Does a vegetable-enriched Nothing Else™ bread have benefits to health?

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Abstract

Bread is one of the most consumed foods, world-wide and in New Zealand. Currently, the paradigm of new bread and functional food development offers the opportunity for incorporation into wheat flour plant-based ingredients such as vegetables, seeds and whole grains to allow for the making of health claim on a food product. In addition, there is a call for food to be less processed and to contain sustainable ingredients, and for food labels to be simplified and clean. The Auckland University of Technology (AUT) Nothing Else™ front-of-pack brand can only be used on products with eight or fewer wholesome ingredients and would be appropriate for a healthier bread. The overarching research question that drove this research was "How can food science and nutrition inform the development of a vegetable-enriched Nothing Else™ bread, accepted by consumers, with potentially verifiable health claims?" Six chapters describe the body-of-work undertaken to answer this question.

The first part of the review of literature (**Chapter 2**) focuses on the effect of the enrichment of bread with functional ingredients on its physical and sensory attributes, including the swallowing of bread. Swallowing was investigated because as a population ages the prevalence of chewing and swallowing difficulties increases. In addition, many older people have discretionary funds which potentially increase their ability to buy palatable and nourishing bread which is easy to swallow.

In collaboration with an advisory support group, two breads (VB75 and VB100) were formulated and produced in-house at the AUT School of Hospitality kitchens with eight ingredients including drum-dried pumpkin and sweet corn flours, flaxseed, and sprouted wheat and wholemeal wheat flours (**Chapter 3**).

The first experimental study (**Chapter 4**) evaluated the physical, subjective ease of chewing and swallowing and sensory attributes for four breads of increasing

healthiness: commercial white (WB) and wheatmeal (WMB) $1 breads, and the two in-house breads enriched with mixes of pumpkin and sweet corn flours (VB75 and VB100). Objective texture measures (TA.XT.plus texture analyser, Stable Microsystems, Surrey, UK) were assessed for four 11.5 mm thick bread crumb slices of the WB, WMB, VB75 and VB100 breads. Fifty physically active volunteers aged 50+ years rated on Likert scales liking and ease of bite and getting the bread into the mouth, ease of chewing and swallowing, and ease of bread bolus movement through the throat. Number of chews before swallowing were also recorded. The VB75 and VB100 were liked almost twice as much as the WB and WMB in all the sensory attributes assessed. Overall liking of all breads was correlated with ease of swallowing: VB75 r=0.597, 95% CI (0.382, 0.751); VB100 r=0.665, 95% CI (0.474, 0.796); WB r=0.422, 95% CI (0.163, 0.627); and WMB r=0.475, 95% CI (0.227, 0.665). Objectively, the breads were not different in hardness but differed in measures of chewiness, resilience, cohesion, springiness and gumminess. Subjectively and objectively, WB and WMB recorded the highest chewiness values (50% higher than VB75 and VB100). The vegetable-enriched breads required less chewing and were rated twice as easy to chew by the participants. After swallowing, the vegetable-enriched breads were perceived by the participants to move almost twice as easily through the throat compared to the commercial breads.

The second literature review (**Chapter 5**) was a systematic search for published literature in the databases PubMed, Web of Science and Cochrane Library via Wiley and OVID on reports of the validation of “health claims” or “health effects” of bread enrichment with “functional ingredients”. The main published finding was that functional bread has the potential to attenuate glycaemic response and reduce appetite which may reduce risk for diet-related non-communicable diseases.

In the second experimental study (**Chapter 6**), the VB75 (VB) from the first study (**Chapter 4**) was selected and investigated for its glycaemic and appetite suppression potential when compared with commercial WMB and WB over a 120-minute period. After an overnight fast, 10 apparently healthy, young participants (23±7 years, 70% Pacific) received in random order on three separate days, 75g of the three breads WB, WMB and VB. Blood was sampled before and 15, 30, 45, 60, 90 and 120 minutes after the bread consumption. The incremental-area-under-the-curve (iAUC) for glucose and insulin was determined and an appetite questionnaire completed by participants at each timepoint. The mean glucose iAUC were not different among breads. However, the mean insulin iAUC for the VB was significantly less than for the WB and WMB; VB and WB 12415pmol/L*minutes (95% CI 1918, 22912pmol/L*minutes, p=0.025) and VB and WMB 13800pmol/L*minutes (95% CI 1623, 25976 p=0.031). Over the 120-minute period following consumption, compared to the WB and WMB, VB stimulated a stronger fullness feeling in the participants.

The VB (β-carotene 236.78µg/100g and fibre= 7.2/100g) could be an effective carrier for the delivery of carotenoids and fibre if consumed every day. The third and final experimental pilot study (**Chapter 7**) primarily sought to investigate whether this vegetable-enriched bread, when consumed every day (200g/day) over two weeks, would increase plasma carotenoid concentration and improve lipid profile. A secondary analysis was carried out for criterion validation of the Veggie meter™ reflection spectroscopy measures of skin carotenoid scores against laboratory-based plasma carotenoid concentration, and for construct validation against a six-item carotenoid food frequency questionnaire. The daily consumption of 200g of VB over two weeks showed no measurable change in the plasma carotenoids or lipid concentrations. There was a strong correlation (r=0.845 and 95% CI 0.697, 0.924) between the plasma carotenoid concentration and the Veggie meter™ scores (n=30). A positive correlation was observed for participants (n=10) average baseline and post-intervention vegetable intake and their plasma carotenoid measure (r=0.446, 95% CI -0.255, 0.840 and r=0.255, 95% CI -0.446, 0.762 respectively) and skin carotenoid reflection score (ρ=0.489 and Ϯ=0.689). Reported compliance with consumption of the bread was 100%.

In conclusion, this proof-of-principle work (**Chapter 8**) has demonstrated that, when compared to WB and WMB, vegetable-enriched breads have the potential to improve nutrition status and 'ease of swallowing' especially in older people and those who are susceptible to dysphagia. The consumption of VB was associated with a lower insulin release and higher satiety which may be related to the higher fibre and less-chewy texture of the VB. Finally, the consumption of 200g VB for two weeks caused no substantial changes in plasma carotenoid and lipid concentrations. Future works should focus on the long term consumption of the VB and its association with any changes in glucose tolerance, weight, lipid profile and gut microbiome. Additionally, the need to investigate the economics and commercial viability of bread production using novel functional ingredients such as dried vegetables and clean-label branding such as the Nothing Else™ brand is encouraged.

Keywords: Vegetable-enriched bread, white bread, wheatmeal bread, physical quality, liking, swallowing, glycaemic response, appetite suppression, carotenoids, lipid profile, Veggie meter™

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Abbreviations

AUTEC	Auckland University of Technology Ethics Committee (AUTEC)
BMI	Body Mass Index
CE	Catechin equivalence
CI	Confidence Interval
FFQ	Food frequency questionnaire
GAE	Gallic Acid Equivalent
GI	Glycaemic index
GIP	Gastric inhibitory polypeptide
GL	Glycaemic load
GLP-1	Glucagon-like peptide 1
HDLc	High density lipoprotein cholesterol
HSRCS	Health Star Rating Calculator Score
IA	Isaac Amoah
iAUC	incremental-area-under-the-curve
IDDSI	International Dysphagia Diet Standardisation Initiative
LDLc	Low density lipoprotein cholesterol
NPSC	Nutrient Profile Scoring Criterion
NPS	Nutrition Profiling Score
SE	Standard Error
SEM	Standard Error of the Mean
T2DM	Type 2 diabetes mellitus
VB	Vegetable-enriched bread
WB	White bread
WMB	Wheatmeal bread

Glossary

Acceptability: Used synonymously for the liking of bread.

Carotenoid reflection score: A score between 0 to 800 of the amount of light reflected from the fingertip at 455nm.

Consumer: Somebody who eats bread.

Dysphagia: Difficulty with swallowing.

Functional ingredients: Ingredients when utilised in bread-making allow for the making of a health claim on bread besides providing nutritional nourishment.

Functional bread: Bread with health benefits beyond normal nutrition.

Flour: Dried powder from an edible food with lower moisture content and good shelf storage properties. Powder and flour have been synonymously used in some parts of the thesis.

Lipaemia: Increased turbidity or cloudiness in blood samples due to high triglyceride concentration.

Obesity: A form of malnutrition characterised by accumulation of excessive fat.

Pomace: By-product of fruit and vegetable after juice has been extracted and removed.

Physical properties of bread: The intrinsic and physical attributes of bread.

Presbyphagia: This describes changes in swallowing dynamics of older people due to age-associated changes in the mechanisms of swallowing which may increase risk for aspiration and regurgitation.

Triacylglycerol: This is used to refer to triglyceride.

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 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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(Isaac Amoah, BSc, MPhil)

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Co-authored works presented at conferences

Amoah, I., Cairncross, C., & Rush, E. (2019, November). Beyond nutrition: From conceptualization to development—A case study of a bread. *Multidisciplinary Digital Publishing Institute Proceedings*, 37(1), 12. https://doi.org/10.3390/proceedings2019037012. Oral presentation at the *New Zealand Nutrition Society Conference* at Eastern Institute of Technology, Napier, New Zealand.

Amoah, I., Cairncross, C., & Rush, E. (2019, October). Evaluation of the glycaemic and appetite suppression potential of a vegetable-enriched bread. Poster presented at *Food Structures, Digestion and Health International Conference* at Novotel Lakeside Rotorua, New Zealand.

Amoah, I. (2019, August). Bread with vegetables: for that feel full feeling and regulation of blood sugar. Paper presented at the *Auckland University of Technology 3 Minute Thesis Finals*, Auckland, New Zealand.

Amoah, I., Cairncross, C., & Rush, E. (2018, July). Objective and subjective measures of vegetable-enriched bread in relation to ease of chewing and swallowing. Poster presented at the *Riddet Institute Conference 2018* at InterContinental Hotel, Wellington, New Zealand.

Amoah, I., Cairncross, C., & Rush, E. (2017, December). Development and validation of a functional "Nothing Else™" bread. Oral presentation at *Auckland Nutrition Research Network* at University of Auckland, New Zealand.

Amoah, I., Cairncross, C., & Rush, E. (2017, November). Developing a commercially viable functional bread: the synergistic impact of bringing together expertise from varied backgrounds (case study). Oral presentation at the *Postgraduate and Early Career Nutrition Conference* at Tamaki Yacht Club, Auckland, New Zealand.

Amoah, I., Cairncross, C., & Rush, E (2017, November). Health effects of functional bread – A review. Poster presented at the *7th International Symposium on Delivery of Functionality in Complex Food Systems*, Grand Millennium Hotel, Auckland, New Zealand.

Researcher’s practical contribution towards this work

Concept and creation of bread (Chapter 3)

- Networking with Professor Elaine Rush and members of the AUT food network, Bakers Research Institute, Cedenco NZ Ltd, Dave Brown, Professor Owen Young prior to formation of an advisory network for the research process.
- Facilitation of the delivery of bread ingredients for bread-making
- Co-designing with Arno Sturny, the vegetable-enriched breads were developed

Physical qualities, liking and ease of swallowing study (Chapter 4)

- Preparation and processing of ethics form for approval by Auckland University of Technology Ethics Committee (AUTEC) for the study
- Facilitation of the delivery of bread ingredients for bread-making
- Involved in the vegetable-enriched bread preparation
- Conducting the participants’ subjective liking and ease of swallowing study
- Evaluation of the physical qualities of the bread including colour, specific volume, and using the texture analyser to objectively measure the textural attributes of the bread crumb

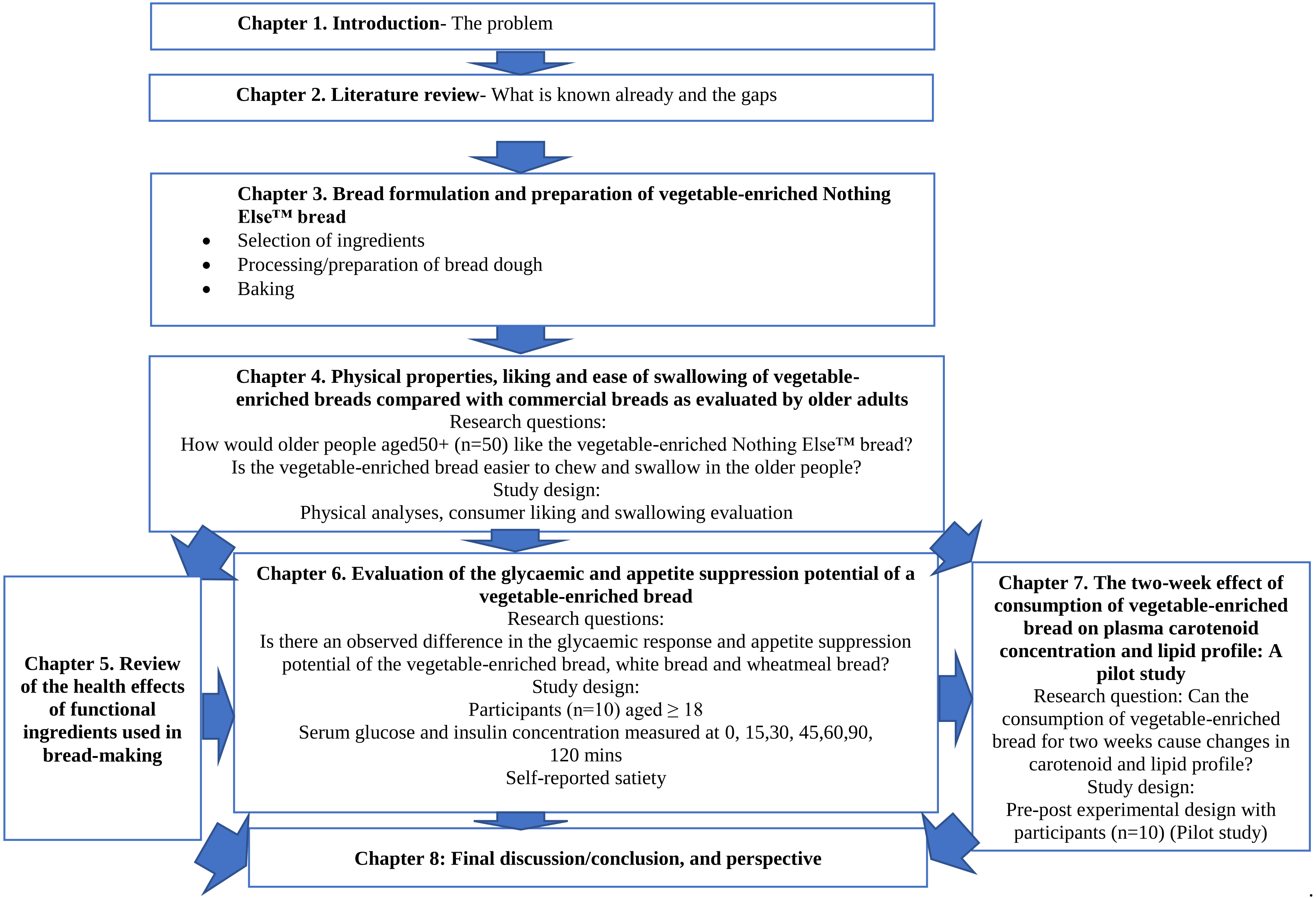
Glycaemic and appetite response study (Chapter 6)

- Preparation and processing of ethics form for approval by AUTEC for the study
- Facilitating the ordering and purchasing of blood sampling devices including syringes, vacutainers, canula, IV leur, posiflush and tegaderm
- Coordination and organisation of participants for the trial
- Centrifugation and separation of serum aliquot from participants’ drawn blood
- Laboratory investigation of serum glucose and insulin concentration
- Evaluation of participants’ subjective liking and swallowing perceptions of the bread

Carotenoid and lipid profile study (Chapter 7)

- Preparation and processing of ethics form for approval by AUTEC for the study
- Facilitating the ordering and purchase of blood sampling devices including syringes and vacutainers
- Coordination and recruitment of participants for the trial
- Centrifugation and separation of plasma aliquot from participants' drawn blood for carotenoid and lipid profile analyses
- Plasma carotenoid analysis was carried out by Canterbury Health Laboratory in Christchurch. My role was to prepare the sample for courier delivery to the laboratory in Christchurch for the analysis of carotenoid concentration

Thesis Flow Chart



Chapter 1. Introduction

The problem

Globally, the prevalence of diet-related non-communicable diseases such as type 2 diabetes mellitus, cardiovascular disease, and obesity is increasing (H. Wang et al., 2016). In the case of New Zealand, the prevalence of self-reported type 2 diabetes mellitus for adults aged 25years and over stood at 6.5% in 2017 (Ministry of Health, 2019d). Disparity amongst non-communicable diseases prevalence exists within the various ethnicities in New Zealand. For example the prevalence of type 2 diabetes mellitus is higher for Pacific people 15.7% compared with Māori 9.3%, Asian 7.6% and New Zealand European/Others 5.2% in 2017 (Ministry of Health, 2019d). Obesity (which is a measured body mass index of 30+ for adults aged 18 years and over, or equivalent for those less than 18 years) prevalence amongst New Zealand adults was 32.2% in 2017 (Ministry of Health, 2019a). There however exists variations in obesity prevalence amongst the various ethnicities with Pacific adults recording 65%, Māori adults 47.5%, European/Other adults 30.7% and Asian adults 15.1% in 2017 (Ministry of Health, 2019a). In regards to cardiovascular disease, the prevalence of high blood pressure amongst New Zealand adults aged 15+ years is 16.5% in 2017 (Ministry of Health, 2019b). In the context of New Zealand, evaluation of high blood pressure prevalence involved using data from individuals who have been informed by their doctor to have high blood pressure and those currently taking medication regularly for high blood pressure management (excluding pregnant women) (Ministry of Health, 2019b). Despite European/Other group recording a higher prevalence of 17.3%, Pacific and Māori adults who recorded prevalence of 13.7% and 15.4% respectively were more at risk of developing high blood pressure in 2017 (Ministry of Health, 2019b). The prevalence of high blood cholesterol (treated with medication) amongst New Zealand adults was 10.9% in 2017 (Ministry of Health, 2019c). In terms of disparity amongst ethnicities, adults of Pacific recorded 10.7%, Māori origin 9.4%, Asian origin 6.7% and those of European/Other ethnicities recorded 11.3% in 2017 (Ministry of Health, 2019c). Globally, mortality attributed to diet-related non-communicable diseases is 80% (World Health Organization, 2018). The high mortality (80%) is related to the quality and quantity of the food supply, dietary patterns (Mohammadifard et al., 2017; Schulze, Martinez-Gonzalez, Fung, Lichtenstein, & Forouhi, 2018) and the cost of foods (Pearson-Stuttard et al., 2017). It is recommended that for good health, a variety of wholesome foods in sensible quantities should be consumed each day (Food and Agriculture Organization of the United Nations, 2018). But one of the main concerns is the low consumption of vegetables as consumers are not meeting the recommendation in the food-based dietary guidelines. For example, in New Zealand, 60% of adults do not consume the recommended three or more servings of vegetables a day (Ministry of Health, 2016). At the time of writing this Thesis, starchy and non-starchy vegetables were not separated in the questionnaire used for the New Zealand health survey (Ministry of Health, 2016). In addition to diet impacting on the development of non-communicable diseases, lack of physical activity is associated with chronic disease development (Wahid et al., 2016; Warburton & Bredin, 2017). In the case of New Zealand, the prevalence of physically active adults who exercised for at least 30 minutes on five or more days is at 48% (Ministry of Health, 2016).

Apart from mortality, diet-related non-communicable diseases adversely affect the economies of most countries. In 2006, NZ$624m of New Zealand's total health care expenditure was spent treating obesity, overweight and their comorbidities (Lal, Moodie, Ashton, Siahpush, & Swinburn, 2012). In addition to healthcare, there is the cost of loss of productivity of the workforce and the social cost of caring for those whose wellness is impaired. The proportion of older adults in the New Zealand population is increasing (Statistics New Zealand, 2015), and the prevalence of comorbidities associated with ageing such as impaired ingestion and digestion of food (Popman, Richter, Allen, & Wham, 2018; Wham et al., 2017), sarcopenia and frailty is increasing (Hoogendijk et al., 2019).

Rationale and significance of the study

In 2009, bread and bread products contributed the highest proportions of energy (11%), protein (11%), carbohydrate (17%) and dietary fibre (17%) to the New Zealand diet (Ministry of Health, 2011). The same survey also revealed that bread-based dishes, such as sandwiches, were the most frequently (41%) consumed convenience food. Accordingly, the bakery industry in New Zealand is a large part of the food market with bread and bread products that range from artisan to bulk commercial, whole grain to refined white wheat, and low- to high-cost breads. In New Zealand, bread has been chosen for fortification because it is consumed by a large proportion of the population. For example, salt which is an ingredient in sliced bread in New Zealand is fortified with iodine (Food Standards Australia New Zealand, 2015) and there is an active proposal to add folic acid to bread flour (L. A. Houghton, 2014). Therefore, bread is one food that could be reformulated to improve the intake of nutrients at a population level, and it appeals to the commercial market for high-value nutrition products with associated health claims.

Between 2012 and 2017, increased consumer demand for healthier foods that provide essential health benefits beyond basic nutrition has led to an increased purchase of functional bread products in New Zealand such as wholegrain breads (NielsenScantrack, 2017). A functional bread has several health benefits beyond normal nutrition which may include appetite suppression (Ekstrom, Bjorck, & Ostman, 2016; Sandberg, Bjorck, & Nilsson, 2016), blunting glucose and insulin release into the blood (Rosen, Ostman, & Bjorck, 2011; Sandberg et al., 2016), improved diastolic blood pressure (Hobbs et al., 2013), improved blood lipid profiles (Sereni et al., 2017) or decreased oxidative stress (Butnariu & Caunii, 2013). Also, several studies have suggested that replacing refined grain with whole grains and other functional food ingredients in bread could improve appetite (Bazzano et al., 2005; Keogh, Atkinson, Eisenhauer, Inamdar, & Brand-Miller, 2011; Lee et al., 2006; Vuksan et al., 2010).

Although functional ingredients such as whole grains may contain beneficial compounds that allow for the making of a health claim on bread, their incorporation into bread do not always demonstrate health effects when the bread is consumed (Turpeinen, Juntunen, Mutanen, & Mykka Ènen, 2000) who compared over 4 weeks the consumption of wholemeal rye bread with low fibre wheat bread and found no change in hemostatic factors. Thus specific health effects need to be validated in humans. This is warranted as factors including how ingredient-ingredient interact may either synergistically improve or impair the health effect potential of the bread. For example, certain inherent antinutrients in food impair the absorption of certain essential nutrients in food products (Rousseau, Kyomugasho, Celus, Hendrickx, & Grauwet, 2019). Additionally, the amount of essential nutrients required to demonstrate physiological effect that would result in a beneficial health effect may not be high enough in newly developed food products. Consequently, in New Zealand, health claims can only be made for developed food products when there is a body of scientific evidence to support the claim (Food Standards Australia New Zealand, 2017).

A recent proof-of-concept report explored the feasibility of developing a low-cost healthier bread in New Zealand (Wilson et al., 2016). Wilson and his team used linear programming software to explore the prospects of developing a low-cost heart-healthy bread. In this optimization study, the authors enriched bread with a selection of carefully selected, nutritionally-dense ingredients with evidence of cardiovascular disease prevention. These ingredients included wholemeal flour, potassium salt, nuts such as walnuts and seeds such as linseed, sunflower, pumpkin and sesame. Nutritional superiority was considered in terms of sodium, potassium, fatty acid ratio and fibre. The cost of the bread was between $1.5-3.0/600g loaf. In addition, the potential to use novel and nutritious ingredients such as vegetables for functional lower-cost bread-making is promising. Vegetables are rich in health-influencing bioactive compounds (Shashirekha, Mallikarjuna, & Rajarathnam, 2015), including fibre, vitamins and minerals which are essential to address hidden hunger (micronutrient deficiencies) (Shashirekha et al., 2015). The high moisture content and water activity of vegetables and other plant foods (Maltini, Torreggiani, Venir, & Bertolo, 2003) creates a favourable environment for mould growth and spoilage, which limits shelf-life. However, vegetables including pumpkin and sweet corn can be processed into purees and drum-dried into flours that have lower moisture content and better shelf-storage properties (Galaz et al., 2017; Pua et al., 2010). The formulation of bread with drum-dried vegetable flours is a viable way of ensuring that beneficial bioactive nutrients in vegetables including carotenoids could be made available to consumers during lean seasons which can contribute towards enhanced nutritional status. The challenge lies in formulating healthier bread that would be acceptable to be purchased and consumed by the public.

Drivers of successful commercialisation include a clean label and brand (Mueller & Szolnoki, 2010). A clean label tag on a food product from a consumer perspective imparts the idea of a food product being natural, additive- or preservative-free and organic (Ingredion, 2014). Branding and telling a story about food products is one way of influencing the buying pattern of consumers (Deliza & MacFie, 1996). Also, the performance of a food product brand in terms of its financial growth is not only influenced by the logo (C. W. Park, Eisingerich, Pol, & Park, 2013) but the philosophy behind the brand. In that regard, in 2010, as a way to promote sustainable consumption of food products, Auckland University of Technology (AUT), New Zealand, developed the "Nothing Else™" brand (Brown et al., 2015). The Nothing Else™ brand may be placed on foods with eight or fewer natural ingredients and has been used to market water and nuts/dried fruit since 2011, and a healthier snack bar since 2013 (Brown et al., 2015). The brand is a front-of-pack label that lists, in descending order, the eight or fewer ingredients contained in a product. Part of the appeal of the Nothing Else™ brand is clean labelling. Communicating the ingredients in a packaged Nothing Else™ vegetable-enriched bread through a front-of-pack label is a way of creating a supportive environment for consumers to have the personal agency that enables healthy food choices.

Figure 1.1. Nothing Else™ with the list of wholesome ingredients displayed on the front of the pack.

In addition to the mandatory back-of-pack nutrition information panel (NIP), front-of-pack labels and health claims are the major sources of nutrition information about a food product for consumers (Talati et al., 2016). The Nutrient Profile Scoring Criterion (NPSC) is a profiling system used in assessing the suitability of a food product to make a health claim and to determine the Health Star Rating, based on the nutrient composition of the product (Food Standards Australia New Zealand, 2017, 2019). Developing a vegetable-enriched Nothing Else™ bread with a Health Star Rating of 4 or above, and which is reasonably priced, could be a strategic approach to improving the nutritional quality of the food supply while ensuring consumers can afford to buy the bread in New Zealand.

In other countries including Ghana, which is the home of the author of this thesis, bread, is also one way of using wheat alternatives and other plant products such as vegetable by-products and underutilised or low-cost vegetables (Gomez & Martinez, 2018; Martins, Pinho, & Ferreira, 2017). Earlier research undertaken by the author in Ghana included bread formulated with wheat and pumpkin (Adubofuor, Anomah, & Amoah, 2018) and ripe banana (Adubofuor, Amoah, Batsa, Agyekum, & Buah, 2016). A breakfast cereal formulated with soya bean, maize and pumpkin flours was also developed (Asaam, Adubofuor, Amoah, Apeku, & Yildiz, 2018). The sensory properties of these products were judged acceptable by panellists in terms of taste and aftertaste; however, there was no further testing of the bioavailability of nutrients or whether these foods could be an acceptable part of the dietary pattern. In this present PhD study, the choice of pumpkin for the bread formulation was based on my past research experience in Ghana. Pumpkin contains total carotenoid concentrations ranging between 234 and

405 μg/g carotenoids (de Carvalho et al., 2012) and fibre of 11g/100g (Perez Gutierrez, 2016). A β-carotene concentration of 73 μg/g fresh weight has also been reported for pumpkin (de Carvalho et al., 2012). Carotenoids are antioxidants and have the potential of addressing hidden hunger (vitamin A deficiency) (Moran, Mohn, Hason, Erdman, & Johnson, 2018). Theoretically, bread could be formulated from wheat flour (500g), yeast (15g), salt (15g), tap water (100g) and enriched with pumpkin powder (75g) and sweet corn powder (20g). At a yield of 87%, 100g of the bread will provide beta carotene and fibre at concentration of 382μg/100g and 5.4g/100g respectively. The consumption of 4 standard slices (200g) will contribute 764 μg of beta carotene and fibre concentration of 10.8g. This will contribute towards meeting the RDI of β-carotene (700μg) for women and about 36% of the adequate intake of fibre for men (30g/day) and women (25g/day) (National Health and Medical Research Council (Australia), 2006). In the case of the sweetcorn, its use in the bread formulation was through a serendipitous means as it was generously given by Cedenco. New Zealand grows sweetcorn but this is mainly fed to cows. It is also another underutilised vegetable for human consumption. Thus, it utilisation in bread formulation could contribute towards addressing food security through improving the nutritional status of consumers.

Research question

How can food science and nutrition inform the development of a vegetable-enriched Nothing Else™ bread, accepted by consumers, with potentially verifiable health claims?

Design of the studies

Three linked studies explored the physical properties, sensory perceptions and possible health effects of the vegetable-enriched Nothing Else™ bread. Factors which were assessed included acute enhancement of the ease of swallowing, acute blood glucose and insulin response, appetite suppression, and the effect of regular consumption of the selected vegetable-enriched Nothing Else™ bread on the bioavailability of the carotenoids in participants' blood and lipid profile.

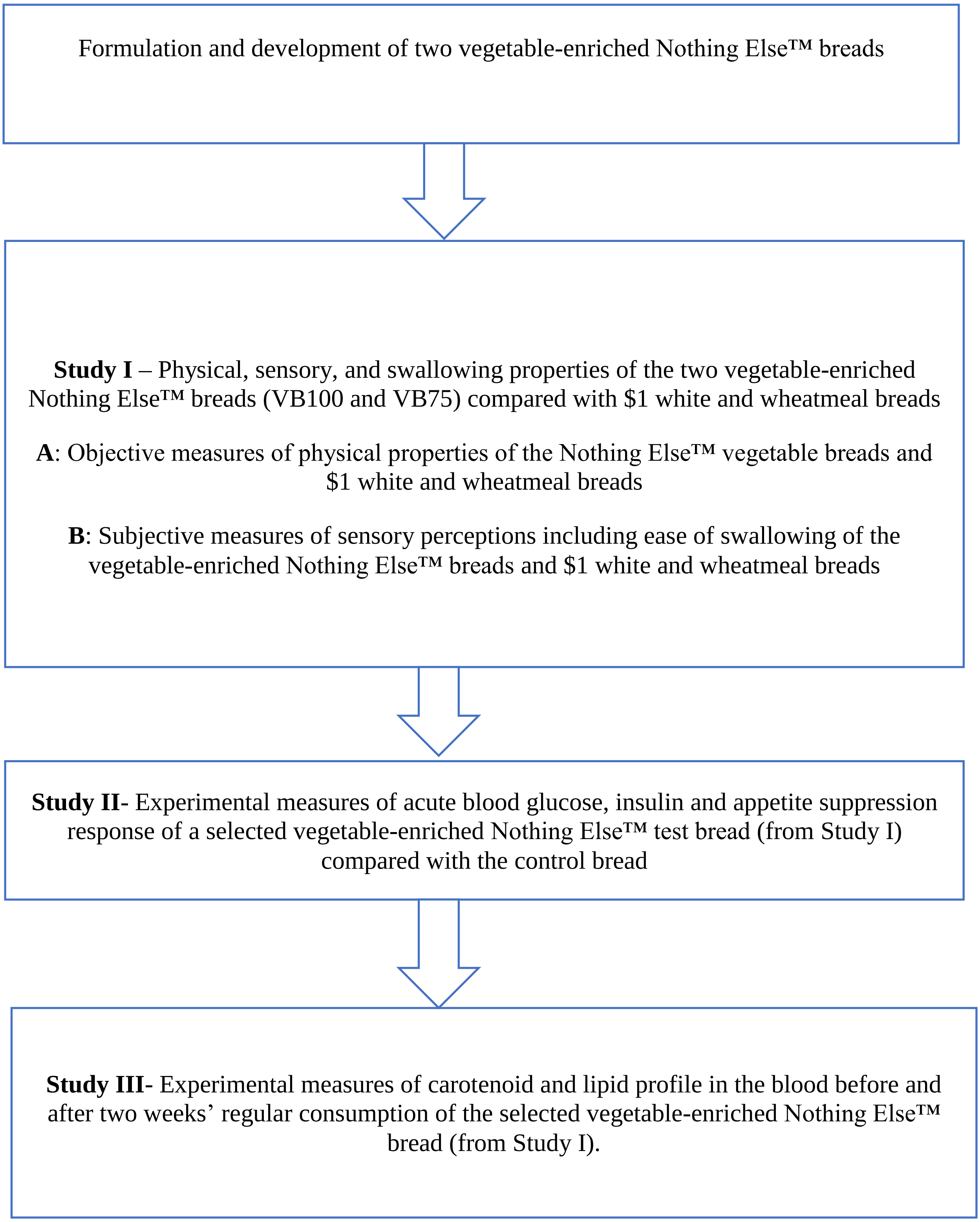


Figure 1.2. Flow chart of the experimental process.

Table 1.1. Summary of research procedures, outcomes and hypothesis

Research process		Outcomes	Primary outcome	Hypothesis
1. Formulate and develop bread		Vegetable-enriched Nothing Else™ test breads VB75 and VB100	Vegetable-enriched Nothing Else™ test breads	
2a. Physical characterisation	Objective means	Colour Specific volume Hardness Chewiness Cohesiveness Springiness Resilience	Hardness	The vegetable-enriched Nothing Else™ bread VB100 will be softer than the VB75 and control white and wheatmeal breads due to its high pumpkin flour level
2b. Sensory perceptions	Subjective means	Colour Aroma Taste Texture Mouthfeel Ease of chew Ease of swallowing Ease of bite and getting into mouth Ease of movement though the throat Sticking in the throat	Ease of swallowing Preferred vegetable-enriched Nothing Else™ test bread selected	Vegetable-enriched Nothing Else™ test bread VB100 will be easy to swallow due to its high pumpkin flour levels
3. Health effects		Blood glucose and insulin response	Glucose and insulin	The mean blood glucose and insulin iAUC following the consumption

Research process		Outcomes	Primary outcome	Hypothesis
		Appetite suppression response		of the vegetable-enriched Nothing Else™ bread will be lower than the control white and wheatmeal breads
4a. Health effects	2 weeks pre-post experimental test design	Carotenoid (β-carotene) in the vegetable-enriched Nothing Else™ bread. Carotenoid in blood plasma and skin carotenoid concentration using a Veggie meter™ of participants will also be measured	Carotenoid in the blood	Plasma carotenoid concentration will be higher after two weeks of vegetable-enriched Nothing Else™ test bread consumption
4b. Health effects	2 weeks pre-post experimental test design	Lipid profiles of participants	Lipid profiles	Lipid profile levels will be improved after two weeks of vegetable-enriched Nothing Else™ test bread consumption

Note. VB75 (lower vegetable content) and VB100 (higher vegetable content)

Chapter 2. Literature review

Bread is one of the most consumed staple food items across the globe (Kourkouta et al., 2017). Consequently, it promises to be an ideal vehicle for the delivery of bioactive compounds that have the ability to demonstrate health effects when formulated from composite flour containing functional ingredients (Amoah, Cairncross, Sturny, & Rush, 2018; Birch & Bonwick, 2018; Gomez & Martinez, 2018; Martins, Pinho, & Ferreira, 2017). The consumption of bread dates back to the first agricultural revolution, with the first bread prepared around 10,000 BC from a mixture of grain flour and water (Mondal & Datta, 2008). Bread intake rate differs among European countries. Turkey and Bulgaria have the highest intake rate (approximately 104 and 95 kg/head/year respectively) whilst the United Kingdom has the lowest (approximately 32 kg/head/year) (International Association of Plant Bakers, 2015).

In New Zealand, the value of yearly bread sales increased by 3.5% (from $462,491,400 to $479,077,700) between the year to 19/06/2016 and the year to 18/06/2017. Non-white bread and specialty bread also increased by 3.3% and 6.6% respectively in sales value (from $233,008,800 to $241,028,800 and $67,855,300 to $72,670,200 respectively) over that same trading year (NielsenScantrack, 2017) (**Table 2.1**). On average, non-white bread costs 50% more than white ($3.00 per unit compared with $2.00). Speciality breads averaged $4.50 per unit. This means that sales of white bread were, in unit-volume, 56% of the sales of non-white bread in 2017. In contrast, in 2009, it was reported by New Zealand adults that 30% consumed white bread and 63% light or heavy grain bread (Ministry of Health, 2011). Bread and bread products were the largest contributor of energy (11%), protein (11%), carbohydrate (17%) and fibre (17%) to the New Zealand diet (Ministry of Health, 2011).

Table 2.1. Statistical data on bread trade in New Zealand supermarkets

	Year ending June, 2017	Year ending June, 2016	Year ending June, 2015
	Val Sales ($'000)	Val Sales ($'000)	Val Sales ($'000)
Bread	479,078	462,491	455,081
Non-white Bread	241,029	233,009	236,118
White Bread	91,543	92,213	93,710
Specialty Bread	72,670	67,855	60,603
Buns & Rolls	37,025	32,530	29,912
Indian Bread	3,728	3,459	3,036

Note. Val = Value. Statistics as reported by Nielsen ((NielsenScantrack, 2017))

The classification scheme used by NielsenScantrack and the bread industry is based on ingredients, size and shape of the bread products. Non-white bread though open to discussion includes wheatmeal bread in New Zealand. White bread is mainly formulated from strong white flour and water. Specialty bread are mainly artisanal style breads that contain vegetables, use gluten-free ingredients, are seeded and prepared as sourdoughs. Buns and rolls are of smaller form/size (smaller portion) and may be enriched with dried fruits and spice such as hot cross buns for Easter or may contain eggs, sugar, nuts, chocolate pieces. The Indian bread are unleavened and include chapatti and roti.

Existing breads and bread products on the commercial market range between artisan and bulk commercial and whole grain to refined white wheat. Bulk commercial white breads are the least costly. Commercially produced breads include white bread, wheatmeal bread, rolls and buns and specialty breads. Breads that are made from wholegrains must include every part of the grain including the outer layers, bran and germ (Food Standards Australia New Zealand, 2016b), and are more nutritionally-dense than from refined flours. Wholegrains, seeds and nuts in bread improve the nutritional profile particularly with reference to fibre in the bran and B vitamins and healthy fats in the germ plus phytochemicals are present which are lost during the refining process (Seal, Nugent, Tee, & Thielecke, 2016).

In New Zealand there is a duopoly in supermarket chains, Progressive (45%) and Foodstuffs (55%). Progressive operates under the brandname Countdown. A search on New Zealand Countdown supermarket online (Countdownonline, 2020) for commercially sold breads returned 8 white, 50 brown, grain and seeded bread, 3 fruit breads and 26 gluten free and "free from" breads, a total of 87 items. Bread enriched with seeds including Burgen® toast bread enriched with soy and linseed, Burgen® toast bread enriched with pumpkin seeds and chia, Ploughmans® bakery toast bread with soy and linseed, Vogels® toast bread enriched with soy and linseed and Vogels® toast bread with spelt and flaxseed. Bread enriched with wholegrains that have the potential to demonstrate health effects including Vogels® toast bread containing mixed grain, Burgen® toast bread ancient grains, Nature fresh® toast bread wheatmeal, Freyas® toast bread wholemeal grain, Vogels® harvest grains toast bread with sprouted whole grains, Ploughmans® bakery toast bread farmhouse wholemeal, Ploughmans® bakery toast bread wholemeal grains, Woolworths® New Zealand's essentials sliced white and wheatmeal bread, Burgen® sliced bread wholemeal and seeds (Countdownonline, 2020) are also commercially available. Commercially available specialty breads including sourdough bread available are Ploughmans® bakery toast bread sourdough, Vogels® gluten free sliced bread wholemeal and Vogels® gluten free sliced bread white are commercially available. For bread rolls, Countdown® bread rolls long and Countdown® bread rolls long sesame are available commercially (Countdownonline, 2020). Additionally, other in-store produced breads include in-store bakery sliced bread high fibre low GI, in-store bakery traditional loaf four seed loaf and in-store bakery Vienna loaf sesame.

A careful review of bread available from Countdownonline showed that the practice of bread enrichment with ingredients that have the potential to demonstrate health effect including seeds, sourdough and healthier grains such as chia is a common practice commercially in the baking industry. Breads formulated with wholegrain flours have been investigated for their ability to attenuate glycaemic response and suppress appetite in the acute state compared with refined white flour (Kristensen et al., 2010). The authors reported that though no changes in glucose was observed following the consumption of breads, whole grain bread suppressed appetite compared to their refined white bread counterparts in the acute state. The fortification of bread with legumes including lupin (Keogh et al., 2011; Lee et al., 2006), oilseeds (e.g hazelnuts) (Devi et al., 2016), sprouted grains in the form of sourdough (Tucker et al., 2014), vitamins (folic acid and vitamin B-12) (Winkels, Brouwer, Clarke, Katan, & Verhoef, 2008) and minerals (eg. Calcium)(Martin, Weaver, Heaney, Packard, & Smith, 2002) have been reported. Bread fortification with lupin, hazelnuts, sprouted sourdough resulted in attenuation of glycaemic response (Devi et al., 2016; Keogh et al., 2011; Tucker et al., 2014) and suppressed appetite (Lee et al., 2006). Bread co-fortified with folic acid and vitamin B-12 caused serum folate concentration to increase by 45%, and 49% for serum vitamin-B12 compared to the placebo group (Winkels et al., 2008). Martin et al. (2002) conducted randomised crossover study using a two study approach involving healthy postmenopausal women who were fed with bread fortified with calcium sulfate and three salts (calcium lactate, calcium carbonate, and calcium sulfate). In the first study, there was no significant difference in the absorption concentration of calcium from the three salts. However, in the second study, the absorption of fractional calcium was significantly greater from fortified bread compared to its milk counterpart thus highlighting the effectiveness of bioavailability of calcium sulphate as a fortificant for white bread. Nutritional density of bread and therefore healthiness can be increased by the use of wholegrain flours, the addition of nuts, seeds, minerals and vitamins. Other ingredients that could be added to bread include vegetables and fruits. In particular, the potential of using flour from vegetables to fortify bread is promising but there are few existing bread products that contain vegetables, with the exception of cornbread and there is very little research concerning vegetable-enriched bread as discussed in the next paragraphs.

Despite the different bread types addressed, it must be emphasized that this research focused on bread enrichment with vegetables. Bread enrichment with vegetables including corn, particularly in North America (Gwirtz & Garcia-Casal, 2014), in cladodes (Msaddak et al., 2017) in Tunisia and freeze-dried carrot, carrot, tomato, beetroot or broccoli (Ranawana, Raikos, et al., 2016) in Scotland has been explored. In New Zealand, there has however been some attempt to enrich bread with vegetable flour at the commercial level. For example, Ploughmans® bakery enriches bread with a small amount of pumpkin flour (1%) and seeds (4%). The need for bread enrichment with vegetables is to improve the nutritional profile of bread. Fresh and frozen vegetables are high in moisture (Maltini et al., 2003) content and thus are highly perishable. This compromises their economic value and adversely impact on food security. However, drum drying can be used to process vegetables including pumpkin and sweet corn into purees and flours that have lower moisture content and better shelf-storage properties (Galaz et al., 2017; Pua et al., 2010). Thus utilizing vegetables in bread could be a viable way of sustainably ensuring they are in season even during lean seasons. From a technological perspective, the incorporation of these vegetables into bread may adversely impacts on the qualities.

On average New Zealand adults only consume half the recommended daily intake of fibre. Bread formulated with refined wheat flour is a relatively poor source of dietary fibre and a known high glycaemic food (Brennan & Cleary, 2007; Ho et al., 2013; Sandberg, Bjorck, & Nilsson, 2017; Zafar et al., 2015).

In New Zealand, efforts geared towards developing healthier bread have been explored in the research context. Examples include incorporating hazelnuts and its flour into bread to promote nut consumption (Devi et al., 2016), addition of apple pectin and kiwifruit, blackcurrant or apple polyphenol extract into bread (Sun-Waterhouse et al., 2011), enriching bread with white button, shiitake, and porcini mushroom powders (X. Lu, Brennan, Serventi, & Brennan, 2018) and bread fortification with salmon fish (*Oncorhynchus tschawytscha*) powder (Desai et al., 2018). However, no study has reported on the incorporation and validation of the health effects of New Zealand-grown vegetables like drum-dried pumpkin and sweet corn flours in bread for New Zealanders.

Bread-making and effect of ingredients addition on the physical properties of bread

Bread is traditionally prepared from refined wheat flour. However, variations in bread exist due to differences in the selection, processing procedures employed, quantity and mix of ingredients (Lau, Soong, Zhou, & Henry, 2015). The need to promote the utilisation of other non-wheat ingredients in composite preparation with wheat flour is encouraged due to the benefits, including cutting down wheat importation by countries that are not wheat-growing (Noorfarahzilah, Lee, Sharifudin, Fadzelly, & Hasmadi, 2014) and enabling consumers to benefit from other bioactive compounds trapped in non-wheat ingredients (Hobbs et al., 2013). That notwithstanding, the need to use wheat flour as a base ingredient in bread formulation is essential owing to its glutenin and gliadin content that eventually develops into a gluten network during mixing with water (Anton & Artfield, 2008) producing a viscoelastic dough. The gluten network subsequently retains carbon dioxide gas produced through the fermentation action of yeast and lactic acid bacteria on sugars, a property essential to leaven the bread (Wieser, 2007).

Processes involved in bread-making

The bread-making process involves ingredient scaling, mixing, bulk fermentation, folding, dividing, pre-shaping, dough resting, shaping, last stage fermentation, baking and cooling (Guerrini, Parenti, Angeloni, & Zanoni, 2019). In the bakery business ingredients other than flour, such as sugar and yeast, are expressed as a percentage by weight of the flour (Mondal & Datta, 2008).

There are two main dough preparation processes: the straight dough (Y. Lu et al., 2014) and indirect methods. In the straight dough method, bread ingredients are mixed, kneaded and fermented and the dough baked. However, in the indirect method, wheat flour and water are usually mixed and allowed to rest after which pre-fermented ingredients are added, kneaded, fermented and baking allowed to proceed. In recent times, sourdough application technology has received a wider acceptance amongst bakers. The process involves the addition of lactic acid-fermentation bacteria, lactobacillus, to complement yeast during the fermentation process (Rizzello, Lorusso, Montemurro, & Gobbetti, 2016). Although the application of sourdough technology on breads impacts on the protein digestibility of bread, a commonly known effective way of improving the nutritional profile of bread is through the addition of functional ingredients to wheat flour to create a functional bread. A functional bread is bread with health-beneficial properties beyond basic nutrition (Różyło et al., 2014). The substitution of functional ingredients in bread impacts on the physical properties and sensory attributes of the bread.

Physical attributes of functional bread

Evaluation of the physical properties of bread is essential as it impacts on the qualities and sensory properties of the bread. Properties of importance include colour, loaf weight, specific volume, hardness, springiness, cohesiveness, chewiness and resilience (Longoria-García et al., 2018; Martins, Pinho, & Ferreira, 2017). These properties shed light on the processes involved in the bread-making such as leavening and the water absorption properties of the dough. The determination of the weight and volume of bread, for example, is essential in determining the specific volume (mL/g) which is useful in the quality control of bread-making (Ananingsih, Gao, & Zhou, 2013). Crust and crumb colour provide essential sensory attributes to both the breadmaker and consumer. It indicate the extent of browning (Maillard reaction), baking temperature and time to the baker. Too much browning impacts on the taste and crust and crumb hardness to the consumer.

The physical properties of dough are impacted by the ingredients used in the bread formulation. Struck et al. (2018) studied the wheat macromolecules, glutenin and gliadin and their interaction with flour from dried blackcurrant pomace in a dough. The authors reported that the development of gluten network is impaired when flour from dried blackcurrant pomace is incorporated resulting in a dough with lower stickiness, resistance and extensibility. Exploring further at the microscopic level, the authors observed the disruption of the gluten network in the risen/proofed dough enriched with the pomace fibre. Other studies that incorporated fibre-rich ingredients into bread and their effect on bread qualities have been reported.

Bread enrichment with fibre from pomaces of lemon (0-9%) (Chang, Li, & Shiau, 2015), apple/pear/date (Bchir, Rabetafika, Paquot, & Blecker, 2014) and pineapple (at 10% substitution) (Chareonthaikij, Uan-On, & Prinyawiwatkul, 2016) resulted in increased bread crumb hardness (Chang et al., 2015; Chareonthaikij et al., 2016) and reduced specific volume (4.60, 4.30 and 3.0 cm^3/g for the "control", 5% and 10% pineapple-fibre enriched bread respectively) and (2.9 cm^3/g for the control and 2.7/2.8 cm^3/g for the date, pear, and apple pomace fibre-enriched breads respectively) (Bchir et al., 2014; Chareonthaikij et al., 2016) (**Table 2.2**). Additionally, bread enrichment with fibre from mango peel (at 1, 3 and 5%) (Pathak, Majumdar, Raychaudhuri, & Chakraborty, 2017) and artichoke by-product (3, 6, 9 and 12%) (Frutos, Guilabert-Antón, Tomás-Bellido, & Hernández-Herrero, 2008) resulted in a general decrease in loaf volume and specific volume (Frutos et al., 2008; Pathak et al., 2017) whereas crumb moisture and loaf density increased as the substitution increased (Pathak et al., 2017) (**Table 2.2)**. The artichoke by-product (stem and leaves) was chosen because of their high content of the fructooligosaccharide, inulin, which is a prebiotic fibre (Frutos et al., 2008). The stems and leaves were collected from artichoke canning factories, were washed and liophilized to eliminate all water content (Frutos et al., 2008). The liophilized sample was milled in a grinding mill, and passed through a sieve with a mesh of 0.210mm. The product contained 55% fibre (Frutos et al., 2008). Fibres vary in their ability to hold water in dough and bread matrices and this depends on the source and type of the fibre, with some having good water-holding capacity whereas others are poor (Elleuch et al., 2011). Although fibre generally weakens the gluten network in bread dough, fibres with high levels of hydrophilic compounds have good water-retaining property and tend to produce bread with soft crumb attribute than their poor water-holding counterparts.

Table 2.2. Effect of bread enrichment with pomaces and other food-by products as functional ingredients on physical properties

Functional ingredients	Substitution levels	Optimal substitution level	Effects on physical properties of bread	References
Grape pomace flour (GPF)	6%, 10% and 15%	6%	↓ brightness, ↑ firmness and ↓ loaf volume	(Sporin, Avbelj, Kovac, & Mozina, 2018)
Lemon pomace fibre	0-9%	3%	↑ crumb hardness ↓ cohesiveness and springiness	(Chang et al., 2015)
Pineapple pomace fibre	5 or 10%	5%	↑ specific volume and ↓ crumb firmness	(Chareonthaikij et al., 2016)
Fibre concentrate prepared from apple, pear, and date pomaces	2% of fibre	2%	Enhanced L* of the control, pear and date fibre except for the apple fibre	(Bchir et al., 2014)
Grape seed flour	2.5, 5, 7.5, and 10%	2.5-5%	↓ loaf brightness and volume, ↑hardness and porosity	(Hoye Jr & Ross, 2011)
Grape seed extract	300mg, 600mg and 1g	300mg, 600mg and 1g	↓ L* values, ↑a* and b* values. No adverse effect on crumb hardness	(Peng et al., 2010)
Pomegranate seed flour	5%, 7.5% and 10%	Not observable	↓ loaf volumes, heights and brightness of breads, ↑ a* and b* values, hardness and chewiness	(Gül & Şen, 2017)
Rice bran	10%	10% with *L. mesenteroides*	↑ crumb firmness, ↑ higher loaf volume from sourdoughs fermented by *L. mesenteroides*	(Farahmand, Razavi, Yarmand, & Morovatpour, 2015)

Functional ingredients	Substitution levels	Optimal substitution level	Effects on physical properties of bread	References
Middling fraction (M) of wholegrain (WM) and pearled (PM) barley	15%, 30%, 45% and 60%	30% WM and 15% PM	↑ crumb hardness with ↑ levels of barley flour substitution. Loaf volume not significantly affected for 30% WM and 15% PM-substituted breads	(Sullivan, O'Flaherty, Brunton, Arendt, & Gallagher, 2011)
Oat bran	10, 20 and 30%		↑ specific volume and ↓ crumb hardness with xylanase and sourdough addition	(Banu, Măcelaru, & Aprodu, 2017)
Buckwheat (*Fagopyrum Esculentum Moench*) bran	20%	20% with sodium stearoyl-2-lactylate (SSL) + transglutaminase (TG) (0.5% + 0.4%)	↓ bread volume and ↑ bread crumb lightness, volume, crumb softness and the crust for combination of SSL + TG to the bran	(Atalay, Bilgicli, Elgün, & Demir, 2013)
Rootlets	5%, 10%, 15% and 20%	5%	↓ crumb hardness and ↑ chewiness	(Waters et al., 2013)
Brewers' spent grain	5, 10, 15 and 20%	5%	↓ crumb hardness	(Waters, Jacob, Titze, Arendt, & Zannini, 2012)
Hemp seed cake	5,10 and 20%	5 and 10%	↓ bread volume	(Pojić, Dapčević Hadnađev, Hadnađev, Rakita, & Brlek, 2015)

Functional ingredients	Substitution levels	Optimal substitution level	Effects on physical properties of bread	References
Cupuassu peel flour	3, 6, and 9% cupuassu peel flour	NA	↓ specific volume ↑ crumb L* values for 3% cupuassu peel flour, followed by the sample with 6% cupuassu peel flour ↑ yellowish colour	(Salgado, Rodrigues, Donado-Pestana, dos Santos Dias, & Morzelle, 2011)

Note. ↑ = higher and ↓ = lower. L=Lightness, a*-redness and b*-yellowness. NA- Not applicable

Table 2.3. Effect of bread enrichment with whole functional ingredients on physical properties

Functional ingredients	Substitution levels	Optimal substitution level	Effects on physical properties of bread	References
Cladodes powder	2.5%, 5%, 7.5% and 10%	7.5%	↓ crust and crumb colour (L*, a* and b*) and ↑ specific volume	(Msaddak et al., 2017)
Turmeric (*Curcuma longa* L.) powder	2%, 4%, 6% and 8%	Not clearly observable	↑ hardness and crumb colour (a* and b* values) ↓specific volume and crumb lightness (L*) colour	(Lim, Park, Ghafoor, Hwang, & Park, 2011)
Doum fruit (*Hyphaenethebaica* L.) flour (DFF)	5 %, 10 %, 15% and 20 %	5% (resilience, cohesiveness, springiness and crust L*)	↓ loaf volume, cohesiveness, resilience, springiness and ↑ hardness and chewiness	(Aboshora et al., 2016)

Note. ↑ = higher and ↓ = lower. L=Lightness, a*-redness and b*-yellowness. NA- Not applicable.

Nutritional profile enhancement of functional bread

The nutritional profile of bread is enhanced in most cases following bread enrichment with functional ingredients. This is because functional ingredients in addition to their rich bioactive composition have other intrinsic nutrients including fibre, protein, minerals and other non-bioactive vitamins trapped within them (Martins, Pinho, & Ferreira, 2017). It is these attributes of functional ingredients which impact on their definition as providing health benefits beyond normal nutrition (Birch & Bonwick, 2018). Generally, enrichment of wheat flour with plant-based products including seeds, brans or pulp increases the fibre composition of bread (Martins, Pinho, & Ferreira, 2017). de Lamo and Gómez (2018) carried out an extensive review on the effect on the nutrient composition of bread enriched with oilseeds including pumpkin seed flour, sunflower seeds, sesame seeds, chia seeds and flaxseed. The authors posited that bread enrichment with the seeds enhanced the protein, fibre, omega-6, omega-3 and mineral (calcium, iron, magnesium, phosphorous, potassium, sodium, zinc) composition of the bread.

The nutritional profile of bread could be enhanced through the addition of functional ingredients, either whole or in crushed form. For example, chia can be added to bread in whole form or in ground form as powder (de Lamo & Gomez, 2018). The addition of the ground form increases the surface area and easily releases the essential nutrients from the seed into the bread. The challenge with adding ground seeds rather than whole seeds into bread is that baking temperatures may adversely impact on the heat-labile essential nutrients present in it, reducing its bioavailability and bioaccessibility. It must, however, be mentioned that the formation of bread crust could also be protective towards the preservation of nutrients present in the crushed seeds. Baking normally adversely impacts on bioactive nutrients including polyphenols (Pasrija, Ezhilarasi, Indrani, & Anandharamakrishnan, 2015; Sun-Waterhouse et al., 2011) and carotenoids including β-carotenes (Thatte, Indrani, & Lakshmi A. J., 2011) which are precursors of vitamin A. These are heat-labile compounds that are easily degraded by heat (Pasrija et al., 2015).

Table 2.4. Effect of bread enrichment with functional ingredients on the nutritional profile of the bread

Ingredients	Substitution amounts	Effects on bread nutritional profile	Most acceptable/liked substitution	References
Pomegranate seed flour	5%, 7.5% and 10% levels	↑ fibre content	5%	(Gül & Şen, 2017)
Wheat bran and germ mixture	15%	↑ protein scores, essential amino acid index and biological values and ↑ dietary fibre	NA	(Pontonio, Lorusso, Gobbetti, & Rizzello, 2017)
Fibre from defatted rice bran	5 and 10%	↑ higher fibre concentration	NA	(Abdul-Hamid & Luan, 2000)
Buckwheat (*Fagopyrum Esculentum Moench*) milling products	20%	↑ fibre, protein, ash, mineral and fat contents		(Atalay et al., 2013)
Perilla seed	1, 3 and 5%	↑ protein, ash and fat contents	1%	(Vieira da Silva, Vieira da Silva, Bonafé, Evelázio de Souza, & Visentainer, 2016)

Note. ↑ = higher and ↓ = lower. NA-Not applicable.

Sensory perceptions of functional bread

Consumers buy bread formulated with ingredients that demonstrate health effects that also has sensory attributes crucial for bread liking (Birch & Bonwick, 2018). There is therefore the need to ensure a balance in the proportion of functional ingredients and other ingredients used to produce bread with an acceptable sensory and enhanced nutritional profile. Although, traditionally, sensory attributes of bread are usually evaluated subjectively by panellists, recent advances in technology has allowed for the discrimination of certain attributes of food products objectively using electronic nose and tongue (Marengo et al., 2018). Subjective attributes evaluated on bread mostly include colour, taste, aroma, texture and overall liking.

The colour, taste and aroma of bread depend on the combination of ingredients, temperature and time of baking, and the extent of Maillard browning (Michalska, Amigo-Benavent, Zielinski, & del Castillo, 2008). Consumers want bread with a taste and smell that appeals to the palate of the mouth and overall flavour experience. Aroma sensations are perceived through oronasal signals and interpreted by the brain and perceived as smell. Functional ingredients have aromatic compounds trapped in their cell walls. Consequently, when used in composite formulation with wheat flour, baking degrades the cell walls of functional ingredients and allows the release of these aromatic compounds into the bread matrix (Ribas-Agusti, Martin-Belloso, Soliva-Fortuny, & Elez-Martinez, 2017). This aroma may influence the consumers' perception of the smell of the bread. As well as taste and smell, the mouthfeel or textural perceptions of bread are usually impacted by the fibre content of the ingredients (Gomez & Martinez, 2018). The textural perceptions of bread are usually impacted by fibre-rich ingredients. Fibre, though essential for its health beneficial properties including preventing bile acid recycling (Blackwood, Salter, Dettmar, & Chaplin, 2000), has the potential of adversely impacting on bread crumb hardness, dryness and ease of manipulating in the mouth which may negatively impact on consumers (Martins, Pinho, & Ferreira, 2017). The incorporation of fibre-rich ingredients into bread dough results in gluten dilution consequently resulting in bread with poor textural properties (Struck et al., 2018). To overcome this challenge in the bakery industry, most commercial bakers add emulsifiers into the bread dough to produce bread with textural attributes that appeals to consumers (Stampfli & Nersten, 1995). The enrichment of bread with functional ingredients derived from fruits (Chang et al., 2015; Chareonthaikij et al., 2016), cereals/grains (Atalay et al., 2013; Sullivan et al., 2011) and oil seed cakes(Tarek-Tilistyák et al., 2014) impacted on the sensory attributes of the bread. In most cases, formulations with the addition of up to 5% by weight of functional ingredients resulted in bread that has acceptable sensory attributes as subjectively evaluated by consumers.

Table 2.5. Effect of ingredient incorporation in bread on sensory perceptions

Functional ingredient(s)	Substitution amounts	Effects on sensory perceptions	Most acceptable/liked substitution	Reference
Fermented (FCP) and unfermented (UCP) citrus peels	2, 4 and 6%	↑ acceptability for bread with UCP. ↑acceptability for bread formulated with 4 and 6% unfermented citrus peels treated with hot dry air at 150°C and 100°C respectively	4 and 6% unfermented citrus peels treated with hot dry air at 150°C and 100°C respectively	(Shyu, Lu, & Lin, 2014)
Grape pomace flour	6, 10 and 15%	↑ intensity of aftertaste and sand feeling in the mouth	NA	(Sporin et al., 2018)
Grape seed flour	2.5, 5, 7.5 and 10%	↓ astringency and sweetness for bread with ≥7.5%	Up to 5%	(Hoye Jr & Ross, 2011)
Lemon pomace fibre (LPF)	0-9%	↓ flavour, texture, and overall acceptability attributes for LPF at 6 and 9% substitution	3%	(Chang et al., 2015)
Pomegranate seed flour	5%, 7.5% and 10% levels	↑ acceptability for 5% substitution	5%	(Gül & Şen, 2017)

Functional ingredient(s)	Substitution amounts	Effects on sensory perceptions	Most acceptable/liked substitution	Reference
Pineapple pomace fibre (PPF)	5 or 10%	↓ liking for PPF addition at 10% but ↑ for 5% PPF substitution	5%	(Chareonthaikij et al., 2016)
"Middling" fraction (M) of wholegrain (WM) and pearled (PM) barley (BM)	15%, 30%, 45% and 60%	No significant difference was found in the acceptability of WM and PM breads	Up to 30% BM	(Sullivan et al., 2011)
Buckwheat (*Fagopyrum Esculentum Moench*) milling products	20%	↓ overall acceptability		(Atalay et al., 2013)
Rootlets	5%, 10%, 15% and 20%	↑ liking for 5% rootlets, or fermented rootlets substitution	5% fermented rootlet	(Waters et al., 2013)
Brewers' spent grain	5, 10, 15 and 20%	↑ liking for either brewers' spent grain or fermented brewers' spent grain (using the lactic acid bacteria, *Lactobacillus plantarum FST 1.7*) up to a 10% substitution	10%	(Waters et al., 2012)

Functional ingredient(s)	Substitution amounts	Effects on sensory perceptions	Most acceptable/liked substitution	Reference
Nejayote solids	3, 6, 9%	↑ scores for overall acceptability and subjective and objective textural attributes	Up to 9%	(Acosta-Estrada, Lazo-Vélez, Nava-Valdez, Gutiérrez-Uribe, & Serna-Saldívar, 2014)
Cake from naked pumpkin seed, sunflower seed, yellow linseed, and walnut	5% and 10%	↑ brown colour for walnut seed cake and partly-brown bread for yellow linseed cake	5% sunflower seed cake	(Tarek-Tilistyák et al., 2014)

Note. ↑ = higher and ↓ = lower. NA-Not applicable.

Diet-related non-communicable disease and food

The most common population determinant of malnutrition in first world countries is a measure of weight relative to height, the body mass index (BMI) (kg/m^2). Obesity prevalence in New Zealand is high with one in three adults classified as obese (BMI ≥ 30 kg/m^2) (Ministry of Health, 2016). In 2006, the cost incurred from the treatment of overweight and obesity was estimated to be NZ$624m and accounted for 4.4% of New Zealand's total health care expenditure (Lal et al., 2012). The increased obesity prevalence in New Zealand is partly attributed to the food supply which has readily available, inexpensive, relatively energy-dense, nutrient-poor foods (Vandevijvere, Molloy, Hassen de Medeiros, & Swinburn, 2018; Vandevijvere, Waterlander, Molloy, Nattrass, & Swinburn, 2018). Obesity is a form of malnutrition and the prominent cause remains a lack of balance between energy intake and its expenditure (Ricci-Cabello, Herrera, & Artacho, 2012). An association between the consumption of energy-dense nutrient-poor bread and diet-related non-communicable diseases including obesity development has been reported (Ellulu, Abed, Rahmat, Ranneh, & Ali, 2014). Fibre-poor foods trigger proinflammatory reactions in the body and are an independent risk factor for obesity development (Strasser, 2017). It is against this background that fibre-rich foods have been promoted for their consumption. Fibre increases gastric distension, delays gastric emptying and favourably impacts on the stimulation of satiety hormones including cholecystokinin, pancreatic polypeptide and glucagon-like peptide (L. Chambers, McCrickerd, & Yeomans, 2015). The satiety hormones bind to the receptors in the satiety centres of the hypothalamus of the brain (Perry & Wang, 2012). The sensation of fullness is consequently experienced, impairing consumers craving for more food. This remains one plausible mechanism underlying fibre-rich food intake and obesity regulation. In a study where fibre-rich bread was served to participants, the authors posited that the consumption of fibre-rich bread increased subjective satiety sensations in the participants (Ekstrom et al., 2016). Bread is a commonly consumed food in New Zealand. Therefore, improving the food supply by developing fibre-rich, nutrient-dense vegetable-enriched Nothing Else™ bread is part of a viable approach to addressing nutrition-related non-communicable diseases which are comorbidities of obesity.

Comorbidities of obesity refer to the accompanying chronic diseases whose development is associated with obesity and include chronic diseases such as type 2 diabetes mellitus and dyslipidaemia. About 80% of deaths world-wide are attributed to these obesity comorbidities which are diet-related non-communicable diseases (World Health Organization, 2018).

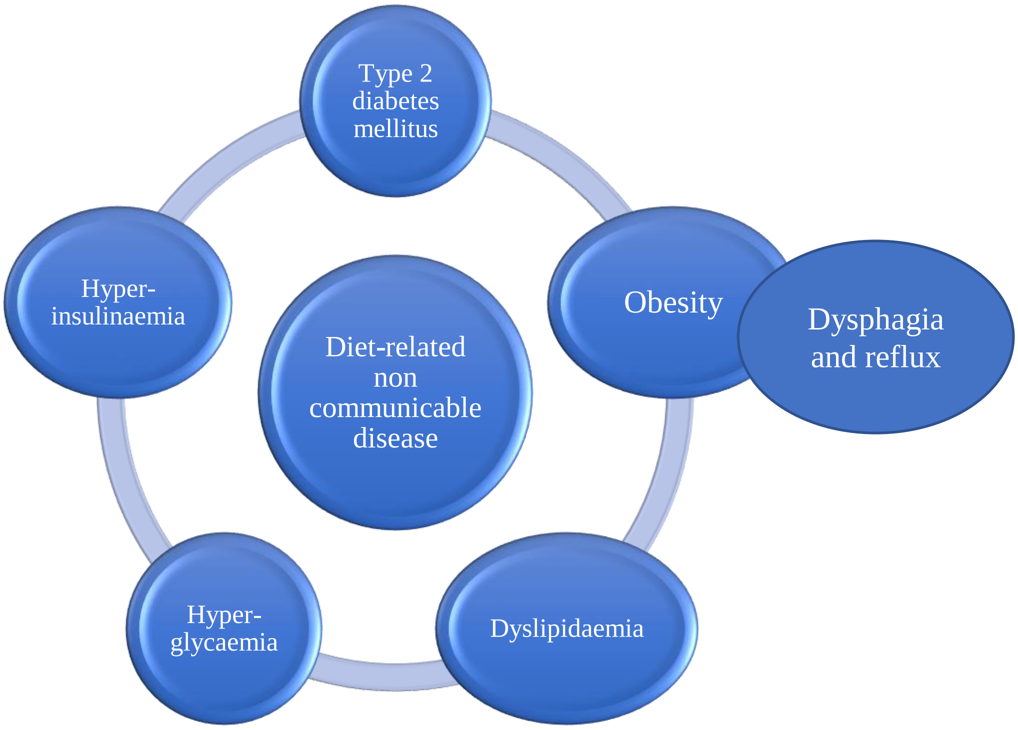


Figure 2.1. The interplay between diet-related non communicable diseases.

The interrelationship between obesity and its comorbidities can be seen in (**Figure 2.1**, above). Obesity results from the accumulation of excess fat cells and is associated with type 2 diabetes mellitus, hyperlipidaemia and hypertension (Edwards et al., 2019; Yu et al., 2019). The accumulation of excess fat is associated with the onset of insulin resistance (Ye, 2013). Insulin resistance is one major attributable cause of type 2 diabetes mellitus and results in increased concentration of circulating blood glucose (Viljoen & Sinclair, 2011). This causes increased circulation of insulin released from the beta cells of the pancreas, resulting in hyperinsulinemia (Ye, 2013). The production of low-grade chronic inflammation is associated with excess body fat. Consequently, an increased expression of inflammatory cytokines and infiltration of immune cells in adipocytes are found to characterise obesity (Rosa, Zulet, Marchini, & Martinez, 2012). Dyslipidaemia, which is an abnormality in lipid profile, is also associated with obesity. Obesity and its comorbidities are dependent on poor nutrition across the life course and usually improve with body weight reduction and improved nutrition. In addition, malnutrition affects the mechanics of ingestion and digestion of food. Bread may be a means of improving the nutritional quality of the diet. For example, the acute (one day) consumption of 200g red beetroot-enriched bread (containing 100 g red beetroot that made up 50% of the total weight of the dough) served as a sandwich with Philadelphia cheese reduced systolic blood pressure (Hobbs et al., 2013) and bread enriched with chickpea (Zafar et al., 2015) and lupin (Keogh et al., 2011) flours attenuated glycaemic response.

In addition, malnutrition and aging affect the mechanics of ingestion and digestion of food. The improvement of nutritional status depends on the nutritional quality and bioavailability of the foods eaten. Consequently, bread reformulation as a tool to improve the food supply is one that can be harnessed.

Dysphagia and reflux

In New Zealand, 12.6% of adults are morbidly obese (Ministry of Health, 2019a). Dysphagia and reflux are associated with obesity (Mohamed & Attwood, 2011). Difficulty in swallowing occurs predominantly in older and morbidly obese people and can potentially compromise their nutritional status (Morris, 2006) as limited food is ingested. In the case of the morbidly obese individuals, the effect of the limited food intake could result in a weight loss but the effect of sustaining the potential weight loss in the long term without potential adverse effect on health outcomes may need to be investigated. In people with dysphagia, swallowing, chewing and food pocketing difficulties in the mouth occurs (Dalton, Caples, & Marsh, 2011). Omeprazole is a medication prescribed for patients with swallowing difficulty secondary to acid reflux. The New Zealand's Pharmaceutical Management Agency (PHARMAC), involved in facilitating affordable access to medicines through a combination of aggressive price negotiations, has reported that, omeprazole is the third most commonly prescribed drug for patients (Gleeson, Lopert, & Reid, 2013; PHARMAC, 2019). This attests to how common dysphagia is, particularly amongst older people in New Zealand.

Table 2.6. Top five medications by number of prescriptions in New Zealand in 2018

Medicine	Prescriptions	Rank
Paracetamol	2,940,000	1
Atorvastatin	1,430,000	2
Omeprazole	**1,410,000**	**3**
Amoxicillin	1,210,000	4
Aspirin	1,180,000	5

(PHARMAC, 2019)

The International Dysphagia Diet Standardisation Initiative (IDDSI) has thus proposed a classification system that groups the textural properties of food products in relation to their ease of swallowing. The classification system involves 0=Thin drinks, 1=Slightly thick, 2=Mildly Thick, 3=Moderately Thick (Liquidised), 4=Extremely Thick (Pureed), 5=Minced and Moist, 6=Soft and Bite-sized and 7=Regular (International Dysphagia Diet Standardisation Initiative, 2016). In this classification scheme, the transitional foods are classified from 5-7 according to IDDSI. Transitional foods start from 5-7 on the IDDSI framework and refers to foods that undergo a change in textural dynamics from for example firm solid texture into another textural form following the application of external agents including water or saliva or temperature changes including for example heating (International Dysphagia Diet Standardisation Initiative, 2019). The framework used by IDDSI allows for the provision of acceptable terms that highlight the description of food thickness and textural attributes. The application of IDDSI tests reveals the textural and flow attributes of the specific food products, yet attention to testing conditions including temperature is warranted (International Dysphagia Diet Standardisation Initiative, 2016). Generally, because soft and smooth foods appear easiest to swallow (Morris, 2006), people with dysphagia require soft diets (Nazarko, 2008). Anecdotally (Dennisen, 2018), consumers with presbyphagia seek soft foods at the supermarket but there is a lack of these foods. In the case of bread, the textural attributes impact on its swallowing properties. Bread developed from very refined white wheat flour clogs and impairs swallowing in older adults (anecdotally). The clogging ability of refined white bread following consumption was shared anecdotally by the participants that participated in this present study. This however needs further investigations. In a study, participants could be asked to spit out the chewed refined white wheat bread bolus prior to swallowing and the bolus characteristics including its stickiness measured instrumentally. There is, therefore, the need to modify the texture of breads to enhance swallowing. This has the potential to address some of the challenges confronted by older people with presbyphagia during the swallowing of food. There are, however, no published criteria for assessing the subjective ease of swallowing of food or how this relates to the physical properties of the food. Criteria that subjectively relate to ease of swallowing would be a useful biomarker for healthier foods.

A vegetable-enriched Nothing Else™ bread with soft textured crumb may not only enhance ease of swallowing but may improve the biomarkers of health effects including glycaemia, antioxidant status and lipid profile.

Glycaemic response

The consumption of food with high glycaemic index and load has been reported to have a causal effect on the risk of type 2 diabetes mellitus development (Livesey et al., 2019). The glycaemic index (GI) concept of food, introduced in 1981 (Jenkins et al., 1981), remains a classification system that deals with the measurement of the blood glucose-raising potential of carbohydrate foods. It is the incremental-area-under-the-blood glucose-curve (iAUC) recorded for a 50g available carbohydrate portion of a test food expressed as a percentage of the response to 50g available carbohydrate of a reference food consumed on another day by the same participant (Food and Agriculture Organization of the United Nations and the World Health Organization, 1998). The glycaemic load is the GI of a food multiplied by the number of grams of carbohydrate in that food as consumed. The causal relation between the consumption of foods with high glycaemic index and load and the risk of type 2 diabetes mellitus development to consumers (Livesey et al., 2019) has necessitated that, the consumption of whole or minimally processed grains are encouraged for use in bread-making as they reduce the risk of type 2 diabetes mellitus (Salmeron et al., 1997), partially related to the lower GI.

Diets high in soluble fibres elicit a lower postprandial glycaemic response as the availability of starch to α-amylase is reduced, the absorption of glucose produced from starch hydrolysis is delayed and gastric emptying time is increased (Borczak, Sikora,

Sikora, Rosell, & Collar, 2012; Guillon & Champ, 2000; Rokka, Ketoja, Jarvenpaa, & Tahvonen, 2013). The health effects of foods, such as wholegrain breads, which reduce the glycaemic load of the diet are also attributed to factors including the presence of bioactive compounds including polyphenols which inhibits the activity of amylases involved in carbohydrate digestion, and thus, favourably affect health (Sacks et al., 2014).

Lipid profile

Diet is a modifiable risk factor that impacts on blood lipid profile (Nicklas, 1995). In the blood, cholesterol transport is carried out by lipoprotein complexes including low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein and chylomicrons (Soliman, 2018). Triglyceride is one of the main constituents of lipoproteins hydrolysed by lipoprotein lipases in the blood vessels to free fatty acid and glycerol (Tada, Nohara, & Kawashiri, 2018). Laboratories routinely measure total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol. An association has been established between the consumption of foods formulated from refined carbohydrates and hypertriglyceridaemia courtesy of denovolipogenesis (Riccardi, Vaccaro, Costabile, & Rivellese, 2016). Consumption of large quantities of refined white flour bread and other carbohydrates such as rice and sugar may increase triglyceride synthesis while conversely wholegrains may reduce.

Goff, Cowland, Hooper, and Frost (2013), in a systematic review and meta-analysis of randomised controlled trials, reported that an improvement in lipid profile is associated with the consumption of diets with low glycaemic index, particularly when it is fibre enriched. Fibre promotes the reduction of plasma low density lipoprotein levels by inhibiting the absorption of bile acids and cholesterol as well as suppressing lipogenesis in the liver, eventually leading to a reduction in triglyceride concentrations (Romero, 2002). Soluble fibre in particular has been established as an ideal candidate that favourably impact on lipid profile. Bread rich in soluble fibre therefore has the potential to reduce triglyceride. Thus, the need to promote the development of fibre-rich bread is justified.

Appetite/satiety suppression

Satiety connotes the state of fullness one feels in which further eating is impaired, and is achieved subsequent to eating food (J. Slavin & Green, 2007). The perception of fullness is triggered by interaction of neurological and hormonal signals that regulate appetite (Perry & Wang, 2012).

Foods that induce a strong feeling of fullness are beneficial for weight control (L. Chambers et al., 2015). The bulking and textural properties of fibre are associated with slowed macronutrient absorption and increased satiety.

Diets rich in fibre from wholegrains, vegetables and fruits have reduced energy density and may induce the fullness feeling. The viscous nature of soluble fibres enables them to prolong the intestinal phase of digestion (J. Slavin & Green, 2007). Factors such as wheat flour characteristics (variety and particle size) and the processing technique employed during the bread-making process may help regulate postprandial glycaemic and insulinaemic responses, satiety, release of appetite regulating hormones and food intake (Burton & Lightowler, 2006; Rokka et al., 2013). In addition, plant ingredients contain phytochemicals that may influence digestion, absorption and bioavailability of essential nutrients.

Health effects of phytochemicals and antioxidants – a focus on carotenoids

Phytochemicals are plant-derived compounds including carotenoids that may have the ability to exert biological activity as part of the human diet and may include compounds that are essential vitamins in the human diet (V.M. Koistinen, Katina, Nordlund, Poutanen, & Hanhineva, 2016). Diets rich in vegetables and whole grains are rich in phytochemicals which may be associated with prevention of chronic disease (Aune et al., 2016). Whole, unrefined vegetables are rich in fibre and antioxidants such as carotenoids and may serve as functional ingredients for the food industry (Lunn & Buttriss, 2007).

Around 600 carotenoids have been identified in plants (Krinsky and Johnson, 2005). Carotenoids are biologically-active, fat-soluble pigmented compounds made up of 40 carbon atoms that are formed through covalent bonding of eight isoprene units. The presentation of the structures can be totally linear or may have rings at either one or both ends, and the rings can contain hydroxyl groups, ketones or epoxies (Elvira-Torales et al., 2019). The constituent of dietary carotenoids are α-carotene, β-carotene, lycopene and the xanthophylls, or oxygen-containing carotenoids, β -cryptoxanthin, lutein and zeaxanthin (Krinsky and Johnson, 2005). The consumption of carotenoid-rich foods may lead to yellowing of skin due to the deposition of carotenoids in the subcutaneous fat (Pezdirc et al., 2016). In terms of function, some carotenoids such as α and β-carotene are involved in the formation of vitamin A. Generally, as antioxidants, carotenoids scavenge free radicals thus mitigating against oxidative stress. The bioavailability of carotenoids is impacted by factors including dosage, form of carotenoid, the interaction of the carotenoid in the food matrix, type of food processing method applied and particle size (Elvira-Torales et al., 2019). Consequently, β-carotene absorption from foods of plant origin ranges from approximately 7% to 65% in humans (Haskell, 2012). Specifically, when carrot and spinach-based meals are co-served with enough concentration of dietary fat (approximately 10-40g/100g), β carotene absorption from carrot ranges from approximately 7% to 65% while that of spinach ranges from approximately 5% to 26% for spinach (Haskell, 2012).

Free radicals are chemical agents generated in the body due to biological reactions; they destroy relevant biomolecules, often through oxidation. They are associated with most nutrition-related non-communicable diseases particularly when the antioxidant capacity of the body is exceeded. This necessitates the need for intake of exogenous sources of antioxidants from vegetables into the body. The role of antioxidants in the prevention of diet-related non-communicable diseases including type 2 diabetes mellitus, cardiovascular diseases and cancer has been established through human and molecular studies (Fan, Zhang, Yu, & Ma, 2006).

A specific vegetable example is pumpkin which has been targeted for its higher β-carotene content (Murkovic, Mülleder, & Neunteufl, 2002). The orange pulp of pumpkin contains promising levels of carotenoids, especially β-carotene which has pro-vitamin A activity. de Carvalho et al. (2012) consequently reported that pumpkin is one of the crops targeted to help address vitamin A deficiency challenges in Brazil. The same authors reported β-carotene content ranging from 141.95 to 244.22 μg/g for the *Cucurbita moschata* pumpkin variety (de Carvalho et al., 2012). Sweet corn is another vegetable rich in the antioxidant lutein (Kurilich & Juvik, 1999). Development of a vegetable-enriched Nothing Else™ bread including pumpkin and sweet corn flours as parts of the ingredients could be a strategic way of improving the vitamin A stores of consumers. Due to the increasing public awareness of health issues, there is potential for consumer acceptance of vegetable-enriched bread with enhanced antioxidant properties (Nanditha & Prabhasankar, 2009). For older people, due to the increase in oxidative stress related to ageing, carotenoids, which are antioxidants delivered through vegetable-enriched breads, could promote reduction in the oxidative stress triggered by free radicals.

For vegetable-enriched breads to be commercially viable and reach the target market of older people, bakers need to prioritise improving the shelf life, making it affordable and ensure the taste of the bread is appealing. Marketing would also be required.

Shelf life, cost and taste consideration in bread choice

Consumers' liking and eating of bread is largely dependent on the quality parameters of palatability, nutrition and wholesomeness (Lawless & Heymann, 2010). Eating quality attributes such as the aroma, taste, aftertaste and colour of the bread remain factors that appeal to consumers and, to have a market edge, breadmakers must ensure these attributes meet consumers' liking and preference (Singh-Ackbarali & Maharaj, 2014). Consumers' liking of the sensory attributes of bread is primarily based on the responses of sensory organs.

Sensory evaluation provides cues that are essential to the understanding of consumers' liking of newly developed food products such as bread and are perceived through the sense of touch, sight, smell, taste and hearing (Sidel & Stone, 1993). External subjective factors including bread shape or crust colour also impact on consumers' liking of bread (Nagyová, Rovný, Stávková, Uličná, & Mad'arová, 2009). Additionally, Skořepa & Pícha (2016) reported that bread appearance was one key factor considered by consumers during bread buying. Bakers are therefore admonished to focus on being innovative, developing bread with variations in appearance, taste, smell and texture that meets consumers' interest but not at the expense of price.

Bread products with functional ingredients that demonstrate health effects should be sold at a reasonable price (Moslehpour, Aulia, & Masarie, 2015) as price remains crucial in influencing consumers' buying patterns (Mueller & Szolnoki, 2010). Chládková and Kudová (2008) reported that bread consumption in the Czech Republic and the European Union is on a decline and attributed one of the reasons to the increasing price of bread. Wilson et al. (2016) used linear programming software and, through a proof-of-concept study, posited that there exists the potential to formulate breads that are nutritionally superior to white breads, as well as being reasonably priced in New Zealand. An efficient way to develop a low-priced but healthier bread could be through the addition of locally-grown underutilised vegetable crops. The low-cost nutrient-dense bread must remain stable on the shelf as well.

The two major factors that compromise the storage of bread are mould spoilage and staling. This limits the shelf-life of bread to about 3-4 days in the absence of added preservatives (Muizniece-Brasava et al., 2012). Fungal growth therefore remains a critical factor in predicting the shelf-life of bread. The moisture content of bread remains around 40% with water activity (aw) of 0.96, thus making bread susceptible to mould growth (Cioban, Alexa, Sumalan, & Merce, 2010).

Bread staling depends on factors including the bread-making process, storage conditions (room temperature, relative humidity, storage with or without crust) and baking conditions (Besbes, Jury, Monteau, & Le Bail, 2014). Retrogradation of starches plays a critical role in bread staling. Additionally, cross-linking between starch and protein, partial drying and moisture transformation from crumb to crust of bread are other factors also involved in bread staling (Baik & Chinachoti, 2000; Besbes et al., 2014; Gray & Bemiller, 2003). Effective anti-staling strategies suggested to extend the shelf-life of bread include the use of emulsifiers and preservatives in bread formulations. Yet, consumers are moving away from food products enriched with synthetic additives such as emulsifiers and preservatives. A promising natural way to overcome this challenge is to enrich bread with functional ingredients including vegetables. Bread enriched with vegetables has enhanced antioxidant composition. Consequently, agents of bread spoilage including lipid and protein oxidation, are impaired (Amoah et al., 2018).

Additionally, the antioxidants act as anti-microbial agents inhibiting the microbial reactions in bread that lead to bread spoilage (Amoah et al., 2018). Also, recent technologies involving the sudden interruption of the bread-making process have been successfully employed to supply freshly baked bread to consumers. These technologies employed include refrigerated or frozen dough, frozen proofed dough, par-baked bread and fully-baked bread (Best, 1995).

Summary of literature review: The prospect of bread enrichment with functional ingredients that demonstrate health effects is possible. However, their addition in bread impacts on the physical, nutritional and sensory properties of the bread. There is therefore the need to ensure the right proportion of functional ingredients is used in composite preparation with wheat flour for bread that will be appealing to consumers in terms of the nutritional and sensory qualities. The need to explore the consumer acceptability, ease of ingestion, attenuate glycaemic response, appetite suppression and bioavailability of carotenoids following the consumption of bread enriched with functional ingredients is warranted. There is also the need to investigate the effect of vegetable-enrichment in bread and its impact on shelf life properties of the bread.

Health claims, front-of-pack labels and nutrition information panels

Promoting healthier food choices amongst consumers can be approached through several means. In New Zealand, the use of nutrition labels on food products has remained compulsory since December 2002. It is therefore imperative that food manufacturers display a nutrition information panel on food products, including an ingredient list and allergen declarations, to aid consumers in getting informed on the composition of a food product (Gorton, Ni Mhurchu, Chen, & Dixon, 2009).

Nutrition information panel, front-of-pack labels and health claims are three essential sources of information for consumers regarding the composition and potential health claims of food products marketed in the supermarket (Talati et al., 2016). Thus, food claims take the form of either nutrition claims or health claims.

Nutrition claims are made in reference to the composition of the food and includes comparative and content claims. Health claims, on the other hand, refer to the functional and physiological role the food plays in the human system, such as claim that express a reduction of a disease risk (Verhagen & van Loveren, 2016). According to Food Standards Australia New Zealand (2017), there are three forms of health claim. These include nutrient content claims which informs consumers on the presence or absence of a particular nutrient (e.g., 'rich in β-carotenes'); general-level health claims which establish the direct relationship between a food nutrient and its health function (e.g., 'rich in vitamin A for proper eyesight') and high-level health claims, which relate a particular nutrient in a food to a specific disease (e.g., 'good source of iron to reduce anaemia risk').

Several health claims with regard to fibre and oligosaccharides have been substantiated (Verhagen & van Loveren, 2016). Talati et al. (2016) reported that health claims were more likely to be considered during the assessment of food products by consumers provided they were perceived to be trustworthy, relevant and informative. The NPSC can help make a health claim on a food product.

The NPSC, used in Australia and New Zealand, is a nutrient profiling system used to assess the suitability of a food product to make a health claim based on the nutrient composition of the product (Food Standards Australia New Zealand, 2017). The NPSC helps in the determination of the Health Star Rating of a food product. The Health Star Rating front-of-pack labelling system for packaged foods was introduced to make it easier for consumers to compare the healthiness of products within a product range based on the number of stars (Dunford et al., 2017). The higher the number of stars, the healthier the product and the greater the tendency of consumers to purchase the product.

There is an emphasis on the reformulation of foods including bread, and the use of the Health Star Rating on bread (Food Standards Australia New Zealand, 2017), creating the path for the identification of healthier breads by consumers. The Health Star Rating awards positive points for protein, fibre, and fruit, vegetables, legumes and seeds, and negative for total energy, sodium, saturated fat and sugar/100g of product. For example, Homebrand essentials $1 white bread has 3.5 stars and wheatmeal has 4.0 stars. The extra 0.5 star is related to the higher fibre content of the wheatmeal bread. The increasing consumer demand for healthier foods is also driving consumers towards “clean label” and non-white and specialty bread products that are nutritionally superior and devoid of synthetic additives (Birch & Bonwick, 2018; Ranawana, Raikos, et al., 2016). Therefore, developing a reasonably priced Nothing Else™ functional bread from eight wholesome ingredients mainly sourced from New Zealand, with a Health Star Rating of 4 or above, will resonate well with consumers’ interest. On a much smaller, in-house scale, the Nothing Else™ front-of-pack label is also a brand (Brown et al., 2015) marketed previously within AUT as an upfront brand for water and for snacks such as nuts and snack bars.

Branding of food products

Extrinsic product indicators such as packs and branding influence consumers’ choice of food products (Deliza & MacFie, 1996). Mueller and Szolnoki (2010) observed that the style of the label and brand are two key drivers promoting the acceptability of food products by consumers.

A brand logo has the inherent ability to positively influence the perception of a brand of food products for consumers. Several studies have reported that the design attributes of a brand logo that can be visually observed, such as colour (Labrecque & Milne, 2011; Madden, Hewett, & Roth, 2000) and shape (Jiang, Gorn, Galli, & Chattopadhyay, 2016), have the potential to initiate symbolic associations that creates an enabling platform for consumers to relate back to the brand, implicating brand logo design as an influential tool on brand image (Fajardo, Zhang, & Tsiros, 2016). Brands with appealing images have a higher revenue and profit-boosting potential (Bharadwaji & Menon, 1993) and impact positively on the financial performance of the brand (C. W. Park et al., 2013). Examples of such brands include Heinz™ which is used in marketing the widely accepted tomato ketchup and Sanitarium™ which is a widely accepted brand for breakfast cereal in New Zealand.

In 2010, AUT in New Zealand, in its quest to ensure the sustainable consumption of food products, developed the Nothing Else™ brand (Brown et al., 2015). The Nothing Else™ brand uses at least eight natural ingredients and has been used to market a healthier snack bar since 2013. The specific questions asked in this study were:

Can two potential vegetable-enriched Nothing Else™ breads with eight wholesome ingredients, when compared to control $1 WB and WMB, have appealing physical properties, be liked and easier to swallow when consumed?

Can a potential vegetable-enriched Nothing Else™ bread with eight wholesome ingredients, when compared to control $1 WB and WMB, attenuate the insulin and blood glucose response, and improve appetite over a 120-minute period following consumption?

Can a potential vegetable-enriched Nothing Else™ bread with eight wholesome ingredients increase blood carotenoid concentration and improve lipid profile when consumed every day over two weeks?

Chapter 3. Bread formulation and preparation of vegetable-enriched breads

Advisory support group

There was close engagement with an advisory network which was established at the commencement of this study. This created the opportunity to enhance collaboration and harmonisation of input throughout the research process. The role of the individuals and institutions constituting the advisory group is highlighted below (**Table 3.1**).

Table 3.1. Role of individuals and institutions constituting the advisory group

Person/Organisation	Role
Arno Sturny	Master baker, AUT Hospitality and Tourism – Provided expertise on the formulation and bread-making process and access to AUT kitchens
Prof. Owen Young	Provided advice on the use of facilities at the AUT Food Science Laboratory
Tania Watson	Bakers Research Institute – Provided advice on the path to commercialisation aspects
Steph Clout	Provided library support services for systematic reviews
Dr. Fabrice Melian	AUT Roche Diagnostic Laboratory – Provided advice on laboratory investigation of serum samples and access to laboratory
Dave Brown	The designer of the Nothing Else™ brand
Mitali Purohit	AUT Enterprises Ltd (AUTEL) – Provided advice on Intellectual Property (IP) protection
Wilson Huang	AUT Enterprises Ltd (AUTEL) – Provided advice on IP protection
Riddet Institute	Centre of Research Excellence – PhD Scholarship
Cedenco	Supplier of vegetable flours

Rationale for selection of ingredients for the vegetable-enriched Nothing Else™ bread formulation

Flexibility exists around the choice of the main ingredients (flour, yeast, salt) for bread-making, allowing the substitution of wheat flour with other ingredients (Wilson et al., 2016). The use of whole grains (Vuksan et al., 2010), vegetable flours (Ranawana, Campbell, et al., 2016) and oilseeds (de Lamo & Gomez, 2018) in bread to enhance its antioxidant and potential health-beneficial properties has been reported.

The potential high demand for bread with health claims in New Zealand highlights the market potential of the proposed vegetable-enriched Nothing Else™ bread. Developing a reasonably-priced vegetable-enriched Nothing Else™ bread from eight wholesome ingredients mainly sourced from New Zealand, with a Health Star Rating of 4 or above, is a unique initiative to provide a sustainable and healthier bread for the New Zealand market.

Pumpkin powder

Pumpkin belongs to the genus Curcubita and of the family curcubitaceae and includes varieties *Cucurbita pepo*, *Cucurbita moschata* and *Cucurbita maxima* (Murkovic et al., 2002). Total carotenoid concentration ranging from 404.98 and 234.21 μg/g for pumpkin variety *Cucurbita moschata* has been reported (de Carvalho et al., 2012). The same authors reported an α-carotene and β-carotene content ranging from 67.06 to 72.99 μg/g and 141.95 to 244.22 μg/g respectively for the *Cucurbita moschata* variety (de Carvalho et al., 2012). Carotenoids are essential biological compounds derived from vegetables including pumpkin and carrot that act as raw materials for vitamin A formation and demonstrate antioxidant properties (Moran et al., 2018). Carotenoids are broadly grouped into carotenes and xanthophyll, with the carotenes consisting of α and β carotenes (Krinsky & Johnson, 2005). Beta carotene is the most common form though both α and β forms exist in plants. The recommended dietary intake (RDI) of carotenoid for men is 900μg/day and that for women is 700μg/day (National Health and Medical Research Council (Australia), 2006). Also, the presence of certain organic acids, including citric acid, fumaric acid and malic acid, have been identified in pumpkin varieties (Nawirska-Olszanska, Biesiada, Sokol-Letowska, & Kucharska, 2014). These organic acids could impact on the aroma of pumpkin flour when used for new food product development. The pulp of pumpkin contains significant amount of pectin, a water-soluble fibre and a major component of plant cell walls (Fissore, Matkovic, Wider, Rojas, & Gerschenson, 2009). Pumpkin powder significantly decreased glucose, triglycerides, LDL, C-reactive protein (CRP) and cholesterol in alloxan-induced diabetic rats (2g/kg body weight) during a four-weeks intervention (Asgary et al., 2011). In this study, the diabetic rats received pumpkin powders through gavage feeding once a day. The pumpkin powders were produced from dried pumpkin pulp ground into powder using an electric grinder. The authors did not state how the pumpkin was prepared for gavage e.g. if it was mixed with oil or water.

Pumpkin flour has been reported to have a great potential in supplementing cereal flours for the development of baked products (Dhiman, Sharma, & Surekha, 2009; Xanthopoulou, Nomikos, Fragopoulou, & Antonopoulou, 2009). In our study in Ghana where pumpkin flour was enriched with wheat for bread development at substitution (0%, 5%, 15% and 25%), we found that increasing pumpkin enrichment resulted in bread with lower specific volume (Adubofuor et al., 2018). Bread with 5% pumpkin flour was the most acceptable and not different from the control bread (Adubofuor et al., 2018). Similarly, Wongsagonsup, Kittisuban, Yaowalak, and Suphantharika (2015) explored the use of pumpkin flour as a composite ingredient with wheat flour in bread-making and reported that an increase in pumpkin flour substitution resulted in loaf volume reduction and increase in bread crumb yellowness. El-Demery (2011) explored the use of pumpkin flour in bread formulation and observed that loaf weight and water-holding capacity reached it maximum at substitution levels of 15% and 20%.

Sweet corn powder

Sweet corn (*Zea mays* saccharata Sturt) is a corn variety with immature sweet kernels used as food. They are processed into canned, frozen and powdered forms for use in food product development (Tokuji et al., 2009). Sweet corn is a rich source of dietary fibre cellulose and β-glucan (Topping & Clifton, 2001) and the carotenoids lutein and β-carotene (Kurilich & Juvik, 1999). Chun et al. (2005) reported that sweet corn has high levels of total phenols, flavonoids and antioxidants. The health-beneficial compounds in sweet corn makes it an essential food ingredient that can be utilised in functional bread-making.

Flaxseed

Flaxseed (*Linum usitatissimum*) has received wide attention as a functional food ingredient due to its excellent nutrient profile (Marpalle, Sonawane, & Arya, 2014). The postprandial lipaemia, appetite suppression, improved glycaemic response and serum cholesterol lowering following flaxseed intake has been reported (Hutchins et al., 2013; Kristensen et al., 2013; Thakur, Mitra, Pal, & Rousseau, 2009). The health effects of flaxseed could be attributed to its rich content of omega-3 fatty acids, dietary fibre, high protein quality, and antioxidants including phenolics and lignin (Hussain, Anjum, & Alamri, 2011). The presence of fibres including mucilage (Kristensen et al., 2013) and gums (Thakur et al., 2009) in flaxseed has been reported. In a randomised, cross-over study by Hutchins et al. (2013), overweight or obese men and postmenopausal women (n= 25) with pre-diabetes consumed 0, 13, or 26g of ground flaxseed for 12 weeks. Prediabetes was defined by the authors as participants with impaired fasting glucose concentration ranging between 100 and125 mg/dL (5.5 and 6.9 mmol/L) analysed from drawn fasted blood. The authors reported that the consumption of the 13g ground flaxseed resulted in insulin and glucose reduced by a statistically significant but biologically trivial 0.1 mmol/L and 2 mU/L. In another study, the consumption of chapattis containing flaxseed gum (5g) by 62 participants with type 2 diabetes mellitus for three months resulted in a significant decrease in LDL cholesterol from 11,098 mg/dl to 9,299 mg/dl (Thakur et al., 2009). Kristensen et al. (2013) investigated the effect of flaxseed dietary fibre-enriched meal consumption on postprandial lipemia and appetite suppression. In that study, 18 young men took four different flaxseed fibre-enriched meals of same caloric content served after an overnight fast in a double-blind randomised crossover design. The flaxseed dietary fibre-enriched test meal composition included: control, 1.4g/MJ; whole flaxseed, 2.4g/MJ from whole flaxseeds; low-mucilage dose, 2.4g/MJ from flaxseed dietary fibre; and high-mucilage dose, 3.4g/MJ from flaxseed dietary fibre. The authors posited that the consumption of the high-mucilage dose-enriched test meal significantly attenuated triglyceride concentrations by 18% and increased participants' feeling of fullness compared to the control. Insulin release was also significantly attenuated following the consumption of the low- and high-mucilage-enriched test meals. Ibrügger, Kristensen, Mikkelsen, and Astrup (2012) investigated how the consumption of flaxseed dietary fibre supplements impact on appetite and food intake suppression acutely. The study was two-fold and employed a single-blinded randomised crossover design where 24 and 20 participants respectively took a 300ml drink (control) and a 300ml drink with added flax fibre extract (2.5g of soluble fibres) (first study) and a control drink with flax fibre tablets (2.5g of soluble fibres) (second study). The authors reported that the consumption of the flaxseed drink resulted in increased fullness sensation compared to the control, consequently leading to a significant decrease in subsequent energy intake.

Flaxseed flour incorporated into bread enabled the crumb to retain moisture, resulting in bread with a soft crumb (Marpalle et al., 2014). The antioxidant properties of flaxseed originate from its high content of phenolic compounds and could prevent the oxidation of lipids and proteins in bread, a role essential to enhance the shelf-life of the bread

(Kasote, Hegde, & Deshmukh, 2011). Incorporating flaxseed into bread remains a viable approach to providing a healthier bread for New Zealanders.

Sprouted wheat flour

Sprouting enhances the physicochemical, nutritional, functional and organoleptic attributes of grains and cereals naturally (Blandino, Al-Aseeri, Pandiella, Cantero, & Webb, 2003; A. K. Singh, Rehal, Kaur, & Jyot, 2015). Incorporating sprouted wheat flour into regular wheat flour for bread-making improves the amount of carbon dioxide gas produced during leavening, producing bread with high specific volume and soft crumb texture (Marti, Cardone, Nicolodi, Quaglia, & Pagani, 2017). The use of sprouted wheat flour to improve the enzymatic activity of wheat dough has been reported (Marti et al., 2017). Additionally, during sprouting of grains and cereals, production of essential bioactive compounds occurs and may increase the health benefits of whole grains (Nelson, Stojanovska, Vasiljevic, & Mathai, 2013).

Biochemical changes take place during grain sprouting including the breakdown of stored energy reserves from starch, lipids and proteins (Theodoulou & Eastmond, 2012). Anti-nutritional factors which hitherto would have prevented the bioavailability of essential minerals in the cereal grain is reduced during grain and cereal sprouting (Benincasa, Falcinelli, Lutts, Stagnari, & Galieni, 2019). Antinutrients including phytate in grains bind to minerals in a food matrix preventing the minerals from being absorbed from the food matrix (Benincasa et al., 2019). Sprouting a form of processing seed kernel has been reported to reduce phytate concentrations resulting in increased bioavailability of minerals compared to their unsprouted counterparts (Benincasa et al., 2019). The functional properties of cereals is enhanced as a result of the generation of bio-functional compounds, increase in protein solubility, *in vitro* protein digestibility and lowering of glycaemic index during sprouting (A. K. Singh et al., 2015).

Wholemeal wheat flour

Wholemeal wheat flour has gained wide patronage by consumers in recent times. This is attributed to its rich composition of essential compounds that demonstrate health effects, including phytochemicals and antioxidants. Wholemeal wheat flour contains all the essential parts – the bran, the endosperm and germ – in contrast to the refined grains, which has the bran and the germ of the grains removed during the milling process (Gani, Wani, Masoodi, & Hameed, 2012). Carotenoids, phenolics and flavonoids remain the major antioxidants in wheat, mainly concentrated in bran layers, and the amount of antioxidants depends on the grain variety (K. Kim, Tsao, Yang, & Cui, 2006). Consuming bread prepared from minimally processed wheat bran, and particularly the aleurone fraction, increased plasma betaine concentrations which aids in liver function and inactivation of harmful chemicals including drug by-products (Keaveney et al., 2015). The use of wholemeal wheat flour in bread-making is a way of improving the nutrient profile of bread.

Bread development

Based on the preliminary work undertaken to inform this research, it was decided to develop two vegetable-enriched Nothing Else™ breads: one with a higher vegetable concentration (VB100) than the other (VB75).

In collaboration with Arno Sturny and using the AUT School of Hospitality and Tourism training kitchens, prototype breads using eight wholesome ingredients to fit the Nothing Else™ criteria were developed. Through informal testing, taste and palatability were favourably assessed.

All ingredients used for the bread preparation were stored at the School of Hospitality and Tourism until ready to be used. The bread-making process was carried out at the School of Hospitality and Tourism. The indirect bread-making method was used for the breads produced. The indirect method of bread-making was used for the bread development for the liking and swallowing study. A pre-ferment consisting of 225g wholemeal flour, 150g water and 0.5g instant yeast was developed for 2 minutes to tight dough consistency. The mixture was left covered for 12 hours at 20℃ to allow for fermentation. A 150g portion of linseeds was soaked in 180g boiled water for 2 hours at room temperature before placing in chiller for 10 hours. The final dough was prepared by mixing 450g strong wheat flour, sprouted red wheat flour (115g), pumpkin powder (75g), sweet corn dried (20g), salt (15g), instant yeast (15g) and water (600g) for 8 minutes. The dough was developed for 6 minutes. The soaked linseed was added to the final mixture and mixed until incorporated. The dough was allowed to undergo bulk fermentation for one and half hours. The fermented dough was cut and shaped into logs and placed in tins and proved for an hour. The tins of dough were placed in a steam oven and baked at 215℃ for 35 minutes. After baking, the VB75 breads were allowed to cool and packaged in transparent rubber packs. The same procedure was repeated for the VB100 breads except for the amount of pumpkin flour that was scaled up to 100g. It must however be mentioned that, the VB used for the glycaemia and carotenoid study had the sprouted wheat flour reduced to 15 g and extra wholemeal flour added to make up to 325g.The sprouted wheat flour used for VB formulation was different from the sprouted red wheat flour used for the VB75 and VB100 in terms of the places sourced and possibly different physicochemical properties. As seen from (**Table 3.3**), when 115g of the sprouted wheat flour was used for VB formulation, the baked bread collapsed and thus resulting in it reduction to 15g. The ingredients used for the bread formulation and places sourced is presented in (**Table 3.2**).The recipe formulation for the various bread used for the study is presented in (**Table 3.3).**

Table 3.2. Ingredients used in bread formulation and places sourced from

Ingredients	Source	Location, New Zealand
Strong white flour	Champion	Auckland
Wholemeal flour	Champion	Auckland
Flaxseed	Ceres Organic	Auckland
Sprouted red wheat flour/sprouted wheat flour	Huckleberry/Food Source	Auckland
Pumpkin powder	Cedenco	Gisborne
Corn powder	Cedenco	Gisborne
Yeast	Bakels	Auckland
Salt	Cerebos Skellerup	Auckland

Table 3.3. Recipe formulation for bread development

Ingredient	VB75	VB100	VB Trial 1	VB Trial 2
Water	930g	930g	930g	930g
White strong flour	450g	450g	450g	450g
Wholemeal wheat flour	225g	225g	225g	325g
Flaxseed	150g	150g	150g	150g
Sprouted red wheat flour/sprouted wheat flour	115g	115g	115g	15g
Pumpkin powder	75g	100g	75g	75g
Sweetcorn powder	20g	20g	20g	20g
Instant yeast	5.5g	5.5g	5.5g	5.5g
Salt	15g	15g	15g	15g

Note. VB75 and VB100 are bread used for Study 1. VB bread was used for study II and III

Figure 3.1. Preliminary Nothing Else™ functional bread dough ready for baking.

Figure 3.2. Preliminary baked vegetable-enriched Nothing Else™ bread.

The Health Star Rating was calculated using the NPSC (Food Standards Australia New Zealand, 2019). The Health Star Rating Calculator Score (HSRCS) (% food methods) for the two vegetable-enriched breads was -3 and the Health Star Rating of 4 implies that health claim can be made. The nutrition profiling score (NPS) and the health star rating was calculated from the criteria for energy, saturated fat, sodium, sugars, fibre, protein and vegetable content /100g of the bread (Food Standards Australia New Zealand, 2019). Two numbers are derived: the nutrient profiling score which is between -10 and 40 healthy to less healthy and the inversely related health star rating which ranges between 0.5 to 5 stars with 5 stars most healthy (Food Standards Australia New

Zealand, 2019). For the two vegetable-enriched breads the NPS was -3 and the Health Star Rating 4. A health claim can be made on a food if the NPS is less than 4.

Strengths and limitations

The harmonisation of cross-faculty expertise and the availability of diverse facilities at the university enhanced the smooth process of this research from the conceptualisation through to the development phase. The researcher (IA) had previously worked with pumpkin flour for bread and breakfast cereal development and understood the properties and behaviour of pumpkin flour, such as its high-water absorption property promoting dough stickiness and dough with lower extensibility especially with increased pumpkin flour substitution. The harmonisation of expertise from the artisanal baker resulted in the development of bread with high loaf volume, soft crumb and a relatively golden crust.

In contrast, for mainstream food product development, factorial designs are employed with variations in mixes, cooking temperature and time and leavening to optimise the product. In this present study, this was not followed but rather past research work published (Adubofuor et al., 2018; Asaam et al., 2018) coupled with the rich experience of Arno Sturny, an artisanal baking expert, was harmonised in the pre-trial phase leading to the development of the vegetable-enriched bread. However, there was no consideration of cost for the vegetable-enriched bread as the organoleptic and nutritional qualities were prioritised. Considering the commercial prospect of the vegetable-enriched bread, the cost of bulk production and mark-ups across the supply chain would be necessary to present the product to potential investors.

Conclusion

Two breads enriched with vegetables were developed to meet the eight or fewer Nothing Else™ criteria with a Health Star Rating of 4.

Chapter 4. Physical properties, liking and ease of swallowing of vegetable-enriched bread compared with commercial breads as evaluated by older adults

Abstract

Characteristics of food that influence liking and ease of chewing and swallowing are not well understood. Reformulation of bread to improve nutrient density may improve liking, ease of chewing and swallowing which could improve dietary intake particularly with aging. The study aimed to compare objectively and subjectively four breads of increasing nutrient density: $1 white (WB) and wheatmeal (WMB) commercial breads and two in-house formulations of vegetable-enriched breads (VB75 or VB100) which incorporated drum-dried pumpkin and sweet corn flours for physical, sensory and ease-of-chewing and swallowing properties. Each bread underwent instrumental texture analysis. The commercial and vegetable-enriched breads were not different by hardness or springiness but the vegetable breads were up to 25% less cohesive, less gummy and less chewy than the commercial breads. Questionnaires and Likert scale (150mm) responses were completed by 50 physically active volunteers aged 50+ years. Overall liking of the VB75 and VB100 were rated as 40% higher than the white and wheatmeal breads. Vegetable-enriched breads were rated as almost 50% easier to chew (mean±SD; WB 70.53±39.46mm, WMB 77.68±33.13mm, VB75 104.78±30.69mm, VB100 107.58±24.90mm) and swallow (WB 70.29±37.98mm, WMB 77.53±34.88mm, VB75 104.63±28.25mm, VB100 104.90±25.54mm). Vegetable-enriched breads compared to white and wheatmeal breads were instrumentally and subjectively less gummy, cohesive and chewy than commercial breads and have the potential to both improve nutrition and 'ease of swallowing' in older people and maybe those who are susceptible to presbyphagia. New areas of research should explore other underutilised vegetables for bread enrichment and their ability to aid swallowing and improve nutrition status.

Keywords: Bread, older adult, sensory evaluation, swallowing evaluation, crumb texture

Introduction

Consumer demand for food is motivated by cost (Pappalardo & Lusk, 2016), perceptions of taste, healthiness and naturalness (Kraus, 2015) with the last two factors driven by awareness of the importance of diet in health and diet-related non-communicable diseases. Bread, a staple food in New Zealand (Ministry of Health, 2011), contributes 11% of total daily energy intake and older people are more likely to choose wholegrain bread (60%) than younger people (Ministry of Health, 2011). Therefore, the reformulation of bread to improve its nutrition for older people could be attractive to breadmakers and consumers of bread.

In New Zealand, the yearly sales of bread increased between the year to 19/06/2016 and the year to 18/06/2017 by 3.5% (from $462,491,400 to $479,077,700) (NielsenScantrack, 2017). In the same trading period, non-white bread and specialty bread also increased in sales value by 3.3% (from $233,008,800 to $241,028,800) and 6.6% (from $67,855,300 to $72,670,200) respectively (NielsenScantrack, 2017). In addition to the supply of energy and the macronutrients carbohydrate, protein and fat, breads may have health effects and can be called “functional breads” (Birch & Bonwick, 2018). Drum-dried pumpkin and sweet corn powders are two potential functional ingredients that could be utilised in bread formulation. Bioactive compounds including carotenoids (de Carvalho et al., 2012) and essential micronutrients such as potassium are present in pumpkin (Adubofuor, Amoah, & Agyekum, 2016) whilst sweet corn is a source of dietary fibre (I. Singh, Langyan, & Yadava, 2014), which may demonstrate health effects though its consumption by people with renal challenges may be taken with caution due to the adverse effect of potassium on the kidney and those taking drugs that act as ACE inhibitors for the regulation of blood pressure (Momoniat, Ilyas, & Bhandari, 2019). Despite the potential benefits associated with functional bread consumption, the incorporation of functional ingredients in bread will impact on its physical properties including the textural properties of bread crumb and sensory attributes (Longoria-García et al., 2018). Thus, there is the need to ensure a balance between sensory attributes and textural properties.

Particularly for older people, difficulties associated with chewing and swallowing affect the ability to ingest certain foods including bread (Wirth et al., 2016; Woo, Tong, & Yu, 2018) which may result in poor outcomes in their nutrition and health status (Tagliaferri, Lauretani, Pela, Meschi, & Maggio, 2018). In New Zealand, omeprazole, a medication prescribed for patients with swallowing difficulties secondary to acid reflux, is the third most prescribed medication (Gleeson et al., 2013; PHARMAC, 2019), attesting to how common dysphagia is, especially amongst older people. Many older people have discretionary funds which potentially increase their ability to buy palatable and nourishing bread which is easy to swallow. There is therefore the need to evaluate the physical properties, sensory attributes including "ease of swallowing" and demand of breads with the target market of older consumers.

The objective of this study was to evaluate the physical, sensory and swallowing attributes of two vegetable-enriched Nothing Else™ breads compared with controls, two commercially produced breads $1 white and wheatmeal breads, with 50 older physically active adults aged 50+. The hypotheses were that:

a. The vegetable-enriched Nothing Else™ breads will have softer crumb texture than the control breads possibly due to their fibre/pectin composition that has good moisture keeping properties.

b. The higher pumpkin flour content vegetable-enriched Nothing Else™ bread will be easier to swallow than the lower pumpkin flour content vegetable-enriched bread, and both will be easier to swallow than the control commercial breads.

c. The vegetable-enriched Nothing Else™ breads will be more liked and consumers would be willing to eat them at home.

Design and methods

Selected ingredients and bread-processing

This experimental study involved the formulation of two vegetable-enriched Nothing Else™ breads. The physical and subjective (human participant) measures were compared to controls of $1 commercial white and wheatmeal breads. The two breads enriched with drum-dried pumpkin and sweet corn vegetable flours met the eight ingredients or less requirement of the Nothing Else™ brand.

In Ghana, as part of my role as a research assistant, we used solar dried pumpkin powder to formulate pumpkin and wheat composite bread (Adubofuor et al., 2018). Therefore, there was prior knowledge about the proportions of ingredients that would allow bread to rise and have an acceptable colour. In addition, the expertise of Arno Sturny, a Master baker from AUT School of Hospitality and Tourism, combined with that of the researcher (IA), resulted in the two recipes that included the ingredients, in descending order by weight: strong white flour, wholemeal flour, flaxseed, sprouted wheat flour, pumpkin flour, sweet corn flour, instant yeast and salt.

The bread was produced in the kitchens of AUT School of Hospitality and Tourism, Auckland, New Zealand. The two vegetable-enriched breads differed only in the proportion of pumpkin powder where the VB100 contained 25% more pumpkin powder (dry mix) than the VB75. All ingredients were stored in airtight containers in the cold room of AUT School of Hospitality and Tourism prior to formulation and development of the breads.

Selected ingredients and bread-processing

The indirect dough method for bread preparation was used for the bread-making, thus preparation of the pre-ferment and soaker (soaked flaxseed) preceded the preparation of the dough.

Table 4.1. Ingredients source and rationale for their use in bread formulation

Ingredients	Rationale
Strong white flour	Promotes gluten network formation
Wholemeal flour	Enhances the fibre and bioactive content of bread
Flaxseed	Enhances the fibre concentration of bread and attenuates glycaemic response
Sprouted red wheat flour/sprouted wheat flour	Increases resistant starch in bread to attenuate glycaemic response, enzyme improver, enhances enzymatic activity during fermentation and improve bioactive content
Pumpkin powder	Increases carotenoid concentration
Corn powder	Increases carotenoid and fibre concentration
Yeast	Promotes fermentation consequently resulting in the release of CO_2 that promotes the leavening of loaves
Salt	Impacts charges on the gluten proteins enhancing the network formation and augments taste

Table 4.2. Proximate composition of breads

Component	*WB	*WMB	†VB75	††VB100
Moisture (%)	36.57	38.19	39.11	46
Protein (g/100g)	8.5	8.8	ND	6.5
Dietary fibre (g/100g)	2.7	4.6	7.2	6.5
Insoluble fibre (%)	ND	ND	5.5	ND
Soluble fibre (%)	ND	ND	1.7	ND
Fat (g/100g)	1.6	1.7	ND	ND
Carbohydrate (g/100g)	46.7	43.1	ND	36
Sodium (mg/100g)	392	398	380	380
Potassium (mg/100g)	ND	ND	300	277
Energy (kJ/100g)	1020	982	ND	889
β-carotene (μg/100 g)	ND	ND	236.78	ND

*Note.** As reported on the nutrition information panel. †analysis by Asurequality, an Internationally Accredited New Zealand laboratory ††Derived from recipe with the New Zealand Food Composition Tables (Ministry of Health, 2019e). ND= Not determined. WB-White Bread, WM-Wheatmeal bread, VB75 and VB100- Bread with 75 and 100g pumpkin substitution.

Physical analyses

Physical analyses included determination of baking loss, loaf volume, specific volume, colour and textural attributes. Each of these is described in turn below.

Baking loss

All weights were measured in triplicate to a precision of 0.5g. The difference in weight between the weighed bread loaves after baking and the weighed dough used in baking was recorded. Baking loss (%) was calculated according to the following formula (Fan et al., 2006):

Baking loss (100%) = $[1- (\frac{Bread\ weight}{Total\ material\ weight})] \times 100\%$

Loaf volume and specific volume determination

The loaf volume of the vegetable-enriched breads was determined according to the rapeseed method (Shittu, Dixon, Awonorin, Sanni, & Maziya-Dixon, 2008) but with a slight modification with the use of sorghum instead of rapeseed. Briefly, a weighed quantity of sorghum seed was poured into a volumetric flask. The volume occupied by the weighed sorghum seeds was recorded and used as a reference volume in comparison with the volume displaced by the bread loaf. The specific volume was determined by dividing the volume of bread (mL) by the weight of the bread samples (g). Four replicate measures were made for four loaves from each bread batch.

Colour

The colour of the crust of the bread loaf and of the crumb of sliced bread in terms of L* (lightness), a* (redness), and b* (yellowness) values in the CIELAB colour space (McGuire, 1992) were measured directly with a Nix Pro Colour Sensor (Hamilton, Canada) on the sliced bread samples (thickness 11.50 mm). Colour measurement was recorded for at least five replicates for each bread.

Texture analysis

Texture profile analysis (TPA) was determined using a (TA.XT.plus texture analyser, Stable Microsystems, Surrey, UK) with a 5kg load cell. Crumb slices of 11.50 mm were 75% compressed. Parameters used include a pre-test speed of 5.00 mm/sec, test speed of 1.00 mm/sec, post-test speed of 5.00 mm/sec, target mode-strain, time of 5.00 sec and trigger force of 0.010N. A minimum of five replicates from the sliced breads were analysed and averaged. The parameters recorded were hardness, chewiness, cohesiveness, springiness, gumminess and resilience.

Acceptability and swallowing

The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human participants were approved by the Auckland University of Technology Ethics Committee (AUTEC) (New Zealand, 18/22) (**Appendix 1**).

The number of participants required was calculated based on previous work (Hough et al., 2006). The number of participants was 29 when the meaningful difference over a 150mm Likert scale was set at 20%, with an α of 0.05 and β of 0.1.

Participants were 50 older physically active people (50+ years) predominantly European who were registered members of the never2old group (an exercise programme), and regularly attend the Sport and Fitness Centre at the Auckland University of Technology, North Shore campus, Auckland, New Zealand. The participants were advised about this study by the leaders of the never2old programme and the advertisement was posted on the notice boards in the fitness centre (**Appendix 4**). The researcher gave a brief presentation to potential participants prior to a fitness session and information sheets were distributed. Participants who responded to the invitation were recruited in a chronological manner. Participants were excluded if they were receiving drugs that would affect taste (e.g., chemotherapy) or were gluten intolerant or allergic to any of the ingredients. Inclusion criteria were: aged more than 50 years, consumed bread at least once a week, and had no known allergy or intolerance to gluten. After a one-on-one opportunity was provided for participants to ask any questions, an appointment was made for the testing.

Participants attended the sensory evaluation sessions in a sensory room at the fitness centre. Each participant was seated at a table so that they could not see other participants. Participants signed a consent form and were presented with two slices each of each of the four different breads (one slice with crust and a second de-crusted slice of 11.50 mm square). The bread was served in an unrandomised (first 29 participants) and randomised (next 21 participants) order to the consumers on white plates identified with random three-digit numbers. For logistical reasons each portion of bread had been stored sealed and frozen and was allowed to defrost, sealed for a 1-hour period. Water was used to rinse the mouth between breads to minimise any residual effect between samples. Four questionnaires, one for each bread, with 150 mm Likert scales anchored by verbal end points at each end for attributes including "colour", "aroma", "taste", "texture", "mouthfeel", "overall liking" and "willingness to eat at home" and ''ease of biting and getting into the mouth, ease of chewing, ease of swallowing and ease of movement through the throat" were presented to each participant (**Appendices 5a, 5b and 5c**).

Statistical analyses

All the analyses were done using Statistical Package for the Social Sciences (SPSS) version 24.0 software (IBM, New York). The results on physical properties and ease of swallowing of bread were subjected to one-way analysis of variance (ANOVA) at 95% confidence interval. Post hoc Tukey's test was used to compare the mean values and establish significance differences at $p<0.05$. Sigma plot® software was used to visually establish the relationship between the objective and subjective perceptions associated with ease of swallowing of the bread.

Results

The fifty participants were predominantly female; 62% females and 38% males. In terms of ethnic segregation, Europeans constituted 96%, Australians 2% and Asians 2%. In relation to age segregation, 70-79 years (52%), 80-89 years (24%), 60-69 years (20%) and 50-59 years (4%).

Loaf weight, baking loss, loaf volume and specific volume of the VB75 and VB100 breads were not significantly different (**Table 4.3**). The VB75 and VB100 breads had substantially darker (>40% darker) crusts than the WB and WMB. The lightness, redness and yellowness colours of the crusts of the VB75 and VB100 breads were not different.

Physically, the hardness and springiness among the four breads were not significantly different. The WMB was the most resilient to compression and had higher cohesion than the two vegetable-enriched breads but not the WB (**Table 4.3**). Resilience and cohesion of VB75 were higher than VB100 but springiness, gumminess and chewiness were not different between the two vegetable-enriched breads. Objective chewiness was higher for the WMB compared with both the VB75 (1.64 units, 95% CI 0.42, 3.25 p=0.043) and VB100 (1.93 units, 95% CI 0.49, 3.37, p=0.006) but not the WB.

Table 4.3. Physical and textural attributes of the four breads

Characteristics	N	Bread samples				
		WB	WMB	VB75	VB100	p-value
Physical, textural attributes						
Loaf weight (g)	4			420.0±1.6^{a}	421.8±2.5^{a}	0.556
Baking loss (%)	4			10.4±0.3^{a}	10.1±0.5^{a}	0.556
Loaf volume (mL)	4			1027.7±25.1^{a}	1047.3±11.9^{a}	0.381
Specific loaf volume (mL/g)	4			2.5±0.1^{a}	2.5±0.0^{a}	0.498
Loaf crust colour						
L*	7	57.1±6.5^{b}	53.26±1.7^{b}	33.2±1.0^{a}	35.56±4.1^{a}	<0.0001
a*	7	17.5±1.3^{b}	17.4±1.1^{b}	14.3±0.9^{a}	15.4±0.7^{a}	<0.0001
b*	7	35.5±2.9^{b}	33.0±1.3^{b}	19.2±1.2^{a}	22.1±2.6^{a}	<0.0001
Loaf crumb colour						
L*	10	83.7±2.8^{c}	77.8±2.5^{b}	55.0±4.5^{a}	55.4±3.6^{a}	<0.0001
a*	10	-0.1±0.2^{a}	2.0±0.6^{b}	3.2±0.8^{c}	4.3±1.4^{d}	<0.0001
b*	10	10.3±1.0^{a}	15.3±1.8^{b}	36.1±2.5^{c}	40.0±1.8^{d}	<0.0001
Hardness (g)	5-9	8.49±1.74^{a}	8.51±1.00^{a}	8.68±2.23^{a}	10.06±1.09^{a}	0.131
Resilience (%)	5-9	28.78±3.14^{b}	33.41±3.22^{c}	27.06±3.23^{b}	20.58±2.65^{a}	<0.0001
Cohesion	5-9	0.74±0.05^{c}	0.80±0.04^{c}	0.60±0.06^{b}	0.52±0.02^{a}	<0.0001
Springiness (%)	5-9	84.74±17.87^{a}	91.51±1.84^{a}	87.55±4.46^{a}	82.36±5.62^{a}	0.170
Gumminess	5-9	6.21±1.14^{a,b}	6.80±0.80^{b}	5.19±1.36^{a}	5.23±0.51^{a}	0.006
Chewiness	5-9	5.41±1.81$^{a, b}$	6.23±0.79^{b}	4.59±1.33^{a}	4.30±0.43^{a}	0.007

Note. Data is expressed as mean ± standard deviation. Values with different superscript in a row are significantly different (p<0.05). WB = white bread, WMB = wheatmeal bread, VB75 and VB100 = breads with 75 and 100g pumpkin substitution. Means with different superscripts in a column are significantly different (p < 0.05). N= Number of replicates. L* indicates the lightness value, a* indicates the degree of redness and b* indicates the degree of yellowness. A higher the L*, a* and b* value means a higher degree of lightness, redness and yellowness of the bread respectively. A higher hardness, resilience, cohesion, gumminess and chewiness value indicates a higher degree of bread crumb hardness, resilient, cohesion, springinessy, gumminess and chewiness of the bread crumb respectively following the double compression measurements of using the texture analyser probe.

Evaluated by the participants, the VB75 and VB100 breads were liked almost twice as much as the WB and WMB for all the sensory attributes assessed. The participants also stated that they were willing to eat the VB75 and VB100 breads at home (**Table 4.4**). There were no differences between the WMB and the WB for the liking attributes except for the colour which was liked more for the WMB than the WB. Both the VB75 and VB100 recorded scores almost twice those of the WB and WMB for willingness to eat at home (**Table 4.4**).

WB was perceived as more difficult to bite and get into the mouth, chew, swallow and move through the throat compared with the other breads (**Table 4.4**) and also that it sticks in the throat more during swallowing. The VB100 was perceived as the easiest to chew and swallow and moved more easily through the throat with less throat stickiness. The swallowing attributes of VB75 bread were, however, not significantly different from the VB100 in terms of ease of bite and getting into the mouth (p=0.99), ease of chew (p=0.97), ease of swallow (p=1.00), ease of throat movement (p=0.99) and less stickiness in throat (p=0.96). The overall liking of all the breads was strongly correlated with the ease of swallowing: VB75, r=0.597, 95% CI (0.382-0.751); VB100, r=0.665, 95% CI (0.474-0.796); WB, r=0.422, 95% CI (0.163-0.627); and WMB, r=0.475; 95% CI (0.227-0.665)

Table 4.4. Sensory liking and swallowing perceptions of the four breads by participants (n=50)

Characteristics	Bread samples				
	WB	WMB	VB75	VB100	p-value
Sensory attributes: liking (mm/150 mm)					
Colour	54.24±37.23[a]	80.65±34.42[b]	85.74±33.42[b]	87.54±37.66[b]	<0.0001
Aroma	73.12±31.06[a]	76.16±27.49[a]	95.17±27.30[b]	92.16±34.12[b]	<0.0001
Taste	52.77±31.09[a]	60.22±29.27[a]	93.42±30.00[b]	94.10±34.27[b]	<0.0001
Texture	46.70±30.81[a]	60.71±30.93[a]	96.69±28.78[b]	97.60±31.89[b]	<0.0001
Mouthfeel	47.46±30.26[a]	54.34±27.57[a]	94.36±27.57[b]	91.09±36.44[b]	<0.0001
Overall liking	40.85±31.59[a]	52.43±32.80[a]	92.03±34.24[b]	93.58±35.52[b]	<0.0001
Willing to eat at home	30.19±33.40[a]	47.64±34.24[a]	84.67±42.89[b]	86.47±42.21[b]	<0.0001
Swallowing evaluation/ 150mm					
Ease of biting and getting into the mouth	98.31±31.79[a]	99.56±26.53[a,b]	113.29±28.32[b]	111.34±26.47[a,b]	0.012
Ease of chew	70.53±39.46[a]	77.68±33.13[a]	104.78±30.69[b]	107.58±24.90[b]	<0.0001
Ease of swallow	70.29±37.98[a]	77.53±34.88[a]	104.63±28.25[b]	104.90±25.54[b]	<0.0001
Ease of throat movement	77.33±39.75[a]	78.18±34.78[a]	108.61±27.53[b]	110.41±23.03[b]	<0.0001
Less stickiness in throat	72.33±45.45[a]	75.94±39.64[a]	107.62±33.46[b]	111.48±29.18[b]	<0.0001
Number of chews before swallowing	19	21	18	19	

Note. Data is expressed as mean ± standard deviation. Values with different superscript in a row are significantly different (p<0.05). Scoring is on a Likert scale 0–150 mm with 0mm as the least and 150mm as the most acceptable. WB = white bread, WM = wheatmeal bread, VB75 and VB100 = breads with 75 and 100g pumpkin substitution. Means with different superscripts in a column are significantly different (p < 0.05).

Participants reported both the two commercial breads as harder to chew than both the two vegetable-enriched breads (**Figure 4.1**).

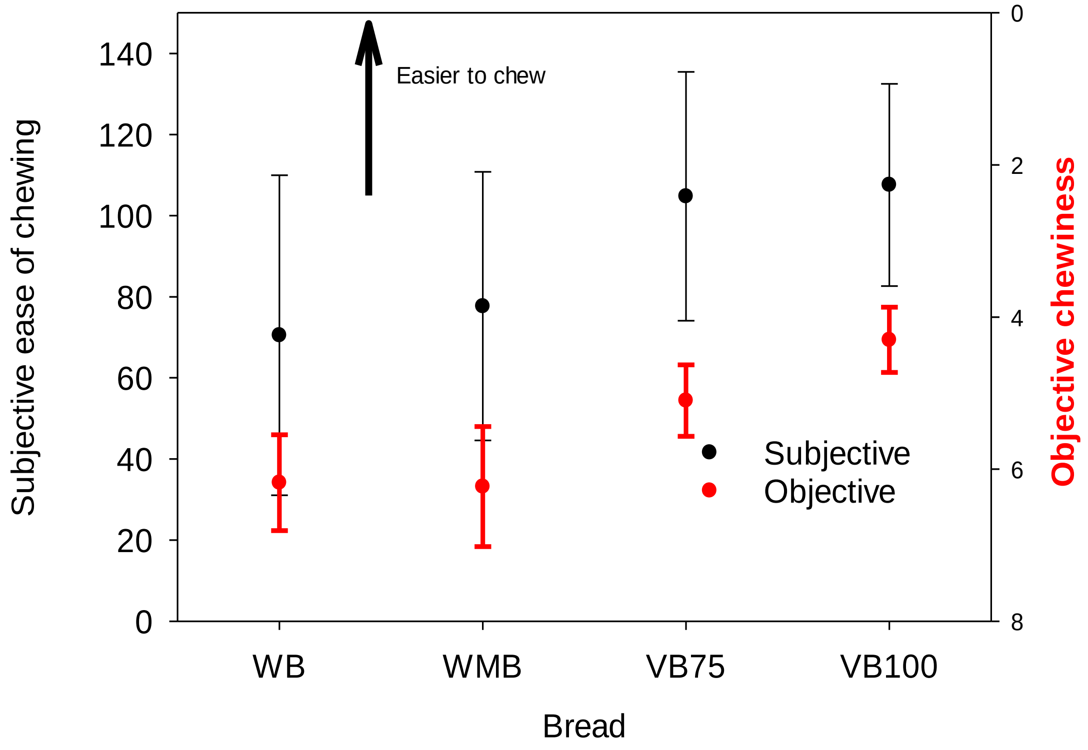


Figure 4.1. Relationship between subjective ease of chewing and objective chewiness of bread samples. WB = white bread, WM = wheatmeal bread, VB75 = bread with 75g pumpkin substitution and VB-100 = bread with 100g pumpkin substitution.

The participants found it easier to bite and get the VB75 and VB100 breads into the mouth than the WB and WMB. The measured hardness of the VB75 and VB100 was not different significantly than the WB and WMB.

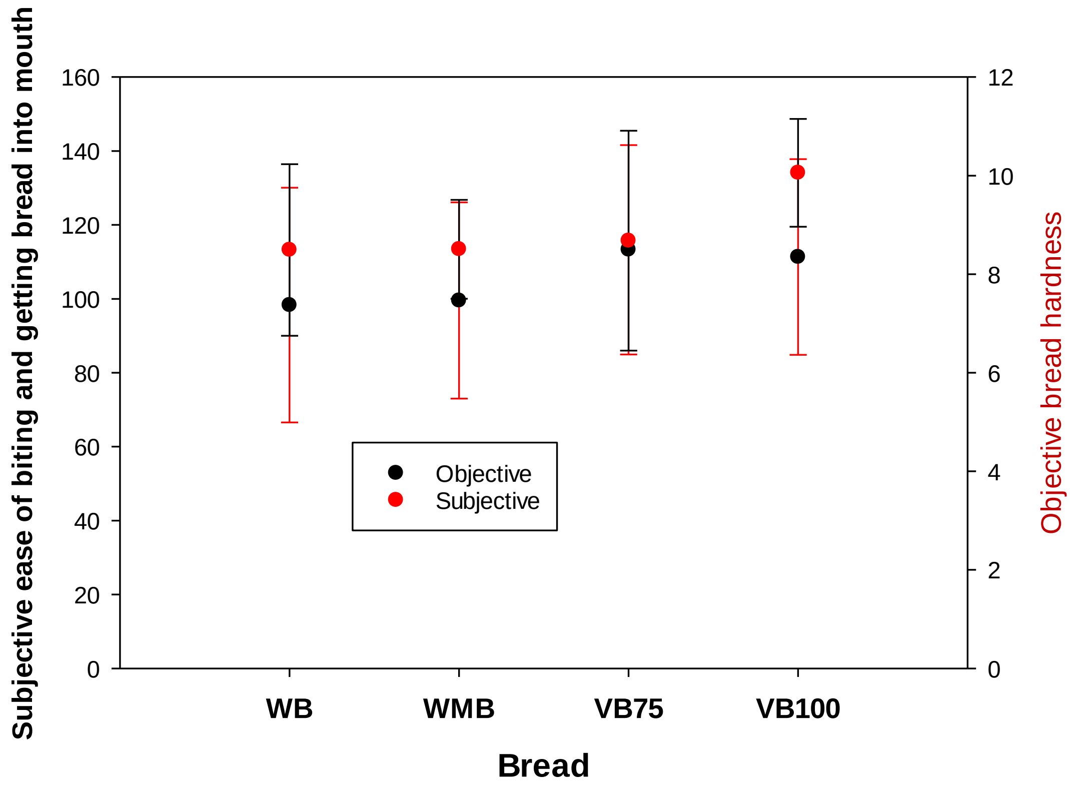


Figure 4.2. Relationship between subjective ease of biting and getting bread into mouth and objective bread hardness. WB = white bread, WM = wheatmeal bread, VB75 = bread with 75g pumpkin substitution and VB100 = bread with 100g pumpkin substitution.

Discussion

This study has shown that older participants substantially preferred the taste, and found the VB75 and VB100 breads easier to swallow compared with the commercial breads and would be willing to eat the vegetable-enriched breads at home. This confirmed the hypotheses that the vegetable-enriched Nothing Else™ test bread with higher pumpkin flour concentration (VB100) will be easier to swallow than the vegetable-enriched bread with lower pumpkin concentration (VB75), and both will be easier to swallow than the commercial control breads (WB and WMB). The objective measure of chewiness was the only physical measure able to differentiate between the commercial breads and the vegetable-enriched breads in the same direction as the participants' perceived ease of chewing. A novel ease-of-swallowing "solid food" questionnaire was checked for construct validity with research team.

Explanations for the easier chewing and swallowing of the vegetable-enriched breads related to an easier formation and passage of the bread bolus. This may be attributed to pumpkin powder containing pectin (Fissore et al., 2009) which is rich in hydrophilic fibres (Einhorn-Stoll, 2018), increasing the bulk of the bread in the mouth and a potential increased saliva stimulation. Saliva promotes the formation of cohesive network between the bread bolus in the mouth, consequently aiding swallowing (Mosca & Chen, 2016). Increased salivation is stimulated by the colour of bread (Q. J. Wang et al., 2019). The colour of food impacts on consumers' choices. Bread with white crumb is perceived to be unhealthy, particularly amongst older people, as indicated anecdotally by the participants. The yellow colour of the VB probably created an impression of healthiness in the minds of the participants, consequently resulting in the higher liking of the VB. Kraus (2015) reported that drivers for consumers' liking of food are dependent on healthiness and naturalness.

The vegetable-enriched breads were more liked for their taste compared to the commercial WB and WMB breads. A plausible reason could be the action of saliva as a medium for the dilution of taste compounds including sugar and salt (Liem & Russell, 2019) from the vegetable-enriched breads. The diluted compounds are subsequently conveyed to taste receptors on the surface of the tongue (Mosca & Chen, 2017; Neyraud, Prinz, & Dransfield, 2003) and are perceived to be appealing by the participants.

An explanation for the liking of the aroma of the vegetable-enriched breads could be in relation to the role of saliva. Mosca and Chen (2017) postulated that saliva increases the availability of aroma compounds from food when the food gets broken into smaller particle sizes during chewing. The released aroma compounds attach themselves to receptors in the mouth while some diffuse into cavities of the nose leading to flavour perception which in turn results in increased salivation (Canon, Neiers, & Guichard, 2018). Aroma released from bread also impacts on the release of saliva. Studies on the flavour volatiles available in sweet corn revealed the presence of aroma compounds including dimethyl sulfide, 2-acetyl-1-pyrroline and 2-acetyl-2-thiazoline (Buttery, Stern, & Ling, 1994). Interestingly, 2-acetyl-1-pyrroline, which is an essential flavour compound produced from Maillard reaction in sweet corn, is noted for its appealing flavour in bread (Adams & De Kimpe, 2006). 2-acetyl-2-thiazoline, on the other hand, is found to generate a roasty popcorn-like favour in bread (Adams & De Kimpe, 2006). Other compounds including hydrogen sulfide, methanethiol, acetaldehyde, ethanol, ethanethiol, dimethyl sulfide impact the aroma of thermally processed sweet corn (Flora & Wiley, 1974). The presence of these compounds in addition to the Maillard reaction that takes place during the baking process possibly resulted in the generation of appealing aromatic compounds in the vegetable-enriched breads. This could be attributed to the degradation and modification of the cell walls of the vegetable ingredients which may result in an aroma favourable to the consumers (Ribas-Agusti et al., 2017). A strong positive association between the liking of food and its aroma composition has been reported (Forde, van Kuijk, Thaler, de Graaf, & Martin, 2013) thus the higher liking score recorded for the vegetable-enriched breads by the participants may be justified. Additionally, the pre-fermentation of the wholemeal flour for 12 hours using yeast possibly improved the textural properties and promoted the release of certain volatile and aromatic compounds in the vegetable-enriched breads.

It is also worth highlighting that during bread chewing, the texture of the bread matrix impacts on the release of aroma compounds (Bonneau et al., 2018). In the present study, participants had a favourable perception of the crumb texture of the vegetable-enriched breads. Consequently, salivation of the bread bolus increased, resulting in the ease of bread swallowing. The vegetable-enriched breads were reported to be easier to chew.

Chewing is a mechanical process that stimulates the release of saliva (Humphrey & Williamson, 2001) consequently promoting increased bread bolus lubrication (Mosca & Chen, 2016). This possibly resulted in easier swallowing and movement of the bread bolus through the throat (Mosca & Chen, 2017).

In New Zealand, people with impaired swallowing which could be attributed to presbyphagia have been reported to be about 32.1% of those aged 65+ (for European ethnicity) and 55+ (for Maori and Pacific ethnicities) admitted to residential care (Wham et al., 2017). Thus, for this segment of the population, the vegetable-enriched Nothing Else™ breads, if consumed, could be favourable for their overall nutritional intake.

The older participants subjectively found white bread more difficult to chew and swallow though there was no difference between its crumb hardness and that of the vegetable-enriched breads as evaluated objectively. This was expected, as white bread is formulated from refined white flour, poor in fibre and contains emulsifiers which improves bread crumb textural attributes (Ajibade & Ijabadeniyi, 2019; Stampfli & Nersten, 1995). Consequently, after bread chewing, bolus swallowing may get impaired as it clogs in the throat, and this was confirmed anecdotally by the participants. The commercial sold white and wheatmeal breads contained emulsifiers 481 (sodium oleyl lactylate, sodium stearoyl lactylate and sodium lactylate) and 472e (diacetyltartaric and fatty acid esters of glycerol) (Food Standards Australia New Zealand, 2016a) to improve the bread crumb textural properties and to cause the bread to feel softer in the plastic bag and therefore appear fresher (Stampfli & Nersten, 1995). This likely contributed to the lower hardness of the white breads when measured with the texture analyser, as the use of emulsifiers has been reported to improve the textural properties of bread (Ajibade & Ijabadeniyi, 2019; Stampfli & Nersten, 1995). The use of food additives including hydrocolloids to enhance the properties of bread, especially in commercially sold bread, is a common and accepted practice. However, particularly amongst the health-conscious older population, consumers are avoiding food products with food additive enhancement as they see them as “unnatural” (E. V. Chambers, Chambers, & Castro, 2018).

There is potentially an adverse effect of the higher moisture content in the vegetable-enriched bread VB100 as higher water activity leads to early spoilage by microbiological organisms, especially mould (Axel, Zannini, & Arendt, 2017; Galic, Curic, & Gabric, 2009; Smith, Daifas, El-Khoury, Koukoutsis, & El-Khoury, 2004). However, in a review paper recently published (Amoah et al., 2018), we posited that functional breads tend to have a longer shelf life than white breads due to antimicrobial and antioxidant bioactivity compounds present in the functional ingredients which impair the growth rate of mould and the oxidation of lipids and fats in the bread matrix.

Strengths and limitations

Only four loaves each of the four breads (WB, WMB, VB75 and VB100) were tested and a limited number of repeats of the objective measures were made on only one batch of each bread. The reliability and accuracy of the objective measures as representative of these breads therefore needs to be confirmed. Fifty participants in the acceptability study is a strength, and clear differences in participants’ perception of the breads were shown. A further strength is that the testing targeted an age demographic who are more likely to experience difficulty in swallowing due to the ageing process. A major limitation was that no data about presbyphagia or medication was collected from the participants. The proof-of-principle that a subjective evaluation of the dynamics of the mechanics of ingestion from biting to movement through the throat assessed by a novel questionnaire was feasible and consistently showed the discrimination between the breads is a strength and needs to be expanded to test and rank more breads. There is very little research on subjective ease of swallowing of foods and, usually, the individual rather than the food is assessed for ability to swallow.

Conclusion

The present study has showed that pumpkin and sweet corn flour enrichment of bread improves its sensory attributes, consequently leading to greater liking amongst a group of older physically active adults. In terms of the physical measure of bread crumb softness, no difference among the breads was found but the enrichment of bread with pumpkin and sweet corn and the pre-ferment dough preparation was apparently associated with improved ease of chewing and swallowing. Future research should focus on relating how the microstructure, particularly the hydrophilic properties of the bread matrix, impacts on the ease of swallowing. Next steps will be to explore different measures of physical properties on swallowability, and whether the vegetable enrichment results in acute and long-term changes in glycaemia, lipidaemia and micronutrient status which could result in a health claim. There is also the need to explore the commercial feasibility of enriching breads and other bakery products with vegetables, and to explore further a subjective swallowability index for foods.

Chapter 5. Review of the health effects of functional ingredients used in bread-making

Functional foods are distinct from food fortification and supplementation in that these food products, taken as part of the usual diet, have benefits to health in addition to the established nutritional effects (Amoah et al., 2018; Birch & Bonwick, 2018). The beneficial effects of the consumption of functional foods, including bread, which is the focus of this review, must be shown with scientific studies that support a health claim.

This systematic review is in two-fold, with the first stage focused on the bioactive properties of functional ingredients used in the formulation of breads and the second stage was to identify the potential health effects of functional breads in human trials. A systematic search for functional ingredients in bread and randomised controlled trials on the potential health effects associated with functional bread consumption in humans was conducted between 1 March and 5 June, 2017 using the electronic databases of the Web of Science and PubMed for the first stage of the review, with the Cochrane library via Wiley and OVID added for the second stage of the review.

The search strategy involved using these key search terms:

1. (Bread OR Loaf) AND (Novel ingredient*)
2. (Bread OR Loaf) AND (Phytochemical*)
3. (Bread OR Loaf) AND (Bioactive* compound*)
4. (Health*) AND (Bread OR Loaf)
5. (Health*) AND (Bread OR Loaf) AND (Develop*)
6. (Health*) AND (Bread OR Loaf) AND (Health effect*)

7. (Health*) AND (Bread OR Loaf) AND (Functional)
8. (Health*) AND (Bread OR Loaf) AND (Functional) AND (Health claim*)
9. (Health*) AND (Bread OR Loaf) AND (Fortify* or enrich*)
10. (Health*) AND (Bread OR Loaf) AND (Fortify* or enrich*) AND (Health claim*)
11. (Bread OR Loaf) AND (Physiological effect* OR Physiological action* OR Physiological function*)
12. (Bread OR Loaf) AND (Medicinal herb* OR Herbal extract*)

A total of 8,507 journal articles and conference proceedings were obtained. The abstracts, the titles and full-text articles were screened for relevance. Thirty-eight studies that looked at key functional ingredients and potential bioactive properties were extracted for the first stage of the review. Forty-nine studies met the criteria for the second stage of the review following the removal of duplicates, reviews, commentaries and studies on mineral fortified breads.

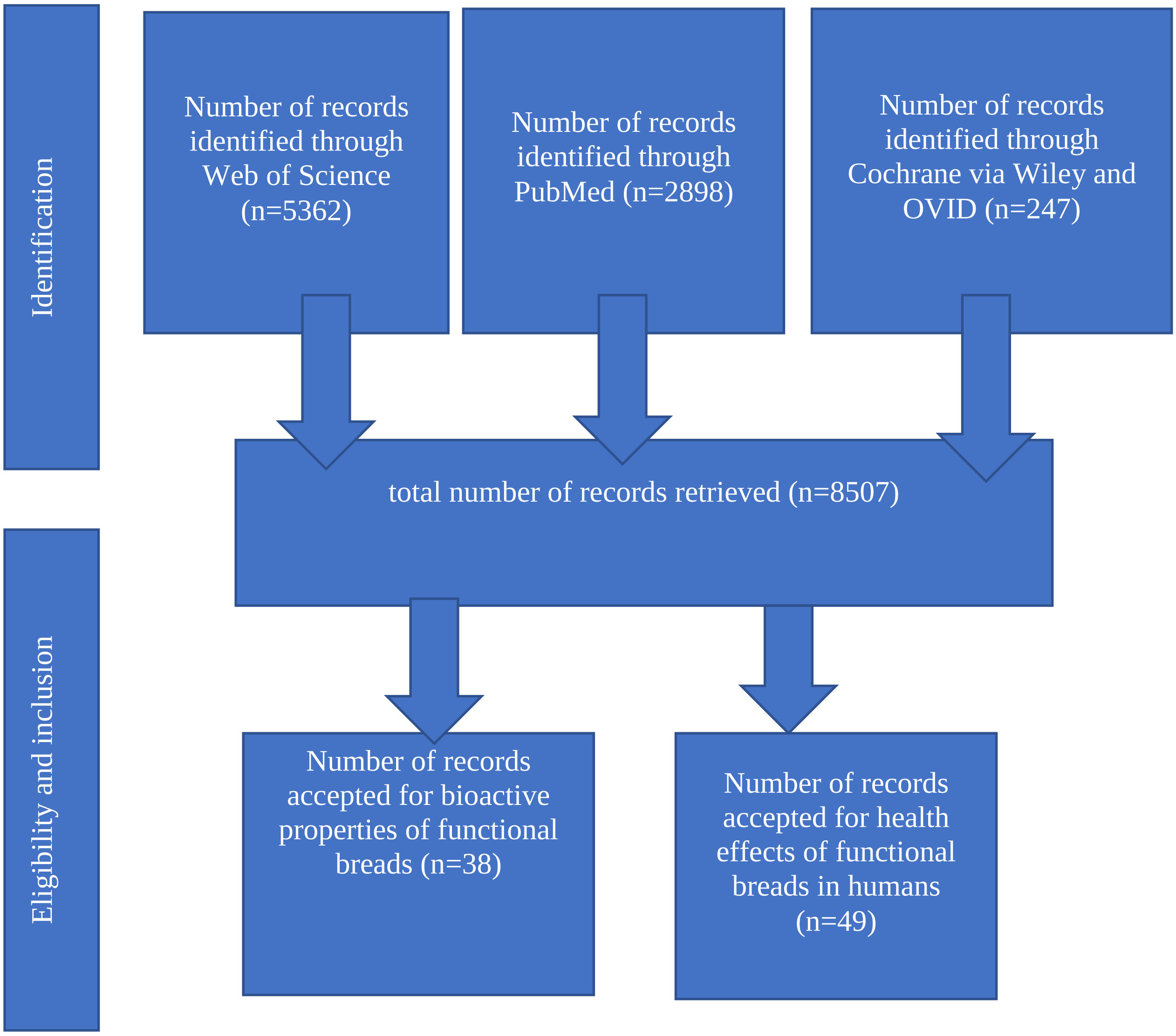


Figure 5.1. Flow chart of processes involved in the selection of published articles for the systematic review.

Bioactive properties of bread formulated with functional ingredients

Of the 38 studies identified that examined the addition of functional ingredients to bread and bioactivity *in vitro*, 13 used cereals, pseudocereals and grains, 11 herbs, weeds and leaf extracts, 6 oilseeds and legumes and beans, 5 vegetables and 3 fruits (**Table 5.1**). Antioxidant activity is measured in several ways in the studies (**Table 5.1**). These include ferric ion reducing antioxidant power (FRAP), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), and 2, 2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) free radical scavenging activity assay, where a statistically meaningful increase was reported as an increase denoted as "↑antioxidant activity" in the table. Bioactive compounds present in functional ingredients, including fruits and vegetables, legumes, nuts, cereals and herbs, impact on their ability to demonstrate significant physiological roles in the bread matrix and, consequently, they impact favourably on human health including showing anti-cancer properties, and militating against degenerative diseases and chronic and acute inflammation (Shashirekha et al., 2015). However, the shorter shelf-life of most of these functional ingredients before incorporation into bread may limit their use. In particular, fruits and vegetables cannot easily be used in their fresh form: they have high water activity which promotes microbial growth and spoilage (Maltini et al., 2003), are bulky to store and are limited in their availability during lean season so drying and processing into flours is necessary. For the bakery industry, utilisation of functional ingredients in bread formulation is warranted due to the extended shelf-life properties of the bread caused by the antimicrobial properties of the bioactive compounds in functional ingredients (Amoah et al., 2018; Culetu, Duta, & Andlauer, 2018) and their concomitant ability to inhibit lipid peroxidation (Ning, Hou, Sun, Wan, & Dubat, 2017). Literature related to the bioactive properties associated with the incorporation of fruits, vegetables and their components, oilseed, tree plant seeds/extracts, legumes/beans, herbs/weeds, leaf extract, tubers, rhizomes, fungi, cereals, pseudocereals and grain ingredients in functional bread formulation is thus reported (**Table 5.1)**.

Functional bread enrichment with fruits and vegetable ingredients

Flours from fruits and vegetables are used in functional bread formulation to enhance the bioactive properties of bread. The use of extracts of baobab fruit, doum (*Hyphaenethebaica L.*) fruit flour and *Garcinia mangostana* pericarp powder in functional bread formulation increased polyphenolic content (S. A. Coe, Clegg, Armengol, & Ryan, 2013), enhanced total phenolics (Aboshora et al., 2016; Ibrahim, Salleh, Suzihaque, & Hashib, 2015), total flavonoid content (Aboshora et al., 2016) and consequently antioxidant activity (Aboshora et al., 2016; Ibrahim et al., 2015) (**Table 5.1**). In terms of vegetables, the enrichment of bread with vegetable flour increased the antioxidant concentration and mitigated against the rapid oxidation of lipids (using carrot, tomato, beetroot and broccoli flour) and proteins (using beetroot, carrot and broccoli flour), consequently leading to an improvement in the shelf-life of the functional bread, particularly by beetroot and broccoli flour (Ranawana, Campbell, et al., 2016; Ranawana, Raikos, et al., 2016). In a similar manner, the total phenolic content, antioxidant properties (Gawlik-Dziki et al., 2014; Msaddak et al., 2017; Swieca, Gawlik-Dziki, Dziki, Baraniak, & Czyz, 2013) and protein digestibility (Swieca et al., 2013) of the breads were also enhanced following the incorporation of cladodes powder (Msaddak et al., 2017), broccoli sprout (Gawlik-Dziki et al., 2014) and onion skin powder (Swieca et al., 2013) into bread. An observation that needs confirmation is that oil-free wheat flour bread fortified with freeze-dried carrot, tomato, beetroot or broccoli flours (Ranawana, Campbell, et al., 2016) exhibited a reduced antioxidant potential compared to that found in another study by the same authors (Ranawana, Campbell, et al., 2016) where the vegetable flour breads included corn oil. This is an example where bioactive properties of the bread are affected by the modification of bread recipes with inclusion or exclusion of ingredients such as oil (Ranawana, Campbell, et al., 2016; Ranawana, Raikos, et al., 2016), the proportion of flours substituted (Das, Raychaudhuri, & Chakraborty, 2013) and the method of bread preparation (Penas, Diana, Frias, Quilez, & Martinez-Villaluenga, 2015). One study reported no dose-response between the addition of broccoli sprouts with antioxidant and phenolic content of bread (Gawlik-Dziki et al., 2014).

The utilisation of oilseed/tree plant seed/extract and legumes/bean as functional ingredients in bread-making

Diverse seeds and legumes have been used in composite formulation with wheat flour for functional bread development. An increase in alpha-linolenic acid, lignans, and total polyphenol was observed when flaxseed (9%) was incorporated into teff-based injera, an indigenous bread of the people of Ethiopia (Girma, Bultosa, & Bussa, 2013). Additionally, bread fortification using powders of herbal plant seeds such as fennel (Das et al., 2013), *Perilla frutescens*, an Asian herb rich in omega 3 fatty acids (Vieira da Silva et al., 2016), and date seed increased the concentration of phenolic compounds (Das et al., 2013; Vieira da Silva et al., 2016) and increased the antioxidant activity of the bread (Das et al., 2013; Vieira da Silva et al., 2016) (**Table 5.1**). Supplementation of white wheat bread with fennel seed powder optimised antioxidant activity and increased total phenolic concentration when 7% fennel seed powder was substituted for wheat flour. In the case of DPPH scavenging ability, a similar trend was observed when 5% fennel seed powder substitution was used (Das et al., 2013).

The utilisation of seeds of the legume lupin increased the polyphenolic, carotenoid and antioxidant content of wheat bread (Villarino et al., 2015). Supplementation of “control” wheat bread with 15% fenugreek seed powder increased the total phenolic concentration from 99 to 413mg GAE/100g; increased the flavonoid contents from 2.31 to 2.91mg CE/g and also enhanced antioxidant activity (Afzal, Pasha, Zahoor, & Nawaz, 2016). Enrichment of wheatmeal flour with 1 and 5% green coffee flour increased the concentration of phenolic compounds and enhanced the lipid-protecting capacity in wholemeal wheat bread (Swieca, Gawlik-Dziki, Dziki, & Baraniak, 2017).

Herbs/weed and leaf extract as functional ingredients in functional bread formulation

Other plant materials including leaf extracts, weeds, and herbs have also been incorporated into bread and the change in bioactivity reported (**Table 5.1**). Potential functional benefits including increased antioxidant activity (Culetu, Fernandez-Gomez, Ullate, del Castillo, & Andlauer, 2016; Ning et al., 2017; Przeor & Flaczyk, 2016; Zhu, Sakulnak, & Wang, 2016) which is related to the content of polyphenols (Przeor & Flaczyk, 2016) and total phenolic compounds (Culetu et al., 2016; Ji, Gang, & Hu, 2013) were reported (**Table 5.1**). Significant reductions in the production of peroxide (Ning et al., 2017) as a result of the utilisation of white mulberry leaf extract (Przeor & Flaczyk, 2016), green tea powder (Ning et al., 2017), *Potentilla anserina* (Ji et al., 2013), black tea (Zhu et al., 2016) and theanine/polyphenol fractions from black tea dust (decaffeinated) (Culetu et al., 2016) in functional bread formulation add to the evidence for the addition of novel plant ingredients to produce functional bread. The increase in total phenolic and antioxidant capacity observed for the polyphenol-enriched bread from the decaffeinated black tea dust was linear between 30 and 70g/100g flour (Culetu et al., 2016). Bread enriched with shatavari (*Asparagus racemosus*) root powder, a popular Indian herb, showed the presence of alkaloids, steroids, terpenoids, and saponins (N. Singh, Jha, Chaudhary, & Upadhyay, 2014) which have bioactive properties.

Tubers, rhizomes and fungi incorporation in bread as functional ingredients

Flours and products from root tubers such as yam flour (*Dioscorea purpurea*) (Hsu, Hurang, Chen, Weng, & Tseng, 2004), purple potato (Sgherri, Micaelli, Andreoni, Baldanzi, & Ranieri, 2016), increased the antioxidant capacity (Hsu et al., 2004) of functional bread formulations (**Table 5.1**). Purple potato powder-enriched bread had higher amounts of free phenolic acids (gallic, chlorogenic acid, protocatechuic, caffeic acid, vanillic, ferulic acid, p-coumaric acid and syringic acids) and higher antioxidant activity in comparison with yellow potato powder-enriched bread (Sgherri et al., 2016). Turmeric powder, showed enhanced antioxidant activity when incorporated into functional bread compared to control bread without turmeric (Lim et al., 2011). The addition of encapsulated curcumin extracted from turmeric into bread increased the bioavailability of the bioactive compound curcuminoid (Vitaglione et al., 2012). The incorporation of *Auricularia auricular* polysaccharide flour improved the antioxidant property of the bread (Fan et al., 2006).

Cereal, pseudocereal and grain ingredients in functional bread development

The use of cereal grain and pseudocereal components including wholemeal wheat sourdough (Novotni et al., 2011; Penas et al., 2015), extracts of barley hull, whole wheat flour, partially debranned grain flour, and refined flour from three coloured wheat varieties (deep purple, light purple, and black) and one white wheat variety (Li et al., 2015) in functional bread formulation has been reported (**Table 5.1**). Additionally, rye flour (Ozoliņa & Kunkulberga, 2010), extruded wheat bran and cereal β-glucan hydrogel (Mikušová et al., 2013), wheat, rye, barley, oat wholegrain flours (Ragaee, Guzar, Dhull, & Seetharaman, 2011), barley flours from three different hulled varieties and whole wheat flours from two wheat varieties (soft and white spring wheat types) (Y. Lu et al., 2015) have been used in functional bread formulation. The use of these cereal grain and pseudocereal components in functional bread formulation resulted in increased antioxidant activity (Hao & Beta, 2012; Holtekjolen, Baevre, Rodbotten, Berg, & Knutsen, 2008; Li et al., 2015; Mikušová et al., 2013; Ozoliņa & Kunkulberga, 2010; Penas et al., 2015; Ragaee et al., 2011), significant increase in free phenolic acids (Ragaee et al., 2011) and bound phenolic acids (Ragaee et al., 2011) and increased flavonoid concentration (Mikušová et al., 2013) in the functional bread. An increase in bioaccessible polyphenols providing higher antiradical activity (Collar & Angioloni, 2014; Hao & Beta, 2012) was also observed following the use of high β-glucan barley flour (Collar & Angioloni, 2014) and extracts of barley hull (Hao & Beta, 2012) in functional bread formulation **(Table 5.1).** Angioloni and Collar (2011) reported that, in regard to bread formulated with a single cereal, total phenol content was significantly higher in buckwheat-enriched bread compared to bread formulated from wheat, oat or rye. The synergistic increase in bioactive compounds when functional ingredients are combined for bread formulation has also been reported. There was a synergistic increase in polyphenol content and its bioaccessibility when bread was formulated with mixtures of cereal flours from barley, oats and rye used in composite formulation with wheat. Similar trends were recorded for antioxidant activity (Angioloni & Collar, 2011). The concentration of bound phenolic compounds in barley-incorporated bread increased, whereas the concentration of free phenolic compounds decreased during baking of the barley-incorporated bread (Holtekjolen et al., 2008). This could be attributed to the degradation or modification of cell wall polysaccharides, proteins and other matrix components, consequently increasing the concentration of extractable phenolic compounds, by the high baking temperature (Ribas-Agusti et al., 2017).

Flour from grains including monascus-fermented rice (anka) flour (Tseng, Yang, Chen, & Mau, 2011) and teff (Alaunyte, Stojceska, Plunkett, Ainsworth, & Derbyshire, 2012) enhanced the antioxidant capacity (Alaunyte et al., 2012; Tseng et al., 2011), showed high g-aminobutyric acid concentration and enhanced the reducing power and scavenging ability of bread (Tseng et al., 2011). The polyphenol content of bread enriched with yellow maize flour was slightly higher than in the white maize-substituted bread formulation, a finding attributed to the greater flavonoid content of the yellow maize varieties (Collar, Balestra, & Ancarani, 2014).

Summary of findings

This review suggests that functional ingredients based on diverse plants used in bread formulations add high quantities of bioactive compounds, and studies have demonstrated strong bioactive properties in functional bread. The following section addresses the question: Are health-promoting effects associated with the consumption of these functional breads?

Table 5.1. Potential health-promoting substances and biological activity contribution of functional ingredients used in functional bread

Functional food ingredient(s) added to bread	Food group	Potential health promoting substance and contribution to bread quality (*in vitro*)	Reference
Fruits			
Baobab fruit extract	Fruit	↑ polyphenol content.	(S. A. Coe et al., 2013)
Doum fruit (Hyphaenethebaica L.) flour	Fruit	↑ phenolic content, ↑ flavonoid content and ↑ antioxidant activity.	(Aboshora et al., 2016)
Garcinia mangostana pericarp powder	Fruit	↑ phenolic content, ↑ antioxidant activity, ↑ colour and ↑ moisture content of the bread.	(Ibrahim et al., 2015)
Vegetables			
Freeze dried carrot, tomato, beetroot and broccoli powders **without corn oil**	Vegetables	↑ antioxidant activity, ↓ lipid (carrot, tomato, beetroot and broccoli) and ↓ protein (beetroot, carrot and broccoli) oxidation. ↑ bread storage time (beetroot and broccoli).	(Ranawana, Raikos, et al., 2016)
Freeze-dried carrot, tomato, beetroot or broccoli **with corn oil**	Vegetables	↑ nutrient density, ↑ bread storage time of the oil-free breads but ↓ antioxidant activity compared to oil-containing bread.	(Ranawana, Campbell, et al., 2016)

Functional food ingredient(s) added to bread	Food group	Potential health promoting substance and contribution to bread quality (*in vitro*)	Reference
Cladodes powder	Vegetable	↑ total phenolic content and ↑ antioxidant activity compared to the control.	(Msaddak et al., 2017)
Broccoli sprout	Vegetable	↑ total phenolic content, ↑ antioxidant activity.	(Gawlik-Dziki et al., 2014)
Onion skin	Vegetable	↑ total phenolic content, ↓ free radicals and ↑ protein digestibility.	(Swieca et al., 2013)
Oilseed/tree plant seed/extract, legumes/bean			
Flaxseed	Seed	↑ dietary fibre, ↑ alpha linolenic acid, ↑ lignans, ↑ proteins and ↑ total polyphenols of anti-oxidant nature.	(Girma et al., 2013)
Fennel seeds	Seed	↑ total phenolic content, ↑ antioxidant activity measured by ferric reducing antioxidant power (FRAP). Above 7% the increase in antioxidant activity was attenuated.	(Das et al., 2013)
Perilla frutescens seeds	Seed	↑ total phenolic content, ↑ antioxidant activity.	(Vieira da Silva et al., 2016)
Lupin (*Lupinus angustifolius*: ASL)	Legume	↑ total phenolic content, ↑ antioxidant activity, ↑ carotenoids.	(Villarino et al., 2015)
Fenugreek seed powder	Legume	15% supplementation: ↑ total phenolic content, ↑ flavonoid, ↑ antioxidant activity compared to “control” bread.	(Afzal et al., 2016)
Green coffee bean	Bean	↑ total phenolic content and ↓ lipid oxidation.	(Swieca et al., 2017)

Functional food ingredient(s) added to bread	Food group	Potential health promoting substance and contribution to bread quality (*in vitro*)	Reference
Herbs/weed and leaf extract			
White mulberry leaf extract	Leaf extract	↑ total phenolic content, ↑ antioxidant activity.	(Przeor & Flaczyk, 2016)
Green tea extract	Herb	↑ antioxidant activity, ↓ production of peroxide.	(Ning et al., 2017)
Potentilla anserina powder	Weed	↑ total phenolic content.	(Ji et al., 2013)
Black tea	Herb	↑ antioxidant activity.	(Zhu et al., 2016)
Decaffeinated tea dust extracts: theanine and polyphenol enriched	Herb	Theanine-enriched fractions increased: ↑ total phenolic content, ↑ antioxidant capacity in comparison with control. Polyphenol-enriched fractions increased: ↑ total phenolic content, ↑ antioxidant capacity in comparison with control and theanine-enriched fractions.	(Culetu et al., 2016)
Shatavari (*Asparagus racemosus*) root powder	Herb	↑ alkaloid, ↑ steroid, ↑ terpenoid and ↑ saponin.	(N. Singh et al., 2014)
Yam (*Dioscorea purpurea*) flour in bread	Stem tuber	↑ antioxidant activity.	(Hsu et al., 2004)

Functional food ingredient(s) added to bread	Food group	Potential health promoting substance and contribution to bread quality (*in vitro*)	Reference
Purple and yellow potato powder	Root tuber	↑ free phenolic acids (gallic, chlorogenic acid, protocatechuic, caffeic acid, vanillic, ferulic acid, p-coumaric acid and syringic acids), ↑ antioxidant activity.	(Sgherri et al., 2016)
Turmeric powder	Rhizomatous herbaceous perennial plant	↑ antioxidant activity.	(Lim et al., 2011)
Curcumin (from turmeric)	Herb	↑ curcuminoid bioavailability during absorption (*in vitro* modelled) and ↑ phenolic acids as the major metabolites of curcuminoids.	(Vitaglione et al., 2012)
Auricularia auricular polysaccharide flour	Fungus	↑ antioxidant activity.	(Fan et al., 2006)
Cereal, pseudocereal and grain			
High beta glucan barley flour	Cereal	↑ bio-accessible polyphenols→↑ antiradical activity.	(Collar & Angioloni, 2014)
Extruded wheat bran, cereal β-glucan hydrogel, and lactobacilli starter culture	Cereal/Probiotic	↑ flavonoids content, ↑ antioxidant activity in wheat-rye bread.	(Mikušová et al., 2013)

Functional food ingredient(s) added to bread	Food group	Potential health promoting substance and contribution to bread quality (*in vitro*)	Reference
Single (oat, rye, buckwheat and wheat) and multigrain breads (blends)	Cereals and Pseudocereal	↑ total phenol content amongst the single bread extracts were significantly higher in buckwheat (808mg GAE/kg). Mixed breads exhibited ↑ polyphenol content, ↑ antioxidant activity and higher polyphenol bioaccessibility with higher degree of wheat replacement.	(Angioloni & Collar, 2011)
Bread from wheat varieties (white Yumai 49-198 (YU), deep purple Jizi 439 (JZ), light purple Shandongzimai 1 (SDZM), and black wheat Heibaoshi 1 (HBS)	Cereal	Antioxidant activities of steamed bread made from whole wheat flour, partially debranned grain flour, and refined flour were 23.5%, 21.1%, and 31.6% lower, respectively, compared to the corresponding values for flour alone.	(Li et al., 2015)
Whole wheat flours	Cereal	Ferulic acid was the most abundant phenolic acid in the dough, crumb, and upper crust made with refined and whole wheat flour in two wheat varieties (Macon and Louise). ↑ phenolic acids in the upper crust fraction was higher than that in the dough and crumb fractions, suggesting that total phenolic acid content, especially ferulic and p-coumaric acids, were not lowered during baking.	(Y. Lu et al., 2015)

Functional food ingredient(s) added to bread	**Food group**	**Potential health promoting substance and contribution to bread quality (*in vitro*)**	**Reference**
Wholemeal wheat sourdough (produced by Lactobacillus brevis CECT 8183 and protease)	Cereal	Inhibition of angiotensin converting enzyme I (ACE) and antioxidant activities of the peptide fraction<3kDa of sourdough bread was 1.7 and 2.6-3.0 times higher than the "control" bread.	(Penas et al., 2015)
Sourdough from wholemeal wheat flour	Cereal	↑ total phenolic content.	(Novotni et al., 2011)
Different barley flour	Cereal	↑ antioxidant activity compared to the "control" bread, dependent on the variety of barley as well as the extraction rate of the flour.	(Holtekjolen et al., 2008)
Wheat, rye, barley, oat wholegrain flours and two fibres namely cellulose (insoluble fibre) and xanthan gum (soluble fibre)	Cereals/ Pseudocereals/Fibres	↑ free and bound phenolics, ↑ antioxidant capacity.	(Ragaee et al., 2011)
Extracts of barley hull	Pseudocereal	↑ total phenolic content and ↑ antioxidant activity.	(Hao & Beta, 2012)

Functional food ingredient(s) added to bread	**Food group**	**Potential health promoting substance and contribution to bread quality (*in vitro*)**	**Reference**
Monascus-fermented rice (anka) flour	Cereal	↑ antioxidant activity, ↑ reducing power and ↑ scavenging ability.	(Tseng et al., 2011)
Teff (*Eragrostis tef*)	Grain	↑ antioxidant capacity.	(Alaunyte et al., 2012)
Yellow maize	Grain	↑ polyphenol content compared with white maize bread samples.	(Collar et al., 2014)

Biomarkers of health effects associated with bread containing functional ingredients

Biomarkers are objective and quantifiable measures that characterise and have been noted to correctly predict essential clinical outcomes across a variety of treatments and populations (Strimbu & Tavel, 2010). The challenge of participants' under- and over-reporting of food frequency intake mars the outcome of findings in nutrition research. This has warranted the need to explore innovative ways of objectively evaluating specific nutrients derived from the consumption of foods of interest. For example, to track consumers' consumption of carotenoid-rich vegetables including pumpkin, carrot, tomato and spinach, an objective way could be to measure the concentration of carotenoids retained in the skin (Rush, Amoah, Diep, & Jalili-Moghaddam, 2020). Recent advances in technology have resulted in the development of a portable device called Veggie meter™ which uses the principle of reflection spectroscopy to measure skin carotenoid scores (Ermakov et al., 2018; Rush et al., 2020). Skin carotenoid is posited to be a biomarker that can be used to track fruit and vegetable intake (Rush et al., 2020). Skin carotenoid is thus a nutritional biomarker as it makes provision for the nutrient status of the body which subsequently gives the nutritional status of the population (Potischman, 2003). The objective skin carotenoid score from the Veggie meter™ can provide an idea of the bioavailability of carotenoid, validate the carotenoid-rich food frequency questionnaire, and measure variations in response to the retention of skin carotenoids according to gender and ethnic differences (Rush et al., 2020).

There is the need to target nutritional biomarkers to evaluate the effect of an intervention in humans (Corella & Ordovas, 2015). Corella and Ordovás (2015) classified biomarkers in the context of nutrition studies. The authors posited that biomarkers of dietary exposure, biomarkers of nutritional status and biomarkers of health exist. Biomarkers of dietary exposure assess nutrients and bioactive compounds derived from food consumption (Pico, Serra, Rodriguez, Keijer, & Palou, 2019). In the case of biomarkers of nutritional status, they reveal other factors aside from nutrient intake including the bioavailability of the nutrients and their ability to influence physiological processes. Lastly, biomarkers of health and disease highlight the health state of an individual and measure abnormally produced compounds and their metabolites, and reveals their association with disease onset (Pico et al., 2019). Examples include serum glucose, insulin and triglyceride concentration.

Health-promoting effects associated with functional bread consumption

The second stage review of literature (as shown in **Table 5.2**) evaluated the health effects associated with functional bread consumption compared to white wheat bread used as a control. Characteristics extracted included year of publication, study design (e.g., randomised controlled trial with white wheat bread as a control), amount of bread or concentration of bioactive compounds in the bread served and health outcomes. Inclusion criteria for selection were as follows: papers published in peer-reviewed journals in English where the key focus was to develop functional bread with health effects. A total of 8,507 journal articles and conference proceedings were obtained. Following the removal of duplicates, reviews, commentaries, studies on mineral fortified breads, studies that used glucose solution as a control sample, studies that only developed functional breads but did not use them in a randomised controlled trial, studies that validated the health effect of only non-standard breads (such as bagels, crispbread) and non-human interventional study using bread. Studies involving mineral and vitamin fortified bread were excluded as that was not the focus of this study. Sixty-five studies were accepted for this second stage of the review. The use of white wheat bread, refined wheat bread, conventional wheat bread and white bread were assumed to be “control” refined white wheat bread, because there is currently no standardised term for refined white wheat bread.

Fruit and vegetable-enriched bread and their health-effects

The bioactive properties of baobab fruit when used in composite preparation with wheat flour for bread development has been studied (**Table 5.2**). Coe and Ryan (2016) reported a blunted insulin requirement for white bread enriched with baobab extract. The authors attributed this observation to the rich polyphenol composition of the baobab extract. In another study, the cardio-protective effect associated with the consumption of beetroot-enriched bread was investigated (Hobbs et al., 2013) (**Table 5.2**). The consumption of beetroot-enriched bread acutely increased endothelium-independent vasodilation and decreased diastolic blood pressure although no improvement in systolic blood pressure was observed. The health-beneficial property of beetroot has been largely attributed to its NO_2-producing properties, NO_2 being a compound found to demonstrate vasodilation effect.

Legumes, seeds and nuts utilisation in bread formulation

In this section of the review, health outcomes associated with the consumption of bread formulated with legumes and their extracts (white bean, chickpea and Australian sweet lupin flour) (S.K. Johnson, McQuillan, Sin, & Ball, 2003; S. K. Johnson, Thomas, & Hall, 2005; Udani, Singh, Barrett, & Preuss, 2009; Zafar et al., 2015), seeds (salba) (Ho et al., 2013; Vuksan et al., 2010) and hazelnut (Devi et al., 2016) are reported (**Table 5.2)**. Ho et al. (2013), in a randomised controlled, dose-response trial, investigated the effect of form (i.e., whole and ground salba seeds, *Salvia Hispanica L.*) on postprandial glycaemia in healthy participants. A significant effect of dosage, but not of form or dose-form interaction, was found for salba seed-enriched bread. The authors posited that ground and whole salba are both equally effective in attenuating blood glucose concentration (Ho et al., 2013) and improved subjective appetite ratings (Vuksan et al.,

2010) in a dose-dependent manner when incorporated into bread. A similar observation was made when 3000mg of white bean extract powder (Phase 2 brand) was incorporated into white bread (Udani et al., 2009). Bread formulated with chickpea flour, a leguminous crop known for its higher phenolic content (Oberoi, Gupta, & Kaur, 2017), attenuated glycaemic response compared to its white bread counterpart though no effect on appetite suppression was established (S. K. Johnson et al., 2005; Zafar et al., 2015).

Health effects associated with the intake of nut (finely sliced hazelnut and semi-defatted hazelnut flour) enriched-breads have been reported (**Table 5.2**). Devi et al. (2016) observed that the area-under-blood glucose-curve was significantly lower for the nut enriched-breads compared to the control bread but with no significant differences recorded between the various nut enriched-breads. Additionally, no significant differences existed in satiety sensations between the bread samples (Devi et al., 2016).

The application of sourdough ingredients and technology in functional bread development, and health effects associated with its consumption

Sourdough ingredients and technology application in the bakery industry improves texture, enhances the shelf-life, flavour and health-promoting qualities of bread (Hansen & Schieberle, 2005). The composition of sourdough starter involves a mixture of flour and water fermented through the activities of yeast and lactic acid bacteria (Pétel,Onno, & Prost, 2017). Bread developed from sourdough are rich in organic acids including lactic and propionic acid or their corresponding salts such as sodium propionate (Liljeberg, Lönner, & Björck, 1995), and demonstrate health-promoting effects. Beneficial health properties reported for sourdough bread include attenuating blood glucose (Lappi et al., 2010; Najjar et al., 2009; Scazzina, Del Rio, Pellegrini, & Brighenti, 2009; Todesco, Rao, Bosello, & Jenkins, 1991; Tucker et al., 2014), insulin (Bondia-Pons et al., 2011; Lappi et al., 2010; Mofidi et al., 2012), GLP-1 (Mofidi et al., 2012; Najjar et al., 2009) and serum apolipoprotein B-100 responses (Tucker et al., 2014) (**Table 5.2).** This could be attributed to the enzymatic degradation of cell walls during dough fermentation resulting in the release of bound bioactive compounds especially the polyphenols (V.M. Koistinen et al., 2016; V. M. Koistinen et al., 2017) that demonstrate health-promoting properties associated with the consumption of sourdough bread.

In another study, the elevation of plasma insulin concentration following the consumption of propionate-rich sourdough bread was reported (Darzi, Frost, & Robertson, 2012). Sodium propionate, a salt of propionic acid, though an anti-fungal agent against moulds in bread, has been reported to lower blood glucose, insulin response (Todesco et al., 1991) and gastric emptying rates (Darwiche et al., 2001). It has been found that differences in the source of the starting food ingredients in sourdough bread impact on the variations in health outcomes. For example, postprandial glycaemic responses in adults with type 2 diabetes was significantly lower following the consumption of bread developed from sprouted grain sourdough compared to bread from normal sourdough and white wheat flour (Tucker et al., 2014) providing a useful insight into the need to use sprouted grains to enhance the health-promoting effect of bread. Sourdough bread's ability to attenuate glycaemic and insulinaemic response following consumption is attributed to the reduced availability of simple carbohydrates as a result of the fermentative activity of the lactic acid bacteria and yeast (Maioli et al., 2008). Additionally, the presence of resistant starch has been found to be more abundant in sourdough bread than in bread leavened with *S. cerevisiae*, thus promoting the lowering of glycaemic response (Scazzina et al., 2009). The profile of volatile organic compounds produced from the consumption of sourdough rye bread was observed to be different from that obtained from refined white bread (Raninena et al., 2017) highlighting the fact that metabolic effects induced by a sourdough bread are at variance with a refined white wheat bread. Certain changes in amino acids and their metabolites have been reported for a low-fibre sourdough bread that induced a lower postprandial insulinaemic effect following its consumption (Bondia-Pons et al., 2011). Aluko (2019) reported that, certain types of food derived peptides have been found to demonstrate a favourable effect on blood pressure regulation potential. Penas et al. (2015) reported that, the angiotensin converting enzyme I (ACE) inhibitory and antioxidant activity of the peptide fraction (less than 3kDa) from a low-sodium wheat bread with 21.00% wholemeal sourdough from wheat (produced by *Lactobacillus brevis* CECT 8183 and protease) were 1.70 and 2.60-3.00 times higher the control bread (Penas et al., 2015). However, no greater beneficial effects on 24-hour blood pressure, endothelial function, or glucose metabolism was apparently found when a low-sodium sourdough bread enriched with soya protein concentrate high in glutamic acid and protease was consumed by pre- or mild-to-moderate hypertensive patients in comparison to the control bread (Becerra-Tomas et al., 2015).

This review has highlighted evidence for health benefits associated with the consumption of functional bread formulated from sourdough, especially in attenuating glycaemic response. Sourdough technology, therefore, has the potential to favourably affect the glycaemic response associated with the intake of low-fibre and nutrient-poor white bread.

Cereal/grain flour utilisation in functional bread development and health effects associated with its consumption

Marques et al.(2007) investigated the glycaemic index of white spelt bread and compared it with white wheat bread in healthy participants (**Table 5.2**). In that study, the breads were baked under the same conditions to mitigate the effect of processing parameters on the breads. The authors observed no difference in the glycaemic response of the participants when white spelt and wheat breads were consumed (Marques et al., 2007). The findings from the study suggested that the processing of grains (milling) adversely impacts on their bioactive properties and, consequently, on their health-promoting effects. In terms of barley flour utilisation in bread formulations, bread formulated with whole barley flour (10%) attenuated glycaemic response and improved satiety sensations in diabetic subjects two hours after consumption compared to standard white bread (Urooj, Vinutha, Puttaraj, Leelavathy, & Rao, 2009). The effect of rye bread consumption on serum and urine enterolactone (ENL) concentrations in healthy volunteers has been investigated. There was significant increase in the mean serum ENL concentrations in both men and women during the rye-bread period over the wheat-bread period compared to the baseline measures. Correspondingly, daily urinary ENL excretion increased significantly during the rye-bread period compared with the wheat-bread period and was 5- and 10-fold higher in men and women respectively in comparison with the amount of plant lignan precursors measured in the rye bread. This findings adds evidence to the bioavailability of enterolactone in rye in the human gut following rye bread intake (K.S. Juntunen et al., 2000). Plant lignans, a class of phyto-oestrogens, are dietary fibre-associated compounds. The lignans such as secoisolariciresinol (SECO) and matairesinol (MAT) are transformed by intestinal bacteria to enterodiol (END) and enterolactone (ENL) respectively, and thereafter END is oxidized to ENL (Borriello, Setchell, Axelson, & Lawson, 1985).

Effects of bread formulated with different grain type/varieties and their effect on health outcomes following consumption

Some ancient grain varieties have been found to have health-promoting benefits compared to modern types (**Table 5.2**). Sereni et al. (2017) reported significant reduction in total cholesterol, LDL-cholesterol and blood glucose concentration in healthy participants following the consumption of bread formulated from ancient wheat varieties when compared with bread formulated from modern wheat grain (Sereni et al., 2017). *Amilo* and *Rekrut* rye breads significantly blunted postprandial glucose and insulin response compared to bread from *Dankowski Zlote, Nikita,* and *Haute Loire Pop* varieties (Rosen, Ostman, Shewry, et al., 2011). A higher feeling of fullness following the consumption of bread formulated from the rye flour from all the varieties compared to white wheat bread was reported. Bread formulated with flour from *Vicello* and *Picasso* rye compared to *Amilo, Evolo,* and *Kaskelott* had a better blood glucose response (Rosen, Ostman, & Bjorck, 2011). Rye bread made from not only *Vicello* and *Picasso* but also *Amilo* and *Kaskelott* significantly blunted insulin incremental peak compared to white wheat bread. The authors posited that rye bread's blood glucose, insulin and appetite response-regulating ability could be attributed to its rich bioactive compound (caffeic, ferulic, sinapic and vanillic) constitution (Rosen, Ostman, Shewry, et al., 2011) coupled with its rich insoluble fibre content (Rosen, Ostman, & Bjorck, 2011). Interestingly, authors of another study reported that the structural difference between rye and wheat bread was the crucial factor for blunting the postprandial insulin, glucose-dependent insulinotropic polypeptide, and C-peptide responses induced following the consumption of rye bread, not necessarily the content of fibre (K.S. Juntunen et al., 2003).

The consumption of bread formulated from barley β-glucan-enriched flour fractions (obtained from barleys with either normal or waxy starch) and its impact on glycaemic control has been investigated. Bread formulated with 100% wheat flour had a higher glycaemic index of 82.80 which markedly reduced to 57.2 following the substitution of wheat flour with 40% β-glucan-enriched barley flour (normal starch). However, the formulation of bread from wheat and β-glucan-enriched flours used as composite flour, with the β-glucan-enriched flour (from waxy barley) constituting 40%, resulted in the reduction of the glycaemic index to 70.10. Despite the reduction of glycaemic index been significant, the study findings led the authors to posit that the ability of β-glucan-enriched flour to reduce glycaemic index is dependent on the type of starch in the barley (Finocchiaro et al., 2012).

Impacts of fibre-enriched bread consumption on health outcomes

Studies involving the incorporation of certain types of purified insoluble fibres, mostly from cereals (oat, wheat, resistant starch), have been explored (M. O. Weickert et al., 2005; M.O. Weickert et al., 2006) (**Table 5.2**). Fibre increases satiety, prevents reabsorption of bile salts consequently promoting the lowering of serum cholesterol, and delay the release of glucose from food into the blood (Blackwood et al., 2000). Bread enriched with purified insoluble cereal fibre from oat, wheat and resistant starch induced different postprandial responses of PYY and ghrelin, with no difference in satiety effect recorded (M.O. Weickert et al., 2006). Additionally, a significant early insulin response, which was associated with an earlier increase of postprandial active GIP values, with no effect on GLP-1 but a significant reduction of postprandial glucose, was recorded the following day, upon ingestion of the control bread (M. O. Weickert et al., 2005). β-glucan is a known soluble fibre in most cereals and grains. Barley β-glucan incorporated into bread regulated appetite in the acute state by modulating sensations and reducing energy intake (Vitaglione, Lumaga, Stanzione, Scalfi, & Fogliano, 2009) and lowering blood glucose response (Jalil, Combet, Edwards, & Garcia, 2016; Pick, Hawrysh, Gee, & Toth, 1998) though the area under the curve for insulin was higher (Pick et al., 1998). The authors posited that the satiety-inducing effect of β-glucan is mediated by ghrelin and PYY (Vitaglione et al., 2009). Also, bread formulations with type-3 resistant starch improved postprandial glycaemic response (Lin, Shyr, & Lin, 2012).

In terms of rye, a number of authors (Gråsten et al., 2007; Gråsten et al., 2000; Hlebowicz et al., 2009; Isaksson et al., 2013; Lankinen et al., 2011; Moazzami et al., 2012; Rosen, Ostman, & Bjorck, 2011; Rosen, Ostman, Shewry, et al., 2011; Sandberg et al., 2017; Turpeinen et al., 2000) increased the fibre enrichment of rye breads through the use of wholegrain/wholemeal rye flour components and reported on their health-promoting effects (**Table 5.2**). Fibre-enriched rye bread increased: plasma enterolactone concentration (Gråsten et al., 2007); faecal output and frequency, reducing mean intestinal time (improved bowel function) (Gråsten et al., 2007; Gråsten et al., 2000); the activities of β-glucuronidase and β-glucosidase in men; urease activity in women (Gråsten et al., 2000); and faecal butyrate concentration in men. However, significantly lower faecal total and secondary bile acid concentrations in both men and women (Gråsten et al., 2000), lower availability of macronutrients for small intestinal digestion and absorption due to increase in ileal excretion of energy and macronutrients (Isaksson et al., 2013), increased concentrations of metabolites that have the potential of mediating positive effects of rye bread on satiety and weight maintenance (Lankinen et al., 2011), and higher betaine levels (Moazzami et al., 2012) have been reported. Lower leucine and isoleucine concentration with a corresponding higher concentration of total- and LDL-cholesterol levels (Moazzami et al., 2012) and no difference in postprandial blood glucose response or gastric emptying were reported following the consumption of rye wholemeal bread compared with white wheat bread (Hlebowicz et al., 2009). No significant variation in the effects of whole rye bread intake on coagulation, fibrinolysis or platelet function was observed compared with "control" wheat bread and a slightly lower concentration of D-dimers, which are fibrin degradation products, was observed after the wheat bread consumption period (Turpeinen et al., 2000).

Timing of fibre-enriched bread intake and its impacts on health

The timing of intake of functional bread products can impact its ability to deliver health benefits. Whole grain rye kernel bread consumed as part of evening meals demonstrated beneficial effects against potential inflammatory markers that could trigger the onset of nutrition-related non-communicable diseases such as diabetes (A. C. Nilsson, Ostman, Holst, & Bjorck, 2008; Sandberg et al., 2016) (**Table 5.2)**. There was enhanced appetite modulation, a favourable effect on glucose, GLP-1 and PYY response the subsequent morning after breakfast intake (Sandberg et al., 2016). An increased concentration of short chain fatty acids and breath hydrogen was reported following the consumption of the rye bread due to the beneficial colonic fermentation of indigested carbohydrates from the rye ingredient in the colon of the large intestines (Sandberg et al., 2016). Consumption of barley kernel-based bread formulated from ordinary, high-amylose or β-glucan-rich genotypes, or white wheat flour bread enriched with a mixture of barley fibre and resistant starch, when consumed by participants as part of an evening meal, blunted glucose response and lowered the release of the inflammatory marker interleukin-6 at the subsequent breakfast. The concentration of the protein hormone, adiponectin, was also elevated (A. C. Nilsson et al., 2008).

Postprandial glucose and insulin responses were attenuated following the consumption of bread meal consumed in the evening with a 1:1 ratio of whole grain rye flour and rye kernels with resistant starch, and increased the gut hormone PYY in plasma the following morning 0–120 min after a standardised breakfast, compared to white wheat flour bread (P = 0.01). The intake of the whole grain rye flour bread resulted in appetite suppression in the participants, consequently decreasing their desire to eat. Decreased hunger feeling was observed for participants following the intake of both whole grain rye flour bread and bread formulated from 1:1 whole grain rye flour and rye kernels. Decreased fasting free fatty acid with increased breath hydrogen concentration following the consumption of the all rye-based evening meals was reported. Energy intake at lunch and the inflammatory marker interleukin-6 were not found to be impacted by the intake of the rye-based evening meals compared to white wheat flour bread following consumption (Sandberg et al., 2017).

Gum-enriched functional bread

The consumption of guar gum-enriched bread reduced the postprandial rise in blood glucose and enhanced cognitive performance (A. Nilsson, Radeborg, & Bjorck, 2012) (**Table 5.2**). A combination of medium-weight guar gum and whole grain corn flour (high in amylose content) used in bread formulation improved glucose, insulin, and subjective appetite of participants following consumption as the content of resistant starch increased in the bread (Ekstrom, Bjorck, & Ostman, 2013). A similar effect was observed for guar gum, whole grain rye and/or high amylose maize starch in white bread, but with the added benefit of stimulating PYY excretion (Ekstrom et al., 2016). No significant differences in the postprandial blood glucose responses were observed between the "control" and guar bread, and all guar breads significantly induced a decrease in the postprandial rise in plasma insulin, findings which were not influenced by large variations in particle size of the guar gum or molecular weight (Ellis, Dawoud, & Morris, 2007).

Conclusion

The formulation and development of bread with functional ingredients results in increased bioactive properties that have potential health-promoting effects. Reported health-promoting effects associated with the consumption of bread enriched with functional ingredients include attenuated glucose and insulin release, improved appetite and lipid profile, and enhanced cognitive function. The subsequent chapters of the thesis will investigate the glycaemic response modulation, appetite suppression, carotenoid bioavailability and lipid profile of participants following consumption of the vegetable-enriched Nothing Else™ bread selected from **Chapter 4.**

Table 5.2. Effects of functional food ingredient addition in bread on health outcomes

Functional food ingredient	Type of study	Duration of intervention	Number of subjects	Amount of bread served per visit/ bioactive present	Health effects	Reference
Fruits and vegetables						
Baobab fruit extract	Randomised crossover design	180 minutes	13 healthy normal or slightly overweight volunteers	50g of available carbohydrate	No ↓ in glycaemic response or hunger, but significant ↓ total and insulin iAUC	(S. Coe & Ryan, 2016)
Beetroot	Acute, randomised, open-label, controlled crossover trial	420 minutes	23 healthy men	200g bread containing 100g beetroot (1.1 mmol nitrate) or 200g control white bread (CB; 0g beetroot, 0.01 mmol nitrate)	↑ increased endothelium-independent vasodilation and ↓ diastolic blood pressure	(Hobbs et al., 2013)
Legumes, seeds and nuts						
White kidney bean extract (Phaseolus vulgaris)	Randomised crossover study	120 minutes	13 healthy adults	Dosages of 1500 mg, 2000 mg, and 3000 mg	↓ glucose response	(Udani et al., 2009)

Functional food ingredient	Type of study	Duration of intervention	Number of subjects	Amount of bread served per visit/ bioactive present	Health effects	Reference
Chickpea flour	Randomised, crossover	90 minutes	13 healthy female subjects	50g supplying 25g available carbohydrate	↓ glycaemic response	(Zafar et al., 2015)
Chickpea flour	Randomised, single-blind, cross-over study	120 minutes	11 healthy subjects	50g available carbohydrate	↓ postprandial glycaemic response	(S. K. Johnson et al., 2005)
Lupin	Randomised controlled crossover design	180 minutes	16 and 17 respectively for study 1 and 2	40g total carbohydrate	↑ satiety and ↓ energy intake	(Lee et al., 2006)
Lupin	Randomised cross-over design	120 minutes	20 healthy adults	36.9 and 37.3g available carbohydrate for burgen and lupin breads respectively	↓ blood glucose and ↑ satiety responses	(Keogh et al., 2011)
Australian sweet lupin (Lupinus angustifolius) kernel fibre	Randomised, crossover design	120 minutes	21 healthy adults	50g available carbohydrate breakfasts / 90g lupin kernel flour	↓ iAUC (18.8%) for insulin response	(S.K. Johnson et al., 2003)

Functional food ingredient	Type of study	Duration of intervention	Number of subjects	Amount of bread served per visit/ bioactive present	Health effects	Reference
Salba flour	Randomised controlled crossover design	120 minutes	13 healthy adults	7, 15 or 24g of whole or ground Salba baked into white bread	↓ blood glucose levels in a dose-dependent manner	(Ho et al., 2013)
Salba	Acute randomised, double-blind, crossover design	120 minutes	11 healthy subjects	50g available carbohydrate with addition of 0, 7, 15 or 24g of Salba	↓ blood glucose and appetite responses	(Vuksan et al., 2010)
Sliced hazelnut and semi-defatted hazelnut flour	Randomised controlled, crossover study	6 days- 120 minutes	32 healthy adults	Breads contained either 30g of finely sliced hazelnuts, 30g semi-defatted hazelnut flour, or 15g of each (amounts per 120g bread)	↓ AUC for blood glucose	(Devi et al., 2016)

Functional food ingredient	Type of study	Duration of intervention	Number of subjects	Amount of bread served per visit/ bioactive present	Health effects	Reference
Sourdough treatment						
3-grain sprouted sourdough	Randomised crossover design	300 minutes	12 adult diabetics	50g available carbohydrate	↓ blood glucose iAUC, second meal plasma insulin iAUC and serum apolipoprotein B-100 iAUC	(Tucker et al., 2014)
Sodium propionate	Randomised crossover acute study	120 minutes	6 healthy subjects	3.3g sodium propionate per 50g available portion of bread	↓ blood glucose response and alters lipid metabolism	(Todesco et al., 1991)
Propionate-rich sourdough	Randomised cross-over balanced design	180 minutes	20 healthy adults	4.8mmol per 100g bread (2.3mg per g propionic acid and 2.98mg per g calcium propionate).	No effect on appetite	(Darzi et al., 2012)
Sodium propionate	Randomised, crossover study	120 minutes	9 healthy subjects	50g available starch (approx.155g bread) that was served with 8.8g	↓ gastric emptying rate and ↓ glycaemic and insulinemic responses	(Darwiche et al., 2001)

Functional food ingredient	Type of study	Duration of intervention	Number of subjects	Amount of bread served per visit/ bioactive present	Health effects	Reference
				butter and 18.9g cheese		
Sourdough	Single blind, randomised, crossover design	300 minutes	10 overweight subjects	50g available carbohydrate	↓ blood glucose response	(Najjar et al., 2009)
Sourdough fermentation of wholemeal wheat	Randomised, crossover design	240 minutes	11 adults with Metabolic syndrome	50g available carbohydrate	↓ postprandial glucose and insulin responses	(Lappi et al., 2010)
Sourdough from wheat flour	Randomised, crossover design	120 minutes	8 healthy volunteers	50g of available carbohydrates	↓ postprandial blood glucose response	(Scazzina et al., 2009)
Sourdough from rye endosperm	Randomised crossover design	40 minutes	16 healthy subjects	50g available carbohydrate	↓ insulin response	(Bondia-Pons et al., 2011)
Sourdough	Randomised, crossover study	180 minutes	12 and 11 overweight or obese male adults for study 1 and 2 respectively	50g available carbohydrate for study 1	↓ blood glucose response	(Mofidi et al., 2012)

Functional food ingredient	Type of study	Duration of intervention	Number of subjects	Amount of bread served per visit/ bioactive present	Health effects	Reference
Sourdough and soya protein concentrate rich in glutamic acid and a protease	Randomised, double-blind, crossover trial	4 weeks	30 subjects with pre or mild-to-moderate hypertension	120g/day of bread	Low-sodium bread, low-sodium wheat bread rich in potassium, g-aminobutyric acid, and angiotensin-converting enzyme inhibitor peptides had no significant effect different from conventional wheat bread or low-sodium wheat bread enriched in potassium on the 24-hour BP, endothelial function, or glucose metabolism.	(Becerra-Tomas et al., 2015)

Functional food ingredient	Type of study	Duration of intervention	Number of subjects	Amount of bread served per visit/ bioactive present	Health effects	Reference
Cereals/grains and pseudocereal						
Spelt (Triticum spelta L.) flour	Randomised crossover	120 minutes	10 healthy adults	50g of bread	Similar glycaemic response to "control" white wheat bread was observed	(Marques et al., 2007)
Barley flour	Randomised, crossover	120 minutes	15 diabetic subjects	50g of breads	↓ postprandial blood glucose response	(Urooj et al., 2009)
Wholemeal rye flour	Randomised crossover design	4 weeks	39 healthy adults	Mean intake of rye bread= 219 and 162g/d and 200 and white bread =153g/d respectively	↑ enterolactone concentrations	(K.S. Juntunen et al., 2000)
Ancient wheat grains	Randomised, double-blinded crossover trial	8 weeks	45 clinically healthy subjects	NA	↓ blood glucose response, total- and LDL-cholesterols concentration	(Sereni et al., 2017)

Functional food ingredient	Type of study	Duration of intervention	Number of subjects	Amount of bread served per visit/ bioactive present	Health effects	Reference
Endosperm and wholegrain rye	Randomised crossover design	180 minutes	12 healthy subjects	40g of available starch	↓ acute insulinaemic responses and improved glycaemic profile	(Rosen, Ostman, Shewry, et al., 2011)
Wholegrain rye from different varieties	Randomised crossover study	180 minutes	20 healthy subjects	50g of available starch	↓ blood glucose and insulin responses but ↑ satiety	(Rosen, Ostman, & Bjorck, 2011)
Rye flour	Randomised crossover design	8 weeks	19 healthy postmenopausal women	50g available carbohydrate	↓ postprandial insulin response to rye bread than to wheat bread	(K.S. Juntunen et al., 2003)
β-glucan-enriched flours, obtained from barleys with either normal or waxy starch	Randomised crossover design	120 minutes	Nine healthy adults	50g of available carbohydrate	↓ glycaemic index especially with the flour containing normal starch	(Finocchiaro et al., 2012)
Purified insoluble fibres (wheat fibre, oat fibre, resistant starch)	Randomised, single-blind, within-subject crossover design	300 minutes	Fourteen healthy women	50g of available carbohydrates	↓ postprandial glucose response and ↑colonic fermentation	(M. O. Weickert et al., 2005)

Functional food ingredient	**Type of study**	**Duration of intervention**	**Number of subjects**	**Amount of bread served per visit/ bioactive present**	**Health effects**	**Reference**
Wheat-fibre	Randomised, single-blind, controlled crossover design	300 minutes	Fourteen healthy adult women	10·5g wheat-fibre or 10·6g oat-fibre per portion	↓ postprandial responses of PYY and ghrelin after the intake of the wheat-fibre enriched bread but not after oat-fibre enriched bread	(M.O. Weickert et al., 2006)
Barley β-glucan	Randomised, cross-over design	180 minutes	Fourteen healthy subjects	A dose of 3g barley β-glucans was provided by the β-GB portion	↓ AUC $_{60–180}$ of plasma ghrelin (23%) and ↑ total AUC (16%) of PYY, ↓ glucose response and improved appetite	(Vitaglione et al., 2009)
β-glucan and black tea	Randomised cross-over design	180 minutes	15 healthy adults	NA	↓ blood glucose AUC for β-glucan period but not for black tea supplementation on glucose and insulin responses	(Jalil et al., 2016)

Functional food ingredient	Type of study	Duration of intervention	Number of subjects	Amount of bread served per visit/ bioactive present	Health effects	Reference
High β-glucan barley flour	Randomised crossover study (24 weeks)	12 weeks	11 Type 2 diabetic subjects	25g barley bread	↓ glucose response AUC blunted but not for insulin	(Pick et al., 1998)
Type 3 resistant starch	Randomised, crossover study	120 minutes	Ten healthy subjects	10, 30 and 60% substitution	↓ postprandial blood glucose	(Lin et al., 2012)
Wholemeal rye	Randomised crossover design	4 weeks	Forty healthy subjects	49 carbohydrate (% energy)	No significant variation in the effects of whole rye bread intake on coagulation, fibrinolysis or platelet function	(Turpeinen et al., 2000)
Whole grain rye (whole grain rye flour bread (RFB) or a 1:1 ratio of whole grain rye flour and rye kernels bread (RFB/RKB), with/without type 2 resistant starch	Randomised crossover overnight study design	210 minutes	Twenty-one healthy subjects	50g available starch	↓ blunted postprandial glucose- and insulin responses (iAUC) and ↑ PYY in plasma following the consumption of RFB/RKB + RS. RFB ↑ subjective satiety and	(Sandberg et al., 2017)

Functional food ingredient	**Type of study**	**Duration of intervention**	**Number of subjects**	**Amount of bread served per visit/ bioactive present**	**Health effects**	**Reference**
					decreased desire to eat, and both RFB and RFB/RKB ↓ feeling of hunger	
Rye whole-meal flour	Randomised, crossover design	90 minutes	10 healthy adults	150g breads	No effect on postprandial blood glucose response or gastric emptying	(Hlebowicz et al., 2009)
Rye flour	Randomised, controlled, crossover design	8 weeks	33 postmenopausal women with elevated serum total cholesterol	A minimum of 4–5 portions of the test bread had to be eaten daily. Each portion of rye bread weighed 24–28g and each portion of wheat bread 21–25g.	No effect on lipid profiles	(Moazzami et al., 2012)

Functional food ingredient	Type of study	Duration of intervention	Number of subjects	Amount of bread served per visit/ bioactive present	Health effects	Reference
Rye flour	Randomised crossover design (8wk intervention)	8 weeks	39 postmenopausal women with elevated serum total cholesterol	A minimum of 4–5 portions of the test breads had to be eaten daily. The portions of rye breads weighed 24–28g and those of wheat breads 21–25g.	No changes in lipidomic profiles	(Lankinen et al., 2011)
High-fibre rye	Randomised crossover design	2 weeks	10 ileostomy subjects	Low-fibre wheat bread (20g dietary fibre per day) for 2 weeks followed by high-fibre rye bread (52g dietary fibre per day) for 2 weeks	↑ ileal excretion of energy and macronutrients	(Isaksson et al., 2013)

Functional food ingredient	Type of study	Duration of intervention	Number of subjects	Amount of bread served per visit/ bioactive present	Health effects	Reference
Whole meal rye flour	Randomised crossover study	4 weeks	17 healthy subjects	A minimum of 4–5 portions of test breads had to be eaten. test bread portions, which were 27.5–40g for rye breads and 22.5–25.0g for wheat breads	↑ faecal output and faecal frequency and shortened mean intestinal time, ↑ activities of β-glucuronidase and β-glucosidase in men while urease activity was ↑ significantly in women during the rye bread period. ↑ faecal butyrate concentration and ↓ faecal total and secondary bile acid concentrations	(Gråsten et al., 2000)

Functional food ingredient	Type of study	Duration of intervention	Number of subjects	Amount of bread served per visit/ bioactive present	Health effects	Reference
High-fibre rye	Randomised crossover trial	8 weeks	39 postmenopausal women	The portions of RBs and WBs weighed 24.1 to 28.1 and 20.8 to 25.0g respectively. A minimum of 4 to 5 portions had to be eaten each day	↑ faecal frequency (improved bowel function) and plasma enterolactone concentration	(Gråsten et al., 2007)
Whole grain rye kernel	Randomised cross-over design	180 minutes	19 healthy young adults	50g available starch	↑ plasma PYY, GLP-1 and fasting short chain fatty acids (acetate and butyrate) concentration. ↓ postprandial blood glucose and serum insulin and fasting free fatty acid concentration. ↑ appetite suppression	(Sandberg et al., 2016)

Functional food ingredient	Type of study	Duration of intervention	Number of subjects	Amount of bread served per visit/ bioactive present	Health effects	Reference
Barley kernel based (ordinary, high-amylose-or-β-glucan-rich genotypes) and refined wheat enriched with a mixture of barley fibre and resistant starch	Randomised crossover design	180 minutes	15 healthy subjects	50g available starch	↑ breath hydrogen excretion ↓ glucose release subsequent to breakfast	(A. C. Nilsson et al., 2008)
Guar gum	Randomised crossover design	240 minutes	40 healthy adults	50g available starch	↑ cognitive function and ↓ glucose response	(A. Nilsson et al., 2012)
Medium weight guar gum and whole grain corn flour	Randomised crossover	180 minutes	12 healthy volunteers	37g of available starch	↓ glucose and insulin response and ↑ appetite suppression	(Ekstrom et al., 2013)

Functional food ingredient	**Type of study**	**Duration of intervention**	**Number of subjects**	**Amount of bread served per visit/ bioactive present**	**Health effects**	**Reference**
Guar gum, wholegrain rye flour or high amylose corn starch	Randomised crossover design	240 minutes	19 healthy adults	50g of available starch	↑ appetite suppression	(Ekstrom et al., 2016)
Guar gum	Randomised crossover design	120 minutes	17 healthy subjects	50g 'available' carbohydrate	↓ postprandial rise in plasma insulin by all the guar breads.	(Ellis et al., 2007)

Note. PYY = peptide tyrosine tyrosine, GLP-1 = glucagon-like peptide 1, AUC = area under curve, iAUC = incremental area under curve.

Chapter 6. Evaluation of the glycaemic and appetite suppression potential of a vegetable-enriched bread

Abstract

Bread is a commonly consumed food in New Zealand. Consequently, enriching bread with vegetables to improve its glycaemic and appetite suppression effects may be a viable way of enhancing its health-promoting potential. This experimental cross-over study with 10 apparently healthy participants aged 23±7 years sought to test the effect of a vegetable-enriched bread (VB) in comparison to commercial white bread (WB) and wheatmeal bread (WMB) on serum glucose, insulin response and subjective appetite suppression over a 120-minute period. In a predetermined random order and on three separate occasions separated by at least a three-day interval, participants visited the laboratory and consumed 75g of WB, WMB or VB following an overnight fast of at least eight hours. Venous blood samples were drawn for the analysis of serum glucose and serum insulin twice before the meal (0 min) and at 15, 30, 45, 60, 90 and 120 min after consumption of the bread. At the same time as each blood sample was drawn, participants rated their subjective feelings of hunger, fullness, satisfaction and desire to eat using an appetite evaluation questionnaire on a 150 mm Likert scale. The distance on the scale was measured using an electronic digital caliper (Warrior®, Cornwall, England) to a precision of 0.01mm. The total incremental area under the curve (iAUC) was calculated for both glucose and insulin responses following the WB, WMB and VB consumption. The mean glucose iAUC were not different among the breads. The mean insulin iAUC for the VB was significantly lower than the WB and WMB; VB and WB 12415pmol/L*minutes (95% CI 1918, 22912pmol/L*minutes, p=0.025) and VB and WMB 13800pmol/L*minutes (95% CI 1623, 25976pmol/L*minutes p=0.031). Over the 120-minute period following consumption, compared to the WB and WMB, the VB stimulated a higher fullness feeling in the participants. There was no difference in the swallowability attributes of all the breads including ease of swallowing. The consumption of VB was associated with less insulin release and higher satiety over 120 minutes which may be related to the higher fibre content and texture of the VB.

Keywords: Vegetable-enriched bread, white bread, wheatmeal bread, glucose, insulin, appetite

Introduction

Globally, the quality and quantity of the food supply, is a major attributable factor for the development of diet-related non-communicable diseases including type 2 diabetes mellitus (Min, Zhao, Slivka, & Wang, 2018). In 2009, bread and bread products contributed the highest proportions of energy (11%), protein (11%), carbohydrate (17%) and dietary fibre (17%) to the New Zealand diet (Ministry of Health, 2011). The commercial bread market is dominated by cheap bread made from highly refined flours, that are usually poor sources of dietary fibre and generally considered a high glycaemic food (Kikuchi et al., 2018; D. Y. Kim, Kim, & Lim, 2018) with a lower ability to suppress appetite (Bo et al., 2017). Yet health-conscious bread consumers seek bread with a higher dietary fibre content than white bread (Sajdakowska, Gebski, Zakowska-Biemans, & Jezewska-Zychowicz, 2019).

The addition of novel ingredients to increase dietary fibre content in bread is known to favourably impact on glycaemic response and satiation (Williams, Mikkelsen, Flanagan, & Gidley, 2019). Novel ingredients that improve glycaemic response and appetite suppression include hazelnuts (Devi et al., 2016), wholemeal rye flour (Ostman et al., 2019), salba seeds (Ho et al., 2013; Vuksan et al., 2010) and chickpea flour (S. K. Johnson et al., 2005). What is not known is whether drum-dried vegetable flours (Galaz et al., 2017) with low moisture content and excellent shelf-life, and nutrient-enriching and phytochemical properties when used to enrich bread, will demonstrate effects that could support a health claim. We have already shown (**Chapter 4**) that bread made of drum-dried pumpkin and sweet corn flours, sprouted wheat flour, wholemeal wheat flour and flaxseed is acceptable to consumers and easier to swallow than commercial $1 white and wheatmeal breads.

Pumpkin flour contains carotenoid compounds, particularly beta carotene, and the hydrophilic fibre pectin (Einhorn-Stoll, 2018; Fissore et al., 2009). Sweet corn flour is a rich source of dietary fibres, cellulose and β-glucan that enhance its ability to promote satiety (Topping & Clifton, 2001).

Other wholesome ingredients selected for the bread included flaxseed and wholemeal flour which have shown suppression of postprandial lipaemia and appetite (Kristensen et al., 2013) and improved glycaemic response (Hutchins et al., 2013).

Another novel ingredient is sprouted wheat which has been reported to have increased bioactivity (Donkor, Stojanovska, Ginn, Ashton, & Vasiljevic, 2012). Recently, it has been found to act as an enzyme improver in bread (Marti et al., 2017). Enzyme improvers are hydrolytic amylases and proteases produced during controlled grain sprouting whose activity can be harnessed to complement the activity of yeasts during dough leavening resulting in improving the concentration of gas produced during dough leavening. This results in bread with high specific volume and lower crumb hardness during storage (Marti et al., 2017; Marti, Cardone, Pagani, & Casiraghi, 2018). The consumption of bread formulated with wholemeal flours improves glycaemia response possibly due to large particle size, and the functional properties of sprouted cereals are enhanced as a result of the generation of bio-functional compounds, and improved glycaemic properties through reduction of available carbohydrates (A. K. Singh et al., 2015). The study therefore sought to test the effect of this vegetable-enriched Nothing Else™ bread, in comparison to commercial $1 white and wheatmeal breads, on serum glucose, insulin response and subjective appetite suppression effect over a 120-minute period after consumption.

Participants

The study was advertised at the AUT South Campus. Ten apparently healthy participants who were relatively sedentary and regular bread eaters (at least three times per week) took part in the study. Exclusion criteria included regular and high intensity activity, following a low carbohydrate diet and gluten intolerance.

The establishment of health claims related to the reduction in postprandial glycaemic response demands that the test bread has a statistically significant decrease (minimum 20%) in incremental area under the blood glucose response curve (iAUC) in comparison to the reference white bread (Health Canada, 2013). The reported mean coefficient of variations (CVs) for testing iAUC for glucose in the literature are in the range of 20-30% (Wolever et al., 2008). Therefore, in this study, which followed the ISO (2010) for a glycaemic index testing study with a predicted minimum 20% decrease in iAUC with a CV of 20%, 10 participants were required to show a 20% difference in glucose iAUC between treatments for a power of 80% with an alpha of 0.05.

Study design and methods

The vegetable-enriched bread used for the glycaemia response study was VB75 (**Chapter 3**). The use of the VB75 for the glycaemia study was because there was no significant difference in the overall liking of the VB75 and VB100 (**Chapter 4**). Also, the consideration of cost as a driver for the purchase of breads drove the choice for the VB75 over VB100 as the amount of pumpkin powder used in VB100 was higher than the VB75. The use of more pumpkin flours incurs extra cost in the production of the bread. Consequently, the VB100 is expected to be higher in cost than the VB75.

The experimental study was approved by the Auckland University of Technology Ethics Committee (AUTEC, approval number 18/147). Participants were asked to follow their normal evening meal and then to fast overnight for at least eight hours before visiting the laboratory at 9am the next day. Consumption of water was allowed. The participants were additionally asked to restrict the intake of alcohol and excessive physical activity the day before each test.

In this experimental, cross-over study, participants in a predetermined random order and on three separate occasions separated by at least a three-day interval consumed 75g of white bread (WB), wheatmeal bread (WMB) or vegetable-enriched bread (VB). The breads were frozen at -16°C but defrosted one hour prior to consumption. The breads were served to participants, after an overnight fast, with 250mL of tapwater. A peripheral venous catheter (BD Venflon, Becton Dickinson, Helsingborg, Sweden) was inserted into the antecubital vein. Two fasting blood samples were taken five minutes apart and then the participants were asked to consume the 75g of bread within 12 min. The subjects were seated comfortably and listened to music of their own choice throughout the procedure.

Measurements

Venous blood samples were drawn for the analysis of serum glucose and insulin before the meal (0 min) and at 15, 30, 45, 60, 90 and 120 min after consumption of the bread. In addition, the participants were asked, at each timepoint when the blood was drawn, to rate their subjective feelings of hunger, fullness, satisfaction and desire to eat on a 150mm visual analogue scale (VAS). The distance on the scale was measured using an electronic digital caliper (Warrior®, Cornwall, England) to a precision of 0.01mm.

The drawn blood sample stood for 20-40 minutes and then was centrifuged for 10 minutes at 3500 rpm (centrifuge Z 150 A, Woodbridge, NJ). Serum was then aliquoted into Eppendorf tubes and immediately frozen at -20°C. The frozen serum was transported in ice to the AUT Roche Diagnostics Laboratory. Glucose and serum concentrations were determined by specific diagnostic assays on an automated clinical chemistry analyser (Cobas Modular P800/E170, Roche Diagnostics New Zealand Ltd, Auckland, NZ). The test principle for the glucose measurement was by enzymatic colorimetric test with limits of measurement being 0.11-41.6mmol/L. For the insulin measurement, the test principle employed was electrochemiluminescence with limits of measurements being 1.39-6945pmol/L. For all assays, the limit of measurement or functional sensitivity represents the lowest measurable analyte level that can be distinguished from zero. Method comparisons, limitations and specific performance data can be found at www.e-labdoc.roche.com.

A questionnaire with a 150mm-long Likert scale anchored by verbal end points (measuring attributes including “colour”, “aroma”, “taste”, “texture”, “mouthfeel”, “overall liking” and “willingness to eat at home” and “ease of biting and getting into the mouth, ease of chewing, ease of swallowing and ease of movement through the throat”) was designed to determine the degree of liking and ease of swallowing of bread. The distance on the scale was measured using an electronic digital caliper (Warrior®, Cornwall, England) to a precision of 0.01mm.

Method

Table 6.1. Ingredients used in white bread (WB), wheatmeal bread (WMB) and vegetable-enriched bread (VB) formulation

WB	WMB	VB
Wheat flour	Wheat Flour	Water
Water	Water	Wheat flour
Baker's Yeast	Wheatmeal Flour	Wholemeal flour
Iodised salt	Baker's Yeast	Flaxseed
Canola oil	Vinegar	Pumpkin powder
Acidity regulator (263)	Iodised Salt	Sweet corn powder
Soy flour	Wheat Gluten	Sprouted wheat flour
Emulsifier (481, 472e)	Acidity Regulator (263)	Salt
Vitamin (Folic Acid)	Roasted Barley Malt Flour	Baker's yeast
	Canola Oil	
	Soy Flour	
	Emulsifiers (481, 472e)	
	Vitamin (Folic Acid)	

Selected nutrients in the vegetable-enriched bread were determined at Asurequality, an Internationally Accredited New Zealand laboratory. Analyses were moisture content (AOAC 925.10), β-carotene (EN 12823-2:2000, COST91, 1986), dietary fibre (insoluble) (AOAC 991.43 Ankom), dietary fibre (soluble) (AOAC 991.43 Ankom), dietary fibre (total) (AOAC 991.43), sodium (AsureQuality Method, ICP-OES) and potassium (AsureQuality Method, ICP-OES). The HSRCS (% foods method) for the VB, WB and WMB was calculated in compliance with FSANZ regulations (Food Standards Australia New Zealand, 2019).

Statistical analyses

Data was checked for normal distribution using the Shapiro-Wilk test (where $p>0.05$ implied normality for the data) due to the small sample size. All the variables followed a normal distribution except the subjective evaluation of "Could you eat more" for WMB, "Are you satisfied "for WB and "Do you want to eat" for WMB". The total incremental area under the curve (iAUC) was calculated for both glucose and insulin responses following WB, WMB and VB consumption. The calculation was carried out using the ISO standard following the trapezoid rule (The International Organization for Standardization, 2010). In using the trapezoid rule, the area of the curve above the fasting baseline was assumed to be a trapezoid and calculated as the sum of the areas of a triangle and a rectangle with the units for glucose of mmol/litre * minutes. Using the general linear model, repeated measures and analysis of covariance were carried out on the total iAUC of the glucose, insulin responses and appetite responses including "Are you hungry?", "Could you eat?", "Are you satisfied?" and "Do you want to eat?" Pearson's correlation was used to establish associations between bread liking and the ease of bread swallowing. Interpretation of the magnitude of effect size of change between the post-treatment and the baseline measures used Cohen's effect size criteria with 0.2 being small, 0.5 being moderate and 0.8 being large (Field, 2009). Unless otherwise stated, all data were analysed using International Business Machines Corporation® SPSS® Statistics Version 25.

Results

Nutrient analysis

Bread (VB) enrichment with drum-dried pumpkin and sweet corn powders increased the nutrient density resulting in higher dietary fibre, potassium and β-carotene compared to the WB and WMB (**Table 6.2**). White bread (WB) had a lower fibre content compared to the VB and the WMB. The WB and WMB breads were also higher in sodium content compared to the VB bread. The moisture content of the VB was slightly higher than the two commercially produced breads.

Table 6.2. Nutrient composition of the test breads

Component	WB	WMB	VB
Moisture	36.6	38.2	39.1
Protein (g/100g)	8.5	8.8	7.5*
Dietary fibre (g/100g)	2.7	4.6	7.2
Insoluble fibre (g/100g)	nd	nd	5.5
Soluble fibre (g/100g)	nd	nd	1.7
Fat (g/100g)	1.6	1.7	4.8*
Carbohydrate (g/100g)	46.7	43.1	33.9*
Sodium (mg/100g)	392	398	380
Potassium (mg/100g)	nd	nd	300
Energy (kJ/100g)	1020	982	932*
β-Carotene (µg/100g)	nd	nd	236.8

Note. nd = not determined, * = sourced from the Plant & Food Research & Ministry of Health (2016), WB = white bread, WM = wheatmeal bread, and VB = bread with 75g pumpkin powder substitution.

Participants

There were 10 apparently healthy participants (9 males, 1 female) mostly of Pacific ethnicity (n=8) with an average age of 23±7 years and BMI of 32.1±4.5 kg/m^{2}.

Table 6.3. Participant characteristics at baseline (n=10, t=0)

Measure	Unit	Mean	SD
Age (years)	years	23.1	7.0
Weight	kg	106.7	18.2
Height	cm	181.9	11.0
BMI	kg/m^2	32.1	4.5
Glucose	mmol/L	5.3	0.4
Insulin	pmol/L	130.9	69.6
HOMA1-IR	mmol/L*pmol/L	31.1	16.7
HOMA1-%B	p/mol/mmol	1498.4	957.2

Note. BMI = body mass index, HOMA1-IR = homeostatic model assessment-insulin resistance, HOMA1-%B = homeostatic model assessment-insulin resistance-beta cell function (Wallace, Levy, & Matthews, 2004).

Liking and ease of swallowing of bread

Participants preferred the VB the most, with WB and WMB following in that order. The taste liking of the VB was almost double that for the WMB and around 1.1 times more than WB. Participants reported they were more willing to eat the VB at home compared to the WB and WMB. There was no difference in the liking of colour, aroma, mouthfeel, ease of swallowing, number of chews and willingness to eat at home among the three breads. A significant positive association was found between the liking and ease of swallowing of the WMB, $r = 0.709$ 95% CI (0.143-0.925), and the VB, $r = 0.816$ 95% CI (0.383- 0.955).

Table 6.4. Sensory liking and swallowing perceptions of test breads (n=10)

Characteristics	Bread			
	WB	WMB	VB	p-value
Sensory attribute (mm/150 mm)				
Colour	95.1±28.4[a]	80.0±33.4[a]	103.5±36.4[a]	0.285
Aroma	93.8±22.2[a]	83.6±25.0[a]	98.8±28.6[a]	0.408
Taste	101.2±20.0[a]	73.8±20.5[a]	113.5±28.3[b]	0.002
Texture	101.3±18.7[b]	73.9±19.3[a]	112.7±29.4[b]	0.003
Mouthfeel	96.4±27.4[a]	83.6±28.2[a]	102.4±32.7[a]	0.361
Overall liking	97.8±32.6[a,b]	75.7±30.2[a]	112.8±23.0[b]	0.026
Willing to eat at home	83.4±48.3[a]	91.6±38.7[a]	111.1±27.8[a]	0.284
Swallowing evaluation (mm/150mm)				
Ease of biting and getting into the mouth	97.8±33.1[a]	81.5±35.4[a]	106.1±24.2[a]	0.584
Ease of chew	83.6±38.2[a]	82.1±39.7[a]	98.2±36.1[a]	0.225
Ease of swallow	82.3±35.1[a]	86.9±40.2[a]	107.2±22.3[a]	0.996
Ease of movement in throat	97.8±33.1[a]	81.5±35.4[a]	106.1±24.2[a]	0.220
Less stickiness in throat	104.0±32.6[a]	92.4±35.1[a]	117.0±21.8[a]	0.214
Number of chews before swallowing	24±6.33[a]	24±5.94[a]	22±6.38[a]	0.675

Note. Likert scale 0-150 mm with 0mm as the least and 150 mm as the most acceptable. WB = white bread, WM = wheatmeal bread, and VB = bread with 75g pumpkin powder substitution. Data is expressed as mean ± standard deviation. Means with the same superscripts in a column are not significantly different ($p > 0.05$). Mean values with the same superscript in a row are not significantly different ($p>0.05$).

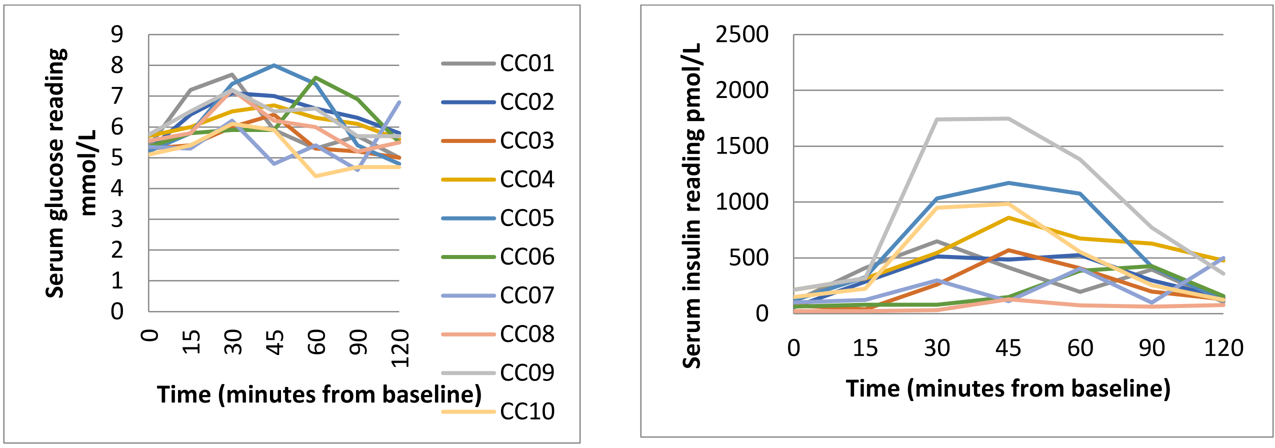
Glucose and insulin response

Generally, after consumption of each bread, the glucose and insulin concentrations were at their highest peak between 15 and 60 minutes and below baseline at 120 minutes, which was associated with an increase in hunger. Consumption of the VB elicited a glucose response that was not different from the WB and WMB (**Table 6.5** and **Figure 6.3**). The mean difference of the iAUC for the VB compared with the WB was 19.1 mmol*min/L (95% CI -14.8, 53.0 mmol*min/L, p=0.235) and WMB was 10 mmol*min/L (95% CI -18.9, 39.4 mmol*min/L, p=0.449).

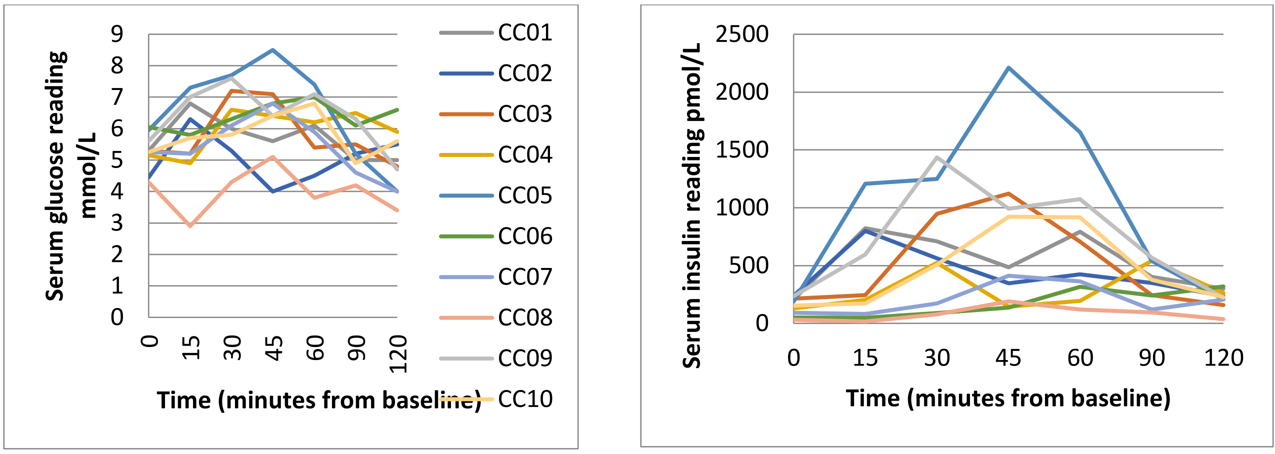
The consumption of the WMB and WB bread elicited significantly higher insulin responses than VB (

Table 6.6 and **Figure 6.3**). The mean insulin iAUC for the VB was almost 38% less than that of the other two breads. The mean difference of the insulin iAUC for the VB compared with the WB was 12415 pmol*min/L (95% CI 1918, 22912 pmol*min/L, p=0.025) and WMB was 13800 pmol*min /L (95% CI 1623, 25976 pmol*min/L, p=0.031).

WB



WMB



VB

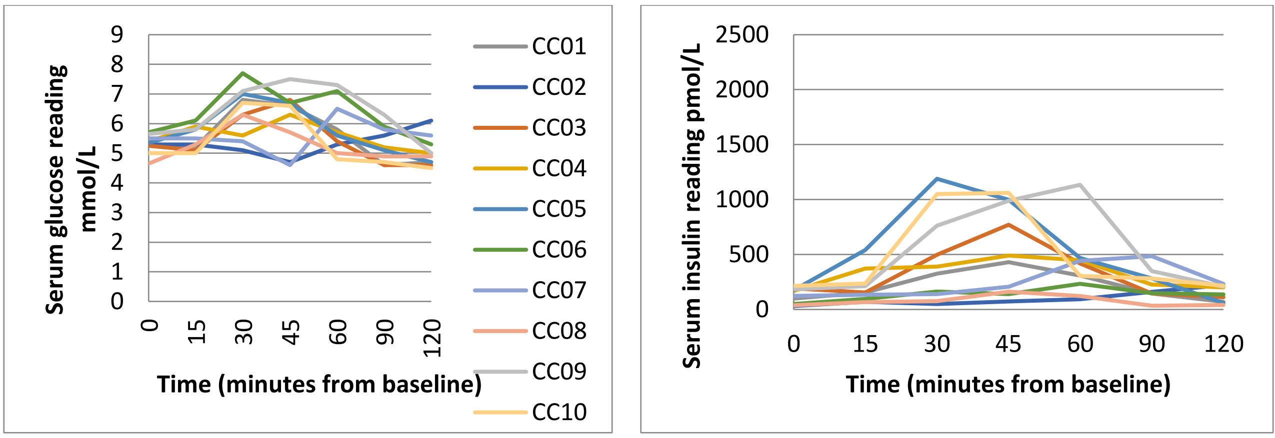


Figure 6.1. Glucose and insulin response following the consumption of bread. WB = white bread, WMB = wheatmeal bread, and VB = bread with 75g pumpkin powder substitution. Time t=0 is the baseline.

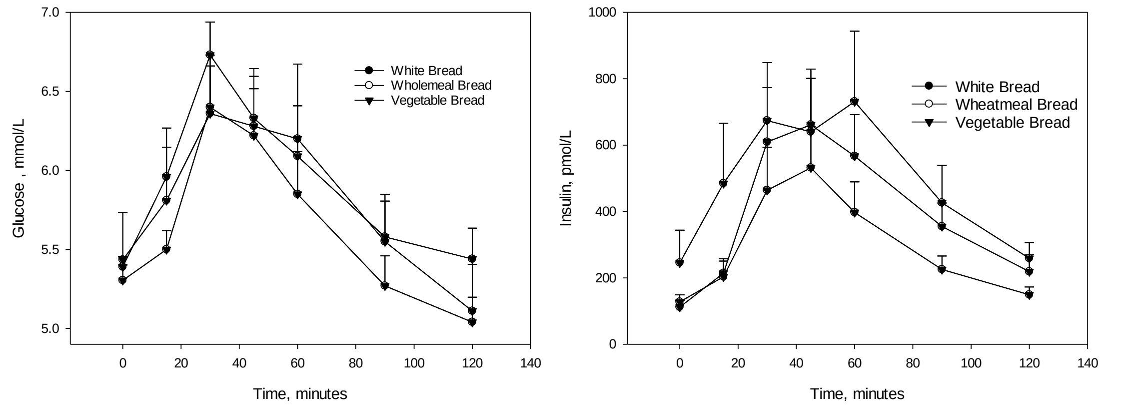


Figure 6.2 Mean (SEM) responses (n=10) of glucose and insulin following consumption of 3 breads

Table 6.5. Participants' serum area under curve, glucose after 120 minutes for test breads

Bread	**Mean/ mmol*min/L**	**Standard Error**	**95% Confidence Interval**	
			Lower Bound	**Upper Bound**
WB	75.9	13.5	45.3	106.4
WMB	67.0	9.9	44.7	89.3
VB	56.8	9.6	35.0	78.5

Note. WB = white bread, WMB = wheatmeal bread and VB = bread with 75g pumpkin powder substitution.

Table 6.6. Participants' serum area under curve, insulin after 120 minutes for test breads

Bread	**Mean pmol.min/L**	**Standard Error**	**95% Confidence Interval**	
			Lower Bound	**Upper Bound**
WB	32892	8058	14663	51121
WMB	34276	8594	14835	53718
VB	20476	4425	10466	30488

Note. WB = white bread, WMB = wheatmeal bread, and VB =bread with 75g pumpkin powder substitution.

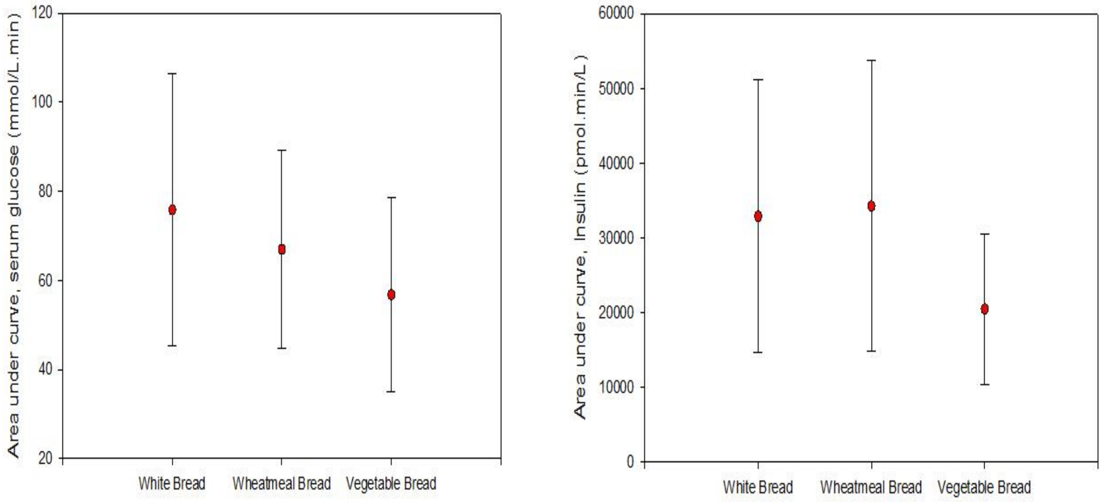


Figure 6.3. Glucose and insulin response following the consumption of test breads.

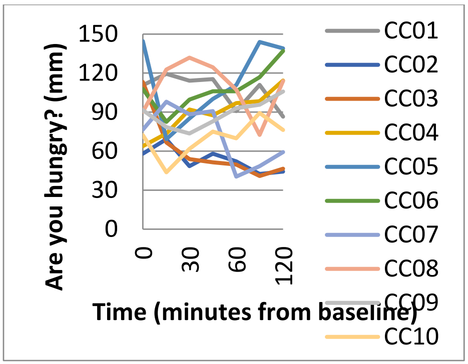
The rating of hunger was lowest for VB throughout the period, while participants reported only a small initial decrease in hunger up to 60 min for the WMB, from which point hunger increased (**Figure 6.4** and Error! Reference source not found.). WB had the least hunger suppressing effect. The mean difference of the change in baseline of the VB compared with the WB and WMB was WB 80.29 (95% CI -65.18, 225.75) and WMB 52.91 (95% CI -111.60, 217.41). There was, however, no significant difference between the hunger suppression effects of the VB, WMB and WB.

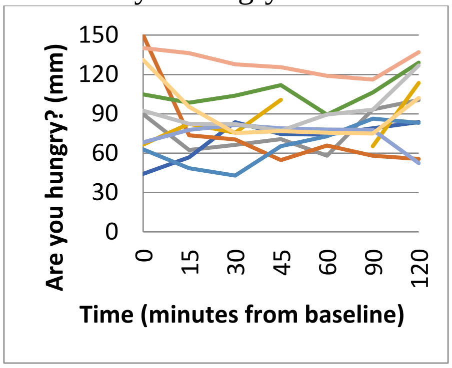
The VB had a higher appetite satisfaction compared to the WB and WMB (**Figure 6.4** and Error! Reference source not found.). A significant difference existed in the ability of the VB to provide satisfaction compared to the WB and WMB. The VB made the participants full and thus they were not wanting to eat more, particularly from the 15th to the 30th minute. The WB recorded the least ability to reduce participants' ability to eat. VB and WB showed a similar effect on participant perception of desiring to eat more.

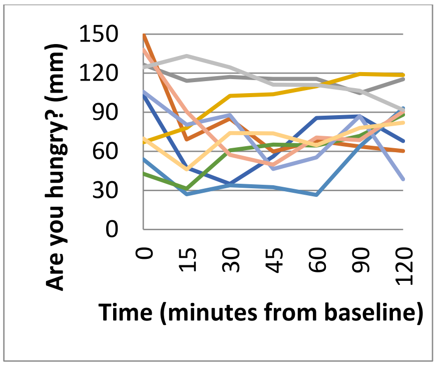
In terms of the participants ability to eat further, the vegetable-enriched bread impaired the participants' ability to eat further. The mean difference of the change in baseline of the vegetable-enriched Nothing Else™ bread compared with the WB and WMB was WB -27.49 (95% CI -94.42, 39.45) and WMB -79.64 (95% CI -213.22, 53.94).

WB **WMB** **PB**

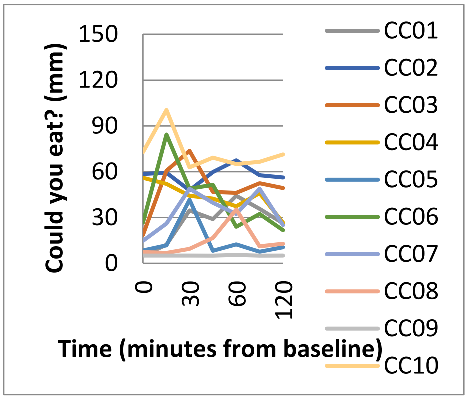
Are you hungry?

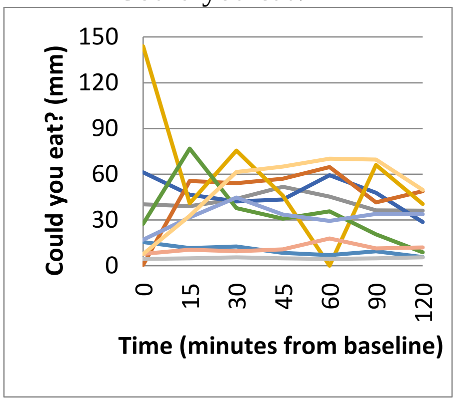


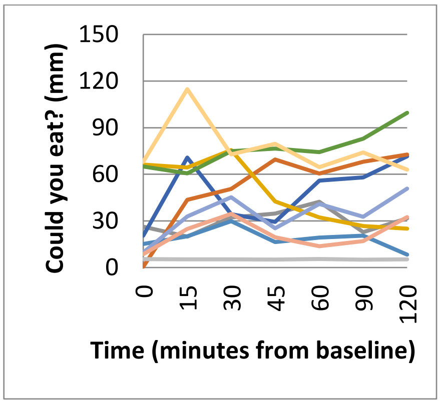




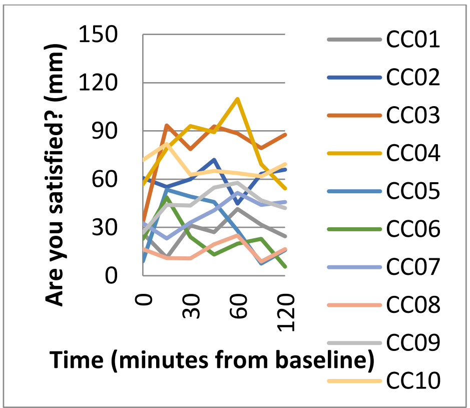
Could you eat?

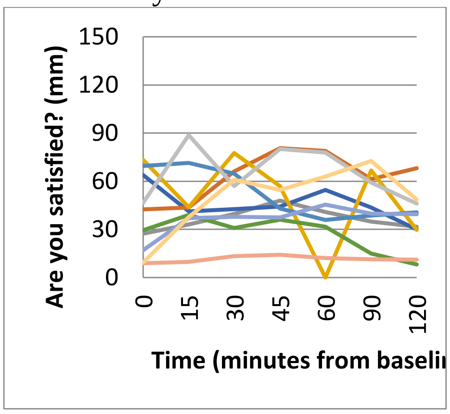


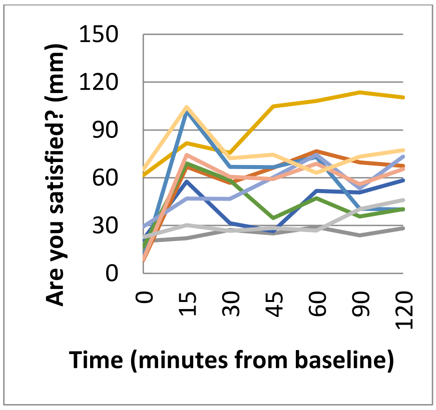




Are you satisfied?







Do you want to eat?

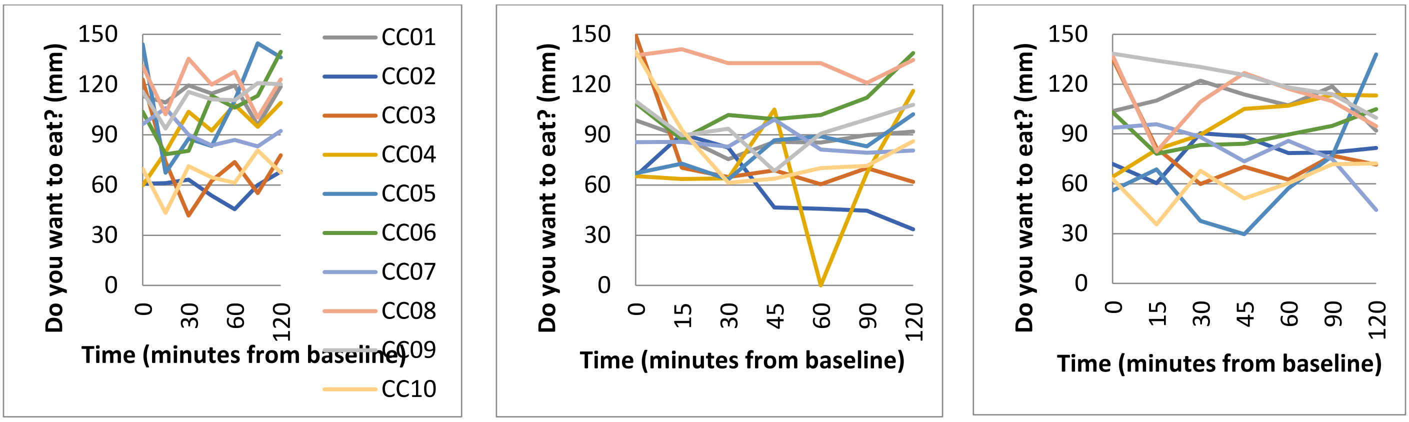


Figure 6.4. Satiety responses following the consumption of breads. WB = white bread, WMB = wheatmeal bread and VB = bread with 75g pumpkin powder substitution.

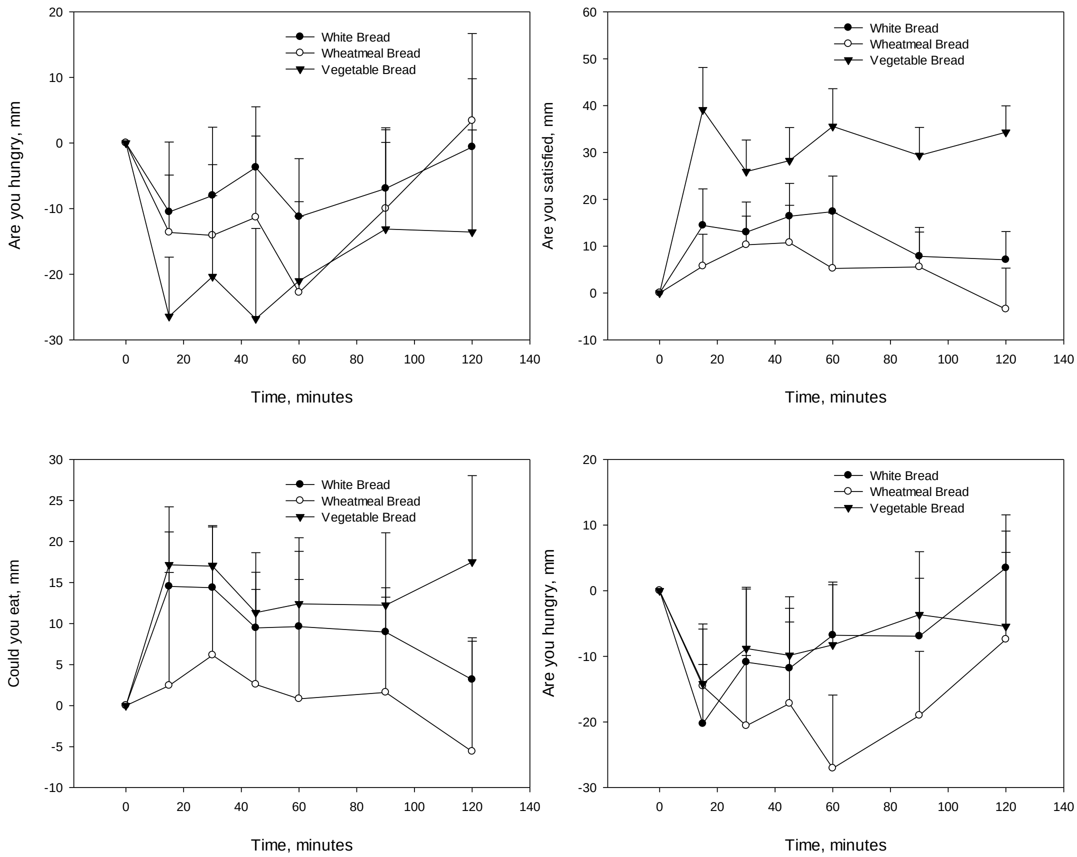


Figure 6.5. Changes in satiety responses (n=10) from baseline following the consumption of breads. Mean SEM error bars

Discussion

The discussion in this chapter will take a three-way approach. Firstly, the primary outcome, which is glucose and insulin response following the consumption of the WB, WMB and VB will be discussed. Secondly, the appetite suppression effect following the consumption of the WB, WMB and VB, as evaluated by the participants, will be addressed. Finally, the relationship between sensory attributes and appetite suppression following the consumption of WB, WMB and VB will be discussed.

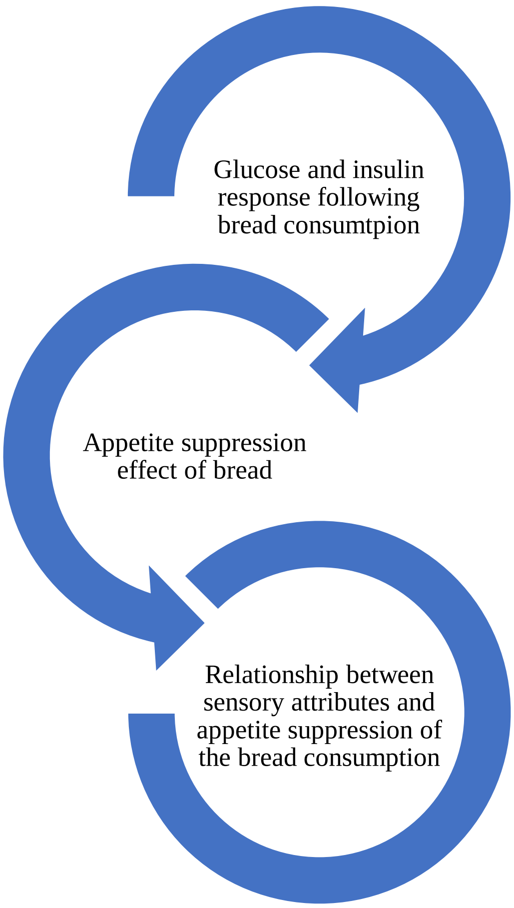


Figure 6.6. Summary of discussion on glycaemic response.

This study has shown that bread enriched with drum-dried pumpkin and sweet corn powders significantly attenuated insulin release by 38% but with no change in glucose release. Bread made with refined flour only, i.e., low fibre, has been reported to elicit higher insulin iAUC concentration compared with wholemeal and grain breads: 12,882 pmol*min/L, 11,203 pmol*min/L and 6,659 pmol*min/L respectively (Holt, Miller, & Petocz, 1997). Coe and Ryan (2016) found that baobab powder added to white bread was not associated with a reduction in glucose release and satiety over a 180-minute period but, similar to this investigation, insulin iAUC was attenuated by about 20%. The authors attributed this observation to the polyphenol compounds in baobab that may reduce the rate and degree of starch digestibility. In another study, the consumption of whole-kernel rye bread, β-glucan-enriched bread and wholemeal pasta could not attenuate glucose response over a 180-minute period compared to white wheat bread (Juntunen et al., 2002). However, postprandial insulin release was significantly attenuated by 41.71% and 21.50% for the whole-kernel rye and β-glucan-enriched breads respectively, compared to the white bread. The whole-kernel rye and β-glucan-enriched breads had a fibre content of 12.8 and 17.1% respectively. The authors posited that the structure and form of food products, which are impacted by the fibre composition, affect the insulin response following food consumption (Juntunen et al., 2002).

As postulated by others, the reduction in the insulin response to the vegetable-enriched bread following consumption could be attributed to three factors. The first and second factors are related to the higher fibre and viscous matrix of the VB, which reduce the rate of bread bolus digestion (Holt et al., 1997), and the structure of the ingredients (K. S. Juntunen et al., 2002) mediating the release of the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide (GLP) (Jones, Bloom, Buenaventura, Tomas, & Rutter, 2018) which in turn reduce the secretion of insulin. Although in this present study, measures of incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide (GLP) was not carried out, it could however be considered for future works. Pectin fibres in pumpkin (Fissore et al., 2009) and gums and mucilage from flaxseed (Kristensen et al., 2013;

Thakur et al., 2009) synergistically enhance the viscous fibre composition of the VB. The consumption of flaxseed gum enriched-chapatti and flaxseed mucilage-enriched meals resulted in significant attenuation of insulin release (Kristensen et al., 2013; Thakur et al., 2009).

What was more interesting about this present study was the higher insulin concentration (4%) released following the consumption of the WMB compared to the WB. Because the WMB contains higher fibre (4.6g/100g) than the WB (2.7g/100g), a greater potential to attenuate insulin more effectively than the WB is expected. Ironically, the insulin release following the consumption of the WB was lower than that of the WMB. The plausible rationale could be due to more emulsifiers used in WMB enrichment. Generally, enriching bread with bran or wholemeal flour in WMB dilutes the gluten and tends to impart poor textural properties to the crumb (Martins, Pinho, & Ferreira, 2017). This compels bakers to add emulsifiers to the bread. The higher insulin release following the consumption of the WMB could be attributed to the presence of emulsifiers 481 (sodium oleyl lactylate, sodium stearoyl lactylate and sodium lactylate) and 472e (diacetyltartaric and fatty acid esters of glycerol) that potentially have an insulin-stimulating effect on the β-cells of the pancreas. The lack of emulsifiers in the VB may have contributed to the significantly lower insulin release following consumption compared to the WMB and WB. However, this needs to be investigated further in future studies.

The last factor that has been implicated in the modulation of glycaemic control includes the presence of polyphenols inhibiting carbohydrate-digesting enzymes that reduce the rate of digestion (Umeno, Horie, Murotomi, Nakajima, & Yoshida, 2016).

L. Chambers et al. (2015) posited that there is an association between the perception of the sensory attributes of food and appetite suppression. The result of the present study is consistent with this hypothesis. The participants found the taste and texture of the VB appealing. This possibly resulted in the stimulation of satiety centres of the hypothalamus in the brain (L. Chambers et al., 2015), consequently leading to a sensation of appetite satisfaction. Another plausible explanation for the postprandial appetite satisfaction sensation elicited by the VB could be its high fibre content due to the pumpkin (Fissore et al., 2009), sweet corn and flaxseed enrichment (Kristensen et al., 2013; Thakur et al., 2009). Fibre impacts on the matrix and texture of the vegetable-enriched bread (Martins, Pinho, Ferreira, Jekle, & Becker, 2017) and increases chewability, consequently inducing appetite suppression sensations(L. Chambers et al., 2015). Additionally, due to the high fibre composition of VB, its consumption reduces gastric emptying time and the distension of the gastrointestinal tracts (Williams et al., 2019). This eventually results in the secretion of gut hormones including PYY, cholecystokinin (CCK), GLP-1, pancreatic polypeptide (PP) (Perry & Wang, 2012; Steinert et al., 2017). The released hormones pass through the blood-brain barrier, bind to receptors in the satiety centres of the hypothalamus in the brain and trigger a satiety signalling cascade that makes the participants feel full. Associations between the release of appetite-suppressing hormones and insulin release regulation and glycaemic control has also been reported. Ibrügger et al. (2012) investigated how the consumption of flaxseed dietary fibre supplements impact on appetite and food intake suppression acutely. The study was in two-fold and employed a single-blinded randomised crossover design where 24 and 20 participants respectively took a 300ml drink (control) and a 300ml with added flax fibre extract (2.5g of soluble fibres) (first study) and control drink with flax fibre tablets (2.5g of soluble fibres) (second study). The authors reported that the consumption of the flaxseed drink resulted in an increased fullness sensation compared to the control, consequently leading to a significant decrease in subsequent energy intake.

Glycaemic index and glycaemic load of foods and the diet profile the blood-glucose raising potential of carbohydrate-based diets. This has important application for both the prevention and management of type 2 diabetes mellitus. It is important to differentiate between association and causality. The present study found an association of an attenuated insulin response with consumption of the VB. Longitudinal studies provide evidence for prevention of T2DM. A recent systematic review and meta-analysis assessed the relationship between dietary glycemic index/glycaemic load and type 2 diabetes for causality. They reported that, dietary GI and GL are implicated as causal factors that impact on T2D incidence as evidence was provided that all nine of the Hill's criteria were met (1) Strength of Association, (2) Consistency, (3) Specificity, (4) Temporality, (5) Biological Gradient, (6) Plausibility, (7) Experimental evidence, (8) Analogy, and (9) Coherence). In addition, neither dietary fibre nor cereal fibre nor wholegrain were found to be a reliable or effective surrogate measures of GI or GL which means that other factors such as polyphenols may be a factor.

Strengths and limitations

The principles of the protocol for glycaemic index testing (The International Organization for Standardization, 2010) and calculation of the incremental areas under the curve were followed, with the number of participants (10) meeting the requirements for sample size. The food portion size was not based on a standard amount of carbohydrate (50g) but on a realistic serving size of each bread (75g). Measurement error was minimised with analysis of glucose and insulin on all stored samples undertaken on one day in one run with an accredited medical laboratory system.

Conclusion

This investigation is a proof-of-principle comparison of glucose and insulin responses to two popular commercial breads, $1 white and wheatmeal breads, and a novel vegetable-enriched bread, which had previously been shown to be palatable, easy to swallow, nutrient-dense and have a high Health Star Rating. Consumption of the vegetable-enriched bread was associated with a reduction in insulin released postprandially and increased satiety. Future studies could better define the participants' glycaemic status and could compare the response of those previously diagnosed with and without type 2 diabetes mellitus.

Chapter 7. The two-week effect of consumption of vegetable-enriched bread on plasma carotenoid concentration and lipid profile: A pilot study

Abstract

Bread is commonly eaten in New Zealand. Consequently, it is a promising vehicle for the delivery of bioactive compounds, including carotenoids and fibre, which have health-promoting functions in the body. The primary aim of the study was to investigate the effect of two weeks of consumption of a vegetable-enriched bread on plasma carotenoid and lipid concentrations. Secondarily, the association of carotenoid and lipid concentrations with the consumption of carotenoid-rich foods in a six-item carotenoid food frequency questionnaire was investigated. A third outcome was to validate carotenoid reflection scores of the non-invasive, point-of-care Veggie meter™ with laboratory-assessed, plasma carotenoid concentrations. The study design was pre-post experimental, where participants (n=10) consumed 200g of the vegetable-enriched bread (VB) for two weeks. Blood was sampled one week prior (week -1) immediately before (week 0) and after two weeks of bread consumption (week 2). Dietary intake of carotenoid-rich foods was assessed using a six-item carotenoid food frequency questionnaire. Fingertip carotenoid reflection scores were measured using the Veggie meter™. Consumption of the VB resulted in no marked changes in the plasma carotenoids and lipids. Frequency of consumption of carotenoid-rich foods was positively correlated with the plasma carotenoid and carotenoid reflection scores. A significant positive correlation (r=0.741, 95% CI 0.209, 0.935) was observed between the consumption of fruit and vegetables with HDL cholesterol (week 2). There was a strong positive correlation between the plasma carotenoid concentrations and the carotenoid reflection scores (r=0.845, 95% CI 0.697, 0.924). The participants' reported compliance with the intervention was 100%. In conclusion, plasma carotenoid and lipid profile concentrations were unchanged with the consumption of 200g VB each day for two weeks. Carotenoid-rich food intake was positively associated with biomarkers of carotenoids, and validation of the carotenoid reflection scores with plasma carotenoid concentrations suggests that the Veggie meter™ has the potential to provide portable (or in-the-field) measurement of carotenoid levels.

Keywords: Bread, carotenoid concentration, carotenoid reflection scores, lipids, Veggie meter™

Introduction

Globally, bread is a staple food (Kourkouta et al., 2017). Enrichment of bread with vegetables can increase the nutritional value including essential nutrients such as carotenoid and fibre content (Ranawana, Campbell, et al., 2016). The addition of vegetables (Shashirekha et al., 2015) and seeds (Veiga, Costa, Silva, & Pintado, 2018) to recipes for frequently consumed foods adds variety, improves the quantity of bioactive nutrients and has the potential to benefit health.

It has previously been shown in this thesis that a bread enriched with pumpkin, sweet corn and flaxseed was not only liked for both taste and texture, but that older adults reported that this bread was easier to swallow than commercial white and wheatmeal breads (**Chapter 4**). In addition (**Chapter 6**) this vegetable-enriched bread attenuated insulin response and induced a greater fullness sensation compared to commercial white and wheatmeal breads. Pumpkins and sweet corn are good sources of the carotenoid β-carotene and lutein (Elvira-Torales, Garcia-Alonso, & Periago-Caston, 2019) and dietary fibres. However, it is not known how much of a bread containing pumpkin, sweet corn, flaxseed and wholemeal flour would need to be consumed and for how long for biomarkers of nutritional status to change.

Several studies have reported on the benefits associated with the consumption of fibre-rich foods. Fibre increases satiety, prevents reabsorption of bile salts consequently promoting the lowering of serum cholesterol, and are fermented in the colon to produce short-chain fatty acids which promote the lowering of colon cancer incidence (Blackwood et al., 2000). Pumpkin contains a significant amount of pectin, a water-soluble fibre which is a major component of plant cell walls (Fissore et al., 2009). Pumpkin powder (2g/kg/bodyweight of rat/day) fed to alloxan-induced diabetic rats over four weeks significantly decreased triglycerides, LDL and total cholesterol (Asgary et al., 2011). Therefore, the enrichment of bread with pumpkin powder may provide an avenue for improving circulating lipids.

Carotenoids act as antioxidants by impairing free radicals and some are precursors of vitamin A (Elvira-Torales et al., 2019; Moran et al., 2018). They are present in vegetables and fruit but also available in animal products including dairy, eggs and liver. Carotenoids are thus biomarkers of the dietary intake of fruits and vegetables (including pumpkin, sweet corn and dark green leafy vegetables) and animal products (including eggs, liver and milk) (Ashton, Pezdirc, Hutchesson, Rollo, & Collins, 2017; Pezdirc et al., 2016). Carotenoids are carried by the blood to sites including skin tissues for storage (Ashton et al., 2017), and reduce free radical production from the action of UV rays from the sun on the skin (Fiedor & Burda, 2014). The "gold standard" for the measurement of carotenoid status involves the measurement of carotenoid concentration in plasma or serum. However, this can be costly and time consuming, and limits participation in research studies because a venepuncture is required. A "Veggie meter"™ (Longevity Link Corporation, Utah) which measures skin carotenoid score by reflection spectroscopy of the fat pad of the index finger has been shown in 54 volunteers to be a valid (r=0.81) and reliable (SD 3.4 – 4.1%) measure of serum carotenoid concentration (Ermakov et al., 2018). The Veggie meter™ is non-invasive, portable, requires no consumable equipment and takes around three minutes per measurement. Validation of the Veggie meter™ would thus allow investigations of the effects of dietary intake of carotenoid-rich foods including vegetable and fruit intake on carotenoid reflection scores in larger studies of dietary interventions.

In this feasibility and pilot study, the primary research aims were:

1. To measure changes in carotenoid and lipid profile concentrations if vegetable-enriched bread was consumed every day over two weeks.

2. To investigate the association of carotenoid concentrations with the consumption of carotenoid-rich foods in a six-item carotenoid food frequency questionnaire.

3. The secondary aim was to assess the utility of the non-invasive, point of care Veggie Meter™.

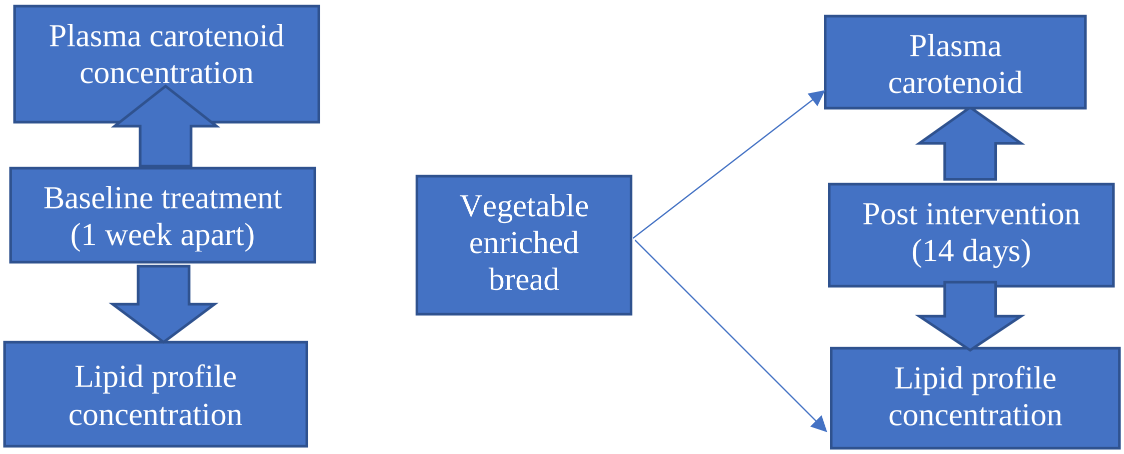


Figure 7.1. Schematic presentation of plasma carotenoid and lipid profile study.

Method

Design

This feasibility and pilot test-retest study was in two parts, a feasibility study to assess if participants would eat the bread every day (compliance), and a pilot study in which it was considered that 10 participants were sufficient, as this would allow estimation of average values and variability and also allow a determination of how many participants would be required for a larger study.

The experimental study was approved by the Auckland University of Technology Ethics Committee (AUTEC, approval number 18/146). Additionally, the trial was registered with the Australian New Zealand Clinical Trial registry, reference ID: ACTRN12618002040246.

Bread analysis

The vegetable-enriched bread VB75 (**Chapter 4**) (VB) was used for this study. The use of the VB75 for the carotenoid and lipid profile study was because there was no significant difference in the overall liking of the VB75 and VB100 (**Chapter 4**). Also, the consideration of cost as a driver for the purchase of breads drove the choice for the VB75 over VB100 as the amount of pumpkin powder used in VB100 was higher than the VB75. The use of more pumpkin flours incurs extra cost in the production of the bread. Consequently, the VB100 is expected to be higher in cost than the VB75.

This bread contained 237μg/100g β-carotene, 7.2g/100g total dietary fibre of which soluble fibre constituted 1.7g/100g and insoluble fibre 5.5g/100g (**Table 6.2**).

Dose

A 200g sample of the bread (daily dose) contained 474μg/100g of β-carotene (79μg Retinol Equivalents) which is 10% of the recommended dietary intake (RDI) for men (900μg) and 16% of the RDI for women (700μg) (National Health and Medical Research Council (Australia), 2006). The 200g sample contained 14.4g of fibre which is almost 50% of the adequate intake (AI) for men (30g/day) and 58% of the AI for women (25g/day) (National Health and Medical Research Council (Australia), 2006).

Ten apparently healthy participants were recruited. A written and verbal explanation of the study was presented to participants and they all signed a consent form. Inclusion criteria included participants who were 18 years or older, regular bread eaters (at least three times per week), and willing to eat four slices (200g) of the vegetable-enriched bread every day for two weeks. Exclusion criteria involved having any liver disease, being allergic to gluten or any of the ingredients in the bread, or taking supplements containing vitamin A. Participants made three visits (2× baseline one week apart, (weeks -1 and 0) and following the intervention (week 2) to the Nutrition and

Metabolism Laboratory at AUT South campus for blood sampling, carotenoid reflection measures using the point-of-care Veggie meter™ and to complete a six-item carotenoid food frequency questionnaire (**Appendix 10**). This questionnaire included the general fruit and vegetable frequency questions as used in the New Zealand National Health Survey (Ministry of Health, 2011), as well as foods selected for their high carotenoid content as reported in the New Zealand Food Composition Tables (Plant & Food Research & Ministry of Health, 2016). The consumption of carotenoid-rich foods including liver, egg, fruits, vegetables, pumpkin and carrots, green leafy vegetables including kale and spinach, milk, cheese and yoghurt was evaluated at weeks -1, 0 and 2. Weekly fruit and vegetable intake was computed by summing the weekly intake of each of the fruits and vegetables. Average baseline fruit and vegetable intake was derived by dividing the sum of fruit and vegetable intake at weeks -1 and 0 by 2. This was repeated for the eggs, carrot/pumpkin, green leafy vegetables, milk/cheese/yoghurt to derive their averages.

At the second baseline visit, participants were provided with 200g portions (4 slices/day) of the vegetable-enriched bread to eat each day for two weeks. After baking and cooling 200g portions of bread were sealed in foil with air excluded then frozen to maintain freshness and defrosted prior to consumption. A preliminary trial conducted showed that defrosting bread for an hour was enough to bring it down to room temperature and to have acceptable freshness. Participants who did not have enough space in their freezer for storage of all the bread were offered the option to collect the second week of the bread after day 7. Participants completed a compliance diary of the date and time of consumption of each portion of bread over the two-week period. Each participant was contacted by phone at least twice during the intervention period to offer support and encourage compliance with the daily bread consumption.

At weeks -1, 0 and 2, for the measurement of plasma carotenoid concentration and serum lipids profile, 5mL samples of non-fasted venous blood were collected by a phlebotomist into a 10ml BD Vacutainer® plain glass tube (Becton Dickinson, Auckland, NZ) (for serum collection) and an EDTA glass tube (for plasma collection). The glass tubes for the plasma samples were covered with aluminium foil to prevent the degradation of the carotenoids by light. Serum and plasma were then isolated from the whole blood by centrifugation at 1500 revs/min at 4°C for 10 min and stored at -80°C in Eppendorf tubes prior to analysis. Plasma samples were couriered at -80℃ to IANZ accredited Canterbury Health Laboratories (CHL), Christchurch, New Zealand, for analysis. For the analysis, plasma proteins were precipitated by addition of ethyl alcohol and carotenoids were extracted into petroleum spirit. Carotenoids were measured colorimetrically using a spectrophotometer (Cary 4000, Agilent, Santa Clara, Ca) at the wavelength 440nm. The method was reported as linear up to 20µmol/L. The lowest measurable concentration of carotenoids by this method was 0.1µmol/L. The reference range for normal used by the laboratory was 1.5-3.0µmol/L, with the current coefficient of variation for this assay 4.4% (Sies, 2019).

Serum total cholesterol, LDL, HDL and triglycerides concentrations were determined by specific diagnostic assays (**Table 7.1**) on a Roche Diagnostics automated clinical chemistry analyser (Cobas Modular P800/E170, Roche Diagnostics New Zealand Ltd, Auckland, NZ) at the AUT Roche Diagnostic Laboratory, Auckland, New Zealand. For all assays, the limit of measurement or functional sensitivity represented the lowest measurable analyte level that can be distinguished from zero. It was calculated as the value lying two or three standard deviations above that of the lowest standard. Method comparisons, limitations and specific performance data for this instrument can be found at www.e-labdoc.roche.com.

Table 7.1. Roche Diagnostics automated clinical chemistry analyser

Analyte	Lower limit of measurement (mmol/L)	Measuring range (mmol/L)	Test principle
Total cholesterol	0.100	0.1-20.7	Enzymatic colorimetric test
Triglycerides	0.078	0.078–14.2	Homogeneous enzymatic colorimetric assay
Low density lipoprotein cholesterol	0.080	0.08–3.10	Homogeneous enzymatic colorimetric assay
High density lipoprotein cholesterol	0.050	0.05-11.3	Enzymatic colorimetric assay

Note. Analysis conducted with Cobas Modular P800/E170, Roche Diagnostics New Zealand Ltd, Auckland, New Zealand.

A portable Veggie meter™ (Longevity Link Corporation, Utah, USA) was calibrated using black and white references according to the manufacturer's instructions. Participants were asked to wash their hands using soap, rinse well, and then dry with a paper towel. The participant's right index finger was placed on the lens of the light, scanned three times and the average of the carotenoid scores recorded. Acceptable precision between the three scans was <10% and the range of possible scores on the meter was 0 to 800.

Statistics

Data was visualised through scatter plots and line charts. Data was checked for normal distribution using the Shapiro-Wilk test (where $p>0.05$ implied normality for the data). Generally, all the data followed a normal distribution, except for triglycerides level, intake of liver, intake of egg, and skin carotenoid score (week -1), baseline daily vegetable intake (week 0), carrot/pumpkin intake (week 2), green leafy vegetables intake, and average baseline Veggie meter™ carotenoid score. Descriptive statistics were used to report the frequency of daily intake of fruit and vegetables, carrot/pumpkin, eggs, green leafy vegetables and dairy products. Intra-individual coefficient of variations between weeks -1 and 0 for outcome measures was determined from the residuals in the one-way ANOVA. Baseline measures were compared (paired T test). Correlations between skin carotenoid reflection scores, plasma carotenoid concentration and frequency of intake of carotenoid-rich foods were explored using Pearson r. The association between fruit and vegetable intake and the lipid profile (total cholesterol, LDL, HDL, triglycerides) was also assessed with Pearson r. Spearman correlation were used in instances where associations were established between a normally distributed variable and a non-normally distributed variable. Kendall's tau-b correlation was used for two non-normally distributed variables. Interpretation of the magnitude of effect size of the change in the post-treatment measure from the baseline measures was carried out using Cohen's effect size system with 0.2 categorised as small, 0.5 being moderate and 0.8 large (Field, 2009). Unless otherwise stated, all data were analysed using International Business Machines Corporation® SPSS® Statistics Version 25.

Results

Baseline

The participants were predominantly male (n=10, male = 8, female = 2), were relatively young (mean age 30 y, median, 25th, 75th; 30, 19, 39) and weight ranged between 61 and 125 kg (mean±SD; 90±20 kg).

Baseline mean plasma carotenoid concentration at week -1 was 1.8±0.9, 1.8±0.8 at week 0, with the mean difference 0.02 (95% CI -0.2, 0.2) and intra-individual coefficient of variation 9%. The mean Veggie meter™ carotenoid reflection score was 334±130 at week -1, 335±126 at week 0, the mean difference -1 (95% CI-18, 17) and the intra-individual coefficient of variation was 5%. Pearson r correlation between the baseline plasma carotenoid concentrations was 0.961, and the baseline mean carotenoid reflection scores was 0.981. Triglycerides varied widely, by 62%, between measurement points (**Figure 7.2**). The intra-individual variability between the baseline measurement points for the cholesterol, LDL and HDL were 5%, 8% and 21% respectively.

At weeks -1 and 0, the number of servings of vegetables and fruit each day was four with range (2.8, 5.3) and (1.75, 5.0) respectively (**Table 7.2**). Green leafy vegetable consumption was low, with an average median of one serving for week -1 and 0. At week -1 and 0, the median daily intake of total carotenoid from carotenoid-rich foods was 5.7 (3.8, 6.7) and 6.4 (3.0, 6.8). The average median daily intake of carotenoid foods at baseline was 6.1 (3.6, 6.6) servings (**Table 7.2**).

Overall, the baseline measures of carotenoid concentration and of carotenoid food intake were not different, and therefore the two baseline measures were averaged to compare with the post-intervention measure.

Table 7.2. Median frequency of consumption of foods containing carotenoids before intervention

	Frequency of consumption		
Food/time	**Week -1**	**Week 0**	**Average of week -1 and 0**
Fruit/day	1.5 (1.0, 2.3)	2.0 (0.75, 2.3)	1.5 (1.0, 2.3) [0,4]
Vegetables/day	2.0 (1.8, 2.3)	2.0 (1.0, 3.0)	2.0 (1.4, 2.6) [0.5, 3.5]
Fruit and vegetables/day	4.0 (2.8, 5.3)	4.0 (1.75, 5.0)	4.0 (2.4, 5.1) [0.5, 6.0]
Carrot or pumpkin/week	1.5 (0.4, 3.3)	0.63 (0.0, 3.0)*	1.0 (0.3, 3.1) [0, 4]
Eggs/week	3.0 (2.5, 4.9)	3.0 (2.5, 4.9)	3.0 (2.5, 4.9) [0.25, 14.0]
Green leafy vegetables/week	0.5 (0.0, 3.5)	1.5 (0.0, 4.3)	1.0 (0.0, 3.5) [0, 5]
Dairy/week	3.0 (2.0, 5.0)	3.3 (1.0, 4.3)	3.0 (2.0, 5.0) [2, 6.0]
*Total carotenoid foods/day	5.7 (3.8, 6.7)	6.4 (3.0, 6.8)	6.1 (3.6, 6.6) [1.1, 8.3]

Note. Data given as median (25^{th}, 75^{th} percentile) [Range], * = significantly different by Willcoxon sign test.

Intervention

The majority of participants reported consuming the bread in the evening, with times ranging from 6pm to 11pm. Overall, compliance to the intervention was 100%, as each of the participants reported consuming 200g of the vegetable-enriched bread each day over the two-week period.

There were no meaningful changes between the baseline and post-intervention measures of carotenoid reflection scores, plasma carotenoid concentration (**Table 7.3)** and lipid profiles (**Table 7.4)**. Two out of 10 participants (20%, #3 and #10) had a small increase in plasma β-carotene concentration. One participant recorded a decrease in skin carotenoid score (10%, #4). Three participants (30%, # 1, #7 and #9) recorded a decrease in total cholesterol, four participants (40%, # 1, #2, and #10) recorded lower triglyceride scores, and two participants (20%, #3 and #10) recorded higher HDL concentration from baseline to post-treatment **(Figure 7.2)**.

Table 7.3. Baseline and post-intervention plasma carotenoid concentration and skin carotenoid reflection score of participants (n=10)

Measure	Mean (n=10)	Standard Deviation	Change	95% CI	p-value
Baseline plasma carotenoid, µmol/L	1.81	0.84	-0.06	(-0.204, 0.084)	0.370
Post-treatment plasma carotenoid, µmol/L	1.87	0.85			
Baseline skin carotenoid score	334.10	127.65	16.37	(-4.984, 37.724)	0.117
Post-treatment skin carotenoid score	318.20	129.01			

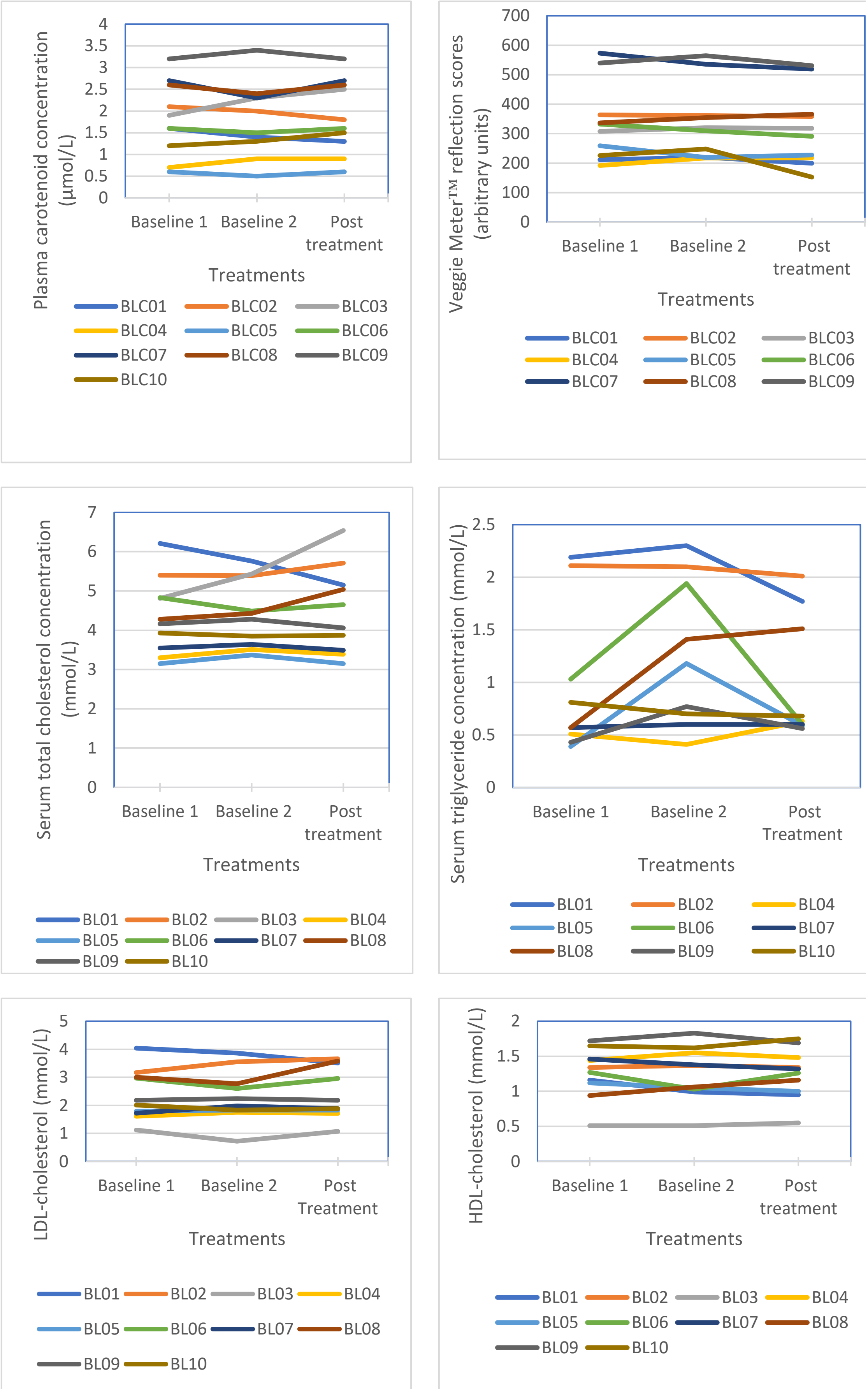


Figure 7.2. Changes in plasma carotenoid measures, skin carotenoid reflection score and lipid concentrations at baseline and post treatment. HDL = high density lipoprotein, LDL = low density lipoprotein.

Post-treatment measures of the total cholesterol, triglycerides and LDL cholesterol were slightly higher (2.6%, 2.9% and 3.7% respectively) **(Table 7.4)** than baseline but this was not statistically significant. There was a significant positive association observed for the baseline and post-treatment measures for total cholesterol, triglycerides, LDL and HDL cholesterol.

Table 7.4. Changes between averaged baseline and post-intervention lipids (n=10)

Parameter	**Mean/ mmol/L** (n=10)	**Standard Deviation**	**Change**	**95% CI**	**p-value**
Chol_Base	4.39	0.90	-0.12	(-0.54, 0.31)	0.552
Chol_Post	4.51	1.11			
TG_Base	1.97	2.77	-0.06	(-0.54, 0.42)	0.789
TG_Post	2.03	3.31			
LDL_Base	2.34	0.90	0.09	(-0.29, 0.11)	0.354
LDL_Post	2.43	0.92			
HDL_Base	1.25	0.37	0.00	(-0.07, 0.07)	1.00
HDL_Post	1.25	0.36			

Note. Chol = Cholesterol, TG = Triglyceride, LDL = Low Density Lipoprotein Cholesterol, HDL = High Density Lipoprotein Cholesterol, Base = Baseline, Post = Post intervention.

Validation of Veggie meter™ skin carotenoid reflection scores

Thirty measures of plasma carotenoid and Veggie meter™ carotenoid reflection score were available from the three measurement points (weeks -1, 0, 2). There was a strong agreement between the plasma carotenoid concentrations and the skin carotenoid reflection scores (**Figure 7.3 and Table 7.5**). All three time point measures of association demonstrated that 72% (r^2 overall 95% CI 49, 85%, SE 68) of the variation in the relationship between the two methods was accounted for. The regression equation to determine plasma carotenoid concentration from carotenoid reflection score was:

Plasma carotenoid concentration (µmol/L) = 0.0056×carotenoid reflection score - 0.0071.

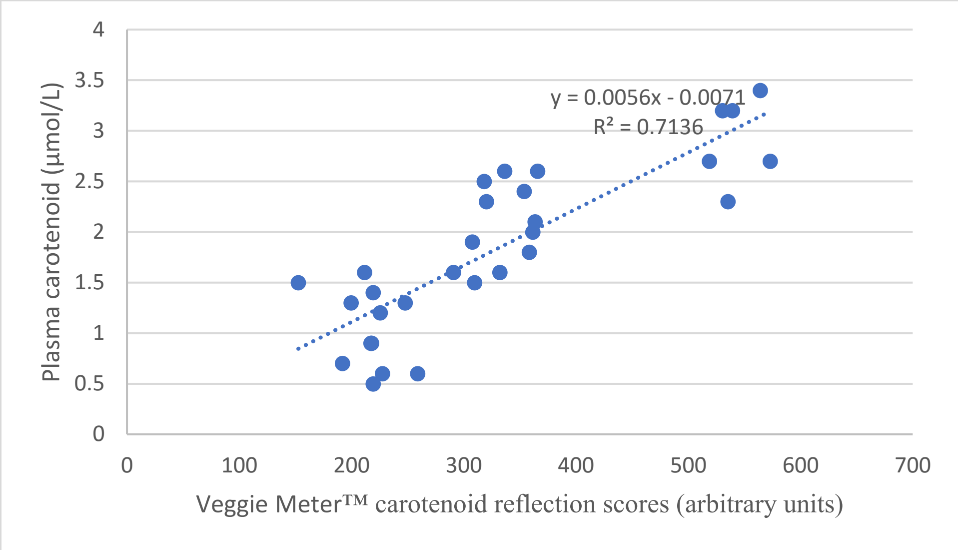


Figure 7.3. Association between non-invasive skin carotenoid scores and invasive plasma carotenoid measure.

Table 7.5. Associations between Veggie meter™ carotenoid reflection scores and plasma carotenoid measures

Time points	**Pearson r (95% CI)**			
	Week -1 (n=10)	Week 0 (n=10)	Week 2 (n=10)	Combined (n=30)
Week -1 (n=10)	0.842* (0.452, 0.962)			
Week 0 (n=10)		0.864* (0.514, 0.967)		
Week 2 (n=10)			0.842* (0.452, 0.962)	
Combined (n=30)				0.845* (0.697, 0.924)

Note. * = Significant association between the Veggie meter™ skin carotenoid reflection scores and the plasma carotenoid measures, CI = Confidence Interval. Combined data from all the participants visits (two baseline and one post intervention).

The weight of the participants was negatively correlated with the plasma carotenoid concentration and carotenoid reflection scores (**Table 7.6**). The correlation was significant at Weeks -1 and 2 for the plasma carotenoid concentration, and at Week 0 for the carotenoid reflection score.

Table 7.6. Associations between participants' weight and plasma carotenoid measures and Veggie meter™ carotenoid reflection scores

	Pearson r (95% CI)					
	Plasma carotenoid week -1	Plasma carotenoid week 0	Plasma carotenoid week 2	Veggie meter™ carotenoid reflection score week-1	Veggie meter™ carotenoid reflection score week 0	Veggie meter™ carotenoid reflection score week 2
Weight	**-0.647**	-0.620	**-0.681**	-0.512	**-0.754**ϯ	-0.565

Note. CI = confidence interval, bold text indicates correlation is significant at 95% CI, ϯ = Spearman correlation.

The total carotenoid concentration calculated from the frequency of consumption of each of the carotenoid-rich foods was totalled, and the mean concentration was positively correlated with the plasma carotenoid concentration and carotenoid reflection scores (**Table 7.7**). The strength of the correlation for the carotenoid reflection scores was generally higher than the plasma carotenoid concentrations and was significant at week -1 and 0.

Table 7.7. Associations between total carotenoids from carotenoid-rich foods and plasma carotenoid concentration and carotenoid reflection score

	Pearson r (95% CI)					
	Plasma carotenoid week -1	Plasma carotenoid week 0	Plasma carotenoid week 1	VM carotenoid reflection score week -1	VM carotenoid reflection score week 0	VM carotenoid reflection score week 2
Grand Beta Carotene	0.533	0.455	0.513	**0.690**	**0.687**ϯ	0.612

Note. Bold text indicates correlation is significant at 95% CI, ϯ = Spearman correlation.

Overall, daily frequency of intake of individual plant-based carotenoid-containing foods (fruits, vegetables, carrot/pumpkin, green leafy vegetables) positively correlated with both the plasma carotenoid and carotenoid reflection scores at baseline (**Table 7.8**). When the participants' fruit and vegetable intake from the two baseline questionnaires were combined and averaged, the strength of correlation increased, particularly for the carotenoid skin reflection measures. The correlation coefficients for the plasma carotenoid concentrations were ~0.5, and for the skin carotenoid reflection scores 0.6 (**Table 7.8**). Weekly consumption of animal-based carotenoid-containing foods (milk, cheese, yoghurt and liver) had a positive correlation with the plasma carotenoid measures and skin carotenoid reflection scores (**Table 7.10**). Egg consumption had a positive correlation with the Veggie meter™ carotenoid reflection scores (**Table 7.10**). However, the baseline consumption of eggs at weeks -1 and 0 had a negative correlation with plasma carotenoid score but not at week 2. There were no significant correlations between the consumption of the animal-based carotenoid-rich foods and the plasma carotenoid concentration and carotenoid reflection scores.

Table 7.8. Association of carotenoid-rich food intake (fruits and vegetables) with biomarker measures of carotenoid status (n=10)

	Fruit week -1	**Fruit week 0**	**Fruit Av**	**Fruit week 2**	**Veg week -1**	**Veg week 0**	**Veg Av**	**Veg week 2**	**Fruit & veg week -1**	**Fruit & veg week 0**	**Fruit & veg Av**	**Fruit & veg week 2**
<u>Plasma carotenoid measure</u>												
Week -1	0.320				0.308				0.396			
Week 0		0.304				0.508				0.462		
Baseline Av			0.326				0.446				0.457	
Week 2				0.514				0.255				0.419
<u>Skin carotenoid reflection scores</u>												
Week -1	0.506				0.402				0.580			
Week 0		0.319†				**0.547†**				0.632		
Baseline Av			0.377†				0.489†				**0.598†**	
Week 2			0.463⸷	**0.676**			**0.689**⸷	0.459			**0.755**⸷	0.624

Note. Veg = vegetables, Av = average, bold text indicates correlation is statistically significant at 95% CI, ⸷ = Spearman correlation, † = Kendall correlation. Fruit, vegetable, fruit and vegetable consumption were reported in daily intakes.

Table 7.9. Association of other plant-based carotenoid-rich food intake with biomarker measures of carotenoid status (n=10)

	Carrot, pumpkin week -1	Carrot, pumpkin week 0	Carrot, pumpkin Av	Carrot, pumpkin week 2	Green leafy vegetables week -1	Green leafy vegetables 0	Green leafy vegetables Av	Green leafy vegetables week 2
<u>Plasma carotenoid measures</u>								
Week -1	0.051				0.407			
Week 0		0.464⸷				0.450⸷		
Baseline Av			0.246				0.423⸷	
Week 2				0.112⸷				0.318
<u>Veggie meter™ Skin carotenoid scores</u>								
Week -1	0.138				0.331⸷			
Week 0		0.368†				0.435†		
Baseline Av			0.232⸷				0.368†	
Week 2				0.006⸷				0.091⸷

Note. Av = Average, ⸷ = Spearman correlation, † = Kendall correlation. Carrot/pumpkin and green leafy vegetable intakes are reported in weekly intakes.

Table 7.10. Association of animal-based carotenoid-rich food intake with biomarker measures of carotenoid status (n=10)

	Milk, cheese and yoghurt week -1	**Milk, cheese and yoghurt week 0**	**Milk, cheese and yoghurt Av**	**Milk, cheese and yoghurt week 2**	**Egg week -1**	**Egg week 0**	**Egg Av**	**Egg week 2**	**Liver week -1**	**Liver week 0**	**Liver Av**	**Liver week 2**
<u>Plasma carotenoid measure</u>												
Week -1	0.372				-0.090ǂ				0.141ǂ			
Week 0		0.161				-0.099ǂ				0.268ǂ		
Baseline Av			0.307				-0.037ǂ				0.241ǂ	
Week 2				0.322				0.037ǂ				0.295ǂ
<u>Veggie meter™ Skin carotenoid scores</u>												
Week -1	0.017				0.166ǂ				0.144ǂ			
Week 0		0.252ǂ				0.070†				0.194†		
Baseline Av			0.132ǂ				0.163†				0.138†	
Week 2				0.401				0.061ǂ				0.245ǂ

Note. Av = Average, ǂ = Spearman correlation † = Kendall correlation. Milk/cheese/yoghurt, egg and liver intakes are reported in weekly intakes

Fruit and vegetable intake generally had a negative correlation with total cholesterol and triglycerides (**Table 7.11**). The correlations were not significant for the total cholesterol and LDL cholesterol. Fruit intake had a significant correlation with triglycerides at week 2. The combined fruit and vegetable intake at week 2 had a significant negative correlation with triglyceride; however, this was not significant at week -1 or 0. Fruit and vegetable intake had a positive correlation with HDL cholesterol. The correlation was significant at week 2 for the intake of fruit, vegetable and combined fruit and vegetable.

Table 7.11. Association of carotenoid-rich food intake with lipid profile (n=10)

	Fruit week -1	**Fruit week 0**	**Fruit Av**	**Fruit week 2**	**Veg week -1**	**Veg week 0**	**Veg Av**	**Veg week 2**	**Fruit & veg week -1**	**Fruit & veg week 0**	**Fruit & veg Av**	**Fruit & veg week 2**
Total Cholesterol												
Week -1	-0.501				-0.055				-0.389			
Week 0		-0.311				-0.105				-0.255		
Base Av			-0.422				-0.082				-0.328	
Week 2				-0.579				-0.317				-0.490
Triglyceride												
Week -1	-0.330ǂ				-0.454ǂ				-0.368ǂ			
Week 0		-0.441ǂ				0.000†				-0.283ǂ		
Base Av			-0.389ǂ				-0.215ǂ				-0.374ǂ	
Week 2				**-0.658**ǂ				-0.564ǂ				**-0.636**ǂ
LDL Cholesterol												
Week -1	-0.260				0.697				-0.110			
Week 0		-0.065				0.116				0.020		
Base Av			-0.161				0.168				-0.019	
Week 2				-0.178				0.161				-0.574
HDL Cholesterol												
Week -1	0.103				0.524				0.358			
Week 0		0.258				0.452				0.403		
Base Av			0.200				0.541				0.423	
Week 2				**0.672**				**0.664**				**0.741**

Note. Av = average, Veg = vegetables, LDL = Low Density Lipoprotein, HDL = High Density Lipoprotein, bold text indicates significant at 95% CI,ǂ = Spearman correlation, † = Kendall correlation.

Discussion

This study showed that compliance and acceptability of the intervention were feasible. The direction of this discussion will focus on why there were no significant marked changes on the measures of carotenoids and lipids following the two weeks' consumption of vegetable-enriched bread. Exploration of the association of participants' reported intake of carotenoid-rich foods with their objectively measured carotenoid status and lipid profile status will be addressed. Lastly, the strong criterion and moderate construct validity of the Veggie meter™ reflection spectroscopy scores as a pragmatic field measurement of vegetable intake will be discussed.

Consumption of the vegetable-enriched bread daily for two weeks was not associated with significant change in the plasma carotenoid or lipid measures. This null finding could be attributed to several factors: the carotenoid dose in the bread over the two-week treatment period was insufficient to change participant's storage status, and the participants' weight was negatively associated with their body carotenoid stores as measured through plasma carotenoid concentration (**Table 7.6**). Thus, as participants' weight increased, the dose of carotenoid required to cause a change increased. Pragmatically, those with higher body weight will require a higher amount of the vegetable-enriched bread to increase their carotenoid concentration than those with lower body weight. However, in the present study, participants consumed equal amounts of bread, which possibly contributed to the lack of change in the carotenoid stores. A search on Scopus and PubMed databases revealed that, to date, no study has used bread as a medium of intervention for carotenoid delivery, so direct comparisons with literature cannot be made. This constrains comparison of this present findings with already published works where bread was used as a medium to improve carotenoid stores in humans. However, certain pertinent factors, including the food matrix used for carotenoid delivery and the duration of consumption of the carotenoid-rich foods, have been reported in previous studies to impact body carotenoid status (**Table 7.12**).

Studies that found a positive carotenoid change following the consumption of carotenoid-rich food used a food matrix that was liquid, with higher carotenoid dosages (Martinez-Tomas et al., 2012; Müller, Bub, Watzl, & Rechkemmer, 1999) (**Table 7.12**).

Table 7.12. Comparison of effect of carotenoid-rich food intake on body carotenoid stores with the vegetable-enriched bread study

Study objective and Reference	**No. of participants and study duration**	**Food ingredients**	**Dose/day**	**Change in carotenes**	**Findings**
Vegetable-enriched bread intake on plasma β-carotenes and lipid profile (present study)	10 (two weeks)	Solid bread enriched with pumpkin powder	474µg/day of β-carotene and 14.4g/day of fibre	0.06µmol/L	No significant change in carotenoid and lipid profile concentration
Fruit and vegetable soup intake on serum β-carotene and lycopene concentration (Martinez-Tomas et al., 2012)	14 (four weeks)	Liquid soup with equal amounts (20%) of carrot, tomato and broccoli with 5% olive oil	4mg	0.45µmol/L	Serum β-carotene increased significantly
Carotenoid-rich food intake and its impact on plasma carotenoid (Müller et al., 1999)	23 and eight weeks (four periods of two weeks) with two weeks' baseline	Liquid juice preparation from tomato, carrot and spinach powder	330mL tomato juice (40mg lycopene), 330mL carrot juice (15.7mg α-carotene and 22.3mg β-carotene) and 10g spinach (11.3mg lutein and 3.1mg β-carotene)	1.45µmol/L	8.6 and 3.2-fold increase respectively for α- and β-carotene after the carrot juice intake. Two-fold increase in β-carotene during spinach intake period

It is challenging to use a baked product such as bread as a vehicle for carotenoid delivery. To increase the carotenoid concentration of the bread requires the addition of increased quantities of carotenoid-rich ingredients such as pumpkin and sweet corn powders. This consequently impacts on bread physical qualities, such as loaf volume, and its sensorial attributes, including colour, which can be viewed negatively by consumers. For example, increasing the carotenoid concentration of bread through the addition of tomato products, which are rich in lycopene, resulted in a lower consumer acceptability and impacted adversely on physical quality through reduction in specific volume (Nour, Ionica, & Trandafir, 2015). Pumpkin and sweet corn are rich sources of carotenoids including β-carotenes and lutein (Elvira-Torales et al., 2019); however, baking temperatures may degrade these important nutrients as they are heat labile (Kopec & Failla, 2018). The degree to which degradation occurred is unknown in this study. We have recently posited the need to promote the use of encapsulation strategies in such ingredients, which allows for the shielding of heat-labile bioactive nutrients (like carotenoids) with a cell wall material such as maltodextrin (Amoah et al., 2018). Therefore, it is necessary to give consideration to encapsulating bioactive ingredients used for bread formulation. The common wall materials used for encapsulation are carbohydrate based, including the polysaccharide maltodextrin and so their use for encapsulation could be seen as a functional ingredient.

Possibly, impaired bioaccessibility and bioavailability of the carotenoids resulted in the reduced dose of carotenoids measured after the intervention. In a previous study, when participants consumed tomato and carrot juice with their main diet over a two-week period, plasma α- and β-carotene concentrations increased 8.6 and 3.2-fold respectively (Müller et al., 1999). In another study, participants were fed 300mL of soup prepared with carrot, tomato and broccoli, with the soup containing 3.9 and 4mg β-carotene and lycopene respectively, for four weeks. Serum β-carotene significantly increased over the baseline measure after week three (0.33 versus 0.69µmol/L) and week four (0.69 versus 0.78µmol/L) (Martinez-Tomas et al., 2012). The common trend running through these two studies is the apparent higher concentrations of β-carotenes present in their liquid-based food source (juices and soup) compared to β-carotene concentration of 236.78µg/100g present in our vegetable-enriched bread (**Table 7.8**) and this may contribute to the null finding of this study.

For the first time, a Veggie meter™ reflection score was used to measure participants' consumption of carotenoid-rich foods and has been validated against plasma carotenoid concentration. The 84% of variability accounted for in this study indicates the Veggie meter™ is a reliable alternative to laboratory assessment. Ermakov et al. (2018) validated carotenoid scores from the Veggie meter™ with serum carotenoids of participants and found a strong association (R=0.81; $p < 0.001$). In a similar vein, in this study, we found a strong association (r^2=0.72). The Veggie meter™ is a convenient device that non-invasively evaluates a person's carotenoid score and has the potential to be used in large population-based studies to track population fruit and vegetable intake during interventions. Challenges related to participants' unwillingness to partake in research studies that invasively sample blood, and the associated ethical challenges, makes this device appropriate for use due to its non-invasive nature and rapid operation.

Enriching the bread with the carotenoid-rich pumpkin and sweet corn powders enhanced the fibre content. An association between the intake of fibre-rich diets and improved lipid profile concentration has been established in several epidemiological studies.

Generally, the cholesterol, triacylglycerol, HDL and LDL concentrations did not record any significant improvement following the intervention period. Similar findings have been recorded with bread fortified with *Palmaria palmata* and alginate. In the case of the alginate bread, its consumption for a period of two weeks enhanced the process of lipolysis and has been targeted for aiding weight loss (D. Houghton et al., 2019). In another study, *Palmaria palmata*-enriched bread increased triglycerides and cytokines (Allsopp et al., 2016). In the present study, the observation of no change in the lipids profile composition of the participants could be attributed to two factors.

Participants in this study had a lower vegetable and fruit intake (**Table 7.2**) than the New Zealand daily recommendations of at least two servings of fruit and three servings of vegetables (Ministry of Health, 2011). Yet, it was established in the correlation matrix that the total carotenoids from the carotenoid-rich foods was positively associated with the plasma carotenoid concentrations and carotenoid reflection scores (**Table 7.7**). In New Zealand, the Ministry of Health reports that only 39% of adults aged 15 years and over meet the recommended fruit and vegetable intake (Ministry of Health, 2011). Participant median daily intake of vegetable and fruit was lower than the recommended servings of least two fruits and three vegetables every day, or 5+ a day and a number of participants reported no intake of the specified foods. In terms of ethnicity, 34% of Pacific, and 28% of the Asian population met the recommended servings, compared to 42% for the European/Other population. In this study, 40% of the participants reported their ethnicity as Pacific, 40% African and 20% Asian. Factors involved in the low consumption of fruit and vegetables in this country include cost (Metcalf, Scragg, & Jackson, 2014) which is largely driven by availability due to seasonal variations. However, there are concerns that New Zealand grows significant amounts of fruit and vegetables which are exported to the global market, while its own people struggle to consume the recommended amounts, primarily due to cost (Rush &

Obolonkin, 2020). In particular, for families in the low-income brackets, achieving the recommendation is taxing on their financial circumstances. For example, in a family of five, meeting the 5+ a day recommendation will require they consume 17.5 kg of vegetables and fruits per week which will create a burden on their finances. There is, therefore, the need for decision-makers to put in place pragmatic approaches to encourage increased public intake of vegetables and fruits as the Australian guideline is for seven servings (five servings of vegetables and two servings of fruits).

When the association between fruit and vegetable consumption and lipid profile was explored, a negative association was found for the total cholesterol and triglyceride concentration. However, positive associations were found between LDL and vegetable intake, and HDL and vegetable and fruit intake. Foods rich in fibre, particularly the soluble type, have been found to improve serum lipid profile. In this present study, though the vegetable-enriched bread had a higher fibre concentration of 7.2g/100g, the soluble fibre fraction constituted a smaller proportion (1.7g/100g) compared to the insoluble fraction (5.5g/100g). The relatively lower fraction of the soluble fibre fraction may have contributed to the lack of change in serum lipid profiles of the participants over the intervention period.

Strength and limitations

A strength of this study is that it adds to library of knowledge on interventions aimed at increasing body carotenoid stores, and validation of a non-invasive method to measure carotenoid levels, and fruit and vegetable intake. Although participants were asked to record their consumption pattern of the 200g of the vegetable-enriched bread daily in a diary, it is not certain that this compliance was achieved. As this is a pilot study, a sample size of 10 is small, and future research should include a larger sample size. A limitation of this study was that β-carotene was measured in the bread matrix; however, total carotenoids were analysed in the plasma samples. This made it difficult to establish the direct association of the bread β-carotenes with the plasma carotenoids. However, the use of the Veggie meter™ reflection scores in this study partially rectified this situation. Pumpkin and sweet corn are rich in lutein and β-carotene, but currently there is not a specific biomarker for lutein. As the Veggie meter™ measures the composite score of all the carotenoids deposited in the skin (Ermakov et al., 2018), carotenoid uptake following bread consumption and its storage status in the body could be determined.

Conclusion

The daily consumption of a vegetable-enriched bread for a two-week period did not significantly increase participants' circulating carotenoid concentrations. In a similar vein, no significant improvement in total cholesterol, triglyceride, LDL and HDL was observed over the intervention period. A strong positive association between HDL cholesterol and fruit and vegetable intake was found. The validation of the non-invasive Veggie meter™ carotenoid reflection score against the invasive plasma carotenoid concentration suggests the Veggie meter™ has the potential to be a useful device for large population-based studies to investigate fruit and vegetable intake.

Chapter 8. Final discussion and conclusion

Original contribution to knowledge of this thesis

The overarching research question was: How can food science and nutrition inform the development of a vegetable-enriched Nothing Else™ bread, accepted by consumers, easier to swallow and with potentially verifiable health claims?

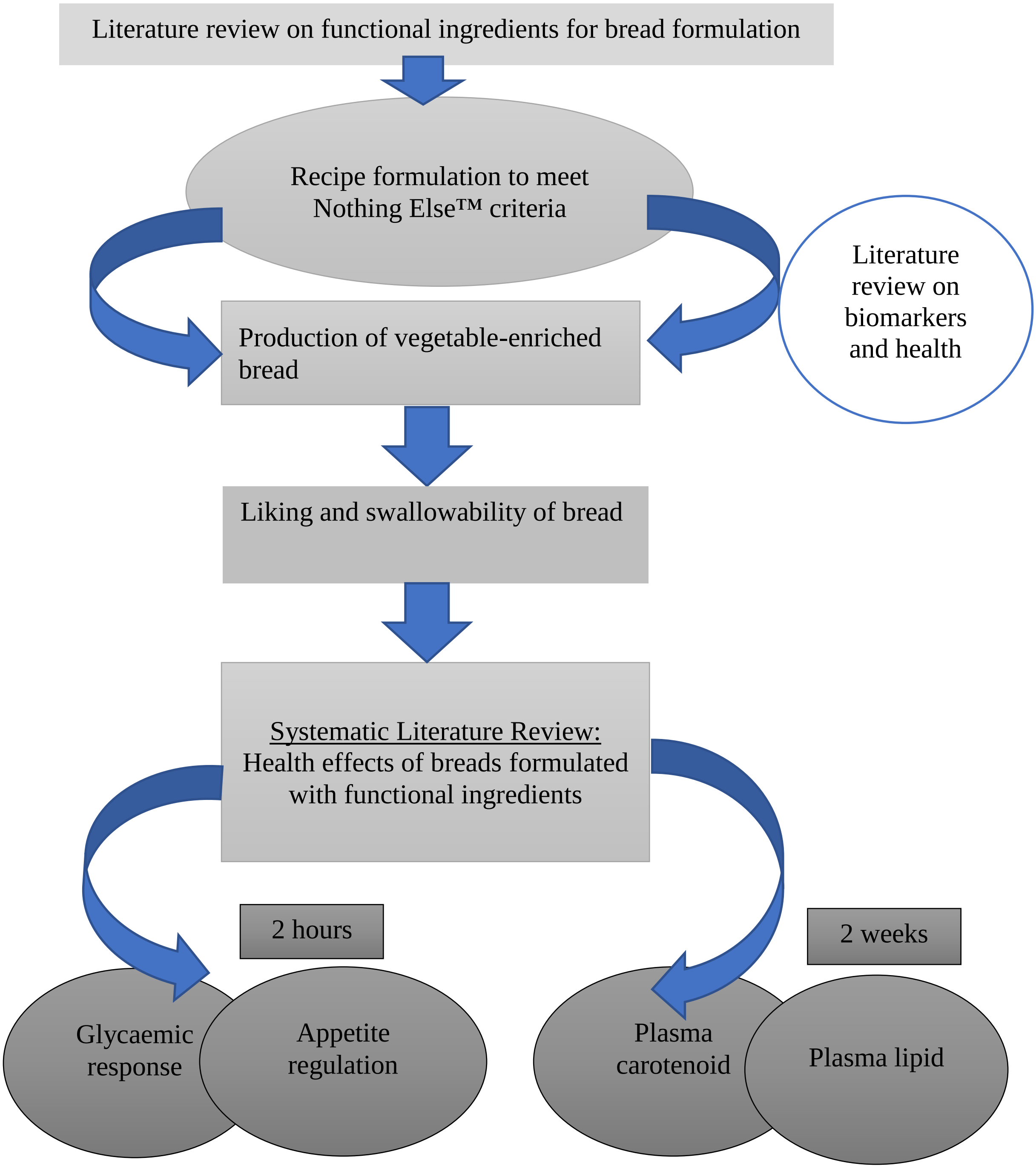


Figure 8.1. Flow chart of the bread development, health claim validation and studies.

This thesis has demonstrated a proof-of-principle project, achieved through harmonisation across three faculties of AUT where culinary, food science and nutrition expertise contributed to the development and testing of a novel vegetable-enriched bread with a validated health claim.

Arguably, the most original and novel measurement was the subjective measure of swallowability that assessed each stage of the swallowing process – biting and getting food into mouth, chewing and swallowing, movement of food bolus through the throat and its stickiness in the throat. The vegetable-enriched bread was easier to swallow than the commercial breads. Ease of swallowing assesses physical sensory properties of the bread but also the physiological responses of salivation and peristalsis going beyond the taste receptors on the tongue to ease of manipulation and passage down the oesophagus.

This study has shown for the first time that older participants are receptive to a novel vegetable-enriched bread with a validated health claim. With the increasing older adult population in New Zealand, the study finding that the novel vegetable-enriched bread was easier to chew and swallow than commercial white bread has positive implications for a vulnerable population group that includes older people with presbyphagia.

The acute insulin response in 10 young adults was attenuated following the consumption of the vegetable-enriched bread. Thus, the consumption of the vegetable-enriched bread would not burden the β-cells of the pancreas to release insulin. This has implications for public health in New Zealand as this country has a very high prevalence of nutrition-related non-communicable diseases including obesity. It has been well established that obesity is associated with type 2 diabetes and hyperinsulinemia. The vegetable-enriched bread has the potential to be a viable prospect in ameliorating hyperinsulinemia. The suppression of appetite following the consumption of the vegetable-enriched bread implies in practical terms that participants' craving for energy-dense and nutrient-poor snacks would be impaired. This could be a viable approach towards addressing obesity.

After two weeks' consumption of the vegetable-enriched bread, there was no change in carotenoid concentration or lipid profile of the 10 participant's blood but the compliance with eating the bread every day was 100%. This finding highlights the consumption of bread alone is not sufficient to increase carotenoid stores.

Why bread and why older people?

Bread is a commonly consumed food in New Zealand and the value has increased since the start of this thesis, over the period 2017-2019 (**Table 8.1**) (NielsenScantrack, 2019). The specialty breads, where functional breads rightly fit in, recorded a percentage increase of 4% in value during the 2017-2018 trading year and 5% during the 2018-2019 trading year (**Table 8.1**). This increase in bread sales provides a greater opportunity for the commercial viability of the vegetable-enriched bread.

Table 8.1. Value of bread trade in New Zealand supermarkets (NielsenScantrack, 2019)

	Year ending June, 2019	Year ending June, 2018	Year ending June, 2017
	Value of Sales ($'000)	Value of Sales ($'000)	Value of Sales ($'000)
Bread	516,077	492,928	488,623
Non-white Bread	241,928	235,121	242,059
White Bread	98,588	94,421	93,607
Specialty Bread	83,130	78,874	75,700
Buns & Rolls	44,360	40,246	38,332
Indian Bread	6,862	5,882	4,864

New Zealand's population is ageing. In New Zealand's population of 4,942,500 (Statistics New Zealand, 2019), the number of older adults aged 65+ is expected to increase to between 988,500 and 1,087,350 by 2032 (Statistics New Zealand, 2018). Ageing is associated with impaired food chewing and swallowing due to poor dental orientation and weakened muscles (H. S. Park, Kim, Lee, & Park, 2017; Xu, 2016).

Difficulty in swallowing occurs predominantly in older and morbidly obese people and can potentially compromise their nutritional status (Morris, 2006) as limited food is ingested. In dysphagia, as well as swallowing challenges, chewing and food pocketing difficulties in the mouth may also occur (Dalton et al., 2011). This has warranted the need to improve bread texture to allow easier chewing and swallowing by older people.

In this thesis, the vegetable-enriched breads (VB75 and VB100) were almost 1.5 times easier to chew and swallow compared to their WB and WMB counterparts. This could be attributed to pectin fibre (Fissore et al., 2009) from the pumpkin powder in the vegetable-enriched breads. Pectin has hydrophilic properties and the higher water content may be the reason the vegetable-enriched breads were easier to chew and swallow (Einhorn-Stoll, 2018). Though the WB was not significantly different from the VB75, VB100 and WMB in terms of its objective hardness measure, the participants found the WB difficult to swallow which, anecdotally, was purported to be due to a clogging effect in the throat. This may be due to the poor fibre content (2.7g/100g). The liking of all the breads was strongly correlated with the ease of swallowing. There is therefore the need to prioritise improving the textural attributes of bread to aid swallowing. particularly when considering older people as the target market.

A literature search on Web of Science, Scopus and PubMed revealed that no study on bread has validated ease of swallowing as an outcome. Others, however, have shown that bread with a higher nutrition value has the potential to be used to address malnutrition in older participants. A search on Web of Science using the words (Bread OR Loaf) AND (Sensory) AND (Older OR Ageing participant*) revealed only two studies where older people participated. In those two studies, the overarching goal was to investigate how breads could be used to address malnutrition in older people, with bread liking evaluated as a co-outcome. Song et al. (2018) enriched rye bread with whey protein hydrolysate, whey protein isolate, and/or soy protein isolate and recruited older people (61 to 83 years old) to evaluate the sensory attributes of the bread. Farouk et al. (2018) enriched bread with beef and served it to 70 older people to solicit their sensory perceptions and reported that, there was no difference in the liking of the meat-enriched bread and the control bread.

An interesting dynamic was observed in terms of the evaluation of sensory attributes by the older participants in the liking and swallowing study (**Study 1**) and the relatively younger participants in the glycaemia and appetite suppression study (**Study 2**). The older people had a stronger dislike for WB whilst the relatively younger participants did not differentiate between the breads. Sandvik et al. (2017) engaged older participants (45-80 years) to evaluate their perception of nine commercial rye breads with reference to chewy flavour and sour taste. The authors posited that participants' liking of rye breads with chewy texture and sour flavour was associated with their level of education and their childhood bread experience. Song et al. (2018) found that sourdough bread formulated with 7% whey protein hydrolysate and soy protein isolate was liked for its taste and modified texture. Similarly, bread enriched with beef was liked by older participants with no significant difference between it and its non-meat control counterpart (Farouk et al., 2018). A previous AUT study with participants from the same exercise programme (never2old) found that these physically active older adults regularly consumed bread in their childhood, which was usually wholemeal or wholegrain (Lucas et al., 2019). They were also conscious of the importance of eating well to support their ageing process. This may contribute to the strong dislike of WB by the older participants in this study.

There is increased consumer demand for bread with health-promoting properties (Birch & Bonwick, 2018). Consequently, breads enriched with vegetables, including freeze-dried carrot, tomato, beetroot or broccoli (Ranawana, Campbell, et al., 2016), cladodes powder (Msaddak et al., 2017) and broccoli sprouts (Gawlik-Dziki et al., 2014), have been researched. However, most of these studies are test tube-based (in vitro). The validation of the health-promoting properties of bread using humans as participants is essential for consumer confidence in newly developed products and health claims. Again, newly developed food products with health claims are more likely to be purchased compared to their counterparts without any claim. Against this backdrop is the rationale behind the investigation of the glycaemic attenuating and appetite suppression potential of the VB, WMB and WB in this thesis. Consumption of the VB elicited a significantly lower insulin release compared to the WMB and WB. The consumption of the WB resulted in 38% and WMB 40% more insulin release compared to the VB. Because the WMB contains higher fibre (4.6g/100g) than the WB (2.7g/100g), it is expected to have a greater potential to attenuate insulin better than the WB. Ironically, the insulin release following the consumption of the WB was lower than that of the WMB. The plausible rationale could be that it is due to the greater amount of emulsifiers in WMB. Generally, enriching bread with bran or wholemeal flour in WMB dilutes the gluten and tends to impart poor textural properties to the crumb (Martins, Pinho, & Ferreira, 2017). This compels bakers to add emulsifiers to bread. The higher insulin release following the consumption of the WMB could be attributed to the presence of emulsifiers that have insulin-stimulating effect on the β-cells of the pancreas. The lack of emulsifiers in the VB may have contributed to the significantly lowered insulin release following consumption compared to the WMB and WB. However, this needs to be investigated further in future studies. Coe and Ryan (2016) found that baobab powder added to white bread was not associated with a reduction in glucose concentration or satiety over a 180-minute period but, similar to this investigation, insulin iAUC was attenuated by about 20%. The authors attributed this observation to the polyphenol compounds in baobab that may reduce the rate and degree of starch digestibility. In another study, the consumption of whole-kernel rye bread, β-glucan-enriched bread and wholemeal pasta could not attenuate glucose response over a 180-minute period compared to white wheat bread (Juntunen et al., 2002). However, postprandial insulin release was significantly attenuated by 42% and 22% for the whole-kernel rye and β-glucan-enriched breads respectively compared to the white bread. The whole-kernel rye and β-glucan-enriched breads had a fibre content of 12.8 and 17.1% respectively. The authors posited that the structure and form of food products impacts on the insulin response following food consumption (Juntunen et al., 2002).

The consumption of the VB made the participants feel full significantly over the 120-minute period of the study compared to the WMB and WB. This could be attributed to the higher fibre content of the VB. Fibre-rich foods have been found to induce satiety sensations as they delay gastric emptying in the stomach and stimulate the release of appetite hormones PYY and cholecystokinin which binds to the satiety centres of the hypothalamus of the brain, inducing the perception of fullness (L. Chambers et al., 2015).

Response over two weeks

In the last study, the daily consumption of the VB for two weeks was associated with no measurable change in plasma carotenoid or lipid concentrations. This could be attributed to a relatively low dose of carotenoids (236.8μg of β-carotene per 100g of bread) present in the bread compared to other studies that used liquid-based medium for carotenoid delivery. In a previous study, when participants consumed a juice preparation of tomato and carrot with their main diet for a two-week period, plasma α- and β-carotene concentrations increased 8.6 and 3.2-fold respectively (Müller et al., 1999). In another study, participants were fed 300mL of soup prepared with carrot, tomato and broccoli, with the soup containing 3.9 and 4mg β-carotene and lycopene respectively, every day for four weeks. Serum β-carotene significantly increased over the baseline measure after week three (0.33 to 0.69μmol/L) and week four (0.69 to 0.78μmol/L) (Martinez-Tomas et al., 2012). The dose of carotenoids was 20 times higher than the present study. This is the first-time drum-dried pumpkin and sweet corn powders have been used together in bread and the health effects tested.

The dose of fibre (14.4g/day) in the bread, whilst high, caused no measurable change in the lipid profile over the two-week period. Participants in this study who consumed more vegetables had a higher HDL concentration. D. Houghton et al. (2019) enriched bread with alginate and, in a double-blind, randomised, controlled cross-over pilot study, demonstrated that circulating triacylglycerol was reduced following the consumption of alginate-enriched bread compared to control. This was a two-week intervention. This is one of many reasons for the recommendation of increased intake of a variety of vegetables.

Secondary outcomes

In terms of the validation of the skin carotenoid data with the plasma carotenoid data, strong agreement (72%, r= 0.85) was seen with the laboratory measure validating the Veggie meter™ as a reliable device for measurement of carotenoid status. A regression equation for plasma carotenoid concentration from the Veggie meter™ reading was derived. Our agreement is consistent with findings by Ermakov et al. (2018) who also validated carotenoid reflection scores with serum carotenoid levels with 64% of the variation accounted for (r=0.81). A positive correlation was observed for participants' (n=10) average baseline and post-intervention vegetable intake and their plasma carotenoid measure (r=0.446, 95% CI -0.255, 0.840 and r=0.255, 95% CI -0.446, 0.762, respectively) and skin carotenoid reflection score (ρ=0.489 and Ŧ=0.689). Subsequently the Veggie meter™ has been employed in a survey of the carotenoid status of 571 Auckland residents aged from 16-85 years (Rush et al., 2020).

Strengths and limitations

There are several factors that strengthen the findings of this thesis and the series of investigations presented. The opportunity to work closely with the multidisciplinary advisory/support group strengthens this research work. Throughout this study, there was close engagement with a diverse advisory group which included talking with the Pure Food Company (https://thepurefoodco.com/), New Zealand Baking Industry Research Trust (https://www.bakeinfo.co.nz/), the Nothing Else™ branding and business advisors, AUT food network (https://foodnetwork.aut.ac.nz/). This created the opportunity to harmonise expertise and provided unrestricted access to the use of equipment that was essential towards the smooth running of the research. The large number of older participants recruited for the acceptability and swallowing study is one of the strengths. In the glycaemia and appetite study, the principles of the protocol for glycaemic index testing (ISO, 2010), the number of participants (10) and the calculation of areas under the curve was followed, the food portion size was not based on a standard amount of carbohydrate but on a realistic serving size for each bread. The small sample size (n=10) for carotenoid and lipid profile study is a limitation. In the carotenoid study, a limitation was the fact that β-carotene rather than total carotenoid were measured in the bread. However, the biomarker measured in the blood was plasma carotenoid and the Veggie meter™ measured skin carotenoids as well. Though the β-carotenes present in the drum-dried pumpkin and sweet corn powders are carotenoids, evaluation of the total carotenoid rather than β-carotene in the bread could have strengthened the relationship between the bread carotenoids and the plasma carotenoids. There are a number of measurements that, on reflection, could have been undertaken. The microstructure of the

VB, WMB and WB could be examined using a confocal microscope and related to how the breads aid swallowing. The rationale for this is because ingredient combination and interaction in the bread crumb matrix impacts on the microstructure and could consequently impact on the subjective ease of chewing and swallowing of the bread bolus.

Future work recommended for consideration

The research has highlighted that a swallowability index for breads could have utility. In particular, bread formulated with other underutilised or low-value vegetables may help increase vegetable intake. The research question would be: Similar to the glycaemic index that identifies the blood glucose raising potential of food, would a swallowability index of several bread products consumed by people with presbyphagia discriminate those foods that are easier to swallow? The study design ideally would include ethnic and socioeconomic diverse groups. In designing this study, consideration to video recording of participants' buccal activity will need to be considered to guard against subjective under and over-reporting of participants' bread number of chews.

The long-term effect of the consumption of the VB on insulin response should be investigated. The research question would be: Would the daily consumption of VB, and not white bread, reduce fasting insulin (and glucose) over one, two or three months? In designing this study, pre-screening of the participants prior to the participation in the study to allow comparison of those with hyperinsulinemia with those without it. In the present study, 70% of the participants were of Pacific origin and a high BMI with a higher risk of T2DM. Future studies must give consideration to equal participation of participants of other ethnicities including European, Maori and Asian backgrounds with considerations to varying body size, waist to height ratio, women versus men and active versus inactive.

The role of food emulsifiers and the amount in the bread involved in stimulating insulin release must be investigated further. This study could be carried out using animal models (rat). The research question would be: Would the consumption of bread enriched with/without emulsifiers over one, two or three months by rats increase insulin circulation in the blood? The WMB and WB elicited a higher insulin release compared to the VB. Emulsifiers such as lecithin and glycerides are added to these commercial breads to improve the textural properties. The need to investigate effect of the addition of these emulsifiers on insulin release is also warranted as the VB was devoid of them.

The long term effect of the VB consumption on weight reduction, lipid profile and carotenoid concentration could also be considered. The research question would be: Would the consumption of VB for one, two or 3 months improve weight reduction, lipid profile and carotenoid in the blood? In designing this study, adjustment of participants' weight with bread dosage must be considered.

The effect of the VB on the gut microbiome warrants future investigation. The research question would be: Would the consumption of VB for one, two or three months promote the growth of beneficial microorganisms in the gut? The soluble fibre pectin in VB has the potential to act as a prebiotic and stimulate the growth of the gut microbiome.

The use of VB as a vehicle to increase the consumption of carotenoid-rich foods, in particular vegetables, for example in sandwiches, should be explored. The research question would be: Would the consumption of VB sandwiched with carotenoid-rich foods including spinach or carrots and eggs increase plasma carotenoid concentration when consumed for one, two or three months? The rationale for this is because the carotenoids in the dark green leafy vegetables, pumpkin and carrots and eggs could complement the carotenoids in the VB and enhance the carotenoid status of the consumers.

Perspective

From conceptualisation to development

In food product development, ingredient combination impacts on the sensory properties including swallowability of the food product. The balance to achieve bread with appealing sensory properties and enhanced ability to improve plasma carotenoid and lipid profile concentrations requires a holistic approach. A lot of times, in mainstream food product development, attention is paid to incorporation of various ingredients and the sensory perceptions. However, the acceptance and liking of the sensory perceptions of a product does not guarantee it has a favourable health effect. Interestingly for this study, though the vegetable-enriched bread was liked by consumers, it could not significantly improve plasma carotenoids and lipid profile concentration. The antidote to this could be to increase the concentration of pumpkin in the bread formulations. However, from a technological point of view, increasing the concentration of pumpkin, aside from having adverse impact on the gluten network, will also impact on the physical qualities particularly the volume and colour. A decrease in bread volume from a commercial point of view implies loss of value. An adverse effect on the perceived quality of the bread implies acceptability to consumers will be impaired. There is therefore the need to explore appropriate ways of delivering carotenoid-rich ingredients into bread. In that regard, encapsulation has been suggested to be an effective way of incorporating carotenoid into bread matrix without significant degradation (Amoah et al., 2018). A supportive environment must also be provided. In that regard, the carotenoid-enriched bread needs to be available at a reasonable cost so that consumers can purchase it.

Food security, public health and the need for food reformulation and product development

As part of the United Nations Sustainable Development Goals 2030, Goal 2 advocates for zero hunger, and interventions geared towards achieving food security and improved nutrition (United Nations, 2015). Yet, climate change continues to threaten food security. Food security implies that adequate nutrient-dense foods are available at all times to households (Rush, Puniani, Snowling, & Paterson, 2007). New Zealand grows enough vegetables and fruits to feed the population (Rush & Obolonkin, 2020). However, the prices of vegetables in New Zealand during the off-season is expensive, compelling consumers on low incomes to substitute vegetable intake with other nutrient-poor foods (Amoah, Cairncross, & Rush, 2017). The shorter shelf life of vegetables warrants their processing into flour to ensure their availability during lean seasons. That notwithstanding, the processing of vegetables and fruits into juices results in the generation of food by-products which create nuisance in the environment.

In recent times, the effort to promote interventions geared towards promoting food security has stimulated interest in the valorisation of food industry by-products from vegetable and fruit processing for new food product developments, including bread. Vegetable by-products including broccoli by-products (Lafarga et al., 2018), tomato skin and seeds (Nour et al., 2015) and artichoke by-products (Frutos et al., 2008) have been incorporated into bakery products including bread and crackers. In a recent study by Rush et al. (2020) where skin carotenoid reflection scores of participants were measured, the authors posited that 82% of the participants (n=571) were at moderate risk of carotenoid deficiency based on a review of health outcomes. The outcome of that study highlighted a potential vitamin A deficiency amongst Aucklanders. This is not surprising as hidden hunger (micronutrient deficiencies) appears to be increasing. Carotenoids are precursors of vitamin A and are present in higher quantities in vegetables including pumpkin, carrots and green leafy vegetables. New Zealand food waste constitute 17% of all waste (Reynolds, Mirosa, & Clothier, 2016). In terms of the capacity generated, 224,000 and 103,000 tonnes of food waste are generated by households and industries in New Zealand respectively in 2011. In that same year, the value of New Zealand food waste stood at NZ$568 million (Reynolds et al., 2016). There is an opportunity to utilise vegetable by-products and low-value abnormally sized vegetables in food from New Zealand's agro-industrial processors and farmers respectively. The way forward is to turn these vegetable by-products and excess, rejected and odd-sized vegetables into flour. The need to explore cheaper means of drying could be explored. In that regard, solar dryers could be used, and the dried materials turned into flour for their incorporation into bread, a commonly consumed staple. At present, apple pomaces are being turned into high-value products, mainly flour, for the provision of fibre in bakery products but there are no reports of similar uses of vegetables or other nutrient-dense plants such as legumes. Specialty and whole grain breads, which include vegetable-enriched breads, are expensive in New Zealand. Consequently, consumers in the lower income brackets are compelled to purchase white bread which is nutrient poor. Promoting the reformulation of bread with flour from solar dried vegetable by-products and odd-sized vegetables would result in reduction in the cost of the bread. This has the potential to promote commercially viable cheap vegetable-enriched breads on the bread market. The public health implications of this would be improvements in the micronutrient status of consumers, and the promotion of food diversity and food security.

Conclusion

This series of feasibility and pilot studies has proven the viability of developing vegetable-enriched breads. The vegetable-enriched breads were more liked for their taste, colour, and aroma compared to commercial WB and WMB. Compliance with the intervention was good, with the participants willing to eat the vegetable-enriched breads over a two-week period. Participants reported that the vegetable-enriched bread was easier to chew, and swallow compared to the commercial breads, and these findings may inform bread producers targeting functional breads for the older adult population. The glycaemic and appetite control validation carried out on the VB, WMB and WB was essential to reveal the health-promoting effects of the bread. The uniqueness of this study lies in the point that the WMB and WB are two commercially produced breads which, due to their low cost of $1, get a wider patronage. Yet, the study findings revealed a higher insulin release associated with the consumption of the WMB and WB. The consumption of the VB made the participants full over a 120-minute period. This in a practical sense implies the participants will have a lower desire for snacking on nutrient-poor foods that have the potential to increase obesity risk. This is different from fibre-poor breads such as WB which appear to increase consumers' appetite drive. Consumption of the VB for two weeks caused no changes in plasma carotenoid and lipid profile concentration. There was a positive correlation between the usual reported consumption of vegetables and the HDL cholesterol concentration. Compliance of the VB intake was 100%. There was a strong correlation between the invasive carotenoid concentration and the carotenoid reflection scores. The economics and commercial viability of bread production using novel functional ingredients such as dried vegetables and clean-label branding such as the Nothing Else™ brand needs to be investigated further.

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Appendices

Appendix 1. AUTEC ethics approval for liking and swallowing study

13 March 2018

Elaine Rush
Faculty of Health and Environmental Sciences

Dear Elaine

Re Ethics Application: **18/22 Does a vegetable-enriched 'Nothing Else' bread have benefits to health**

Thank you for providing evidence as requested, which satisfies the points raised by the Auckland University of Technology Ethics Committee (AUTEC).

Your ethics application has been approved for three years until 13 March 2021.

Standard Conditions of Approval

1. A progress report is due annually on the anniversary of the approval date, using form EA2, which is available online through http://www.aut.ac.nz/researchethics.
2. A final report is due at the expiration of the approval period, or, upon completion of project, using form EA3, which is available online through http://www.aut.ac.nz/researchethics.
3. Any amendments to the project must be approved by AUTEC prior to being implemented. Amendments can be requested using the EA2 form: http://www.aut.ac.nz/researchethics.
4. Any serious or unexpected adverse events must be reported to AUTEC Secretariat as a matter of priority.
5. Any unforeseen events that might affect continued ethical acceptability of the project should also be reported to the AUTEC Secretariat as a matter of priority.

Please quote the application number and title on all future correspondence related to this project.

AUTEC grants ethical approval only. If you require management approval for access for your research from another institution or organisation then you are responsible for obtaining it. You are reminded that it is your responsibility to ensure that the spelling and grammar of documents being provided to participants or external organisations is of a high standard.

For any enquiries, please contact ethics@aut.ac.nz

Yours sincerely,

Kate O'Connor
Executive Manager
Auckland University of Technology Ethics Committee

Cc: isaac.amoah@aut.ac.nz

Appendix 2. AUTEC ethics approval for glycaemic and appetite suppression amended

3 October 2018

Elaine Rush
Faculty of Health and Environmental Sciences

Dear Elaine

Re: Ethics Application: **18/147 Does a vegetable-enriched Nothing Else bread have benefits to health - Part Two**

Thank you for your request for approval of an amendment to your ethics application.

The amendment to personnel taking blood sample is approved.

Non-Standard Conditions of Approval

1. Insert advice concerning cannulation (not venous sample in PIS dated 1 October 2018)

Non-standard conditions must be completed before commencing your study. Non-standard conditions do not need to be submitted to or reviewed by AUTEC before commencing your study.

I remind you of the **Standard Conditions of Approval**.

1. A progress report is due annually on the anniversary of the approval date, using form EA2, which is available online through http://www.aut.ac.nz/research/researchethics.
2. A final report is due at the expiration of the approval period, or, upon completion of project, using form EA3, which is available online through http://www.aut.ac.nz/research/researchethics.
3. Any amendments to the project must be approved by AUTEC prior to being implemented. Amendments can be requested using the EA2 form: http://www.aut.ac.nz/research/researchethics.
4. Any serious or unexpected adverse events must be reported to AUTEC Secretariat as a matter of priority.
5. Any unforeseen events that might affect continued ethical acceptability of the project should also be reported to the AUTEC Secretariat as a matter of priority.

Please quote the application number and title on all future correspondence related to this project.

AUTEC grants ethical approval only. If you require management approval for access for your research from another institution or organisation then you are responsible for obtaining it. If the research is undertaken outside New Zealand, you need to meet all locality legal and ethical obligations and requirements.

For any enquiries please contact ethics@aut.ac.nz

Yours sincerely,

Kate O'Connor
Executive Manager
Auckland University of Technology Ethics Committee

Cc: isaac.amoah@aut.ac.nz; Carolyn Cairncross

Appendix 3. AUTEC ethics approval for carotenoid and lipid profile study amended

14 November 2018

Elaine Rush
Faculty of Health and Environmental Sciences

Dear Elaine

Re: Ethics Application: **18/146 Does a vegetable-enriched Nothing Else bread have benefits to health**

Thank you for your request for approval of amendments to your ethics application.

The amendment to the exclusion criteria (participants taking supplements containing vitamin A) and the additional study measure (veggie meter) is approved.

I remind you of the **Standard Conditions of Approval**.

1. A progress report is due annually on the anniversary of the approval date, using form EA2, which is available online through http://www.aut.ac.nz/research/researchethics.
2. A final report is due at the expiration of the approval period, or, upon completion of project, using form EA3, which is available online through http://www.aut.ac.nz/research/researchethics.
3. Any amendments to the project must be approved by AUTEC prior to being implemented. Amendments can be requested using the EA2 form: http://www.aut.ac.nz/research/researchethics.
4. Any serious or unexpected adverse events must be reported to AUTEC Secretariat as a matter of priority.
5. Any unforeseen events that might affect continued ethical acceptability of the project should also be reported to the AUTEC Secretariat as a matter of priority.

Please quote the application number and title on all future correspondence related to this project.

AUTEC grants ethical approval only. If you require management approval for access for your research from another institution or organisation then you are responsible for obtaining it. If the research is undertaken outside New Zealand, you need to meet all locality legal and ethical obligations and requirements.

For any enquiries please contact ethics@aut.ac.nz

Yours sincerely,

Kate O'Connor
Executive Manager
Auckland University of Technology Ethics Committee

Cc: isaac.amoah@aut.ac.nz; Carolyn Cairncross

Appendix 4. Advertisement notice for liking and swallowing study

Which bread do you like?

50 Volunteers Required

We are looking for volunteers who eat bread at least once a week, aged 65 or older and with no known allergy or intolerance to gluten, to rate their liking of four different bread samples.

All that will be required of you is that you are able to attend one tasting session at the AUT fitness centre, Akoranga campus between 10:30 am and 11 am. The tasting session will take 30 minutes.

If you would like to participate, please respond before the 25th of February, 2018 by contacting the phone number and e-mail address below:

Phone: Isaac Amoah - 021 0865 9403

Email: isaac.amoah@aut.ac.nz

This project has been reviewed and approved by AUTEC, Auckland University of Technology Ethics Committee

Which bread do you like
and why?
Isaac Amoah
02108659403
isaac.amoah@aut.ac.nz

Which bread do you like
and why?
Isaac Amoah
02108659403
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Which bread do you like
and why?
Isaac Amoah
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Which bread do you like
and why?
Isaac Amoah
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Which bread do you like
and why?
Isaac Amoah
02108659403
isaac.amoah@aut.ac.nz

Appendix 5a. Consumer liking and swallowing questionnaire

ID#__________ Date / Month / 2018

Consumer Testing

Are you (please tick)

- ☐ Male
- ☐ Female

What is your ethnic group(s) (please tick)

O European O Māori O Pacific Peoples O Asian
O Other Ethnicity ....

Do you have any allergies associated with

nuts	☐Yes	☐ No
gluten	☐Yes	☐ No
egg	☐Yes	☐ No
milk	☐Yes	☐ No
food colouring	☐Yes	☐ No

Any other allergies? Please name ....

** If you have any allergies associated with food ingredients that may be in this sample please discuss this with the researcher and do not proceed with this test*

How often do you eat bread?

- ☐ Once per month
- ☐ Once per week
- ☐ Several times per week (number____)
- ☐ Once per day
- ☐ Several times per day (number____)

Instructions:

You will be asked to examine and taste four different breads, one at a time.
The testing of each bread will be in 2 steps – (1) Sensory, (2) Chewing and swallowing – then we will ask you to compare the bread samples.
You will fill out a questionnaire page for each step.
Each bread will have an identification code on the plate and on the questionnaire.

Appendix 5b. Consumer liking and swallowing questionnaire

ID#__________ Date / Month / 2018

Part 1 – Sensory Evaluation

Bread sample …………

Please rinse your mouth.

We are interested to know how you find this bread using your senses – please look, smell, feel and then taste the sample, and please put a circle or mark on each line scale.

How do you like the……..

Colour — Extremely Dislike |————————————————| Extremely Like

Smell (Aroma) — Extremely Dislike |————————————————| Extremely Like

Taste — Extremely Dislike |————————————————| Extremely Like

How it feels (Texture) — Extremely Dislike |————————————————| Extremely Like

How it feels in your mouth (mouthfeel) — Extremely Dislike |————————————————| Extremely Like

How much do you like this bread overall? — Extremely Dislike |————————————————| Extremely like

Would you be willing to eat this bread at home? — Extremely Unwilling |————————————————| Extremely willing

Appendix 5c. Consumer liking and swallowing questionnaire

ID#__________ Date / Month / 2018

Part 2 - Swallowing evaluation

Bread sample ....

Please rinse your mouth.

We are interested in how you find this bread to chew and swallow. We ask you to count the number of chews it takes to eat this bread.

Please take one normal bite of the bread, and count the number of chews before you swallow, and write this here.

Number of chews ....

Please put a circle mark on the line scale to answer the questions below.

How easy was it to chew the bread? — Extremely Difficult |————————| Extremely Easy

How easy was it to swallow the bread? — Extremely Difficult |————————| Extremely Easy

How easy was it to bite and get into your — Extremely Difficult |————————| Extremely Easy

How easily did the bread move down — Extremely Hard |————————| Extremely Easy

Did the bread stick in your throat? — Yes, a lot |————————| Not at all

Thank you very much for participating in this project, we appreciate your time.

Appendix 6a. Appetite reducing evaluation questionnaire

ID#__________ Date / Month / 2018

Appetite reducing Testing

Are you (please tick)

- ☐ Male
- ☐ Female

What is your ethnic group(s) (please tick)

O European O Māori O Pacific Peoples O Asian
O Other Ethnicity ....

Do you have any allergies associated with

nuts	☐Yes	☐ No
gluten	☐Yes	☐ No
egg	☐Yes	☐ No
milk	☐Yes	☐ No
food colouring	☐Yes	☐ No

Any other allergies? Please name ....

** If you have any allergies associated with food ingredients that may be in this sample please discuss this with the researcher and do not proceed with this test*

How often do you eat bread?

- ☐ Once per month
- ☐ Once per week
- ☐ Several times per week (number____)
- ☐ Once per day
- ☐ Several times per day (number____)

When did you last eat food or drink anything rather than water? ....

When did you last exercise or did an activity which made you huff and puff? ....

Appendix 6b. Appetite reducing evaluation questionnaire

ID#__________   Date / Month / 2018

Instructions:
You will be required to indicate how you feel in terms of how the bread impacts on your appetite at a particular time measurement by marking a line on the scale before and after eating the bread.

Appetite effect Testing   **Time:..........mins**

Bread sample ....

Are you hungry?
Not Hungry ———————————— Extremely Hungry

Could you eat?
Can eat more ———————————— Too full, cannot eat any more

Are you satisfied?
Less satisfied ———————————— Extremely satisfied

Do you want to eat?
Less desire to eat ———————————— Extreme desire to eat

Appendix 7. Advertisement notice for glycaemia and appetite study

Which bread can improve appetite and blood sugar?

10 Volunteers Required

We are looking for volunteers who eat bread at least 3 times a week, aged 18 years or older and with no known allergy to wheat flour, wheat bran, sweetcorn, canola oil, emulsifiers, sprouted wheat flour, soy flour, acidity regulator, pumpkin flour, iodised salt, yeast, flaxseed and wholemeal wheat flour to test three different bread samples.

You will be required to report at the **Nutrition and Metabolism Laboratory-AUT South Campus** at least 12 hours after your last eating occasion to attend three sessions of bread consumption between 9 am and 11 am. A session will take two hours. You will be required to indicate how the bread improves your appetite and will have your blood taken by a qualified phlebotomist.

You are allowed to bring your laptop and work on it while there if you wish to. A $25 voucher will be given as a token to **appreciate** you for your **time**.

If you would like to participate, please respond before the end of April, 2018.

Phone: Isaac Amoah - 021 0865 9403

Email: isaac.amoah@aut.ac.nz

This project has been reviewed and approved by AUTEC, Auckland University of Technology Ethics Committee

Appendix 8. Record form for participants evaluation for glycaemia and appetite suppression study.

BREAD AND GLYCAEMIA STUDY

Name	
Ethics explained	
Consent signed	
Weight	
Height	

ID#	
Date	
Time last ate or drank	
Age	
BMI	
Time exercised	

Treatment randomised	
Visit 1	
Visit 2	
Visit 3	

Time	Blood sample ID	Event

Appendix 9. Advertisement notice for carotenoid and lipid profile study

Flyer

Which bread can improve your immune system and lipid profile?

10 Volunteers Required

We are looking for volunteers who eat bread at least 3 times a week, aged 18 years or older for a study on how bread affects your immune system and lipid profile.

You will be required to report at the Nutrition and Metabolism Laboratory- AUT South Campus. You will be asked to make two baseline visits, and one extra visit after eating four slices of vegetable-enriched bread a day for two weeks. At each visit, blood will be drawn by a qualified phlebotomist and measured for beta carotenes and lipid profile concentration. Participants will walk in, have any questions answered, have their blood taken and walk out. A session will take a maximum of 30 minutes. A $20 voucher will be given as a token in appreciation of your time.

If you would like to participate, please contact Isaac Amoah.

Phone: Isaac Amoah - 021 0865 9403

Email: isaac.amoah@aut.ac.nz

This project has been reviewed and approved by AUTEC, Auckland University of Technology Ethics Committee

Appendix 10. Food frequency survey for carotenoid and lipid study

Survey for Veggie Meter Foods DATE_____________

ID#_____________

Choose the one best answer by placing a circle around it. A guess is better than leaving a blank.

Are there any **foods that you do not** eat? Yes No Don't know

If yes what are they?

Do you eat **liver** (including liver pate)? Yes No Don't know.

If yes how often every day, most days, every week, every month, once a year

Do you eat **eggs**?

If yes how often every day, most days, every week, every month, once a year

Vegetables and fruits

A serving is a handful. Can be fresh, frozen or canned. Do not count fries or hot chips as a vegetable.

How many servings of **vegetables** do you eat **in one day?**

Circle one	0		1		2		3		4		5		6		7+		Don't know

How many servings of **fruit** do you eat **in one day?**

Circle one	0		1		2		3		4		5		6		7+		Don't know

Which vegetables?

How many servings of **carrot or pumpkin** or do you eat **in one week**?

Circle one	0		1		2		3		4		5		6		7+		Don't know

How many servings of dark **green leafy vegetables (silverbeet, spinach)** do you eat **in one week?**

Circle one	0		1		2		3		4		5		6		7+		Don't know

Dairy foods

How many servings of **milk, cheese, yoghurt** do you eat **in one week**

Circle one	0		1		2		3		4		5		6		7+		Don't know

Do you take any **vitamin supplements**? Yes No

If yes

Name(s) of supplement ……………………………………………………. How often ?______________