ± 0.009^{b}	2.323 ± 0.037^{a}	2.299 ± 0.073^{a}
Pinobanksin*	1.863 ± 0.017^{a}	1.702 ± 0.060^{b}	1.654 ± 0.040^{b}
Ellagic acid*	0.697 ± 0.026^{a}	0.647 ± 0.011^{a}	0.587 ± 0.027^{b}
p-Hydroxybenzoic acid**	0.357 ± 0.0.18^{c}	0.638 ± 0.014^{a}	0.564 ± 0.026^{b}
Chrysin**	0.420 ± 0.001^{a}	0.378 ± 0.006^{b}	0.360 ± 0.004^{c}
Kaempferol**	0.263 ± 0.002^{c}	0.354 ± 0.009^{a}	0.327 ± 0.005^{b}
Luteolin**	0.254 ± 0.002^{c}	0.342 ± 0.009^{a}	0.316 ± 0.005^{b}
Quercetin	0.118 ± 0.002	0.119 ± 0.008	0.113 ± 0.008
Sakuranetin	0.017 ± 0.001	0.016 ± 0.001	0.016 ± 0.000
Acacetin	0.003 ± 0.000	0.003 ± 0.001	0.003 ± 0.000

* Significant differences existed between batches at the .01 probability level. Values with different superscript letters are significantly different.

** Significant differences existed between batches at the .001 probability level. Values with different superscript letters are significantly different.

3.4.5 Antioxidant activity

The antioxidant activities of NZHDH, as determined by the FRAP, CUPRAC, and phosphomolybdenum assays, are presented in **Table 3.4**. Significant differences existed between the different batches of NZHDH for the FRAP assay ($p = 0.011$), with batch 3 having significantly higher antioxidant activity than batch 1. Each assay gave different results for the antioxidant activity of NZHDH. This can be attributed to the fact that each assay relies on a different chemical reaction, and as such each antioxidant has different activity for each of these reactions (Tafulo, Queirós, Delerue-Matos, & Sales, 2010). Additionally, according to Tafulo et al. (2010), coexisting compounds in the samples will interfere differently across different methods.

A study on Polish honey determined the antioxidant activity of nectar, coniferous, and leafy HDH using the FRAP assay (Dżugan, Tomczyk, Sowa, & Grabek-Lejko, 2018). These HDH varieties had antioxidant activities of 50.40 ± 18.05, 53.90 ± 16.62, and 50.55 ± 14.39 mg Trolox equivalents (TE) / 100 g respectively, which are very similar to the results obtained for NZHDH. A separate study on Turkish honey determined the antioxidant activity of pine, sunflower, and chestnut HDH by use of the CUPRAC assay (Guzelmeric, Ciftci, Yuksel, & Yesilada, 2020). Their results ranged from 102.89 ± 2.23 to 261.5 ± 6.48 mg TE / 100 g, and results for NZHDH in the current study fall within this range. Another study on Turkish honey determined the antioxidant activity of pine (192.30 ± 18.03 mg GAE/100g), chestnut (212.06 ± 12.41 mg GAE/100g), and oak (209.00 ± 32.66 mg GAE/100g) HDH using the phosphomolybdenum assay (Silici & Ülgen, 2019). However, no study could be found in the literature which expressed results from the phosphomolybdenum assay in terms of Trolox equivalents and therefore comparison to results found in the current study was not possible.

Studies carried out by Morroni et al. (2018), Pentoś, Łuczycka, Oszmiański, Lachowicz, and Pasternak (2020), and Viteri, Zacconi, Montenegro, and Giordano (2021) determined the antioxidant activity of New Zealand Mānuka honey to be 31.74 ± 1.86, 519.76 ± 3.56, and 35.04 ± 0.00 mg TE/100g respectively according to the FRAP assay. In the literature, Mānuka honey has been used as the "gold standard" for antioxidant activity, and has been found to be superior to a variety of honeys sourced from Germany, Algeria, Saudi Arabia, Scotland, Malaysia, and Poland, among others (S. Ahmed & Othman, 2013; Alvarez-Suarez, Gasparrini, Forbes-Hernández, Mazzoni, & Giampieri, 2014; Pentoś et al., 2020). The antioxidant activity of NZHDH, as determined in this study using the FRAP assay was higher than that reported for Mānuka honey by Morroni et al. (2018) and Viteri et al. (2021). However, it was approximately ten times lower than that reported for Mānuka honey by Pentoś et al. (2020).

Table 3.4 Antioxidant activity (Trolox equivalents, TE) of New Zealand honeydew honey (NZHDH) (n=9).

Antioxidant assay	mg TE/100g NZHDH Batch 1	mg TE/100g NZHDH Batch 2	mg TE/100g NZHDH Batch 3
FRAP*	47.43 ± 2.09[a]	51.27 ± 1.63[ab]	53.77 ± 1.38[b]
CUPRAC	158.63 ± 3.21	157.67 ± 1.21	163.56 ± 14.13
Phosphomolybdenum	103.16 ± 15.43	110.18 ± 21.74	113.41 ± 13.02

* Significant differences existed between batches at the .05 probability level. Values with different superscript letters are significantly different.

3.4.6 Amino acid profile

Of the 34 free amino acids present in the amino acid standard A9906, 17 were quantified in NZHDH. The results are presented in **Table 3.5**. This is the first time that free amino acids have been profiled in NZHDH. The total concentration of free amino acids ranged from 491.601 ± 16.839 mg/100g (Batch 1) to 568.962 ± 53.165 mg/100g (Batch 2), with proline being the most abundant. L-aspartic acid, L-glutamic acid, L-alanine, and L-phenylalanine were the next most abundant amino acids. These results are in agreement with the literature, which identified proline as the major amino acid in HDH, with aspartic acid, glutamic acid and phenylalanine among those present at greatest concentration (Iglesias et al., 2004; Pérez et al., 2007). According to Hermosín, Chicón, and Dolores Cabezudo (2003), the amino acid composition of honey is attributable to both insect and botanical origin, with pollen being the major contributor; however, proline is unique as it originates from the honeybee.

Table 3.5 Amino acid profile of New Zealand honeydew honey (NZHDH) (n=9).

Amino acid	mg/100g NZHDH Batch 1	mg/100g NZHDH Batch 2	mg/100g NZHDH Batch 3
L-Proline	413.077 ± 16.704	478.504 ± 53.148	446.477 ± 35.215
L-Aspartic acid***	18.591 ± 1.314^{b}	29.034 ± 1.055^{a}	28.769 ± 1.205^{a}
L-Glutamic acid***	8.212 ± 0.752^{b}	15.317 ± 0.320^{a}	15.422 ± 0.531^{a}
L-Alanine***	12.787 ± 0.698^{a}	7.739 ± 0.379^{b}	7.187 ± 0.674^{b}
L-Phenylalanine	5.431 ± 0.036	5.443 ± 0.072	6.452 ± 0.704
Sarcosine**	4.736 ± 0.333^{b}	5.289 ± 0.096^{b}	6.110 ± 0.382^{a}
L-Threonine	4.091 ± 0.941	2.658 ± 0.321	2.810 ± 0.109
L-Valine**	4.028 ± 0.413^{a}	2.693 ± 0.110^{b}	2.906 ± 0.184^{b}
β-Alanine*	2.908 ± 0.431^{b}	3.572 ± 0.11^{b}	3.848 ± 0.078^{a}
L-Lysine***	3.318 ± 0.261^{a}	2.071 ± 0.162^{b}	2.314 ± 0.052^{b}
Glutamine***	1.658 ± 0.174^{b}	2.856 ± 0.036^{a}	3.098 ± 0.099^{a}
L-Tyrosine	3.021 ± 0.217	2.561 ± 0.048	2.674 ± 0.276
L-Leucine	2.620 ± 0.259	2.344 ± 0.140	2.802 ± 0.162
Hydroxy-L-proline*	2.061 ± 0.294^{b}	2.389 ± 0.061^{ab}	2.717 ± 0.128^{a}
L-Isoleucine*	2.582 ± 0.299^{a}	2.131 ± 0.206^{ab}	1.892 ± 0.099^{b}
Asparagine*	1.397 ± 0.068^{b}	2.472 ± 0.447^{a}	1.705 ± 0.258^{b}
γ-Amino-n-butyric acid***	1.082 ± 0.086^{c}	1.889 ± 0.060^{b}	2.346 ± 0.203^{a}

* Significant differences existed between batches at the .05 probability level. Values with different superscript letters are significantly different.

** Significant differences existed between batches at the .01 probability level. Values with different superscript letters are significantly different.

*** Significant differences existed between batches at the .001 probability level. Values with different superscript letters are significantly different.

3.5 Conclusion

This research has provided a comprehensive chemical profile of NZHDH produced from nectar exuded by the scale insect *Ultracoelosotoma assimile,* which feeds on the Southern beech. In terms of proximate analysis, sugar was the major component of NZHDH, while moisture, protein, and ash content were all consistent with the literature reported for HDH. Potassium was identified as the major mineral present in NZHDH, with significant amounts of phosphorus, magnesium, and sodium. Fructose and glucose were the major sugars present in NZHDH, with sugars unique to NZDH like palatinose, turanose, erlose, and melezitose also present. The dominance of erlose over melezitose is consistent with the literature for NZHDH. The major phenolic compounds quantified in NZHDH were pinocembrin, abscisic acid, and pinobanksin. Of the phenolic compounds quantified in this study, only p-hydroxybenzoic acid has previously been quantified in NZHDH. The antioxidant activity of NZHDH was comparable to results obtained in the literature for Mānuka honey, which is considered to be the "gold standard" among honeys in terms of antioxidant activity. Amino acids have not previously been quantified in NZHDH. Proline was the major amino acid present in NZHDH; L-aspartic acid, L-glutamic acid, L-alanine, and L-phenylalanine were the next most abundant. The chemical comparison of NZHDH to other varieties of HDH profiled in the literature, and to Mānuka honey in terms of antioxidant activity, suggest that it would have similar health-promoting benefits and therefore be of interest to both the food industry and to consumers. However, further study would be required to determine any in vitro and in vivo health-promoting effects of NZHDH. Further work to determine the sensory properties of NZHDH is also recommended.

4 A comprehensive chemical and nutritional analysis of New Zealand yacon concentrate

4.1 Prelude

Chapter 4, *A comprehensive chemical and nutritional analysis of New Zealand yacon concentrate,* examines the chemical profile of yacon concentrate (YC) and other yacon-related materials in scientific literature and compares it to experimental data generated for New Zealand yacon concentrate (NZYC). The consumption of yacon, an herbaceous perennial plant, can be dated back as far as 500 A.D., and yacon has also been used in traditional medicines for treatment of diabetes, digestive disorders, and renal disorders. However, yacon was only introduced to New Zealand in the 1980s. The soil and climatic conditions in New Zealand are substantially different to those of South America where yacon originates from, so it is to be expected that the chemical profile of yacon grown in New Zealand and products produced from New Zealand yacon (such as NZYC) will differ from other varieties of yacon and YC.

Interest in yacon has grown around the world due to its potential as a functional food, which can be related to its unique profile of bioactive and prebiotic compounds. Yacon is known for being particularly high in fructooligosaccharides (FOS), which are non-digestible prebiotic carbohydrates and thus have low calorific value. Consumption of FOS has also been linked to a variety of health benefits such as increased local immune response, reduction in colon pH, suppression of inflammation, reduction in risk of colorectal cancer, and supported growth of beneficial gut bacteria. YC, a sweet syrup with similar sensorial and physical properties to honey, is one emerging product that is considered to be a nutraceutical food and has the potential for development of novel food products and new diet therapy applications. However, given the novelty of yacon in New Zealand, information on the chemical composition of NZYC remains scant. Therefore, it is important to develop a comprehensive chemical profile of NZYC to allow researchers, manufacturers, and the general public to better understand the potential health benefits of NZYC.

This chapter, similar to the previous chapter, **Chapter 3** fills this gap in the literature. The chemical composition of NZYC has been determined with respect to its proximate composition, mineral profile, sugar profile, phenolic profile, antioxidant activity, and amino acid profile. Furthermore, the potential nutritional benefits of NZYC have been explored by glycaemic indexing of NZYC. The FOS content of NZYC, determined semi-quantitatively, ranged from 17.625 ± 0.325 g/100g to 52.276 ± 0.808 g/100g. FOS content

of yacon is known to vary due to several factors such as plant age, harvest conditions, and post-harvest conditions, among others. NZYC was also determined to have a low glycaemic index (40 ± 0.22), supporting the classification of NZYC as a nutraceutical food and its potential for future diet therapy applications. The potential health benefits of NZYC have been highlighted by this research and would be of interest to researchers, manufacturers, and the general public, potentially increasing the market potential of NZYC.

4.2 Introduction

Yacon (*Smallanthus sonchifolius*) is an herbaceous perennial plant which forms tuberous roots (Ojansivu et al., 2011; M. R. Yan et al., 2019). The roots can be consumed raw (although they can be boiled or baked), and have texture and a sweet taste similar to apple and pear; in French, they are named 'poire de terre', which translates to 'pear of the earth' (Ojansivu et al., 2011). Juice can also be extracted from the roots for drinking or production of syrups or concentrates (Douglas et al., 2005; Ojansivu et al., 2011). Young stems of the yacon plant can be consumed similarly to celery, while the leaves can be used to make herbal teas (Douglas et al., 2005; Ojansivu et al., 2011). In addition to food applications, yacon has traditional medicinal uses such as treatment of diabetes, digestive disorders, and renal disorders (Ojansivu et al., 2011; M. R. Yan et al., 2019).

Yacon is an ancient Andean crop grown from Colombia to North-western Argentina; depictions dating to 500-1200 A.D. have been found in an archaeological deposit of Nazca, while the first written record is dated to 1615 A.D. (Douglas et al., 2005; Ojansivu et al., 2011). It is native to the lower latitudes of South America (0-25°S), and grows in subtropical and warm temperate environments at altitudes between 600 and 3500 m (Douglas et al., 2005). Optimum growth occurs between 18 and 25°C, while temperatures below 10 to 12°C can be detrimental to growth (Douglas et al., 2005).

In the early 1980s, yacon was imported into New Zealand as a novel vegetable; from New Zealand, it was introduced to Japan, and from Japan to Korea and Brazil (Douglas et al., 2005; Ojansivu et al., 2011). An unpublished study carried out in New Zealand planted yacon in four sites at latitudes from 37 to 45°S. The two South Island sites (Lincoln and Mosgiel) had very low yields compared to the North Island sites of Pukekohe and Hamilton. This can be attributed to the climatic differences between the North and South Islands, wherein the higher temperatures and longer seasons in the North Island match more closely to the

Andean conditions (Douglas et al., 2005). One study on the chemical composition of seven different yacon cultivars from around the world showed that yacon tubers of the 'New Zealand' cultivar had the lowest sum of glucose, fructose, and sucrose, and the lowest total phenolic content, total flavonoid content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and ferric reducing antioxidant power (FRAP); however, 'New Zealand' yacon tubers did have the highest 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity (Khajehei et al., 2018).

In recent years, there has been growing global interest in yacon due to its potential as a functional food, which can be related to its unique profile of bioactive and prebiotic compounds, including antioxidants and phenolic compounds (Douglas et al., 2005; Ojansivu et al., 2011; M. R. Yan et al., 2019). Of special interest is the concentration of fructooligosaccharides (FOS) in the yacon tubers, comprising between 40 and 70% of dry matter by weight, which is higher than that of any other plant matter (Ojansivu et al., 2011; M. R. Yan et al., 2019).

Fructooligosaccharides are short-chain oligosaccharide fructans with a degree of polymerisation (DP) lower than 9, classed as non-digestible prebiotic carbohydrates. They are non-digestible as amylase enzymes are unable to hydrolyse β-(2,1) bonds; similarly, salivary and internal digestive enzymes are unable to hydrolyse FOS (Genta et al., 2009; M. R. Yan et al., 2019). Thus, FOS have a low calorific value for humans and pass undigested through to the colon. In the colon they are fermented by anaerobic bacteria, forming short-chain fatty acids (SCFA). Animal studies suggest that the formation of SCFA can increase local immune response and reduce colon pH, suppressing inflammation and reducing the risk of colorectal cancer (M. R. Yan et al., 2019). Furthermore, consumption of FOS supports the growth of beneficial gut bacteria (particularly those of the genus *Bifidus* and *Lactobacillus*), which may aid in the easing of constipation and reduction of lipid and glucose in the blood (Genta et al., 2009; M. R. Yan et al., 2019). Conversely, in the few clinical studies carried out on yacon, only bloating and flatulence have been reported as adverse effects attributable to FOS content, and only in levels of daily intake above 0.14 g FOS/kg body weight; the only significant concern related to consumption of yacon is one reported case of anaphylaxis (Genta et al., 2009). Yacon roots may also contain inulin; like FOS, inulins are fructan-type oligosaccharides – however, they can have DP of up to 60. Inulins are known to have similar health-promoting effects to those of FOS (M. R. Yan et al., 2019).

One product that has recently arisen in the market as a result of growing interest in yacon is yacon syrup or yacon concentrate (YC); it is associated with physical and sensorial characteristics similar to that of honey or sugar cane syrup (Genta et al., 2009). Unlike other sweet syrups, YC is hypocaloric owing to its high FOS content; furthermore, dietary supplementation of YC has been shown to enhance satiety sensation, aiding in weight loss (Genta et al., 2009). Thus, YC may be considered as a nutraceutical food, and has potential for development of novel food products and new diet therapy applications (Genta et al., 2009; M. R. Yan et al., 2019).

In terms of proximate composition, carbohydrates make up 65-70% of YC by weight, while water accounts for around 25%, protein for 1-2%, and fat for around 0.1% (Manrique et al., 2005b). The only significant micronutrient in YC is potassium, which accounts for around 1% of the concentrate total weight (Manrique et al., 2005b). The carbohydrate profile of YC can vary widely between varieties of cultivars; for instance, YC produced from cultivar CLLUNC118 contains 10.9% FOS, 15.5% free glucose, 25.4% free fructose, and 12.2% free sucrose, while YC produced from cultivar AMM5163 contains 47.6% FOS, 2.6% free glucose, 7.9% free fructose, and 20.0% free sucrose (Manrique et al., 2005b).

Phenolic compounds are secondary metabolites in plants which are of interest primarily due to their antioxidative properties, as well as their anti-inflammatory and anti-carcinogenic properties (Khajehei et al., 2018; M. R. Yan et al., 2019). Yacon tubers, from which YC is derived, contain a notably high level of phenolic compounds – approximately 200 mg per 100 g fresh weight (Manrique et al., 2005b). Chlorogenic acid has been identified as a major phenolic compound present in yacon tubers (Manrique et al., 2005b; X. Yan et al., 1999); other phenolic compounds identified include quercetin, ferulic acid, quinic acid, 3,5-dicaffeoylquinic acid, caffeic acid, and three ester derivatives of caffeic acid (Manrique et al., 2005b; Ojansivu et al., 2011; Takenaka et al., 2003). Amino acids, similar to phenolic compounds, are of interest primarily due to their antioxidative properties. The major amino acid identified in YC in the literature is L-tryptophan; glutamine, arginine, alanine, threonine, and valine have also been identified as present in YC (M. F. G. Silva et al., 2018; X. Yan et al., 1999). M. F. G. Silva et al. (2018) also identified the organic acids formic acid, fumaric acid, citric acid, and malic acid as being present in YC.

Glycaemic index (GI) is defined as the incremental blood glucose area following ingestion of food of interest, expressed as a percentage of the corresponding area following ingestion of the equivalent amount of

carbohydrate from a standard reference product (Arvidsson-Lenner et al., 2004). According to Arvidsson-Lenner et al. (2004), the glycaemic response of a food, and therefore the insulin response, depends on the rate of gastric emptying, the rate of digestion, and the rate of absorption of carbohydrates from the small intestine. Various food factors have been demonstrated to affect glycaemic response; these include overall structure, cell wall integrity, starch composition, and granular structure, the fructose-glucose ratio, and the presence of gel-forming types of dietary fibre, organic acids, or amylase inhibitors (Arvidsson-Lenner et al., 2004). Low GI foods are those for which the breakdown of carbohydrates into glucose and subsequent absorption into the blood takes relatively longer and have GI values of 55 or less. Low GI diets have been shown to improve overall blood glucose and lipid control for normal and diabetic individuals and can aid in weight loss, reduction of circulating triglycerides, and improvement of blood pressure (Radulian, Rusu, Dragomir, & Posea, 2009; Wolever et al., 1992).

The aim of this research is to produce a comprehensive chemical and nutritional profile of YC derived from the 'New Zealand' yacon cultivar (NZYC). This will confirm the potential of NZYC as a nutraceutical food product and as an ingredient to add value to other products such as functional drinks.

4.3 Materials and methods

4.3.1 Materials

The NZYC used in this study was produced from yacon cultivar 'New Zealand'. Three different production batches were sourced from Yacon New Zealand Ltd. Yacon crops are grown in the North Island of New Zealand and typically harvest in the mid-year. After harvest, the yacon roots are graded, milled, pressed to juice, and concentrated to 80° Brix.

All reagents used in this study are ≥99% in purity, unless otherwise stated. Ultrapure water (UPW) was produced using a Purite Select Fusion water deionisation unit (Suez Water Technologies & Solutions, USA). Glacial acetic acid, maleic acid, and sodium carbonate were sourced from Ajax FineChem, Australia. Chlorogenic acid (≥95%) was sourced from Alfa Aesar, United States. 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate was sourced from Apollo Scientific, UK. Sucrose (purity not stated), malic acid, succinic acid, and tartaric acid were sourced from BDH Laboratory Supplies, UK. Potassium sulphate was sourced from ECP Ltd, New Zealand. Ferulic acid (≥98%), kaempferol, and kaempferol-3-O-rutinoside (≥98%) were sourced from Extrasynthese, France. Methanol, ethanol, acetonitrile, ammonium acetate, d-

glucose anhydrous, chloroform, and formic acid were sourced from Fisher Scientific, UK. Gallic acid was sourced from LobaChemie, India. Copper sulphate pentahydrate was sourced from Merck KGaA, Germany. Xylitol was sourced from Nirvana Organics, Australia. D(-)-Fructose was sourced from Panreac, Spain. Strata C18-E (500 mg/3 mL) SPE cartridges were sourced from Phenomenex, USA. Sodium tetraborate decahydrate (borax) was sourced from PureScience, New Zealand. Iron (III) chloride was sourced from Scharlau Chemie, Spain. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, 97%), 2,4,6-tripyridyl-S-triazine (TPTZ), Amino Acid Standard A9906, neocuporine (≥98%), ellagic acid (≥95%), Multielement Standard Solution 6, Folin & Ciocalteu's phenol reagent, L-alanine-2,3,3,3-d_4, dry acetonitrile, 1-kestose (≥98%), nystose (≥98%), catechin, epicatechin (≥90%), caffeic acid (≥98%), citric acid, p-coumaric acid (≥98%), rutin trihydrate (≥94%), isorhamnetin (≥95%), myricetin (≥96%), quinic acid, malonic acid, fumaric acid, salicylic acid, 2,2-Di(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH), and sodium acetate (anhydrous) were sourced from Sigma-Aldrich, USA. Sodium acetate trihydrate was sourced from Thermo Fisher Scientific, New Zealand. Sulphuric acid (95-98%), hydrochloric acid (36%), nitric acid (70%), boric acid, and sodium hydroxide (97%) were sourced from Univar, USA.

4.3.2 Proximate analysis

Protein content of NZYC was determined by the Kjeldahl method (Bicsak & Collaborators, 1993). NZYC sample (0.50 g) was mixed with copper sulphate (0.50 g) and potassium sulphate (7.00 g) in a digestion vessel, to which 10 mL of concentrated sulphuric acid was added. Samples were digested for 60 minutes at 420°C using a DK 20 heating digester (Velp Scientifica, Italy) equipped with a JP reticulating water aspirator (Velp Scientifica, Italy). After digestion, samples were cooled to room temperature. Samples were then distilled with 80 mL 35% sodium hydroxide, 80 mL 4% boric acid, and 100 mL distilled water using 100% steam power, and titrated with 0.01 M hydrochloric acid using a VAP 450 distillation unit (Gerhardt, Germany) coupled with a Titroline easy titrator (SI Analytics, Germany).

Moisture content was determined by refractive index using a J57 automatic refractometer (Rudolph Research, USA) according to the method described by Chessum, Chen, Hamid, and Kam (2022). Ash content was determined by mass difference using a Perfect Fire HDTP-56-55 furnace (Canadian Instrumentation Company, Canada) according to Chessum et al. (2022). Carbohydrate content was determined by mass difference once protein, moisture, and ash content were determined.

4.3.3 Mineral profile

The mineral profile of NZYC was determined by microwave plasma atomic emission spectrometry (MP-AES) according to the methods described by Chessum et al. (2022). Digestions were carried out in Multiwave Go microwave digestion system (Anton-Paar, Austria), while standards and samples were analysed using the Agilent 4200 MP-AES system (Agilent Technologies, US).

4.3.4 Sugar profile

4.3.4.1 Determination of monosaccharides by high-performance liquid chromatography (HPLC) with evaporating light scattering detector (ELSD)

The concentrations of fructose, glucose, and sucrose present in NZYC were determined by HPLC/ELSD according to the method described by Chessum et al. (2022), using 0.20 g of NZYC rather than 0.15 g of honey.

HPLC/ELSD analysis was carried out using a Shimadzu LC-10AT liquid chromatogram coupled to an Agilent 385-ELSD. Samples were injected with a Shimadzu SIL-10A auto injector. The column was a Luna Omega 3µm Sugar 100Å 250 x 4.6mm (Phenomenex 00G-4775-E0) with a SecurityGuard Cartridge (Phenomenex AJ0-4495). The HPLC parameters were as follows: the mobile phase was acetonitrile:UPW (80:20, v/v), isocratic elution, pump flow rate of 0.5 mL/minute, analysis time of 75 minutes, injection volume of 10 µL, sample loop volume of 50 µL, temperature of 25°C. The ELSD parameters were as follows: evaporator temperature was 80°C, nebuliser temperature was 50°C, and the inert gas flow rate (N_2) was 1.20 standard litres per minute (SLM).

4.3.4.2 Determination of fructooligosaccharides and inulins by high performance liquid chromatography (HPLC) with evaporating light scattering detector (ELSD)

The concentrations of fructooligosaccharides and inulins (degree of polymerisation [DP] 3-13) were determined by HPLC-ELSD. A mixed standard of 1-kestose (DP3) and nystose (DP4) was prepared at a series of concentrations from 1.5 to 0.03125 ppm in UPW. Approximately 0.3 g NZYC was dissolved in 10 mL of UPW. 3 mL of sample was washed three times with 3 mL of chloroform to remove all non-polar

organic compounds. 1.2 mL of the remaining aqueous layer was then centrifuged at 4,466 xG for 5 minutes, and 1 mL was transferred into a 2 mL GC vial for HPLC/ELSD analysis.

HPLC/ELSD analysis was carried out using a Shimadzu LC-10AT liquid chromatogram coupled to an Agilent 385-ELSD. Samples were injected with a Shimadzu SIL-10A auto injector. The column was a Luna Omega 3µm Sugar 100Å 250 x 4.6mm (Phenomenex 00G-4775-E0) with a SecurityGuard Cartridge (Phenomenex AJ0-4495). The HPLC parameters were as follows: Mobile phase A was UPW, and mobile phase B was acetonitrile. The pump flow rate was 1.0 mL/minute, analysis time of 62 minutes, injection volume of 20 µL, sample loop volume of 50 µL, temperature of 25°C. The ELSD parameters were as follows: evaporator temperature was 80°C, nebuliser temperature was 50°C, and the inert gas flow rate (N_2) was 1.20 standard litres per minute (SLM).

The gradient program was as follows: The initial percentage of mobile phase B (B%) was 75% and was decreased to 45% over 50 minutes. B% was then increased to 75% over two minutes, and was held at 75% for 10 minutes, and the run was complete.

4.3.5 Phenolic profile

4.3.5.1 Total phenolic content

The total phenolic content of NZYC was determined by the Folin-Ciocalteu (FC) assay according to the method described by Chessum et al. (2022), with minor modifications. 500 µL of FC phenol reagent was added to 20 µL of prepared NZYC sample and diluted with 980 µL UPW.

4.3.5.2 Quantitative phenolic profile by liquid chromatography – mass spectrometry (LC-MS)

NZYC samples were prepared and the phenolic profile was determined according to the procedure described by Chessum et al. (2022). For the standards, a 100 mg/L stock solution of a mixture of phenolic compounds (chlorogenic acid, gallic acid, catechin, epicatechin, caffeic acid, p-coumaric acid, ferulic acid, ellagic acid, rutin, kaempferol, kaempferol rutinoside, isorhamnetin, and myricetin) was prepared in methanol, and diluted with methanol to yield standards at concentrations ranging from 0 to 20 mg/L. Stock and standard solutions were stored in the dark at 4°C.

LC-MS analyses were conducted using an Agilent 1260 Infinity Quaternary LC System (Santa Clara, CA 95051 USA). The LC-MS system consisted of the following components: 1260 quaternary pump (model number: G1311B), 1260 infinity ALS sampler (model number: G1329B), 1260 infinity TCC column component (model number: G1316A), 1260 infinity diode array detector (DAD) (model number: G4212B), connected to a 6420 triple quadrupole LC/MS system with electrospray ionisation (ESI) source (model number: G1948B). The column used was a Kinetex Evo C18 (2.1 x 150 mm, 1.7 µm) (Phenomenex, USA).

The MS ionisation source conditions were as follows: capillary voltage of 4 kV, drying gas temperature of 300°C, drying gas flow of 10 L/min, nebulizer pressure of 40 psi. The negative and positive ionisation modes were performed with multiple reaction monitoring (MRM) for quantitative analysis. The LC-MS conditions were as follows: Mobile phase A was 0.1% formic acid in UPW. Mobile phase B was 0.1% formic acid in acetonitrile. The flow rate was 0.3 mL/min.

The gradient program was as follows: The initial percentage of mobile phase B (B%) was 5% and held for 0.5 minutes. B% was then raised to 15% over 1.5 minutes. B% was then raised to 20% over 7 minutes; then to 50% over two minutes and held at 50% for a further two minutes. B% was then raised to 80% over one minute and held at 80% for a further two minutes. B% was then lowered to 5% over the course of one minute and held until the run time of 28 minutes was complete.

4.3.6 Ferric reducing antioxidant power (FRAP), cupric ion reducing antioxidant capacity (CUPRAC), and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assays

6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) standards were prepared within the concentration range of 5 to 160 mg/L in 75% ethanol. NZYC sample was prepared by making 1.0 g of NZYC up to 10 mL with UPW.

The antioxidant activity of NZYC was determined by the FRAP assay according to the procedure described by Chessum et al. (2022), with minor modifications. Instead of adding 100 µL of prepared NZYC sample to the reaction mixture, 10 µL of NZYC sample was added with 90 µL UPW.

The antioxidant activity of NZYC was determined by the CUPRAC assay according to the procedure described by Chessum et al. (2022), with minor modifications. Instead of adding 1 mL of NZYC sample to the reaction mixture, 10 µL of NZYC sample was added with a further 990 µL UPW.

The antioxidant activity of NZYC was determined by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay, using a method developed from the procedures described by Blois (1958); Kedare and Singh (2011). A 0.1 M sodium acetate buffer was prepared by dissolving sodium acetate in UPW. A buffered ethanol solution was then prepared by mixing 0.1 M sodium acetate buffer with ethanol in a 2:3 ratio by volume. A 0.3 mM DPPH solution was prepared by dissolving DPPH in methanol; this solution was stored at 4°C in the dark as it was light and temperature sensitive. A 100 mg/L Trolox stock solution was prepared by dissolving Trolox in buffered ethanol, from which a series of standards were produced at concentrations from 2 to 40 mg/L. NZYC stock solutions were prepared by dissolving one gram of NZYC in 25 mL buffered ethanol; sample solutions were prepared by further dilution with buffered ethanol, ranging from ten-fold to two-fold dilutions.

To perform the assay, 0.20 mL of diluted sample, standard, or buffered ethanol (control) was mixed with 2 mL of DPPH, then 2 mL of methanol was added. For the blank, 0.20 mL of buffered ethanol was mixed with 4 mL of methanol. The reaction mixtures were kept in the dark at room temperature for 30 minutes before absorbance was read at 517 nm against the blank.

DPPH inhibition was calculated according to **Equation 4.1**, where A_c is the absorbance of the control and A_s is the absorbance of the sample or standard. Maximum inhibition percentage was determined as 50% inhibition; above 50%, the absorbance reading is meaningless.

$$Inhibition\ of\ DPPH\ (\%) = \left(\frac{A_c - A_s}{A_s}\right) * 100 \qquad \textbf{Eq. 4.1}$$

4.3.7 Amino acid profile

The amino acid profile of NZYC was determined by the AccQTag (6-aminoquimolyl-N-hydroxysuccinimidyl carbamate) derivatisation method and analysed using liquid chromatography-mass spectrometry (LC-MS), according to the method described by Chessum et al. (2022). For sample preparation, 0.1 g of NZYC was dissolved in 10 mL of UPW (rather than 0.3 g), and all other steps were the same.

LC-MS analyses were conducted using an Agilent 1260 Infinity Quaternary LC System (Santa Clara, CA 95051 USA). The LC-MS system consisted of the following components: 1260 quaternary pump (model number: G1311B), 1260 infinity ALS sampler (model number: G1329B), 1260 infinity TCC column

component (model number: G1316A), 1260 infinity diode array detector (DAD) (model number: G4212B), connected to a 6420 triple quadrupole LC/MS system with electrospray ionisation (ESI) source (model number: G1948B). The column used was a Poroshell 120 EC-C18 (2.1 x 150 mm, 2.7 µm) (Agilent, USA).

The MS ionisation source conditions were as follows: capillary voltage of 4 kV, drying gas temperature of 300°C, drying gas flow rate of 10 L/min, and nebuliser pressure of 30 psi. The positive ion mode was performed with multiple reaction monitoring (MRM) for quantitative analysis. The LC-MS conditions were as follows: Mobile phase A was 0.1% formic acid in UPW. Mobile phase B was 0.1% formic acid in acetonitrile. The flow rate was 0.3 mL/min, and the column temperature was 25°C.

The gradient program was as follows: The initial percentage of mobile phase B (B%) was 5% and held for 1 minute. B% was then raised to 10% over four minutes, then to 15% over five minutes. B% was then raised to 45% over five minutes, then to 80% over two minutes and held at 80% for a further two minutes. B% was then lowered to 5% over one minute and held at 5% until the run time of 29 minutes was complete.

4.3.8 Quantitative organic acid profile by LC-MS

A quantitative organic acid profile of NZYC was produced using LC-MS. A mixed standard (citric, succinic, malic, maleic, malonic, fumaric, tartaric, pyruvic, quinic, and salicylic acids) was prepared at 500 ppm. Via serial dilution, a series of mixed standards was produced at concentrations from 10 to 0.3125 ppm. NZYC (approximately 1.0 to 1.5g) was dissolved in 10 mL of UPW and diluted down a hundredfold in UPW. Samples were then centrifuged at 4,466 xG for 5 minutes, and the supernatant and mixed standards were used for analysis by LC-MS.

LC-MS analyses were conducted using an Agilent 1260 Infinity Quaternary LC System (Santa Clara, CA 95051 USA). The LC-MS system consisted of the following components: 1260 quaternary pump (model number: G1311B), 1260 infinity ALS sampler (model number: G1329B), 1260 infinity TCC column component (model number: G1316A), 1260 infinity diode array detector (DAD) (model number: G4212B),

connected to a 6420 triple quadrupole LC/MS system with electrospray ionisation (ESI) source (model number: G1948B). The column used was a Kinetex Evo C18 (2.1 x 150 mm, 1.7 μm) (Phenomenex, USA).

The MS ionisation source conditions were as follows: capillary voltage of 4 kV, drying gas temperature of 300°C, drying gas flow of 10 L/min, nebulizer pressure of 40 psi. The negative and positive ionisation modes were performed with multiple reaction monitoring (MRM) for quantitative analysis. The LC-MS conditions were as follows: Mobile phase A was 0.1% formic acid in UPW. Mobile phase B was 0.1% formic acid in acetonitrile. The flow rate was 0.2 mL/min, and the column temperature was 40°C.

The gradient program was as follows: The initial percentage of mobile phase B (B%) was 5% and held for 0.5 minutes. B% was then raised to 20% over two and a half minutes, then to 50% over two minutes and held at 50% for a further four minutes. B% was then lowered to 5% over three minutes and held at 5% until the run time of 25 minutes was complete.

4.3.9 Glycaemic index (GI), in vivo test

NZYC was tested for its glycaemic index to evaluate its health-related benefits. The test was conducted at the Sydney University's Glycaemic Index Research service. Glycaemic index was measured in vivo using the international standard method ISO 26642:2010(E) (International Organisation for Standardisation, 2010) (n = 10). A group of 10 healthy, non-smoking people were recruited for the test. A weighted portion of the NZFOS+ sample containing 25 g of available carbohydrate or a drink containing 25 g of glucose was consumed within 10 minutes on two separate occasions. Participants capillary blood samples were analysed for glucose at 0 (baseline), 15, 30, 45, 60, 90, 120 minutes after the start of the ingestion of a test food. GI was calculated as the area under the response curve and above the baseline (International Organisation for Standardisation, 2010). The GI test was review and approved by the Human Research Ethics Committee of the University of Sydney (2017/801). Informed consent was obtained from all participants involved in the test.

4.3.10 Statistical analysis

Statistical analysis was carried out using Microsoft Excel version 2104 and R Studio version 1.1.463. Proximate composition, sugar profile, phenolic profile, antioxidant activity, amino acid, and organic acid measurements were done in triplicates from different batches of NZYC and the results were reported as mean values ± standard deviation as calculated in Excel and analysed by one-way analysis of variance (ANOVA) using R Studio. Post hoc analysis was carried out in R studio using Tukey's honestly significant difference (HSD) test with a 95% family-wise confidence interval. Mineral profile measurements were done in triplicates from a single batch of NZYC, and results were reported as mean values ± standard deviation as calculated in Excel.

4.4 Results and discussion

4.4.1 Proximate analysis

The results from the proximate analysis of NZYC are presented in **Table 4.1**. The protein content, determined by the Kjeldahl method, ranged from 4.744 ± 0.650 to 6.634 ± 0.158 g/100g. The moisture content, determined by refractive index, ranged from 9.648 ± 0.320 g/100g to 11.943 ± 0.046 g/100g. The ash content, determined by mass difference after incineration at 550°C, ranged from 2.642 ± 0.130 g/100g to 3.364 ± 0.410 g/100g. The carbohydrate content, determined by mass difference, ranged from 79.454 ± 0.387 to 80.671 ± 0.664 g/100g. The water content of NZYC is much lower than that of around 25% by weight as reported in the literature (Manrique et al., 2005b); however, this could be due to differences in processing methodology, as Manrique et al. (2005b) developed their process with the aim of achieving a final product with 73° Brix, while NZYC used in this research claims to have 80° Brix. This claim is consistent with the determined carbohydrate content of approximately 80 g/100g. Conversely, the determined protein content was two to three times higher than the literature value of 1-2% (Manrique et al., 2005b). This difference may be attributable to a number of factors, such as differences in processing methodology, variety of yacon used, harvest time, soil conditions when cultivating yacon or climatic conditions when nurturing the crop.

Table 4.1 Proximate analysis of New Zealand yacon concentrate (NZYC) (n=9).

Macronutrient	g/100g NZYC	g/100g NZYC	g/100g NZYC

	Batch 1	Batch 2	Batch 3
Carbohydrate	80.671 ± 0.664	79.454 ± 0.387	80.354 ± 0.544
Moisture***	11.943 ± 0.650[a]	11.890 ± 0.361[a]	9.648 ± 0.320[b]
Protein**	4.744 ± 0.650[b]	5.675 ± 0.070[ab]	6.634 ± 0.158[b]
Ash*	2.642 ± 0.130[b]	2.981 ± 0.122[ab]	3.364 ± 0.410[a]

* Significant differences existed between batches at the .05 probability level. Values with different superscript letters are significantly different.

** Significant differences existed between batches at the .01 probability level. Values with different superscript letters are significantly different.

*** Significant differences existed between batches at the .001 probability level. Values with different superscript letters are significantly different.

4.4.2 Mineral profile

Fifteen minerals were quantified in NZYC by MP-AES, and the results are presented in **Table 4.2**. Potassium was the most abundant mineral (658.366 ± 5.927 mg/100g), followed by phosphorus (93.121 ± 0.500), calcium (44.225 ± 4.478), and magnesium (36.628 ± 0.907). These findings are consistent with the literature for YC, in that potassium is the only major micronutrient identified by Manrique et al. (2005b), although the potassium content identified in this research is slightly lower than 1% by weight.

The mineral profile of NZYC obtained in this research is similar to the mineral profile of Brazilian yacon syrup as determined by M. F. G. Silva et al. (2018). Potassium was the major mineral (691.00 ± 33.96 mg/100g), followed by phosphorus (162.00 ± 2.65), magnesium (45.67 ± 10.12), sulfur (42.00 ± 2.65, not quantified in this research), calcium (40.67 ± 3.79) and sodium (17.00 ± 1.73) (M. F. G. Silva et al., 2018). M. F. G. Silva et al. (2018) also quantified copper, iron, zinc, and manganese at relatively low concentrations.

Table 4.2 Mineral profile of New Zealand yacon concentrate (NZYC) (n=3).

Mineral	mg/100g NZYC
Potassium	658.366 ± 5.927
Phosphorus	93.121 ± 0.500
Calcium	44.225 ± 4.478
Magnesium	36.628 ± 0.907
Aluminium	31.581 ± 0.269
Iron	23.809 ± 0.199
Sodium	9.035 ± 0.152
Nickel	0.530 ± 0.047
Zinc	0.461 ± 0.023
Copper	0.107 ± 0.006

Lead	0.050 ± 0.003
Titanium	0.001 ± 0.000
Antimony	n.d.
Boron	n.d.
Cobalt	n.d.

4.4.3 Sugar profile

The sugar profile of NZYC is presented in **Table 4.3**. Fructose was the major sugar quantified, comprising between 27.783 ± 2.830 g/100g and 36.463 ± 2.757 g/100g. In batches 1 and 3 of NZYC, the concentrations of glucose and sucrose were approximately double that of nystose; however, in batch 2, the concentration of nystose was higher than that of glucose and sucrose. This corresponds with batch 2 having significantly lower fructose concentration than batches 1 and 3. Additionally, the HPLC-ELSD profile for batch 2 contained more peaks than either batch 1 or batch 3 **(Figure 4.1)**. As fructooligosaccharides and inulins are fructans (chains of fructose joined together with a terminal glucose molecule), the observed inverse relationship between fructose and FOS/inulin concentration is not unsurprising.

The chromatographic peaks DP5-13 were assigned based on the generally accepted assumption that the retention time of structurally similar carbohydrates will increase as DP increases (Borromei, Cavazza, Merusi, & Corradini, 2009). However, it should be noted that commercial standard/reference compounds with DP higher than four were not obtained, and so quantification based on the standard curve developed for nystose (DP4) is only semi-quantitative for FOS with DP5+.

The sum of FOS/inulin in the NZYC samples was as follows: 24.225 ± 0.809 g/100g (batch 1), 52.276 ± 0.808 g/100g (batch 2), and 17.625 ± 0.325 g/100g (batch 3). According to M. F. G. Silva et al. (2018) the FOS content of yacon syrup ranges from 10.9 to 47.6% depending on a number of factors, including cultivar, physiological conditions such as plant age, harvest and post-harvest conditions, and others. The results obtained in this research fall either within this range or just above, which may be due to any of the aforementioned factors, or the semi-quantitative method used for FOS/inulin with DP5+.

Table 4.3 Sugar profile of New Zealand yacon concentrate (NZYC) (n=9).

Sugar	g/100g NZYC	g/100g NZYC	g/100g NZYC

	Batch 1	Batch 2	Batch 3
Fructose**	35.798 ± 1.871[a]	27.783 ± 2.380[b]	36.463 ± 2.757[a]
Glucose	8.582 ± 0.730	8.334 ± 0.401	8.069 ± 0.546
Sucrose*	10.327 ± 0.475[a]	9.166 ± 0.492[b]	9.016 ± 0.410[b]
1-Kestose (DP3)***	3.299 ± 0.187[b]	4.883 ± 0.077[a]	2.767 ± 0.046[c]
Nystose (DP4)***	5.397 ± 0.424[b]	9.552 ± 0.148[a]	4.699 ± 0.224[b]
DP5***	4.083 ± 0.291[b]	7.843 ± 0.107[a]	3.435 ± 0.113[c]
DP6***	3.237 ± 0.308[b]	6.636 ± 0.137[a]	2.788 ± 0.143[b]
DP7***	2.588 ± 0.250[b]	5.963 ± 0.078[a]	1.949 ± 0.105[c]
DP8***	2.179 ± 0.319[b]	5.260 ± 0.100[a]	1.987 ± 0.097[b]
DP9***	1.515 ± 0.258[b]	4.247 ± 0.258[a]	n.d.
DP10***	1.201 ± 0.058[b]	3.068 ± 0.241[a]	n.d.
DP11***	0.726 ± 0.163[b]	2.076 ± 0.358[a]	n.d.
DP12	n.d.	1.517 ± 0.420	n.d.
DP13	n.d.	1.231 ± 0.387	n.d.

* Significant differences existed between batches at the .05 probability level. Values with different superscript letters are significantly different.

** Significant differences existed between batches at the .01 probability level. Values with different superscript letters are significantly different.

*** Significant differences existed between batches at the .001 probability level. Values with different superscript letters are significantly different.

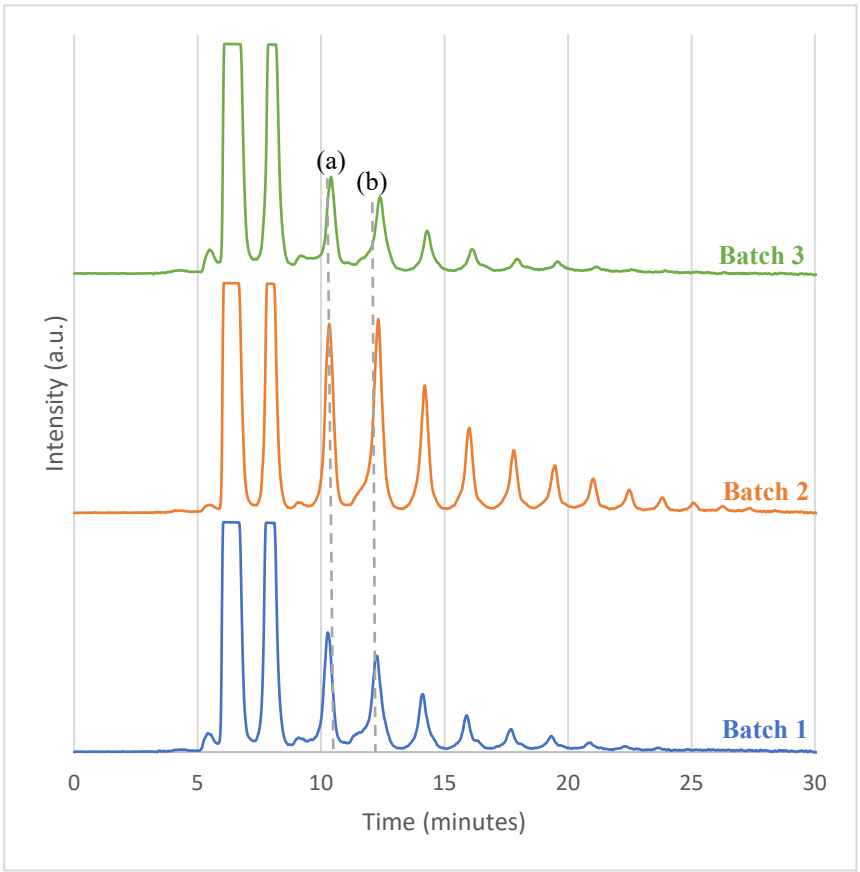

Figure 4.1 HPLC-ELSD chromatogram of time (minutes) vs intensity (arbitrary units (a.u.)) for New Zealand yacon concentrate (n=3). Peak (a) = 1-Kestose (DP3); (b) = Nystose (DP4).

4.4.4 Phenolic profile

The total phenolic content (TPC, measured in gallic acid equivalents [GAE]) and individual phenolic profile of NZYC is presented in **Table 4.4**. The total phenolic content of NZYC ranged from 564.97 ± 9.28 mg GAE/100g to 785.11 ± 43.14 mg GAE/100g. Gomes da Silva et al. (2017) found the TPC of yacon syrup obtained from a local market in Brazil to be 120.225 ± 3.005 mg GAE/100g, while Yuanita et al. (2021) produced yacon syrup from tubers obtained in Wosobo, Indonesia and found TPC to range from 90.765 to 105.056 mg GAE/100g. These results are surprising as the discussed literature indicated that yacon tubers of the 'New Zealand' variety had lower TPC than other yacon tubers, yet the NZYC analysed in this research had higher TPC than other yacon syrup samples discussed in literature. This could be attributed to differences in processing parameters – for instance, the yacon syrup produced by Gomes da Silva et al. (2017) was concentrated to 71° Brix, while the NZYC used in this research is concentrated to 80° Brix - or various other factors such as soil and climatic conditions, and harvest and post-harvest conditions.

The results for the individual phenolic compounds are in agreement with the discussed literature, in that chlorogenic acid is the major phenolic compound identified in YC, with caffeic acid and ferulic acid also being identified as present (Manrique et al., 2005b; Ojansivu et al., 2011; Takenaka et al., 2003; M. R. Yan et al., 2019). Various studies on the benefits of chlorogenic acid have suggested that chlorogenic acid could attenuate cognitive decline and reduce the risk of neurodegeneration, and has potential to protect against oxidative damage, positively affect glucose tolerance, and decrease blood pressure (Heitman & Ingram, 2017). Caffeic acid has been suggested to have anti-inflammatory, anticancer, antiviral, anti-depressive and antihyperglycemic effects (Birková, Hubková, Bolerázska, Mareková, & Čižmárová, 2020), while ferulic acid has been shown to exert antimicrobial, anti-inflammatory, antidiabetic, and anticancer effects; studies suggest it may also be used to moderate blood pressure and provide neuroprotective effects (Batista, 2014). p-Coumaric acid, kaempferol, and isorhamnetin were also identified as present in NZYC; however, kaempferol and isorhamnetin were only identified as present in very low concentrations or were not detectable. All other phenolic compounds listed in the materials and methods section were not detected in this research.

Table 4.4 Phenolic profile of New Zealand yacon concentrate (NZYC) (n=9).

Phenolic compound	mg/100g NZYC Batch 1	mg/100g NZYC Batch 2	mg/100g NZYC Batch 3
Chlorogenic acid*	9.302 ± 1.203[a]	10.361 ± 1.398[a]	6.187 ± 0.221[b]
Caffeic acid	1.479 ± 0.040	1.540 ± 0.150	1.302 ± 0.069
p-Coumaric acid*	0.141 ± 0.006[a]	0.137 ± 0.009[a]	0.106 ± 0.006[b]
Ferulic acid*	0.012 ± 0.002[b]	0.018 ± 0.002[a]	0.011 ± 0.001[b]
Kaempferol**	0.002 ± 0.000[b]	0.010 ± 0.001[a]	0.001 ± 0.000[c]
Isorhamnetin**	0.001 ± 0.000[b]	0.002 ± 0.000[a]	n.d.
FC total phenolic content**	648.95 ± 10.39[b]	564.97 ± 9.28[c]	785.11 ± 43.14[a]

* Significant differences existed between batches at the .01 probability level. Values with different superscript letters are significantly different.

** Significant differences existed between batches at the .001 probability level. Values with different superscript letters are significantly different.

4.4.5 Antioxidant activity

The antioxidant activity of NZYC was determined by three different methods; the FRAP, CUPRAC, and DPPH assays. The results are presented below in **Table 4.5**. Each assay gave different results for the antioxidant activity of NZYC. This can be attributed to the fact that each assay relies on a different chemical reaction, and as such each antioxidant has different activity for each of these reactions (Tafulo et al., 2010). Additionally, according to Tafulo et al. (2010), coexisting compounds in the samples will interfere differently across different methods.

According to the FRAP assay, the antioxidant activity of NZYC ranges from 1084.14 ± 18.63 to 1442.54 ± 259.11 mg TE/100g. New Zealand Mānuka honey, considered to be the "gold standard" amongst honeys in terms of antioxidant activity, has been found in the literature to have antioxidant activity ranging from 31.74 ± 1.86 to 519.76 ± 3.56 mg TE/100 g according to the FRAP assay (Morroni et al., 2018; Pentoś et al., 2020; Viteri et al., 2021). Goji berries, another nutraceutical food of interest, have been shown to have antioxidant activity ranging from 133.26 ± 5.28 to 486.54 ± 4.58 mg TE/100 g, according to the FRAP assay (Ilić et al., 2020). In terms of the FRAP assay, NZYC has antioxidant activity two to three times higher than other foods that are of interest due to their antioxidant activities.

According to the CUPRAC assay, the antioxidant activity of NZYC ranges from 2735.31 ± 235.47 to 3085.78 ± 637.45 mg TE/100 g. According to M. R. Yan, Permal, Quach, Chessum, and Kam (2022), the antioxidant activity of New Zealand Mānuka honey as determined by the CUPRAC assay is 52.6 mg

TE/100g, while goji berries have been shown to have antioxidant activity ranging from 154.35 ± 0.83 to 264.66 ± 1.78 mg TE/100g according to the CUPRAC assay (Ilić et al., 2020). In terms of the CUPRAC assay, NZYC has antioxidant activity at least ten times greater than other foods of interest due to their antioxidant activities.

According to the DPPH assay, the antioxidant activity of NZYC is approximately 1550 mg TE/100g. Studies on New Zealand Mānuka honey found antioxidant activity to range from 11.17 ± 0.80 to 28.41 ± 1.72 mg TE/100g according to the DPPH assay (Bundit, Anothai, Pattaramart, Roongpet, & Chuleeporn, 2016; Pentoś et al., 2020), while goji berries have been shown to have antioxidant activity ranging from 111.03 ± 1.03 to 255.92 ± 0.90 mg TE/100g according to the DPPH assay (Ilić et al., 2020). In terms of the DPPH assay, NZYC has antioxidant activity several times greater than other foods of interest due to their antioxidant activity.

Table 4.5 Antioxidant activity (Trolox equivalents, TE) of New Zealand yacon concentrate (NZYC) (n=9).

Antioxidant assay	mg TE/100g NZYC Batch 1	mg TE/100g NZYC Batch 2	mg TE/100g NZYC Batch 3
FRAP	1084.14 ± 18.63	1100.17 ± 135.66	1442.54 ± 259.11
CUPRAC	2735.31 ± 235.47	2970.97 ± 90.18	3085.78 ± 637.45
DPPH	1551.38 ± 334.70	1536.08 ± 64.34	1549.04 ± 251.17

4.4.6 Amino acid profile

Of the 34 free amino acids present in the amino acid standard A9906, 16 were quantified in NZYC. The results are presented in **Table 4.6**. The most abundant amino acids present in NZYC were L-arginine, L-glutamic acid, L-proline, L-aspartic acid, and asparagine. Significant differences existed between the three batches of NZYC for all amino acids except glycine. In each instance where significant differences existed, the concentration in batch 2 was significantly higher than the other two batches apart from L-tyrosine, where batch 2 was not significantly different to batch 3. Batch 3 only had significantly greater concentration than batch 1 for L-proline, L-tyrosine, and L-valine.

Interestingly, although L-tryptophan is identified in the literature as a major amino acid in YC, it was not detected in this research. M. F. G. Silva et al. (2018) and X. Yan et al. (1999) identified glutamine, arginine, alanine, threonine, and valine as being present in yacon and in YC; each of these amino acids were

quantified in this research. L-aspartic acid, L-glutamic acid, and L-proline were not identified in the literature but were present in relatively high concentration (>500 mg/100g in batch 2) in this research. This could be due to differences between cultivars used in the literature and NZYC.

Table 4.6 Amino acid profile of New Zealand yacon concentrate (NZYC) (n=9).

Amino acid	mg/100g NZYC Batch 1	mg/100g NZYC Batch 2	mg/100g NZYC Batch 3
L-Arginine**	468.02 ± 53.71^{b}	1369.68 ± 42.04^{a}	554.30 ± 54.09^{b}
L-Glutamic acid**	213.87 ± 7.19^{b}	586.15 ± 11.94^{a}	62.25 ± 3.48^{c}
L-Proline**	139.60 ± 5.69^{c}	539.36 ± 11.47^{a}	418.52 ± 25.80^{b}
L-Aspartic acid**	329.15 ± 21.14^{b}	525.23 ± 10.22^{a}	212.50 ± 6.22^{c}
Asparagine**	204.63 ± 12.72^{b}	503.00 ± 22.75^{a}	137.43 ± 10.66^{c}
L-Alanine**	191.23 ± 8.57^{b}	253.72 ± 17.67^{a}	94.46 ± 3.06^{c}
L-Serine**	92.12 ± 1.91^{b}	199.81 ± 9.36^{a}	37.97 ± 7.99^{c}
L-Isoleucine**	80.76 ± 1.44^{b}	123.59 ± 2.42^{a}	68.61 ± 4.19^{c}
L-Threonine**	51.43 ± 1.84^{b}	105.13 ± 4.73^{a}	19.88 ± 1.79^{c}
L-Valine**	48.87 ± 2.71^{c}	71.55 ± 0.39^{a}	60.85 ± 4.24^{b}
γ-Amino-n-butyric acid**	32.70 ± 3.79^{b}	65.00 ± 1.79^{a}	8.05 ± 0.75^{c}
Glutamine**	16.44 ± 1.57^{b}	35.24 ± 1.99^{a}	16.58 ± 0.17^{b}
Glycine	17.80 ± 1.27	30.32 ± 6.99	20.12 ± 7.23
L-Leucine*	13.55 ± 1.19^{b}	18.08 ± 1.28^{a}	12.90 ± 0.49^{b}
Ethanolamine**	4.33 ± 0.28^{b}	16.67 ± 0.83^{a}	2.20 ± 0.32^{c}
L-Tyrosine*	10.64 ± 1.05^{b}	14.88 ± 1.08^{a}	18.21 ± 2.20^{a}
β-Alanine*	9.30 ± 1.34^{b}	10.92 ± 0.43^{a}	7.40 ± 0.13^{c}

* Significant differences existed between batches at the .01 probability level. Values with different superscript letters are significantly different.

** Significant differences existed between batches at the .001 probability level. Values with different superscript letters are significantly different.

4.4.7 Organic acid profile

The results from the quantification of organic acids in NZYC are presented in **Table 4.7**. Quinic, malic, malonic, succinic, fumaric, maleic, and citric acid were detected; tartaric acid and salicylic acid were not. Citric acid was by far the most abundant organic acid, present at more than ten times the concentration of malic acid, the next most abundant organic acid. Quinic acid and fumaric acid were present at similar concentrations to malic acid; the concentration of succinic acid was lower, while maleic acid and malonic acid were present at very low concentrations or were not detected.

In the literature, M. F. G. Silva et al. (2018) identified formic acid, fumaric acid, citric acid, and malic acid as being present in YC. Formic acid was not quantified in this research, while fumaric acid, citric acid, and malic acid were all quantified.

Table 4.7 Organic acid profile of New Zealand yacon concentrate (NZYC) (n=9).

Organic acid	mg/100g NZYC Batch 1	mg/100g NZYC Batch 2	mg/100g NZYC Batch 3
Citric acid	2547.5 ± 268.9	2347.9 ± 75.5	2700.6 ± 84.6
Malic acid*	178.39 ± 20.14^{b}	197.38 ± 6.94^{b}	235.33 ± 6.88^{a}
Quinic acid*	113.52 ± 14.39^{b}	89.11 ± 1.10^{c}	139.75 ± 4.65^{a}
Fumaric acid**	77.96 ± 13.23^{b}	145.43 ± 9.37^{a}	57.64 ± 9.33^{b}
Succinic acid*	15.27 ± 2.91^{b}	22.46 ± 1.05^{a}	17.85 ± 0.77^{b}
Maleic acid	1.824 ± 0.521	1.588 ± 0.199	1.981 ± 0.369
Malonic acid	n.d.	6.799 ± 0.604	n.d.

* Significant differences existed between batches at the .01 probability level. Values with different superscript letters are significantly different.

** Significant differences existed between batches at the .001 probability level. Values with different superscript letters are significantly different.

4.4.8 Glycaemic index (GI), in vivo test

Glycaemic index is a measure of the effect of the consumption of carbohydrate foods on blood glucose levels. The results from the Sydney University's Glycaemic Index Research service revealed that NZYC has a low glycaemic index (GI = 40 ± 0.22). Although other natural sweeteners such as maple syrup, coconut sugar and molasses can be classified as low-GI foods, they have higher GI values, typically around 54-55; honey is classified as a medium-GI food (GI between 56-69). This supports the classification of NZYC as a nutraceutical food and its potential for future diet therapy applications.

4.5 Conclusion

This research has provided a comprehensive chemical profile and glycaemic indexing of NZYC. In terms of proximate analysis, sugar was the major component with content consistent to the product claim of 80° Brix. Ash content was consistent with the literature, while moisture and protein content were lower and higher than literature values respectively. Potassium was the most abundant mineral, which is consistent with the literature, with significant amounts of phosphorus, calcium, and magnesium also identified. Fructose was the major sugar present in NZYC; in batch 2, where fructose concentration was significantly lower than the other two batches, the concentrations of the FOS 1-kestose and nystose were significantly higher. FOS and

inulins with higher DP (DP5-13) were also identified as present according to the HPLC spectra, following the assumption that retention time of structurally similar carbohydrates will increase as DP increases. Chlorogenic acid and caffeic acid were the two most abundant phenolic compounds identified in the present study, in agreement with the discussed literature. Ferulic acid, also identified in the literature, was quantified in the present study, along with p-coumaric acid, kaempferol, and isorhamnetin. Three different methods were used to determine the antioxidant activity of NZYC; the CUPRAC assay, the FRAP assay, and the DPPH assay. Each assay showed NZYC to have antioxidant activity at least two to ten times greater than literature values for other foods of interest due to their antioxidant activity; namely, Mānuka honey and goji berries. The most abundant amino acids present in NZYC were L-arginine, L-glutamic acid, L-proline, L-aspartic acid, and asparagine. Citric acid was the major organic acid identified in NZYC, while quinic acid, malic acid, and fumaric acid were also present in relatively high concentrations.

Although yacon has been cultivated for centuries in South America, it has only been introduced relatively recently to the Western world and gained interest as a potential functional food. YC is one novel product that has arisen as a result of growing interest in yacon; however, it has not been well-discussed in the literature. This present study has added significant knowledge to the literature in terms of the chemical composition of NZYC. The high phytochemical contents in NZYC, including phenolic and flavonoid compounds with proven antioxidant capacities, as well as the low glycaemic index value, could support its classification as a nutraceutical food product for future new diet therapy applications.

5 Developing a novel flavoured low alcohol beer using New Zealand honeydew honey and yacon concentrate

5.1 Prelude

Chapter 5 – *Developing a novel flavoured low alcohol beer using New Zealand honeydew honey and yacon concentrate* – aims to develop an innovative way to utilise NZHDH and NZYC in a functional low alcohol beverage, utilising a brewing technique readily available to homebrewers. NZHDH (**Chapter 3**) and NZYC (**Chapter 4**) were chemically profiled earlier in this thesis; the purpose of **Chapter 5** is to develop a novel food product which utilises the potential health benefits highlighted by the earlier work. This experimental chapter was conducted in two parts: (1) chemical profiling of the developed low alcohol beverages, and (2) sensory evaluation of the developed low alcohol beverages.

The results from part one showed that low alcohol beverages were successfully produced, with ethanol content ranging from 0.61 ± 0.02 % to 0.86 ± 0.03 % ABV. The presence of fructooligosaccharides was identified in NZYC-containing samples but not in other samples, supporting the incorporation of NZYC into LABs to develop a functional beverage, particularly with respect to gut health. Certain volatile compounds associated with 'floral' and 'earthy' flavours were detected at much higher levels in NZHDH than in NZYC, while certain volatile compounds associated with 'floral' flavours were detected at much higher levels in NZYC than in NZHDH.

The results from part two showed no significant differences between low alcohol beverages in terms of overall liking, or liking of appearance, aroma, mouthfeel, or flavour, and no significant interactions between low alcohol beverages and panellist age, gender, or frequency of beer consumption. However, panellists did differentiate the samples by appearance by way of Check-All-That-Apply questions. Penalty analysis revealed that overall liking of low alcohol beverages may be improved by increasing sweetness, hoppiness, and bitterness. Overall, the results from part two support reformulation to increase overall liking and to increase differentiation between samples.

5.2 Introduction

According to Food Standards Australia New Zealand (2023a), a beer may be labelled as 'low alcohol' in New Zealand if it contains no >1.15 % alcohol by volume (ABV). Demand for low alcohol beer (LAB) and non-alcoholic beer (NAB, typically defined as <0.50 % ABV in literature) is growing around the world as consumer attitudes towards alcohol consumption shift. Steady declines in adolescent alcohol use have been observed between 2002 and 2014 in most high-income European nations as well as Australia, New Zealand, Canada, Japan, and the United States (Vashishtha et al., 2020). In the European Union approximately 1.38 billion litres of NAB was produced in 2019, representing 3.8 % of beer volume and 4.1 % of beer value; although overall market share was low, the growth of NAB volume and value between 2013 and 2019 was greater than that for regular beer (Kokole, Jané Llopis, & Anderson, 2022). Possible drivers for growth in consumer interest in NAB and LAB include health consciousness, and social responsibility (for instance, sober driving) (Kokole et al., 2022).

Industrial processes for LAB and NAB production are classified as either physical (thermal treatments or membrane separation) or biological. Physical processes are carried out after full fermentation; thermal treatments use heat to completely or partially remove alcohol while membrane separation processes selectively remove alcohol across semi-permeable membranes (Salanță et al., 2020). Membrane separation processes have some advantages over thermal treatments (mild operating temperatures, low energy consumption, little or no need for enhancing agents, and reduced operating costs); however, both membrane separation and thermal treatments require specialised equipment not found in standard breweries (Salanță et al., 2020). Biological processes are carried out during fermentation, and many do not require the use of specialised equipment; however, modifications to the fermentation process can result in undesirable changes to the sensorial characteristics of the beer (Salanță et al., 2020). Using a higher quantity of non-fermentable sugar will lead to an unpleasant sweetness and wort-like flavour, arrested or limited fermentation can lead to deficiencies in aromatic compounds, and cold-contact brewing generates aldehydes which contribute to off-flavours (Salanță et al., 2020). Although industrial methods to produce LAB and NAB are well-described in the literature, research on the production of LAB or NAB in a home-brew context is non-existent. Most industrial processes are inaccessible to home-brewers due to the need for specialised equipment or inputs only available to industry (e.g. specialised yeasts). The most accessible method to home brewers – thermal

distillation – is disregarded in the literature, as the removal of alcohol under higher temperatures has been found to have significant adverse effects on beer taste (Brányik et al., 2012).

Honeydew honey (HDH) is produced from nectar exuded from insects such as aphids and scale insects; most commercial New Zealand HDH (NZHDH) is produced from nectar exuded by *U. assimile* which feed on trees in the Nelson beech forest (see **Chapter 3.2**). HDH typically has a stronger flavour and darker colour than blossom honey, and has distinct amino acid, polyphenol, and sugar profiles (Pita-Calvo & Váquez, 2017; Seraglio et al., 2019; Vasić et al., 2019). Despite its high antioxidant activity and comparable chemical profile to other varieties of HDH (see **Chapter 3.5**), NZHDH remains an underutilised resource due to the dominance of Mānuka honey.

Yacon concentrate (YC) is a sweet syrup derived from juice extracted from the roots of the yacon plant (*Smallanthus sonchifolius*) and has physical and sensorial characteristics similar to honey or sugar cane syrup. However, unlike other syrups, YC is hypocaloric owing to its high concentration of fructooligosaccharides (FOS) and inulin, which are non-digestible oligosaccharide fructans that cannot be utilised by *Saccharomyces cerevisiae* (Genta et al., 2009; S. A. Wang & Li, 2013; M. R. Yan et al., 2019). The bioactive profile and total FOS content of yacon roots, and therefore YC, varies between cultivars. New Zealand YC (NZYC) is produced from the roots of yacon cultivar 'New Zealand', which was developed as recently as the 1980s (Douglas et al., 2005). The FOS content of NZYC varies widely, ranging from 17.625 ± 0.325 to 52.276 ± 0.808 g/100 g (see **Chapter 4.4.3**).

Functional foods are commonly defined as those which contain, in addition to nutrients, other components that may be beneficial to health (C. G. Kumar, Sripada, & Poornachandra, 2018; Temple, 2022). Yacon has previously been described in the literature as a functional food due to its biologically active components, particularly FOS, inulin, and phenolic compounds (M. R. Yan et al., 2022). NZYC has recently been incorporated into functional beverages containing collagen, blackcurrant, or vitamin C (M. Yan, Chessum, Nand, Terzaghi, & Kam, 2023).

This work aims to fill a gap in the literature by developing an innovative way to utilise NZHDH (an underutilised resource) and NZYC (a recent introduction to the market) in a functional LAB beverage, utilising a brewing technique (thermal distillation) readily available to homebrewers. Their high

concentrations of fermentable sugars make NZHDH and NZYC excellent alternatives to priming sugars during secondary fermentation. However, as stated, thermal distillation is typically disregarded in the literature due to its significant adverse effects on beer taste. It is hypothesised that utilising NZHDH and/or NZYC during secondary fermentation will introduce acceptable flavours which mask the adverse effects of thermal distillation, either through their pre-existing volatile profiles or by the generation of new volatiles via yeast metabolism, while keeping alcohol content below 1.15 % ABV.

5.3 Materials and methods

5.3.1 Materials

All reagents used in this study are ≥99 % in purity unless otherwise stated. NZHDH (three different production batches over the course of one year) was sourced from Streamland Honey Group Ltd, Rotorua, New Zealand. Three different production batches of NZYC were sourced from Yacon New Zealand Ltd., Auckland, New Zealand. Ultrapure water (UPW) was produced using a Purite Select Fusion water deionisation unit (L300760, Purite, Oxford, England).

Butan-1-ol (>95 %) (AJA107–2.5GL) and hydrochloric acid (36 %) (AJA1367–2.5 L) were sourced from AJAX FineChem, New South Wales, Australia. Methyl-2-methyl butyrate (8219AL) was sourced from AK Scientific, San Francisco, USA. 6-aminoquimolyl-N-hydroxysuccinimidyl carbamate (BIB6284) was sourced from Apollo Scientific, Stockport, England. Sodium chloride (BDH9286–500 G) was sourced from BDH Chemicals, Poole, England. Disodium phosphate (47,201) was sourced from ECP Ltd, Auckland, New Zealand. Acetonitrile (A998–212), chloroform (C607–4), ethanol (10,428,671), formic acid (AC270480010), and methanol (A452SK-4) were sourced from Fisher Scientific, Loughborough, England. Rice hulls, Gladfield wheat malt (PLU-107), Mangrove Jack's Carbonation Drops, Gladfield ale malt (PLU-100), rolled wheat flakes, Safale US-05 Dry Ale Yeast, and Whirlfloc were sourced from Brewshop, Hamilton, New Zealand. Pacific Jade hops, Mangrove Jack's amber crown top beer bottles, and Mangrove Jack's crown seals were sourced from Hauraki Home Brew, Auckland, New Zealand. Sodium citrate dihydrate (1–3646) was sourced from J.T. Baker Chemical Co., Phillipsburg, USA. Mannitol (M150/18/89) was sourced from May and Baker Ltd., Dagenham, England. Fructose (14,278) was sourced from Panreac Química SLU, Barcelona, Spain. Sodium tetraborate dihydrate (borax) (N1017515–500) was sourced from Pure Science, Wellington, New Zealand. 1-kestose (72,555–100MG), Amino acid standard (A9906), C7 – C30 Saturated Alkanes

(49,451-U), dry acetonitrile (271,004–1 L), l-alanine-2,3,3,3-d4 (485,845–1 G), and nystose (56,218–100MG) were sourced from Sigma-Aldrich, Burlington, USA.

5.3.2 Methods

5.3.2.1 Brewing of low alcohol beer

5.3.2.1.1 Brewing

The LABs were brewed according to the following procedure. 2 kg of wheat malt, 2.56 kg ale malt, 0.55 kg flaked wheat, and 0.5 kg of rice hulls were milled using a three-roller grain mill with a 1 mm gap. The milled grains were then mashed with 20 kg of water at 75 °C for 1 hour in a Grainfather mash tun model no 10,191 (Grainfather, Auckland, New Zealand); the temperature was then increased to 80 °C and held for 10 min to mash out. The spent grains were then sparged with 8 kg of water at 72 °C. The temperature of wort was raised to 100 °C and boiled for 1 hour. A Mangrove Jack's hop spider (Grainfather, Auckland, New Zealand) was placed in the mash tun and three grams of Pacific jade hops were added at the beginning of the boil; a further seven grams were added along with one Whirlfloc tablet within the last 10 min of the boil. After the boil phase was completed, the hop spider was removed. The wort was then pumped through a Grainfather counterflow chiller (Grainfather, Auckland, New Zealand) to cool to 40 °C, and was then whirlpooled to oxygenate.

5.3.2.1.2 Primary fermentation

The cooled wort was then transferred to a Grainfather conical fermenter model no 10,162 (Grainfather, Auckland, NZ) and pitched with one packet of Safale US-05 Dry Ale Yeast. The conical fermenter was then closed and sealed with an airlock. The beer was fermented at 18 °C for 7–10 days, or until bubbling was no longer observed through the airlock.

5.3.2.1.3 Distillation and secondary fermentation

Once fermentation was complete, the yeast slurry was removed from the conical fermenter and set aside in a refrigerator set to 5 °C. The beer was transferred back into the mash tun and a T500 distillation column (Still Spirits, Wellington, New Zealand) was attached (**Appendix A**). The beer was distilled at 100 °C until ethanol was no longer observed dripping from the condensing tube. 750 mL glass bottles were prepared according to **Table 5.1** and were then filled with distilled beer. The addition of NZYC, NZHDH, and/or sucrose at this stage was both for flavour and for secondary fermentation - to re-carbonate the beer while keeping alcohol content below 1.15 % ABV. Sucrose was added in the form of carbonation drops; one carbonation drop is equivalent to 3.33 g of sucrose. After beer samples were prepared, bottles were capped and conditioned in a dark cupboard at room temperature for three weeks. After conditioning, beer samples were stored in a refrigerator set to 5 °C.

Table 5.1 Formulation of low alcohol beers.

Sample	Beer volume (mL)	Yeast slurry (mL)	Sucrose (g)	NZHDH (g)	NZYC (g)
LoHDH	750	1	3.33	4.7	0
HiHDH	750	1	0	9.4	0
LoYC	750	1	3.33	0	4.7
HiYC	750	1	0	0	9.4
Control	750	1	6.66	0	0
HDHYC	750	1	0	4.7	4.7

'Lo' denotes a lower concentration of New Zealand honeydew honey (NZHDH) or New Zealand yacon concentrate (NZYC); 'Hi' denotes a higher concentration of NZHDH or NZYC. 'HDHYC' beer contains a lower concentration of both NZHDH and NZYC.

5.3.2.2 Determination of ethanol content

5.3.2.2.1 Standard preparation

A working standard of ethanol was prepared at 1 % concentration by adding 500 µL of ethanol to 25 mL of UPW in a 50 mL volumetric flask and making up to the mark with UPW. A 5 % butan-1-ol solution (internal standard) was prepared by adding 2.5 mL of butan-1-ol to 25 mL of methanol in a 50 mL volumetric flask and making up to the mark with UPW. Calibration standards of ethanol were prepared at concentrations from 1 % to 0.03125 % ABV in UPW by serial dilution.

5.3.2.2.2 Sample preparation

980 µL of standard, beer sample, or UPW (blank) were added to 1.5 mL short thread amber glass vials (P/N THC1109250, Thermo Fisher Scientific, Waltham, USA) and spiked with 20 µL of internal standard. Vials were capped with 9 mm short thread screw caps (P/N 09 15 0838, Thermo Fisher Scientific, Waltham, USA). Samples were then analysed using the following instrumentation and parameters.

5.3.2.2.3 Standard and sample analysis

Ethanol content of the LABs was determined by gas chromatography coupled with a flame ionisation detector (GC-FID). An Agilent 7890A GC-FID (Agilent, Santa Clara, USA) equipped with a J&W DB-FATWAX Ultra Inert column (30 *m* × 250 µm × 0.25 µm) (Agilent, Santa Clara, USA) was used for separation. The oven temperature program was set at 50 °C for 3 min, followed by an increase of 80 °C/min to 220 °C, then held for 6 min with a constant column flow of 1.505 mL/min of hydrogen gas. The injector temperature was set at 250 °C and the split ratio was 20:1. The detector temperature was set at 280 °C, with a hydrogen flow rate of 30 mL/min, an airflow rate of 400 mL/min, and a makeup flow rate of 3.495 mL/min. The injection volume was 0.5 µL.

5.3.2.2.4 Standard curve

A standard curve for ethanol was generated with concentration ranging from 0.03125 % to 1 % ABV. The curve equation was $Y = 1233.13064X - 16.99745$, where Y represents peak area and X represents %ABV. The R^2 value was 0.99826.

5.3.2.3 Qualitative determination of fructooligosaccharides and inulins by acid hydrolysis and determination of fructose

5.3.2.3.1 Reagent and standard preparation

A citric acid buffer was prepared by mixing 30.7 mL of 0.2 M citric acid with 19.3 mL of 0.2 M disodium phosphate and making it up to 100 mL with UPW. An internal standard of mannitol was prepared in UPW at a concentration of 50,000 ppm, and a stock solution of fructose was prepared in UPW at a concentration of 1500 ppm. Fructose standards were then prepared from the stock solution by dilution in UPW at concentrations ranging from 100 to 1500 ppm.

5.3.2.3.2 Sample preparation

LAB samples (30 mL) were transferred to 50 mL centrifuge tubes (339,652, Thermo Fisher Scientific, Waltham, USA) and degassed in an Elmasonic S 10 H ultrasonic bath (Elma, Singen, Germany) using the degas function. Degassed LAB samples were then adjusted to pH 4.0 with citric acid buffer. pH was measured using a Eutech pH 700 meter (Thermo Fisher Scientific, Waltham, USA). Eight mL of LAB samples were then transferred into 10 mL amber vials (THC18 09 1310, Thermo Fisher Scientific, Waltham, USA), capped (THC18 03 1578, Thermo Fisher Scientific, Waltham, USA), and heated in a 120 °C oven for 90 min. After 90 min, glass vials were placed on ice to stop the hydrolysis and cooled to room temperature. Three millilitres of hydrolysed LAB samples were then transferred to 15 mL falcon tubes (339,650, Thermo Fisher Scientific, Waltham, USA), washed with 3 mL of chloroform, and centrifuged at 2500 relative centrifugal force (RCF) for 5 min. The aqueous layer was used for subsequent analysis.

One mL of standard, sample, or blank (UPW) were transferred to 1.5 mL short thread amber glass vials (P/N THC1109250, Thermo Fisher Scientific, Waltham, USA) and spiked with 20 µL of mannitol internal standard. Vials were capped with 9 mm short thread screw caps (P/N 09 15 0838, Thermo Fisher Scientific, Waltham, USA). Fructose was then determined by high-performance liquid chromatography (HPLC) with an evaporating light scattering detector (ELSD) using the following instrumentation and parameters.

5.3.2.3.3 Standard and sample analysis

HPLC/ELSD analysis was conducted using a Shimadzu LC-10AT liquid chromatogram (Shimadzu, Auckland, New Zealand) coupled with an Agilent 385-ELSD (Agilent, Santa Clara, USA). Samples were injected with a Shimadzu SIL-10A auto-injector (Shimadzu, Auckland, New Zealand). The column was a Luna Omega 3 µm Sugar 100 Å 250 × 4.6 mm (P/N 00G-4775-E0, Phenomenex. Torrens, USA) with a SecurityGuard Cartridge (P/N AJ0–4495, Phenomenex, Torrens, USA).

The HPLC parameters were as follows: the mobile phase was acetonitrile:UPW (80:20, v/v), isocratic elution, pump flow rate of 0.5 mL/minute, analysis time of 75 min, injection volume of 10 µL, sample loop volume of 50 µL, temperature of 25 °C. The ELSD parameters were as follows: evaporator

temperature was 80 °C, nebuliser temperature was 50 °C, and the inert gas flow rate (N_2) was 1.20 standard litres per minute (SLM).

5.3.2.3.4 Standard curve

A standard curve for fructose was generated with concentration ranging from 100 to 500 ppm. The curve equation was $Y = 0.000315996X + 64.0396$, where Y represented concentration and X represented peak area. The r^2 value was 0.9938321.

5.3.2.4 Determination of volatile compounds

Volatile compounds present in the beer samples, NZHDH, and NZYC were extracted by solid-phase microextraction (SPME), analysed by gas chromatography – mass spectrometry (GCMS), and measured in terms of internal standard response ratio (ISRR).

5.3.2.4.1 Sample preparation

To prepare the NZHDH and NZYC, 1.0 g was dissolved in 5 mL UPW; LAB samples were used undiluted. 2 mL of sample or UPW (blank) were added to a 10 mL amber vial (THC18 09 1310, Thermo Fisher Scientific, Waltham, USA) along with 0.5 g sodium chloride. Internal standard (1 ppm) was prepared by dissolving one µL of methyl-2-methyl butyrate into 100 mL of UPW. Ten µL of internal standard was added to each vial; samples were then capped (THC18 03 1578, Thermo Fisher Scientific, Waltham, USA) and vortexed for 30 s using a Benchmark Benchmixer (BV1000, Benchmark Scientific, Sayreville, USA).

5.3.2.4.2 Sample analysis

The headspace vials were incubated at 60 °C for 15 min. During incubation, samples were agitated in cycles of 60 seconds on and 10 seconds off, with an agitator speed of 1300 rpm. The SPME fibre used for this study was Supelco 50 mm/30 µm DVB/CAR/PDMS, Stableflex, 24GA Fibre Assembly (P/N 57,329-U, Sigma-Aldrich, Burlington, USA). The extraction period was 25 min, and the desorption time was 5 min. SPME volatile analysis was performed on an Agilent 7890 GC system (Agilent, Santa Clara, USA) coupled with an Agilent 5977B MSD (Agilent, Santa Clara, USA), equipped with a Gerstel MultiPurpose Sampler (Gerstel, Linthicum, USA). The column used was a J&W DB-FATWAX Ulta Inert column (0.25 µm film thickness, 0.25 mm internal diameter, 30 m length) (Agilent, Santa Clara, USA). The carrier gas was helium

with a constant flow rate of 1.1 mL/min. The injection was splitless, with the inlet temperature for the injection port set at 250 °C. For the chromatographic conditions, the temperature was set at 40 °C, held for 2 min, increased to 240 °C at a rate of 8 °C/min, and held for 3 min. The total run time of mass spectrometry (MS) was 30 min. The MS conditions were set at a source temperature of 250 °C and a quad temperature of 150 °C, with the electron ionization energy set at 70 eV. The total MS total ion chromatogram scanned masses ranged from 29 to 450 *m/z*.

5.3.2.4.3 Compound identification

To aid in the identification of unknown compounds, C7 – C30 saturated alkane reference material was run using the same GC–MS method used for sample analysis. The retention times for alkanes from heptane to triacontane were uploaded to the MassHunter Unknowns software program (Agilent, Santa Clara, USA) to augment the NIST14 library compound identification with retention index (RI) values.

The spectra and semipolar column RI values and spectra for compounds from the existing NIST database were compared to the experimentally obtained spectra and RI values. If (a) spectra were similar and (b) RI values were within twenty points, this was identified as a positive hit. If a positive hit was not identified, then the RI values of spectrally similar compounds in the NIST database were manually compared to the experimentally obtained RI value to obtain a hit. Once a hit was obtained, the compound was added to the MassHunter library. The completed library was then run against the entire sample set to obtain relative concentrations, normalised to the response of the internal standard (methyl-2-methyl butyrate). As such the results are semi-quantitative. GCMS quantification was not performed, and absolute concentration was not obtained.

5.3.2.5 Determination of colour

5.3.2.5.1 Sample preparation

10 mL of LAB samples were placed into beakers and degassed by sonication in ice water for 10 min using an Elmasonic S 10 H ultrasonic bath (Elma, Singen, Germany). Degassed LAB samples were then transferred to 15 mL centrifuge tubes (339,650, Thermo Fisher Scientific, Waltham, USA) and centrifuged at 5000 rpm for 15 min.

5.3.2.5.2 Sample analysis

Samples were transferred to plastic cuvettes. Absorbance was measured at 430 nm in triplicates against a blank (UPW) using an Ultrospec 7000 UV–Vis spectrophotometer (11,608,853, Thermo Fisher Scientific, Waltham, USA). Colour (in EBC units) was obtained by multiplying absorbance at 430 nm by twenty-five.

5.3.2.6 Determination of amino acids

5.3.2.6.1 Reagent and standard preparation

AccQTag reagent was prepared by warming and sonicating 2.8 mg 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate in 1 mL dry acetonitrile to dissolve using a Quantrex 90H sonicator (L&R Ultrasonics, Kearny, USA). Sodium citrate buffer was prepared by dissolving 5.88 g sodium citrate dihydrate in 100 mL UPW. Borate buffer was prepared by warming and sonicating 7.63 g of sodium tetraborate decahydrate in 90 mL UPW and 10 mL acetonitrile and adjusting pH to 8.8 using 0.1 M hydrochloric acid. pH was measured using a Eutech pH 700 meter (Thermo Fisher Scientific, Waltham, USA). A neutralising solution was prepared by mixing 10 mL formic acid with 90 mL UPW. Internal standard spiked methanol (ISSM) was prepared by dissolving 1 mg d4-alamine in 100 mL methanol.

Standards were prepared ranging from 200 µM to 0.781 µM by diluting amino acid standard A9906 in UPW. 50 µL of ISSM was added to 50 µL of each standard.

5.3.2.6.2 Sample preparation

Samples were prepared by mixing 1 mL of LAB or UPW (blank) with 5 mL of 6 M HCl in a 20 mL glass vial (320018R-2375, JG Finnergan, Vineland, USA). The mixtures were purged under nitrogen gas, capped (THC18 03 1578, Thermo Fisher Scientific, USA) and digested overnight in a drying oven (MOV-112, Sanyo, Osaka, Japan) at 110 °C. The digested samples were filtered using 9.0 cm Whatman 40 filter paper (144,090, W&R Balston Ltd., Kent, England), and 100 µL of each sample or blank was transferred into a microcentrifuge tube. Hydrochloric acid was dried off at 100 °C in a drying oven (MOV-112, Sanyo, Osaka, Japan), and the dried samples and blank were resuspended in 500 µL of 200 mM sodium citrate buffer. 40 µL of resuspended sample or blank was added to 40 µL of ISSM in microcentrifuge tubes, which were then centrifuged at 4466 xG for 5 min. The supernatant was used for AccQTag derivatisation.

5.3.2.6.3 AccQTag derivatisation

10 µL of sample, standard, or blank was added to 70 µL of borate buffer in a microcentrifuge tube, followed by 10 µL of AccQTag reagent. The tubes were vortexed immediately, capped, and incubated at 55 °C in an Agilent 7890A GC-FID oven (Agilent, Santa Clara, USA) for 15 min. Neutralising solution (400 µL) was then added to each tube. Solutions were transferred to 0.7 mL short thread micro-vials (P/N 11 19 1706, Thermo Fisher Scientific, Waltham, USA) and capped with 9 mm short thread screw caps (P/N 09 15 0838, Thermo Fisher Scientific, Waltham, USA).

5.3.2.6.4 Sample and standard analysis

Liquid chromatography-mass spectrometry (LC-MS) analyses were conducted using an Agilent 1260 Infinity Quaternary LC System (Agilent, Santa Clara, USA). The LC-MS system consisted of the following components: 1260 quaternary pump (model number: G1311B), 1260 infinity ALS sampler (model number: G1329B), 1260 infinity TCC column component (model number: G1316A), 1260 infinity diode array detector (DAD) (model number: G4212B), connected to a 6420 triple quadrupole LC/MS system with electrospray ionisation (ESI) source (model number: G1948B). The column used was a Kinetex Evo C18 (2.1 × 150 mm, 1.7 µm) (Phenomenex, Torrens, USA).

The MS ionisation source conditions were as follows: capillary voltage of 4 kV, drying gas temperature of 300 °C, drying gas flow rate of 10 L/min, and nebuliser pressure of 30 psi. The positive ion mode was performed with multiple reaction monitoring (MRM) for quantitative analysis. The LC-MS conditions were as follows: Mobile phase A was 0.1 % formic acid in UPW. Mobile phase B was 0.1 % formic acid in acetonitrile. The flow rate was 0.225 mL/min, and the column temperature was 25 °C.

The gradient program was as follows: The initial percentage of mobile phase B (B%) was 5 %, and held for 3 min. B% was then raised at a rate of 1 % per minute to 10 %, and was held for 2 min. B% was then raised at a rate of 1.2 % per minute to 17 %, then raised at a rate of 42 % per minute to 80 % and held for 0.5 min. B% was then lowered at a rate of 50 % per minute to 5 % and the run was complete.

5.3.2.6.5 Standard curve

The analyte concentration ranges, curve equations, and fits are presented in **Table 5.2**.

Table 5.2 Standard curve information for amino acid analysis.

Analyte	Concentration range (μM)	Equation	r^2 value
L-Proline	0.78 - 200	$Y = 0.011366X$	0.9995
L-Glutamic acid	0.78 - 200	$Y = 0.003799X + 0.003717$	0.9994
L-Histidine	0.78 - 200	$Y = 1.4528*10^{-4}X$	0.9901
Glycine	0.78 - 100	$Y = 0.006435X + 0.004427$	0.9991
L-Aspartic acid	0.78 - 100	$Y = 0.004764X + 0.003803$	0.9981
L-Alanine	0.78 - 200	$Y = 0.003604X + 0.001898$	0.9998
L-Serine	0.78 - 100	$Y = 0.005368X$	0.9976
L-Leucine	0.78 - 100	$Y = 0.022675X + 0.003198$	0.9989
L-Valine	0.78 - 50	$Y = 0.008518X$	0.9993
L-Arginine	0.78 - 100	$Y = 1.3617*10^{-4}X$	0.9957
L-Threonine	0.78 - 50	$Y = 0.006420X$	0.9983
L-Phenylalanine	0.78 - 50	$Y = 0.013880X – 0.005159$	0.9987
L-Lysine	0.78 - 50	$Y = 0.005488X$	0.9989
L-Tyrosine	0.78 - 50	$Y = 0.010623X – 0.003470$	0.9989
L-Isoleucine	0.78 - 50	$Y = 0.018771X – 0.004326$	0.9994
Glutamine	0.78 - 50	$Y = 8.2846*10^{-4}X$	0.9928
γ-Amino-n-butyric acid	0.78 - 100	$Y = 0.008709X$	0.9997
L-Cystine	0.78 - 50	$Y = 0.005466X$	0.9949
L-Methionine	0.78 - 50	$Y = 0.009534X$	0.9986
Asparagine	0.78 - 100	$Y = 2.800*10^{-4}X – 3.9303*10^{-4}$	0.9964
Ethanolamine	0.78 - 200	$Y = 0.006561X$	0.9992
Hydroxy-L-proline	0.78 - 100	$Y = 0.06742X$	0.9991
L-Ornithine	0.78 - 50	$Y = 0.005230X$	0.9997
β-Alanine	0.78 - 100	$Y = 0.005159X$	0.9997

Y = Relative response, X = Concentration (μM)

5.3.2.7 Sensory evaluation

5.3.2.7.1 Study protocol

The sensory evaluation was presented in a questionnaire format including unstructured line scales, just-about-right (JAR) scales, check-all-that-apply (CATA) questions, and sociodemographic questions. An information sheet about the experiment, a consent form, and a quick-response (QR) code linking to the questionnaire (on Qualtrics) were presented to the subject upon arrival at the Food Science Laboratory (Auckland University of Technology). Subjects were instructed to answer the questions according to the order presented on the questionnaire. For each LAB, a randomised three-digit code was presented at the top of each section of the questionnaire, and subjects were required to answer the questions specifically to the LAB with the corresponding randomised three-digit code. The subjects first evaluated acceptance using unstructured line scales, then the appropriateness of the level of a specific attribute using JAR scales, and

finally CATA questions to understand consumer perception of the LABs. In between LABs, subjects were instructed to eat some unsalted crackers and drink some water to cleanse the palate. After all samples were tasted, subjects answered the sociodemographic questions.

5.3.2.7.2 Subjects

Subjects were recruited from the Auckland area through posters and word-of-mouth. Subjects were screened for the following criteria: (1) the subject was at least 20 years of age (due to conditions of ethics approval), (2) the subject had no food allergies (such as wheat, yacon, honey, barley, hops, and alcohol), (3) the subject was in good health and not pregnant or trying to conceive a child, (4) the subject was not operating heavy machinery or driving within two hours of completing the sensory test, and (5) the subject was a regular (at least once a month) consumer of beer. Fifty-four subjects attended the sensory evaluation; however, one was removed due to the subject withdrawing from the sensory evaluation. 58.5 % of subjects were male, the largest age group was 20–29 (41.5 %), and most subjects drank beer on a fortnightly-to-monthly basis (71.7 %). A summary of the subject's sociodemographic details is presented in **Table 5.3**. This sensory evaluation was reviewed and approved by the AUT Ethics Committee with an approval number of 23/265.

Table 5.3 Sociodemographic information of subjects with percentages including age, gender, and frequency of beer consumption.

Characteristic		Sample *n* (%)
Age	20-29	22 (41.5%)
	30-39	16 (30.2%)
	40+	15 (28.3%)
Gender	Male	31 (58.5%)
	Female	22 (41.5%)
Beer consumption	At least once a week	15 (28.3%)
	Fortnightly-to-monthly	38 (71.7%)

5.3.2.7.3 Sample presentation and sensory evaluation procedure

The sensory evaluation took place in the Food Laboratory at the AUT city campus with the temperature set at 22 °C. White fluorescent light was used in the Food Laboratory. A researcher was available to explain the experiment and how to answer the questionnaire to the subjects. Subjects were instructed to consume the LABs one at a time following the order of the randomised three-digit codes

provided on the questionnaire and answered the survey questions accordingly. Samples were poured into a 30-mL transparent portion cup on a subject-by-subject basis to prevent loss of carbonation. LABs were stored in a refrigerator set at 5 °C before the sensory evaluation.

5.3.2.7.4 Scales and questionnaire

The sensory evaluation consisted of (1) unstructured line scales labelled from “dislike extremely” to “like extremely” for determining the overall liking, as well as liking of appearance, aroma, flavour, and mouthfeel, of the LABs, (2) JAR scales labelled from “Much too little” to “Much too much” for determining whether hoppiness, bitterness, or sweetness could be reformulated or adjusted to increase product overall liking, and (3) CATA questions to determine which sensory terms were relevant or perceived by the subjects.

Terms used in the CATA questionnaire were formulated by a semi structured focus group. Ten subjects were recruited from Auckland University of Technology; all were regular beer drinkers, and eight had previous experience with sensory evaluation. Based on the results from the focus group, eight appearance terms (hazy, clear, carbonated, foamy, golden, amber, yellow, brown), four taste terms (bitter, sour, sweet, umami), eight flavour terms (earthy, floral, grassy, crisp, hoppy, metallic, wooden, citrus), and six mouthfeel terms (light, medium, persistent, carbonated, astringent, tongue-coating) that best described the LABs were selected. A list of terms and their corresponding definition were provided to subjects when evaluating samples in terms of the CATA sensory attributes. Subjects were asked to select all the terms that they considered best to describe each LAB evaluated.

5.3.2.8 Statistical analysis

Statistical analysis was conducted using R Studio version 2023.09.0. R packages used in this paper include ‘agricolae’ for Tukey's honestly significant difference (HSD) test; ‘factoextra’, ‘FactoMineR’, and ‘gplots’ for correspondence analysis; ‘rcompanion’, ‘DecTools’, and ‘coin’ for Cochran's *Q* test; and ‘FactoMineR’ and ‘SensoMineR’ for penalty analysis.

For sensory evaluation, results from unstructured line scales were analysed by one-way and two-way analysis of variance (ANOVA) using R studio. Statistical significance was defined to exist at $p < 0.05$ for both one-way and two-way ANOVA. Post hoc analysis was conducted using Tukey's HSD test with a 95 % family-wise confidence interval. Results from CATA questions were analysed using Cochran's *Q* test, with

statistical significance set at $p<0.05$. When statistical significance was reached for CATA terms, McNemar's test was used for post hoc testing. In addition, correspondence analysis was conducted to visualise the CATA terms. For JAR analysis, the mean drop in overall liking was calculated from unstructured line scales (0 to 100), JAR results were further processed by categorising attributes into "not enough" (e.g., 1 and 2), "just-about-right" (e.g., 3), and "too much" (e.g., 4 and 5) categories from the 5-point scale used in the sensory survey. JAR questions were analysed using SensoMineR, which produced the penalty score and degree of significance.

Ethanol, fructose, colour, and amino acid measurements were done in triplicates from three separate production batches; results were reported as mean values ± standard deviation as calculated in Excel and analysed by the Kruskal-Wallis test using R Studio. Post hoc analysis was conducted in R studio using Dunn's test of multiple comparisons with the Holm method. Volatile compound measurements were done in triplicates; results were reported as mean values ± standard deviation as calculated in Excel and analysed by the Kruskal-Wallis test using R Studio.

5.4 Results and discussion

5.4.1 Ethanol content in beer samples

The ethanol contents of the LABs, as determined by GC-FID, are presented in **Table 5.4**. All results are lower than the 1.15 % ABV threshold for a beer to be labelled as 'low alcohol' in New Zealand. The amount of NZYC and NZHDH to be added to the LABs was determined by the amount of fermentable sugars present in these samples (see **Chapters 3.4.3** and **4.4.3**).

Control 1 had significantly lower ethanol content than HiHDH 1, HiHDH 3, and LoHDH 3. No other significant differences were identified between the LAB samples when correcting for multiple comparisons. This suggests that the levels of fermentable sugars in the NZHDH used for this work may be higher than that identified in the literature. These results also demonstrate that it may be possible to add higher levels of NZHDH and/or NZYC to LABs without exceeding the 1.15 % ABV threshold; however, this could lead to over-carbonation of the beer.

Table 5.4 Ethanol content (ABV%) of low alcohol beers (n=3)

Sample	ABV% Batch 1	ABV% Batch 2	ABV% Batch 3
Control	0.61 ± 0.02^{a}	0.65 ± 0.01^{ab}	0.65 ± 0.04^{ab}
HDHYC	0.67 ± 0.01^{ab}	0.69 ± 0.03^{ab}	0.71 ± 0.07^{ab}
LoHDH	0.72 ± 0.05^{ab}	0.77 ± 0.02^{ab}	0.84 ± 0.05^{b}
HiHDH	0.85 ± 0.02^{b}	0.84 ± 0.02^{ab}	0.86 ± 0.03^{b}
LoYC	0.66 ± 0.02^{ab}	0.72 ± 0.00^{ab}	0.70 ± 0.00^{ab}
HiYC	0.68 ± 0.04^{ab}	0.69 ± 0.02^{ab}	0.69 ± 0.02^{ab}

Significant differences existed between samples at the .05 probability level. Values with different superscript letters are significantly different.

'Lo' denotes a lower concentration of New Zealand honeydew honey (NZHDH) or New Zealand yacon concentrate (NZYC); 'Hi' denotes a higher concentration of NZHDH or NZYC. 'HDHYC' beer contains a lower concentration of both NZHDH and NZYC.

5.4.2 Fructooligosaccharides and inulins in the beer samples

A preliminary trial was conducted to quantitatively determine FOS and inulins using the method from **Chapter 4.3.4.2**. However, the HPLC spectra (**Appendix B**) had a high number of peaks, and it could not be determined which peaks corresponded to FOS, inulins, and other carbohydrates. In the method laid out in **Chapter 4.3.4.2**, two FOS standards (1-kestose and nystose) are used, and subsequent peaks are assigned to higher-order FOS and inulins based on the commonly accepted assumption that the retention time of structurally similar carbohydrates increases as the degree of polymerisation increases. This method of peak designation could not be used in the present study for the aforementioned reasons.

According to Navarro, Vela, and Navarro (2012), around 90 % of the carbohydrates that are present in beer post-fermentation are dextrins, while around 10 % are polysaccharides that originate from the cell walls of the grains. It is assumed that these dextrins and other polysaccharides were also present in the LABs. To break down these dextrins and polysaccharides to their constituent sugar units to yield a cleaner HPLC chromatogram, acid hydrolysis was conducted. However, acid hydrolysis also breaks down FOS and inulins to their constituent sugar units (**Appendix C**). As dextrins and these other polysaccharides are comprised of glucose units, while FOS and inulins are comprised of fructose units with a terminal glucose unit, it was hypothesised that if FOS and/or inulins were present in the LABs, the LABs would be characterised by the presence of fructose. The results from the determination of fructose are presented in **Table 5.5**.

Table 5.5 Results from fructose analysis (n=3).

Sample	mg/L fructose (batch 1)	mg/L fructose (batch 2)	mg/L fructose (batch 3)
LoYC	193.35 ± 5.57^{ab}	195.33 ± 15.98^{ab}	196.48 ± 18.99^{ab}
HiYC	262.73 ± 23.63^{b}	243.72 ± 19.08^{b}	206.98 ± 40.54^{ab}
HDHYC	186.94 ± 20.05^{ab}	146.12 ± 30.49^{ab}	122.36 ± 7.60^{a}
LoHDH	n.d.	n.d.	n.d.
HiHDH	n.d.	n.d.	n.d.
Control	n.d.	n.d.	n.d.

Significant differences existed between samples at the .05 probability level. Values with different superscript letters are significantly different.

'Lo' denotes a lower concentration of New Zealand honeydew honey (NZHDH) or New Zealand yacon concentrate (NZYC); 'Hi' denotes a higher concentration of NZHDH or NZYC. 'HDHYC' beer contains a lower concentration of both NZHDH and NZYC.

These results support the hypothesis that FOS and/or inulin were present in the LABs, as those samples that contained NZYC did have fructose present following acid hydrolysis, while those samples that did not contain NZYC contained no fructose. Samples HiYC 1 and HiYC2 contained significantly more fructose than sample HDHYC 3 after hydrolysis, which suggests that these samples may also have contained significantly more FOS and/or inulin than HDHYC 3.

These results also support the claim that *Saccharomyces cerevisiae* is unable to metabolise FOS and inulin. Hence, FOS is a viable option to be incorporated into beer as a hypocaloric sweetener and thereby yield a functional beverage. Studies show that daily FOS intake between 4 and 15 g is sufficient to reduce constipation (Sabater-Molina, Larqué, Torrella, & Zamora, 2009), while various studies on the effect of daily FOS intake on satiety and appetite reduction were inconclusive using doses from 8 to 21 g (Hess, Birkett, Thomas, & Slavin, 2011). In the study carried out by Hess et al. (2011), daily doses of 10 or 16 g of FOS resulted in dose-dependent increases in breath hydrogen response, indicating fermentation of FOS in the gut which has been associated with digestive benefits and improved gut health.

5.4.3 Volatile compounds

With respect to volatiles, the most significant compounds in beer fall into the following categories: aldehydes, higher alcohols, esters, vicinal diketones, sulfur compounds, and hop-derived compounds (Blanco et al., 2016; Piornos et al., 2023; Riu-Aumatell et al., 2014). Aldehydes are formed mainly from sugars and amino acids via the Maillard reaction and Strecker degradation during mashing and boiling stages and are

additionally formed by yeast metabolism during fermentation (Piornos et al., 2023; Riu-Aumatell et al., 2014). During normal fermentation, aldehydes are subsequently converted to higher alcohols via the anabolic pathway of yeast metabolism, and to esters by an enzyme-catalysed reaction between acetyl-CoA and higher alcohols (Blanco et al., 2016; Piornos et al., 2023). There is a relationship between the concentrations of higher alcohols and esters; in lagers, the ideal ratio of higher alcohols to esters falls between 4:1 and 4.7:1 (Blanco et al., 2016). Vicinal diketones are a byproduct of the synthesis of some amino acids, sulfur compounds form as a result of a variety of reactions including the light-induced degradation of isoα-acids from hops, while the essential oil of hops may contain over 1000 compounds which can also be converted to other volatiles by yeast metabolism (Blanco et al., 2016; Piornos et al., 2023; Riu-Aumatell et al., 2014).

As thermal distillation was conducted in this work to produce LAB samples, a significant proportion of volatile compounds present after primary fermentation would have been lost. For many LABs and NABs, the final volatile profile is only determined when flavours are added at the end of production (Piornos et al., 2023), so it was theorised that the addition of NZHDH and/or NZYC would play a significant role in the aroma and flavour of the final beers. For instance, yeast metabolism of NZHDH and NZYC during secondary fermentation could lead to the formation of aldehydes, and subsequently higher alcohols and/or esters. Additionally, volatile compounds present in NZHDH and NZYC may have contributed to the final aroma and flavour.

SPME analysis was conducted for the beer samples, as well as for neat NZHDH and NZYC. For the beer samples, principal component analysis was conducted, along with hierarchical cluster analysis and k-means clustering (**Appendix D**). However, there was no clear separation of beer samples along the principal components, nor did any clear trends emerge by either method of cluster analysis. This may be due to the low concentration of NZHDH and NZYC in the beers; the volatile compounds common to beer would be binding competitively with the NZHDH and NZYC volatiles to the SPME fibre, thus making it harder to distinguish NZHDH beers from NZYC beers.

A total of forty-nine volatile compounds were detected in the neat NZHDH and/or NZYC samples by SPME-GC–MS, including nine alcohols, six aldehydes, two carboxylic acids, eight esters, eleven ketones, five terpenoids, and eight others. These compounds are detailed in **Table 5.6**. Of the forty-nine identified compounds, forty-four were identified in the NZHDH samples. A previous study by Revell, Morris, and

Manley-Harris (2014) identified 23 volatile compounds in NZHDH of the southern beech (*Nothofagus* spp.) variety; 11 of these compounds were identified in the present study. These compounds include 3,7-dimethylocta-1,6-dien-3-ol (linalool), (5E)−3,7-dimethylocta-1,5,7-trien-3-ol (hotrienol), phenylmethanol (benzyl alcohol), 2-phenylethanol, benzaldehyde, phenylacetaldehyde (benzeneacetaldehyde), 1-phenylethan-1-one (acetophenone), 1-(2-methoxyphenyl)ethenone (*o*-methoxyacetophenone), 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol (terpineol), cycloocta-1,3,5,7-tetraene, and 2-methyl-1-benzofuran. Revell et al. (2014) analysed a total of ten types of New Zealand monofloral kinds of honey and found that linalool was present in significantly greater concentration in NZHDH than all other varieties, although it was also found in some mānuka, pōhutukawa, clover, kāmahi, thyme, southern rātā, and tāwari honey samples. Therefore linalool, a floral and spicy terpene alcohol, may be a poor marker for southern beech HDH (Howe, 2020; Revell et al., 2014).

Table 5.6 Volatile profile of New Zealand honeydew honey (NZHDH) and New Zealand yacon concentrate (NZYC) (n=3).

Volatile	ITSD response ratio (NZHDH)	ITSD response ratio (NZYC)	Aroma notes
Alcohol			
Hexan-1-ol	0.01 ± 0.00	0.01 ± 0.00	Herbaceous, grassy[4]
2-Ethylhexan-1-ol*	0.08 ± 0.00	0.11 ± 0.00	Mild, oily, sweet, floral-rose, fruity[6]
3,7-Dimethylocta-1,6-dien-3-ol*	1.31 ± 0.10	0.13 ± 0.01	Sweet, tender, fresh floral[2]
(5E)-3,7-Dimethylocta-1,5,7-trien-3-ol*	0.62 ± 0.05	N.D.	Floral[5]
Decan-1-ol*	0.01 ± 0.00	0.00 ± 0.00	Fatty, waxy[7]
Undecan-1-ol*	0.05 ± 0.00	0.02 ± 0.00	-
Phenylmethanol*	0.03 ± 0.00	0.00 ± 0.00	Green, pungent[8]
2-Phenylethanol*	0.66 ± 0.16	0.29 ± 0.17	Honey, sweet, yeast, floral, spicy, herbal, rose[1]
Dodecan-1-ol*	0.24 ± 0.01	0.07 ± 0.01	Waxy, soapy[7]
Aldehyde			
Benzaldehyde*	0.36 ± 0.03	0.06 ± 0.01	Almond, cherry, stone[3]
5-Methylfuran-2-carbaldehyde*	0.05 ± 0.00	0.31 ± 0.01	Almond, caramel[9]
(2S)-2-[(2S,5S)-5-Ethenyl-5-methyloxolan-2-yl]propanal*	0.06 ± 0.01	N.D.	Floral[10]
Phenylacetaldehyde*	0.32 ± 0.02	0.08 ± 0.01	Rose, floral, peach[2]
3,4-Dimethylbenzaldehyde*	0.06 ± 0.00	0.10 ± 0.02	Almond[11]
4-Methoxybenzaldehyde*	0.37 ± 0.06	N.D.	Hawthorn, aniseed[12]
Carboxylic acid			
Acetic acid	0.02 ± 0.00	0.03 ± 0.01	Sour, vinegar, pungent[1]
3-Methylbutanoic acid*	0.36 ± 0.03	N.D.	Cheesy[7]
Ester			
Ethyl acetate*	0.05 ± 0.01	0.01 ± 0.00	Fruity, solvent-like[3]
Methyl benzoate*	0.04 ± 0.00	0.05 ± 0.01	Fruity, herbal, floral[13]
Ethyl decanoate*	0.00 ± 0.00	N.D.	Floral[14]
Methyl-2-hydroxybenzoate*	0.03 ± 0.00	N.D.	Wintergreen, mint[15]
Ethyl-2-phenylacetate*	0.13 ± 0.01	0.01 ± 0.00	Floral, honey[7]

2-Phenylethyl acetate	0.20 ± 0.09	0.10 ± 0.05	Rose, floral, fruity, sweet, honey[1]
2-Phenylethyl butanoate*	0.00 ± 0.00	0.00 ± 0.00	Floral, fruity[7]
Bis(2-methylpropyl) benzene-1,2-dicarboxylate*	0.04 ± 0.00	0.02 ± 0.01	-
Ketone			
3-Methylbutan-2-one*	0.02 ± 0.00	0.06 ± 0.00	-
4-Methylpent-3-en-2-one*	0.07 ± 0.01	N.D.	-
1-(Furan-2-yl)ethan-1-one*	0.06 ± 0.00	0.85 ± 0.03	Coffee, caramel, sweet[16]
1-(Furan-2-yl)propan-1-one*	0.02 ± 0.00	0.07 ± 0.00	Roasty, nutty[13]
3,5,5-Trimethylcyclohex-2-en-1-one*	0.06 ± 0.01	0.01 ± 0.00	Rooibos-woody[17]
1-Phenylethan-1-one*	0.04 ± 0.00	0.05 ± 0.01	Orange blossom, jasmine[18]
2,6,6-Trimethylcyclohex-2-ene-1,4-dione*	0.57 ± 0.05	N.D.	Seaweed[17]
1-(1H-pyrrol-2-yl)ethenone*	0.00 ± 0.00	0.07 ± 0.00	Nutty[17]
1-(2-Methoxyphenyl)ethanone*	2.15 ± 0.18	N.D.	-
1-(Furan-2-yl)-2-hydroxyethanone*	N.D.	0.02 ± 0.00	Sweet[19]
1-(2-Hydroxy-5-methylphenyl)ethanone*	0.01 ± 0.00	N.D.	Floral, herbaceous[20]
Terpenoid			
(1R,2R,4S)-1,3,3-Trimethyl-2-norbornanol*	N.D.	0.04 ± 0.00	Camphor[21]
4-Methyl-1-(propan-2-yl)cyclohex-3-en-1-ol*	N.D.	0.07 ± 0.00	Terpene-like, musty[21]
2-(4-Methylcyclohex-3-en-1-yl)propan-2-ol*	0.16 ± 0.01	0.52 ± 0.01	Pine, terpene, lilac, mint, floral, citrus, orange[15]
(2E)-3,7-Dimethylocta-2,6-dien-1-ol*	0.01 ± 0.00	0.02 ± 0.00	Floral, geranium[12]
(6E)-3,7,11-Trimethyldodeca-1,6,10-trien-3-ol*	N.D.	0.00 ± 0.00	Herby, woody[22]

Other			
Cycloocta-1,3,5,7-tetraene*	0.01 ± 0.00	N.D.	-
2-[(2S,5R)-5-ethenyl-5-methyloxolan-2-yl]propan-2-ol*	0.16 ± 0.02	0.01 ± 0.00	Floral, earthy[23]
1,2,3,4-tetramethylbenzene*	N.D.	0.10 ± 0.01	-
2-methyl-1-benzofuran*	0.02 ± 0.00	N.D.	Burnt phenolic[24]
(Furan-2-yl)methanol	0.05 ± 0.00	0.05 ± 0.00	Burnt sugar, fermented, creamy, caramel[1]
2-methoxyphenol*	0.01 ± 0.00	N.D.	Smokey[25]
5-pentyloxolan-2-one	0.01 ± 0.00	0.01 ± 0.00	Coconut[7]
2,4,5-trimethylphenol*	0.02 ± 0.00	N.D.	-

[1]Alves et al. (2020), [2]Ji (2021), [3]I. M. Ferreira and Guido (2018), [4]Olaniran, Hiralal, Mokoena, and Pillay (2017), [5]Pino and Fajardo (2011), [6]Api et al. (2016), [7]Herkenhoff, Brödel, and Frohme (2024), [8]Y. Zhao et al. (2014), [9]Lee, Jo, and Kim (2010), [10]Schneider, Dötterl, and Seifert (2013), [11]C. Zhao, Fan, and Xu (2021), [12]Evans et al. (1999), [13]Bettenhausen et al. (2018), [14]Bettenhausen et al. (2020), [15]Rajendran, Silcock, and Bremer (2023), [16]Dusart et al. (2022), [17]du Preez, de Beer, Moelich, Muller, and Joubert (2020), [18]Soucy (2014), [19]Kameoka (1986), [20]J. Wang et al. (2024), [21]Schieberle and Grosch (1988), [22]Satora and Pater (2023), [23]Monacci et al. (2024), [24]Food and Agricultural Organization of the United States (2024), [25]Sterckx, Missiaen, Saison, and Delvaux (2011).

*Significant differences existed between samples at the .05 probability level

Another study by Brown (2013) analysed the headspace of honeydew (nectar exuded by other insects which are collected by bees and processed into HDH) and black sooty mould found on black beech trees (*Nothofagus solandri*) in Arthur's Pass National Park on the South Island of New Zealand. Eleven compounds were identified, seven of which were identified in NZHDH in the present study. These compounds include phenylmethanol, 2-phenylethanol, benzaldehyde, phenylacetaldehyde, methyl-2-hydroxybenzoate (methyl salicylate), ethyl 2-phenylacetate, and 2-phenylethyl acetate.

Of the remaining 30 compounds identified in NZHDH in the present study, a further 16 have been identified in other varieties of HDH in the literature (Castro-Vázquez, Díaz-Maroto, & Pérez-Coello, 2006; Duru, Taş, Çayan, Küçükaydın, & Tel-Çayan, 2021; Janoskova, Vyviurska, & Špánik, 2014; Jerković & Marijanović, 2010; Karabagias, 2022; Karabagias, Badeka, Kontakos, Karabournioti, & Kontominas, 2014;

Lušić, Koprivnjak, Ćurić, Sabatini, & Conte, 2007; von Eyken Bonafonte, 2019; Yang, Battesti, Costa, Dupuy, & Paolini, 2018; Yildiz et al., 2022), while to the best of the author's knowledge 14 have not been previously identified in literature. Those compounds not previously identified include undecan-1-ol, dodecan-1-ol, 5-methylfuran-2-carbaldehyde, 3,4-dimethylbenzaldehyde, 4-methoxybenzaldehyde, methyl benzoate, 2-phenylethyl butanoate, 1-(furan-2-yl)propan-1-one, 2,6,6-trimethylcyclohex-2-ene-1,4-dione, 1-(1H-pyrrol-2-yl)ethenone, 2-[(2S,5R)−5-ethenyl-5-methyloxolan-2-yl]propan-2-ol, 2-methoxyphenol, 5-pentyloxolan-2-one, and 2,4,5-trimethylphenol. Common aroma descriptors associated with these compounds include floral (with hawthorn and rose specified), fruity, and earthy (see **Table 5.6**). Interestingly, 2,6,6-trimethylcyclohex-2-ene-1,4-dione (also known as 4-oxoisophorone) has been associated with the aroma of seaweed (du Preez et al., 2020).

Of the forty-nine volatile compounds detected, thirty-five were identified in NZYC, of which nine had previously been identified in yacon-related materials in the literature. Benzaldehyde and acetic acid have been identified in the roots of fresh Colombian yacon (Cuervo, Benitez, & Castellanos, 2018). 4-methyl-1-propan-2-ylcyclohex-3-en-1-ol, (2E)−3,7-dimethylocta-2,6-dien-1-ol, acetic acid, ethyl acetate, methyl benzoate, 2-phenylethanol, 3,7-dimethylocta-1,6-dien-3-ol, and benzaldehyde have been identified in a mixed juice containing yacon, litchi, and longan (Chen et al., 2019). Phenylmethanol has been identified in essential oil extracted from the leaves of the yacon plant (Li, Liu, Lan, Zheng, & Rong, 2009). Literature concerning the volatile compounds present in yacon or YC is limited with the remaining twenty-six compounds found in the current study not previously identified. Aroma descriptors for these compounds include sweet, floral, almond, caramel, coffee, pine, and citrus, among others (see **Table 5.6**). However, bis(2-methylpropyl) benzene-1,2-dicarboxylate, also known as diisobutyl phthalate, is a plastic-related contaminant that has previously been identified in honey and so likely originates from the packaging material rather than being botanical in nature (von Eyken Bonafonte, 2019).

It is important to note that the method used to identify volatile compounds in the present study was semi-quantitative. Results are expressed in terms of internal standard response ratio, which cannot be used to determine concentration and can only be used to compare the relative concentration of each compound between samples that have been treated equally. Therefore, no inferences can be made on whether the

concentration of these compounds is sufficient to be detected by humans. They also cannot be used to compare the concentration of different compounds within the same sample.

Compounds which have a much greater internal standard response ratio in NZHDH than in NZYC include 3,7-dimethylocta-1,6-dien-3-ol, (5E)−3,7-dimethylocta-1,5,7-trien-3-ol, 2-phenylethanol, dodecan-1-ol, benzaldehyde, phenylacetaldehyde, 4-methoxybenzaldehyde, 3-methylbutanoic acid, ethyl-2-phenylacetate, 2,6,6-Trimethylcyclohex-2-ene-1,4-dione, 1-(2-Methoxyphenyl)ethenone, and 2-[(2S,5R)−5-ethenyl-5-methyloxolan-2-yl]propan-2-ol. Aroma notes for these compounds are detailed in **Table 5.6**; common terms include sweet, floral, honey, rose, and earthy. Compounds which have a much greater internal standard response ratio in NZYC than in NZHDH include 5-methylfuran-2-carbaldehyde, 1-(furan-2-yl)ethan-1-one, 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol, and 1,2,3,4-tetramethylbenzene. Aroma notes for these compounds are detailed in **Table 5.6**; descriptors include almond, caramel, coffee, pine, and floral. Of these terms, floral and earthy were identified by the semi structured focus group. Hence, if the concentration of compounds associated with ‘floral’ and ‘earthy’ were above the detection limit in the final LABs, then differences may have been identified in sensory analysis.

5.4.4 Colour in beer samples

The results from the colour analysis are displayed in **Table 5.7**. Low EBC colour corresponds to a lighter or paler beer; high EBC colour corresponds to a darker beer. As the production method for the different LAB samples was identical up to the addition of NZHDH, NZYC, and/or carbonation drops, it was theorised that any differences in the colour of the LAB samples would be the result of these additions. The colour of honey is determined by both its botanical origin and its mineral composition; the L* (or lightness) score for honeydew honeys has been correlated to the concentration of arsenic, cadmium, iron, sulfur, lead, and calcium (Jara-Palacios et al., 2019; Pita-Calvo & Váquez, 2017). Additionally, some researchers have found correlation between honey colour and antioxidant activity (Bergamo et al., 2019), suggesting that phenolic compounds may play a role in determination of colour. It is assumed that the mineral content and phenolic profile of yacon concentrate is also determinative of colour.

Table 5.7 Results from European Brewery Convention (EBC) colour analysis (n=3).

Sample	EBC (batch 1)	EBC (batch 2)	EBC (batch 3)
Control	13.76 ± 0.03^{k}	14.10 ± 0.02^{j}	14.20 ± 0.02^{j}
HDHYC	17.81 ± 0.03^{b}	17.26 ± 0.03^{c}	16.83 ± 0.04^{e}
LoHDH	14.76 ± 0.03^{i}	15.38 ± 0.10^{g}	14.60 ± 0.18^{i}
HiHDH	15.18 ± 0.04^{h}	14.77 ± 0.01^{i}	13.55 ± 0.09^{l}
LoYC	17.34 ± 0.05^{c}	17.04 ± 0.04^{d}	16.18 ± 0.01^{f}
HiYC	20.23 ± 0.00^{a}	17.95 ± 0.02^{b}	16.70 ± 0.10^{e}

Significant differences existed between samples at the .05 probability level. Values with different superscript letters are significantly different.

'Lo' denotes a lower concentration of New Zealand honeydew honey (NZHDH) or New Zealand yacon concentrate (NZYC); 'Hi' denotes a higher concentration of NZHDH or NZYC. 'HDHYC' beer contains a lower concentration of both NZHDH and NZYC.

Although there was noticeable difference in colour of NZYC and NZHDH, with NZYC being significantly darker than NZHDH, few significant differences between LAB samples were identified. HiHDH3 had significantly lower EBC colour (i.e. was significantly lighter in colour) than HiYC1 and HiYC2, while control 1 had significantly lower EBC colour than HiYC1. Although significant differences were not observed across all LAB samples, the observed significant differences are consistent with the respective colours of NZHDH and NZYC. It is possible that, at higher concentrations of NZHDH and NZYC, more significantly different colours may be achieved between LAB samples.

5.4.5 Amino acids in beer samples

Twenty-four amino acids were quantified in the present study and are detailed in **Table 5.8**. Although significance ($p < 0.05$) was identified by the Kruskal Wallis test for many of the amino acids, post-hoc analysis using Dunn's test for multiple comparisons with the Holm method only identified one significant difference across all amino acids; LoHDH 1 had significantly lower l-arginine content (68.43 ± 2.80 mg/L) than HiYC 1 (113.09 ± 2.30 mg/L) ($p = 0.0429$).

Table 5.8 Amino acid profile of low alcohol beer (LAB) as determined by liquid chromatography – mass spectrometry (n=3).

Amino acid	mg/L LAB Control batch 1	mg/L LAB Control batch 2	mg/L LAB Control batch 3	mg/L LAB HDHYC batch 1	mg/L LAB HDHYC batch 2	mg/L LAB HDHYC batch 3	mg/L LAB LoHDH batch 1	mg/L LAB LoHDH batch 2	mg/L LAB LoHDH batch 3	mg/L LAB HiHDH batch 1	mg/L LAB HiHDH batch 2	mg/L LAB HiHDH batch 3	mg/L LAB LoYC batch 1	mg/L LAB LoYC batch 2	mg/L LAB LoYC batch 3	mg/L LAB HiYC batch 1	mg/L LAB HiYC batch 2	mg/L LAB HiYC batch 3
L-Proline*	721.84 ± 59.98	698.04 ± 25.11	668.55 ± 75.02	699.44 ± 57.42	710.93 ± 25.40	637.20 ± 16.49	673.58 ± 24.26	719.16 ± 3.85	706.45 ± 26.81	704.23 ± 54.63	765.86 ± 49.68	663.61 ± 34.13	657.27 ± 42.88	677.13 ± 38.36	634.59 ± 50.30	783.52 ± 35.39	718.06 ± 43.90	697.99 ± 24.77
L-Glutamic acid*	694.61 ± 59.81	721.38 ± 30.94	671.17 ± 75.09	708.29 ± 70.05	714.46 ± 28.54	622.80 ± 12.23	646.75 ± 13.21	733.60 ± 1.09	690.78 ± 33.82	688.93 ± 52.95	747.08 ± 47.64	633.18 ± 37.93	655.12 ± 41.88	707.36 ± 40.84	622.74 ± 65.35	783.28 ± 22.41	739.12 ± 49.56	707.61 ± 40.82
L-Histidine*	207.66 ± 17.54	196.97 ± 7.75	147.17 ± 71.17	189.60 ± 15.67	199.46 ± 14.13	173.72 ± 11.79	197.93 ± 26.51	221.67 ± 7.73	187.32 ± 21.33	187.29 ± 12.25	228.26 ± 7.90	184.18 ± 14.95	201.74 ± 18.17	178.43 ± 14.04	181.16 ± 16.38	224.61 ± 29.30	215.65 ± 27.44	169.02 ± 6.78
Glycine*	159.13 ± 13.51	165.94 ± 5.98	157.06 ± 24.82	154.17 ± 15.49	161.34 ± 2.12	139.98 ± 3.95	145.12 ± 4.00	162.85 ± 2.08	153.58 ± 4.91	150.64 ± 17.05	167.86 ± 8.71	145.27 ± 6.16	140.48 ± 9.56	150.89 ± 12.23	137.73 ± 12.53	167.15 ± 10.79	153.61 ± 8.98	147.56 ± 6.05
L-Aspartic acid	126.87 ± 10.24	131.93 ± 5.38	125.39 ± 14.73	125.08 ± 12.34	133.32 ± 5.79	117.27 ± 1.37	118.70 ± 2.10	134.15 ± 0.81	128.46 ± 4.92	124.76 ± 10.01	141.80 ± 10.39	120.20 ± 7.75	119.10 ± 8.30	126.34 ± 8.15	116.70 ± 9.73	137.12 ± 5.88	131.17 ± 8.36	125.91 ± 10.20
L-Alanine*	125.33 ± 10.60	132.68 ± 7.01	122.64 ± 13.87	118.88 ± 11.02	126.68 ± 3.65	109.95 ± 1.04	114.50 ± 2.22	134.56 ± 2.41	125.94 ± 3.74	122.73 ± 11.59	138.16 ± 8.83	115.79 ± 7.27	111.86 ± 7.35	119.92 ± 6.58	110.45 ± 10.52	131.38 ± 6.36	123.87 ± 10.40	118.92 ± 7.56
L-Serine**	122.10 ± 9.71	126.46 ± 9.33	111.52 ± 13.51	115.06 ± 9.87	116.93 ± 3.60	101.15 ± 3.64	110.19 ± 3.58	124.90 ± 1.30	116.08 ± 2.40	118.61 ± 16.04	133.76 ± 8.47	111.02 ± 6.27	104.62 ± 8.16	112.08 ± 7.29	103.75 ± 7.97	125.21 ± 5.75	109.25 ± 3.31	106.18 ± 5.70
L-Leucine**	117.83 ± 10.49	122.53 ± 2.84	112.92 ± 11.21	112.65 ± 9.79	116.84 ± 3.50	101.82 ± 3.07	111.57 ± 3.35	122.06 ± 1.77	115.70 ± 1.46	115.48 ± 8.93	134.51 ± 7.72	108.22 ± 5.34	105.40 ± 7.35	110.80 ± 6.20	101.09 ± 8.65	121.25 ± 5.61	109.66 ± 5.89	103.85 ± 3.76
L-Valine*	100.95 ± 7.75	104.74 ± 2.51	99.43 ± 11.75	95.77 ± 10.07	101.08 ± 3.98	88.33 ± 2.68	92.77 ± 3.43	103.52 ± 1.30	98.85 ± 4.30	97.41 ± 7.71	112.29 ± 7.19	92.17 ± 4.23	89.69 ± 6.33	97.26 ± 5.88	87.77 ± 7.03	106.15 ± 4.40	97.25 ± 7.28	92.39 ± 3.21
L-Arginine*	82.51 ± 6.27^{ab}	91.47 ± 8.02^{ab}	92.37 ± 9.22^{ab}	97.02 ± 10.85^{ab}	111.86 ± 5.59^{ab}	85.65 ± 0.56^{ab}	68.43 ± 2.80^{b}	96.78 ± 6.39^{ab}	82.68 ± 11.20^{ab}	82.54 ± 22.19^{ab}	107.92 ± 4.39^{ab}	83.95 ± 16.54^{ab}	89.29 ± 8.12^{ab}	97.95 ± 10.70^{ab}	77.60 ± 20.75^{ab}	113.09 ± 2.30^{a}	98.44 ± 20.02^{ab}	97.85 ± 8.14^{ab}
L-Threonine*	91.07 ± 6.93	94.99 ± 4.40	87.51 ± 9.27	87.69 ± 7.60	90.84 ± 4.29	80.23 ± 3.69	84.40 ± 2.16	94.34 ± 1.28	90.93 ± 0.97	88.24 ± 8.52	103.21 ± 5.81	85.09 ± 4.36	81.79 ± 5.26	86.06 ± 4.43	79.06 ± 8.12	97.22 ± 4.15	88.68 ± 6.02	87.33 ± 3.68
L-Phenylalanine*	87.25 ± 6.79	89.06 ± 3.67	84.64 ± 9.16	86.24 ± 6.33	87.05 ± 4.29	75.63 ± 1.68	82.30 ± 1.80	86.84 ± 2.88	82.22 ± 1.54	84.97 ± 8.40	93.26 ± 6.21	81.66 ± 4.24	79.35 ± 5.22	81.35 ± 6.25	73.60 ± 5.03	97.54 ± 7.17	84.61 ± 5.27	82.93 ± 3.28
L-Lysine*	89.14 ± 6.08	88.32 ± 5.95	80.84 ± 9.24	76.82 ± 5.82	87.08 ± 2.92	71.65 ± 2.03	81.34 ± 2.24	89.85 ± 1.18	84.23 ± 6.50	84.96 ± 5.37	92.37 ± 10.46	76.12 ± 2.65	76.41 ± 5.36	78.95 ± 5.09	72.84 ± 6.33	81.56 ± 3.98	77.78 ± 4.75	73.69 ± 7.97
L-Tyrosine*	80.91 ± 6.42	89.75 ± 5.50	81.84 ± 9.35	80.86 ± 6.19	88.62 ± 3.61	74.91 ± 1.36	76.31 ± 1.73	84.24 ± 3.93	82.52 ± 1.63	79.42 ± 7.37	93.73 ± 5.19	80.54 ± 2.35	74.65 ± 5.94	81.76 ± 6.87	75.20 ± 5.27	92.67 ± 8.27	85.64 ± 3.48	82.62 ± 5.30
L-Isoleucine**	76.35 ± 6.68	78.28 ± 4.20	72.57 ± 8.51	69.74 ± 4.98	73.37 ± 2.01	64.62 ± 0.13	68.87 ± 1.45	78.14 ± 0.84	73.32 ± 3.16	69.86 ± 6.82	83.11 ± 2.98	68.71 ± 3.47	66.74 ± 3.90	72.24 ± 5.78	64.41 ± 3.67	77.37 ± 3.25	70.26 ± 4.93	66.63 ± 2.23
Glutamine	72.16 ± 33.73	62.58 ± 6.33	64.70 ± 12.96	71.75 ± 9.02	82.69 ± 10.19	54.10 ± 6.65	53.67 ± 9.78	55.16 ± 1.95	52.33 ± 11.60	57.63 ± 27.89	60.61 ± 15.40	72.54 ± 6.36	43.82 ± 2.07	61.58 ± 9.97	48.31 ± 3.53	74.50 ± 9.66	52.90 ± 11.71	62.87 ± 7.54
γ-Amino-n-butyric acid***	19.00 ± 2.41	26.43 ± 2.38	25.43 ± 2.88	21.94 ± 1.89	26.08 ± 1.49	22.45 ± 0.80	19.34 ± 0.44	27.18 ± 0.63	26.82 ± 1.45	21.90 ± 1.65	27.49 ± 2.43	23.71 ± 1.55	20.87 ± 1.39	27.60 ± 2.78	23.25 ± 2.22	24.31 ± 1.20	28.33 ± 1.93	25.79 ± 1.70
L-Cystine*	20.69 ± 1.54	23.29 ± 7.49	18.79 ± 1.75	22.98 ± 4.45	22.57 ± 1.18	17.50 ± 1.28	23.26 ± 2.41	16.89 ± 1.38	22.65 ± 1.25	18.15 ± 1.92	20.32 ± 1.66	18.23 ± 1.74	27.41 ± 4.12	22.50 ± 1.17	20.24 ± 2.70	18.65 ± 7.10	22.72 ± 1.71	23.34 ± 1.25
L-Methionine*	18.75 ± 2.12	17.85 ± 0.58	18.49 ± 2.44	16.70 ± 1.22	17.02 ± 0.87	15.28 ± 0.87	16.65 ± 0.87	19.52 ± 0.50	19.05 ± 0.67	17.59 ± 1.35	19.67 ± 0.93	17.17 ± 1.10	16.01 ± 0.81	17.65 ± 2.42	16.58 ± 1.24	18.64 ± 0.97	16.80 ± 1.12	16.04 ± 0.57
Asparagine	13.69 ± 1.30	12.77 ± 0.38	12.74 ± 1.90	13.10 ± 2.13	10.74 ± 1.95	13.62 ± 0.90	12.99 ± 0.48	14.42 ± 1.75	11.84 ± 1.92	13.51 ± 1.58	11.83 ± 1.23	15.05 ± 3.64	12.36 ± 1.02	15.08 ± 1.53	12.38 ± 1.42	14.87 ± 2.46	13.68 ± 0.69	17.15 ± 3.73
Ethanolamine**	12.43 ± 0.97	12.33 ± 0.80	10.79 ± 0.68	11.58 ± 1.04	11.31 ± 0.47	10.13 ± 0.42	11.17 ± 0.65	12.16 ± 0.46	11.65 ± 0.61	11.99 ± 0.86	13.06 ± 1.03	10.91 ± 0.20	10.22 ± 0.57	11.62 ± 1.14	10.38 ± 0.66	12.47 ± 0.32	11.53 ± 0.93	10.95 ± 0.68
Hydroxy-L-proline*	6.82 ± 0.88	6.35 ± 0.94	6.43 ± 1.01	7.63 ± 1.09	7.59 ± 0.49	6.82 ± 0.18	7.47 ± 0.31	7.95 ± 0.37	6.77 ± 0.14	7.08 ± 1.00	8.19 ± 0.51	7.29 ± 1.10	7.47 ± 0.65	8.21 ± 0.89	7.01 ± 0.90	9.05 ± 0.83	8.58 ± 0.19	8.72 ± 0.37
L-Ornithine	6.28 ± 1.63	7.25 ± 0.79	6.63 ± 0.77	6.08 ± 0.76	6.76 ± 0.18	6.18 ± 0.21	5.48 ± 0.41	7.56 ± 0.88	6.87 ± 0.62	7.19 ± 1.27	6.23 ± 1.67	6.38 ± 0.52	5.41 ± 0.66	6.42 ± 0.89	5.90 ± 1.81	6.24 ± 0.54	6.56 ± 0.47	6.86 ± 1.24
β-Alanine	3.68 ± 0.13	3.72 ± 0.21	3.37 ± 0.52	3.92 ± 0.86	3.37 ± 0.25	3.10 ± 0.24	3.32 ± 0.13	3.42 ± 0.26	3.90 ± 0.25	3.48 ± 0.20	3.30 ± 0.08	3.36 ± 0.36	3.42 ± 0.65	3.85 ± 0.59	3.11 ± 0.15	3.61 ± 0.19	3.37 ± 0.33	3.45 ± 0.50

* Significant differences existed between batches at the .05 probability level according to the Kruskal Wallis test. Values with different superscript letters are significantly different according to Dunn's test for multiple comparisons with Holm's method.

** Significant differences existed between batches at the .01 probability level according to the Kruskal Wallis test.

*** Significant differences existed between batches at the .001 probability level according to the Kruskal Wallis test.

'Lo' denotes a lower concentration of New Zealand honeydew honey (NZHDH) or New Zealand yacon concentrate (NZYC); 'Hi' denotes a higher concentration of NZHDH or NZYC. 'HDHYC' beer contains a lower concentration of both NZHDH and NZYC.

Amino acids present in the LAB would have mainly originated from the grains used for brewing. While most of the amino acids are metabolised during fermentation, as will be later discussed, around 30 % of nitrogen compounds derived from barley are present in the final beer (Fontana & Buiatti, 2009). In the literature, amino acids such as alanine, arginine, cysteine, gamma aminobutyric acid, leucine, proline, threonine, and valine have been reported at concentrations in excess of 100 mg/L, with proline typically the most abundant (Fontana & Buiatti, 2009). In the present study, proline and L-glutamic acid were by far the most abundant amino acids at concentrations of around 700 mg/L. L-histidine, glycine, L-aspartic acid, and L-serine were all reported at concentrations in excess of 100 mg/L. While these concentrations are higher than those identified in the literature, they have all been previously identified at concentrations of 50 mg/L or higher in various beers (Fontana & Buiatti, 2009).

Essential and non-essential amino acids themselves typically have negligible taste, although L-alanine and L-tryptophan are associated with sweet and bitter taste respectively (I. M. Ferreira & Guido, 2018). However, when metabolised they significantly influence the development of the aroma and taste profile (Hazelwood, Daran, van Maris, Pronk, & Dickinson, 2008; Wong et al., 2023). Yeasts such as *Saccharomyces cerevisiae* metabolise amino acids into volatile compounds such as aldehydes, fusel alcohols, and fusel acids via the Ehrlich pathway, and these volatile compounds do have a significant impact on the flavour of LAB (Wong et al., 2023). Similarly, *Saccharomyces cerevisiae* can metabolise non-essential amino acids such as glycine, alanine, and serine to pyruvate, which is then broken down into esters via the anabolic pathway (Wong et al., 2023). Amino acids also participate in the Maillard reaction; some products of the Maillard reaction such as furfural and 5-hydroxymethylfurfural are correlated with bitter and stale flavours and aromas (I. M. Ferreira & Guido, 2018). Strecker degradation, a reaction between amino acids and α-dicarbonyls formed in the Maillard reaction, forms Strecker aldehydes, which also contribute to flavour and aroma (I. M. Ferreira & Guido, 2018).

It was hypothesised that the different amino acid profiles of NZHDH and NZYC would result in different flavour profiles in the LABs, as different volatile compounds would be formed by the metabolic action of *S. cerevisiae*, as well as the Maillard reaction and Strecker degradation. However, SPME analysis of the LABs suggested otherwise, with principal component and cluster analyses yielding no significant

results as previously discussed. This may be because the concentration of NZYC and NZHDH added to the LABs was too low to cause significant difference.

This is supported by the lack of significant difference in the amino acid profile as shown in **Table 5.8**. As stated earlier, around 30 % of nitrogen compounds remain in beer after fermentation. Assuming the composition of the remaining 30 % is representative of the initial composition, it would theoretically be possible to identify any significant differences arising from the addition of NZYC and NZHDH, if significant differences did exist.

5.4.6 Sensory evaluation

One-way ANOVA showed no significant differences ($p > 0.05$) between any of the LABs in terms of appearance, aroma, flavour, or mouthfeel. Significant differences were identified in terms of overall liking ($p = 0.0491$). However, Tukey's HSD test showed no significant differences between any of the paired means. Two-way ANOVA showed that the interactions between the LABs and panellist age, gender, or frequency of beer consumption were not significant ($p > 0.05$) for overall liking, appearance, aroma, flavour, or mouthfeel. As stated, few significant differences were identified between LAB samples in terms of colour analysis, and this was reflected in the liking of the appearance of the LABs with no significant differences identified. In terms of aroma and flavour, the lack of significant differences between the LABs is supported by the results from SPME analysis, where the volatile profiles of the different LABs were similar or identical. The mean scores for overall liking, appearance, aroma, flavour, and mouthfeel are detailed in **Table 5.9**. All scores had broad standard deviations with mean values that tended to fall between 50 and 70 on unstructured line scales, indicating that samples were not strongly liked but tended to be more liked than disliked. With respect to overall liking, the highest score was for the control (64.11 ± 18.28), while the lowest was for LoHDH (54.60 ± 26.83).

Table 5.9 Results from unstructured line scale questions (n=53).

Sample	Overall liking	Appearance	Aroma	Flavour	Mouthfeel
LoHDH	54.60 ± 26.83	62.45 ± 21.06	48.58 ± 25.20	51.83 ± 28.75	59.34 ± 23.81
HiHDH	63.87 ± 21.90	70.09 ± 21.08	60.53 ± 24.29	59.55 ± 26.49	60.57 ± 26.25
LoYC	63.23 ± 21.01	69.36 ± 20.95	57.74 ± 22.72	60.32 ± 23.58	63.51 ± 23.80
HiYC	57.77 ± 20.77	65.83 ± 21.65	53.94 ± 20.14	53.19 ± 22.16	57.02 ± 22.02
Control	64.11 ± 18.28	67.28 ± 22.36	53.45 ± 22.98	60.09 ± 22.99	60.79 ± 21.57
HDHYC	54.49 ± 24.28	64.25 ± 21.70	52.92 ± 25.35	51.38 ± 28.73	56.91 ± 25.08

'Lo' denotes a lower concentration of New Zealand honeydew honey (NZHDH) or New Zealand yacon concentrate (NZYC); 'Hi' denotes a higher concentration of NZHDH or NZYC. 'HDHYC' beer contains a lower concentration of both NZHDH and NZYC.

Seven CATA sensory attributes were statistically significant between LABs using Cochran's *Q* test. Four of these terms were appearance terms - brown, carbonated (appearance), foamy, and yellow; two were mouthfeel terms – carbonated (mouthfeel), and tongue-coating; and one was a taste term – sour.

32.1 % of panellists selected 'brown' for LoYC, which was significantly higher than for LoHDH (9.4 %), HiHDH (7.5 %), HiYC (13.2 %), and the control (3.8 %), and was not significantly different to HDHYC (18.9 %). This is somewhat in agreement with the results from colour analysis; although significant differences were not achieved across all samples, the EBU colour for LoYC samples was higher (i.e., darker) than LoHDH, HiHDH, and control samples, and was similar to HDHYC samples. However, HiYC samples tended to have similar or higher EBU colour than LoYC even though significantly more panellists selected 'brown for LoYC than HiYC. 9.4 % of panellists selected 'yellow' for HiYC, which was significantly less than LoHDH, HiHDH, and the control (all 28.3 %), while not significantly different to LoYC (20.8 %) or HDHYC (15.1 %). These results agree with the overall trend observed in colour analysis, where LoHDH, HiHDH, and control samples had lower EBU colour (i.e., were lighter) than HiYC (although significance was not determined between all samples), while LoYC and HDHYC tended to have more similar EBU colour to HiYC.

64.2 % and 50.9 % of panellists selected 'foamy' as a descriptor for the appearance of HiYC and LoYC samples respectively which was significantly higher than all other samples, the highest of which was LoHDH (18.9 %). Conversely, only 26.4 % and 30.2 % of panellists selected 'carbonated' as a descriptor for the appearance of HiYC and LoYC samples respectively, significantly lower than all other samples except LoHDH (49.1 %), which was not significantly different to LoYC. Significantly more panellists selected 'sour' as a descriptor for the taste of HDHYC (69.8 %) than HiHDH (41.5 %), with no other significant differences identified.

Although statistical significance was determined by Cochran's *Q* test for carbonated (mouthfeel) and tongue-coating, McNemar's test identified no significance between the samples. The contingency table for check-all-that-apply terms is presented in **Table 5.10**.

Table 5.10 A contingency table of the fraction of consumers (n=53) selecting the 20 terms from the Check-All-That-Apply questionnaire to describe low alcohol beer.

Attribute	Significance	LoHDH	HiHDH	LoYC	HiYC	Control	HDHYC
Appearance							
Amber	*0.887*	0.340	0.377	0.302	0.396	0.321	0.340
Brown	*<0.001****	0.094^{a}	0.075^{a}	0.321^{b}	0.132^{a}	0.038^{a}	0.189^{ab}
Carbonated	*<0.001*[1]***	0.491^{bd}	0.642^{ab}	0.302^{cd}	0.264^{c}	0.774^{a}	0.660^{ab}
Clear	*0.285*	0.283	0.226	0.321	0.321	0.358	0.208
Foamy	*<0.001*[6]***	0.189^{a}	0.057^{a}	0.509^{b}	0.642^{b}	0.057^{a}	0.094^{a}
Golden	*0.943*	0.453	0.528	0.509	0.472	0.472	0.472
Hazy	*0.697*	0.528	0.491	0.453	0.528	0.472	0.547
Yellow	*0.005***	0.283^{a}	0.283^{a}	0.208^{ab}	0.094^{b}	0.283^{a}	0.151^{ab}
Flavour							
Camphor	*0.407*	0.226	0.132	0.113	0.132	0.170	0.132
Citrus	*0.193*	0.245	0.358	0.245	0.283	0.340	0.396
Crisp	*0.584*	0.245	0.340	0.340	0.396	0.321	0.340
Earthy	*0.156*	0.358	0.170	0.283	0.226	0.283	0.245
Floral	*0.968*	0.226	0.226	0.264	0.208	0.264	0.245
Grassy	*0.969*	0.208	0.245	0.208	0.189	0.189	0.226
Hoppy	*0.078*	0.358	0.225	0.396	0.226	0.283	0.226
Metallic	*0.467*	0.151	0.113	0.358	0.208	0.113	0.208
Wooden	*0.281*	0.132	0.038	0.094	0.170	0.075	0.132
Mouthfeel							
Astringent	*0.846*	0.151	0.151	0.132	0.208	0.189	0.189
Carbonated	*0.019**	0.208^{a}	0.321^{a}	0.226^{a}	0.302^{a}	0.434^{a}	0.396^{a}
Light	*0.404*	0.358	0.528	0.434	0.415	0.491	0.472
Medium	*0.464*	0.434	0.302	0.358	0.396	0.321	0.434
Persistent	*0.582*	0.226	0.132	0.226	0.170	0.151	0.132
Tongue-coating	*0.024**	0.189^{a}	0.113^{a}	0.019^{a}	0.151^{a}	0.057^{a}	0.151^{a}
Taste							
Bitter	*0.409*	0.566	0.377	0.491	0.453	0.453	0.434
Sour	*0.006***	0.642^{ab}	0.415^{a}	0.604^{ab}	0.604^{ab}	0.491^{ab}	0.698^{b}
Sweet	*0.921*	0.208	0.283	0.245	0.283	0.245	0.245
Umami	*0.304*	0.170	0.264	0.170	0.189	0.189	0.113

Note: Letter within each row represents a statistical difference between each sample.
No asterisk represents statistical significance was not reached.
*Statistical significance was reached at $p < 0.05$
**Statistical significance was reached at $p < 0.01$
***Statistical significance was reached at $p < 0.001$

'Lo' denotes a lower concentration of New Zealand honeydew honey (NZHDH) or New Zealand yacon concentrate (NZYC); 'Hi' denotes a higher concentration of NZHDH or NZYC. 'HDHYC' beer contains a lower concentration of both NZHDH and NZYC.

Further analysis of CATA sensory attributes was carried out using correspondence analysis (CA) to visualise the association between sensory attributes and LAB, as shown in **Figure 5.1**. The CA biplot map explains 73.53 % of the total variance (59.12 % in the first dimension and 14.41 % in the second dimension). The results displayed in **Figure 5.1** show that LoYC and HiYC are closely grouped in the negative scores of the first dimension and correlated with appearance terms of foamy and brown. According to Blasco, Viñas, and Villa (2011), foaming in beer occurs mainly as a result of the interactions between proteins and hop acids. Although barley proteins are the major contributors to beer foaming, with yeast proteins playing a secondary role (Blasco et al., 2011), it may be that the proteins present in NZYC contributed to the strong correlation of the 'foaming' variable with the LoYC and HiYC samples. The protein content of NZYC ranges from 4.744 ± 0.650 to 6.634 ± 0.158 g/100 g (see **Chapter 4.4.1**), while the protein content of NZHDH – 0.47 ± 0.04 g/100 g - is approximately ten times lower (see **Chapter 3.4.1**). NZYC is also darker in colour than NZHDH, which may explain why the 'brown' variable is strongly correlated with the LoYC and HiYC samples, while the 'yellow' variable is strongly correlated with the other samples. Control and HiHDH samples had high positive scores along dimension one and were correlated with attributes of yellow and carbonated. These results are somewhat supported by the results from colour analysis as previously discussed; furthermore, the results from the determination of ethanol content weakly support the correlation of HiHDH with the carbonated attribute. Although Dunn's test of multiple comparisons using Holm's method identified few significant differences between samples, HiHDH LAB samples tended to have higher ethanol content and would therefore have the highest content of carbon dioxide.

The results from the second dimension are less significant, as they only explain 14.41 % of the total variance, and most of the descriptor terms lie close to the origin. However, HiYC and HDHYC samples had relatively high negative scores and were correlated with flavour terms of wooden, metallic, and citrus, and mouthfeel terms astringent, carbonated, and tongue-coating. Of these flavour terms, citrus is associated with 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol, a compound with a higher internal standard response ratio in NZYC than in NZHDH.

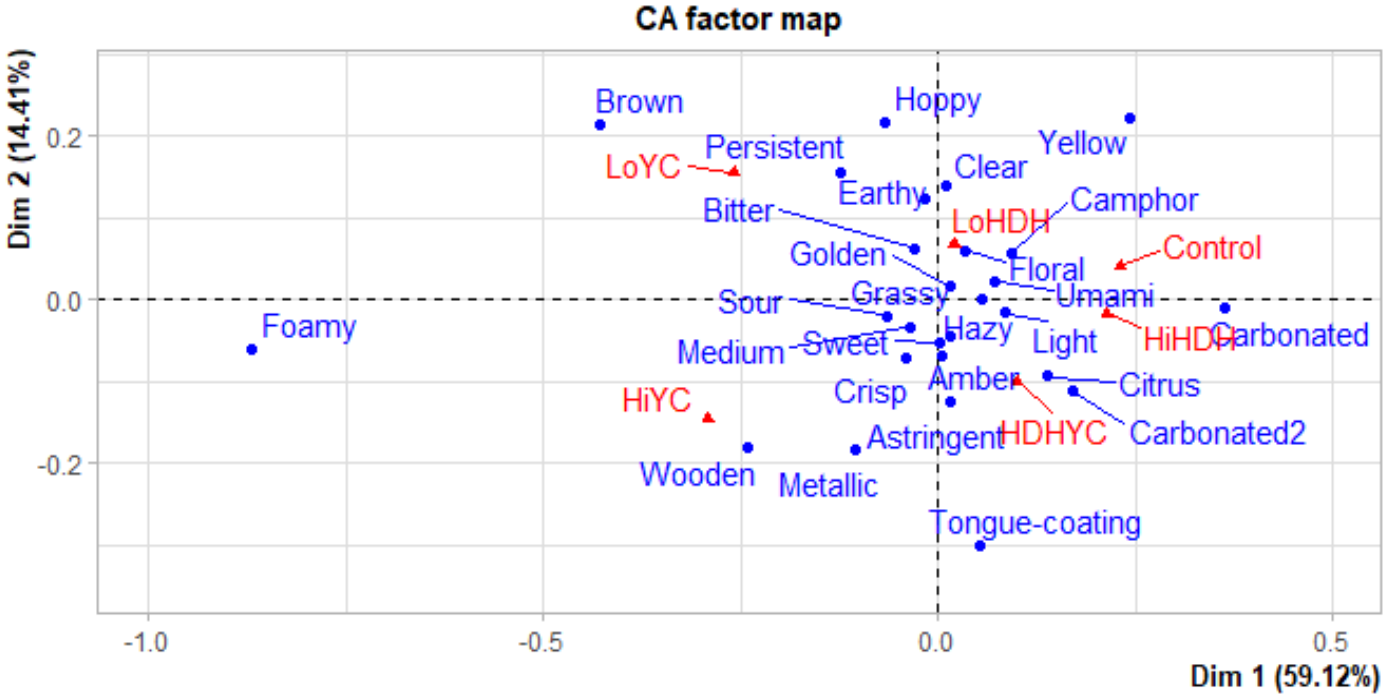

Figure 5.1 Correspondence analysis of the Check-All-That-Apply terms and the cluster distribution of low-alcohol beer samples.

Note: 'Carbonated' = appearance term; 'Carbonated2' = mouthfeel term. 'Lo' denotes a lower concentration of New Zealand honeydew honey (NZHDH) or New Zealand yacon concentrate (NZYC); 'Hi' denotes a higher concentration of NZHDH or NZYC. 'HDHYC' beer contains a lower concentration of both NZHDH and NZYC.

Figure 5.2 summarises the penalty analysis results for the LABs. Frequency, plotted on the x-axis, refers to the percentage of panellists who indicated either too much or too little of each attribute. Penalty, plotted on the y-axis, refers to the mean drop in overall liking as a result of that attribute having too little or too much intensity. Two attributes – not enough sweetness and not enough hoppiness – contributed significantly to the overall liking score for all six samples. Not enough sweetness had frequency ranging from 41.5 % in the control (**Figure 5.2f**) to 54.7 % in both the HDHYC (**Figure 5.2e**) and LoHDH (**Figure 5.2a**) samples, with a penalty of 15.3 ± 2.4. Not enough hoppiness had frequency ranging from 34.0 % in HiHDH (**Figure 5.2b**) to 52.8 % in HiYC (**Figure 5.2d**), with a penalty of 12.6 ± 2.6. Not enough bitterness also contributed significantly to almost all samples except LoHDH (**Figure 5.2a**), where too much bitterness had a higher frequency (24.5 % compared to 17.0 %). Excluding LoHDH, the frequency for not enough bitterness ranged from 26.4 % in HiHDH (**Figure 5.2b**) to 43.4 % in the control (**Figure 5.2f**), with a penalty

of 10.6 ± 2.7. Too much sweetness, too much hoppiness, and too much bitterness (except for LoHDH) had low frequency and thus did not contribute significantly to the overall liking of the LABs.

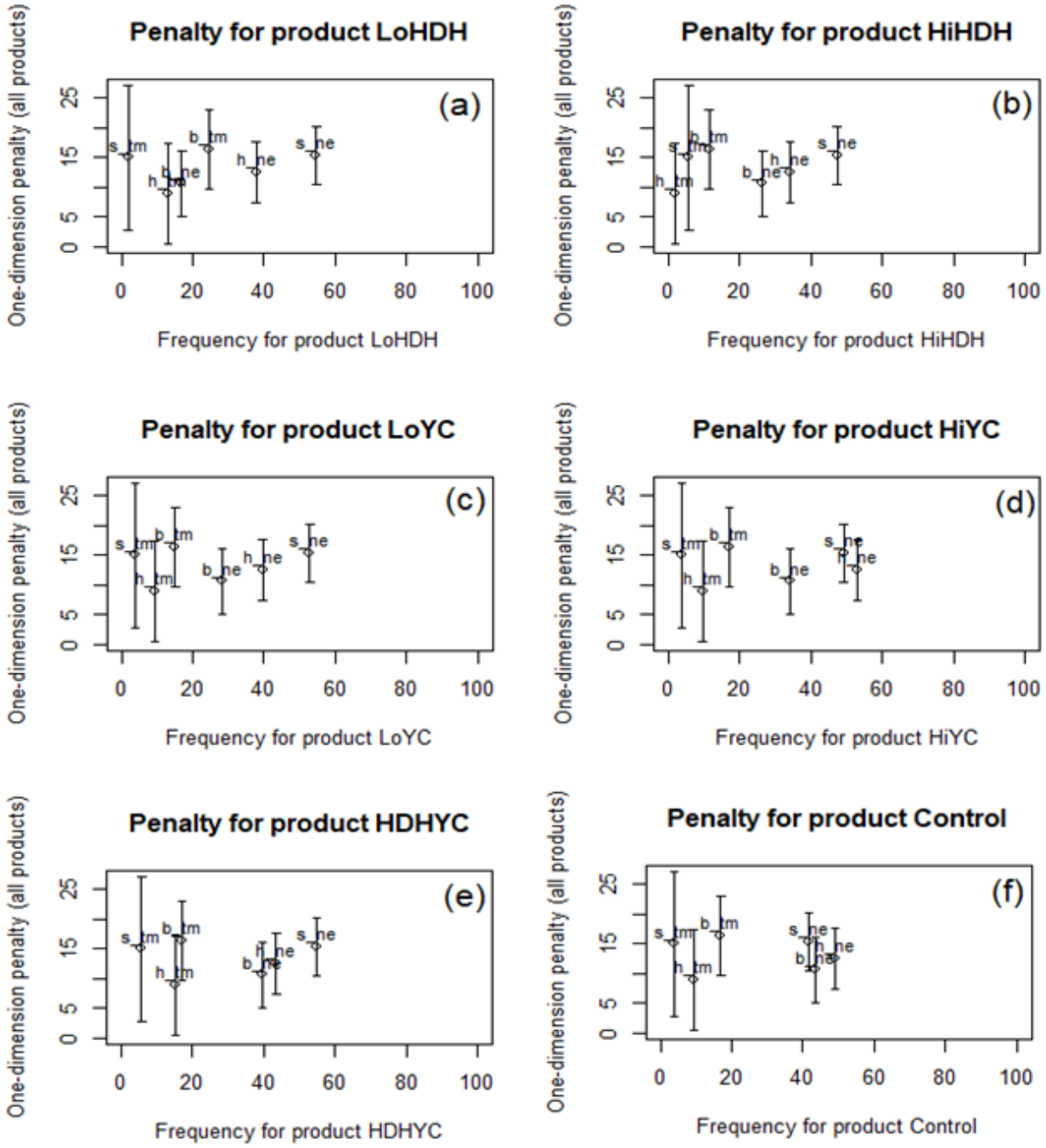

Figure 5.2 Penalty analyses of low-alcohol beer samples. (a) LoHDH, (b) HiHDH, (c) LoYC, (d) HiYC, (e) = HDHYC, (f) = Control. 'Lo' denotes a lower concentration of New Zealand honeydew honey (NZHDH) or New Zealand yacon concentrate (NZYC); 'Hi' denotes a higher concentration of NZHDH or NZYC. 'HDHYC' beer contains a lower concentration of both NZHDH and NZYC.

Sensory attribute is denoted to the left of the underscore: b = bitterness, h = hoppiness, s = sweetness. Intensity of the attribute is denoted to the right of the underscore: ne = not enough, tm = too much.

Reformulation could be considered to improve the overall liking scores for the LABs. Not enough bitterness had highest frequency for HiYC, HDHYC, and Control LABs, so it would be of most interest to increase the bitterness of these LABs. To increase bitterness, more hops could be added at the start of the boil phase to increase the concentration of iso-alpha acids, while more hops could also be added towards the end of the boil phase to increase the concentration of volatile hop compounds and so increase hoppiness. Not enough hoppiness had frequency of 34.0 % or greater for all LABs, so it would be of interest to increase the hoppiness of all LABs. Not enough sweetness had frequency of 41.5 % or greater for all LABs, so it would be of interest to increase the sweetness of all LABs. To increase the sweetness of the beer, non-fermentable sugars such as lactose or artificial sweeteners could be added to not generate additional alcohol or carbon dioxide. Starchier grain could also be used for brewing to increase the concentration of dextrin in the beer; however, changing the mash ingredients would change the flavour profile of the final beer depending on which grains were selected (Cadenas, Caballero, Nimubona, & Blanco, 2021). FOS could also be added to the beer. In addition to increasing the sweetness of the beer, this would raise FOS content closer to the required dose to classify the LABs as functional beverages. Although increasing the bitterness of the beer may have an adverse effect on overall liking due to the high penalty of too much bitterness, it is important to note that sweetness does have a masking effect on bitterness and that the frequency of not enough bitterness tended to be approximately double that of too much bitterness, except in the LoHDH sample.

5.5 Conclusion

This study successfully produced LABs with respect to ethanol content, which ranged from 0.61 ± 0.02 % ABV (Control 1) to 0.86 ± 0.03 % (HiHDH 3). This also suggests that higher levels of NZHDH and/or NZYC may be added without exceeding the 1.15 % ABV threshold. After acid hydrolysis, fructose (indicating the presence of FOS) was detected in NZYC-containing samples but was not detected in other samples. This supports the incorporation of NZYC into LABs to develop a functional beverage, particularly with respect to gut health. Volatile analysis of NZHDH identified compounds associated with ‘floral’ and ‘earthy’ flavours (terms identified in LAB samples by the semi-structured focus group) at much higher levels than NZYC, while analysis of NZYC identified compounds associated with ‘floral’ at much higher levels than NZHDH. Although secondary fermentation results in chemical changes to volatile compounds,

incorporating higher concentrations of NZHDH and NZYC may result in more significantly different flavours between LAB samples.

Scores for overall liking, appearance, aroma, mouthfeel, and flavour on unstructured line scales were not significantly different between LAB samples, nor were interactions between LAB samples and panellist age, gender, or frequency of beer consumption. At their existing concentration, the additions of NZHDH and NZYC did not successfully differentiate flavoured LABs from the unflavoured controls, although samples were somewhat liked by panellists. CATA analysis showed that 'brown' was selected significantly more to describe the appearance of LoYC than all other samples except HDHYC, while 'yellow' was selected significantly less to describe the appearance of HiYC than LoHDH, HiHDH, and the control. 'Foamy' was selected significantly more, while 'carbonated' was selected significantly less, to describe the appearance of LoYC and HiYC samples. This suggests the addition of NZHDH and NZYC did have some effect on the appearance of LAB samples. Foaming properties may be related to the higher protein content of NZYC than NZHDH. Penalty analysis showed that all samples were not sweet enough and not hoppy enough, while all samples except LoHDH were not bitter enough. This result, along with results from unstructured line scales and CATA analysis, support reformulation of LAB beverages to increase liking and to differentiate samples more significantly from one another.

6 An investigation into potential health benefits of New Zealand honeydew honey and New Zealand yacon concentrate

6.1 Prelude

Chapter 6 – *An investigation into potential health benefits of New Zealand honeydew honey and New Zealand yacon concentrate* aims to investigate potential health benefits of phenolic compounds found in New Zealand honeydew honey (NZHDH) and New Zealand yacon concentrate (NZYC), as well as of NZHDH and NZYC in general. These phenolic compounds were identified earlier in this thesis – in **Chapter 3** for NZHDH and in **Chapter 4** for NZYC. The purpose of **Chapter 6** is to expand on the chemical profiling of NZHDH and NZYC to potentially prove a health benefit.

This experimental chapter was conducted in three parts; (1) an investigation on the inhibitory effects of NZHDH and NZYC phenolics on the formation of acrylamide in the Maillard reaction, (2) an investigation into the anti-diabetes activity of NZHDH and NZYC phenolics and the anti-obesity activity of NZHDH and NZYC, and (3) an investigation into the inhibitory effects of NZHDH and NZYC phenolics on *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa.* The results from study one showed that none of NZHDH, NZYC, or pure phenolic compounds found in NZHDH and NZYC had inhibitory effects on the formation of acrylamide at the concentrations evaluated. The results from study two showed that NZHDH and NZYC phenolic extracts exhibit minor anti-diabetes effects, while crude NZYC exhibited significant anti-obesity effects. The results from study three showed that NZHDH and NZYC phenolic extracts did not inhibit the growth of *Staphylococcus aureus, Escherichia coli,* or *Pseudomonas aeruginosa.*

6.2 Introduction

6.2.1 Study one: Reduction of acrylamide

The Maillard reaction is the non-enzymatic browning process that occurs in food, involving the condensation of amino acids and reducing sugars to form intermediates which polymerise to form brown pigments (Isleroglu et al., 2012). It can be observed in a variety of products, including caramels, condensed milk, roasted meats, and baked goods. In baked goods, it is responsible for the formation of colour and aroma in the crust (Isleroglu et al., 2012). One product of the Maillard reaction of interest is acrylamide, which mainly forms in foods as a result of the condensation of free asparagine and glucose. Condensation of methionine and glutamine with glucose have also been shown to result in acrylamide formation in model systems; however, the degree of acrylamide formation is much lower than that for asparagine, which is the major driver of acrylamide formation in foods (Becalski, Lau, Lewis, & Seaman, 2003; Isleroglu et al., 2012; Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002). The major and minor reaction pathways for the formation of acrylamide in food are shown below **(Figure 6.1)**.

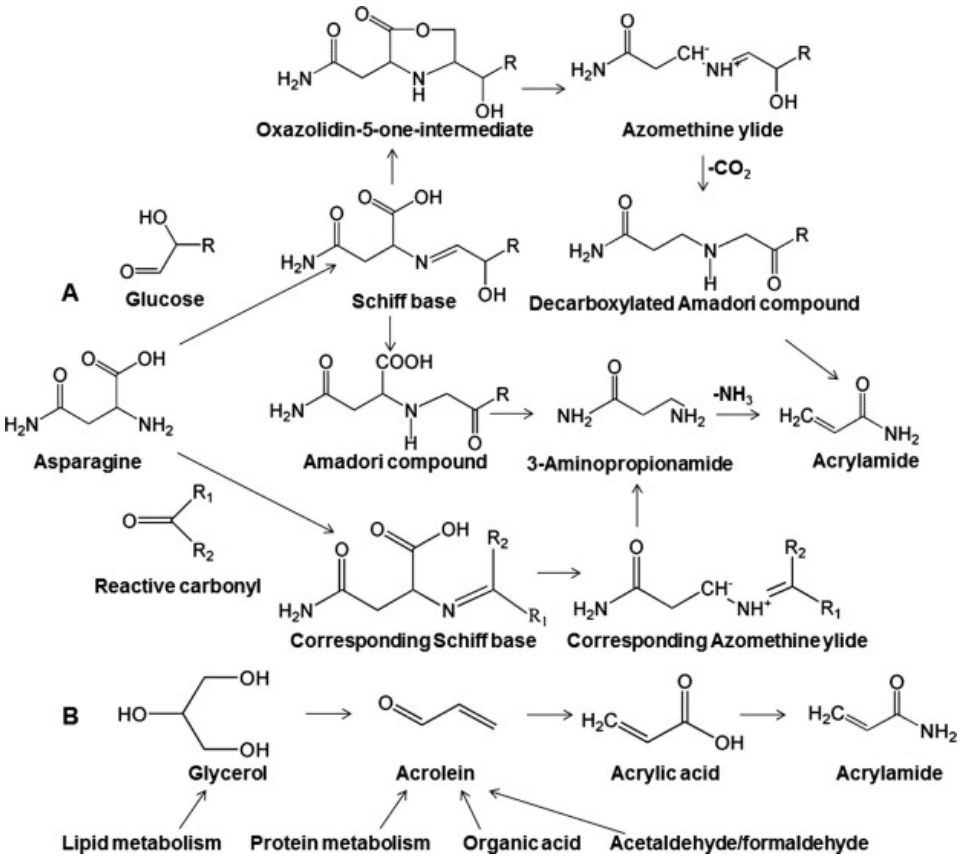

Figure 6.1 Pathways for acrylamide formation in food. (A) Major acrylamide formation pathway. (B) Minor acrylamide formation pathway (Y. Xu et al., 2014).

There are several factors that influence the formation of acrylamide; these include temperature, time, pH, water activity (a_w), the food matrix, availability of asparagine, and storage conditions (Isleroglu et al., 2012; Keramat et al., 2011; Sadd et al., 2008). Higher temperatures and cooking times favour the formation of acrylamide, while lower pH inhibits formation. According to De Vleeschouwer et al. (2007), intermediate water activity is favourable for the Maillard reaction as all reactants are dissolved; increases or decreases in moisture affect the mobility and viscosity of the system and so decrease Maillard reaction rate. Acrylamide levels in food are the net result of simultaneously occurring formation and elimination reactions; according to De Vleeschouwer et al. (2007) the rate constant for acrylamide formation did not vary significantly in model systems in the range of 0.34 to 0.92 a_w while the rate constant for acrylamide elimination is minimal around 0.82 a_w. Thus, the apparent rate of acrylamide formation is maximal around 0.82. The formation of acrylamide in foods is of potential concern as it is a known neurotoxin (Smith & Oehme, 1991), and was classified as "probably carcinogenic to humans" by the International Agency for Research on Cancer (IARC) (International Agency for Research on Cancer, 1994). Furthermore, the European Union has prescribed benchmark levels for acrylamide in certain foods, and has recognised that the setting of maximum levels for acrylamide in certain foods should be considered (see **Table 6.1**) (Commission, 2017).

Table 6.1 Benchmark levels for the presence of acrylamide in foodstuffs (Commission, 2017).

Food	Benchmark level (µg/kg)
French fries (ready-to-eat)	500
Potato chips from fresh potatoes and from potato dough Potato-based crackers Other potato products from potato dough	750
Soft bread (a) Wheat based bread (b) Soft bread other than wheat based bread	 50 100
Breakfast cereals (excl. porridge) (a) Bran products and whole grain cereals, gun puffed grain (b) Wheats and rye based products (1) (c) Maize, oat, spelt, barley, and rice based products (1)	 300 300 150
Biscuits and wafers	350
Crackers with the exception of potato based crackers	400
Crispbread	350
Ginger bread	800
Products similar to the other products in this category	300
Roast coffee	400
Instant (soluble) coffee	850
Coffee substitutes (a) Coffee substitutes exclusively from cereals	 500

(b) Coffee substitutes from a mixture of cereals and chicory (c) Coffee substitutes exclusively from chicory	[2] 4000
Baby foods, processed cereal based foods for infants and young children excluding biscuits and rusks [3]	40
Biscuits and rusks for infants and young children [3]	150

[1] Non-whole grain and/or non-bran based cereals. The cereal present in the largest quantity determines the category.

[2] The benchmark level to be applied to coffee substitutes from a mixture of cereals and chicory takes into account the relative proportion of these ingredients in the final product.

[3] As defined in Regulation (EU) No 609/2013

A range of methods have been proposed to reduce the formation of acrylamide in baked goods; however, many have only been tested in model systems (Sadd et al., 2008). These mitigation strategies tend to focus on reducing or diluting precursors to the Maillard reaction such as free asparagine, for instance by addition of enzymes, yeasts, or amino acids, by binding asparagine with a complexing agent, removing accelerants such as ammonium salts, lowering the pH, or minimising heating temperature and time (Sadd et al., 2008).

Another method through which the formation of acrylamide may be reduced is by the addition of phenolic compounds; however, the literature is inconclusive on this. One study investigated the effects of pure phenolic compounds (cinnamic, gallic, ferulic, coumaric and caffeic acids, catechin and epicatechin) on the formation of acrylamide in a model system, and found that the compounds investigated had no mitigation effects (Bassama et al., 2010). However, another study investigated the effects of the flavonoid naringenin on acrylamide formation in a model system, and found that naringenin did inhibit acrylamide formation by forming adducts with asparagine degradation products (Cheng et al., 2009). Studies have also shown that polyphenolic extracts from certain natural sources such as grape pomace, mint, cumin seeds, or star anise have greater inhibitory effects than pure phenolic compounds (C. Xu et al., 2015; Zhu et al., 2009). Zhu et al. (2009) did note that some plant extracts (*Ilex cornuta Lindl. et Paxt*.) and pure phenolic compounds (ferulic acid and hesperetin) appeared to increase acrylamide formation in a model system.

C. Xu et al. (2015) found that one mechanism through which phenolic extracts inhibit acrylamide formation may be the trapping of sugar fragments and other intermediates at high temperature, but stated further research was needed. Zhu et al. (2009) stated that, for plant extracts, the different types and contents

of phenolic compounds, as well as the chemical structures of these compounds, resulted in different effects on acrylamide formation depending on synergistic or antagonistic interactions.

6.2.2 Study two: Anti-diabetes and anti-obesity activity

6.2.2.1 Anti-diabetes activity

In recent years, there has been a concerning rise in diagnoses of diabetes, to the point where it can be considered epidemic (Benalla et al., 2010). Type 1 diabetes is an autoimmune disease where pancreatic beta-cells which produce insulin are destroyed, causing inulin deficiency which leads to chronic hyperglycaemia (high blood sugar), while type 2 diabetes (which makes up 90-95% of all diabetes cases) is a group of metabolic diseases similarly characterised by chronic hyperglycaemia, caused by a combination of insulin resistance and impaired insulin secretion (Benalla et al., 2010; Dirir et al., 2022; Jaber, 2023). Hyperglycaemia is the major cause of diabetes complications which include heart disease, nerve, kidney, and eye damage, skin and mouth conditions, Alzheimer's disease, and depression (Dirir et al., 2022); type 1 diabetes is typically treated by insulin injection, while type 2 diabetes is treated with drugs which hinder glucose absorption, suppress hepatic gluconeogenesis, and inhibit the reabsorption of glucose by the kidneys. Alpha-glucosidase inhibitors are a common treatment for type 2 diabetes as they reversibly and competitively bind with alpha-glucosidase, delaying digestion of carbohydrates and so reducing blood glucose levels (Bhatia et al., 2019; Dej-Adisai et al., 2021).

However, alpha-glucosidase inhibitors (which include acarbose, miglitol, and voglibose) are known to have adverse side-effects. These include diarrhoea, abdominal pain, and flatulence, which are caused by fermentation of undigested carbohydrates by gut bacteria resulting in increased intestinal gas production (Dej-Adisai et al., 2021; Dirir et al., 2022). In recent years, there has been growing interest in the use of natural products in a variety of therapeutic treatments, including for type 2 diabetes. Natural products have a number of advantages; they have been used for centuries in traditional medicines around the world and so are trusted by consumers, can have decreased toxicity and severity of side effects, and can be lower in cost than conventional drugs (Bhatia et al., 2019; Dej-Adisai et al., 2021; Dirir et al., 2022).

The phenolic compounds found in honey are unique and specific to the type of honey, and determine characteristics such as flavour, colour, and functional properties (Peláez-Acero et al., 2022). Previous studies

have shown that phenolic honey extracts have the ability to inhibit alpha-glucosidase, with IC_{50} values ranging from 55 to 153 μg/mL (Ali et al., 2020; Zaidi et al., 2019). Inhibition of alpha-glucosidase can be achieved by the binding of hydrogen to the active site of the enzyme, and so the hydroxyl group of phenolic compounds may be responsible for their inhibitory activity (Zaidi et al., 2019).

6.2.2.2 Anti-obesity activity

Similar to diabetes, there has been a rapid global rise in obesity. Obesity causes abnormal physiological metabolism, adversely affecting physiological, psychological, and social well-being (T.-T. Liu et al., 2020). Particularly, obesity is known to be a significant risk factor for conditions such as heart disease, hypertension, hyperlipidaemia, diabetes, and cancer (T.-T. Liu et al., 2020). Pancreatic lipase is an enzyme responsible for breaking fat down into glycerol and fatty acids, which are then absorbed by the body and used for metabolism (T.-T. Liu et al., 2020). Pancreatic lipase inhibitors are therefore used as drug treatments for obesity as they limit the level of lipids entering the blood. Honey has been shown in the literature to inhibit the activity of pancreatic lipase, with several varieties of arid land honey outperforming orlistat (a drug conventionally used to treat obesity) (Habib, Kheadr, & Ibrahim, 2021).

6.2.3 Study three: Anti-bacterial activity

Honey is well-known for its anti-bacterial activity, and has been used in traditional medicine since ancient times for treatment of bacterial infections, coughs and colds, and various infectious diseases (Almasaudi, 2021; Kwakman & Zaat, 2012; Mandal & Mandal, 2011). Hydrogen peroxide, methylglyoxal, and bee defensin-1 (an antimicrobial peptide) have been identified as important anti-bacterial components of honey; however, after these compounds have been neutralised or degraded, honey has still been shown to exert anti-bacterial activity against certain microorganisms (Almasaudi, 2021; Kwakman & Zaat, 2012; Mandal & Mandal, 2011). The high phenolic content of honey has been proposed as one possible explanation, but the concentration of individual phenolic compounds tends to be too low for substantial anti-bacterial activity to occur (Almasaudi, 2021; Kwakman & Zaat, 2012). If phenolic compounds are responsible for some of the anti-bacterial activity of honey it would be due to synergistic interactions between these compounds (Kwakman & Zaat, 2012). Yacon has also been used in traditional medicine for centuries, and recent studies have isolated anti-bacterial substances and insecticides from the leaves of the yacon plant (de Almeida, Viana, Costa, Silva, & Feitosa, 2019).

6.2.4 Overall rationale and aims

Honeydew honey (HDH) is honey which is produced by honeybees who feed on nectar (honeydew) exuded by insects such as aphids. In New Zealand, most commercial HDH is produced from honeydew exuded by the scale insect *Ultracoelostomoa assimile* feeding on Southern beech trees (*Nothofagus* spp.) in the Nelson beech forest (Chessum et al., 2022). According to Chessum et al. (2022), the total phenolic content of New Zealand HDH (NZHDH) is 62.30 ± 6.31 mg gallic acid equivalents (GAE) per 100 grams, and the most abundant phenolic compounds are pinocembrin, abscisic acid, and pinobanksin. Of these, pinocembrin has been shown to have moderate inhibitory effects on maltase and sucrase enzymes, with IC_{50} values of 90 and 100 μg/mL respectively (Potipiranun, Adisakwattana, Worawalai, Ramadhan, & Phuwapraisirisan, 2018). To the extent of the author's knowledge, none of pinocembrin, abscisic acid, or pinobanksin have previously been tested for phenolic lipase inhibition in the literature. Pinocembrin has been shown to exert anti-bacterial activity in the literature by induction of cell lysis (Almasaudi, 2021).

Yacon concentrate (YC) is a syrup with physical and sensorial characteristics similar to honey or cane sugar syrup, produced from juice extracted from the roots of the yacon plant (*Smallanthus sonchifolius*) (Chessum, Chen, Kam, & Yan, 2023). In the early 1980s yacon was imported into New Zealand as a novel vegetable, giving rise to the 'New Zealand' cultivar of yacon (Douglas et al., 2005; Ojansivu et al., 2011). According to Chessum et al. (2023), the total phenolic content of New Zealand YC (NZYC) ranges from 565 to 785 mg GAE per 100 g, and the most abundant phenolic compounds are chlorogenic acid, caffeic acid, and *p*-coumaric acid. Both caffeic acid and chlorogenic acid have been shown to inhibit alpha-glucosidase, with IC_{50} values of 4.98 and 9.24 μg/mL respectively (Oboh, Agunloye, Adefegha, Akinyemi, & Ademiluyi, 2015). Chlorogenic acid is an ester of caffeic acid and quinic acid; the substitution of quinic acid for a hydroxyl group may be responsible for chlorogenic acid's higher IC_{50} value (Oboh et al., 2015). Conversely, p-coumaric acid has been shown in the literature to have very low alpha glucosidase inhibition potential (Aleixandre, Gil, Sineiro, & Rosell, 2022; Malunga, Joseph Thandapilly, & Ames, 2018). Chlorogenic acid, caffeic acid, and p-coumaric acid have all been shown in the literature to inhibit the activity of pancreatic lipase (Buchholz & Melzig, 2015). Chlorogenic acid exerts anti-bacterial activity by increasing membrane permeability, inducing cytoplasmic and nucleotide leakage; caffeic acid exerts anti-bacterial activity through

oxidative stress; p-coumaric acid exerts anti-bacterial activity by disrupting the cell membrane and binding to bacterial DNA (Almasaudi, 2021).

This research aims to investigate the potential health benefits of NZHDH and NZYC, and relate them to the phenolic profile of these products described in **Chapters 3** and **4**. There are three objectives to this research: (1) investigate the potential inhibitory effects of NZHDH and NZYC phenolic extracts on the formation of acrylamide in the Maillard reaction, (2) investigate the potential anti-diabetes and anti-obesity activity of NZHDH and NZYC phenolic extracts, and (3) investigate the potential anti-bacterial activity of NZHDH and NZYC phenolic extracts.

6.3 Materials and methods

6.3.1 Materials

The NZHDH used in this study was produced from nectar exuded by the scale insect *Ultracoelostoma assimile*, which feeds on the Southern beech (*Nothofagus* spp.), and was sourced from Streamland Honey Group Ltd, New Zealand. NZHDH samples were collected from three different production batches over a period of one year. The NZYC used in this study was produced from yacon grown in New Zealand; three different production batches were sourced from NZFOS+, New Zealand. Ultrapure water (UPW) was produced using a Purite Select Fusion water deionisation unit (Suez Water Technologies & Solutions, USA).

All reagents used in this study are ≥98% in purity, unless otherwise stated. Calcium chloride (93%+) and lipase AY30 (294760250) were sourced from Acros Organics, Belgium. Sodium carbonate was sourced from Ajax Finechem, Australia. 4-nitrophenyl alpha-D-glucopyranoisde (J50243), acarbose (2193AH), and p-nitrophenyl acetate (J98614) were sourced from AK Scientific, USA. Chlorogenic acid (J60457) was sourced from Alfa Aesar, USA. Difco™ nutrient broth was sourced from BD, USA. Sodium chloride was sourced from BDH Chemicals, England. D-glucose anhydrous was sourced from BioLab (Aust) Ltd, Australia. Costar 96 well culture plates (3599) were sourced from Corning Incorporated-Life Sciences, China. Cultures of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Saccharomyces cerevisiae* were sourced from ESR, New Zealand. Formic acid (F/1900/PB15) and methanol (A4524) were sourced from Fisher Chemical, USA. Acetonitrile and dimethyl sulfoxide (D/4121/PB17) were sourced from Fisher Scientific, UK. Mueller Hinton Agar plates (1290) were sourced from Fort Richard Laboratories, NZ. Gallic acid was

sourced from Loba Chemie, India. Meropenem 10µg disks and vancomycin 30µg disks were sourced from Mast Group Ltd., UK. Strata C-18 Solid Phase Extraction (SPE) cartridges were sourced from Phenomenex, USA. Pinocembrin was sourced from Phytolab, Germany. Acrylamide-d3 Standard solution (72384), acrylamide (A9099), acetonitrile (271004), alpha glucosidase from *Saccharomyces cerevisiae* (65003), L-asparagine (A0884), caffeic acid (C0625), p-coumaric acid (C9008), abscisic acid (90769), pinobanksin (≥95%, 68530), dipotassium phosphate, potassium dihydrogen phosphate, and tris hydrochloride were sourced from Sigma-Aldrich, USA. Hydrochloric acid (36%) was sourced from Univar, USA. Whatmann 40 filter paper was sourced from W&R Balston Ltd, England.

6.3.2 Reduction of acrylamide

The formation of acrylamide was monitored in a model system using a method adapted from that used by Gökmen and Şenyuva (2007). An internal standard was prepared by diluting deuterated acrylamide to a concentration of 10 µg/mL in acetonitrile. A stock solution (A) of acrylamide was prepared at a concentration of 1 mg/mL in UPW, from which a stock solution (B) of acrylamide was prepared at a concentration of 10 µg/mL. Acrylamide standards spiked with 10 µL internal standard were prepared from stock solution (B) at concentrations ranging from 1 to 0.01 µg/mL.

A negative control system was prepared by dissolving 0.901 g glucose in 10 mL UPW. Sample systems were prepared by dissolving 1.482 g NZHDH or 2.183 g NZYC in 10 mL UPW. These masses were used to reach 0.901 g of reducing sugars (i.e. to standardise the amount of sample available for reaction with asparagine) based on the sugar profiles produced in **Chapter 3** and **Chapter 4**. Solutions containing individual phenolic compounds were prepared by dissolving 0.901 g glucose and 5.3 µmol of phenolic compound in 10 mL UPW. Gallic acid was used as a positive control based on results obtained by Zhu et al. (2009), who found that gallic acid mitigated acrylamide formation by 47.7 ± 1.2%.

400 µL of each system was mixed with 26.44 mg asparagine and 400 µL UPW and was sonicated to dissolve. Capless 10 mL GC vials containing 200 µL of reaction mixtures were placed in an oven at 180°C in triplicates. After heating for 10 minutes, the vials were immediately placed on ice.

Pyrolysates were suspended in 5 mL of UPW, and aqueous extract was obtained by vortexing for one minute. 1 mL of aqueous extract was transferred to a microcentrifuge tube and was centrifuged for 5

minutes at 10,000 RPM. 500 µL of clear supernatant was transferred to a 1.5 mL GC vial for analysis and was mixed with 490 µL UPW and 10 µL internal standard.

LC-MS analyses were conducted using an Agilent 1260 Infinity Quaternary LC System (Santa Clara, CA 95051 USA). The LC-MS system consisted of the following components: 1260 quaternary pump (model number: G1311B), 1260 infinity ALS sampler (model number: G1329B), 1260 infinity TCC column component (model number: G1316A), 1260 infinity diode array detector (DAD) (model number: G4212B), connected to a 6420 triple quadrupole LC/MS system with electrospray ionisation (ESI) source (model number: G1948B). The column used was a XSelect CSH C18 (2.1 x 100 mm, 3.5 µm) (Waters, USA).

The MS ionisation source conditions were as follows: capillary voltage of 4 kV, drying gas temperature of 300°C, drying gas flow of 10 L/min, nebulizer pressure of 40 psi. The positive ionisation mode was performed with multiple reaction monitoring (MRM) for quantitative analysis. The LC-MS conditions were as follows: Mobile phase A was 0.1% formic acid in UPW. Mobile phase B was methanol. The flow rate was 0.25 mL/min.

The gradient program was as follows: The initial percentage of mobile phase B (B%) was 5% and was held for 1.5 minutes. B% was then raised to 90% over 0.5 minutes and was held at 90% for 2 minutes. B% was then lowered to 5% over 0.5 minutes, and held at 5% for 9 minutes, and the run was complete.

6.3.3 Extraction of phenolics

Phenolic extracts were prepared for the anti-diabetes, anti-obesity, and anti-bacterial assays according to the procedure described by Chessum et al. (2022), with minor modifications. Extraction was conducted for both NZHDH and NZYC; approximately 25 g of each was dissolved separately in 25 mL of UPW. The remaining procedure was as described, with three SPE cartridges used for each sample. After the phenolic compounds were eluted with acetonitrile and centrifuged, supernatants for each sample were pooled in 25 mL round-bottom flasks. Acetonitrile and any residual water were then removed by rotary evaporation using a Hei-VAP Advantage rotary evaporator (Heidolph, Germany) coupled with a V-100 vacuum pump and I-100 interface (Buchi, Switzerland). Samples were then re-suspended in 20% methanol to a concentration of 0.8 mg extract/mL, or in 2% dimethyl sulfoxide (DMSO) to a concentration of 10 mg extract/mL.

6.3.4 Anti-diabetes and anti-obesity assays

6.3.4.1 Anti-diabetes assay

A 100 mM phosphate buffer was prepared by adding 0.817 g of potassium dihydrogen phosphate and 0.697 g of dipotassium phosphate and adjusting to pH 6.8 with HCl. Alpha glucosidase enzyme was dissolved in the prepared phosphate buffer at a concentration of 0.1 mg/mL. A 5 mM solution of 4-nitrophenyl alpha-D-glucopyranoside was prepared by dissolving 0.016 g in 10 mL of UPW. A 0.1 M sodium carbonate solution was prepared by dissolving 0.106 g sodium carbonate in 10 mL of UPW. Acarbose (positive control) was dissolved in DMSO to a concentration of 0.1 mg/mL.

To the first well of each row in a 96-well plate was added 40 µL of sample (10 mg/mL NZHDH phenolic extract or NZYC phenolic extract), positive control, or blank (10 mg/mL NZHDH or NZYC phenolic extract). 20 µL of DMSO was then added to seven subsequent wells in each row. 20 µL of sample, positive control, or blank was then taken from the first well and added to the second well, which was then mixed. This was repeated until the final well, from which 20 µL was taken and discarded. For the negative control, 20 µL of UPW was added to a single well. To each well was then added 50 µL of phosphate buffer and 10 µL of alpha-glucosidase solution. The plate was then incubated at 37°C for 15 minutes in a Heraeus Heracell Incubator 150 (Thermo Fisher Scientific, New Zealand). After incubation, 20 µL of 4-nitrophenyl alpha-D-glucopyranoside was added to the sample, positive control, and negative control wells, and 20 µL of UPW was added to the blank wells. The plate was then incubated at 37°C for a further 20 minutes. After incubation, 50 µL of sodium carbonate was added to each well. After shaking for 30 seconds, absorbance was measured at 405 nm using a Spark 10M fluorescence plate reader (Tecan, Switzerland) and Spark Control v3.2 (Tecan, Switzerland).

To account for the colour of the sample, the absorbance of the 'blank' was subtracted from the absorbance of the 'sample' at each dilution level. The percentage inhibition was then calculated using the following equation:

$$Inhibition\% = \frac{Abs_{negative\ control} - Abs_{sample}}{Abs_{negative\ control}} * 100$$

6.3.4.2 Anti-obesity assay

A buffer containing 13 mM tris hydrochloride, 1.3 mM calcium chloride, and 150 mM sodium chloride was prepared by dissolving 0.3937 g tris hydrochloride in about 200 mL UPW, then adding 0.0361 g calcium chloride and 2.1915 g sodium chloride and stirring to dissolve. The buffer was then adjusted to pH 8.5 with NaOH and made up to a final volume of 250 mL using UPW. Lipase enzyme was dissolved to achieve concentration of 3 mg/mL in the prepared buffer solution, and was then centrifuged at 4000 RPM for 10 minutes at 20°C. p-nitrophenyl acetate (p-NPA) was dissolved to achieve concentration of 2 mg/mL in DMSO. NZYC was prepared by dissolving in UPW to a concentration of 20 µg/mL; NZHDH was prepared by dissolving in UPW to a concentration of 200 µg/mL.

To the first well of each row in a 96-well plate was added 100 µL of sample (20 µg/mL NZYC in UPW or 200 µg/mL NZHDH in UPW) or blank (20 µg/mL NZYC in UPW or 200 µg/mL NZHDH in UPW). 50 µL of UPW was then added to seven subsequent wells in each row. 50 µL of sample or blank was then taken from the first well and added to the second well, which was then mixed. This was repeated until the final well, from which 50 µL was taken and discarded. 50 µL of lipase solution was then added to each well. For the negative control, 50 µL of buffer and 50 µL of lipase solution was added to a single well. The plate was then incubated at 37°C for 10 minutes in a Heraeus Heracell Incubator 150 (Thermo Fisher Scientific, New Zealand). After incubation, 50 µL of p-NPA solution was added to the sample and negative control wells; 50 µL of DMSO was added to the blank wells. The plate was then incubated at 37°C for a further 20 minutes. The plate was then shaken for 30 seconds, and absorbance was measured at 405 nm using a Spark 10M fluorescence plate reader (Tecan, Switzerland) and Spark Control v3.2 (Tecan, Switzerland).

To account for the colour of the sample, the absorbance of the 'blank' was subtracted from the absorbance of the 'sample' at each dilution level. The percentage inhibition was then calculated using the following equation:

$$Inhibition\% = \frac{Abs_{negative\ control} - Abs_{sample}}{Abs_{negative\ control}} * 100$$

6.3.5 Anti-bacterial activity

The prepared methanolic phenolic extracts were diluted down in UPW to a final concentration of 100 µg extract/mL, yielding a final methanol concentration of 2.5%, while the DMSO extract was used

undiluted. Sterile water, nutrient broth, and disks (hole-punched Whatmann 40 filter paper) were prepared by autoclaving at 121°C for 15 minutes. Extracts were then analysed for anti-bacterial activity by the disk diffusion assay using the following procedure.

Cultures of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were incubated for 16 hours in nutrient broth at 35°C. Plates of Mueller Hinton agar were inoculated with 150 µL of culture in triplicates, and the spread plate method was used to create a bacterial lawn on each plate. Using alcohol-dipped and flamed forceps, disks were picked up. For pre-impregnated positive control disks (Vancomycin for *S. aureus* or Meropenem for *E. coli* and *P. aeruginosa*), the disks were then placed onto the inoculated agar surface and gently pressed to ensure even contact. For the negative control (sterile water) and the phenolic extracts, the edge of the disk was dipped into solution and impregnated by capillary action before being placed onto the inoculated agar surface. Each plate contained four disks: the positive control, negative control, and the two phenolic extracts. Plates were incubated at 35°C for 24 hours and then examined for growth inhibition.

6.3.6 Statistical analysis

IC_{50} values for anti-obesity and anti-diabetes assays were calculated using Graphpad Prism 8. Statistical analysis was carried out using Microsoft Excel version 2104 and R Studio 2023.09.0+463. Acrylamide formation and IC_{50} values were reported as mean values ± standard deviation as calculated in Excel and, where possible, were analysed by one-way analysis of variance (ANOVA) using R Studio. Post hoc analysis was carried out in R studio using Tukey's honestly significant difference (HSD) test with a 95% family-wise confidence interval.

6.4 Results and discussion

6.4.1 Reduction of acrylamide

The results from analysis of acrylamide formation are presented in **Table 6.2** and are visualised in **Figure 6.1**. Significant differences were identified between the samples ($p = 0.00011$); however, none of the samples were significantly different from the negative control (asparagine and glucose with no addition of phenolic compound). p-Coumaric acid had significantly higher acrylamide formation than abscisic acid, caffeic acid, and the positive control (gallic acid); caffeic acid and the positive control also had significantly lower acrylamide formation than pinocembrin, chlorogenic acid, and pinobanksin.

The positive control did achieve a mean reduction of 21.9% relative to the negative control, substantially lower than the 47% reduction reported by Zhu et al. (2009). There were some differences between the protocol followed by Zhu et al. (2009) and the protocol followed in the present work; for instance, Zhu et al. (2009) utilised a longer heating time and higher concentration of phenolic solutions.

The results for abscisic acid and caffeic acid are of note, as although they were not significantly different from the negative control, it is possible that at higher concentrations significant reduction of acrylamide may be observed. As identified in **Chapters 3 and 4**, abscisic acid is a major phenolic compound in NZHDH (0.962 to 2.323 mg/kg), and caffeic acid is a major phenolic compound in NZYC (13.02 to 15.40 mg/kg). Conversely, pinocembrin (2.325 to 2.659 mg/kg) and pinobanksin (1.654 to 1.863 mg/kg) are also major phenolic compounds in NZHDH, and at higher concentrations significant increases in acrylamide formation may be observed. Similarly, chlorogenic acid (61.87 to 103.61 mg/kg) and p-coumaric acid (1.06 to 1.41 mg/kg) are major phenolic compounds in NZYC, and at higher concentrations significant increases in acrylamide formation may be observed.

Several mechanisms have been proposed in the literature for phenolic inhibition of acrylamide formation; these include the trapping of reactive intermediates, antioxidant activity, and asparagine binding and competition. Kotsiou, Tasioula-Margari, Capuano, and Fogliano (2011) observed that the degree of mitigation could be positively correlated to the number of hydroxyl phenyl groups, which would support antioxidant activity as the mechanism of action. It is conferred that the number and position of hydroxyl phenyl groups are directly correlated to antioxidant activity. In the present work, this correlation was observed except for abscisic acid, which only contains one hydroxyl phenyl group yet was one of three compounds to inhibit acrylamide formation. p-Coumaric acid, with the greatest degree of acrylamide promotion, also contains only one hydroxyl phenyl group, while gallic acid, with the greatest degree of acrylamide inhibition, contains three. All other compounds evaluated contain two hydroxyl phenyl groups. However, this positive correlation was not observed in a separate study carried out by Mildner-Szkudlarz, Różańska, Piechowska, Waśkiewicz, and Zawirska-Wojtasiak (2019).

The structure of phenolic compounds has been shown in the literature to have an effect on the formation of heterocyclic amines (HA; another product of the Maillard reaction). In one study carried out by Monti et al. (2001), fresh virgin olive oil (VOO) extract had greater inhibitory effects on HA formation than

one-year-old VOO extract; the fresh VOO extract was found to be richer in *o*-diphenolic compounds than the old VOO extract. However, when oleuropein was added to the model system, there was a slight increase in HA formation. Although oleuropein contains *o*-diphenol reactive groups, it also contains a carbohydrate moiety and as such is more hydrophilic. The polarity of antioxidant compounds such as polyphenols affects their antioxidant capacity; according to Monti et al. (2001), polarity can also influence their effect on HA formation in the Maillard reaction. It should be noted that gallic acid has greater water solubility than all other phenolic compounds evaluated in the present study and exhibited the greatest inhibition of acrylamide.

Monti et al. (2001) also noted a concentration-dependent effect of the VOO extracts on HA formation. At 100 ppm, only a slight decrease in HA formation was observed; at 1100 ppm, HA formation was decreased by 20 to 38%; at 11100 ppm, HA formation was increased relative to the control. Monti et al. (2001) attributed this to the pro-oxidant effect observed for many antioxidants at high concentrations. Similar concentration-dependent effects on acrylamide formation have been reported by Fujiwara et al. (2011); Yuan, Shu, Zhou, Qi, and Xiang (2011); Y. Zhang and Zhang (2008). Fujiwara et al. (2011) attributed the promoting effects of phenolic compounds at high concentrations to the generation of hydrogen peroxide, which participates in acrylamide formation.

Another mechanism through which polyphenols may contribute to acrylamide formation is the provision of carbonyl groups for direct reaction with asparagine, with the degree of acrylamide formation inversely related to the melting point of the carbonyl compound (Hamzalıoğlu & Gökmen, 2012). For example, the carbonyl group of curcumin has been shown to react with the α-amine group of asparagine, generating a Schiff base which is subsequently decarboxylated leading to the formation of acrylamide (Hamzalıoğlu, Mogol, Lumaga, Fogliano, & Gökmen, 2013). Phenolic compounds may also increase the apparent acrylamide formation rate by inhibiting reactions which eliminate acrylamide. One mechanism through which acrylamide is eliminated is polymerisation induced by oxidative radicals; the scavenging of free radicals by phenolic compounds may lead to a decrease in acrylamide elimination and thereby increase the apparent acrylamide formation rate (Y. Liu et al., 2015).

As the degree of acrylamide formation in model systems containing NZHDH and NZYC was not significantly different from the degree of acrylamide formation in the negative control, it was decided that this research would not be continued. While, as stated above, individual phenolic compounds may achieve

significant reductions or increases in acrylamide formation at higher concentrations, the potentially synergistic or antagonistic interactions of these compounds in NZHDH and NZYC overall result in no significant reduction or increase.

Table 6.2 Degree of acrylamide formation in model systems (n=3).

Sample	µg acrylamide / g reducing sugar
p-Coumaric acid	238.12 ± 30.49[a]
Pinocembrin	236.14 ± 21.97[ab]
Chlorogenic acid	221.01 ± 3.07[ab]
Pinobanksin	219.50 ± 14.22[ab]
Negative control[1]	204.64 ± 16.38[abc]
NZYC	199.89 ± 14.15[abc]
NZHDH	199.48 ± 12.48[abc]
Abscisic acid	179.62 ± 9.83[bc]
Caffeic acid	163.58 ± 27.57[c]
Positive control (Gallic acid)	159.85 ± 5.90[c]

Significant differences existed between samples at the .001 probability level. Samples with different superscript letters are significantly different.

[1] Model asparagine-glucose system with no addition of phenolic compounds.

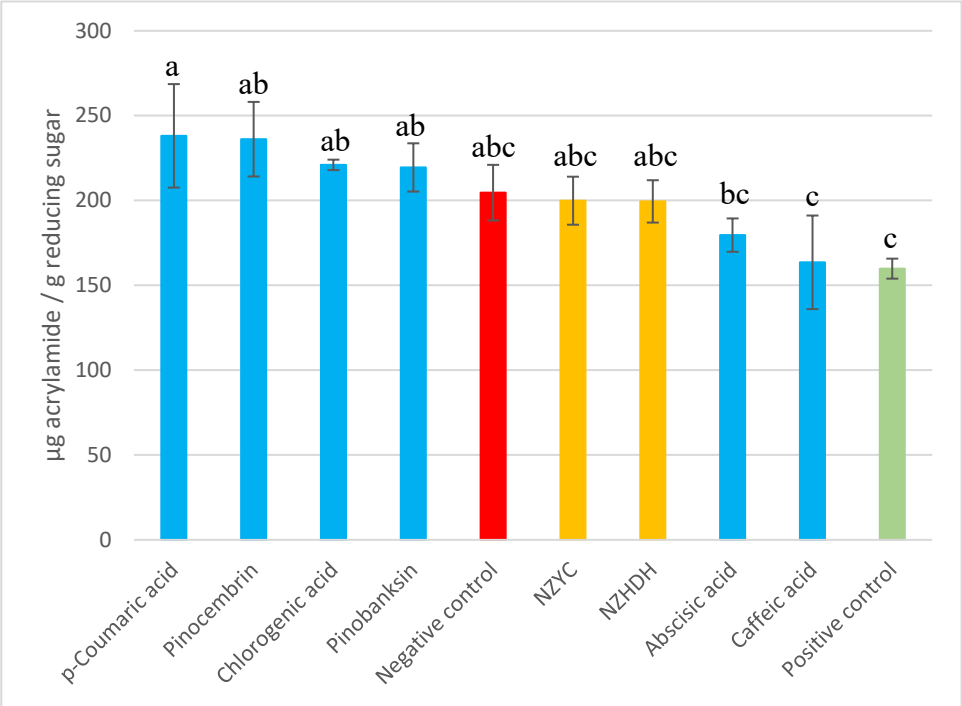

Figure 6.2 Degree of acrylamide formation in model systems (n=3). Samples with different letters are significantly different (p=0.00011).

6.4.2 Anti-diabetes and anti-obesity assays

The results from the alpha glucosidase anti-diabetes assay and lipase anti-obesity assay are presented in **Table 6.3.** Significant differences existed between the IC_{50} values for NZYC and NZHDH phenolic extracts in terms of the alpha glucosidase anti-diabetes assay. One-way ANOVA could not be conducted for the lipase anti-obesity assay as the IC_{50} value could not be determined for two of the three triplicates for NZHDH. To determine IC_{50} value, at least one data point at which a minimum of 50% inhibition is achieved is required; triplicates A and B achieved maximum inhibition of 47.1% and 46.6% respectively at 200 mg/mL and so had reported IC_{50} values of >200 (see **Table 6.3**). However, it is reasonable to conclude that the IC_{50} values for NZYC and NZHDH in terms of the lipase anti-obesity assay are significantly different, given that they differ by a full order of magnitude and the standard deviation for NZYC is 1.86 mg/mL.

For the alpha glucosidase anti-diabetes assay, phenolic extracts of NZYC and NZHDH were used as literature discussed in **Chapter 2.4.2.2** shows that honey phenolic extracts have exhibited IC_{50} values ranging from 55 to 153 µg/mL (Ali et al., 2020; Zaidi et al., 2019). However, for the lipase anti-obesity assay, crude NZYC and NZHDH were used to assess the claim that consumption of yacon concentrate may support blood glucose management. Pancreatic lipase breaks fat down into fatty acids and glycerol, the latter of which may be converted to glucose via gluconeogenesis. As such, inhibition of pancreatic lipase may be a mechanism through which NZYC supports blood glucose management.

The IC_{50} value is a measure of the concentration of a drug, compound, or substance required to inhibit a biological or biochemical process by 50%. In terms of the alpha glucosidase assay, NZYC and NZHDH phenolic extracts had IC_{50} values of 5.56 ± 0.28 and 6.69 ± 0.07 mg/mL respectively, or 5560 and 6690 µg/mL. These values are one to two orders of magnitude higher than the aforementioned IC_{50} values identified for stingless bee honeys of various botanical origins (Ali et al., 2020) and Algerian honeys of various botanical origins (Zaidi et al., 2019), indicating that NZHDH and NZYC phenolic extracts are far less effective at inhibiting alpha glucosidase than phenolic extracts of these varieties of honey.

According to the results presented in **Chapter 3.4.4**, the major phenolic compounds in NZHDH are pinocembrin, abscisic acid, and pinobanksin. According to Lianza et al. (2022), pinocembrin has an IC_{50} value of 156 ± 1.3 µM and demonstrates mixed inhibition of alpha glucosidase from *Saccharomyces cerevisiae* (i.e. both competitive and non-competitive inhibition). According to Amador, Nieto-Camacho,

Ramírez-Apan, Martínez, and Maldonado (2020), at a concentration of 100 µM pinobanksin isolated from *Lippia graveolens* (Mexican oregano) showed weak-to-moderate inhibition (32.54%) on yeast alpha glucosidase type I, while isolated pinocembrin showed weak inhibition (16.26%). The two hydroxy groups of pinocembrin are located at positions 5 and 7 while the three hydroxy groups of pinobanksin are located at positions 3, 5, and 7; according to Tadera et al. (2006), hydroxylation at the 3 and 5 point of flavonones may promote the inhibition of alpha glucosidase. The presence of an additional hydroxy group at the 3 position of pinobanksin, compared to pinocembrin, may explain why pinobanksin exhibited greater inhibition than pinocembrin in the study carried out by Amador et al. (2020).

According to the results presented in **Chapter 4.4.4**, the major phenolic compounds in NZYC are chlorogenic acid and caffeic acid. Both chlorogenic acid and caffeic acid have been shown in the literature to exert antidiabetic effects; Oboh et al. (2015) determined that chlorogenic acid and caffeic acid have IC_{50} values of 9.24 and 4.98 µg/mL respectively on alpha glucosidase from *Saccharomyces cerevisiae*. According to S. Wang et al. (2022), chlorogenic acid has an IC_{50} value of 12.76 ± 0.17 µmol/mL and demonstrates mixed inhibition of alpha glucosidase from *Saccharomyces cerevisiae*. Chlorogenic acid and caffeic acid are both examples of hydroxycinnamic acids. According to Ishikawa et al. (2007), the presence of a phenolic hydroxyl group at the benzene ring of hydroxycinnamic acids may promote inhibition of alpha glucosidase; chlorogenic and caffeic acid was seen to inhibit alpha glucosidase while compounds such as *o*-, *m*-, and *p*-coumaric acids, and *trans*-cinnamic acid – which lack this phenolic group – did not.

One possible explanation for the significantly higher IC_{50} values for NZYC and NZHDH phenolic extracts compared to honey extracts identified in the literature is the inefficiency or poor execution of the extraction method. It may be that other compounds besides phenolic compounds, particularly sugars, were not completely removed by SPE; if this were the case, then the true concentration of phenolic compounds in the extract would be lower than reported. As rotary evaporation under vacuum was used to concentrate the phenolic compounds, it is unlikely that thermal degradation occurred during the preparation of the extracts.

According to Habib et al. (2021), honey may inhibit lipase through covalent attachment of its bioactive compounds, such as polyphenols and peptides, to serine in the active site of the lipase enzyme. Pinocembrin, one of the major phenolic compounds in NZHDH, is 3.33 times more effective than orlistat (the standard anti-obesity drug) at inhibiting pancreatic lipase; similarly, cucumber mesocarp powder (which

contains pinocembrin) is 3.44 times more effective than orlistat (Sari, Lestari, Mariana, & Subandi, 2021). To the extent of the author's knowledge, no discussion in the literature exists concerning the potential inhibitory effects of abscisic acid or pinobanksin, the other major phenolic compounds in NZHDH, on lipase.

According to S. A. Ahmed, Salau, Khan, Saeed, and Ul-Haq (2022), chlorogenic acid (one of the major phenolic compounds in NZYC) has a better binding affinity with human pancreatic lipase (in a crystal complex form) in comparison to orlistat. Chlorogenic acid forms 9 interactions (6 strong hydrogen bonds and 3 weak electrostatic interactions) with the human pancreatic lipase receptor, while orlistat forms 6 interactions (3 strong hydrogen bonds and 3 weak electrostatic interactions (S. A. Ahmed et al., 2022). One potential adverse effect of chlorogenic acid is that it would likely bind to estrogen receptors, affecting normal female sexual development; however, further drug preparation processes would likely be able to control for this effect (S. A. Ahmed et al., 2022). S. A. Ahmed et al. (2022) concluded that chlorogenic acid was non-toxic, satisfied all drug criteria, and had greater structural flexibility, stability, hydrogen bond stability, surface area, and compactness than orlistat, making it a better, safer, and cheaper alternative to the standard drug. Similarly, Cao et al. (2020) found that chlorogenic acid was an effective inhibitor for endogenous grass carp muscle lipase, with an IC_{50} value of 0.58 mg/mL. Chlorogenic acid was shown to be a competitive inhibitor, binding to the catalytic site and changing the enzyme conformation (Cao et al., 2020).

Caffeic acid has been reported in the literature to have an IC_{50} value of 401.5 ± 3.21 μM for porcine pancreatic lipase, approximately 100 times higher than that reported for orlistat (4.0 ± 1.0 μM) (Martinez-Gonzalez et al., 2017). According to Martinez-Gonzalez et al. (2017), caffeic acid has greater affinity for the enzyme-substrate complex than for the enzyme itself, demonstrating mixed-type inhibition, and binds closely to the active site of the enzyme.

The lipase inhibitory effects of chlorogenic acid in particular, as well as caffeic acid, may account for the substantially lower inhibitory effects of NZYC (14.25 ± 1.86 mg/mL) than NZHDH (approximately 200 mg/mL). These results support the claim that consumption of yacon concentrate may support blood glucose management and show that this may be attributed to inhibition of pancreatic lipase thereby reducing the availability of glycerol for gluconeogenesis.

Table 6.3 IC_{50} values for anti-diabetes and anti-obesity assays

Sample	Alpha glucosidase IC_{50} value (mg/mL)*	Lipase IC_{50} value (mg/mL)
NZYC		14.25 ± 1.86
NZHDH		>200 (A) >200 (B) 192.3 (C)
NZYC phenolic extract	5.56 ± 0.28^{b}	
NZHDH phenolic extract	6.69 ± 0.07^{a}	

*Significant differences existed between samples at the .01 probability level.
Samples with different superscript letters are significantly different.

It is worth noting that the methods utilised in determination of anti-diabetes and anti-obesity activity are *in vitro* (i.e., conducted outside of a living organism). *In vitro* methods do provide some advantages over *in vivo* methods (conducted inside living organisms); they are conducted inside controlled environments, are typically more cost-effective, and avoid ethical concerns associated with *in vivo* methods. However, the results from *in vitro* methods do not necessarily translate directly to *in vivo* results, as *in vitro* methods do not replicate the complexity of biological systems or account for the effects of metabolism. Therefore, *in vivo* methods are required for evaluation of drug efficacy, toxicity, and safety before progressing to clinical trials.

Results from *in vitro* methods may be extrapolated to *in vivo* results; however, this requires in-depth understanding of the biological systems and metabolism in question. For instance, in the present work the anti-obesity activity of NZHDH and NZYC was carried out at a single pH; *in vivo*, the pH would change progressively through the digestive tract, and the low pH of the stomach could lead to degradation of certain phenolic compounds. Even with in vitro in vivo extrapolation (IVIVE), it is necessary to verify predicted results with results from actual *in vivo* testing.

6.4.3 Anti-bacterial activity

The results from the disk diffusion assay are presented in **Figures 6.3** and **6.4**. One plate for *S. aureus* in the trial using methanolic extracts (**Figure 6.3**) did not have a disk for the negative control (sterile water) as one disk was dropped while conducting the experimental procedure and extra disks were not prepared. However, all other plates showed no inhibition of bacterial growth for the negative control. All plates showed inhibition of growth by the positive controls; interestingly, in the trial using methanolic extracts (**Figure 6.3**) individual *S. aureus* colonies were observed growing in the zone of inhibition for the

vancomycin which may suggest that a resistant strain was isolated. However, the vancomycin disks used for the assay were from an older stock so may not have been fully effective.

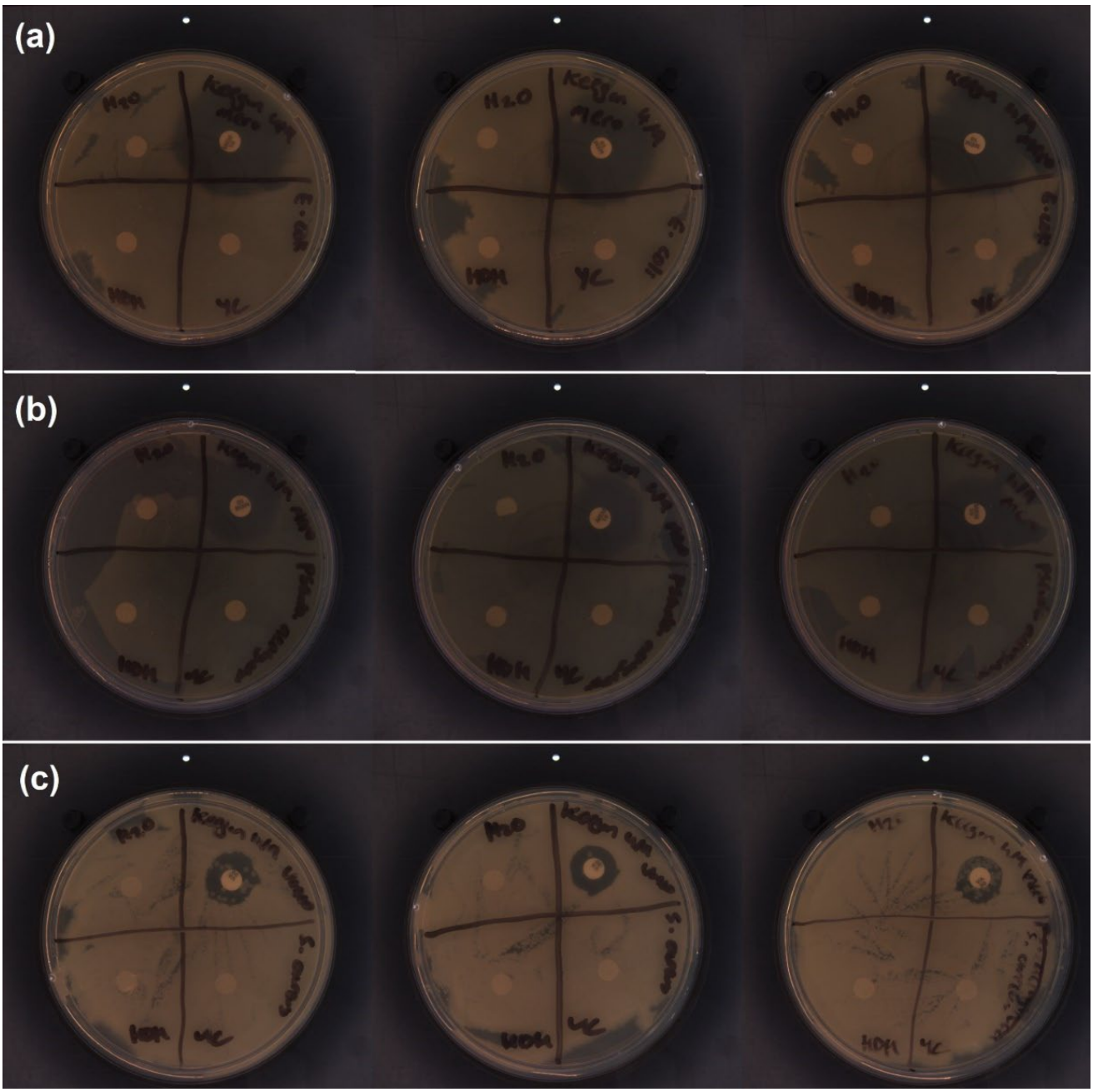

Figure 6.3 Photos of the Mueller Hinton Agar plates used for the disk diffusion assay. (a) = *Escherichia coli*; (b) = *Pseudomonas aeruginosa*; (c) = *Staphylococcus aureus*. Top left quarter = Negative control (sterile water); Top right quarter = Positive control (Meropenem for *E. coli* and *P. aeruginosa*, Vancomycin for *S. aureus*); Bottom left quarter = New Zealand honeydew honey extract in 2.5% methanol; Bottom right quarter = New Zealand yacon concentrate extract in 2.5% methanol.

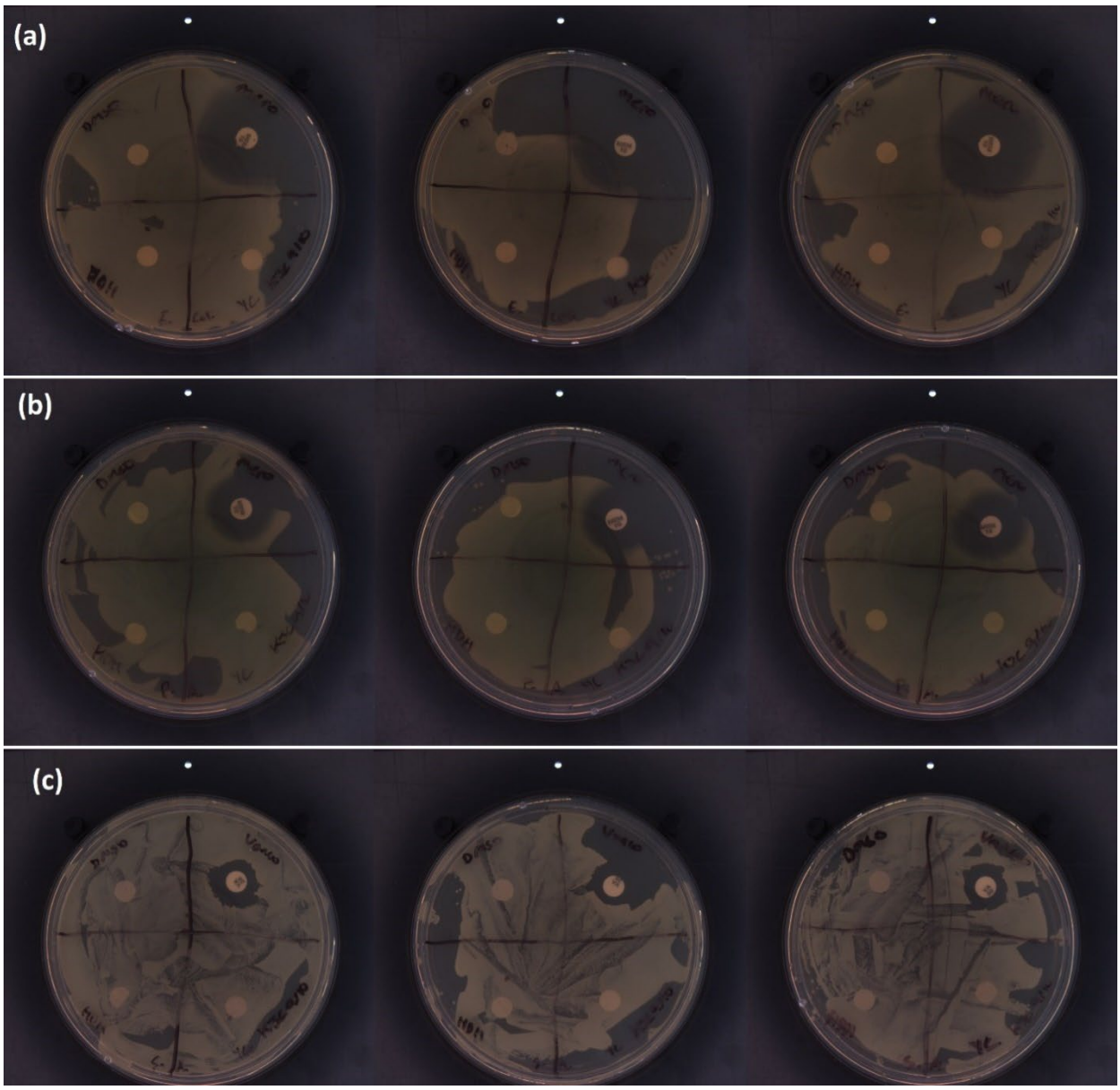

Figure 6.4 Photos of the Mueller Hinton Agar plates used for the disk diffusion assay. (a) = *Escherichia coli*; (b) = *Pseudomonas aeruginosa*; (c) = *Staphylococcus aureus*. Top left quarter = Negative control (2% DMSO in sterile water); Top right quarter = Positive control (Meropenem for *E. coli* and *P. aeruginosa*, Vancomycin for *S. aureus*); Bottom left quarter = New Zealand honeydew honey extract in 2% DMSO; Bottom right quarter = New Zealand yacon concentrate extract in 2% DMSO.

Although photographs show poor or inconsistent spread plate technique, likely due to the researcher's lack of recent practice, each photograph does show a clear zone of inhibition around the positive control disks and no zone of inhibition around the negative control (sterile water or 2% DMSO in sterile water) or NZHDH and YC extracts at either 0.8 or 10 mg/mL, indicating that NZHDH and NZYC phenol extracts exert no anti-bacterial activity on *E. coli*, *P. aeruginosa*, or *S. aureus.*

In one study carried out by Kassym, Kussainova, Semenova, and McLoone (2024), a range of phytochemicals found in honey (3-phenyllactic acid, *p*-coumaric acid, and phloretin) had no activity on *E. coli* at either 100 or 350 µg per disk in the disk diffusion assay, nor at 700 µg per well in the well diffusion assay; however, 3-phenyllactic acid did significantly inhibit the growth of *E. coli* at both 7 and 2 mg/mL in the broth culture assay. Another study carried out by Leyva-Jimenez, Lozano-Sanchez, Borras-Linares, Cadiz-Gurrea, and Mahmoodi-Khaledi (2019) assessed the antimicrobial activities of 33 honey phenolic extracts; *P. aeruginosa* was the most resistant microorganism, followed by *E. faecalis* and *S. aureus*, with *E. coli* being the least resistant. Only five of the thirty-three extracts displayed greater inhibitory effects than whole honey; these extracts were found to contain phenyl lactic acid, *p*-hydroxybenzoic acid, caffeic acid, and derivatives, pinocembrin, and pinobanksin. Pinocembrin and pinobanksin are among the most abundant phenolic compounds identified in NZHDH.

As discussed in **Chapter 6.4.2**, it is possible that the extraction method used for the phenolic extracts was not fully effective, and thus the true concentration of phenolic compounds in the extracts may be lower than calculated. However, the discussed literature suggests that the antimicrobial effects of phenolic compounds present in honey is negligible, and in most cases are only identified at concentrations greater than that at which they exist in whole honey.

6.5 Conclusion

This research demonstrated that, in a model system, the phenolic compounds present in NZHDH and NZYC do not significantly inhibit the formation of acrylamide compared to the negative control of glucose. At the concentration evaluated, individual phenolic compounds identified in NZHDH and NZYC did not result in significant differences in acrylamide formation relative to glucose, although some significant differences between individual phenolic compounds were observed. At greater concentrations, it is possible that significant differences between the negative control and some model systems containing individual

phenolic compounds could be observed; however, based on the results for NZHDH and NZYC further investigation was not conducted.

The results from the alpha-glucosidase anti-diabetes assay suggest that the phenolic extract may not have been prepared properly, as the IC_{50} values for the NZHDH extract (5.56 ± 0.28 mg/mL), as well as that for the NZYC extract (6.69 ± 0.07 mg/mL), were one to two orders of magnitude higher than values reported in literature for other varieties of honey, indicating they were significantly less effective at inhibiting alpha-glucosidase. Pinobanksin, pinocembrin, chlorogenic acid, and caffeic acid – some of the major phenolic compounds in NZHDH and NZYC – have all been shown in the literature to inhibit various types of alpha-glucosidase with varying degrees of effectiveness. However, the results from the lipase anti-obesity assay demonstrate that NZYC has the potential to inhibit lipase with an IC_{50} value of 14.25 ± 1.86 mg/mL. Chlorogenic acid, the major phenolic compound in NZYC, has been shown in the literature to be a more effective inhibitor for human pancreatic lipase than orlistat, the standard drug used in the treatment of obesity.

The results from the disk diffusion assay show that the phenolic extracts of NZHDH and NZYC were unable to inhibit the growth of *P. aeruginosa*, *S. cerevisiae*, or *E. coli*. This is in agreement with the cited literature, with antimicrobial effects of honey attributed to non-phenolic phytochemicals, and honey phenolic extracts often only displaying some inhibitory effects at concentrations greater than that which they exist at in whole honey.

7 Overall conclusion and future research directions

The overall aim of this research was to expand our understanding of NZHDH and NZYC, and to incorporate these foods into novel food products and applications. In this thesis, four studies were conducted to achieve the objectives listed in Chapter 1. These objectives include (1) producing comprehensive chemical and/or nutritional analyses of NZHDH, NZYC, and functional LAB beverages (2) comparing flavour and sensory attributes of different LAB samples using chemical analysis and sensory evaluation, (3) understanding the consumer acceptance of the formulated LAB samples among New Zealand consumers, and (4) evaluating acrylamide-reducing, anti-diabetes, anti-obesity, and anti-bacterial effects of NZYC, NZHDH, or their phenolic constituents.

Each chapter fulfils at least one or more of the objectives stated and was conducted in a manner to further expand our understanding of the physicochemical, sensory, or health-promoting properties of the NZHDH, NZYC, and/or LAB beverages. Below is a summary of each experimental chapter:

7.1 Summary and implications of Chapter 3

The proximate composition, mineral, sugar, phenolic, and amino acid profiles, and antioxidant activity of NZHDH were reported in this study. The proximate composition was consistent with the discussed literature. Potassium was the major mineral (238.6 ± 10.5 mg/100g), with total mineral content comparable to ash content. The combination of fructose and glucose ranged from 55.239 to 56.575 g/100g, acceptable values for HDH, while erlose content was higher than melezitose - consistent with literature for NZHDH produced by *Ultracoelostoma assimile*. Total phenolic content was 62.30 ± 6.261 mg gallic acid equivalents per 100 g; pinocembrin, abscisic acid, and pinobanksin were the most abundant phenolic compounds. L-proline was the major amino acid. The antioxidant activities of NZHDH, determined by the FRAP, CUPRAC, and phosphomolybdenum assays, were comparable to results obtained in the literature for Mānuka honey, which is considered to be the "gold standard" among honeys in terms of antioxidant activity

Overall, the novelty of this research includes comprehensively chemically profiling NZHDH, including the first analysis of amino acids in NZHDH and the first identification of the phenolic compound sakuranetin in NZHDH. From these results, it is suggested that NZHDH has similar health-promoting benefits to other varieties of HDH more well-discussed in the literature, as well as to Mānuka honey.

The purpose of **Chapter 3** is to comprehensively chemically profile NZHDH. Food manufacturers and researchers now have a greater understanding of the chemical composition of NZHDH, and how it relates to potential health benefits. As NZHDH is an underutilised resource, particularly in comparison to Mānuka honey, further utilisation of NZHDH should be considered to increase consumer awareness and market share.

7.2 Summary and implications of Chapter 4

The proximate composition, mineral, sugar, phenolic, amino acid, and organic acid profiles, antioxidant activity, and glycaemic index of NZYC were reported in this study. The moisture content of NZYC was lower than values discussed for YC in the literature, while protein content was higher; however, the total carbohydrate content of around 80 g/100g was consistent with the product claim of 80° Brix. Potassium was the major mineral (658.4 ± 5.9 mg/100g). Fructose was the major sugar, while total FOS and inulin content (determined semi-quantitatively) varied widely between batches (17.6 ± 0.3 to 52.3 ± 0.8 g/100g). Total phenolic content ranged from 565.0 ± 9.3 to 785.1 ± 43.1 mg gallic acid equivalents per 100 g; chlorogenic acid was the major phenolic compound. L-arginine, L-glutamic acid, L-proline, L-aspartic acid, and asparagine were the major amino acids, while citric acid was the major organic acid. The antioxidant activities of NZYC, determined by the FRAP, CUPRAC, and DPPH assays, were several times higher than results obtained in the literature for Mānuka honey and goji berries, two foods of interest for their purportedly high antioxidant activities. The glycaemic index was 40 ± 0.22, which classifies NZYC as a low-GI food.

Overall, the novelty of this research includes adding significant knowledge to the literature in terms of the chemical composition of NZYC. The high phytochemical contents in NZYC, including phenolic and flavonoid compounds with proven antioxidant capacities, as well as the low glycaemic index value, could support its classification as a nutraceutical food product for future new diet therapy applications.

The purpose of **Chapter 4** is to comprehensively chemically profile NZYC and provide information on its glycaemic index. Food manufacturers and researchers now have a greater understanding of the chemical composition of NZYC, and how it relates to potential health benefits. As NZYC is a recent product to the market and has particularly high (although variable) FOS content, further utilisation of NZHDH should be considered to increase consumer awareness and market share.

7.3 Summary and implications of Chapter 5

This study seeks to formulate functional LAB beverages containing NZYC and/or NZHDH by means of thermal distillation, a method accessible to home brewers but disregarded in the literature. Chemical analysis and sensory evaluation were conducted to establish the different physicochemical, sensory, and flavour properties of the six different LAB samples. All six samples were below the 1.15% ABV threshold to be classified as LABs in New Zealand. Samples containing NZYC were found to contain fructose after acid hydrolysis, indicative of the presence of FOS, while samples without NZYC did not. Chemical analysis using SPME-GC-MS was employed to understand the volatile compounds present in NZHDH and NZYC; certain compounds associated with 'floral' and 'earthy' aroma had higher internal standard response ratios (ISSRs) in NZHDH, while certain compounds associated with 'floral' aroma had higher ISSRs in NZYC. Although not all results were significant, the colour of samples containing NZYC tended to be darker than samples which did not. Only one significant difference was identified across all LAB samples with respect to amino acids; LoHDH 1 had significantly lower L-arginine content (68.43 ± 2.80 mg/L) than HiYC 1 (113.09 ± 2.30 mg/L).

Sensory evaluation was conducted using unstructured line scales, Check-All-That-Apply (CATA), and Just-About-Right (JAR). Fifty-three subjects (n = 31 males) completed the sensory evaluation and were analysed for this study. On the unstructured line scales, no significant differences were identified with respect to overall liking, or liking of appearance, aroma, flavour, or mouthfeel. No significant interactions were identified between LABs and subject age, gender, or frequency of beer consumption. All scores had broad standard deviations with mean values that tended to fall between 50 and 70 on unstructured line scales, indicating that samples were not strongly liked but tended to be more liked than disliked. From CATA, attributes such as brown, carbonated (appearance), foamy, yellow, and sour were statistically significant when describing LABs. Correspondence analysis showed that LoYC and HiYC were closely grouped in the negative scores of the first dimension and correlated with appearance terms of foamy and brown, while Control and HiHDH samples had high positive scores along dimension one and were correlated with attributes of yellow and carbonated. For JAR, a high proportion of subjects indicated that bitterness, hoppiness, and sweetness were not enough in all six LAB samples.

Research on LAB produced using thermal distillation is scant in the literature, and this study gives insight into how LABs may be produced using this method. The presence of FOS was confirmed in samples flavoured with NZYC, indicating that it may be possible to produce a functional LAB beverage containing NZYC. Sensory participants in this study indicated that the LABs lacked sufficient bitterness, hoppiness, and sweetness. When launching functional LAB beverages to the New Zealand market, practitioners need to optimise these variables to increase overall liking.

7.4 Summary and implications of Chapter 6

This study explored the potential health benefits of NZHDH and NZYC for the reduction of acrylamide formation, anti-diabetes, and anti-obesity effects, and anti-bacterial effects against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. For the reduction of acrylamide, neither crude NZHDH, crude NZYC, nor individual phenolic compounds present in NZHDH or NZYC resulted in significantly reduced acrylamide relative to the negative control. However, systems prepared with certain individual phenolic compounds contained significantly more acrylamide than the positive control (gallic acid), while caffeic acid and abscisic acid resulted in non-significant decreases in acrylamide relative to the negative control.

Phenolic extracts of NZHDH and NZYC exerted moderate anti-diabetes effects in terms of the alpha glucosidase assay, while crude NZYC exerted significant anti-obesity effects in terms of the lipase assay. These results support the claim that consumption of YC may support blood glucose management and suggest that this may be due to inhibition of pancreatic lipase. Phenolic extracts of NZHDH and NZYC were unable to inhibit the growth of any of the tested bacteria, implying that phenolic compounds are not responsible for any anti-bacterial effects that may be caused by NZHDH and/or NZYC, which agrees with the discussed literature.

7.5 Future direction of the research

The results from the chemical profiling of NZHDH and NZYC (**Chapters 3** and **4**) support further investigation into the health benefits of NZHDH and NZYC and their incorporation into novel food products. The results from the sensory evaluation of formulated LAB beverages suggest that reformulation would be required before further work can be conducted, particularly to increase the bitterness, hoppiness, and

sweetness of the LABs. After reformulation, if the overall liking of the LABs is successfully increased, descriptive analysis or quantitative descriptive analysis could be conducted to identify the intensity of specific attributes that make the formulated LAB beverages unique. Results from **Chapter 5** can aid the process of generating sensory terms and attributes that are likely to be important in the descriptive analysis process. Additionally, researchers may consider combining sensory data (such as CATA) with physicochemical data (such as volatile profile) to establish correlations between these two factors. Finally, results from the lipase anti-obesity assay suggest that NZYC may be able to inhibit pancreatic lipase and thereby support blood glucose management. Clinical trials on these potential effects may be considered by researchers.

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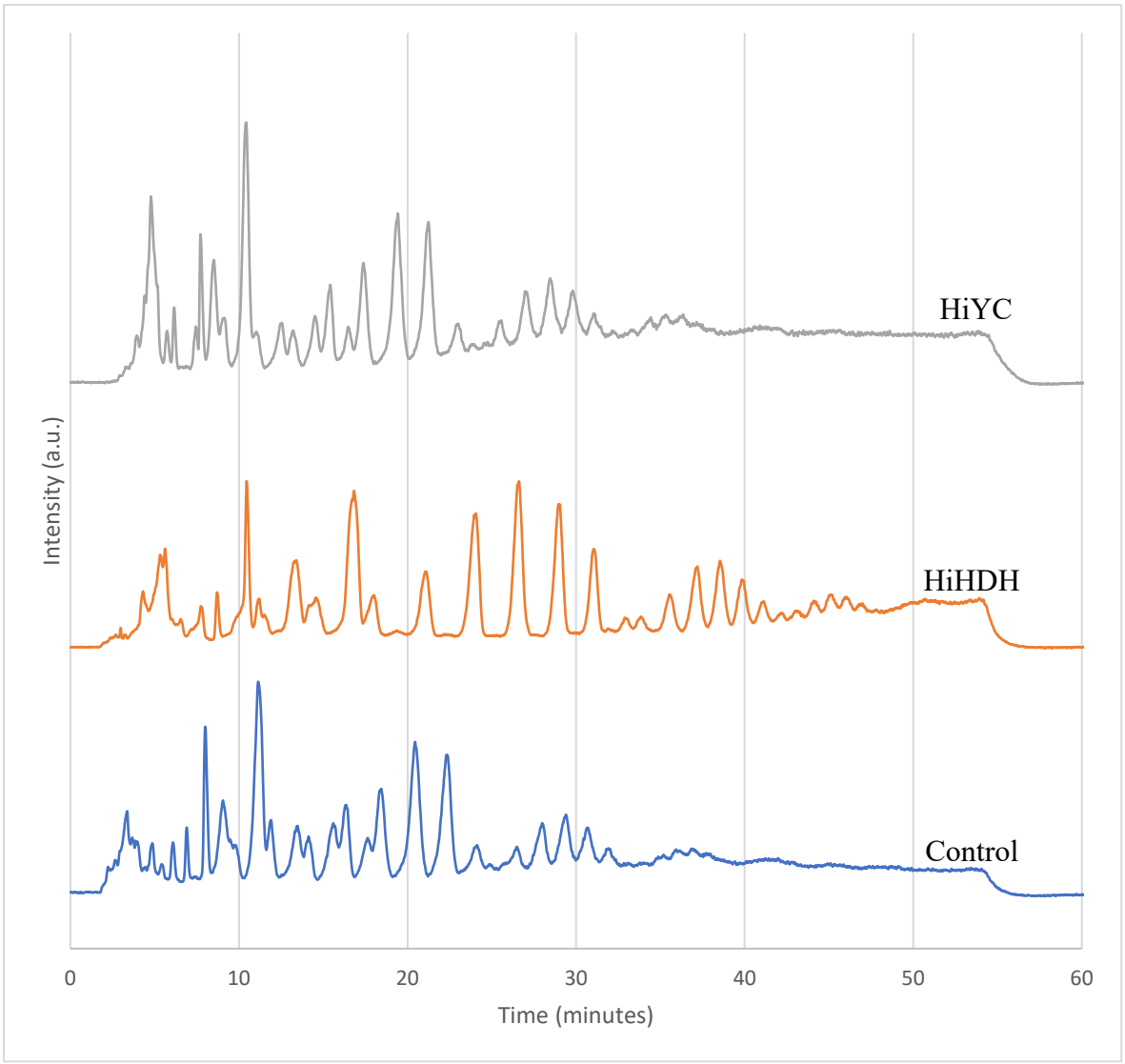
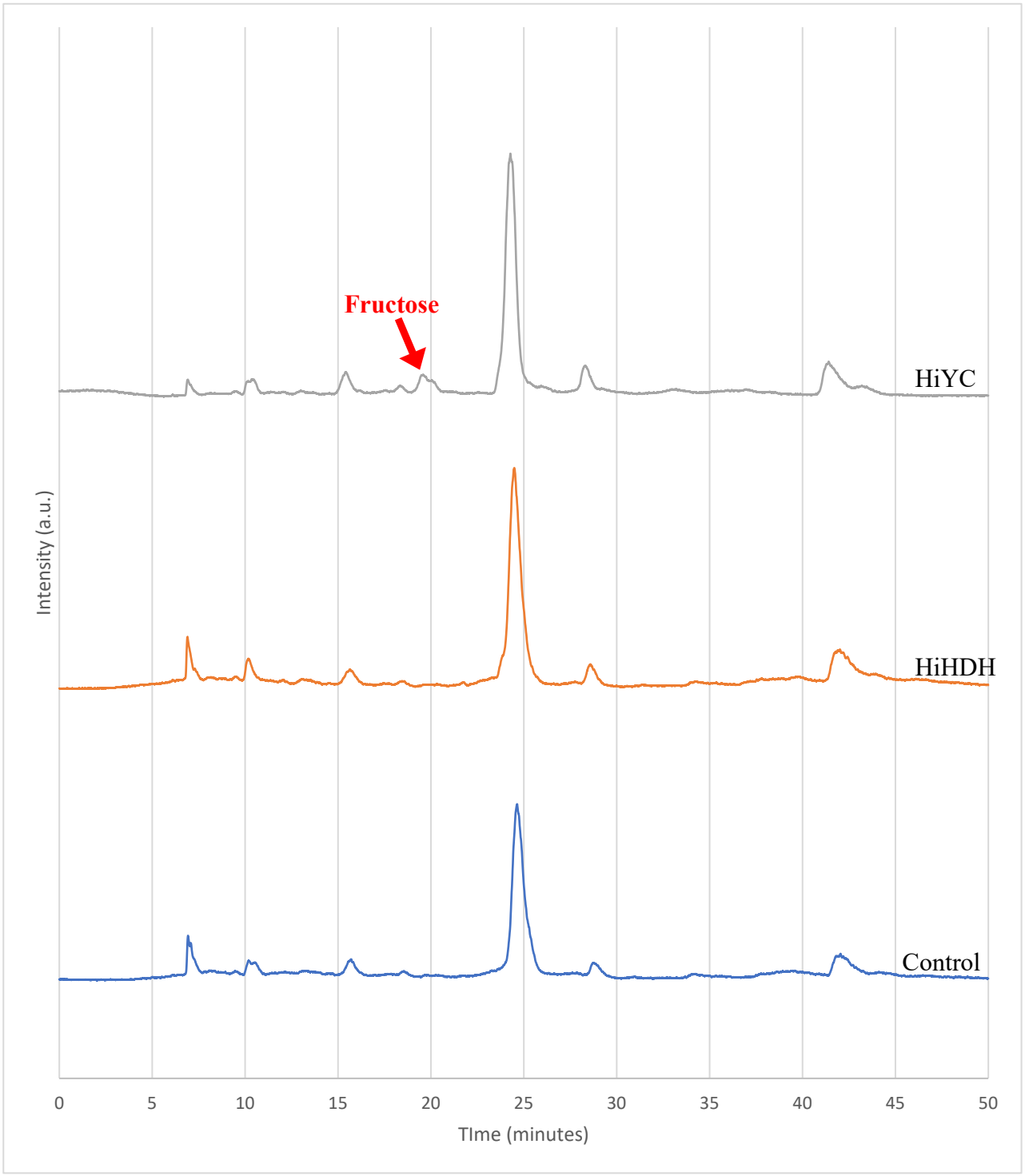
Appendix A. Photo of the Grainfather mash tun model no. 10191 with a T500 distillation column

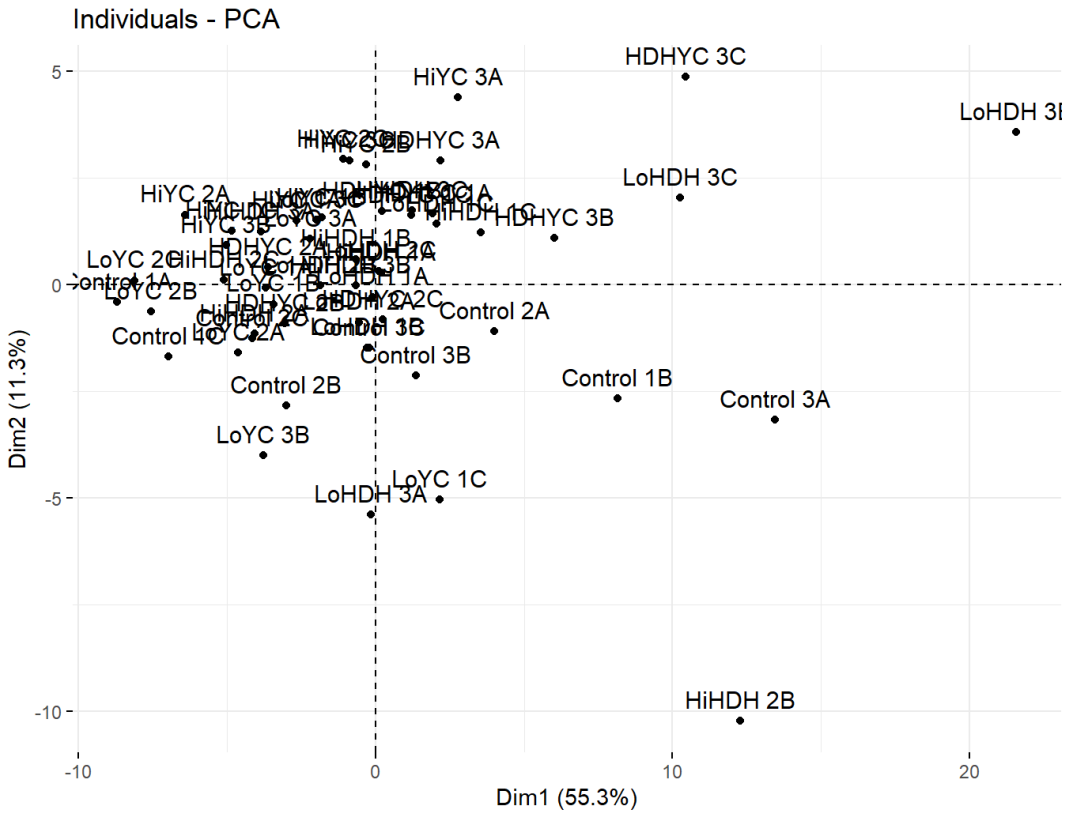
Appendix B. HPLC-ELSD chromatogram of time (minutes) vs intensity (arbitrary units) for samples 'Control 1a', 'HiHDH 1a', and 'HiYC 1a' prior to acid hydrolysis.



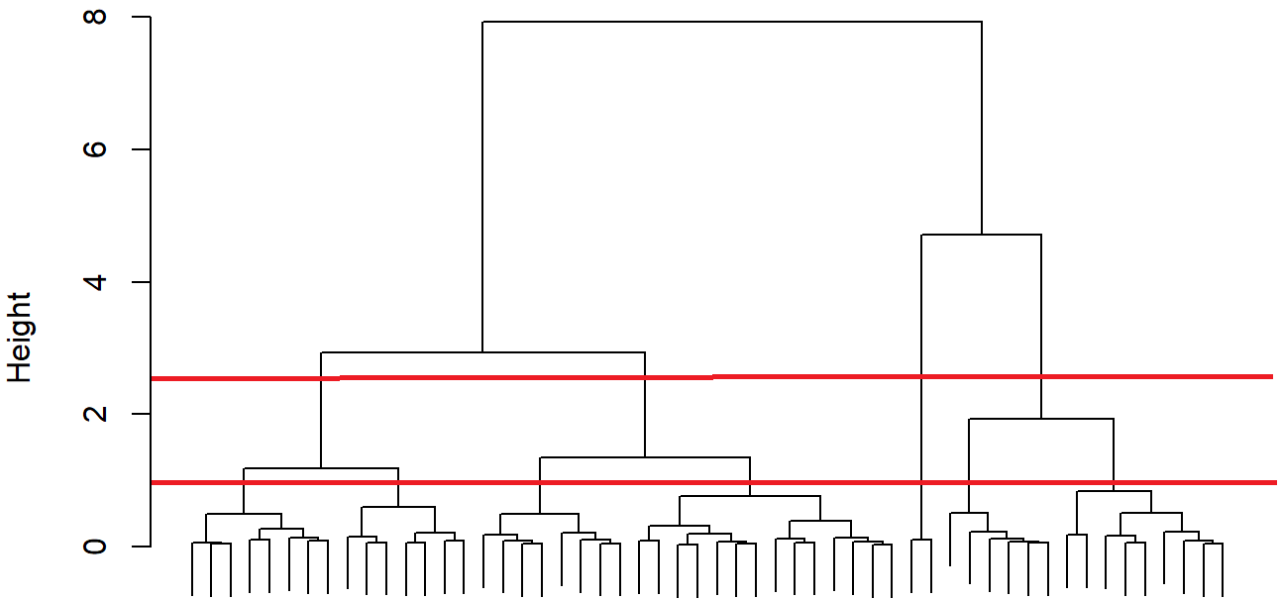
Appendix C. HPLC-ELSD chromatogram of time (minutes) vs intensity (arbitrary units) for samples 'Control 1a', 'HiHDH 1a', and 'HiYC 1a' after acid hydrolysis, showing a distinct fructose peak at 20 minutes.



Appendix D. Principal component analysis, hierarchical cluster analysis, and k-means clustering for low alcohol beer samples



Results of the principal component analysis of three production batches (1, 2, 3) of six low alcohol beer samples (Control, LoYC, HiYC, LoHDH, HiHDH, HDHYC) with triplicates (A, B, C) from the SPME-GC-MS analysis.



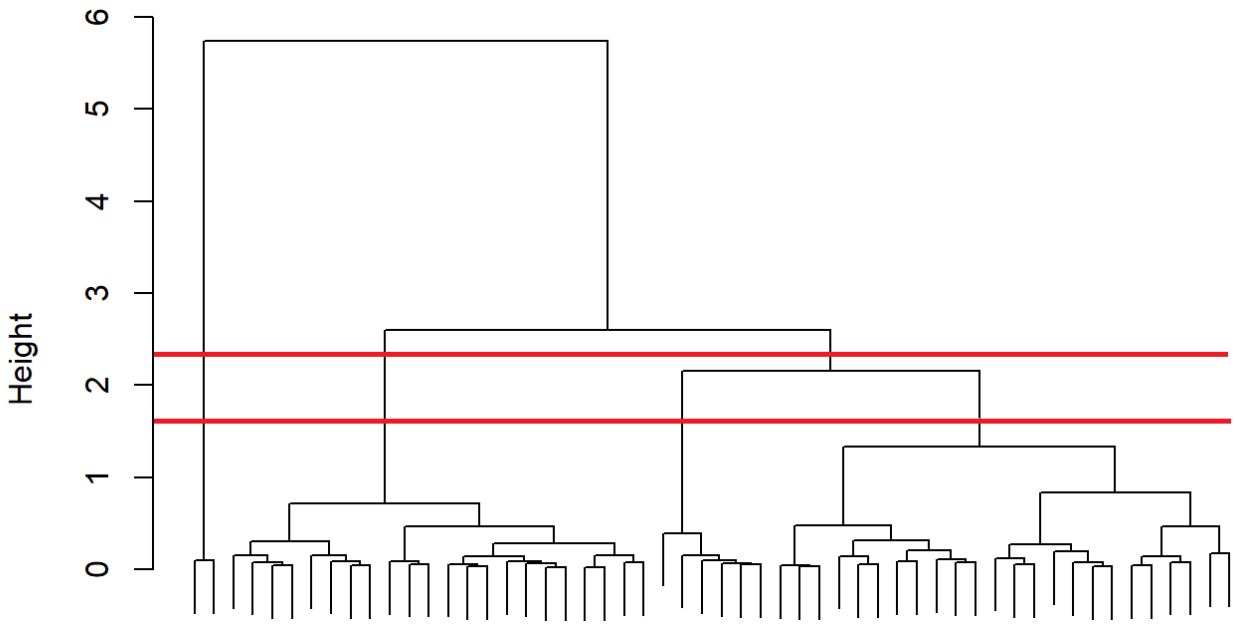
Cluster dendrogram for complete-linkage hierarchical cluster analysis of six low alcohol beer samples (Control, LoYC, HiYC, LoHDH, HiHDH, HDHYC) with triplicates from the SPME-GC-MS analysis.

Composition of the clusters from complete-linkage hierarchical cluster analysis, cut at four clusters.

Cluster	Control	HDHYC	HiHDH	HiYC	LoHDH	LoYC
1	4	3	2	2	4	0
2	2	0	2	2	1	8
3	2	6	4	5	4	1
4	1	0	1	0	0	0

Composition of the clusters from complete-linkage hierarchical cluster analysis, cut at seven clusters.

Cluster	Control	HDHYC	HiHDH	HiYC	LoHDH	LoYC
1	2	2	1	1	3	0
2	1	0	1	0	0	5
3	1	0	1	2	1	3
4	1	3	1	2	0	1
5	1	3	3	3	4	0
6	1	0	1	0	0	0
7	2	1	1	1	1	0



Cluster dendrogram for average-linkage hierarchical cluster analysis of six low alcohol beer samples (Control, LoYC, HiYC, LoHDH, HiHDH, HDHYC) with triplicates from the SPME-GC-MS analysis.

Composition of the clusters from average-linkage hierarchical cluster analysis, cut at three clusters.

Cluster	Control	HDHYC	HiHDH	HiYC	LoHDH	LoYC
1	6	3	4	4	5	8
2	2	6	4	5	4	1
3	1	0	1	0	0	0

Composition of the clusters from average-linkage hierarchical cluster analysis, cut at four clusters.

Cluster	Control	HDHYC	HiHDH	HiYC	LoHDH	LoYC
1	4	2	3	3	4	8
2	2	6	4	5	4	1
3	1	0	1	0	0	0
4	4	2	1	1	1	0

Composition of the clusters from k-means clustering, cut at six clusters.

Cluster	Control	HDHYC	HiHDH	HiYC	LoHDH	LoYC
1	2	0	2	2	1	8
2	2	2	1	1	3	0
3	1	0	1	0	0	0
4	1	3	2	2	1	1
5	1	3	2	3	3	0
6	2	1	1	1	1	0