Nutritional and sensory properties of protein enriched bread

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Nutritional and sensory properties of protein enriched bread

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Table of Contents

List of Figures

List of Tables

Attestation of Authorship

I hereby declare that this submission is my own work and that, to be the best of my knowledge and belief, 'Nutritional and sensory properties of protein enriched bread', 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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Abstract

Bread is one of the most consumed foods worldwide. It is high in carbohydrate and low in protein content. Nowadays, an increasing number of people prefer healthier food with balanced nutrients. Red meat is known to possess high quantity of complete protein and also rich in micronutrients. Navy bean is rich in fibre and plant protein as well, and it is also a low glycaemic index food. In the present study, I seek to develop a new bread product with rich protein content from red meat and fibre-rich navy bean to improve the nutritional values. The objectives of this research were to investigate physicochemical, sensory attributes and digestibility of wheat bread substituted with red meat and navy bean at different levels. The hypothesis of this study was that the fortification of bread with red meat and navy bean will improve the nutritional values of bread with improved physicochemical, sensory and digestibility. In my study, bread formulations with 25% and 50% of raw beef by weight (containing 66g and 132g meat slurry, respectively), navy bean powder (15% and 30%), and mixed meat slurry and navy bean powder (10M30NB[1], 18M15NB, 20M30NB and 35M15NB) were developed. All samples were then evaluated for their physical, nutritional and sensory attributes. In addition, static *in vitro* digestion model was used to analyse the effects of the new ingredients on the digestibility of the enriched bread. *In vivo* the glycaemic index of bread samples was measured in healthy subjects.

All of the fortified bread samples, except 15NB and 30NB, showed significant increase ($p < 0.05$) in protein content, whereas carbohydrate content was found to decrease significantly ($p < 0.05$) in all bread samples. Substitution of wheat flour with meat and navy bean resulted in significantly lower ($p < 0.05$) loaf volume with an increased loaf weight as compared to the control. Fortified breads were noted to significantly decrease ($p < 0.05$) in the lightness and increase redness (red meat) and yellowness according to the L*, a*, b* colour system. Texture results showed that the 25M, 50M and 35M15NB breads were significantly increased ($p < 0.05$) in hardness as compared to the control. Scanning electron microscopy (SEM) pictures of control bread had more randomly distributed gluten matrix as compared to the fortified breads, which

[1] Samples are expressed as percentage meat (M) and navy bean (NB) content. For example, 10M30NB refers to the bread sample containing 10% meat and 30% navy bean.

had less starch granules embedded into larger protein matrix. Sensory evaluation showed that 15NB bread had the highest score for overall liking attribute. Addition of meat and navy bean contributed to increment the deficient essential amino acids in bread, such as lysine, threonine and leucine. Fortified breads showed relatively lower glycaemic index (GI) value in bread than the control.

Overall, the results showed that the incorporation of meat and navy bean changed the nutritional and physicochemical properties and digestibility of bread and GI values as well. The outcome of the present study will have greater impact on extending the use of meat to develop healthier food products and aligns with the AgResearch programme “Red Meat Combifoods: End-to-end management of protein nutrition” supported by the Crown Research Institute Core Fund of AgResearch Ltd.

Abbreviation

AUTEC – AUT Ethics Committee

EGI – Estimated Glycaemic Index

FAO/WHO – Food and Agriculture Organisation/World Health Organisation

FSANZ – Food Standards Australia New Zealand

GI – Glycaemic Index

HI – Hydrolysis Index

M – Meat

NB – Navy Bean

PDCAAS – Protein Digestibility Corrected Amino Acids Score

PM – Project Mapping

RDI – Recommened Daily Intake

RS – Resistant Starch

SSF – Simulated Salivary Fluid

SGF – Simulated Gastric Fluid

SIG – Simulated Intestinal Fluid

SEM – Scanning Electron Microscopy

TE – micromoles of Trolox equivalents (TE) per 100 gm of sample or Trolox units per 100 gm.

TPA – Texture Profile Analysis

TS – Total Starch

WHC – Water Holding Capacity

Chapter 1 Introduction

Bread, a traditional staple food of western countries, has become increasingly popular worldwide during the last decade (Cauvain, 2005). Bread had been one of the essential elements in daily diet because of its pleasurable flavour, low price, nutritional values and easy storage. Bread is made by mixing dough of flour that is proofed with the addition of yeast, followed by baking. The crust of bread is usually golden brown with white crumbs. Moisture and other chemical components, including gluten-starch, lipids, whey protein, casein, shorten the shelf life of bread (Adeleke & Odedeji, 2010).

Consumers are increasingly demanding for bread with higher quality, freshness, and enhanced nutritional and sensory properties (Lambert et al., 2009). Therefore, improvements in bread making technology and the nutrition value of bread are of importance to meet the demand. In terms of nutrition, bread is an excellent source of carbohydrates, to provide energy. However, other nutritional ingredients such as proteins, vitamins and minerals are relatively poor compared to other food commodities. There is an increasing demand for high protein and low carbohydrate foods by people who are conscious of their health. One of the largest contributing factors for an increase in demand for protein rich food is related to weight management. Moreover, an increasing trend of low GI bread is seen globally. White bread is classified as a high GI food where postprandial blood glucose upon consuming bread spikes within a short period of time, disrupting the metabolism of carbohydrates. This in turn is related to type-2 diabetes, obesity and high blood pressure (Hodge, English, O'Dea, & Glies, 2004).

Food fortification is a good way to meet the demand, and is considered as one of the most efficient way to treat micronutrient malnutrition (Crandall et al., 2013). According to the Codex Alimentarius Commission (1987), food fortification is defined as 'the addition of one or more essential nutrients to a food whether or not it is normally contained in the food for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients' (Lawrence, 2013). Preedy, Watson, and Patel (2011) reported that the fortification of foods has been implemented for many years to prevent nutritional

deficiency related diseases, with particular emphasis on decreasing the extent of micronutrient deficiencies, such as iron and iodine.

Fortification of bread with additional ingredients, such as plant protein, sweet potato flour (Hathorn, Biswas, Gichuhi, & Bovell-Benjamin, 2008) and legume flour (Miñarro, Albanell, Aguilar, Guamis, & Capellas, 2012), have been made in the past. Yet, no study has used red meat and/or navy bean to increase the nutritional value, particularly the protein content of bread. Therefore, this study aims to use red meat, lean beef, and navy bean to improve the nutritional value of bread. A further aim is to examine the modified physicochemical, sensory and digestion properties of the value-added bread and whether the concept of adding red meat and navy bean to bread is feasible or not.

Red meat is from mammals such as beef, sheep and goat (Williams, 2007). McNeill and Van Elswyk (2012) reported that red meat contains a high amount of the proteins with all of the nine essential amino acids and key micronutrients required for the humans. The addition of red meat in bread would not only help close the gap in iron deficiency but also provide high quality proteins and other key micronutrients, such as zinc, selenium, phosphorus, potassium and vitamins B and D (Wood & Rowlings, 2011). It also contains essential fatty acids such as linolenic and linoleic acids. Red meat is known to have a high Protein Digestibility Corrected Amino Acids Score (PDCAAS) of 0.9, where the maximum score is 1.0. In contrast to plant proteins with a PDCAAS between 0.5 to 0.7 (Williams, 2007), red meat is an excellent source of proteins which is well utilised and digested by ~~the~~ humans.

Navy bean (*Phaseolus vulgaris*), also known as haricot bean, has been a traditional food in human diet. It is a good source of proteins and carbohydrates, but also rich in vitamins, minerals and polyunsaturated free fatty acids and is low in fat (Rehman & Shah, 2005). Navy bean is considered to have a low GI value compared to other types of cereals, hence the postprandial glucose from consuming navy beans is low. This has been observed by Jenkins et al. (1981) who have compared dried legumes (31 ± 3%) with other foods such as vegetables (70 ± 5%), breakfast cereals (65 ± 5%), biscuits (60 ± 3%), and fruit (50 ± 5%). They concluded that navy beans were preferred choice of energy, because

the postprandial glucose level remained low. Furthermore, navy beans comprise high levels of starch, including a considerable amount of resistant starch (RS) in the small intestine, which is known for its prebiotic properties (Vargas-Torres et al., 2004). White bread is known to have GI value of 71. Therefore, incorporation of navy beans into bread could enhance the overall protein content and lower the GI.

In order to evaluate the changes of digestibility in foods, scientists have started to use *in vitro* digestion models. The protein content in meat is highly digestible, around (94%) compared with the digestibility of beans (78%) and in whole wheat (86%) (Osorio-Díaz, Tovar, Paredes-López, Acosta-Gallegos, & Bello-Pérez, 2005). Recently, an international consensus on a standardised static *in vitro* digestion method suitable for food has been published (Minekus et al., 2014). Although this method is simple, it can be used to understand the overall digestibility pattern, the changes of structure and the release of food constituents under simulated gastrointestinal conditions (Hur, Lim, Decker, & McClements, 2011). *In vitro* digestion models are used as an alternative tool to animal and human models for screening foods or delivery systems that vary with composition and structure (Hur et al., 2011). Results from *in vitro* digestion of bread has indicated that bread structure and its moisture content influence the overall rate of bread digestion. Bornhorst and Singh (2013) have shown that white bread had slower disintegration compared to almond, barley and wheat bread.

The hypothesis of this research was that addition of red meat and navy bean will increase the nutritional value of bread with improved physical, chemical and sensory properties, as well as digestibility. The objective of this project was to study the effects of red meat and navy bean on the physicochemical, sensory properties and digestibility of fortified breads and thereby help to develop a palatable, high-protein bread.

This thesis is composed of five parts. Chapter two is a literature review summarising the past studies of fortified breads and their physicochemical, sensory and digestion properties and glycaemic index. Chapter three shows the materials and methodology used in the project, including the experimental design and statistical data analysis. In chapter four, results obtained from the study are presented and discussed. The final conclusion

chapter summarises the main findings, explaining the research questions, and suggests avenues of future related research.

Chapter 2 Literature Review

2.1 Bread

Bread is a staple food for the world population (Cauvain, 2005). It is usually made by kneading a wheat-flour into a dough and baking in an oven. Bread is well accepted worldwide, due to its ease of preparation, low cost, pleasant sensory attributes and nutritional values. It is one of the largest food commodities with annual production over 9 billion kg (Venturi & Andrich, 2013). This demand has been driven by consumers' seeking convenient and fresh products that provide a source of nutritional value.

Bread not only provides required daily energy for humans, but also plays a critical role in supplying nutrients for the human body. It is rich in carbohydrates and a good source of plant proteins, B group vitamins and minerals such as magnesium, calcium and iron (Isserliyska, Karadjov, & Angelov, 2001). It is no surprise that bread is defined as an essential part of a healthy diet by nutrition experts. Across many different cultures, types of bread eaten differs. For example, baguettes from France, corn bread from the United States of America, crumpets from the United Kingdom and roti from India, to name a few. Appearance, taste, texture, ingredients and processing methods differ amongst different types of breads. However there is an increase in demand for specialty bread with replacement and/or addition of ingredients with higher nutritional values than bread wheat (Wang, Rosell, & Benedito de Barber, 2002). The term, 'bread', used in this thesis refers to 'white bread' in a loaf form, which is typically made with flour from wheat, water and leavening agent.

2.1.1 Basic ingredients of bread

Wheat flour, water, yeast or other leavening agents, salt and sugar are the five fundamental ingredients of bread (Martin, 2004; Sluimer, 2005). Among these ingredients, flour and water have the most significant effect on determining the texture and crumb of bread when baked. In order to improve the quality of bread by increasing nutritional value and/or to ease the processing, additional ingredients have been added and/or replaced as shown by Jackel (1994) and Sluimer (2005).

2.1.1.1 Flour

Flour is a fine powder usually made by grinding the endosperm portion of cereal grains (seeds) to a powder consistency. It provides structure, texture, and flavour to baked products. Flour for making bread loaves is usually from hard, high-protein wheat. This is because wheat flour is able to give a light, pleasant and well-risen loaf of bread compared to other types of flour. Flour made from oat, rye, barley, maize, and other grains are also used for making bread (Brown, 2010). Each of these grains provides starch and protein needed to form bread. Flour is mainly comprised of starch (70-75%), protein (10-12%) and water (14%), and it also contains lipids (2%), pentosans (~2%) and small amounts of vitamins, minerals and enzymes (Charley & Weaver, 1998).

The protein content of the flour is known to be the best indicator of the quality of the bread dough and the baked bread. While bread can be made from all-purpose wheat flour, specialty bread flour, containing more protein (12–14%), is used for high-quality bread. Table 1 summarises the protein content across different types of wheat flour. Hard wheat flour with high protein content, is divided into whole wheat flour, durum wheat flour, and bread flour. These are preferred for making yeast bread (Carson & Edwards, 2009).

Wheat flour contains three water-soluble protein groups (albumin, globulin, and proteoses) and two water-insoluble protein groups (glutenin and gliadin) (Figure 1). When flour is mixed with water, the water-soluble proteins dissolve, leaving the glutenin and gliadin in the dough. When the dough is kneaded, glutenin and gliadin combine to yield the protein complex gluten. Gluten enables the dough to expand with the inner pressure of gasses from air, steam, and carbon dioxide. The expansion is caused by the combination of elastic property from glutenin and viscous property from gliadin (Aamodt, Magnus, Hollung, Uhlen, & Faergestad, 2005). During kneading, gluten absorbs water and partially unfolds. Unfolding of gluten proteins facilitates hydrophobic interactions with sulphhydryl (–SH) and disulphide (S-S) interchange, resulting in formation of thread-like polymers. These linear polymers interact with one another via H-bonding, hydrophobic interactions and s-s bonds to form a sheet-like film that can entrap and retain gas (Giannou, Kessoglou, & Tzia, 2003). The capability of wheat flour dough to retain gas produced

during fermentation and baking will translate to a well-expanded loaf of bread with a light and even crumb as an end product.

Carson and Edwards (2009) reported that the ability of baked product to rise is directly affected by its protein content. Wheat flour has the highest concentration of the proteins that form gluten, hence it provides light, airy textures to baked products. Therefore it is the most often used type of flour for baking (Brown, 2010). Hard wheat flour, which contains strong gluten and high protein content, has better ability to trap and retain carbon dioxide gas and resulted in higher volume of bread (Cauvain, 2003). Bread flour with higher gluten content is ideal for making yeast breads that require more elastic gluten for rising (Brown, 2010). Compare to the hard wheat, soft wheat has the least protein and the highest starch content, which is ideal for the tender, fine crumb of crackers, cakes, and pastries (Carson & Edwards, 2009). When flour with lower protein content (9–11%) is used, the dough mixing time to develop gluten strength is reduced. An extended mixing time is known to lead to oxidization of the dough, resulting in a whiter crumb, like a cake, instead of the cream colour for artisan breads (Hamelman, 2004).

Too much flour in the dough results in a lower bread volume, an increased number of “tunnels,” and drier and tougher crumb. However a baked product made with insufficient flour often has a course texture and a weak, possibly collapsible, structure (Brown, 2010). Bhattacharya, Erazo-Castrejón, Doehlert and McMullen (2002) have shown that 20% waxy wheat flour could substitute for use of shortening to achieve desirable crumb softness and to retard staling upon storage. Therefore the use of an appropriate amount of flour in bread dough is essential to produce high quality bread.

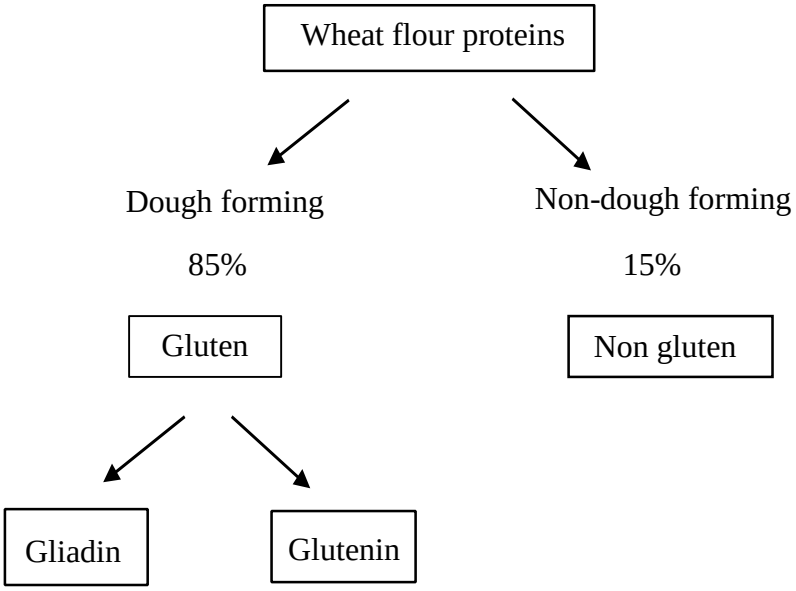


Figure 1 The formation of wheat flour protein

Table 1 The percentage of protein in different wheat flours (Brown, 2010)

Flour		% Protein
Gluten		41
Hard flour	Whole wheat	14
	Durum wheat	13
	Bread	11
All-purpose		10
Soft flour	Pastry	9
	Cake	8

2.1.1.2 Yeast

The traditional way of baking bread is to mix water with flour, salt and the leavening agent (baker's yeast or sourdough starter). Arloris, Coisson and Martelli (1999) reported that baker's yeasts, like *Saccharomyces cerevisiae* strains, have been used for many years because of their desirable dough-leavening characteristics.

The major role of yeast is to convert fermentable carbohydrates into CO_2 and ethanol (Figure 2), under anaerobic condition. The baker's yeasts are sold in the form of dry, fresh

(compressed), or instant yeast. Some of the carbohydrates such as sugars (glucose, fructose, sucrose and maltose) in the flour are fermented by this type of yeasts.
Compared to other strains, baker's yeasts produce uniform, quick and reliable leavening of dough and contribute towards the flavour and support gluten network of bread (Cauvain, 2005).

Yeast（without oxygen) + $C_6H_{12}O_6$ (glucose) $\rightarrow$ 2 C_2H_5OH (ethanol) + 2 CO_2 (gas)

Figure 2 A simplified equation showing the fermentation process

Yeast strains become activated when they are hydrated in water at an optimal temperature, which varies according to the type of yeasts. The optimal temperature for yeast to multiply ranges from 68 to 81°F (20 to 27 °C), while the fermentation is optimal from 81 to 100 °F (27 to 38 °C), more specifically at 95 °F (35 °C) (Brown, 2010). Once activated by the warm water, they are added to the flour mixture. After they are mixed in, the dough is kneaded and allowed to sit in a warm place as carbon dioxide generated by the growing yeast makes the dough rise. As the yeasts multiply and ferment in this warm environment, a small amount of sugar is sometimes added to serve as a food source for them. The mixed dough is allowed to rise for a half an hour under controlled humidity. This is known as proofing. The second rising is optional depending on the type of bread being baked.

The fermentation reaction during dough proofing is highly sensitive to temperature. The fermentation increases with the rising temperature to 40 – 43 °C and becomes inactivated at 55 °C. They also reported that the baker's yeast is able to grow at temperatures 25 to 42 °C under anaerobic condition (Fang, 2008).

2.1.1.3 Water

Water is used to form the flour into a dough or paste. It is also important for the formation of the gluten complex (Larsson & Eliasson, 1991). The weight of liquid used for bread varies between different bread formulae. It is common that a ratio of 3 parts of liquid to 5 parts of flour is used for regular breads (Hamelman, 2004). Water does not

count towards energy or nutrition, therefore contributing in lowering the bread's energy-density (Evaluation & Lilleberg, 2012).

2.1.1.4 Salt

Salt (sodium chloride) contains no calories, proteins or carbohydrates, although unrefined salt does contain traces of other minerals. The sodium and chloride of salt are electrolytes that help the kidneys to regulate the body fluid levels. It is a vital constituent of the human body (Whitney & Rolfes, 2002). Salt carries various functions in bread baking. These include strengthening the gluten network to enhance the dough elasticity and other characteristics such as loaf volume, dough handling, texture, and prolonging the shelf-life by binding water to restrict microbial growth (Salovaara, 2009; Whitley, 2009), improving of the flavour and sensory qualities of bread. Salt is commonly added to bakery formulas at levels from 1 to 2.5% of the flour weight (Fang, 2008). As a staple food in most European countries, bread is one of the main contributors to daily sodium intake (World Health Organization, 2013; Belz, Ryan, & Arendt, 2012). There are initiatives to reduce salt levels in bread across several European countries, ranging from a 10-15% reduction target in Austria and Italy, and up to 30% in Croatia.

One of the most important functions that salt has in the formation of bread dough is to inhibit the yeast to slow down the rate of fermentation (Hamelman, 2004). A small amount of salt prevents yeast from over-fermentation and over-proofing the dough. Salt inhibits the action of flour proteases, which otherwise would depolymerize proteins of the gluten complex. Yeast dough without salt is sticky and is difficult to handle. A low volume, uneven structure, lack of colour, and a bland taste also result from low salt content. In frozen dough products, salt slows the production of carbon dioxide by yeast by delaying the fermentation process (Charley & Weaver, 1998).

Salt helps to strengthen the gluten network. This contributes to making the dough to stretch easier and thus improving the viscoelastic property and texture of the finished bread. Excessive amount of salt results in a firm dough with a low volume because of partially inhibited fermentation, dense cells, and salty taste (Schunemann & Treu, 1988). In a study by Salovaara (2009), additional 2 percent of salt, based on total flour weight, was found

to reduce the CO_2 -formation by approximately 20 percent. Although a small amount is needed, salt is one of the vital ingredients in bread.

2.1.1.5 Sugar

Sugar not only contributes sweetness, but also influences the volume, tenderness, colour, moistness, appearance, and caloric content of bread (Brown, 2010). Sucrose is a disaccharide composed of a unit of dextrose plus a unit of fructose, and is derived from sugar cane or sugar beet (Cauvain & Young, 2006). Sugars contribute to volume by providing food for the yeast, and normally used by yeast during the early stages of fermentation. Later, extra sugars may be added to enhance completion of fermentation by yeast to help rise, to improve the crust colour and to sweeten the bread. Reducing sugars (maltose, fructose and glucose) react will amino acids from protein during baking, which is called Maillard reaction. This reaction contributes to attractive brown colour of bread crust. The higher the concentration of the reducing sugars present, the darker the brown colour produced (Fang, 2008).

Other functions of sugar in flour mixture are to increase moistness and tenderness, and help to improve the shelf life of baked products by delaying staling through the hygroscopic (water retaining), and nature of sugar (Brown, 2010). However too much sugar may result in a lower bread volume, a coarse grain, a gummy texture, and an excessively browned crust for bread. When the sugar content is too low, the baked bread may be drier with reduced browning, lower bread volume, and less tenderness.

2.2 Fortification of bread

The terms “fortification” or “enrichment” were defined by the Codex Alimentarius Commission (1987) as ‘the addition of one or more essential nutrients to a food whether or not it is normally contained in the food for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups’. Food fortification has been used to address nutrient deficiencies around the world in the first half of the 20th century (Dwyer et al., 2014). For instance, the addition of iodine to salt can reduce the risk of goitre; the addition of micronutrients (iron, thiamine, niacin

and riboflavin) to wheat flour and other cereal products is contributed to reduce the risk of iron-deficiency anaemia, beriberi, pellagra and riboflavin deficiency, respectively (Backstrand, 2002).

Bread, as a traditional staple food, provides energy in the form of carbohydrates and most of the nutrients and important micronutrients, such as vitamins and minerals. According to the European Food Safety Authority (2010), at least 50% of daily energy should come from carbohydrates, mainly in the form of starch. However, changes in consumer eating patterns and more conscious of health aspect, have resulted in the demand of the modification and innovations of bread to improve the nutritional values and address population-wide nutrition deficiencies. As a staple food worldwide, bread in particular those made with wheat flour, is one of the ideal vehicles for fortification in different cultures (Preedy et al., 2011).

Bread made with wheat flour is high in carbohydrate, and relatively low in protein, vitamins and minerals. Compared to milk, soybean and pea proteins, wheat protein is poor in nutritional quality with some of the essential amino acids, such as lysine and threonine, being deficient (Bakke et al., 2007 and Jideani et al., 2009). High dietary GI and intake of white bread and starch were associated with increased risk of type 2 diabetes (Hodge et al., 2004). There is a need to improve the nutritional value of bread. Various research has been conducted to enhance the nutritional value of bread. These include increasing dietary fibre (Shittu, Raji, & Sanni, 2007), plant sterol esters (Clifton et al., 2004), antioxidants activity (Jensen, Ostdal, Skibsted, & Thybo, 2011), omega-3 fatty acids (De Conto, Porto Oliveira, Pereira Martin, Chang, & Steel, 2012) and mineral contents (Sanz-Penella, Wronkowska, Soral-Smietana, & Haros, 2013).

In an attempt to fortify bread, many studies have incorporated additional ingredients into wheat flour. These include legumes (section 2.3.1) (Levent & Bilgiçli, 2012; Mubarak, 2001; Ndife, Abdulraheem, & Zakari, 2011; Shin, Kim, & Kim,
2013; Viswanathan & Ho, 2014), cereals (section 2.3.2) (Ameh, Gernah, & Igbabul,
2013; El-Adawy, 1997; Sciences, 2010; Torbica, Hadnadev, & Dapčević, 2010), herbs (section 2.3.3) (Das, Raychaudhuri, & Chakraborty, 2012, 2013), fruits (Hossain, 2014;

Sun-Waterhouse et al., 2011; Zabidi & Yunus, 2014) or vegetables (El-demery, 2011; Hathorn, Biswas, Gichuhi, & Bovell-Benjamin, 2008) (section 2.3.4), to boost the nutritional value of bread. Table 2 further summarizes fortified breads in the literature with changes in physicochemical, nutritional and sensory properties. In this study, increasing the protein content of the wheat flour by adding red meat (lean beef) and navy bean will markedly improve its nutritional value.

Table 2 A table summarizing value-added breads and the changes in physicochemical, nutritional and sensory properties

Bread ingredients	Main type	Added ingredients % (w/w)	Change in physicochemical properties	Change in nutritional properties	Change in sensory properties	References
Legumes						
White bread	Wheat flour	14,16,18 & 20% Sesame seed protein	Loaf volume decreased; Longer dough development time	Increase in protein, minerals, total essential amino acids content, with improved protein digestibility and computed biological value	N/A	El-Adawy, 1997
Wheat bread	Wheat flour	3,6,9 and 12% Sweet lupin seed flour	Water absorption increased	Increase in protein and total essential amino acids, with improved protein digestibility	N/A	Mubarak, 2001
Wheat bread	Wheat flour	5,10,15 and 20% Soy flour (full fat and defatted)	Water absorption increased; loaf volume decreased; Longer shelf life with defatted soy flour supplementation	Protein and fat content increased; Total lysine content of 10% level increased	10% soy flour most acceptable	Dhingra & Jood, 2002
Wheat bread	Wheat flour	Carob fibre; inulin; pea fibre	Specific loaf volume decreased	Fibre content increased	All addition of three fibre are acceptable	Wang et al., 2002
Wheat bread	Wheat flour	1 and 3% Defatted palm date seeds	Bread volume decreased; crumb firmness slightly increased	The dietary fibre content increased	N/A	Bouaziz et al., 2010
Whole wheat bread	Whole wheat flour	10, 20 and 30% Soybean flour	N/A	Protein, crude fibre and mineral content increased	10% soybean flour most acceptable	Ndife et al., 2011

Flat bread	Wheat flour	30% lupin, buckwheat and oat flours and their blends (10% each)	Buckwheat increased darkness and redness in colour	Protein and minerals content increased	Higher acceptability for 30% oats flour and the blends	Levent & Bilgiçli, 2012
Soy bread	Soy flour	Raw, germinated, steamed, roasted soy flour	Germinated had the highest loaf volume and softest texture	Steamed soy flour had the highest moisture content. Heat-treated had higher isoflavone contents	Steamed and roasted improved flavour, higher acceptability	Shin et al., 2013
White flat bread	Strong white flour	5, 15 and 25% Sprouted red kidney bean	N/A	1% increase in protein; 12% decrease in protein digestibility	N/A	Viswanathan & Ho, 2014
Cereal						
Pan bread and Balady bread	Wheat flour	0,5,10,15, and 20% Whole or decorticated sorghum-wheat composite flours	Decreased specific volume; colour was increased in grayish, reddish and yellowish. Hardness and gumminess were increased	N/A	20% was most acceptable	Abdelghafor et al., 2011
Wheat bread	Wheat flour	5, 10, and 15% Stabilized undefatted rice bran	Loaf weight increased; loaf volume decreased	Increased moisture, protein, fat, fibre and ash content; Decreased carbohydrates content	100% wheat had better acceptability scores	Ameh et al.,2013
Wheat bread	Wheat flour	Up to 40g/100g Whole amaranth flour	An increase in crumb hardness and elasticity	Protein, lipid, ash, dietary fibre and mineral contents increased	N/A	Sanz-Penella et al., 2013

Herbs						
White bread	Wheat flour	1, 3, 5, and 7% coriander leaf powder	Better crumb moisture	Sharply increased antioxidant content	3 and 5% had the highest acceptability	Das et al., 2012
Wheat bread	Wheat flour	3, 5, 7, 10, and 15% fennel seed powder	Loaf volume decreased; L* value decreased and a* and b* values increased for the crumb	Crumb moisture increased; antiocidant activity inceased up to 7.0%	Fennel seed between 5 and 7% had the highest acceptability	Das et al., 2013
Fruit and vegetables						
Whole-wheat bread	Whole-wheat flour	50, 55, 60 and 65% Sweet potato flour	Fluctuated moisture contents;	Bread with 50% sweet-potato flour was highest in protein content.	N/A	Greene & Bovell-benjamin, 2004
Wheat bread	Wheat flour	0, 5, 10 and 15% Pumpkin flour	Loaf volume and specific volume were significantly different compared to control	Increased ash and crude fibre; decreased protein and fat content.	5% pumpkin flour most acceptable	See et al., 2007
Wheat bread	Wheat flour	5, 10 and 15% Jackfruit rind flour	Loaf volume decreased; hardness and darkness increased.	N/A	5% incorporated bread had the highest overall acceptance.	Feili et al., 2013
Wheat bread	Wheat flour	25, 35, 45 and 55% Jackfruit seed flour	Decreased colour, flavour, and texture.	Protein, crude fibre and ash content increased.	25% jackfruit seed flour most acceptable	Hossain et al., 2014
White bread	Wheat flour	0,5,10, and 15% Matured soursop flour	5% had the lowest hardness.	Increase in fibre, protein and ash content; Decrease in fat and carbohydrate content.	5% soursop had good acceptability	Zabidi & Yunus, 2014

Others						
White bread	White flour	0,0.1,0.2, 0.4 and 0.8% Lysine Ⅱ	N/A	The protein content improved with lower lysine content; The growth rate of rats improved	N/A	Rosenberg & Rohdenburg, 1952
Wheat bread	Wheat flour	0.017, 0.034, 0.069, 0.10, and 0.14% Maitake mushroom powder	0.14% maitake and EDTA has increased loaf volume	Protein content increased.	N/A	Seguchi et al., 2001
Wheat bread	Wheat flour	5,10,15 and 20% Tilapia Fish Flour	N/A	Protein and fat content were increased; carbohydrates was decreased	N/A	Adeleke & Odedeji, 2010
White bread	Wheat flour	3, 6% pectin; 3% phenolic extract	Breads weight slightly increased; Colour changed.	Bread with added phenolic extracts had greater antioxidant activity and higher extractable phenolic	N/A	Sivam et al., 2011
White pan bread	Wheat flour	0.00-5.00g/100g omega-3; 0.000-0.100g/100g rosemary extract	Specific volume and lightness decreased and firmness and colour saturation increased (the addition of omega-3)	N/A	All had acceptable scores (>5)	De Conto et al., 2012
Wheat bread	White flour	Vitamin A: 30000 and 70000 retinol equivalents (RE) kg^{-1}	Stable	N/A	NS	Crandall et al., 2013

N/A: data is not available. NS: no significant change

2.2.1 Legumes

Legumes, which includes beans, peas and other podded plants, are rich in protein, carbohydrates, minerals and vitamins (Messina, 1999). Protein content in legume is high (15-30%), and they are rich in lysine content but are deficient in sulphur-containing amino acids (cysteine and methionine) (Preedy et al., 2011).

In general, bread protein is an incomplete source because it lacks lysine, which is one of the nine essential amino acids. Breads can become a complete source of protein when they are combined with other plant foods like pulses and legumes, to make up for the deficient lysine. Schuszter (2004) reported that legume seeds are promising materials as nutritional ingredients for food industry. In addition, the isolated protein and fibres of legume seeds have great physicochemical and health protecting values. Fortification of bread with richer protein sources such as soy flour (Dhingra & Jood, 2002; Ndife et al., 2011), and sweet lupin seed (Mubarak, 2001), sesame seed protein (El-Adawy, 1997), and red kidney bean (Viswanathan & Ho, 2014) have been studied previously.

Protein deficiency is a serious problem for vegetarians who depend on cereal or starchy food. For example, a deficiency of L-histidine may cause anaemia; a shortfall of isoleucine lead to headaches and giddiness; undersupply of protein C and protein S are cause abnormal blood clotting. On the severe side, cachexia is a disease that causes the weakening of the skeletal muscles and reduction of protein（Dewettinck et al., 2008）. Fortification of bread with legume flours has been found to increase the protein and mineral content. Dhingra and Jood (2002) supplemented bread with soybean flour. Up to 20% soy flour was incorporated into bread, resulting in increased protein content (13.81%) and lysine content (3.05g/100g) compared with control bread (11.47% and 2.36g/100g, respectively). A study conducted by Nidife et al. (2011) reported a similar result of increased protein and mineral contents with 30% soybean flour-enriched bread having higher protein content (11%) than the control bread (8%), and increased mineral content from 2.5% to 5%. In addition, Mubarak (2001), Levent & Bilgiçli (2012) and Viswanathan & Ho (2014) reported that incorporation of lupin seed, buckwheat, oat flours and red kidney bean in bread increased the content of protein and total essential amino

acids, especially lysine. Rastogi and Singh (1989) have found that the profile of essential amino acids in soybean complemented the deficient essential amino acids of cereals when soybean was added to cereal-based products.

Legume-enriched bread has been reported to change the physical properties, especially loaf volume. Dhingra and Jood (2002) observed a significant reduction in loaf volume as the level of substitution with soy flour increased. This is due to dilution effect on gluten with the addition of non-wheat with no gluten to wheat flour with gluten. Gluten content is a direct indicator of flour strength and viscoelastic properties of bread (Dhingra & Jood, 2002). Therefore a decrease in gluten would have contributed to a decrease in retention of CO_2 gas, causing a decrease in the loaf volume (Sharma & Chauhan, 2000). This would further result in a denser and more compact structure in the resulting bread.

Addition of legumes to bread has been found to improve the digestibility of proteins. El-Adawy (1997) reported that the low protein digestibility of wheat flour could be improved by mixing with highly digestible protein such as sesame protein. Similarly, another study was conducted by Mubarak (2001) also found that the incorporation of lupin products to bread increased its protein digestibility over control samples. On the contrary, a study on fortification of bread with red kidney bean reported that the protein digestibility decreased 12% compared with control bread (Viswanathan & Ho, 2014).

As shown in Table 2, fortified bread with legumes varied in their acceptability and sensory attributes. Bread incorporated with 10% soy flour (Dhingra & Jood, 2002; Ndife et al., 2011) had good acceptability. Another study carried out on bread with 30% oat flour and blends of 10% lupin, 10% buckwheat and 10% oat flour had higher acceptability scores than the controls (Levent & Bilgiçli, 2012). Therefore, the fortification of bread with legumes not only changes the nutritional properties but also can have a significant influence on consumer preference and acceptance.

2.2.2 Cereal

Cereal grains are staple crops which grow in greater quantities and provide more food energy worldwide (Cordain, 1999). However, the nutritional and sensory values of

cereal grain products or cereal-based diets are inferior to those of animal-based diets (products). Nevertheless, Preedy et al. (2011) indicated that genetic engineering, fortification with certain amino acids and nutrients, and complementation with other proteins (notably legumes) can be used to improve the nutritive value of breads. Use of traditional grains and novel flour such as amaranth (Sanz-Penella et al., 2013), rice bran (Ameh et al., 2013) and sorghum (Abdelghafor et al., 2011), to improve the nutritional value of bread and also in order to meet the demands or requirements of targeted groups with special food needs exists in the literature (Preedy et al., 2011).

The amaranth grain can be milled into flour and consumed by humans or used as a functional ingredient in cereal products, such as bread, cookies, noodles and muffins (Sanz-Penella et al., 2013). Amaranth grains have high protein content (11-17%) with a large amount of lysine than most of cereal grains, and they also can complement essential amino acid composition (Oszvald et al., 2009). Moreover, the protein digestibility corrected amino acid score (PDCAAS) of amaranth whole flour is higher (0.64) compared to other cereal grains. Sanz-Penella et al. (2013) studied the incorporation of amaranth flour (up to 40g/100g) in bread, which resulted in a significant increase in protein, lipid, ash, dietary fibre and mineral contents. Bodroza-Solarov et al. (2008) also reported similar results with the use of amaranth flour in bread. They found protein content increased up to 4.4g/100g with maximum levels of substitution of 20g/100g.

Generally, white bread has a low mineral content and should be supplemented to meet the daily requirements for different elements (Skrbic & Filipcev, 2008). Another study conducted by Dyner et al. (2007) showed that the bread and pasta made with wheat and amaranth flours increased mineral and dietary fibre contents in bread at levels up to 20g/100g. Furthermore, Ameh et al. (2013) incorporated stabilized undefatted rice bran in bread, resulting significantly increased protein, fat and fibre contents from 12.04% to 13.10%, 1.57% to 3.77% and 1.76% to 2.91%, respectively. Importantly, incorporated rice bran also increased B-group vitamin content, such as thiamine and Niacin. This is due to rice bran being a rich source of B-vitamins (Farrell, 1994 and Dale, 1997).

As summarised in Table 2, fortification of bread with new cereal flours has also been reported to change the loaf volume like legumes do. Preedy et al. (2011) indicated that composite breads tend to have lower volume and height, resulting in denser and more compact structure due to the reduction in gluten. A study conducted by Abdelghafor et al. (2011) indicated that pan and flat breads supplemented with sorghum flour significantly decreased specific volume of bread. A previous study (Perten et al., 1980) reported a similar result that the addition of sorghum to wheat flour negatively affect (decrease) the volume of bread. In recent research, bread with rice bran also decreased the loaf volume from 655.2 ml to 586.0 ml while loaf weight increased (Ameh et al., 2013). Oladunmoye et al. (2010) speculated that composite flour blends contain less gluten and this results in improper rising of bread through fermentation and proofing with lower retaining ability to retain CO_2 gas.

With regards to sensory attributes and fortification, bread with sorghum-wheat composite flour has been accepted by consumers at level up to 20% level (Abdelghafor et al., 2011). In addition, FAO (1995) reported that acceptability studies conducted by Food Research Centre (Khartoum, Sudan), showed that fortification of bread with composite flour of 70% wheat and 30% sorghum was found to be the most acceptable from consumer test. In contrast, the result of Ameh et al. (2013) indicated that 100% wheat flour bread was more acceptable than composite wheat-rice bran bread with different levels of rice bran supplementation. The eating habit and pattern may have contributed to this result.

2.2.3 Herbs

Herbs have good nutritional values such as high minerals and vitamins contents, natural antioxidants and flavouring agents (Das et al., 2013). It is a new trend to improve nutritional value and sensory properties of common white bread by adding herbal ingredients (Das et al., 2012, 2013).

Research conducted by Das et al. (2012, 2013) indicated that fortified bread with herbal ingredients such as coriander leaf powder and fennel seeds not only significantly increased antioxidant activity, but substantial improvements in sensory characteristics

was also observed. More specifically, Das et al. (2012) reported fortified bread with coriander leaf powder greatly improved taste of bread with a spicy flavour and enhanced the antioxidant activity from 3.052 to 8.767 (TE/100 grams) at the level of 7% supplemented bread. In their study of 2013, Das and his colleagues used fennel seed powder to partly substitute wheat flour, and reported that this supplementation achieved higher consumer acceptability compared to the control. Similarly, antioxidant activity showed an increase in bread with a 7% supplement of fennel seed powder by weight.

2.2.4 Fruit and vegetables

Besides using legumes, cereals, and herbal ingredients, fruits and vegetables such as jackfruit seed (Hossain et al., 2014), soursop flour (Zabidi & Yunus, 2014), sweet potato flour (Greene & Bovell-benjamin, 2004), and pumpkin flour (See et al., 2007) have been added to bread to improve nutritive and sensory properties.

Substitution of part of the wheat flour with non-wheat flours has been advocated for nutritional enrichment of bread. Although most research has focused on increasing the protein content, the addition of carotenoid-rich flours has recently been suggested (Preedy et al., 2011). Greene and Bovell-Benjamin (2004) studied bread supplemented with sweet potato flour (50%, 55%, 60% and 65%). Bread with 50% substitution to sweet potato flour bread had the highest protein value, and the β-carotene content increased as the levels of sweet potato flour increased. However, this fortification had a negative influence on the loaf volume of bread. Inadequate amount of gluten was present to support the structure and loaf volume and that resulted in dropping the quality of bread. Rangel et al. (2008) evaluated the use of bio-fortified sweet potato flour (10, 20 and 30%) in the production of bread (sandwich loaves), and observed that the bread loaves produced with sweet potato flour had lower loaf volumes and darker colour with characteristic flavour as the sweet potato flour levels increased. Substitution of 10-15% of wheat flour with sweet potato flour has been reported in the literature as the most acceptable. The decrease of loaf volume was observed by See et al. (2007) as well when pumpkin flour was used to substitute wheat flour in bread. However, the protein and fat contents in the supplemented bread with pumpkin flour showed lower values compared to the control,

which was due to higher protein content of wheat flour (14.27%) than pumpkin flour (9.65%).

More recently, Jackfruit seed flour (Hossain et al., 2014), jackfruit rind flour (Feili et al., 2013) and matured soursop flour (Zabidi & Yunus, 2014) have been used to develop high fibre bread. Those fortifications resulted in significantly higher protein and fibre contents. In addition, they also improved the sensory characteristics. The score of sensory evaluation showed that 25% jackfruit seed flour bread, 5% jackfruit rind flour and 5% soursop flour bread received good acceptability and was compatible with the control.

2.2.5 Others

Other ingredients such as mushroom powder, fish flour, omega-3 and vitamin A and iron have also been reported to supplement into bread as additional ingredients.

Maitake mushroom has many health benefits for people. For instance, antitumor activity from β-D-glucan (Mizuno et al., 1986) to ameliorate glucosuria with its various bioactive components (Ohtsuru et al., 1999) and better control of blood glucose and water consumption (Horio & Ohtsuru, 1995) for diabetic patients. Fish flour has attractive colour, pleasant flavour, with low moisture content and relatively high protein content. Nutritional studies have reported that fish protein concentrate can be added to the diet of weaning infants and nursing mothers, which helps to improve the nutritional values of their diet (Adeleke & Odedeji, 2010). As shown in Table 2, incorporation of maitake mushroom powder, tilapia fish flour in bread has increased nutritional properties, especially protein content (Seguchi et al., 2001; Adeleke and Odedeji, 2010).

Additionally, Wang et al. (2002) reported that fibre plays a vital role on the reduction of coronary heart-related diseases and diabetes. Hence, they have reported the use of various fibre sources, such as carob fibre, inulin, pea fibre, as fibre-enriching agents in bread making. The results revealed that fibre supplementation decreased the loaf volume, while increased the daily intake of fibre with overall acceptability of the resulting breads.

As shown in Table 2, research have also introduced apple pectin fibre and phenolic antioxidants, omega-3 and rosemary extract, and vitamin A and iron source (Sivam et al., 2011; De Conto et al., 2012; Crandall et al., 2013) as fortified ingredients in order to increase nutritive values of bread.

2.3 Nutritional value of red meat and navy bean

The purpose of this study was to explore the effects of red meat (lean beef) and navy bean powder in addition to bread formulation to improve its nutritional values. Therefore, this section reviews the nutritional values and health benefits of the two protein sources used in this study.

2.3.1 Red meat

According to the Food Standards Australia New Zealand (FSANZ) Food Standards Code, the definition of meat is 'the whole or part of the carcass of any buffalo, camel, cattle, deer, goat, hare, pig, poultry, rabbit or sheep, slaughtered other than in a wild state, but does not include avian eggs, or fetuses or part of fetuses.' In North America and Europe, meat from herbivores such as beef cattle, sheep, and swine serves as an important source of complete protein (Brown, 2010).

Meat has been traditionally considered a highly nutritious food essential for optimal growth, human health and development. It is associated with good health and prosperity. As a protein rich and low carbohydrate food, meat is classified as a low glycaemic index food (Biesalski, 2005). Diets with a high glycaemic index are thought to be associated with or to favour insulin resistance (Frost, Leeds, Trew, Margara, & Dornhorst, 1998). Red meat has a low glycaemic index and may not contribute to the metabolic syndrome as long as its fat/energy content does not contribute primarily to the daily energy intake (Biesalski, 2005).

Biesalaski (2005) and Williamson (2005) reported that red meat is a significant contributor to the intake of key nutrients in a healthy diet. In addition, meat is a valuable

source of dietary protein and the best sources of bioavailable iron in Australian diet (National Health and Medical Research Council, 2003). Red meat is not only an excellent source of high biological value protein, but it is also an important source of a wide range of micronutrients such as iron, selenium, zinc, vitamin A, B6, B12, niacin, phosphorus and folic acid (Biesalski, 2005; Williams, 2007). These micronutrients are either not present in plant derived food or have a poor bioavailability and they are needed for good health in humans (Williamson et al., 2005).

Red meat contains a range of fats, including essential omega-3 polyunsaturated fats (Williams, 2007). In addition, raw red meat contains about 20-25 % of protein, and cooked red meat has around 28-36 % of protein due to the decrease of water content and more concentrated nutrients during cooking. Bhutta (1999) reported that the protein in meat is highly digestible (94%), compared to the beans (78%) and whole wheat (86%). Red meat is a rich source of essential amino acids and have a high Protein Digestibility-Corrected Amino Acid Score (PDCAAS) of 0.9, where the maximum score is 1.0. Plant proteins have a PDCAAS between 0.5 to 0.7 (Williams, 2007). It can be of particular benefit to those where muscle tissue is being rebuilt, e.g. athletes, post-surgery. It is also rich in taurine, essential in new-born infants who are less able to synthesize this amino acid from cysteine (Higgs, 2000).

Table 3 shows typical nutrient composition of lean red meat (beef and lamb), based on recent analyses of Australian retail samples (Sinclair et al., 1999; Williams et al., 2007), and compares this with the new Australian recommended dietary intakes (RDI) (NHMRC, 2006).

Table 3 General Nutritional composition (per 100g) of red meat (NHMRC, 2006)

Nutrients	Lean beef[a]	Lean lamb[b]	Adult RDI[c]
Energy (kJ)	757.00	855.00	5,600-18,600
Protein (g)	30.40	27.50	46-81
Fat (g)	6.60	10.50	-
Cholesterol (mg)	50.00	66.00	-
Sodium (mg)	55.00	78.00	460-920
Calcium (mg)	5.00	19.00	1000-1300
Potassium (mg)	400.00	281.00	2800-3800
Iron (mg)	3.80	2.50	8-27
Magnesium (mg)	25.00	28.00	310-420
Selenium (μg)	8.70	6.00	60-75
Zinc (mg)	6.30	4.70	8-14
Thiamin (mg)	0.50	0.17	1.1-1.4
Riboflavin (mg)	0.21	0.39	1.1-1.6
Niacin (mg)	5.00	5.20	14-16
Pantothenic acid (mg)	0.35	0.74	4-6
Vitamin A (μg)	＜5.00	8.60	700-900 μg RE[d]
Vitamin B_6 (mg)	0.34	0.32	1.3-2.0
Vitamin B_{12} (μg)	2.60	2.40	2.4-2.8

[a]Mean values for diced, stir-fry, round, rump, topside, silverside, fillet, sirloin, scotch fillet, T-bone, blade and chuck steak.
[b]Mean values for diced, stir-fry, leg roast, easy-carve roast, mini-roast, chump chop, loin chop, cutlet and easy-carve shoulder. [c]RDI = recommended dietary intake. [d]RE = retinol equivalents (=1 μg retinol or 6 μg or beta-carotene)

In general, lean red meat has a relatively low fat of less than 7%. It is a great source of protein, niacin, vitamin B6, vitamin B12, phosphorus, zinc and iron, with 100g providing more than 25% of the adult males RDI of these nutrients. It also provides more than 10% RDI of riboflavin, pantothenic acid, and selenium (Droulez & Levy, 2006; Williams, 2007). According to the 1995 Australia Nutrition Survey, the consumption of red meat provided less than 5% of the average adult energy intake, but over 10% of the protein, and more than 13% of dietary zinc intake (McLennan & Podger, 1998).

It is not only important to understand the nutritional values of meat, but also the functional properties of meat. Pedersen et al. (2003) stated that lean meat is a complex biological system, which contains up to 75% water, with the balance being made of 20% protein, around 2% fat and about 3% minor components such as minerals, phosphorous compounds and vitamins. Grau and Hamm (1956) defined the water-holding capacity (WHC) as the ability of meat to retain both inherent water and added water. The WHC of

meat is one of the important functional properties relevant to this study, as it has a significant influence on product quality such as appearance, juiciness and tenderness (Cheng & Sun, 2008). It can lead to poor acceptability of product and decrease the consumption if water content decreases severely. Accordingly, improving WHC of meat is becoming important in the meat industry (Maribo et al., 1998). Some ingredients have been used to improve the WHC of meat based on their functional properties. For instance, addition of salt, functional animal protein, functional plant protein and vegetable oils. In present study, meat added into the bread was in the form of a slurry in order to get a consistent dough mixture. The meat slurry was prepared by adding water and salt. Thus the effect of addition of salt is more important for this study.

The addition of salts, such as NaCl, produces an increase in the water-binding capacity of the meat, being this the basis for cured and emulsified products like sausages. As chloride ions bind to the protein, a repulsion force is exerted between filaments causing the swelling of the structure (Hamm, 1960). Additionally, sodium chloride also weakens the binding of actin and myosin (Offer & Trinick, 1983). Sodium chloride/salt has the ability to adjust the iconic strength of meat with the purpose of improving the WHC of meat. Sodium chloride plays an important role in the solubilisation of myofibrillar proteins for subsequent denaturing/aggregation to give good water retention and acceptable rigidity/elasticity of the meat gels (Gordon & Barbut, 1992).

2.3.2 Navy bean

Navy beans, also known as haricot beans (*Phaseolus vulgaris*), are widely grown and consumed around the world (Chung, Liu, Peter Pauls, Fan, & Yada, 2008). They are excellent sources of proteins (20-25%), complex carbohydrates (50-60%), and good sources of minerals, vitamins and polyunsaturated free fatty acids (Rehman, Salariya, & Zafar, 2001; Reyes-Moreno & Paredes-Lopez, 1993). Beans contain a large amount of resistant starch and fibres that are resistant to digestion in the small intestine. There are passed to the large intestine where they act as the substrate for bacterial fermentation producing short chain fatty acids. They prevent blood glucose levels from rising rapidly

after a meal, resulting in reduced glycaemic and insulinemic responses, and thus beans have been considered as low GI foods (Foster-Powell & Brand Miller, 1995).

Consumption of low GI foods could contribute to reduced incidence and prevalence of heart disease, cardiovascular disease, diabetes, obesity, and also some forms of cancer. The reduced digestibility of legumes including beans has been attributed to their high content of viscous soluble dietary fibres, the presence of various anti-nutrients and relatively high amylose content in starch.

Navy beans are small, pea-sized beans that are creamy white in colour. They are mild-flavoured, dense and smooth. Like other common beans, navy beans are one of 13,000 species of the family of legumes, or plants that produce edible pods. Combined with whole grains such as rice, navy beans provide virtually fat-free high quality protein.

Navy bean offers extraordinary health benefits. It is loaded with antioxidants (Table 4) and provides a good supply of detoxifying molybdenum, and it is also a good source of fibre and protein. In addition, navy bean delivers a good source of magnesium, a mineral with multiple health benefits.

Table 4 The World's Healthiest Foods Rating of nutrients in navy bean

Navy Beans, cooked, 1.00 cup, 182.00 grams, Calories: 255
GI: low

Nutrient	Amount	DRI[a]/DV (%)	Nutrient Density	World's Healthiest Foods Rating
fibre	19.11 g	76	5.4	excellent
folate	254.80 mcg	64	4.5	very good
manganese	0.96 mg	48	3.4	very good
copper	0.38 mg	42	3.0	good
phosphorus	262.08 mg	37	2.6	good
vitamin B1	0.43 mg	36	2.5	good
protein	14.98 g	30	2.1	good
magnesium	96.46 mg	24	1.7	good
iron	4.30 mg	24	1.7	good

[a]DRI: Dietary reference intake.

Navy bean is shown to contain significant amounts of slowly digestible starch (SDS) and resistant starch (RS) (Chung et al., 2008; Sandhu & Lim, 2008). It is considered a low glycaemic index food, because it is rich in slow-digesting carbohydrates, protein and fibre.

In my study, navy bean was used as an additional ingredient in bread to improve the nutritional value. Navy bean is considered as a functional ingredient to improve nutritional quality of a variety of food products.

2.4 Sensory analysis

Food quality is closely related to the sensory properties of food products (Civille, 1991). It is defined as the acceptance for the sensory attributes of a product by the regular users of the product. Sensory analysis is becoming more crucial in new product development because of its value of sensory techniques and the consumer industry increases its use of sensory evaluation (Choi, Phillips, & Resurreccion, 2007).

Even though there is an increasing number of consumers interested in the health aspect of food, about 80% consumers are still concerned about the sensory attributes of bread, which they consider the most important quality criteria. Hence it is necessary to understand liking or preference of consumers using sensory evaluation. Sensory perception plays a vital role in the preference of consumer and purchase of bread (Elía, 2011).

2.4.1 Consumer testing

Consumers are becoming more conscious about the relationship between nutrition and health. In the present, innovations in bread are mainly focused on nutritionally improving bread through enrichment or the use of different flours and ingredients (Preedy et al., 2011). Hence, consumer testing provides an important tool in understanding the consumer expectations of different bread varieties in order to help product developers and manufacturers to achieve their target and produce a successful product (Choi et al., 2007).

For consumers, the sensory quality, especially “taste”, is reported to be the most important purchase criterion (Torjusen et al., 2001).

2.4.2 Projective mapping

Sensory profiling is a class of method for the description and quantification of sensory attributes as perceived by humans. Pagès, Cadoret and Lè (2010) stated that traditional methods of sensory analysis involved *discrimination* (between products), *description* (of products within a group) and *hedonic judgments* of food products. Descriptive analysis (DA) is a common method used for product development, quality evaluation and marketing (Abdi &Valentin, 2007). Although most sensory analyses still use trained assessors in an expert panel, it is a time-consuming and financially expensive method. There is increasing awareness on consumers’ perception of food rather than just on the level of expert panels (Giacalone, 2010/2011). New sensory descriptive methods should not only give sensory data, but should also reflect consumer perceptions. Projective mapping (PM) is a fast alternative to traditional DA, and is becoming more popular among sensory scientists to obtain a quick overview of (dis)similarities among a certain sample set (Hopfer & Heymann, 2013).

Risvik et al. (1994) stated that ‘PM is a two-dimensional descriptive method, which requires the untrained consumers to place products on a two dimensional surface, based on their own choice of perceived similarities and differences in the samples.’ They also suggested that the technique could be a potential tool to help relate sensory analysis and consumer research. An advantage of projective mapping over descriptive analysis is that it can be used to evaluate the importance of product attributes to consumers (Kennedy & Heymann, 2009).

PM methods have been used in many studies on different kinds of food including chocolate (Risvik et al., 1994), blueberry soups (Risvik et al., 1997), snack bars (King et al., 1998), ewes milk cheeses (Barcenas et al., 2004), wines (Pagès, 2005, Perrin et al., 2008, Perrin & Pages, 2009, Ross et al., 2012, Torri et al., 2013), citrus juices (Nestrud & Lawless, 2008), milk and dark chocolates (Kennedy & Heymann, 2009), apple and cheese

(Nestrud & Lawless, 2010), fruit smoothies (Pagès et al., 2010), granola bars (Kennedy, 2010), lemon ice tea (Veinand et al., 2011), bread and yoghurt (Normann, 2012), probiotic yoghurt (Cruz et al., 2013), fruit and vegetables (Mielby et al., 2014), and cheese pies (Marcano et al., 2015).

Table 5 summarises different food products that have been characterized based on their sensory properties using projective mapping. In most of these studies, projective mapping methods were compared with other methods (descriptive analysis, sorting, and conventional profiling). Projective mapping was found to be reliable when carried out with different sample size and panellists (trained and untrained panel), thereby providing accurate results or similar results to other methods.

Table 5 Summary of projective mapping (PM) studies on food products

Products	Aims	Number of panellists	Statistical analysis	Findings	References
5 Chocolates	To compare maps obtained from PM, CP, (dis)similarity scaling techniques	9 trained panellists	GPA, RV coefficient to compare methods	PM is able to link consumer and sensory studies	Risvik et al., 1994
7 Blueberry soups	To compare maps obtained from PM using naive consumers and DA from trained panellists.	8 consumers in PM; 12 trained subjects	PCA on normalized PM data; RV coefficient to compare methods	High agreement between DA and PM in the first dimension only Consumers pulled out major differences similar to DA, but not smaller ones For each PM replicate large individual differences were found	Risvik et al., 1997
18 Commercial snack bars	To compare maps obtained from sorting, structured & unstructured PM using different analysis	2 untrained panels (24 judges, 1 for sorting, 1 for PM)	MDS, GPA for unstructured PM and sorting; CA on structured PM; RV coefficient to compare structured and unstructured PM methods	MDS or GPA for unstructured PM and sorting were more effective than CA analysis for structured PM. PM procedure is more easier for panellists to change their minds	King et al., 1998

8 Ewes milk cheese	To compare maps obtained from PM using trained and untrained panellists.	8 trained consumers for DA and 12 untrained panellists for PM	INDSCAL (individual difference scaling) to check for panel reproducibility and discrimination power; Correlation coefficient between replicate sessions stress and RSD	Trained panel is better than consumer panel using PM Lower correlation for consumers than for DA panel	Barcenas et al., 2004
10 White wine	To evaluate a new method of data collection and to determine the dimensions of perception of a panel about a set of Touraine wines	2 panels (11 wine professionals and 8 wine tasting experts)	MFA to analyse two groups of data	The direct collection of perceived inter-products distance by each subject can be related to free choice profiling, and provides more information easier	Pagès, 2005
11 Citrus juices	To compare PM and traditional attribute scaling	14 chefs and 16 consumers	MFA, GPA for PM and Scaling data; PCA also for Scaling data	Consumer panel produced similar maps in both PM and scaling methods, but chefs did not	Nestrud & Lawless, 2008
10 Wine	To compare CP, UFP and FP to complete a wine Napping	17 judges for DA; 12 wine professionals for PM; 12 wine professionals for FP	HMFA on all three methods; MFA on PM	All three methods produced similar product maps	Perrin et al., 2008
14 Milk and dark chocolate	To compare product maps obtained from PM and DA by untrained panellists	3 panels (9,9,8 subjects)	PCA, MFA used for PM and DA; RV coefficient to compare methods and panels	Good agreement between PM and DA; Similar results obtained from panels;	Kennedy & Heymann, 2009

				The content of cacao is the main factor contributing to the separation of product	
10 Red wines	To compare maps obtained from PM with UFP and DM; to assess the expertise of the judges	14 wine experts for PM with UFP; 8 wine professionals for DA	CA and PCA on attributes; MFA on coordinates; RV coefficient used to compare PM and DM methods	Similar maps were obtained from UFP and DA methods	Perrin & Pagès, 2009
10 Apple and 10 cheeses	To compare maps obtained from PM and sorting	19 and 21 untrained panellists, respectively	MFA for PM data; MDS for sorting data	Similar product maps from PM and sorting methods; Subjects had more difficulty with the apples than cheeses	Nestrud & Lawless, 2010
8 Smoothies	To present a new approach (sorted	23 students and 1 employee	HMFA for "sorted napping" data analysis MFA for tablecloths; MCA for categorizations	The sorted napping method is feasible in practice; The sorted napping data can be handled by HMFA, which manages efficiently mixture of qualitative and quantitative data by balancing the part of the napping and the categorization for each panellist	Pagès et al., 2010
8 Granola bars	To evaluate the similarity of individual maps and compare maps obtained over	1 untrained panel (15 judges)	MFA, HMFA and PMFA used for data analysis; RV coefficient to compare results	Similarity was showed between maps in consumers	Kennedy, 2010

	multiple evaluation sessions			Good agreement on maps over replicate sessions	
8 Lemon ice tea	To compare sensory maps obtained form RG, UFP, and PM	30 consumers for PM with UFP, 43 consumers for FP, 42 consumers for RG	GPA on coordinates; MANOVA for quality of the product discrimination; RV coefficient to compare methods	Similar product sensory maps obtained from all three methods; Comparing PM with RG and FP is difficult because different numbers of dimensions	Veinand et al., 2011
5 Bread and 5 yoghurt	To characterize bread and yoghurt using the PM or Napping method using an untrained consumer panel	7 panellists (5 women and 2 men)	MA, PCA	The partial Napping method is hard to introduce without guidance for consumers; Not enough useful information can be received from the new method	Normann, 2012
6 Red wine	To examine the specific sensory attributes of red wine served at three temperatures (10,16,22 ^{o}C) using PM	12 trained subjects	MFA on the standardized location coordinates; RSQ for the strength of clustering	Greater variability between replicate maps at 10 and 16 ^{o}C; Replicate evaluations is important for a small number of panellists using Napping	Ross et al., 2012
6 Probiotic yoghurt	To compare the performance of check-all-that-apply (CATA), projective mapping, sorting and intensity scales on	30 regular yogurt consumers	HCA for group separations; MFA used for data analysis	Sorting and intensity scales had better results on separation of products	Cruz et al., 2013

	consumers perception of probiotic yoghurts				
11 Italian red wine	To compare differences between experts and consumers using PM in the aroma of Italian red wines	1 trained panel (9 subjects) ,1 expert panel (13 subjects) and 81 consumers	PCA used for intensity data from the trained panel; GPA for mapping data analysis; Linear least square regressions for the relationship between the product coordinates	Experts had better performances on separation of products than consumers; The liking data from consumer tests are more evident than those from PM	Torri et al., 2013
32 Fruit and vegetables	To evaluate the ability of DA, PM and sorting to describe differences among visual stimuli	2 panels (11 assessors each)	MDS for sorting data; MFA on coordinates; DISTATIS	PM and sorting had similar results compared to DA; The separation level of samples obtained from PM and sorting is higher than DA	Mielby et al., 2014
8 Cheese pies	To compare the differences between global PM and partial PM based on texture and flavour	3 groups of consumers (47 for global PM, 53 for partial PM on flavour, 61 for partial PM on texture)	MFA on coordinates of consumers; RV coefficient to compare methods; ANOVA on difference among samples	No large differences on the vocabulary used for description between two methods; T-PM and F-PM can provide more details than G-PM in each specific modality	Marcano et al., 2015

PM, Projective Mapping; DA, Descriptive analysis; PCA, Principal component analysis; MFA, Multi-factor analysis; GPA, Generalized procrustes analysis; MDS, Multidimensional scaling; CA, Coordinate averaging; INDSCAL, Individual difference scaling; CP, Conventional profile; UFP, Ultra-flash profile; FP, Free profile; RV, Regression vector; RSQ, Squared correlations; HMFA, Hierarchical multi-factor analysis; FP, Flash profile; RG, Repertory grid; PN, Partial napping; GN, Global napping; CATA, Check-all-that-apply; HCA, Hierarchical cluster analysis.

2.4.3 Sensory quality of bread

Sensory quality plays a vital role in the total product quality. In the case of bread, flour composition is an important factor which has a significant influence on bread quality. Bread baked with different types of flour results in different quality attributes such as texture, flavour, aroma and colour. Texture is important in quality perception of bread. It is mainly affected by protein content and quality (Finney & Barmore, 1948). Bread quality depends in part on textural properties, and flour possessing extra strong dough mixing characteristics gave a strong bread crumb (Scanlon et al., 2000). Starch is important for the structure of crumb during fermentation (e.g., nutrition to the yeast, stability for the foam lamellae of dough), baking (water absorption, colour of the crust), and at the end of baking, as a factor stabilizing the bread structure (Eliasson & Larsson, 1993).

2.5 *In vitro* digestion

2.5.1 *In vitro* digestion models

In recent years, there has been an increasing interest in the production of many different types of functional foods with respect to health and nutrition. The efficacy and safety of such products are being assessed prior to marketing by *in vivo* and/or *in vitro* studies (Yoo, 2009). Traditional *in vivo* studies with animals or humans provide the most accurate view of digestibility of foods. However, *in vivo* trials require excessive time, cost and labour, as well as ethical approvals with subject to humans or animals in some instances. Hence many efforts have been given to develop *in vitro* digestion procedures and models in order to understand their effects in the humans (Boisen & Eggum, 1991). *In vitro* methods simulating gastro-intestinal digestion are widely used to study the structural changes, digestibility, and bioavailability of foods (Minekus et al., 2014). *In vitro* digestion models are suit for mechanistic studies and hypothesis building due to the advantages of reproducibility, choice of controlled conditions and easy sampling at the site of interest. Once the model is fully validated, accurate and reproducible data can be achieved without any ethical constraints (Yoo & Chen, 2006). These models should be

flexible, accurate, and reproducible. Currently available *in vitro* digestion models can be classified in different types, such as static or dynamic. A wide range of *in vitro* digestion models is available from static mono-compartmental to dynamic multi-compartmental models (Guerra et al., 2012).

The majority of models have been reported in literature are static models, which refer to exposure of the test material to secretion at fixed concentrations of enzymes and bile salts, with controlled pH and temperature over a fixed period of time (Boisen & Eggum, 1991). Dynamic models include flows of and variable pH with respect to time (Savalle et al., 1989). Differences in settings used include time of digestion, concentrations and types of enzymes, use of inorganic and organic salts and bile acids, pH of various digestion stages. In practice, static models provide a feasible and inexpensive and particularly relevant for large pre-screening approaches. Dynamic multi-stage continuous models facilitate long-term studies and probably come the closest to the human conditions (Alminger et al., 2014).

Static models include a limited number of simulated parameters, and are dedicated to often just one particular application (Hur *et al*., 2011). Several models have been designed to assess the digestibility of protein (Kaur et al., 2010; Butts et al., 2012), starch (Englyst, et al., 1996; Zabidi & Aziz, 2009), and the bioaccessibility of micronutrients, such as carotenoids (Garrett et al., 1999). Holm et al. (1985) studied the *in vitro* and *in vivo* digestibility of wheat and found the incubation with pepsin had to be included in the *in vitro* assay using α-amylase to obtain good agreement with *in vivo* results. The *in vitro* digestion protocol was later named "multi-enzymatic protocol" (Osorio-Díaz et al., 2005), and been used extensively to study the rate of starch hydrolysis.

Lima bean, navy bean, common bean and faba bean are commonly digested by pepsin and amylase (Sayago-Ayerdi et al., 2005; Bello-Pérez et al., 2007; Berg et al., 2012), and the reducing amylolysed products are measured to determine the hydrolysis rate of starch. From these studies, the carbohydrates in those legumes have shown a slow hydrolysis rate due to the high content of fibre.

However, these static models are over-simplified compared to the *in vivo* situation. Guerra et al. (2012) reported that none of these static models reproduce the dynamic

processes occurring during human digestion such as gastric emptying or continuous changes in pH and secretion flow rates. Therefore, further efforts and technological developments are needed to improve *in vitro* models and keep up with the growing interest of industry and researchers.

2.5.2 Digestion of foods in human body

The human digestive system, also called the gastrointestinal tract, is a complex process involving breaking down of ingested food into nutrients. These nutrients can be used by the body for growth, cell maintenance or repair, and energy, followed by excretion of waste. Human gastrointestinal tract contains a series of organs and glands that processes food. Food disintegration mainly occurs in the mouth and stomach, whereas enzymatic digestion and absorption of nutrients and water take place mainly in the small and large intestines (Tortora & Derrickson, 2006).

The human digestive system starts in the mouth (oral cavity). Food is partly broken down into small molecules by the process of chewing and mixes with saliva containing alpha-amylase. The secretion of saliva helps to produce a bolus which can be swallowed to pass down the oesophagus and enter into the stomach (Tortora & Derrickson, 2006). With the aid of gastric acids and enzymes (pepsin), food in the stomach is partly digested and turned into chyme which slowly enters the small intestine (duodenum, jejunum and ileum). The small intestine is a key step responsible for most absorption of the active molecules (Fonseca, Fryer, & Bakalis, 2011). In the small intestine, food would be further digested with the help of bile (produced in the liver and stored in the gall bladder), pancreatic enzymes, and other digestive enzymes (lipase). The body then absorbs most of the digested nutrients, as well as water and minerals. These pass through the walls of the small intestine into the bloodstream, which delivers them to the rest of the body. Waste products of digestion pass through the large intestine and out of the body as a solid matter called stool (Dietary Guidelines for Americans, 2010).

Table 6 shows the parts of the digestive process performed by each digestive organ, including how foods are transferred from one organ to another, GI secretions and

composition, and how food particles break down by each organ. In the present study, carbohydrates and protein digestion are as the main studying object.

Table 6 Overview of the digestive process and enzymes in human digestion system

Organ	Motility	Enzyme	Secretion	Substrates	Final Products
Mouth	Mastication and mixing	Salivary amylase	Saliva	Starches (polysaccharides)	Maltose, maltotriose and α-dextrins
Oesophagus	Propulsion by peristalsis	None	Mucus		None
Stomach	Peristalsis, 3 cycles per minute	Pepsin, Gastric lipase	Stomach juice (gastric lipase, pepsin and hydrochloric	Proteins, triglycerides (fats and oils)	Peptides, fatty acid and monoglycerides
Small intestine	Peristalsis	Pancreatic amylase, Trypsin, Chymotrypsin, Carboxypeptidase	Small intestine juice	Starches (polysaccharides), proteins, amino acid at carboxyl end of peptides	Monosaccharides, free amino acids and peptides, free fatty acids and monoglycerides

(Dietary Guidelines for Americans, 2010)

The GI tract breaks down nutrients from food into carbohydrates, protein, fats, and vitamins. In the present study, carbohydrates and protein digestion are the main focus of this study.

2.5.2.1 Digestion of carbohydrates

Carbohydrates provide the human body with energy as nutrients. They must be hydrolysed to the basic units, called monosaccharides, prior to absorption in the small intestine. The digestion of carbohydrates, in particular starches, starts in the mouth with saliva with mastication. Starch is hydrolysed to maltose and maltotriose by aids of alpha-amylase and alpha-dextrinase. As soon as the bolus enters the stomach, the acidic pH of the stomach inactivates salivary amylase. Digestion of carbohydrates does not resume until the digested food enters the duodenum which is the first part of the small intestine (Tortora & Derrickson, 2006). Then the dietary disaccharides are hydrolysed to monosaccharides by enzymes, such as maltase, sucrose and lactase in small intestinal.

In the typical Western diet, digestion and absorption of carbohydrates is fast and takes place usually in the upper small intestine. However, when the diet contains carbohydrates not easily digestible, for example, resistant starch from potato, bean, oat, wheat flour, digestion and absorption take place mainly in the ileac portion of the small intestine.

2.5.2.2 Digestion of protein

Protein is an important nutrient for human body for growth and cell repair. Proteins can be utilized by body as smaller molecules, called amino acids. Amino acids are used to rebuild or replace damaged protein in the body. A large protein molecule is broken down into smaller pieces by mastication and lubrication in the mouth. Protein digestion begins in the stomach, where proteins are fragmented into peptides by the action of gastric pepsin. Pepsin breaks the peptide bonds that hold the protein molecules together to form polypeptides which are chains of amino acids linked together. Acidic chyme is transferred to the duodenum, and proteins, in the form of polypeptides, are broken down by the activity of pancreatic enzymes. These include trypsin, chymotrypsin, and carboxypeptidase (Krehbiel & Matthews, 2003). Each of these pancreatic enzymes has different each one splits specific peptide bonds between amino acids. For instance, trypsin and chymotrypsin cleave peptide bonds between specific amino acids; carboxypeptidase splits off the amino acid at the carboxyl end of a peptide (Tortora & Derrickson, 2006). These enzymes catalyse the breakdown of proteins and polypeptides. After that, the digested proteins and/or amino acids travel through the liver before entering into the main bloodstream. Undigested portion will continue to the large intestine and travel through the kidneys as a waste (urea), and then discharge from the body as urine. Whether the digestive enzymes could readily interact with proteins to cleave peptide bonds or not would be the key component to determine the digestibility of proteins in foods because proteins are folded in structure, in tertiary or quaternary forms (Tortora & Derrickson, 2006).

2.5.3 *In vitro* digestion and enzymes

The types of enzyme included within an *in vitro* digestion model tend to reflect the major food component(s) being investigated. For example, amylases for starch digestion,

proteases for protein digestion, and lipases for lipid digestion (Hur *et al*., 2011). Most of the available models and procedures require the use of digestive enzymes, either from animal sources (e.g. pepsin from porcine) or fungal sources (eg. salivary amylase from *Aspergillus oryzae*). The concentration and composition of enzymes are also very important factors to consider when designing *in vitro* digestion models. Typically, higher enzyme concentrations accelerate digestion or degradation of food components, and so it is important to use physiologically relevant levels. The enzymes are usually added sequentially and pH is adjusted to simulate the conditions of different digestive organs in most of the static models (Hur, et al., 2009). Table 6 summarises the functions of different enzymes in human digestion system.

2.6 Glycaemic Index

The concept of glycaemic index (GI) was first introduced by Jenkins et al. (1981) as a means of classifying different sources of carbohydrate (CHO) and CHO-rich foods in the diet, depending on their impact on postprandial glycaemia (Ferrer-Mairal et al., 2012). Food and Agriculture Organisation/World Health Organisation (FAO/WHO) defined this index as 'the incremental area under the blood glucose response curve of a 50g carbohydrate portion of a test food expressed as a percentage of the response to the same amount of carbohydrate from a standard food taken by the same subject' (FAO/WHO, 1998).

Glycaemic index is a scale ranging from 0 to 100, which helps in ranking the carbohydrate rich foods, depending on how they affect blood glucose levels in a span of 2 hours after having the food. The GI of a food can be ranked into low, medium and high (Table 7). The food having a lower GI takes a longer time to get digested and absorbed and resulting in slower and gradual changes in blood sugar level, whereas high GI food are rapidly digested and absorbed and show a high glycaemic response (Brouns et al., 2005).

Table 7 Standard Glycaemic Index Ranking

Glycaemic Index Ranking	Glycaemic Index Range
Low GI	55 or less
Medium GI	56-69
High GI	70 or more

Wolever et al. (1991) gave the definition of GI as the incremental area under the blood glucose response curve elicited by a 50g of available carbohydrate portion of a food. The values are expressed as a percentage with respect to the blood glucose values obtained from consuming a 50g of carbohydrate from a reference food, taken by the same subject. Available carbohydrates as defined by the Glycaemic Carbohydrate Definition Committee of the AACC is 'carbohydrate released from a food by digestion and which is absorbed as monosaccharides and metabolised by the body (Brooks et al., 2006).' (Woolnough, Monro, Brennan, & Bird, 2008). Table 8 further summarises the GI values of commonly consumed grains and legumes.

The GI is becoming increasingly recognised as a guideline for making healthy food choices, helping consumers to choose the right type of carbohydrate for their health and lifestyles. There is a growing interest in GI from research, public health and industrial bodies. Current global trends relating to type 2 diabetes incidence make evermore necessary the capacity to predict with accuracy and precision a given food's likely glycaemic effect (Woolnough et al., 2008).

Low GI diet have been shown to improve both glucose and lipid levels in people with diabetes (type 1 and type 2). They have benefits over weight control, with better control of appetite and hunger. Low GI diet also reduce insulin levels and insulin resistance and serum lipids in people with hypertriglyceridemia (Wolever et al., 1991).

Table 8 The average GI of grains and legumes from International (Tables of Glycaemic Index and Glycaemic Load, 2002)

Food	GI (glucose=100)	GI category (high/med/low)
Grain, cereal		
White bread	75 ± 2	high
Wholemeal bread	69 ± 2	med
Multi-grain bread	43-55 ± 2	low
Brown rice	68 ± 4	med
White rice	73 ± 4	high
Dried legumes		
Beans (haricot)	31 ± 6	low
Beans (soya)	15 ± 5	low
Beans (kidney)	29 ± 8	low
Peas (chick)	36 ± 5	low
Peas (blackeye)	33 ± 4	low

The GI values of foods with negligible carbohydrate content, such as meats and egg, are zero in GI value with negligible effect on postprandial blood glucose level.

Bread falls into medium-to-high (GI) categories (> 55) due to their richness in sugar and the use of white flour. The GI of bread has been lowered by substituting parts of wheat flour with other types of flours or grains with lower GI or by adding fibres (Akerberg, Liljeberg, & Bjorck, 1998; Angioloni & Collar, 2010). Many factors such as particle size, cooking, processing and starch structure affect the GI (Wolever et al., 2003).

Chapter 3. Materials and methods

3.1 Experimental design

Based on a preliminary study, an experimental mixture design was created with three key ingredients, which were flour (50% to 100%), actual meat (0 to 50%) and navy bean (0 to 30%), at different levels using Minitab (v17, Minitab Inc). The % range of each ingredient was chosen based on preliminary trials where the bread dough wasable to form with at least 50% wheat flour. A formulation containing 100% flour was used as control. All bread samples are listed in Table 9.

Table 9 Bread sample formulated with varying amounts of flour, meat and navy bean using a mixture design

Sample code	Beef slurry(g)	Actual Beef (%)	Navy bean (g)	Navy Bean (%)	Flour(g)	Flour (%)
Control	0	0	0	0	150	100
15NB	0	0	22.5	15	127.5	85
30NB	0	0	45	30	105	70
25M	66	25	0	0	112.5	75
50M	132	50	0	0	75	50
10M30NB	26.4	10	45	30	90	60
18M15NB	46.2	18	22.5	15	101.25	67
20M30NB	52.8	20	45	30	75	50
35M15NB	92.4	35	22.5	15	75	50

M refers to minced beef and NB refers to navy bean powder.
% shown are based on w/w.

3.2 Preparation of bread samples

The main ingredients, such as high grade flour (Homebrand), dried yeast (Edmonds), caster sugar and table salt (Homebrand) were purchased from a New Zealand supermarket. In addition, olive oil (Olivani) and tap water were also used. Lean minced beef was provided by AgResearch Ltd. New Zealand, beef was obtained from New Zealand dairy bulls (18 - 24 months old,). Dry navy beans (*Phaseolus vulgaris*), were purchased in a New Zealand supermarket.

In order to make bread dough, beef mince was made to slurry by adding water and

salt. For 200g of minced raw beef, 150g of water and 2g of table salt were added and homogenized at 6000 rpm for 15 minutes using a homogenizer (L5M-A Laboratory Mixer, Silverson®). The beef slurry was stored in a refrigerator (4 ºC) and used on the same day to bake bread. Dry navy beans were soaked in tap water for 10 hours and boiled for 2 hours in order to deactivate the trypsin inhibitor (Wagner & Riehm, 1967). They were drained, cooled and dried overnight by using a food dehydrator (Hawest Maid Dehydrator. DELUXE FD 1000). Then the dried beans were ground to a powder using a food processor (KENWOOD, multi pro.) at high speed.

The standard formula used for making the control bread sample was: 150g flour, 10g sugar, 3g salt, 3g yeast, 80 ml water and 4 ml olive oil. The weighed ingredients were mixed together using an electronic bread maker (Breville, Baker's Oven). To prepare for other types of samples, flour, navy bean powder and/or beef slurry of varying concentrations were weighed and mixed in a large bowl. Then part of the sugar, salt and olive oil were added to the mixture. For all of the samples, yeast was pre-activated and added at the last to the final dough mixture. Yeast was mixed with warm water and part of sugar and allowed to set for 10 minutes.

The bread maker had pre-set controlled functions for mixing, kneading, resting, and proofing of the bread dough. The total time it took to make one bread loaf was 90 minutes including 2 min 1st knead, 28 min 2nd knead, 10 min 1st rise, 10 seconds punch down, 50 min and 2nd rise.

For the rest of samples, flour, beef meat emulsion and navy beans were combined according to the formulation in Table 9 to make bread dough. Because meat emulsion contains 0.57% water, extra flour was added into meat samples. For example, 1/4 cup and 1/2 cup flour were added into 25% and 50% meat samples, respectively. In addition, extra water was added to navy bean dough because of the greater water absorption of the navy bean powder.

Bread dough from the bread maker was then kneaded by hand for 10 minutes, and fermented in the oven for another 30 minutes at 40 ºC. At last, bread dough was baked for 15 minutes at 180 ºC and 15 minutes at 150 ºC in the oven. After baking, the bread

was cooled down at room temperature for 2 hours, then double wrapped by using the plastic bags and stored in a freezer (-20 ºC) for further analyses.

3.3 Physical evaluation of bread

Physical evaluation was carried out for all of the nine bread samples made from three different batches (Table 9). All samples were analysed in triplicate.

3.3.1 Loaf volume

Loaf volume was measured by the method of displacement of millet seeds (Griswold, 1962). Samples were measured and weighed 1 h after removal from the oven to cool down. Millet seeds were poured into a container of known volume until the bottom of the container was covered. The test bread was then placed inside the container, followed by more millet, which were levelled across the top with a spatula. The volume of millets displaced by the bread loaf was considered as the loaf volume. The specific volume of bread was then calculated using the following formula as mentioned on AACC 1005.01 (See, Wan Nadiah, & Noor Aziah, 2007). Triplicate measurements were taken.

$$\text{Specific volume} = \text{Loaf volume } (cm^3) / \text{Loaf weight (g)}.$$

3.3.2 Bake loss

The dough was weighed before baking, and the breads were weighed after baking (Kim et al., 2003). The percent weight loss of the test bread samples was calculated as: A-B/A*100, where A and B were the weights of the dough and the baked bread, respectively.

3.3.3 Colour of crumb

A Hunter lab (45/0, Colourflex) was used to measure the crumb colour of bread samples. A half slice of bread was crumbled into a petri dish, which was covered and the lens of the Chroma meter placed on top. The colour was measured from 3 different positions. Before measuring, the Chroma meter was calibrated with a white tile and checked for recalibration in between measurements. Colour values were recorded as L*

(0 = black, 100 = white), a* (-a* = greenness and +a* = redness), and b* (-b* = blueness, +b* = yellowness). The parameter the cylindrical coordinates C* (chroma) was calculated using the below formula (Minolta, 1994).

$$C^* = (a^2 + b^2)^{½}$$

3.3.4 Texture analysis

Texture Profile Analysis (TPA) was performed to determine bread texture in terms of hardness, springiness, cohesiveness, chewiness, gumminess and resilience.

All samples were prepared and baked on the day of the test. Each bread sample was cut using a knife to obtain a cube (2cm *2cm* 2cm). The cube was taken from the middle of each loaf to evaluate the crumb texture. TPA was performed using a Stable Micro Systems TA.XT plus Texture analyser (Surry, UK) equipped with a Film Support Rig (HDP/FSR) on a heavy duty platform (HDP/90) (Figure 3). Each bread sample was placed centrally on the base plate and beneath the p/50 cylindrical aluminium probe (50mm) in order to meet with a consistent flat surface. The probe was calibrated according to the instruction before the test. Then the probe was programmed to slowly move down to a lower level in order to touch the sample, the probe pressed in two cycles with a 5s delay. The strain required for 50% compression was recorded using the following settings: pre-test speed: 10.0 mm/s, test speed: 0.5 mm/s, post-test speed: 10.0 mm/s, time 30s; load cell: 50 Kg; trigger force: auto-10g.

Figure 3 Texture Analyser instrument

Six textural parameters were determined and details of each parameter are listed below in Table 10.

Table 10 Six textural parameters used for TPA and their definition (Abdelghafor et al., 2011)

Parameters	Definition
Hardness	The force needed to compress the objects using a given amount. The peak force during the first compression cycle, expressed in 1b or N
Springiness	The elasticity of materials that can be recovered when the compressive force is removed. The time duration between the end of the first bite and the start of the second bite. It is calculated by the time duration of second compression by the time duration of first compression
Cohesiveness	The strength of the internal bonds in the sample. It is defined as the ratio of the positive force area during the second compression to that during the first compression.
Gumminess	The energy needed to break down a semi-solid food ready for swallowing. It is calculated by hardness multiply cohesiveness.
Chewiness	The energy needed to chew a solid food into a state for swallowing. It is calculated by gumminess multiply springiness.
Resilience	The ability required to return to the original position after being compressed. It is calculated by Area 5/ Area 4

3.3.5 Image analysis using Scanning Electron Microscopy (SEM)

In order to prepare for the SEM examination, bread samples were frozen (-80 ºC) and freeze dried using a freeze dryer (ALPHA 2-4 LDplus). The freeze-dried pieces of the bread samples were cut transversely into sizes of about 1*1*0.5 cm using a razor blade. The interior surface of the bread samples was mounted onto a stainless steel metal disc using double-sided carbon conductive adhesive tape. A carbon coating was then applied using the E-1045 ion sputter coater (Figure 4c) in order to produce high resolution. The prepared bread samples were then observed using a field emission scanning electron micrograph apparatus (SU-70 Schottky, Hitachi, Japan) at an accelerating voltage of 5 kV. SEM was used to view the microscopic structure of bread samples to visualize the starch granules, consistency of baking/for quality control purpose.


a)

b)

c)

d)

Figure 4 a) and b) Sample preparation for SEM; c) the E-1045 ion sputter coater; d) the Hitachi SU-70 Schottky field emission SEM

3.4 Nutritional analysis of bread

Moisture, fat, protein, ash content were analysed for all of the nine samples made from three different batches (Table 9). Each sample was analysed in triplicate.

3.4.1 Moisture content

Moisture content was analysed using the oven drying method according to AOAC 925.10 (AOAC, 2005). 10 g of baked bread samples were dried in an oven (SANYO convection oven) at 105ºC until constant weight (dry weight) was achieved. The samples were cooled down in a desiccator. The moisture content was calculated as follows:

$$\%Moisture = \frac{weight\ loss\ of\ moisture}{original\ weight\ of\ sample} \times 100$$

3.4.2 Fat content

Total fat content of bread was analyzed using the Soxhlet extraction method AOAC 996.01 (AOAC, 2000). 5 g of dried sample was ground into fine powder and put in an extraction thimble, with porosity permitting a rapid flow of solvent. The weight of a pre-dried boiling flask was recorded. 150 mL of petroleum ether (AnalaR NORMAPUR® ACS, analytical reagent) was then added into the boiling flask. The boiling flask, Soxhlet extractor, and condenser were assembled as shown on Figure 5. Lipid was extracted using a Soxhlet extractor at a rate of five or six drops per second by condensation for approximately 4 hours, by heating solvent in a boiling flask with boiling chips. Boiling flask with extracted fat was dried in an oven (SANYO convection oven) at 100ºC for 30 minutes, cooled in a desiccator, and then weighed. Fat content was calculated according to the equation below:

$$\%Fat\ (dry\ basis) = \frac{weight\ of\ fat\ in\ sample}{weight\ of\ dried\ sample} \times 100$$

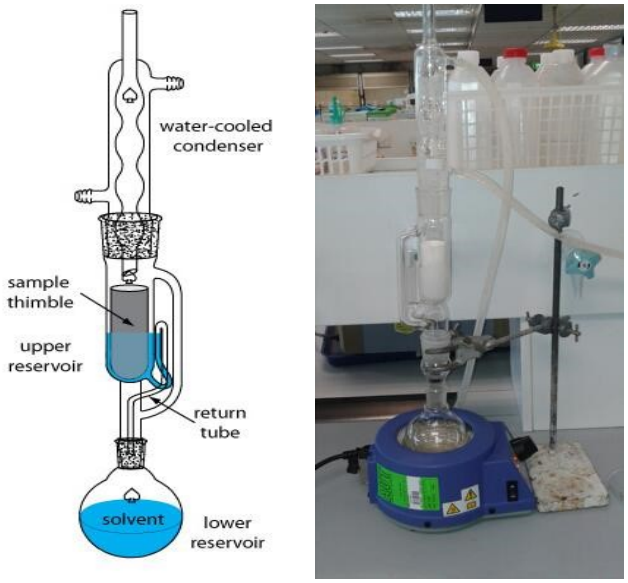


Figure 5 Soxhlet extractor

3.4.3 Protein content

Total protein analysis was done using Kjeldahl method 945.18-B (AOAC 2005). 500 mg of the dried ground sample was accurately weighed, wrapped in nitrogen-free paper, and then placed in a 250 mL digestion tube. A mixture of potassium 7g sulphate

and 0.5g copper sulphate was used as catalyst. 10 ml concentrated sulphuric acid was added and the tube content was carefully mixed prior to digestion. The sample was digested at 420 ºC for 60 minutes using a Velp DK 20 heating block (Figure 6). The level of liquid was monitored during the digestion. When the level dropped significantly, an extra 5-10 mL of concentrated sulphuric acid was added. After 60 minutes, the tube was allowed to cool for 5 minutes and 20 mL of distilled water was added. The liquid in the test tube was clear and colourless.

After the digestion, the sample was distilled using a VelpUDK 139 distillation unit (Figure 6). The digestion tube and the sample were attached to the distillation unit, and the automatic distillation process started. The digest was made alkaline with 50 mL of 40 % (w/w) sodium hydroxide, and the released ammonia was steam distilled into a receiver filled with 20 mL of 4 % (w/w) boric acid. When distillation finished, 5-10 drops of mixed Kjeldahl indicator was added to the receiver flask, and the content was titrated with 0.1 M HCl. Nitrogen content was calculated according to the equation below:

$$Nitrogen\ content\ (mg/g) = \frac{v1 - v2 \times C \times 14}{W}$$

v1 - titrated volume of standard acid for sample, mL; v2 -

titrated volume of standard acid for reagent blank, mL;

C- concentration of standard hydrochloric acid, mmol/mL;

14 = Molar mass of N, mg/mmol

W - sample weight, g

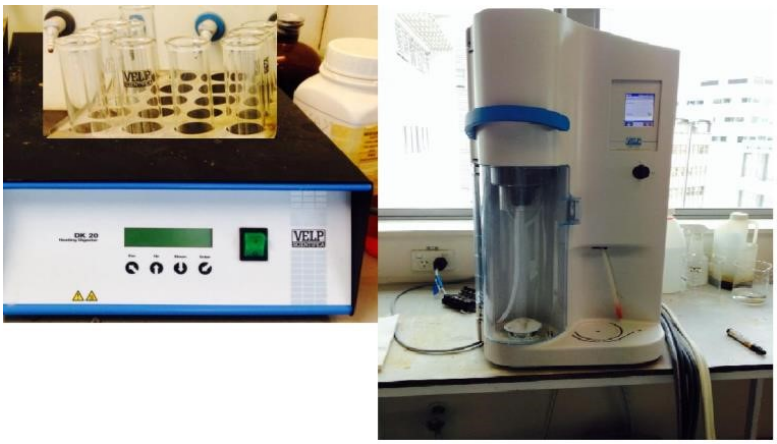


Figure 6 Velp DK 20 heating block for digestion and VelpUDK 139 distillation unit used for protein analysis

3.4.4 Ash content

The ash content of bread samples was measured according to AOAC 923.03 (AOAC, 2005). Dried and ground bread sample was placed into a crucible and it was placed in a cool muffle furnace (Model 200, McGregor Kiln Furnace). The muffle furnace (Figure 7) was ignited for 10 – 12 hours (or overnight) at approximately 550 ºC. The muffle furnace was turned off and cooled down upon the completion of ashing. The door was carefully opened to avoid loss of ash. The crucible was transferred quickly from the muffle furnace to a desiccator. The crucible was covered and the desiccator closed to allow crucibles to cool prior to weighing. At the end of the cooling period, the desiccator cover was removed gradually by sliding to one side to prevent a sudden in rush of air. The cool crucible containing ash was weighed. The ash content was calculated as follows:

$$\%ash\ (dry\ basis) = \frac{weight\ after\ ashing - tare\ weight\ of\ crucible}{dried\ sample\ weight} \times 100$$

Figure 7 Muffle furnace set at 550°C for ashing of bread samples

3.4.5 Carbohydrates content

The proximate carbohydrate content was estimated according to Fraser and Holmes (1958). Carbohydrates determination was done by taking a difference between all of the proximate constituents (total fat, protein and ash) and 100.

3.5 Sensory analysis of bread

Sensory analysis was performed at The Sensory Analysis Centre, Auckland University of Technology (Auckland, New Zealand). Bread samples prepared using the mixture design (Minitab17, Minitab Inc) were used for consumer testing and sensory projective mapping. Ethics application for this study was approved by the Auckland University of Technology Ethics Committee (AUTEC) on 9 April 2014 (Appendix 8a).

3.5.1 Bread samples for consumer testing and projective mapping

Nine bread samples including the control sample were prepared a day before sensory testing and kept frozen at -15 ºC before use. All the samples were unfrozen at room temperature 4 hours before serving to panellists.

Approximately 5*5 cm^2 of each bread sample were put in individual 25 mL plastic containers coded with three-digit random numbers. The order of samples was randomised

to avoid sample order and carry-over effects (Macfie et al., 1989). Filter water was served as a palette cleanser and testing was conducted under red light to make panellists focus more on flavour and texture attributes.

The sample bread slices were trimmed of crust, cut into 2 cm cubes, given a three-digit code and presented at room temperature in plastic cups with lids. Prior to the test, a warm-up sample was served. Panellists were allowed a 10 min break between sample presentations and were asked to clean their palate with crackers and water. Sensory analysis was conducted in an individual test booth under controlled lighting. Test samples were served to the panellists according to a completely randomised design.

3.5.2 Consumer Testing

Fifty-five panellists (aged 20–31 years old), who consumed bread at least once a month, assessed the sensory attributes of bread samples at the AUT University Sensory Lab. They were asked to evaluate each sample for odour (e.g. beany and yeasty), taste or flavour (e.g. beany, salty and meaty), texture (e.g. hardness and softness) and over all liking, using a 100 mm unstructured line scale ranging from left (extremely dislike) to right (extremely like). There was a 30 seconds break in between sample and panellists were provided clean filtered water to rinse their mouth. To prevent bias, no information about the samples was given to the panellists. FIZZ sensory software (2.46B, Biosystems, 2010) was used to design the questionnaire for consumer testing. The questionnaire used is attached in Appendix 1.

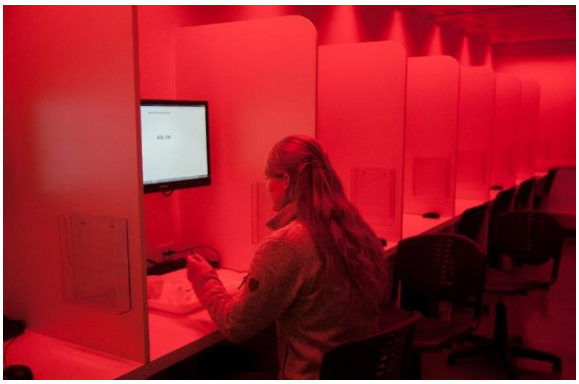


Figure 8 Consumer testing of bread samples

3.5.3 Projective mapping

Like consumer testing, 17 panellists were recruited on the basis that they were regular consumers of bread. Those eligible were invited to attend projective mapping sessions at the AUT Sensory Lab on three consecutive Mondays between 10 am and 3 pm. Panellists were given verbal instructions before entering the sensory booths. The same set of instructions (Appendices 1 & 2) was also displayed on the computer terminals using a FIZZ programmed sensory projective mapping test (FIZZ Network v2.46C, Biosystemes). Panellists then tasted the randomised and coded samples in the sensory booths, in the order presented from left to right.

Panellists grouped the samples according to their similarities and differences, with those grouped close together being more similar to each other. Additionally, they were asked to write descriptors and/or attributes that corresponded to their groupings. Products were positioned on the computer screen and sensory attributes associated with each product were keyed in by the panellists and recorded using the FIZZ Network v2.46C system. Analysis of results was performed using Multifactorial Analysis (MFA) to obtain overall product maps, General Procrustes Analysis was carried out to obtain overall product coordinates, and Principal Component Analysis to obtain product and attribute biplots using Addinsoft XLSTAT-MX version 2012.4.02. Sensory attributes that occurred a minimum of five times across panellists per product were included in the PCA biplots.

As summarised in Table 11, a total of 28 sensory attributes in terms of odour, texture and texture were developed by the panel during the orientation and training sessions. The use of reference materials reduced panel variability.

Table 11 The sensory attributes constituted breads sensory profiles.

Attributes	Definition	Food Reference
Odour		
Yeasty	Aroma of fresh baked bread. Odour associated with aromatic exchange from yeast fermentation	Active yeast, fresh baked yeast bread
Fermented	Fermented Characteristic aroma of fermented dough	Overripe pineapple
Rancid	Aroma typical of rancid nut oil	
Butter-like	Aroma of butter with slightly rancid overtones	Butter
Texture		
Soft	Softness Feeling perceived by touting the bread crumb.	N/A
Hard	Force required to bite completely through sample placed between the molars	N/A
Dry	Dryness Degree of drying effect, amount of saliva absorbed by the sample	N/A
Springiness	Force which the sample returns to its original size and shape after partial compression between the lips	N/A
Dense	The size and ratio of air cells to solid product evaluated during compression of sample with molars on the first bite	N/A
Flavour		
Yeasty	A fermented, yeast-like aromatic	Active yeast, fresh baked yeast bread
Beany	A slightly brown, musty, slightly nutty, starchy flavour associated with cooked dry beans; a green, vegetable aromatic characteristic of green beans or pea pods	N/A
Salt	The basic taste, perceived on the tongue, stimulated by sodium salt, especially sodium chloride	Sodium chloride solutions in spring water
Sweet	The basic taste, perceived on the tongue, stimulated by sugars and high-potency sweeteners.	Sucrose solutions in spring water
Yeasty sour	Yeasty sour flavour produced by yeast mixed with clean, sour notes typical of white vinegar.	N/A
Sour	The basic taste, perceived on the tongue, stimulated by acids such as citric acid.	Citric acid solutions in spring water

3.6 *In vitro* digestion of bread

All trials for digestion were carried out on the different bread samples made from two different batches for each of the five bread samples, which depended on the sensory consumer testing scores. Prior to initiating the digestion experiment, salivary amylase, pepsin, and pancreatin were tested for their enzymatic activity, in order to adjust the amount for correct use.

3.6.1 Enzymes activity assay

The activity of α-amylase (EC 3.2.1.1) was determined according to Sigma-Aldrich (Appendix-3). The activity of pepsin from porcine stomach mucosa (Sigma-Aldrich P-7000, EC 3.4.23.1) was determined using a method based on the stop-point assay of haemoglobin degradation developed by Anson (1938) and published by Sigma® (Appendix-4). Pancreatin activity was based on trypsin activity at 100 U/ml. Porcine trypsin (EC 3.4.21.4) was determined using Nα-Benzoyl-L-arginine ethyl ester (BAEE) as a substrate and published by Sigma (Appendix-5).

3.6.2 Static *in vitro* digestion protocol

3.6.2.1 Procedure of static *in vitro* digestion

The static *in vitro* digestion model used was a modified version of that described previously (Minekus et al., 2014; Hur et al., 2015). The procedure of static *in vitro* digestion model with details of enzyme and pH conditions are shown in Figure 9. Five samples were taken at different time points: after 5min in oral phase, 60 and 120 min in gastric phase, and 60 and 120 min in small intestinal phase, respectively. At each time point, the sample taken from the reactor was snap froze in liquid nitrogen immediately to inactive the enzymes after the reaction for further analysis, which included starch analysis and amino acid profile.

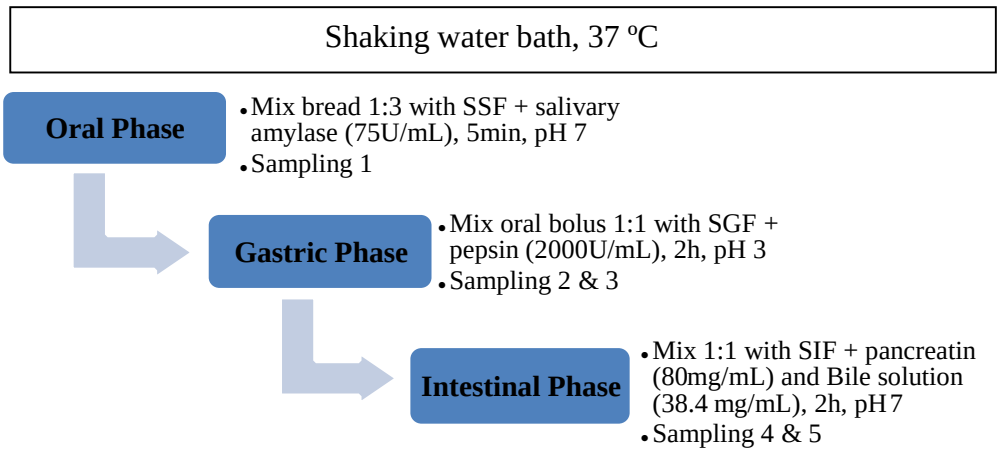


Figure 9 A flow diagram of a static *in vitro* digestion protocol. SSF, SGF, SIG refer to Simulated Salivary Fluid, Simulated Gastric Fluid and Simulated Intestinal Fluid, respectively. The reactor was incubated in a shaking water bath at 37 °C

In the oral phase, 5g of bread sample was mixed with 11ml of electrolyte stock solution of Simulated Saliva Fluid (SSF) (Table 12) to create a thin paste-like consistency by using a homogenizer (L5M-A Laboratory Mixer, Silverson®). Then the mixture sample was transferred into a 50 ml capped glass bottle (digestion reactor) and heated to 37 ºC. pH was adjusted to 7 by adding 50 µl $CaCl_2(H_2O)_2$ and 2950µl water. 1ml of salivary α-amylase (1500 U/ml, α-amylase from *Aspergillus oryzae*, ≥ 150 units/mg protein biuret, Sigma) was added to the mixture to achieve 75 U/ml in the final mixture. The mixture was incubated in a shaking water bath for 5 min at 37 ºC to simulate the oral digestion. At the end of 5 min, 1 M HCl was added to decrease the pH from 7 to 3, in order to inactivate the amylase activity. All reagents were pre-warmed at 37 ºC throughout the digestion period.

In the gastric phase, the bolus was mixed with 15 ml Simulated Gastric Fluid (SGF) stock solution (Table 12). 3.2 ml of pepsin solution (25000 U/ml, pepsin from porcine gastric mucosa, 3200-4500 units/mg protein, Sigma), 10 µl of $CaCl_2(H_2O)_2$ and 1390 µl of water were mixed to make the final pepsin concentration of 2000U/ml. Then the digestion reactor was further incubated for 2h at 37 ºC. 6 M HCl was used to adjust the pH to reach 3.0.

In the following step, Simulated Intestinal Fluid (SIF) electrolyte solution (Table 12) was added to the digestion reactor. 80 µl of $CaCl_2(H_2O)_2$ and 2620 µl water were not added to the electrolyte stock solutions directly. 31 ml of a pancreatin solution (80 mg/ml, pancreatin form porcine pancreas, 4 x USP, Sigma) and bile solution 38.4 mg/ml were added into the digestion reactor and incubated for another 2h at 37ºC. The pH was adjusted to 7 with 1 M NaOH to simulate the pH of small intestine before the shaking water bath started. At the end of 2h incubation, the pH was adjusted to 1.9 by addition of 1M HCl to halt further enzymatic reaction. The compositions of the simulated saliva, gastric, intestinal fluid are listed in Table 12.

Table 12 Composition of the stock solutions used in the static *in vitro* digestion model

		SSF	SGF	SIF
		pH 7	pH 3	pH 7
Constituent	Stock concentration	Volume of stock		
KCl	37.3 g/l	15.1ml	6.9ml	6.8ml
KH_2PO4	68 g/l	3.7ml	0.9ml	0.8ml
$NaHCO_3$	84 g/l	6.8ml	12.5ml	42.5ml
NaCl	117 g/l	-	11.8ml	9.6ml
$MgCl_2(H_2O)_6$	30.5 g/l	0.5ml	0.4ml	1.1ml
$(NH_4)_2CO_3$	48 g/l	0.06ml	0.5ml	-
*$CaCl_2(H_2O)_2$	44.1 g/l	0.05ml	0.01ml	0.08ml

SSF, SGF, SIG refer to Simul ated Saliv ary Fluid, Simul ated Gastric Fluid and Simulated Intestinal Fluid, respectively.

*$CaCl_2(H_2O)_2$ was not added to the electrolyte stock solutions. Instead, it was added to the final mixture of simulated digestion fluid and bread sample.

3.6.2.2 Starch analysis

The total starch content was carried out by using a total starch assay procedure kit (K-TSTA 04/2009, Megazyme International Ireland Ltd., Ireland). This kit is based on the use of amyloglucosidase (McCleary, Gibson, & Mugford, 1997) and has been adopted by AOAC 996.11 (AOAC, 2000). The materials were provided in the starch kit was listed in Appendix 6. The solutions and reagents were prepared according to Appendix 6. The steps of this procedure are also presented in Appendix 6.

The rate of starch digestion was expressed as the percentage of total starch (TS) hydrolysed at different times. Each sample was analysed in triplicate.

3.6.2.3 Glucose analysis

Glucose was measured using a D-glucose assay procedure (GOPOD-FORMAT, K-GLUC 07/2011, Megazyme International Ireland Ltd., Ireland) immediately after the *in vitro* digestion step. Each sample was preserved by using ethanol, and was mixed with a vortex shaker for 10 s. The test tubes were centrifuged at 4000 rpm for 15 min. Supernatant (0.1 ml) of sample was measured according to the instructions in the D-glucose assay (Appendix 7).

3.6.2.4 Essential amino acid profile

1ml of digested sample was collected at each sampling point from digestion period, which were at 5 min in oral phase; 60 and 120 min in gastric phase; 60 and 120 min in intestinal phase. 6M HCl was used to adjust the pH to 1.9-3. Essential amino acid in the samples were determined using a free amino acid GC-FID kit (EZ:faast^{TM}, Phenomenex®, USA). The Zebron ZB-AAA GC column (10 m and 0.25 mm in diameter) which is included in the kit was used. The derivatised amino acids were injected into a GC-FID instrument (Shimadzu GC-17A) equipped with an auto-sampler. Nitrogen was used as a carrier gas. The pressure was set to 43 kPa, and column flow rate was 1.46 ml/min. Column temperature was increased from 50 °C to 120 °C at 50 °C/min, then increased to 165 °C at 5 °C/min, and held at 320 °C for 2 min. The split ratio was 1:15.

3.7 Glycaemic Index (GI) testing of bread

The procedures and requirements for the determination of the glycaemic index (GI) in valued added bread were modified from the study by Ferrer-Mairal and others (2012). According to the overall-liking scores (from high to low) in the consumer test, a total of five different bread samples (control, 15NB, 25M, 10M30NB, 18M15NB) were made available to test for GI test. Each type of bread samples was tested by eight subjects and average values were used to report the findings.

Between March and June 2015, eight subjects were recruited to assess GI of five samples (control, 15NB, 25M, 10M30NB and 18M15NB) containing meat and/or navy bean in a greater Auckland region. The inclusion criteria were that the subject should be healthy males or females aged between 18 and 50 years old, and be willing to fast overnight (7pm to 9am), and be willing to give blood samples over a 2-hour period via finger prick. The exclusion criteria included those with medical conditions (including type 2 diabetes), those on medications interfering with glucose metabolism, those who are allergic or intolerant of beef, navy bean and gluten. Each subject gave written consent to participate. The approval letter was obtained from the Auckland University of Technology Ethics Committee (AUTEC) on 28 January 2015 (Appendix 8b).

Subjects were required to present to the reception of School of Applied Sciences (Level 5, WS building, AUT, Auckland) by in the morning after a 10-12 hour fast. Finger prick samples were obtained with lancets (ACCU-CHEK Safe-T-Pro Plus) at 0, 15, 30, 45, 60, 90, 120 min from hands warmed between electric blankets to ensure good blood flow. 26 subjects (25.75 ± 2.95 years; 22.68 ± 1.95 kg/m^2 of BMI) were included in the study for blood glucose testing.

The first blood sampling was done using finger-prick in Roche lab (Level 5, WS building). Then they were taken into the food lab (Level 5, WS building) to consume one slice of bread (50 g) with 250 ml drink water within 10-15 min. As soon as the consumption of bread was completed, finger-prick capillary blood was collected at 0, 15, 30, 45, 60, 90, and 120 min in Roche lab. Blood glucose was determined by using a portable blood glucose monitor (Figure 10).

Each bread sample was compared with the results obtained from the reference food (50 g of anhydrous glucose). These blood samples were used to construct a blood glucose response curve for the two-hour period. The Incremental Area under the Curve (IAUC) for blood glucose was calculated for each bread sample using the trapezoidal method (Wolever & Jenkins, 1986), and reflected the total rise in blood glycose levels after eating the bread samples. The IAUC for each bread sample taken by each panellist was expressed as a percentage of the mean IAUC for the reference food (glucose) taken by the same subject, and the mean of the resulting values was the bread GI.

The GI has been defined as formula below:

$$GI(\%) = \frac{\text{Area under the curve for 50g carbohydrate from test food}}{\text{Area under the curve for 50g carbohydtare from the reference food (glucose)}} \times 100$$

Figure 10 Blood glucose testing tools

3.8 Statistical analyses

At least 3 replicate determinations were obtained for each of the 3 production batches at different dates. Results were expressed by means of values ± standard deviation of three separate determinations. One way analysis of variance (ANOVA) was examined for significance and in case of significance, mean separation was accomplished by Tukey's (HSD) test or Fisher (LSD) test at 5% significance level. All statistical analyses were performed using the Statistical Analysis System – XLSTAT-MX version 2012.4.02 (Addinsoft, New York, NY, USA).

Chapter 4 Results and Discussion

4.1 Physical evaluation of bread

4.1.1 Loaf volume

Loaf volume is an important criterion to determine the quality of bread (Penfield & Campbell, 1990) where it can be translated as a quantitative measurement of baking performance (Tronsmo, Faergestad, Schofield, & Magnus, 2003). The loaf volume is proportional to the gluten content (Van Hal, 2000). Gluten is the main structure-forming protein in flour which is responsible for the elastic and extensible properties to produce good quality bread (Gallagher et al, 2003).

Table 13 A table summarizing the results of loaf weight, loaf volume and bake loss of bread.

Sample	Loaf weight(g)	Loaf volume(mL)	Specific volume(mL/g)	Bake loss (%)
Control	247.5 ± 0.35^{bc}	490 ± 1.41^{a}	1.98 ± 0.03^{a}	10.15 ± 0.71^{a}
15NB	252.5 ± 1.06^{bc}	515 ± 0.71^{a}	2.04 ± 0.06^{a}	8.01 ± 1.15^{a}
30NB	257 ± 0.99^{ab}	277.5 ± 1.07^{b}	1.08 ± 0.01^{b}	7.72 ± 0.05^{a}
25M	249 ± 1.27^{bc}	210 ± 1.41^{c}	0.84 ± 0.01^{c}	9.35 ± 2.58^{a}
50M	278 ± 1.13^{a}	140 ± 1.32^{d}	0.50 ± 0.03^{e}	9.12 ± 1.94^{a}
10M30NB	248.3 ± 0.27^{bc}	150 ± 1.53^{d}	0.60 ± 0.05^{de}	6.61 ± 1.49^{a}
18M15NB	220.5 ± 0.70^{c}	187.5 ± 1.02^{cd}	0.85 ± 0.05^{c}	8.33 ± 1.03^{a}
20M30NB	244.5 ± 0.77^{bc}	157.5 ± 1.22^{cd}	0.64 ± 0.02^{de}	7.74 ± 0.72^{a}
35M15NB	257.8 ± 0.39^{ab}	185 ± 2.12^{cd}	0.71 ± 0.07^{cd}	7.24 ± 2.06^{a}
p value	< 0.0001	< 0.0001	< 0.0001	0.450

*Data represent as mean ± standard deviation (S.D.) of triplicate measurements.
Different superscripts (a-e) within the same column indicate significant differences at $p < 0.05$ using Tukey's (HSD) test.
Samples are expressed as percentage meat (M) and navy bean (N) content.

Results of loaf volumes and weights are shown in Table 13. The loaf volume of control bread was 490 ml, which not significantly different ($p < 0.05$) with 15NB bread sample. However, a significant decrease ($p < 0.05$) in loaf volume was observed as the level of substitution with added meat and navy bean increased compared to the control, resulting in a more compact bread. This finding is consistent with the results of Ragaee et al. (2011), they reported that partial substitution of wheat flour with some grains such as

barley, cellulose and oat caused a reduction in volume of loaves of bread. In this study, the decreased loaf volume could be explained by the addition of meat and navy bean powder to wheat flour, which lowered the overall content of gluten and consequently, the ability of the dough to rise has decreased, resulting in lesser retention of CO_2 gas (Sharma & Chauhan, 2000). Furthermore, Anil (2007) indicated that reduced loaf volume may also be caused by physical and chemical interactions among fibre components, water, and gluten. The specific loaf volume (Figure 11) also follows a consistent trend with the loaf volume of bread. There was a significant decrease ($p < 0.05$) in all fortified bread samples except for 15NB when compared to control.

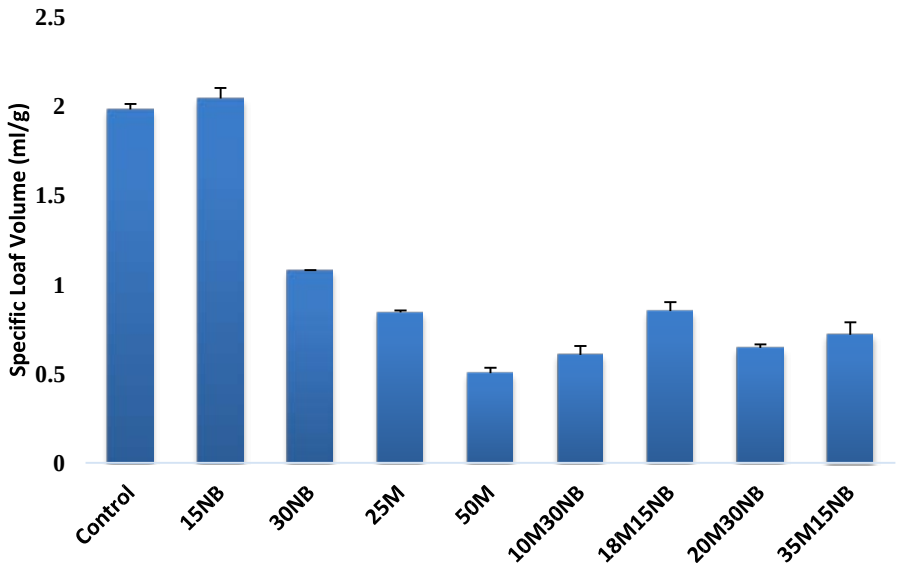


Figure 11 Specific loaf volumes of different breads supplemented with meat (M) and navy bean (NB).

In order to improve the negative impact of non-wheat ingredients on loaf volume of bread, suggestions to increase the lowered loaf volume have been made. Hung et al. (2005) suggested that increase in water content of dough can be helpful to solve the negative effect of substitution and gluten protein dilution on the loaf volume.

As shown on Table 13, the loaf weight had an opposite trend to loaf volume. It showed a significant increase ($p < 0.05$) in all the sample as the level of meat and navy bean increased compared to the control bread, except 18M15NB and 20M30NB. In the same way, Das et al. (2012) reported that loaf weight increased as the level of substitution with coriander leaf powder increased. They indicated that this trend may be caused by the increased amount of water absorbed by the coriander leaf powder. Therefore, the increase

in the loaf weight in this study may be related to the water binding ability of navy bean powder during the dough making process and its ability to retain water to a large extent during the baking process. According to FAO/INFOODS Density Database (version 2.0, 2012), the density of flour (0.48 g/ml) is much lower compared to bean powder (0.69-0.77 g/ml) and raw meat (0.96 g/ml). Hence, the results showed that 50M fortified bread was significantly heavier ($p < 0.05$) than control bread.

4.1.2 Bake loss

Weight loss is unavoidable during the baking process of bread, which is caused by loss of moisture. Bake loss is an important factor related to bread quality because they are highly related with the firming process in starch-based systems (Minarro et al., 2012). As shown on Table 13, no significant differences were observed. The mean bake loss ranged from 6.61% being lowest and 10.15% as the highest in 10M30NB and control samples, respectively.

4.1.3 Colour

Food appearance is one of the factors that define its quality and the first impression the consumer gets directly from foods. Colour, as one aspect of appearance, is an important component of quality in agricultural and food industry.

The colour of breads is a very important factor in their marketing and is directly influenced by the raw materials used in the formulation and also by the baking conditions (Silva et al., 2009). In general, high values of L* indicate a higher light reflectance, resulting in a light colour. High values for a* and b* are translated for samples with strong red colour and yellow or golden colour, respectively (Esteller et al., 2004).

Table 14 Colour parameters of crumbs of bread supplemented with meat and navy bean at different levels.

Sample	Colour parameters			
	L*	a*	b*	C*
Control	79.05 ± 0.18^{a}	1.09 ± 0.04^{e}	19.93 ± 0.18^{c}	19.96
15NB	71.50 ± 0.05^{b}	2.62 ± 0.06^{b}	22.54 ± 0.12^{a}	22.69
30NB	68.74 ± 0.55^{c}	2.94 ± 0.08^{a}	22.65 ± 0.19^{a}	22.84
25M	54.50 ± 0.29^{g}	2.59 ± 0.03^{b}	21.98 ± 0.14^{b}	22.13
50M	46.28 ± 0.57^{i}	2.47 ± 0.10^{b}	19.51 ± 0.55^{c}	19.66
10M30NB	61.85 ± 0.15^{d}	1.88 ± 0.09^{d}	19.21 ± 0.19^{d}	19.30
18M15NB	58.49 ± 0.07^{f}	2.45 ± 0.10^{bc}	22.17 ± 0.23^{ab}	22.05
20M30NB	59.43 ± 0.15^{e}	2.39 ± 0.06^{c}	21.72 ± 0.15^{b}	21.85
35M15NB	52.55 ± 0.28^{h}	2.82 ± 0.14^{a}	21.84 ± 0.33^{b}	22.02
p value	< 0.0001	< 0.0001	< 0.0001	

Data represent mean ± standard deviation (S.D.) from triplicate analysis.
Mean values with different superscripts (a-f) within the same column are significantly different ($p < 0.05$) on Tukey's (HSD) test.
Samples are expressed as percentage meat (M) and navy bean (N) content.

As can be seen from Table 14, the crumb colour lightness (L*), redness (a*), and yellowness (b*) were all affected significantly ($p < 0.05$) by supplementation. The control bread sample was white and slightly yellow. Bread samples containing meat and/or navy bean were darker with reddish or yellow colour compared to the control one when observed with the naked eye (Figure 12). The outcome was consistent with Hunter L*, a*, b* values shown on Table 14. L* value significantly ($p < 0.05$) decreased as the level of meat incorporation increased, in samples that contained meat and/or navy bean. In contrast, the colour of all fortified bread samples had a significant increase ($p < 0.05$) in redness (a* value) and yellowness (b* value) compared to control. Figure 13 showed the bar chart of L*, a*, and b* values of nine bread samples.

The colour of crust (data not shown) is associated with the Maillard reaction. Increase in protein with meat and navy bean addition can result in a darker brown colour with increased Maillard reaction (Gomez et al., 2003). The dark red crumb colour in the fortified breads containing meat may also be due to the presence of myoglobin in meat, which is responsible for the red colour. Myoglobin is present in meat and is responsible for the purplish red muscle colour. The red colour can be observed in the depth of the

muscle when the meat is freshly cut, and changes quickly to bright red oxymyoglobin with the presence of air. Moreover, the colour of meat will change to dark brown when denatured (Mancini & Hunt, 2005). Therefore, the lightness (L*) decreased and redness (a*) increased with increasing amount of meat in the fortified bread samples. The fortified bread (30NB) with higher amount of navy bean was found to be significantly more ($p < 0.05$) yellow compared to control. This is probably due to the slight yellow colour of navy bean compared to flour.

To simplify interpretation of these three dimensional colour values, the Hunter L*, a*, and b* values for each sample were transformed to C* (Chrom), which is an indicator of saturation. The C* value was plotted against the L* value to give two dimensional plots of bread samples as shown in Figure 14. The saturation of bread samples ranged from 19.3 (10M30NB) to 22.84 (30NB). Both redness (a*) and yellowness (b*) affected the saturation.

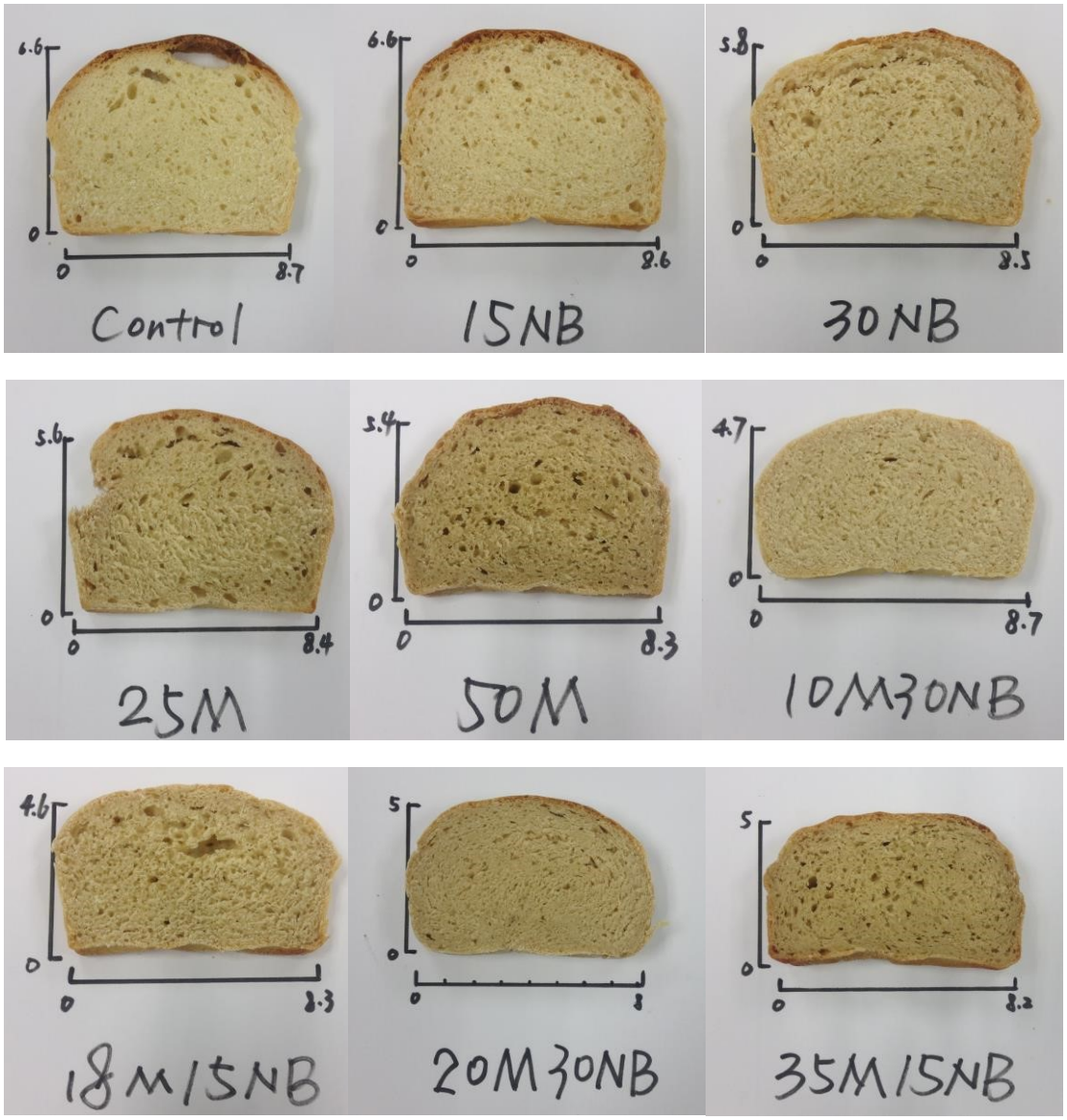


Figure 12 Photograph of bread samples with different concentrations of meat and navy bean.

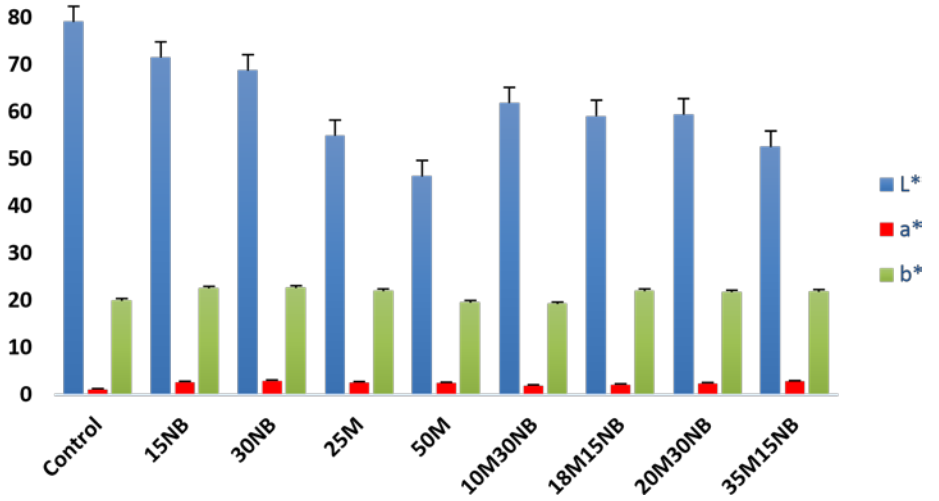


Figure 13 L* a* b* values of fortified bread samples

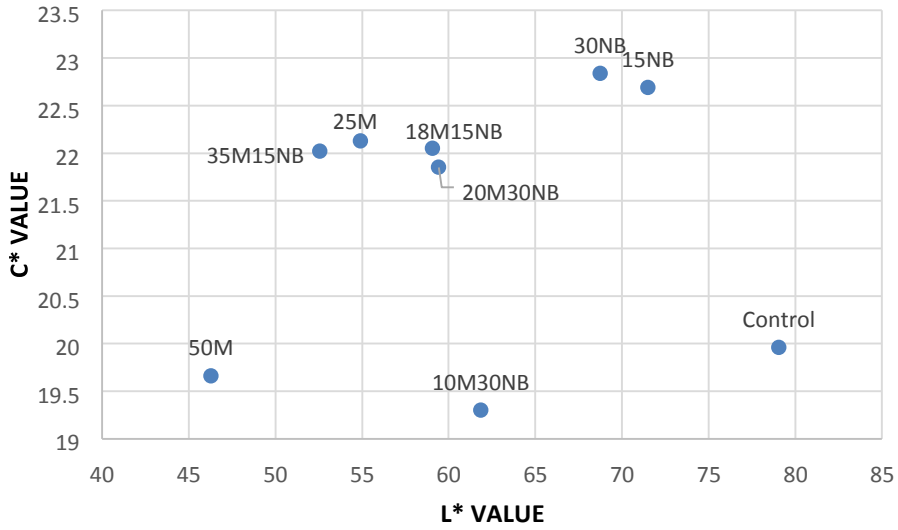


Figure 14 The L* and C* values for control sample and bread samples containing meat and navy bean

4.1.4 Texture characteristics of bread

Instrumental texture evaluation is important when developing new products (Dubost et al., 2003). The texture of fortified bread was determined as hardness, cohesiveness, springiness, gumminess, chewiness, and resilience using Texture profile analysis (TPA).

Table 15 A table summarizing results of texture parameters in bread

Sample	Hardness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
Control	6.89 ± 0.24^{c}	0.94 ± 0.04^{a}	0.87 ± 0.04^{a}	5.99 ± 0.45^{b}	5.62 ± 0.62^{ab}	0.50 ± 0.02^{a}
15NB	7.28 ± 0.24^{bc}	0.93 ± 0.04^{a}	0.83 ± 0.03^{a}	6.09 ± 0.11^{b}	5.64 ± 0.15^{ab}	0.48 ± 0.03^{a}
30NB	7.77 ± 0.17^{abc}	0.94 ± 0.02^{a}	0.84 ± 0.06^{a}	6.52 ± 0.59^{ab}	6.13 ± 0.69^{ab}	0.44 ± 0.04^{a}
25M	8.75 ± 0.23^{a}	0.94 ± 0.08^{a}	0.90 ± 0.04^{a}	7.68 ± 0.51^{a}	7.25 ± 1.09^{a}	0.47 ± 0.03^{a}
50M	8.83 ± 0.62^{a}	0.95 ± 0.04^{a}	0.86 ± 0.05^{a}	7.61 ± 0.33^{a}	7.22 ± 0.39^{a}	0.49 ± 0.04^{a}
10M30NB	7.07 ± 0.12^{bc}	0.79 ± 0.02^{b}	0.89 ± 0.07^{a}	6.33 ± 0.54^{ab}	5.01 ± 0.33^{b}	0.50 ± 0.04^{a}
18M15NB	7.38 ± 0.30^{bc}	0.94 ± 0.05^{a}	0.84 ± 0.07^{a}	6.20 ± 0.72^{b}	5.82 ± 0.98^{ab}	0.44 ± 0.04^{a}
20M30NB	7.55 ± 0.17^{bc}	0.89 ± 0.06^{ab}	0.76 ± 0.04^{a}	5.76 ± 0.41^{b}	5.13 ± 0.71^{ab}	0.40 ± 0.02^{a}
35M15NB	8.12 ± 0.79^{ab}	0.91 ± 0.01^{ab}	0.82 ± 0.07^{a}	7.30 ± 1.07^{ab}	6.08 ± 1.05^{ab}	0.42 ± 0.04^{a}
p value	0.0001	0.016	0.171	0.001	0.018	0.054

Different superscript letters in column are significantly different using one-way ANOVA and Tukey's (HSD) test ($p<0.05$).
Samples are expressed as percentage meat (M) and navy bean (N) content.

The results of texture profile analysis are shown in Table 15. It is evident that all texture parameters of bread samples, except for cohesiveness and resilience, were significantly different ($p < 0.05$) compared to the control bread. The results showed that as the addition of meat and navy bean increased, the hardness of bread crumb in 25M, 50M and 35M15NB significantly ($p < 0.05$) increased compared to control (Figure 15). However no significant differences were observed in other fortified bread samples. This may be due to the dilution of gluten content by the substitution of meat and navy bean, which usually results in hardening of the crumb structure as demonstrated by Sanz-Penella et al., (2013). Similar findings have been reported by Bodroze-Solarov et al. (2008), where a significant increase in hardness was shown with increased levels of

substitution with amaranth flour, along with significantly decreased loaf specific volume. Hardness is not only attributed to the matrix of amylose and amylopectin, but also caused by the interactions between gluten and fibrous materials (Schiraldi & Fessas, 2000; Gomez et al., 2013).

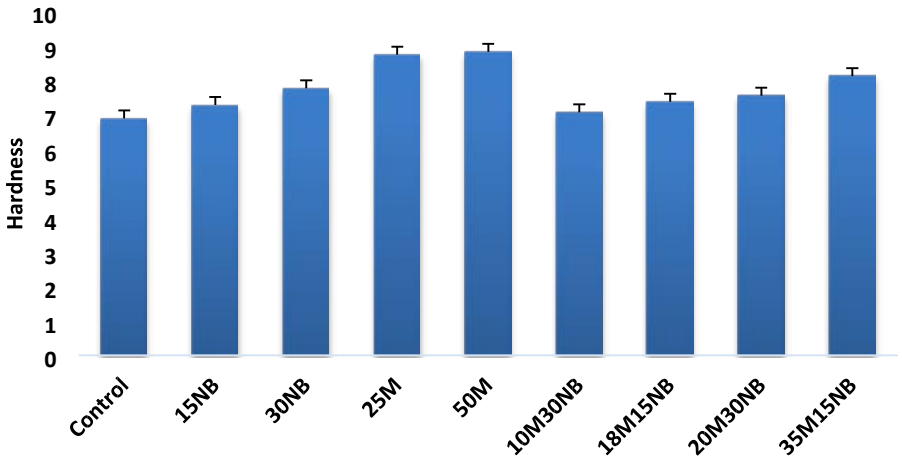


Figure 15 Hardness of bread samples containing meat and navy

In the case of springiness (Figure 16), a significant ($p < 0.05$) decrease was observed in between 10M30NB and the control bread. Springiness is mainly affected by the interaction between gelatinized starch and gluten dough, and it can form continuous sponge structure in bread after heating, which results in a more elastic bread dough (Hoseney et al., 1994). Hence, increase in supplementation causing dilution of gluten structure would have also reduced the ability to hold gases, resulting in a reduced springiness in bread as suggested by Pyler (1988). The results of springiness is also supported by the decrease in loaf volume and specific loaf volume (Table 13) with increased supplementation.

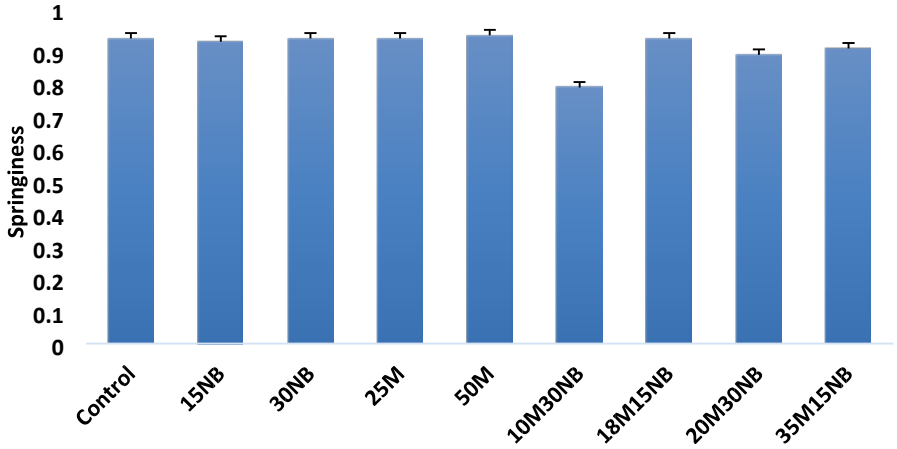


Figure 16 Springiness bread samples containing meat and navy

Chewiness is one of the important factor to indicate the quality of bread. It is calculated by multiplying gumminess and springiness (Abdelghafor et al., 2011). However, the presence of meat and navy bean did not produce meaningful changes in chewiness. Only 25M showed a significant increase ($p < 0.05$) in chewiness compared to 10M30NB. Gumminess is determined by multiplying hardness and cohesiveness (Abdelghafor et al., 2011). Bread samples substituted with only meat at 25% and 50% levels showed a significant ($p < 0.05$) higher value of gumminess compared to control, while other bread samples had similar gumminess values with control bread (Figure 17).

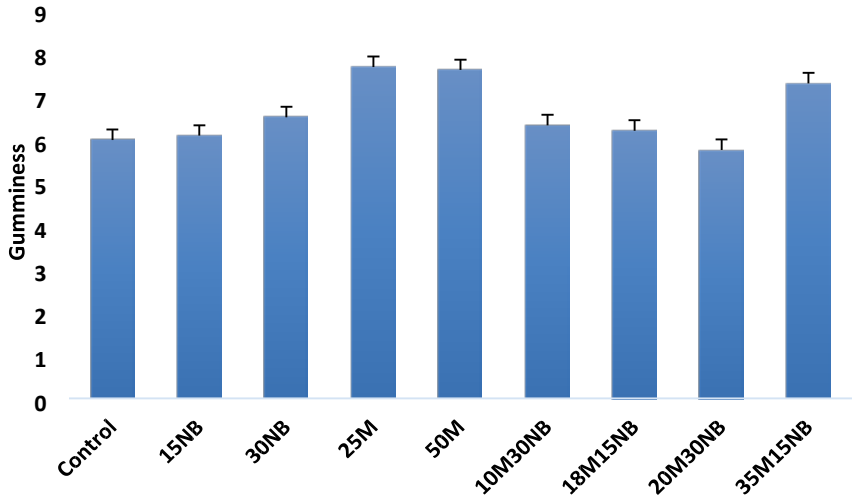
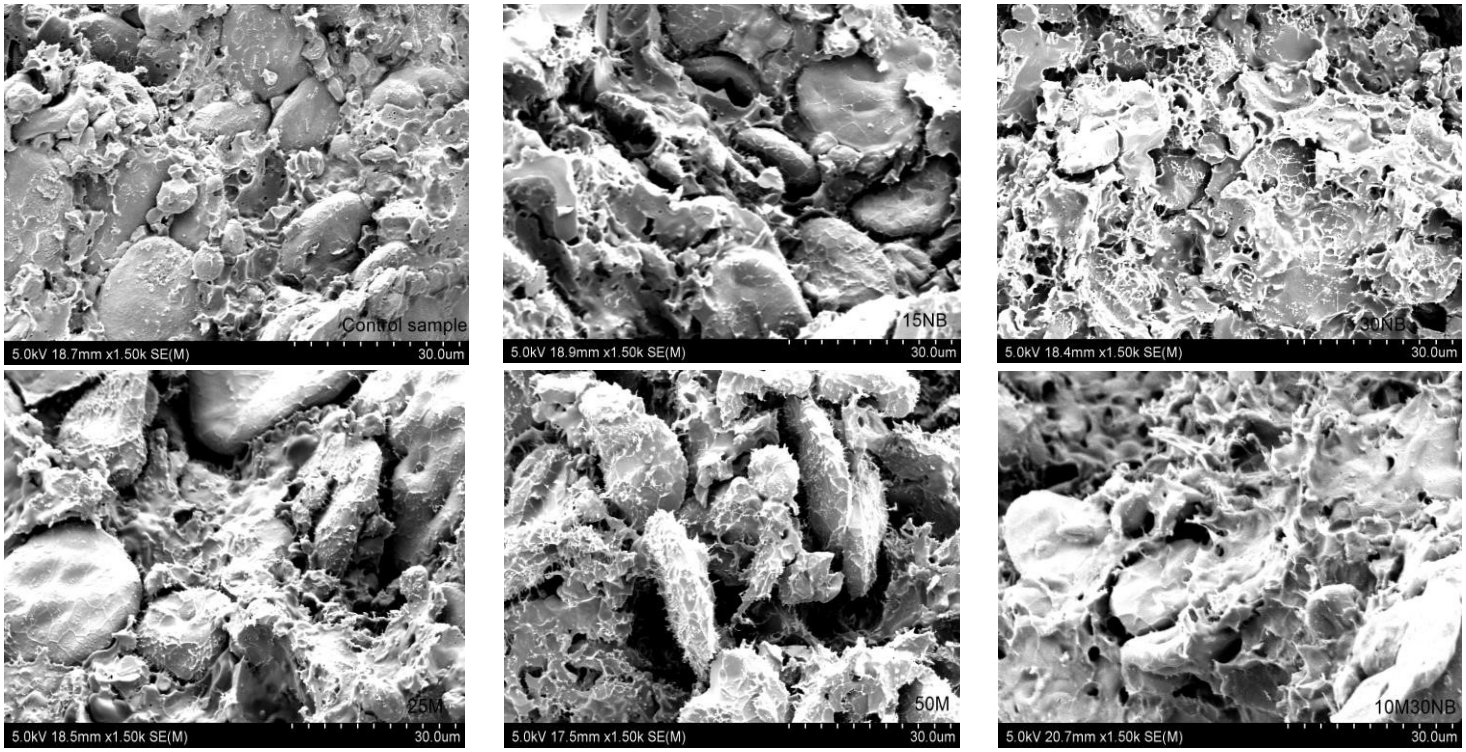


Figure 17 Gumminess of bread samples containing meat and navy bean

4.1.5 Scanning Electron Microscope (SEM)

The relationships between ingredients and products, in terms of structure and function, play an important role in designing new products (Mosharraf, Kadivar, & Shahedi, 2009). SEM observations have been reported to be a useful tool for observing the rheological properties and inter-structure of food (Aranyi & Hawrylewicz, 1969), but also for understanding the interactions of starch and protein molecules during the baking process of breads. Prabhasankar et al. (2003) indicated that porosity in structure, which can be seen from SEM observations, presents the extent of starch gelatinization and protein structure. These in turn play key roles in determining the appearance and texture of the final cereal products.

Micrographs of bread samples with different levels of substitution with meat and navy bean compared to the control are shown in Figure 18 (A-I). Starch granules are discontinuously distributed throughout the surface of bread crumb in the control sample (Figure 18A). The surface has some gas vacuoles and looks coarse. This is probably due to gelatinization of starches. Cumming and Tung (1975) observed the gluten in wheat flour bread dough formed thin sheets over the starch granules and fibre-like structures on the surface, and they suggested that the fibre formation may be caused by the rupturing and rolling back of a sheeted structure. 25M (Figure 18D) shows a smooth surface with only small gas vacuoles, resulting in a small bread volume. As can be seen from Figures 18 (B) to (I), starch granules are embedded and enmeshed in the protein fibrils, which have a lot of vacuoles on their surface. As the replacement of wheat flour by meat and navy bean increased, the protein matrix became larger and more extensive compared to the control one.


Control sample
5.0kV 18.7mm x1.50k SE(M)
30.0um
15NB
5.0kV 18.9mm x1.50k SE(M)
30.0um
30NB
5.0kV 18.4mm x1.50k SE(M)
30.0um
25M
5.0kV 18.5mm x1.50k SE(M)
30.0um
50M
5.0kV 17.5mm x1.50k SE(M)
30.0um
10M30NB
5.0kV 20.7mm x1.50k SE(M)
30.0um

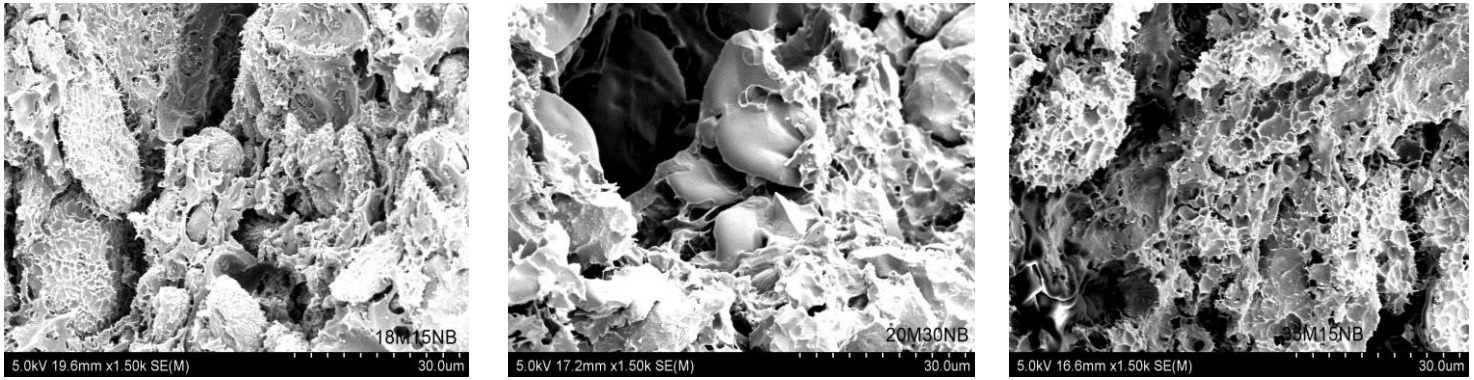


Figure 18 The image of bread samples with different concentrations of meat and navy bean.

4.2 Nutritional analysis of bread

The results of nutrition analysis of the breads supplemented with different percentages of meat and navy bean are presented in Table 16.

Table 16 Nutritional composition of bread samples containing meat and navy bean

Sample	Component (g/100g wet basis)					
	Moisture	Protein	Fat	Ash	Carbohydrates	Energy(kJ)
Control	37.37 ± 1.66^{c}	4.53 ± 0.52^{e}	0.49 ± 0.21^{b}	1.37 ± 0.10^{b}	56.24 ± 0.38^{a}	1037.69
15NB	42.77 ± 0.65^{bc}	5.62 ± 1.14^{de}	0.78 ± 0.35^{ab}	1.58 ± 0.08^{b}	49.25 ± 1.47^{b}	874.16
30NB	46.22 ± 0.56^{abc}	6.48 ± 0.30^{cde}	0.72 ± 0.33^{ab}	1.84 ± 0.16^{ab}	44.74 ± 0.35^{c}	733.18
25M	43.72 ± 0.96^{bc}	7.86 ± 1.09^{bc}	0.79 ± 0.17^{ab}	1.84 ± 0.73^{ab}	45.79 ± 1.74^{bc}	1081.64
50M	47.71 ± 1.57^{abc}	10.53 ± 0.98^{a}	1.04 ± 0.38^{ab}	1.88 ± 0.53^{ab}	38.84 ± 1.03^{d}	1125.59
10M30NB	40.20 ± 1.45^{bc}	8.13 ± 0.69^{bc}	1.06 ± 0.21^{ab}	1.77 ± 0.17^{ab}	48.84 ± 0.63^{c}	868.07
18M15NB	51.89 ± 1.02^{ab}	7.03 ± 0.95^{bcd}	0.61 ± 0.15^{ab}	2.18 ± 0.26^{ab}	38.29 ± 0.11^{c}	974.85
20M30NB	44.46 ± 1.00^{bc}	7.87 ± 1.17^{bc}	1.10 ± 0.45^{a}	2.20 ± 0.55^{ab}	44.2 ± 2.05^{c}	885.65
35M15NB	57.41 ± 1.26^{a}	8.77 ± 0.75^{ab}	0.67 ± 0.02^{ab}	2.84 ± 0.30^{a}	30.31 ± 0.83^{d}	1005.62
p value	0.0001	0.0001	0.013	0.011	0.011	

Different superscript letters in column are significantly different using one-way ANOVA and Tukey's test ($p < 0.05$).
Samples are expressed as percentage meat (M) and navy bean (NB) content.
Energy (kJ/g) was calculated using the Nutritional Panel Calculator (FSANZ, 2011).

4.2.1 Moisture content

As shown on Table 16, moisture values of nine bread samples ranged from 37.37% to 57.41% with sample 35M15NB having the highest value. In addition, a significant increase ($p < 0.05$) in moisture content was seen in 18M15NB and 35M15NB bread samples compared to the control sample. Relatively high moisture content observed for the fortified samples might be due to the meat slurry and the high fibre content in navy bean. Meat is high in water content. Raw lean beef contains 56% - 64% of water (USDA, 2013). High fibre content in navy bean is able to increase the water binding capacity and water absorption by interacting with a large amount of water through the hydroxyl groups in the fibre structure (Wang et al., 2000). The results are in agreement with Mansour et al. (1999) and See et al. (2007). Both studies have reported that supplementation of pumpkin flour to wheat flour could increase water absorption, resulting in a higher value of moisture content in bread samples.

4.2.2 Protein content

As expected, there was a significant ($p < 0.05$) increase in protein content of bread with increase in concentrations of meat and navy bean and a combination of both. The protein content of fortified bread samples ranged between 5.62% and 10.53%. All fortified breads except 15NB and 30NB were significantly higher ($p < 0.05$) than control bread. The supplemented bread with 50% meat had the highest protein content (10.53%). The protein content of beef (diced, fully-trimmed, and raw) is 25-30 % (FSANZ, 2013). In the present study, the meat slurry contained 57% (wet weight) of raw beef and 43% of water (section3.2). The high protein content of meat is mainly responsible for the increasing amount of protein content in bread samples. Similar results have been reported by Adeleke and Odedeji (2010). Substitution of flour with tilapia fish flour, which contains higher protein content than flour on its own, resulted in increased protein content of bread overall. However, navy bean (boiled, no added salt, drained) has 8.2% of protein content which is not much different from that of the flour (11%) (FSANZ, 2013), therefore it has not increased the overall protein content of bread much.

4.2.3 Fat content

The fat content of fortified bread samples with meat, navy bean or a combination of the two, ranged from 0.61% to 1.27%. Although the fat content of all bread samples were very low compared to other macronutrients, the fat content was significantly higher ($p < 0.05$) in 20M30NB sample than control (0.49%). Addition of high percentage of meat and navy bean combination contributed towards the significant increase in fat. The beef used in this study was lean with 2.7% fat and navy bean with 0.7% of fat (FSANZ, 2013). Therefore, the fat content in the bread samples was not as high as expected in the bread with a combination of high meat and navy bean content.

4.2.4 Ash content

Ash content refers to the mineral content of bread, and is determined by completely burning a given quantity of bread sample under prescribed conditions and measuring the residue. The ash content of fortified bread samples ranged from 1.58% to 2.84%, which was not significantly different compared to the control bread (1.37%). Sample 35M15NB recorded the highest value 2.84%. Navy bean (boiled and mashed) and raw beef had similar ash contents of approximately 1 % (U.S. Department of Agriculture, 2013b). Therefore, the addition of meat and navy bean did not affect the ash content significantly in the fortified bread samples. Similarly, Adeleke and Odedeji (2010) reported that the ash content of control and bread samples containing tilapia fish flour were similar, with no significant differences among the bread samples.

4.2.5 Carbohydrate content

Carbohydrate content of the control sample had the highest value of 56.85% compared to the supplemented samples, which ranged from 30.31% to 49.25% (Table 16). Addition of meat and navy bean reduced carbohydrate content of bread. This was probably due to bread flour being the main contributor of carbohydrate content in bread, and the effect of starch dilution through the supplementations of meat and navy bean. Meat has negligible amount of carbohydrates present (0.6%) and navy bean has 26% of carbohydrates. Overall, an increase in protein content resulted in a corresponding decrease in carbohydrates for all supplemented samples. The result agrees well with Salama et al.

(1992) who reported a decrease in carbohydrate content of fortified bread with sesame flour. In addition, See et al. (2007) similarly reported a reduction in total carbohydrate content with the addition of pumpkin flour to bread. A bar chart of nutritional value of bread samples is presented in Figure 19.

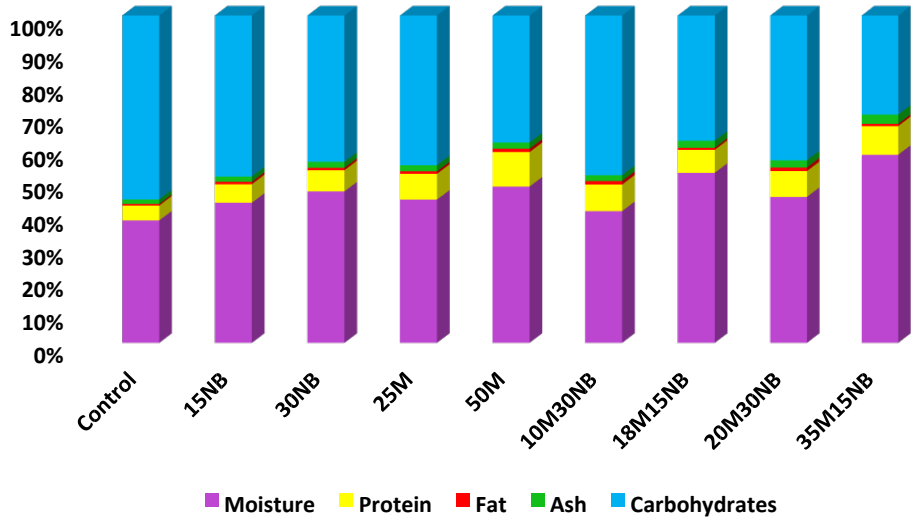


Figure 19 A bar chart of nutritional value of bread samples containing meat and navy bean

4.3 Sensory analysis of bread

4.3.1 Consumer testing

The sensory attributes of nine protein-enriched bread samples were evaluated for different parameters such as, overall liking, texture, odour and flavour. The results are presented in Table 17.

Table 17 Consumer liking data of different bread samples containing meat and navy bean*

Samples	Overall liking	Texture	Odour	Flavour
Control	5.84 ± 1.84^{ab}	5.72 ± 2.16^{a}	5.39 ± 2.00^{a}	6.03 ± 2.03^{a}
15NB	6.08 ± 1.66^{a}	5.70 ± 1.74^{a}	5.61 ± 1.96^{a}	6.01 ± 1.59^{a}
30NB	5.57 ± 1.90^{abcd}	5.33 ± 1.86^{a}	5.52 ± 1.74^{a}	5.43 ± 1.79^{ab}
25M	5.71 ± 2.07^{abc}	5.52 ± 2.12^{a}	5.13 ± 2.05^{a}	5.47 ± 2.38^{ab}
50M	5.13 ± 1.97^{bcd}	5.41 ± 2.26^{a}	4.87 ± 2.25^{a}	5.08 ± 2.40^{b}
10M30NB	5.05 ± 2.40^{cd}	5.09 ± 2.39^{a}	5.30 ± 2.27^{a}	4.91 ± 2.42^{b}
18M15NB	5.36 ± 2.00^{abcd}	4.87 ± 2.14^{a}	5.17 ± 2.07^{a}	5.11 ± 2.27^{b}
20M30NB	4.95 ± 1.95^{d}	4.79 ± 2.20^{a}	4.95 ± 1.79^{a}	5.00 ± 2.07^{b}
35M15NB	4.86 ± 1.98^{d}	4.99 ± 1.96^{a}	5.21 ± 1.90^{a}	5.03 ± 2.12^{b}
p value	0.007	0.185	0.638	0.019

*Hedonic line scale with left end represents 0 (extremely dislike), right end represents 10 (extremely like), and middle represents 5 (neither like nor dislike).
Different superscript letters in column are significantly different using one-way ANOVA and Fisher (LSD) test ($p<0.05$).
Samples are expressed as percentage meat (M) and navy bean (N) content.

Table 17 summarises the mean liking scores of the sensory attributes of eight fortified bread samples and a control sample. Most samples had scores of around 5. There was a significant difference ($p < 0.05$) between the control and the fortified bread samples containing meat and navy bean in terms of overall liking and flavour. However, no significant differences ($p > 0.05$) were observed in liking of texture and odour attributes. In terms of overall liking and flavour liking, breads containing only meat (25M and 50M) or navy bean (15NB and 30NB) were not significantly different to the control sample. Among all bread samples, 15NB had the highest overall liking score. In contrast, other researchers reported that beany flavour and aroma had a negative impact on the sensory attributes of bread. This beany flavour is commonly associated with food legumes (Okoye & Okaka, 2009). Serrem et al. (2011) reported that fortification of bread with defatted soy

flour significantly decreased the roasted soybean flavour, aroma and after taste scores compared to control.

A significant decrease ($p < 0.05$) in overall liking and flavour was found when bread samples were incorporated with a combination of meat and navy bean (10M30NB, 20M30NB and 35M15NB) compared to the control bread. This indicated that a combination of meat and navy bean had a negative impact on overall and flavour liking of bread samples. This is supported from my results on projective mapping that showed a combination of meaty flavour and beany taste in these samples (Figure 20). Flavour was the main factor attributing to overall liking in this study.

4.3.2 Projective mapping

Projective mapping studies have reported panel performance on products of interest (Kennedy, 2010). A total of 17 panellists participated in the projective mapping study over a period of four weeks. The RV coefficients between data from three sessions were used to determine the repeatability of individuals (Kennedy, 2010), and the data obtained are shown in Appendix 9. Panellist performance can be evaluated by their ability to position three duplicated samples close to each other on the projective mapping sheet. For 13 of the 17 panellists, the RV coefficients over the three replicate trials were all more than 0.5 when assessed by MFA. Over the three trials, roughly 10 out of 17 panellists showed an increase in the consensus maps with in replicate sessions. In trials 1 and 2, the RV coefficients of 76% panellists were more than 0.5, and in the third trial, 82% agreement were found among panellists. Products and sensory attribute maps plotted are shown in Figure 20.

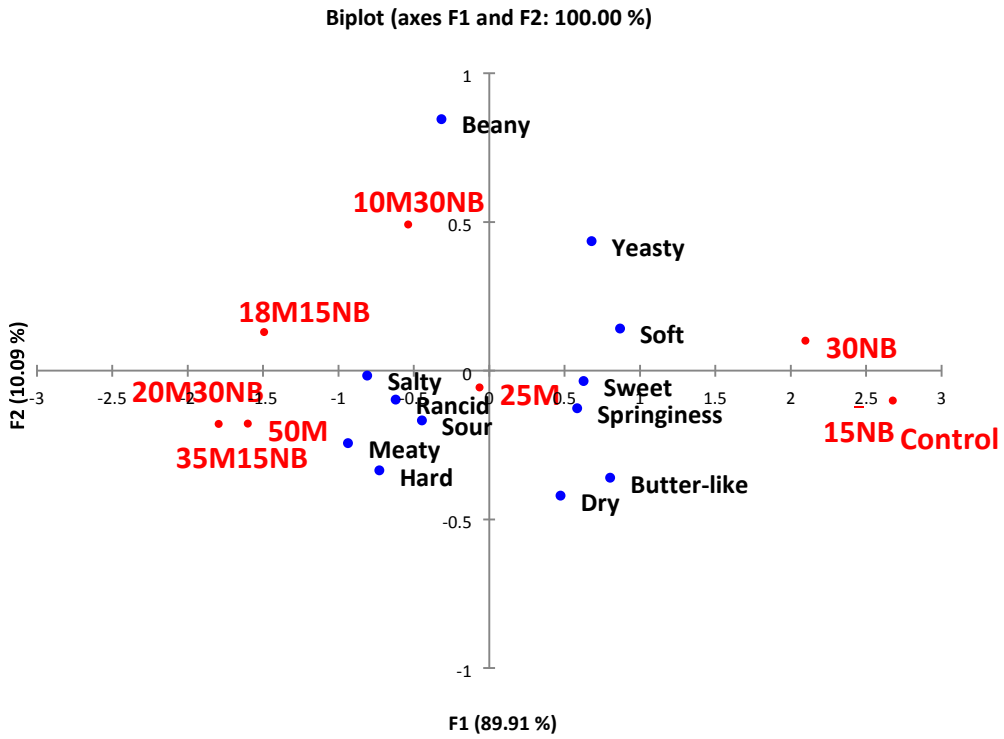


Figure 20 Principal Component Analysis of nine bread samples over the combined three sensory trials

Control and 15NB bread samples shared similar desirable attributes, like sweet, butter-like taste and springiness/chewy texture, which corresponded to the significantly higher ($p < 0.05$) scores for overall liking and flavour compare to other bread samples in consumer testing. Samples 25M, 50M, 20M30NB, and 35M15NB were associated with meaty flavour, hardness texture and salty taste. The meaty flavour of bread sample increased as the concentration of meat slurry increased. The water holding capacity of meat may also affect the appearance and consumer appeal of the sample (Van Laack, 1999). Smith et al. (1988) reported that chicken myofibrillar protein gels had high hardness values due to the denaturation of myofibrillar protein during thermal processing. Moreover, myofibrillar proteins unfold and aggregate when heated to form three-dimensional cross- linked protein network, which traps fat and macroparticulates within the gel matrix (Sun & Holley, 2011). This gel matrix was probably responsible for the hardness of bread samples with high meat content in the present study.

Sample 10M30NB was separated from the other samples due to its strong beany flavour and yeasty taste. The yeasty taste is probably due to the substitution of meat and navy bean with wheat flour. Meat and navy bean contain no gluten which led to the wheat gluten being diluted, and yeast works with gluten to create the structure of bread. This resulted in low loaf volume. Similarly, Ragaee et al. (2011), reported that the substitution of wheat flour with some grains such as barley, cellulose and oat can result in a reduction in volume of bread loaves due to the dilution of gluten and less retention of CO_2 gas to rise the bread dough.

Samples 15NB and 30NB were associated with dry mouth feel and hard crumb texture. Navy bean had higher fibre content which was 10 - 20% of dry mass (Kereliuk & Kozub, 1995). This was probably due to increased fibre from navy bean substitution as reported by Eiman et al. (2008).

4.4 *In vitro* digestion of bread

Five samples (control, 15NB, 25M, 10M30NB and 18M15NB) were selected based on the results of overall liking scores of the consumer testing for digestibility study. Starch hydrolysis and essential amino acids were examined from the samples collected from a static *in vitro* digestion model at five different sampling points over a course of 4hrs.

4.4.1 Starch hydrolysis

The concentration of total starch decreased over the 4hr of digestion period (Figure 21). The concentration indicates the total amount of starch available in the digestion reactor at given times. In other words, it provides information on the transit of the bolus and the chyme through the digestive model. The initial concentration of total starch was the highest for all of the samples, with a range from 22% (18M15NB) to 27% (control). A significant decrease for all samples was observed in the concentration of total starch during the digestion period.

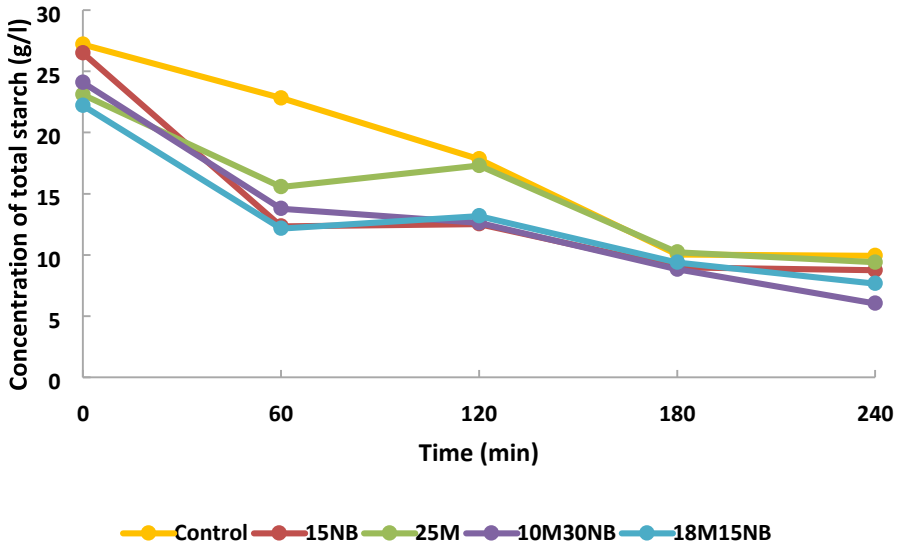


Figure 21 A graph of the concentration of total starch (g/L) available over the digestion period

The result of the starch hydrolysis is shown in Figure 22. % starch hydrolysis was calculated by dividing the concentration of glucose by the concentration of starch at the same sampling time point. A gradual increment in all bread samples was seen as time lapsed. The starch hydrolysis ranged from 18% (10M30NB) to 28% (control), which was in consistent with result from my previous study (Table 16), indicating that carbohydrate content of five samples ranged from 38.29% (18M15NB) to 56.24% (control). With the action of alpha amylase from artificial saliva and HCl from the artificial gastric secretion, starch hydrolysis for all of the bread samples gradually increased up to 60 min mark for all four of the supplemented samples. Post to 60 min, starch hydrolysis remained stable of the supplemented samples. However the control sample showed a different pattern. Rapid starch hydrolysis took place for the first 120 min, and then it stabilised for the next 120 min.

There was no significant difference in the rates of starch hydrolysis in 10M30NB and 18M15NB in the first 60 min of simulated digestion when compared to the control. However, a significantly slower starch hydrolysis was seen in all of the fortified samples from 120 min mark to the end of simulated digestion period. Meanwhile, both 10M30NB and 18M15NB experienced lower values in starch hydrolysis as compared to the other bread samples at all-time intervals. Zabidi and Aziz (2009) reported that all fortified breads with chempedak (*Artocarpus integer*) seed flour at 10%, 20% and 30% level had significantly reduced the starch hydrolysis as compared with control bread. They found the fortified breads had lower estimated glycaemic index (EGI) values corresponded with significantly decreased hydrolysis index (HI), and indicated that dietary fibre could help to reduce the carbohydrate absorption rate by inhibiting starch digestion in the small intestine, resulting in a low GI value (El, 1999, Wolever, 1990). From my results, low meat and high navy bean bread (10M30NB) exhibited a low starch hydrolysis rate due to the high dietary fibre content (10.5%) of navy bean, corresponding with a relatively low GI value compared with other bread samples (section 4.5).

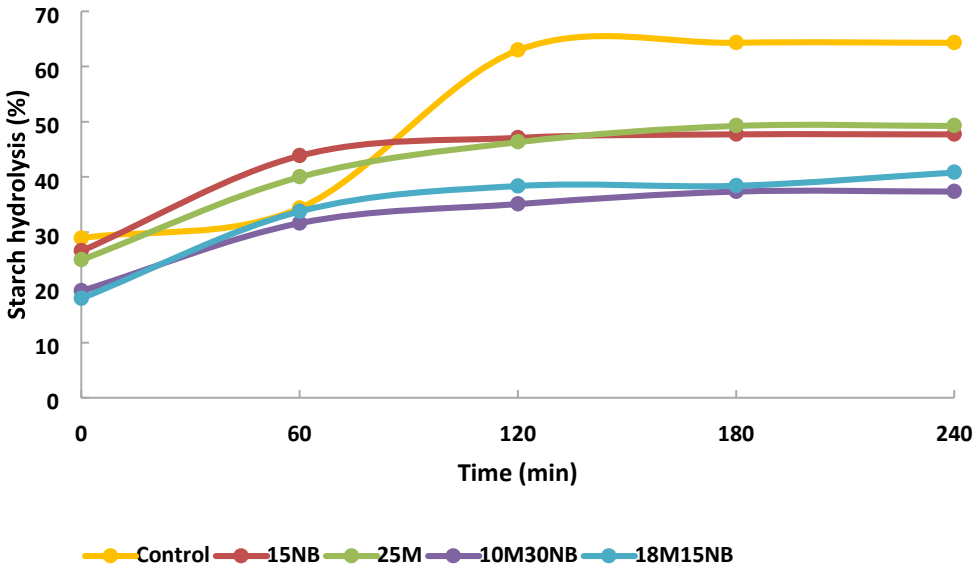


Figure 22 Starch hydrolysis (%) of five bread samples from *in vitro* digestion

4.4.2 Essential amino acid profiles

Bread is poor in protein content, and wheat proteins are deficient in some essential amino acids, such as lysine, threonine and isoleucine (Wrigly & Bietz, 1988; Abdel-Aal & Hucl, 2002). The deficiency of amino acid may cause some severe symptoms and diseases, such as anaemia, irritability and cachexia. Therefore, it is important to increase the amino acid composition in wheat flour by adding protein-enriched ingredients, such as meat and navy bean, which were used in this study. These protein sources are rich in essential amino acid, such as lysine, and have great potential in overcoming protein-calorie malnutrition (Sabanis & Tzia, 2009).

In order to study the digestibility pattern of supplemented breads and to evaluate the impact of added protein-rich ingredients on essential amino acid compositions, we used a static *in vitro* digestion model to simulate the digestion process and collected samples for further analyses. Proteins in the collected samples were hydrolyzed into amino acids and essential amino acid profile was examined. As the time of digestion lapsed, more proteins were digested by enzymes pepsin, chymotrypsin, and trypsin, hence there was an increase

in total amino acid over time for all of the samples (Figure 23). Results obtained from this part were fully digested state. It is clearly shown that 25M fortified bread had the highest value of total amino acid concentration at all times, followed by 18M15NB during the digestion period. This result indicated that addition of higher percentage of meat had more effective impact on the concentration of total essential amino acids.

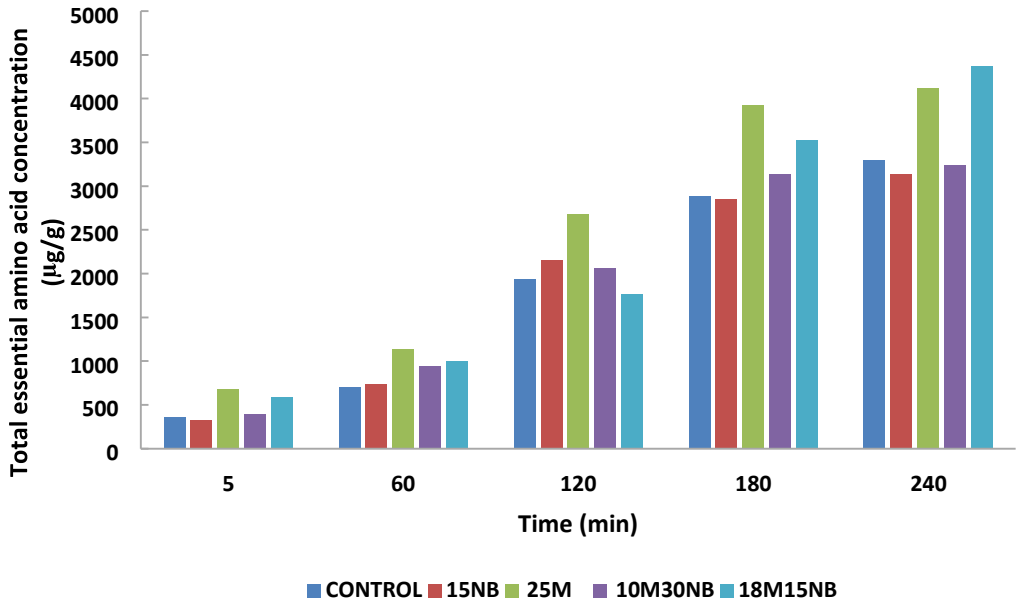


Figure 23 Total essential amino acid concentration during *in vitro* digestion at different time points

Table 18 shows the essential amino acid content in breads after 4 h of simulated digestion. Supplementation of breads with meat and navy bean were significantly different ($p < 0.05$) in the content of all essential amino acids, except isoleucine, phenylalanine and tryptophan. Lysine and threonine contents were significantly increased ($p < 0.05$) in 25M and 18M15NB compared to the control, with 25M being the highest content. This is probably due to the high content of lysine and threonine in beef (Zakhariev et al., 1980). The addition of meat and navy bean also significantly increased ($p < 0.05$) other essential amino acids including valine, leucine, and methionine in 25M and 18M15NB bread samples, which means the meat protein could effectively alleviate the deficiency of essential amino acids in bread. However, results showed a significant

decrease ($p < 0.05$) in histidine content from fortified breads 15NB and 10M30NB over control bread.

Table 18 Essential amino acids (μg/g) found 240 min (end of intestinal digestion) using EZ-Faast method

Essential amino acid (μg/g)	Control	15NB	25M	10M30NB	18M15NB
Valine	341.11 ± 15.99^{a}	362.58 ± 23.51^{a}	520.58 ± 10.8^{b}	371.85 ± 10.3^{a}	523.89 ± 10.06^{b}
Leucine	717.18 ± 28.55^{a}	647.59 ± 26.5^{ab}	877.45 ± 28.2^{c}	665.67 ± 15.7^{ab}	896.17 ± 20.82^{c}
Isoleucine	309.15 ± 17.42^{a}	268.29 ± 17.66^{a}	348.98 ± 7.3^{a}	270.86 ± 8.58^{a}	359.85 ± 12.77^{a}
Methionine	104.16 ± 5.97^{c}	106.67 ± 8.71^{bc}	185.61 ± 5.96^{ab}	119.52 ± 5.14^{abc}	202.21 ± 10.67^{a}
Phenylalanine	534.69 ± 20.17^{a}	537.57 ± 17.6^{a}	670.25 ± 22.8^{a}	575.16 ± 11.98^{a}	762.27 ± 16.06^{a}
Lysine	340.64 ± 19.95^{a}	309.65 ± 15.21^{a}	451.33 ± 13.5^{b}	331.41 ± 10.49^{a}	433.65 ± 12.1^{b}
Histidine	227.52 ± 13.39^{a}	168.93 ± 8.33^{c}	191.99 ± 5.86^{abc}	167.77 ± 13.03^{bc}	214.81 ± 8.71^{ab}
Threonine	503.61 ± 19.33^{a}	502.44 ± 15.58^{a}	599.08 ± 20.6^{b}	528.83 ± 22.39^{ab}	670.13 ± 19.8^{c}
Tryptophan	222.61 ± 8.15^{a}	232.79 ± 8.06^{a}	272.93 ± 6.67^{a}	206.21 ± 11.65^{a}	308.14 ± 8.58^{a}

Different superscript letters in same row are significantly different using one-way ANOVA and Fisher (LSD) test ($p<0.05$).
Samples are expressed as percentage meat (M) and navy bean (N) content.

Similarly, another study on bread enriched with fish mince protein (Ogur, 2014) indicated that the essential amino acids (lysine, isoleucine, threonine, etc.) of breads significantly increased ($p < 0.05$) when the ratio of fish mince to dough increased. They stated that fish mince as animal protein enriches protein and amino acid content for the most part, hence adding fish mince to bread is helpful in elimination of human health problems.

4.5 Glycaemic index

In general, breads belong to medium-to-high GI category due to their high content of starch in flour. The GI of wheat flour bread is varies from 64.2 to 78.7 depending on the different research centres (Wolever et al., 2003). Fardet et al. (2006) indicated that the glycaemic response could be affect by both raw materials and baking processes. Previous studies have been reported that mixing wheat flour with other ingredients which contain fibre could help to reduce the GI of bakery products (Akerberg et al., 1998; Angioloni &

Collar, 2010). Legume flours have high content of soluble dietary fibre, with strong interactions between amylose chains, which could help to lower GI than cereal starches (Tharanathan & Mahadevamma, 2003). Meat is low in carbohydrate and high in protein content, it may be helpful for glycaemic control in people with diabetes and beneficial with respect to overweight (Biesalski, 2005 & Johnson, 2009). In the present study, wheat flour was substituted with navy bean (GI=31) as fibre-rich and meat as protein-rich ingredient in order to reduce the GI of bread.

Glycaemic responses to five bread samples are shown in Figure 24. The fasting glycaemia between fortified breads and control were not significantly different (around 5 mM). The glycaemic peaks for all five bread samples, except 18M15NB, were observed at 30 min post to ingestion, with the highest value of 7.12 ± 0.44 mM for the control and the lowest value of 6.59 ± 0.38 for fortified bread with 10% meat and 30% navy bean.

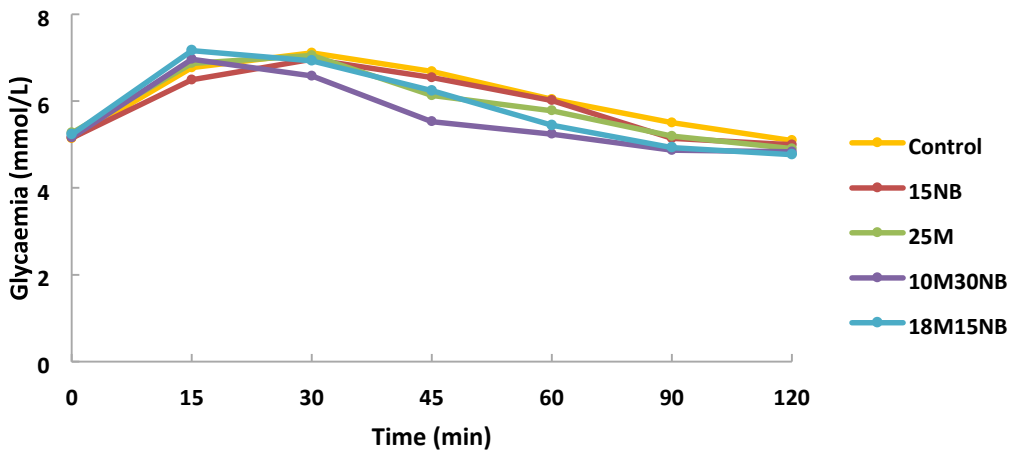


Figure 24 A graph showing averaged blood glucose values (glycaemia, mM) over a period of 2hrs after consumption of a bread sample

The mean IAUC and subsequent GI values for five bread samples are presented in Table 19. A reduction in GI was obtained for fortified breads compared to the control.

Supplementing a combination of meat and navy bean had significant impact on the GI values. A significant decrease in GI ($p < 0.05$) was observed in 10M30NB and 18M15NB bread samples compared to the control bread. However in other bread formula, where either meat or navy bean was added, no significant difference was found in GI compared to the control. There was no correlation between the body mass index and the obtained GI values for any of the bread samples (data not shown). The result is in agreement with a study in which spelt flour was added in bread and showed a significant drop of GI due to higher protein and soluble fibre content compared (Bonafaccia et al., 2000). Moreover, addition of white kidney bean extract to bread at three levels (1500 mg, 2000 mg and 3000mg) have been reported to affect the GI value, but only at 3000mg level of powder was found to significantly reduce the GI value (Udani et al., 2009). In this study, the result showed that bread samples containing higher content of navy bean (10M30NB) showed a significantly lower value in GI than 15NB bread. This indicates that bread supplemented with the appropriate amount of navy bean may be a novel and potentially effective strategy for reducing the GI of bread. Sample 18M15NB also significantly reduced the GI of bread, which is contributed by less carbohydrate content may contribute to lower GI value.

Although a reduction of GI value was observed in fortified breads compared to the control, the GI values for all bread samples were close to one another. All bread samples tested belong to high GI category (70 or more). Five tested samples were chosen from nine samples (Table 9) based on the acceptability to test the feasibility of developing this bread into a commercial product. However with the chosen five samples, extremes of supplementation (for example 50% flour substituted with meat) were not tested for GI. It is speculated that if those samples were tested, a clear difference GI may have been observed. Sample size may have been one of the limitations to the GI testing as well. According to Glycaemic Index Testing & Research at Sydney University, recommended a minimum of 8 subjects are required to test for GI of a food and this recommendation was followed for the current study. However, according to FAO/WHO/ISO standards (2008), at least 10 subjects are needed to determine the GI of a test food. A mixture of female and male subjects were used in this study and sex may have influenced the data. Hence a larger number of subjects should have been recruited for more meaningful results.

Table 19 Glycaemic index values of five bread samples

Sample	IAUC	Glycaemic index
Control	719.59[a]	90.69 ± 5.73[a]
15NB	695.34[ab]	86.89 ± 3.48[ab]
25M	690.20[ab]	86.98 ± 6.14[ab]
10M30NB	657.81[b]	82.90 ± 4.38[b]
18M15NB	671.47[b]	84.62 ± 5.13[b]
Reference(glucose)	793.5	100

Different superscript letters in column are significantly different using one-way ANOVA and Fisher (LSD) test ($p<0.05$).

Chapter 5 Conclusion

In the present study, I used bread as a vehicle to deliver additional nutrition of meat and navy bean, and to explore how meat proteins interact with the macromolecules of other ingredients (e.g., starches and fibre). Actual meat (beef) at 0%, 10%, 18%, 20%, 25%, 35%, 50% and navy bean powder at 0%, 15%, 30% were added into bread samples according to a mixture design (Table 9).

The supplementation of meat and navy bean significantly increased protein and fat content of the bread samples due to meat and navy bean containing high protein content. The moisture content also increased by addition of meat and navy bean, which may be related to the high moisture content of meat and the high fibre content of navy bean. In addition, the physical characteristics of fortified breads were also affected by the addition of meat and navy bean. Loaf volume of fortified bread samples was significantly decreased ($p < 0.05$) except in 15NB sample compared with control bread, because of gluten dilution by adding non-wheat ingredients (meat and navy bean). The texture parameters of the fortified bread (hardness, springiness, gumminess, and chewiness) were significantly affected by the incorporation of meat and navy bean. Moreover, addition of red meat significantly decreased the lightness (L*), while navy bean was significantly increased the yellowness (b*) due to the slight yellow colour of navy bean. SEM was used to investigate the microstructure of bread samples. Enhancement of protein matrix and less starch granules were observed in fortified bread samples compared with the control. Increased protein content and insufficient gelatinization of starch may have led to this result.

Addition of meat and navy bean also affected the consumer acceptability of bread. Significant differences were observed in terms of overall liking, and flavour for almost of all bread samples except only navy bean fortified bread samples (15NB and 30NB) and low meat content (25M). From sensory projective mapping we found that panellists were able to differentiate the fortified bread samples with 15 sensory attributes. Analysis of the projective mapping data helped to explain liking of the reformulated bread samples.

Digestibility was assessed using *in vitro* digestion method, was utilized to examine the digestibility of breads. The results indicated that the addition of the combination of meat and navy bean had a significant impact in the starch hydrolysis rate of breads. 10M30NB and 18M15NB were significantly lower in starch hydrolysis compared with other tested bread samples, corresponding with relatively low GI values. 25M and 18M15NB sample had a significant increase in the content of essential amino acids, such as lysine, threonine, valine, leucine, and methionine. The results demonstrated that this fortification could help to increment the deficiency of amino acids in wheat flour, and balance the essential amino acid content in the fortified bread samples. Glycaemic index was also measured. Only the combination of meat and navy bean samples (10M30NB and 18M15NB) had significant decreased ($p < 0.05$) GI values in subjects.

In this study, proteins from both animal and plant sources were supplemented into a starch food, which was the first investigation that has incorporated combinations of meat and navy bean to increase the nutritional values of bread. Further studies looking at the interaction of combined meat and navy on the nutritional values would be useful to explain the data. Glycaemic index testing should be undertaken on all of the samples prepared in the study with a larger cohort. A dynamic *in vitro* digestion study would be useful to determine effects of reformulated bread on the digestibility. The model would mimic complex conditions occurring in human digestive tract better, and give more accurate and reproducible data.

Overall, we have successfully incorporated meat and navy bean into bread that have acceptable characteristics and additional health benefits. This fortified breads may be helpful to those people who suffering from diabetes and obesity, and will give an idea to study innovations in bread with have a positive implication for human health.

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Appendices

1. Instruction and questionnaire for consumer testing

Please indicate your gender

- ☐ **Male**
- ☐ **Female**

Please indicate your age

- ☐ **Under 20**
- ☐ **20-29**
- ☐ **30-39**
- ☐ **Above 40**

Please indicate if you are allergic to the ingredients listed below

- ☐ **Meat**
- ☐ **Navy Bean**
- ☐ **Gluten**

Are you a vegetarian or culturally sensitive to the presence of meat in bread

☐ **Yes**

☐ **No**

How often do you consume bread?

☐ **Never**

☐ **Once a month**

☐ **Once a week**

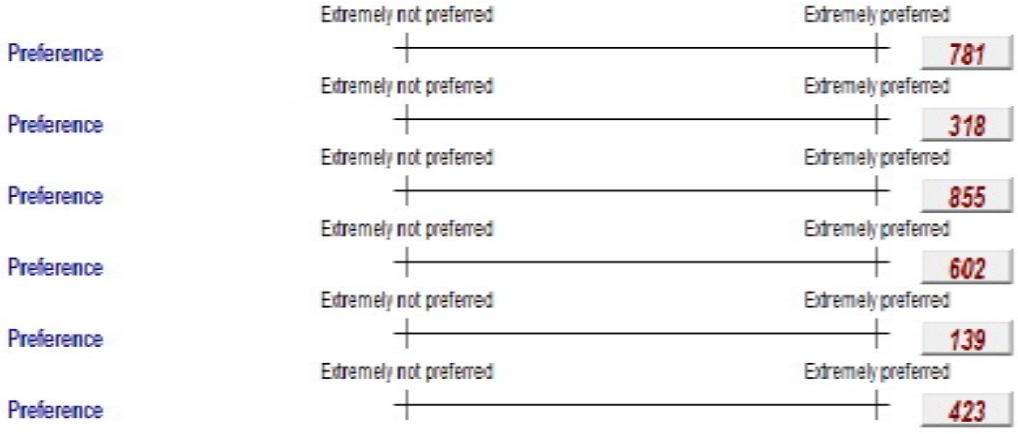
☐ **More than once a week**

Instructions:

Please rate the sample by clicking the line scale given depending on the perceived preference

Please take 30 seconds break per food sample given

Please rinse your mouth with the given filtered water



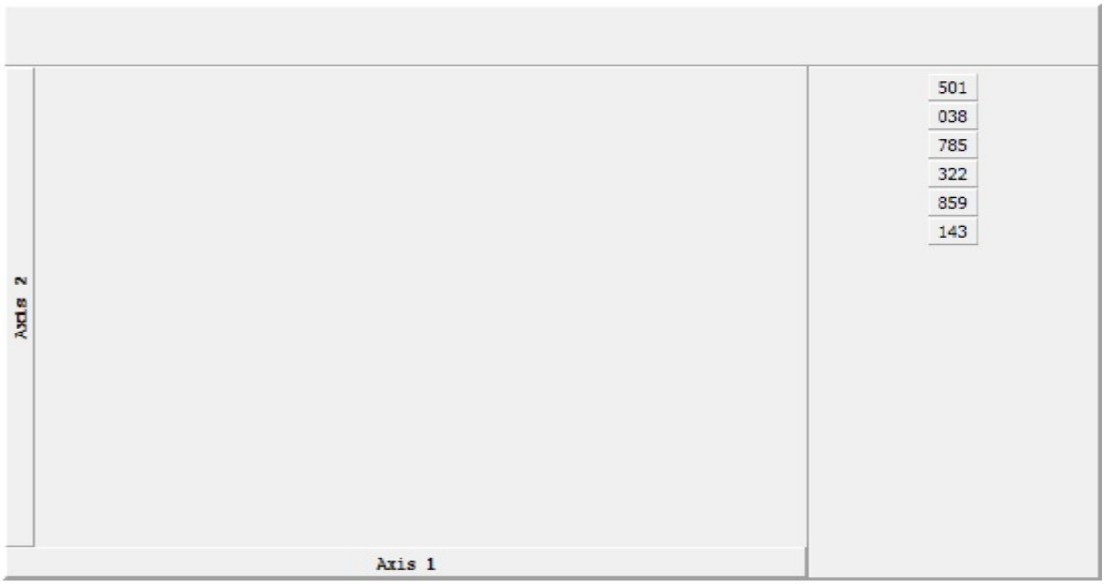
2. Instruction and questionnaire for Projective mapping

Instructions:

Please rate the sample by clicking the line scale given depending on the perceived preference

Please take 30 seconds break per food sample given

Please rinse your mouth with the given filtered water



3. Alpha-amylase activity assay

Enzymatic Assay of α-AMYLASE[1]
(EC 3.2.1.1)

REAGENTS: (continued)

E. Color Reagent Solution (Clr Rgt Soln)
(With stirring, slowly add Reagent C to Reagent D. Dilute to 40 ml with deionized water. If not completely dissolved, the reagents should dissolve when mixed. The solution should be stored in an amber bottle at room temperature. The Color Reagent Solution is stable for 6 months.)

F. 0.2% (w/v) Maltose Standard Solution
(Prepare 10 ml in deionized water using Maltose, Monohydrate, Sigma Prod. No. M-5885.)

G. α-Amylase Solution
(Immediately before use, prepare a solution containing 1 unit/ml of α-Amylase in cold deionized water.)[2]

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable containers:

	Test	Blank
Reagent B (Starch)	1.00	1.00

Mix by swirling and equilibrate to 20°C. Then add:

Reagent G (Enzyme Solution)	1.00	------

Mix by swirling and incubate for exactly 3.0 minutes at 20°C. Then add:

Reagent E (Clr Rgt Soln)	1.00	1.00
Reagent G (Enzyme Solution)	------	1.00

Cap and place in a boiling water bath for exactly 15 minutes, then cool on ice to room temperature and add:

Deionized water	9.00	9.00

Mix by inversion and record the A_{540nm} for both the Test and Blank using a suitable spectrophotometer.

Enzymatic Assay of α-AMYLASE[1]
(EC 3.2.1.1)

PROCEDURE: (continued)

Standard Curve:

A standard curve is made by pipetting (in milliliters) the following reagents into suitable containers:

	Std 1	Std 2	Std 3	Std 4	Std 5	Std Blank
Reagent F (Std Soln)	0.20	0.40	0.60	0.80	1.00	----
Deionized Water	1.80	1.60	1.40	1.20	1.00	2.00
Reagent E (Clr Rgt Soln)	1.00	1.00	1.00	1.00	1.00	1.00

Cap and place in a boiling water bath for exactly 15 minutes, then cool on ice to room temperature and add:

Deionized Water	9.00	9.00	9.00	9.00	9.00	9.00

Mix by inversion and record the A_{540nm} for the Standards and Standard Blank using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

ΔA_{540nm} Standard = A_{540nm} Std - A_{540nm} Std Blank

Plot the ΔA_{540nm} of the Standards vs milligrams of Maltose.

Sample Determination:

Δ_{540nm} Sample = A_{540nm} Test - A_{540nm} Blank

Determine the milligrams of Maltose liberated using the Standard Curve.

$$\text{Units/ml enzyme} = \frac{\text{(mg of Maltose released) (df)}}{\text{(1)}}$$

df = dilution factor
1 = Volume (in milliliter) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

Enzymatic Assay of α-AMYLASE[1]
(EC 3.2.1.1)

CALCULATIONS: (continued)

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will liberate 1.0 mg of maltose from starch in 3 minutes at pH 6.9 at 20°C.

FINAL ASSAY CONCENTRATIONS:

In a 2.00 ml reaction mix, the final concentrations are 10 mM sodium phosphate, 0.50% (w/v) starch, 3.4 mM sodium chloride and 1 unit α-amylase.

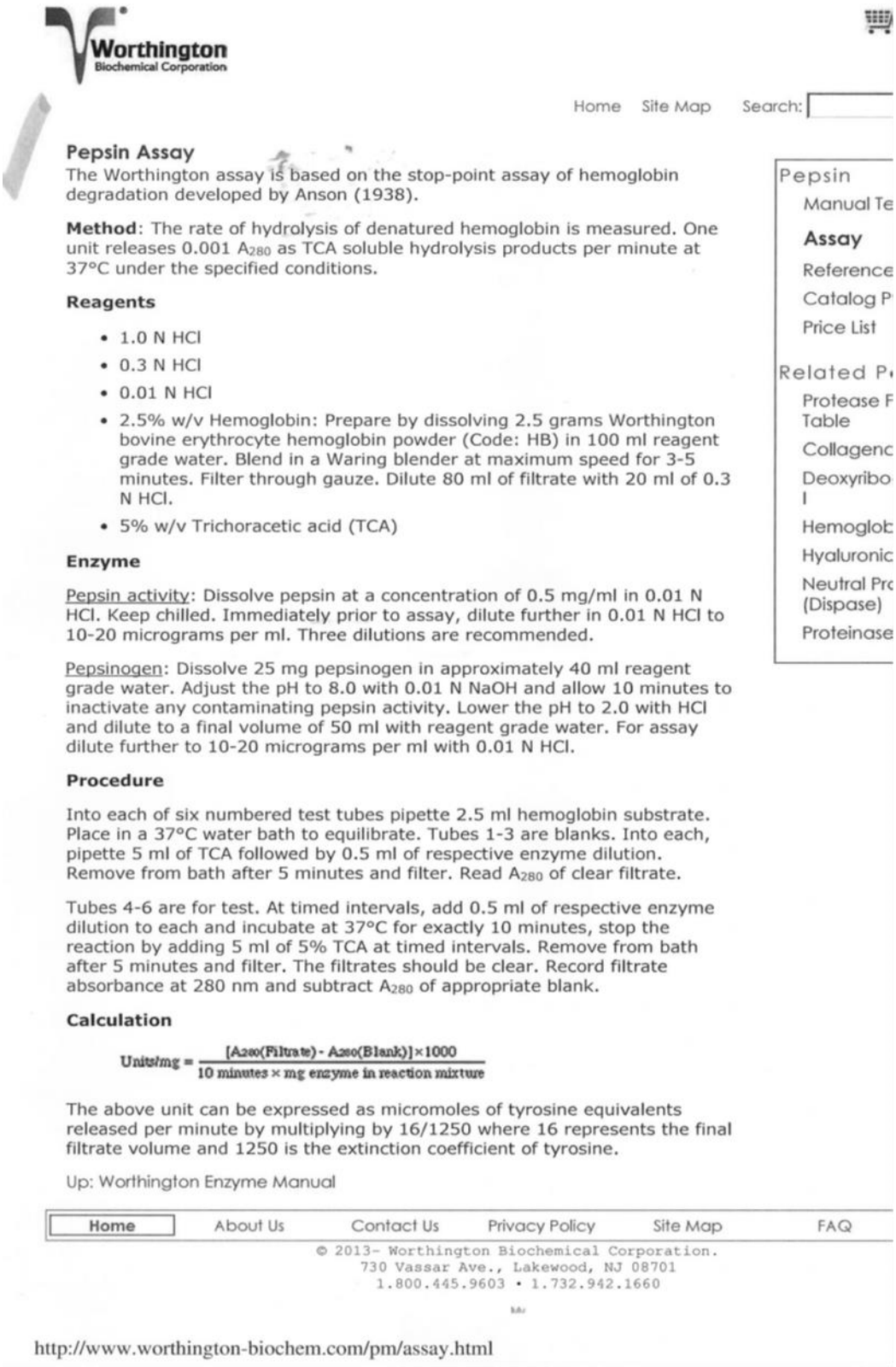
REFERENCE:

Bernfeld, P. (1955) *Methods in Enzymology* **1**, 149-158

NOTES:

1. This enzyme assay is not to be used to assay α-Amylase, Insoluble, Sigma Prod. Nos. A-0909 and A-5386.
2. α-Amylase, Sigma Prod. No. A-4551 is diluted in Reagent A at 20°C rather than in water.
3. This assay is based on the cited reference.
4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

4. Pepsin activity assay (Worthington assay)



Worthington Biochemical Corporation

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Pepsin Assay

The Worthington assay is based on the stop-point assay of hemoglobin degradation developed by Anson (1938).

Method: The rate of hydrolysis of denatured hemoglobin is measured. One unit releases 0.001 A_{280} as TCA soluble hydrolysis products per minute at 37°C under the specified conditions.

Reagents

- 1.0 N HCl
- 0.3 N HCl
- 0.01 N HCl
- 2.5% w/v Hemoglobin: Prepare by dissolving 2.5 grams Worthington bovine erythrocyte hemoglobin powder (Code: HB) in 100 ml reagent grade water. Blend in a Waring blender at maximum speed for 3-5 minutes. Filter through gauze. Dilute 80 ml of filtrate with 20 ml of 0.3 N HCl.
- 5% w/v Trichoracetic acid (TCA)

Enzyme

Pepsin activity: Dissolve pepsin at a concentration of 0.5 mg/ml in 0.01 N HCl. Keep chilled. Immediately prior to assay, dilute further in 0.01 N HCl to 10-20 micrograms per ml. Three dilutions are recommended.

Pepsinogen: Dissolve 25 mg pepsinogen in approximately 40 ml reagent grade water. Adjust the pH to 8.0 with 0.01 N NaOH and allow 10 minutes to inactivate any contaminating pepsin activity. Lower the pH to 2.0 with HCl and dilute to a final volume of 50 ml with reagent grade water. For assay dilute further to 10-20 micrograms per ml with 0.01 N HCl.

Procedure

Into each of six numbered test tubes pipette 2.5 ml hemoglobin substrate. Place in a 37°C water bath to equilibrate. Tubes 1-3 are blanks. Into each, pipette 5 ml of TCA followed by 0.5 ml of respective enzyme dilution. Remove from bath after 5 minutes and filter. Read A_{280} of clear filtrate.

Tubes 4-6 are for test. At timed intervals, add 0.5 ml of respective enzyme dilution to each and incubate at 37°C for exactly 10 minutes, stop the reaction by adding 5 ml of 5% TCA at timed intervals. Remove from bath after 5 minutes and filter. The filtrates should be clear. Record filtrate absorbance at 280 nm and subtract A_{280} of appropriate blank.

Calculation

$$\text{Units/mg} = \frac{[A_{280}(\text{Filtrate}) - A_{280}(\text{Blank})] \times 1000}{10 \text{ minutes} \times \text{mg enzyme in reaction mixture}}$$

The above unit can be expressed as micromoles of tyrosine equivalents released per minute by multiplying by 16/1250 where 16 represents the final filtrate volume and 1250 is the extinction coefficient of tyrosine.

Up: Worthington Enzyme Manual

Pepsin
Manual Te
Assay
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Collagenc
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http://www.worthington-biochem.com/pm/assay.html

5. Pancreatic enzyme (trypsin) activity assay

Procedure for Enzymatic Assay of Trypsin (EC 3.4.21.4)

Description

This procedure is for products with a specification for Trypsin activity using Nα-Benzoyl-L-arginine ethyl ester (BAEE) as a substrate. The procedure is a continuous spectrophotometric rate determination (A_{253}, Light path = 1 cm) based on the following reaction:

BAEE + H_2O $\xrightarrow{\text{Trypsin}}$ Nα-Benzoyl-L-arginine + ethanol

where:
BAEE = Nα-Benzoyl-L-arginine ethyl ester

Unit Definition = One BAEE unit of trypsin activity will produce a ΔA_{253} of 0.001 per minute with BAEE as substrate at pH 7.6 at 25 °C in a reaction volume of 3.20 ml.

Precautions

Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Reagents and Equipment Required

Sodium phosphate, monobasic (Catalog No. **S0751**)

Nα-Benzoyl-L-arginine ethyl ester (BAEE, Catalog No. **B4500**)

1 M Hydrochloric acid (Catalog No. **318949**)

Preparation Instructions

Use ultrapure water (≥18 MΩxcm resistivity at 25 °C) for the preparation of reagents.

Buffer (67 mM Sodium Phosphate Buffer, pH 7.6 at 25 °C) – Prepare a 8.04 mg/ml solution using **sodium phosphate, monobasic** (Catalog No. **S0751**) in ultrapure water. Adjust to pH 7.6 at 25 °C with 1 M NaOH solution.

Substrate Solution (0.25 mM Nα-Benzoyl-L-arginine ethyl ester) – Prepare a 0.086 mg/ml solution using **Nα-Benzoyl-L-arginine ethyl ester** (BAEE, Catalog No. **B4500**) in Buffer.

HCl Solution (1 mM Hydrochloric Acid) – Prepare a 1,000-fold dilution of **1 M Hydrochloric acid solution** (Catalog No. **318949**) in ultrapure water.

Enzyme Solution (Trypsin) – Immediately before use, prepare a solution containing 425-575 units/ml of Trypsin in cold (2-8 °C) HCl Solution.

Procedure

In a 3.20 ml reaction mix, the final concentrations are 62.8 mM sodium phosphate, 0.23 mM Nα-Benzoyl- L-arginine ethyl ester, 0.031-0.063 mM hydrochloric acid, 42.5-115.0 units of trypsin.

1. Pipette the following reagents into suitable quartz cuvettes:

Reagent	Blank (ml)	Test (ml)
Substrate Solution	3.00	3.00
HCl Solution	0.200	0.125

2. Mix by inversion and equilibrate to 25 °C using a suitably thermostatted spectrophotometer. Then add:

Reagent	Blank (ml)	Test (ml)
Enzyme Solution	–	0.075

Note: Final volume in each cuvette must be 3.2 ml per unit definition.

3. Immediately mix by inversion and record the increase in A_{253} for 5 minutes. Using a 1 minute time period and a minimum of 4 data points, obtain the ΔA_{253}/minute using the maximum linear rate for both the Blank and Test.

Results

Calculations

1.

$$\text{BAEE units/ml enzyme} = \frac{(\Delta A_{253}\text{/minute Test} - \Delta A_{253}\text{/minute Blank}) \times (df)}{(0.001) \times (0.075)}$$

Where:
df = dilution factor
0.001 = The change in A_{253}/minute based on unit definition
0.075 = volume (ml) of test sample used in assay
Note: The total volume in the cuvette is not used in the calculation since the unit definition based on 3.2 ml.

2.

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

6. Procedure used for total starch assay Kit

PRINCIPLE:

Thermostable α-amylase hydrolyses starch into soluble branched and unbranched maltodextrins (1).

(1) Starch + H_2O —(α-amylase, pH 7.0 or 5.0, 100°C)→ maltodextrins

Where necessary, **resistant starch in the sample is pre-dissolved by stirring the sample with 2 M KOH at approx. 4°C**, followed by neutralisation with sodium acetate buffer and hydrolysis with α-amylase (2). Alternatively, dissolution in DMSO at 100°C is effective.

(2) Resistant starch + H_2O —(KOH then neutralisation + α-amylase)→ maltodextrins

Amyloglucosidase (AMG) quantitatively hydrolyses maltodextrins to D-glucose (3).

(3) Maltodextrins —(AMG)→ D-glucose

D-Glucose is oxidised to D-gluconate with the release of one mole of hydrogen peroxide (H_2O_2) which is quantitatively measured in a colourimetric reaction employing peroxidase and the production of a quinoneimine dye.

(4) D-Glucose + O_2 + H_2O —(glucose oxidase)→ D-gluconate + H_2O_2

(5) 2 H_2O_2 + *p*-hydroxybenzoic acid + 4-aminoantipyrine —(peroxidase)→ quinoneimine dye + 4 H_2O

Samples containing high levels of D-glucose and maltodextrins are washed with aqueous ethanol (80 % v/v) before analysis.

Analysis of a single sample can be performed within 70 min. Twenty

CALCULATIONS (Liquid samples; mg/100 mL):

$$\text{Starch} = \Delta_A \times F \times \frac{100}{0.1} \times \frac{1}{1000} \times \frac{162}{180} \times 2 \times D$$

$$= \Delta_A \times F \times D \times 1.8$$

where:

Δ_A = Absorbance (reaction) read against the reagent blank.

F = $\dfrac{\textbf{100 (µg of D-glucose)}}{\textbf{absorbance for 100 µg of glucose}}$ (conversion from absorbance to µg)

100 = conversion to 100 mL sample volume.

0.1 = volume of sample analysed.

$\dfrac{1}{1000}$ = Conversion from µg to mg.

$\dfrac{162}{180}$ = Adjustment from free D-glucose to anhydro D-glucose (as occurs in starch).

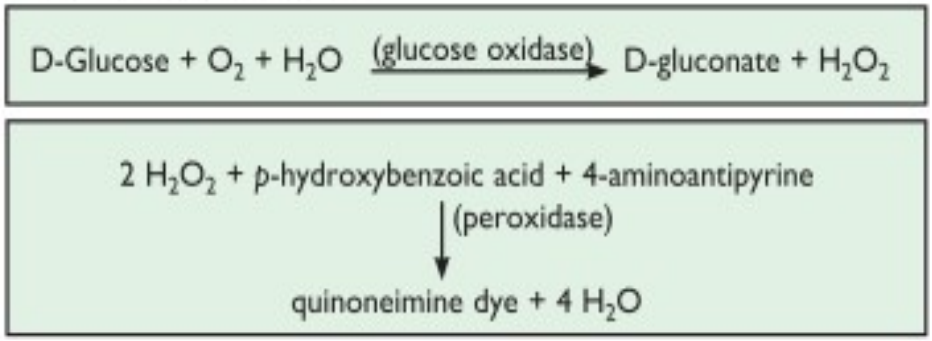
2 = Dilution of the sample solution on incubation with AMG.

D = Further dilution of the incubation mixture (if required).

7. Procedure used for D-glucose assay Kit

PRINCIPLE:

The reactions involved are:



KITS:

Kits suitable for performing 660 assays (3 mL per assay) are available from Megazyme. The kits contain the full assay method plus:

Bottle 1: (x 2) **GOPOD Reagent Buffer.** Buffer (48 mL, pH 7.4), p-hydroxybenzoic acid and sodium azide (0.4% w/v). Stable for > 4 years at 4°C.

Bottle 2: (x 2) **GOPOD Reagent Enzymes.** Glucose oxidase plus peroxidase and 4-aminoantipyrine. Freeze-dried powder. Stable for > 5 years at -20°C.

Bottle 3: D-Glucose standard solution (5 mL, 1.0 mg/mL) in 0.2% (w/v) benzoic acid. Stable for > 5 years at room temperature.

CALCULATION:

$$\text{D-Glucose } (\mu g/0.1 \text{ mL}) = \frac{\Delta A_{\text{Sample}}}{\Delta A_{\text{D-Glucose standard (100 } \mu g)}} \times 100$$

	Reagent blank	Standard	Sample
GOPOD reagent	3.0 mL	3.0 mL	3.0 mL
D-Glucose standard	-	0.1 mL	-
sample	-	-	0.1 mL
buffer or water	0.1 mL	-	-

REFERENCES:

1. Trinder, P. (1969). *Ann. Clin. Biochem.*, **6**, 24.
2. Blakeney, A. B. & Matheson, N. K. (1984). *Starch*, **36**, 265.
3. McCleary, B. V. & Codd, R. (1991). *J. Sci. Food Agric.*, **55**, 303.

Megazyme

8. Letter of Approval from AUT Ethics committee (AUTEC)

a) Ethics Approval letter for sensory evaluations

AUT UNIVERSITY
TE WĀNANGA ARONUI O TAMAKI MAKAU RAU

AUTEC

SECRETARIAT

9 April 2014

Michelle Yoo
Faculty of Health and Environmental Sciences

Dear Michelle

Re Ethics Application: **14/48 Sensory testing of bread with added meat and navy bean.**

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

Your ethics application has been approved for three years until 9 April 2017.

As part of the ethics approval process, you are required to submit the following to AUTEC:

- A brief annual progress report using form EA2, which is available online throughhttp://www.aut.ac.nz/researchethics. When necessary this form may also be used to request an extension of the approval at least one month prior to its expiry on 9 April 2017;
- A brief report on the status of the project using form EA3, which is available online through http://www.aut.ac.nz/researchethics. This report is to be submitted either when the approval expires on 9 April 2017 or on completion of the project.

It is a condition of approval that AUTEC is notified of any adverse events or if the research does not commence. AUTEC approval needs to be sought for any alteration to the research, including any alteration of or addition to any documents that are provided to participants. You are responsible for ensuring that research undertaken under this approval occurs within the parameters outlined in the approved application.

AUTEC grants ethical approval only. If you require management approval from an institution or organisation for your research, then you will need to obtain this.

To enable us to provide you with efficient service, please use the application number and study title in all correspondence with us. If you have any enquiries about this application, or anything else, please do contact us at ethics@aut.ac.nz.

All the very best with your research,

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

Cc: Zhen Guo guozhen1016@gmail.com

b) Ethics Approval letter for *in vivo* glycemic index testing

AUTEC
SECRETARIAT

28 January 2015

Michelle Yoo
Faculty of Health and Environmental Sciences

Dear Michelle

Re: Ethics Application: **14/48 Sensory testing of bread with added meat and navy bean.**

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

I have approved the minor amendment to your ethics application allowing additional testing procedures.

I remind you that as part of the ethics approval process, you are required to submit the following to the Auckland University of Technology Ethics Committee (AUTEC):

- A brief annual progress report using form EA2, which is available online through http://www.aut.ac.nz/researchethics. When necessary this form may also be used to request an extension of the approval at least one month prior to its expiry on 9 April 2017;
- A brief report on the status of the project using form EA3, which is available online through http://www.aut.ac.nz/researchethics. This report is to be submitted either when the approval expires on 9 April 2017 or on completion of the project.

It is a condition of approval that AUTEC is notified of any adverse events or if the research does not commence. AUTEC approval needs to be sought for any alteration to the research, including any alteration of or addition to any documents that are provided to participants. You are responsible for ensuring that research undertaken under this approval occurs within the parameters outlined in the approved application.

AUTEC grants ethical approval only. If you require management approval from an institution or organisation for your research, then you will need to obtain this.

To enable us to provide you with efficient service, please use the application number and study title in all correspondence with us. If you have any enquiries about this application, or anything else, please do contact us at ethics@aut.ac.nz.

All the very best with your research,

Kate O'Connor
Executive Secretary
Auckland University of Technology Ethics Committee Cc:

Zhen Guo guozhen1016@gmail.com

9. The RV coefficient between projective maps and multifactor analysis

Table 1 RV coefficient between projective maps and multifactor analysis (MFA) for week one where 76% of panelists scored > 0.5. Panelists are identified as N1 to N17. Values shown in red indicate poor fit with MFA.

	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13	N14	N15	N16	N17	MFA
N1	1.000	0.224	0.424	0.585	0.381	0.191	0.508	0.057	0.638	0.302	0.427	0.328	0.460	0.342	0.510	0.586	0.469	0.675
N2	0.224	1.000	0.475	0.439	0.327	0.020	0.528	0.225	0.480	0.072	0.720	0.178	0.440	0.483	0.445	0.247	0.445	0.596
N3	0.424	0.475	1.000	0.644	0.718	0.312	0.637	0.458	0.585	0.373	0.545	0.029	0.376	0.519	0.629	0.401	0.814	0.821
N4	0.585	0.439	0.644	1.000	0.637	0.085	0.880	0.182	0.846	0.395	0.763	0.065	0.407	0.388	0.743	0.429	0.686	0.824
N5	0.381	0.327	0.718	0.637	1.000	0.312	0.562	0.452	0.535	0.226	0.429	0.069	0.333	0.360	0.528	0.321	0.621	0.710
N6	0.191	0.020	0.312	0.085	0.312	1.000	0.138	0.471	0.214	0.042	0.030	0.323	0.200	0.161	0.040	0.079	0.116	0.330
N7	0.508	0.528	0.637	0.880	0.562	0.138	1.000	0.304	0.747	0.395	0.797	0.086	0.480	0.432	0.803	0.460	0.650	0.843
N8	0.057	0.225	0.458	0.182	0.452	0.471	0.304	1.000	0.208	0.229	0.165	0.102	0.147	0.193	0.188	0.316	0.234	0.451
N9	0.638	0.480	0.585	0.846	0.535	0.214	0.747	0.208	1.000	0.191	0.602	0.073	0.516	0.490	0.614	0.455	0.558	0.781
N10	0.302	0.072	0.373	0.395	0.226	0.042	0.395	0.229	0.191	1.000	0.246	0.366	0.368	0.043	0.431	0.195	0.371	0.489
N11	0.427	0.720	0.545	0.763	0.429	0.030	0.797	0.165	0.602	0.246	1.000	0.046	0.400	0.447	0.659	0.463	0.601	0.743
N12	0.328	0.178	0.029	0.065	0.069	0.323	0.086	0.102	0.073	0.366	0.046	1.000	0.105	0.093	0.097	0.070	0.032	0.270
N13	0.460	0.440	0.376	0.407	0.333	0.200	0.480	0.147	0.516	0.368	0.400	0.105	1.000	0.471	0.630	0.242	0.534	0.641
N14	0.342	0.483	0.519	0.388	0.360	0.161	0.432	0.193	0.490	0.043	0.447	0.093	0.471	1.000	0.470	0.338	0.513	0.612
N15	0.510	0.445	0.629	0.743	0.528	0.040	0.803	0.188	0.614	0.431	0.659	0.097	0.630	0.470	1.000	0.546	0.799	0.829
N16	0.586	0.247	0.401	0.429	0.321	0.079	0.460	0.316	0.455	0.195	0.463	0.070	0.242	0.338	0.546	1.000	0.440	0.606
N17	0.469	0.445	0.814	0.686	0.621	0.116	0.650	0.234	0.558	0.371	0.601	0.032	0.534	0.513	0.799	0.440	1.000	0.817
MFA	0.675	0.596	0.821	0.824	0.710	0.330	0.843	0.451	0.781	0.489	0.743	0.270	0.641	0.612	0.829	0.606	0.817	1.000

Table 2 RV coefficient between projective maps and multifactor analysis (MFA) for week two where 76% of panelists scored > 0.5.

	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13	N14	N15	N16	N17	MFA
N1	1.000	0.353	0.113	0.546	0.470	0.679	0.382	0.623	0.582	0.602	0.243	0.502	0.533	0.464	0.493	0.177	0.179	0.764
N2	0.353	1.000	0.226	0.390	0.099	0.277	0.395	0.535	0.378	0.351	0.171	0.437	0.250	0.262	0.330	0.217	0.252	0.585
N3	0.113	0.226	1.000	0.222	0.422	0.174	0.209	0.206	0.264	0.433	0.143	0.260	0.029	0.136	0.391	0.244	0.148	0.446
N4	0.546	0.390	0.222	1.000	0.406	0.456	0.372	0.608	0.510	0.334	0.311	0.299	0.472	0.504	0.393	0.172	0.189	0.688
N5	0.470	0.099	0.422	0.406	1.000	0.494	0.243	0.318	0.371	0.572	0.516	0.326	0.367	0.416	0.318	0.149	0.085	0.634
N6	0.679	0.277	0.174	0.456	0.494	1.000	0.354	0.444	0.569	0.468	0.217	0.333	0.695	0.293	0.231	0.057	0.049	0.649
N7	0.382	0.395	0.209	0.372	0.243	0.354	1.000	0.483	0.530	0.403	0.076	0.577	0.337	0.261	0.186	0.279	0.072	0.592
N8	0.623	0.535	0.206	0.608	0.318	0.444	0.483	1.000	0.590	0.602	0.087	0.615	0.261	0.539	0.591	0.270	0.112	0.759
N9	0.582	0.378	0.264	0.510	0.371	0.569	0.530	0.590	1.000	0.590	0.137	0.463	0.442	0.456	0.383	0.376	0.183	0.759
N10	0.602	0.351	0.433	0.334	0.572	0.468	0.403	0.602	0.590	1.000	0.087	0.583	0.357	0.342	0.553	0.281	0.201	0.751
N11	0.243	0.171	0.143	0.311	0.516	0.217	0.076	0.087	0.137	0.087	1.000	0.095	0.220	0.102	0.017	0.089	0.011	0.344
N12	0.502	0.437	0.260	0.299	0.326	0.333	0.577	0.615	0.463	0.583	0.095	1.000	0.343	0.296	0.426	0.499	0.326	0.720
N13	0.533	0.250	0.029	0.472	0.367	0.695	0.337	0.261	0.442	0.357	0.220	0.343	1.000	0.337	0.190	0.208	0.270	0.604
N14	0.464	0.262	0.136	0.504	0.416	0.293	0.261	0.539	0.456	0.342	0.102	0.296	0.337	1.000	0.502	0.200	0.068	0.588
N15	0.493	0.330	0.391	0.393	0.318	0.231	0.186	0.591	0.383	0.553	0.017	0.426	0.190	0.502	1.000	0.284	0.246	0.631
N16	0.177	0.217	0.244	0.172	0.149	0.057	0.279	0.270	0.376	0.281	0.089	0.499	0.208	0.200	0.284	1.000	0.183	0.455
N17	0.179	0.252	0.148	0.189	0.085	0.049	0.072	0.112	0.183	0.201	0.011	0.326	0.270	0.068	0.246	0.183	1.000	0.362
MFA	0.764	0.585	0.446	0.688	0.634	0.649	0.592	0.759	0.759	0.751	0.344	0.720	0.604	0.588	0.631	0.455	0.362	1.000

Table 3 RV coefficient between projective maps and multifactor analysis (MFA) for week two where 82% of panelists scored > 0.5.

	Table1	Table2	Table3	Table4	Table5	Table6	Table7	Table8	Table9	Table10	Table11	Table12	Table13	Table14	Table15	Table16	Table17	MFA
N1	1.000	0.397	0.112	0.295	0.238	0.338	0.126	0.493	0.341	0.506	0.520	0.635	0.401	0.513	0.502	0.453	0.292	0.656
N2	0.397	1.000	0.140	0.321	0.411	0.417	0.087	0.444	0.516	0.582	0.493	0.625	0.250	0.499	0.517	0.329	0.390	0.677
N3	0.112	0.140	1.000	0.397	0.153	0.140	0.113	0.149	0.190	0.140	0.266	0.122	0.244	0.131	0.066	0.122	0.125	0.330
N4	0.295	0.321	0.397	1.000	0.358	0.349	0.115	0.313	0.747	0.605	0.666	0.404	0.533	0.265	0.353	0.186	0.160	0.635
N5	0.238	0.411	0.153	0.358	1.000	0.525	0.226	0.564	0.666	0.533	0.458	0.524	0.410	0.565	0.297	0.204	0.560	0.701
N6	0.338	0.417	0.140	0.349	0.525	1.000	0.247	0.538	0.618	0.625	0.434	0.437	0.149	0.523	0.337	0.181	0.470	0.674
N7	0.126	0.087	0.113	0.115	0.226	0.247	1.000	0.101	0.211	0.294	0.133	0.117	0.055	0.160	0.059	0.151	0.110	0.293
N8	0.493	0.444	0.149	0.313	0.564	0.538	0.101	1.000	0.578	0.515	0.518	0.699	0.340	0.867	0.698	0.285	0.617	0.788
N9	0.341	0.516	0.190	0.747	0.666	0.618	0.211	0.578	1.000	0.795	0.707	0.615	0.330	0.558	0.442	0.195	0.337	0.795
N10	0.506	0.582	0.140	0.605	0.533	0.625	0.294	0.515	0.795	1.000	0.641	0.689	0.282	0.623	0.422	0.371	0.335	0.808
N11	0.520	0.493	0.266	0.666	0.458	0.434	0.133	0.518	0.707	0.641	1.000	0.733	0.348	0.582	0.431	0.338	0.434	0.782
N12	0.635	0.625	0.122	0.404	0.524	0.437	0.117	0.699	0.615	0.689	0.733	1.000	0.268	0.791	0.686	0.414	0.614	0.844
N13	0.401	0.250	0.244	0.533	0.410	0.149	0.055	0.340	0.330	0.282	0.348	0.268	1.000	0.203	0.279	0.170	0.088	0.498
N14	0.513	0.499	0.131	0.265	0.565	0.523	0.160	0.867	0.558	0.623	0.582	0.791	0.203	1.000	0.623	0.224	0.611	0.786
N15	0.502	0.517	0.066	0.353	0.297	0.337	0.059	0.698	0.442	0.422	0.431	0.686	0.279	0.623	1.000	0.194	0.446	0.660
N16	0.453	0.329	0.122	0.186	0.204	0.181	0.151	0.285	0.195	0.371	0.338	0.414	0.170	0.224	0.194	1.000	0.318	0.471
N17	0.292	0.390	0.125	0.160	0.560	0.470	0.110	0.617	0.337	0.335	0.434	0.614	0.088	0.611	0.446	0.318	1.000	0.628
MFA	0.656	0.677	0.330	0.635	0.701	0.674	0.293	0.788	0.795	0.808	0.782	0.844	0.498	0.786	0.660	0.471	0.628	1.000