Physicochemical and sensory characterization of gnocchi and the effects of novel reformulation on *in vitro* digestibility

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Physicochemical and sensory characterization of gnocchi and the effects of novel reformulation on *in vitro* digestibility

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Table of Content

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, 'Physicochemical and sensory characterization of gnocchi and the effects of novel reformulation on *in vitro* digestibility', 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

Conventional gnocchi are small Italian dumplings made from potatoes, flour, and sometimes eggs. They contain a large amount of carbohydrate but are a poor source of protein. In this study, we sought to develop a gnocchi-type food with added nutritional ingredients: navy bean and beef. Our hypothesis is that addition of meat and navy bean will improve the nutritional, physicochemical and sensory properties of the reformulated gnocchi. In this study, gnocchi formulations with added meat and navy bean ingredients at concentrations between 10 - 40% (w/w) were developed. All samples were evaluated for their physicochemical, nutritional and sensory attributes. In addition, Modified *in vitro* Stomach Stir Tank (MISST) was used to analyse the changes caused by addition of the new ingredients on the digestibility of the developed gnocchi.

Samples containing 30 and 40% meat had significantly higher fat and protein content. Addition of meat significantly increased the redness and decreased the lightness of both raw and cooked gnocchi sample according to the L*, a*, b* colour system. The combination of navy bean and meat incorporated into gnocchi formulation significantly increased hardness, springiness and chewiness of most cooked gnocchi samples. Samples with similar texture to the commercial gnocchi sample and a control sample were then subjected to consumer sensory testing and projective mapping. There were no significant differences in overall liking and liking of other attributes (odour, taste, texture and flavour). The product and attribute maps obtained from projective mapping separated the control sample from the other samples due to its soft texture. Samples 40M[1], 30M and 20M10N were associated with a hard texture, while 20M and 10M were associated with chewy, and a neither hard nor soft texture.

Samples 40M, 30M, 10M, 10M30N, and 20M10N were also tested for their *in vitro* digestibility. The pH increased when the food bolus entered the stomach compartment, and then decreased after the feeding period ceased. As the hydrolysis of starch ended in the stomach in our study, the hydrolysis ratio of digested starch vs. total starch were measured. The initial hydrolysis ratio of the gnocchi samples ranged from

[1] Samples are expressed as percentage meat (M) and navy bean (N) content. For example, 20M10N refers to the gnocchi sample containing 20% meat and 10% navy bean.

30% to 41%. The hydrolysis ratio decreased after 50 minutes of *in vitro* digestion. The sample with the highest meat content (40M) had the highest concentration and mass of total water soluble protein at all times, followed by 30M.

Overall, the results showed that the incorporation of meat and navy bean changed the nutritional, physicochemical and the *in vitro* digestibility properties of gnocchi. 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.

Chapter 1. Introduction

Potatoes are a major source of carbohydrate that is largely consumed in the Western diet. They provide not only energy but also micronutrients such as vitamin C, potassium and polyphenols. However potatoes are low in protein content relative to carbohydrates and energy, although the balance of essential amino acids in that protein is good (Dancs, Kondrak, & Banfalvi, 2008). Most potato varieties also fall into the high GI (glycaemic index) range, meaning that the carbohydrate (starch) is quickly converted to glucose (Ek, Brand-Miller, & Copeland, 2012).

Gnocchi are small Italian dumplings usually made from potato, flour (traditionally buckwheat flour) and egg, and shaped into small ovals with a ridged pattern on one side. Traditional gnocchi is a rich source of carbohydrate but is poor in protein. The demand for low carbohydrate and high protein food is on the increase with people more conscious of their health and wellbeing. Therefore, this study replaced a proportion of the potato in gnocchi with red meat and navy bean.

Red meat provides key micronutrients and proteins to the world population (McNeill & Van Elswyk, 2012). Meat proteins provide all essential amino acids and have no limiting amino acids. The Protein Digestibility Corrected Amino Acids Score for red meat like beef is around 0.9 (with the maximum score of 1), and for most plant foods, it is between 0.5 - 0.7 (Williams, 2007). Red meat also plays a vital role in reducing the risk of stunting which is a common disease among children from developing country (Krebs et al., 2011; Sari et al., 2010). Red meat consumption can also reduce the risk of certain micronutrients deficiency, such as iron and zinc (McNeill & Van Elswyk, 2012).

Navy (haricot) beans (*Phaseolus vulgaris*) are a rich and low-cost source of protein (20 - 30% of dry matter) and carbohydrate. The starch of legumes contains a considerable amount of resistant starch (fibre), which has a low digestion rate. Intake of navy beans may increase blood sugar slowly resulting in reduced glycaemic postprandial responses (Gallegos-Infante et al., 2010). Hence incorporation of navy bean flour into gnocchi will not only enhance the protein content but also lower the GI

(glycaemic index), thereby improving the nutritional value of gnocchi. Low GI diet has been associated with decreased risk of type 2 diabetes and chronic disease, and improvements in various metabolic risk (Aston, 2006). In addition, pulse protein is complementary with wheat, in that it is rich in the lysine, a limiting essential amino acid in wheat, whilst wheat compensates for the low methionine content of pulses (Dancs et al., 2008).

Ingredients also affect the digestibility of a product. *In vitro* digestion has been applied in food research in recent years. Osorio-Díaz et al. (2005) evaluated the *in vitro* digestibility of starch from *Phaseolus vulgaris*, and predicted its GI based on enzymatic hydrolysis response. They reported that pulse starches were "slow" carbohydrates with a relatively low GI. The carbohydrate digestibility of spaghetti with pulse flour addition was also studied *in vitro*, which has shown lower rates of enzyme hydrolysis for spaghetti with higher levels of bean flour addition (Gallegos-Infante et al., 2010). Our project will be distinctive in that this will be the first investigation of a novel gnocchi-type food made from combinations of potatoes, navy bean flour, and the constituents of red meat. Addition of new ingredients will, however, change the properties of the gnocchi. Therefore the physicochemical and sensory characteristics of gnocchi will be assessed. In addition sensory characterization of the reformulated gnocchi, and *in vitro* digestibility of the new product will also be examined.

Our hypothesis is that addition of meat protein and navy bean flour will improve the nutritional value of gnocchi with improved physicochemical and sensory properties, as well as desirable digestibility. In our study, we are using gnocchi as a vehicle to deliver meat nutrition, and to explore how meat proteins interact with the macromolecules of other ingredients (eg., starches and fibre) to affect the physicochemical, sensory and digestibility characteristics of gnocchi.

Chapter 2. Literature Review

2.1 Pasta

Pasta is a staple food in the diet of people from many countries. From 2009 to 2011, New Zealand imported more than 6,000 tons of pasta annually (IPO, 2012). Pasta products are well accepted by consumers for their sensory attributes, low cost, ease of preparation and transportation. The processing steps involved in pasta manufacture are, essentially, mixing of durum wheat semolina and water, followed by extrusion and dehydration. Durum wheat (*Triticum turgidum L. var*.) is often used for pasta production because of its unique colour, flavour and cooking quality (Martinez et al., 2007).

Pasta cooking quality can be expressed in terms of firmness, stickiness, cooking and over-cooking tolerance, water absorption, degree of swelling, loss of solids to cooking water and colour (Chillo et al, 2008). The pasta cooking quality is determined by the physical competition between protein coagulation in a continuous network and starch swelling with exudate losses during cooking (Cocci et al., 2008). If the former succeed, starch particles are trapped in the network, resulting in increased firmness of cooked pasta. If the latter prevails, the protein coagulates in isolated bulks results in softness and stickiness due to a lack of continuous framework. Stickiness is mostly associated with the formation of a surface layer of exudates (amylose and amylopectin), which gives pasta the tendency to stick together. Gluten content is also reported as a main factor contributing to the pasta cooking quality such as cooking loss (Petitot, 2010). Gluten can form intra- and intermolecular disulphide bonds during processing. This leads to the formation of a three dimensional gluten network responsible for the unique textural properties of pasta.

2.1.1 Fortification of pasta

Pasta is mainly used as an energy source due to its complex carbohydrate content, and is regarded as a product with low GI due to the nature of the starch granules in durum wheat and the effects of extrusion processing. These nutritional advantages,

along with the appeal of pasta amongst consumers, have made pasta a vehicle for nutraceuticals such as vitamins or polyunsaturated fatty acids. In fact, pasta was one of the first foods permitted for vitamin and iron enrichment in the 1940s by the US FDA (Fradique et al., 2010). Pasta products have since been fortified to enhance their nutritional properties with supplements from various high-protein sources, such as soy flours, whey proteins, microalgae, and germinated pigeon pea (Table 2.1.1).

The term GI was first introduced in 1981 by Jenkins et al. as a physiological (*in vivo*) way of classifying foods rich in carbohydrates based on their blood glucose-raising potential (1981). Foods with a GI value above 70 are classified as high GI, foods with a GI of 56-69 are classified as medium GI, and foods that have a GI of 55 and less are classified as low GI (ISO standards, 2010). Consumption of available carbohydrates that are digested and absorbed rapidly results in high postprandial blood glucose levels, which over the long term are associated with increased risks of obesity, and diet-related diseases including type-2 diabetes, cardiovascular disease and certain types of cancers (Ek et al., 2012). Hence, there have been many suggestions to substitute potatoes with a lower GI carbohydrates to reduce the risk of chronic disease (Brand-Miller et al., 2009).

2.1.1.1 Enhancement of nutritional value via fortification

As shown in Table 2.1.1, the incorporation of legume flour increased the protein content and amino acid score of pasta. Shogren and others (2006) studied the composition of soy flour-fortified pasta. Up to 50% soy flour was incorporated into spaghetti, resulting in increased protein content to 33.5% (compared with 15.4% for control without soy), lysine content to 1.75% (compared with 0.41% for control) and threonine content to 1.14% (compared with 0.43% for control). The incorporation of soy flour also increased other essential amino acids (e.g., methionine, isoleucine, leucine) in the resulting spaghetti. Another study (Petitot et al., 2010) on the incorporation of bean in pasta showed that 35% faba bean flour-enriched pasta resulted in higher protein content (18.8%) than durum pasta without bean flour (13.3%). Alireza and Bhagya (2008) incorporated mustard protein isolate (MPI) into pasta at four levels (0%, 2.5%, 5%, and 10%). The most pronounced effect of enrichment on chemical composition of the pasta was the increase in protein content to around 4.5% with supplementation of 5% MPI in pasta formulation. The MPI-enriched pasta also had higher lysine, cysteine, arginine, and histidine content than control.

Some fortified pasta had increased vitamins and minerals content (Table 2.1.1). Petitot et al. (2010) reported that the incorporation of faba bean and split peas increased the vitamins (B1, B5, B6, B9) and minerals (Fe, Mg, P) in the final product. Addition of 35% faba bean flour into pasta resulted in 100% more vitamin B1 and 200% more vitamin B9 than control. Iron content also increased from 1.9 mg/100 g in control to 3.2 mg/100 g, and phosphorus content increased from 233 mg/100g to 372 mg/100g. Pasta fortified with germinated *Cajanus cajan* seeds provided more vitamins B1, B2 and vitamin E than controlled pasta with 100% durum wheat flour (Torres et al., 2006). Other fortified pasta showed increased antioxidant activity (Fradique et al., 2010; Zouari et al., 2011) with the addition of high antioxidant ingredient such as microalgae and blue-green algae. Antioxidant activity enhancement was also observed in the pasta fortified with germinated *Cajanus cajan* seeds (Torres et al., 2006).

2.1.1.2 Texture

Fortification of pasta has been reported to change the physical properties of raw and cooked pasta (Table 2.1.1). Fortification of durum wheat pasta with 35% of split pea or faba bean flour significantly increased pasta hardness by 38% and 43%, respectively (Petitot et al., 2010). Bahnassey and Khan (1986), and Zhao et al. (2005) reported that fortification of pasta with legume flour (navy bean, pinto bean, lentil, green pea) or protein concentrates increased pasta firmness. Protein-fortified pasta tended to have higher firmness than conventional pasta. This has been attributed to the higher number of polypeptide chains associated with higher protein contents, which increase the ability of proteins to form an insoluble network (Chillo et al., 2010).

In contrast, a study on defatted soy flour-fortified pasta (up to 50% defatted soy flour) reported no significant differences in firmness and cohesiveness (Shogren et al., 2006). The texture properties of the soy-fortified pasta were tested using 10 trained panellists. However other studies have reported increased firmness of pasta measured mechanically with increasing level of soy flour (Chillo et al., 2010; Petitot et al., 2010).

2.1.1.3 Cooking quality

Legume-fortified pasta has been reported to increase cooking loss. As summarised in Table 2.1.1, common bean, split pea, faba bean and germinated pigeon pea flour-enriched pasta had a higher cooking loss compared to durum flour pasta (Gallegos-

Infante et al., 2010; Petitot et al., 2010; Torres et al., 2006). This is associated with the formation of a weaker gluten network as a result of the dilution effect of the wheat gluten (Martinez et al., 2007). As a consequence, the starch leaches more easily into the cooking water. Predictably, mustard protein isolate-enriched pasta also had higher cooking loss than control pasta without any protein enrichment (Alireza & Bhagya, 2008).

2.1.1.4 Sensory properties

As shown in Table 2.1.1, fortified pasta can vary in their acceptability and sensory attributes. Pasta incorporated with microalgae resulted in higher consumer acceptance score (Fradique et al., 2010; Zouari et al., 2011) with appealing colour (e.g., orange and green) that consumers found highly acceptable. The addition levels of microalgae however were relatively low (0.5 - 2%), and consequently the changes in structure and pasta taste were hardly detectable by panellists. Descriptive analysis by a trained panel showed that split pea and faba bean (35%) fortified pasta had higher hardness, elasticity, and fracturability, which was consistent with the instrumental texture test (Petitot et al., 2010). The high amount of legume flour in pasta also did not affect its sensory attributes. Other ingredients like peanut flour, soy flour and geminated pigeon pea, however incorporated in pasta showed high acceptance scores (Howard, Hung, & McWatters, 2011; Torres et al., 2006). Therefore, the incorporation of added ingredients into pasta not only increased the overall pasta nutritional profile but also resulted in no change or increased consumer preference and acceptance scores.

Table 2.1.1. Fortification of pasta with various ingredients and their impact on physicochemical and nutritional properties

Pasta type	***Main Ingredient***	***New ingredients***	***level % (w/w)***	***Brightness***	***Texture***	***Cooking Quality***	***Nutritional profile***	***Sensory evaluation method***	***Sensory properties***	***References***
Fresh Pasta	durum semolina flour	*Chlorella vulgaris* and *Spirulina maxima*	0.5, 1, 2	Brightness increased	Hardness decreased	Cooking time and swelling index increased	Antioxidant	Consumer acceptance test	Higher acceptance score	Fradique et al., 2010
Spaghetti	semolina flour	Common bean (*Phaseolus vulgaris L.*)	15, 30, 45	NA	NA	cooking time decreased, cooking loss increased	High protein; Low hydrolysis rate;	NA	NA	Gallegos-Infante et al., 2010
Spaghetti	durum semolina flour	mung bean, soya bean, red lentil or chickpea	10	NS	hardness and adhesiveness increased	cooking time increased; cooking loss and swelling index did not change	No impact on the GI	NA	NA	Chillo et al., 2010
Pasta or spaghetti	durum wheat semolina	Split pea and faba bean	35	decreased brightness	Increasing hardness	cooking time and swelling index decrease while cooking loss increase	Increased nutritional quality in terms of protein, vitamins and minerals	Descriptive analysis	Higher hardness, elasticity and fracturability	Petitot et al., 2010
Spaghetti	semolina	Banana starch	5, 10, 15, 20	decreased brightness	decreased hardness	cooking loss increased	The resistant starch content increased;	Consumer acceptance test	Spaghetti with 15% of banana starch addition received the highest score of acceptability	Hemaindez-Nava et al., 2009

Pasta	semolina	Peanut flour	30, 40, 50	decreased brightness	NS	cooking loss increased	NA	Home use test, consumer acceptance test	30% peanut flour was preferred by panellist;	Howard, Hung, & McWatters, 2011
	sweet potato	Whey protein Defatted soy flour	10, 20	NA	NA	Cooking loss and swelling index increased	Protein content enhancement	NA	NA	Gopalakrishnan et al., 2011
	semolina	Fish powder Germinated pigeon pea	5, 8, 10	NA	NA	cooking time decreased while cooking loss and swelling index increased	protein, fibre mineral and vitamin improved;	Acceptance test	Similar acceptability with control	Torres et al., 2006
	semolina	Mustard Protein Isolate	2.5, 5, 10	brightness decreased	firmness increase while adhensiveness decreased	cooking loss and swelling index decreased	Protein and amino acids enhancement	Preference test (trained)	Higher score in colour, texture	Alireza & Bhagya, 2008
Pasta	semolina	Blue-green algae	1, 2, 3	brightness increased	firmness increased	cooking loss decreased while swelling index increased	Antioxidant activity	Consumer acceptance test	Higher taste and acceptance scores	Zouari et al., 2011
	durum flour	Defatted soy flour	25, 35, 50	NA	NS	NA	Enhanced protein and amino acids	Discrimination and scaling test (trained)	Spaghetti enriched with up to 35% soy flour indicated no significant difference in flavor and texture compared with control	Shogren et al., 2006

NA: data is not available; NS: no significant change

2.2 Gnocchi-type food

2.2.1 Traditional Gnocchi

Gnocchi is typical Italian potato-based fresh pasta. The homemade traditional product is consumed fresh on the day it is produced, whereas the industrially manufactured one is vacuum packed and stored at around 4 °C. It is usually made from potato, flour (traditionally buckwheat flour) and egg, and shaped into small ovals with a ridged pattern on one side.

As the major ingredient of gnocchi, potato nutritional properties determine the nutritional value of gnocchi. Potatoes are the world's third largest food crop and the most extensively consumed root vegetable (FAO, 2008). They have high starch content and are a major source of dietary carbohydrate. Besides being an energy source, potatoes are also rich in micronutrients (vitamin C, B vitamins and potassium), carotenoids and antioxidant phenols (Ek et al., 2012). Although potatoes are low in total protein content, the balance of essential amino acids in that protein is good. There are different potatoes derivatives used in the industry, such as steam-cooked potatoes, potato puree (water added to potatoes flakes) and reconstituted potatoes. Among those diverse materials, steamed –cooked potatoes had the best sensory properties in terms of colour, flavor and texture (Alessandrini et al., 2010). Studies on different cultivars of potatoes using in *vivo* and *in vitro* digestion indicate that cooked potatoes have mostly rapidly digested starch and high GI (Leeman, Bårström, & Björck, 2005).

Compared to pasta, there are fewer studies on reformulations of traditional gnocchi. In this research, the effects of navy bean and red meat addition into gnocchi formulation to improve its nutritional value will be explored.

2.2.2 Navy bean

2.2.2.1 Nutrition

Until decades ago starch was considered to be an available carbohydrate that can be completely digested and absorbed in the small intestine. However, it is now known that there is a starch fraction resistant to enzymic digestion that passes through the small intestine and reaches the large bowel where it may be fermented by the colonic

microflora. This fraction is called resistant starch (RS) and is defined as the sum of starch and the products of starch degradation not absorbed in the small intestine of healthy individuals (Asp, 1992). In recent years, there has been a considerable interest in the possibility of reducing the incidence of diabetes by altering the glycaemic (blood glucose) response of the carbohydrates ingested. A nutritional variable frequently linked to low GI properties is RS. The types of RS identified in foods are: starch physically entrapped within whole or partly milled grains or seeds (RS1), native, ungelatinized granules of B-type starches (RS2), and retrograded starch (RS3) (Sajilata et al., 2006).

Raw and processed legumes have been shown to contain significant amounts of RS in comparison with other products such as cereals, tubers and unripe fruits. Therefore, starch digestion rate and the release of glucose into the blood stream are slower after the ingestion of legumes, resulting in a reduced glycaemic and insulinemic postprandial responses in comparison with cereal grains or potatoes (Osorio-Díaz et al., 2005). Navy (haricot) bean (*Phaseolus vulgaris*) is a rich and low-cost source of protein (20 - 30% of dry matter) and carbohydrate. They are beneficial for human health, with a low GI. Intake of navy beans has been reported to increase blood sugar slowly resulting in reduced glycaemic postprandial responses (Gallegos-Infante et al., 2010).

2.2.2.2 Trypsin inhibitor

Trypsin is one of the proteolytic enzymes of the pancreas with a molecular weight of 23,800 Dalton (Gunningham, 1954). It is an endopeptidase, which attack peptide bonds and hydrolyzes proteins to peptides at the location of basic amino acids, yielding breakdown products of arginine or lysine at their C-terminal (Beck, 1973). The inhibition of trypsin activity is reported to decrease protein digestibility and nutritive value (Yuan et al., 2008).

Proteinaceous enzyme inhibitors which inhibit animal, bacterial and fungal proteinases have been reported to occur in many cereals and legumes (Sasi Kiran & Padmaja, 2003). Several trypsin inhibitors have been isolated and purified. Examples are the inhibitors from soybean (Yamamoto & Ikenaka, 1967), and navy bean (Wagner & Riehm, 1967). The presence of trypsin inhibitors in animal feed has been associated with growth suppression (Grant, 1989) and pancreatic hypertrophy (Gumbmann et al., 1989). Although the nutritional effects of trypsin inhibitors in humans are not fully clear,

reduction of the level of trypsin inhibitors by, for example, heat or fractionation is generally applied to legume products (Friedman et al., 1991). Soaking and blanching could decrease and inactivate 20-50% of trypsin inhibitor activity of raw soymilk (Yuan et al., 2008).

2.2.3 Red meat

The Food Standards Australia New Zealand (FSANZ) Food Standards Code defines meat as '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 eggs, or foetuses' (FSANZ, 2002). In Australia and New Zealand, the term "red meat" is used by the meat industry to refer to meat from cattle, sheep and goat (i.e. beef, veal, lamp, mutton and goat meat) (Beilken, Eustace, & Tume, 2007).

2.2.3.1 Nutrition

Red meat contains high biological value protein and important micronutrients that are needed for good health throughout life. It also contains a range of fats, including essential omega-3 polyunsaturated fats. However the nutritional composition will vary somewhat according to breed, feeding regimen, season and meat cut. In general, lean red meat has a relatively low fat content, is moderate in cholesterol, and is rich in protein and many essential vitamins and minerals (Beilken et al., 2007). Table 2.2.3.1 summarises the typical nutrient composition of samples of fat-trimmed Australian beef based on recent analyses of national retail samples (Sinclair, Mann, & O'Connell, 1999), and compares the nutrient composition with recent Australian recommended dietary intakes (RDI) (National Health and Medical Research Council, 2006). In general beef is a particularly good source of protein, niacin, vitamin B6, vitamin B12, phosphorus, zinc and iron, with 100 g providing more than 25% RDI of these nutrients. It also provides more than 10% RDI of riboflavin, pantothenic acid and selenium.

Table 2.2.3.1. Average nutrient composition (per 100 g) of the lean component of Australian beef (Sinclair et al., 1999).

	Beef[a]	Adult RDI[b]
Moisture (g)	73.1	
Protein (g)	23.2	46-64
Fat (g)	2.8	
Energy (kJ)	498	6.5-15.8MJ
Cholesterol (mg)	50	
Thiamin (mg)	0.04	1.2-1.2
Riboflavin (mg)	0.18	1.1-1.6
Niacin (mg)	5	1.4-1.6
Vitamin B6 (mg)	0.52	1.3-1.7
Vitamin B12 (mg)	2.5	2.4
Pantothenic acid (mg)	0.35	4-6
Vitamin A (mg)	<5	700-900 ug RE[c]
Beta-carotene (mg)	10	700-900 ug RE
Alpha-tocopherol (mg)	0.63	7-10
Sodium (mg)	51	460-920
Potassium (mg)	363	2800-3800
Calcium (mg)	4.5	1000-1300
Iron (mg)	1.8	8-18
Zinc (mg)	4.6	8-14
Magnesium (mg)	25	310-420
Phosphorus (mg)	215	1000
Copper (mg)	0.12	1.2-1.7
Selenium (mg)	17	60-70

[a] mean values for diced, stir-fry, round, rump, topside, silverside, fillet, sirloin, scotch fillet, T-bone, blade and chuck steak.
[b] RDI= recommended dietary intake, data retrieved from National health and Medical Research Council (2006).
[c] RE = retinol equivalents (=1 mg retinol or 6 mg or beta-carotene).

2.2.3.2 Protein and amino acids

Raw red muscle meat contains around 20 - 25 g protein/100 g. Cooked red meat contains 28–36 % protein, because the water content decreases and nutrients become more concentrated during cooking. The protein is highly digestible (around 94%) compared to the digestibility of beans (78%) and whole wheat 86% (Bhutta, 1999). Protein from meat provides all essential amino acids (lysine, threonine, methionine, phenylalanine, tryptophan, leucine, isoleucine, valine) and has no limiting amino acids. Protein Digestibility Corrected Amino Acid Score (PDCAAS) is a method of evaluating

the protein quality, with a maximum possible score of 1.0. Animal meat like beef have a score of approximately 0.9, compared with values of 0.5–0.7 for most plant foods (Schaafsma, 2000). The amino acid glutamic acid/glutamine is present in beef in the highest amounts (16.5%), followed by arginine, alanine and aspartic acid.

2.2.3.3 Vitamins, minerals and other meat-based bioactive compounds

As with other animal foods, red meat is an excellent source of bioavailable vitamin B12, providing over two-thirds of the daily requirement in a 100 g serving (Table 2.2.3.1). Up to 25% RDI of riboflavin, niacin, vitamin B6 and pantothenic acid can also be provided by 100 g of red meat. In New Zealand levels of 0.10 mg vitamin D3 and 0.45 mg 25-hydroxyvitamin D3 per 100 g have been reported in beef, and levels of 0.04 and 0.93 mg/100 g respectively in lamb (Schaafsma, 2000).

Beef and lamb meat are rich in minerals iron and zinc, with 100 g providing at least one quarter of daily adult requirements (Table 2.2.3.1). The iron in meat is mostly haem iron, which is well absorbed, and meat protein also appears to enhance the absorption of iron from meat. Similarly, absorption of zinc from a diet high in animal protein is greater than from plant foods, and the requirements for zinc may be as much as 50% higher for vegetarians (National and Medical Research Council, 2006). Red meats are also good sources of selenium, providing over 20% RDI per 100 g serve. Lean meat is low in sodium, with a potassium–sodium ratio of > 5.

In addition to the traditional essential nutrients with defined requirements, there are a number of meat-based bioactive substances that have been studied for their potential beneficial effects (Arihara, 2006). Chemicals found as natural components of foods or other ingestible forms that are beneficial to the human body in preventing or treating one or more diseases or improving physiological performance are known as nutraceuticals (Wildman, 2000). In addition to various nutraceutical compounds found in plants (e.g., vegetables), several attractive meat-based bioactive substances, such as conjugated linoleic acid, carnosine, anserine, L-carnitine, glutathione, taurine and creatine, have been studied for their physiological properties (Arihara, 2004). Conjugated linoleic acid, as representative meat-based bioactive compounds, has antioxidant and immunomodulatory properties and may also play a role in the control of obesity (Arihara, 2006).

2.2.3.4 Red meat in global nutrition

Perception of the role of meat, particularly red meat, in the global diet is dichotomous. In developing nations, meat of all types offers a means for reducing malnutrition and increasing food security, while in the developed world, red meat is often viewed as a culprit in the development of diet-related non–communicable diseases (NCD) (McNeill & Van Elswyk, 2012) . The way food availability data is viewed may be misleading as "the literature tends to compare developed to developing country statistics rather than comparing the consumption in the developing world to acceptable nutrition standards" (FAO, 2011). Similarly, the influence of nutrition guidance from developed countries may overshadow recognition of the key micronutrients and protein contributed by red meat to the global food supply (McNeill & Van Elswyk, 2012).

Primary among the diet-related risk factors for NCD (i.e., cardiovascular disease) is increased consumption of energy-dense, nutrient-poor foods that are high in fat, sugar and salt (WHO, 2004). While recommendations to limit red meat are often rooted in the belief that red meat, as a source of saturated fat, contributes significantly to cardiovascular disease, evidence suggests otherwise. In fact, red meat's contribution of many key nutrients relative to the energy provides support that lean red meat is an important nutrient-rich food to fight against many chronic diet-related NCD.

Studies support the role of beef in the maintenance of micronutrient status in children and adolescents in developed countries. Of particular importance are the results reported by Etcheverry et al. (2006), which indicate that, in adolescents, non-heme iron and zinc absorption from a beef meal is significantly greater than that from a meal containing soy protein. Evidence also suggests that red meat provides a unique mixture of highly bioavailable micronutrients, not readily available in plant-based diets that may support the cognitive development and function of children and adolescents. For example, Halterman et al. (2001) have reported lower standardized math scores among iron-deficient school-aged children and adolescents living in the U.S. For aging adults, the combination of protein and micronutrients provided by lean red meats may be critical to preserving social independence by reducing the risk of sarcopenia and sarcopenic obesity. Sarcopenia refers to the degenerative loss of skeletal muscle mass and strength associated with aging while sarcopenic obesity reflects the replacement of lost skeletal muscle with increasing fat mass. Houston et al. (2008) found that the

nutrient package that lean red meat provides is an ideal vehicle for supporting the intake of key nutrients associated with the decreased risk of sarcopenia.

It is estimated that almost one-third of children below the age of five in the developing world are stunted (UNICEF, 2007). Stunting is caused by long-term insufficient nutrient intake and frequent infections, normally occurs before the age of 2, and is largely irreversible (UNICEF, 2007). A recent cross-sectional study emphasizes the importance of meat in the diets of infants and toddlers in developing countries to decrease stunting. After controlling for influential variables, consumption of meat (including chicken, liver, but not fish) was associated with a 36% decrease in the likelihood of stunting among toddlers (12 - 24 months) living in Guatemala, Democratic Republic of Congo, Zambia, and Pakistan (Krebs et al., 2011).

It is likely that red meat may play a critical role in reducing the incidence of under-nutrition and related disease in developing nations and reducing the global burden of diet-related non-communicable disease.

2.3 *In vitro* digestion

2.3.1 Digestion of foods in the human body

Human digestion is a complex process that renders food materials soluble so that they may be absorbed into the bloodstream to be utilized by the body for growth, cell maintenance, and energy (Guerra et al., 2012). During digestion, two simultaneously occurring processes are: (i) mechanical transformations to reduce the food particles into smaller sizes; and (ii) enzymatic transformations involving hydrolysis to ease absorption into the bloodstream (Guerra et al., 2012). The digestion system consists of the gastrointestinal (GI) tract and associated glandular organs producing secretions in the GI tract. GI tract is composed of mouth, oesophagus, stomach, small intestine, and large intestine (Levy et al., 2006). GI motility enables mixing of ingested foods with GI secretions to digest and absorb nutrients from foods (Levy et al., 2006).

2.3.1.1 Mouth

Digestion begins with chewing in the mouth where the food is mechanically broken down by the grinding action of teeth (Easton, 1974b). Chewing lubricates food

by mixing it with saliva. During mastication, solid food is turned into a bolus made up of particles with a median particle size of up to 3mm (Kong & Singh, 2008b). Human saliva is known to have a pH range from 5 to 7, with saliva enzymes such as α-amylase and lingual lipase (Easton, 1974a) present to initiate hydrolysis of starch in the mouth.

Starch takes up 60–70% of the caloric intake by humans (Butterworth, Warren, & Ellis, 2011). Most starches are composed of two kinds of polysaccharides, a linear *α*-(1→4) linked glucan, called amylose, and an *α*-(1→4) linked glucan with 4.2 to 5.9% of *α*-(1→6) branch linkages, called amylopectin, as shown in Figure 2.3.1.1.

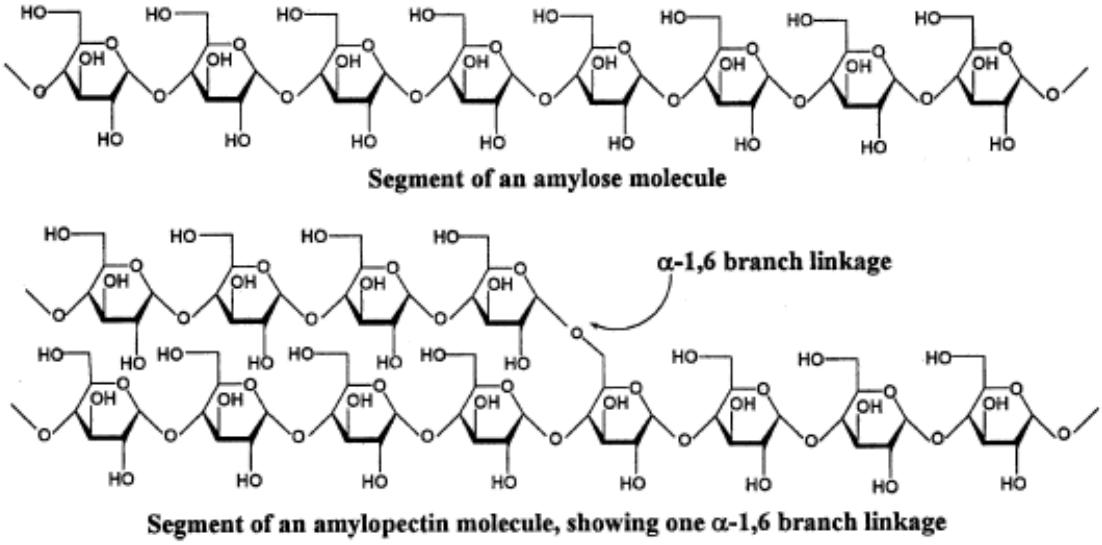


Figure 2.3.1.1. Structure of amylose and amylopectin. Retrieved from (Robyt, 2008)

Starch is the major source of glucose that appears at relatively high concentrations in the blood circulation following intestinal digestion (Apar & Özbek, 2005). It can be classified into rapidly digestible, slowly digestible, or resistant starch depending on its rate of digestibility (Englyst, Kingman, & Cummings, 1992).

The first stage in the metabolism of starch is catalysis by α-amylases, which attack both insoluble starch and starch granules held in aqueous suspension (Apar & Özbek, 2005). They attack the interior parts of the polymeric starch chains, producing a rapid drop in viscosity. Because of such property, they are also known as liquefying enzymes. When α-amylases encounter a starch chain and hydrolyse an α-(1→4) linkage, they produce lower molecular weight products such as maltodextrins, maltotriose,

maltose and glucose by the multiple attack on one of the two chains that were initially cleaved (Robyt, 2008).

The susceptibility of starch to amylase attack depends on the degree of gelatinization and the characteristics of the amylase. Native starch is packaged in semi-crystalline granules with a much larger size than α-amylase molecules. Crystalline areas of starch granules are unfavourable for enzyme attack. The starch granules contain small but variable amounts of proteins and lipids that hinders starch–amylase interaction (Butterworth et al., 2011).

When salivary amylase reaches the stomach, the enzyme is deactivated due to the acidic condition of the stomach. However when sufficient amount of starch and oligosaccharides are ingested, triggering buffering effect from the ingested foods resulting in a higher pH in the stomach, the salivary amylase can be protected from deactivation (Fried, Abramson, & Meyer, 1987). From a study by Fried et al (1997), salivary amylase accounted for 11% of total amylase output in a normal subject after a hamburger meal (Fried et al., 1987).

2.3.1.2 Oesophagus

Oesophagus is a hollow tube, which connects pharynx and stomach. The length in adults range from 25 to 35 cm (Easton, 1974a). The upper oesophageal sphincter opens to let the bolus enter the oesophagus and a series of peristaltic movements further pushes the bolus to the stomach (Mosher, 1927). There is no digestive enzyme secreted in the oesophagus. Mucosal secretion from the wall of the oesophagus lubricates the propulsion of bolus.

2.3.1.3 Stomach

Gastric digestion follows once the bolus reaches the stomach. The proximal part of the stomach, fundus and body, act as a reservoir for food and initiate the contact between the bolus and gastric juice (Guerra et al., 2012). Gastric juice is composed of pepsin and lipase enzymes for protein and lipid digestion, respectively. Hydrochloric acid (HCl) constitutes the gastric juice as well, which leads to a regular fall in gastric pH (approximately from pH 6 to 1.5) prompting protein hydrolysis. Gastric mucus is secreted from the mucosal lining of the stomach wall which protects the stomach from

being digested by the acid (Davenport, 1992) In the distal part of the stomach, antrum, peristaltic waves help to break down large particles by grinding and mixing the gastric content.

Peristaltic waves originate from the stomach wall and spread towards the antrum, mixing and forcing the antral contents toward the pylorus. The frequency of the contractions is approximately 3 cycles per min (Kong & Singh, 2008b). As the peristaltic wave reaches the pylorus, the contraction width increases and indentations deepen, which is known as "terminal antral contraction". Meanwhile, the pylorus contracts and the sphincter narrows, so that the pyloric opening is small on the arrival of the peristaltic wave. The contents are thus squirted back into the stomach through an action called retropulsion. Repeated propulsion, grinding, and retropulsion, together with the function of acids and enzymes, reduce the size of food particles into a softer consistency in a suspension form. In the gastric antrum, solids are ground to particles of a size less than 1 to 2 mm and these are known as chyme (Yoo, 2009).

2.3.1.4 Small and large intestines

The acidic chyme from the stomach is then delivered to the small intestine. Two main roles of the small intestine in digestion are breakdown of macromolecules and absorption of water and nutrients. The acidic chyme is neutralized to pH 6 to 7.5 with bicarbonate ion in the duodenum for optimal enzyme activities. Pancreatic enzymes (a complex mixture of proteases, amylases, and lipases) and other digestive enzymes produced by the lumen of the small intestine act together in the breakdown of food constituents. Water and nutrients are absorbed by villus enterocytes via simple diffusion, facilitated diffusion, or active transport (Bontrager & Lampignano, 2013). Peristalsis propels the chyme through the small and the large intestine (Bontrager & Lampignano, 2013).

2.3.2 *In vitro* digestion models described in the literature

Compared to *in vivo* digestion, *in vitro* digestion studies require shorter time and less cost. Large amount of samples can be obtained with a small amount of test and control products. Once the model is fully validated, accurate and reproducible data can be achieved without any ethical constraints (Yoo & Chen, 2006). A wide range of *in*

vitro digestion models has been designed to study the fate of orally ingested substances, from single static bioreactor to multi-compartmental dynamic systems.

2.3.2.1 Static mono-compartmental models

Static models are the most widespread digestive systems, as summarised in Table 2.3.2.1. The gastric phase of human digestion is mimicked by pepsin hydrolysis of homogenized food, under fixed pH and temperature, for a set period of time (pH 1-2, 37 °C, and 1-3 h) (Guerra et al., 2012). These conditions can be achieved in one bioreactor. The temperature and digestion time can be controlled, as shown in Table 2.3.2.1. 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. In their study, samples were first incubated with pepsin solution (pH 1.5, 37 °C) for 60 minutes, then the pH was adjusted to 6.9 with NaOH and the mixture was incubated with pancreatic alpha amylase solution at 37 °C. This *in vitro* digestion protocol was later called "multienzymatic protocol" (Osorio-Díaz et al., 2005). This multienzymatic protocol has been used extensively to study the starch hydrolysis rate as shown in Table 2.3.2.1.

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; Gallegos-Infante et al., 2010; Filgueras et al., 2011; Berg et al., 2012), and the reducing amylolysis products are measured to determine the hydrolysis rate of starch. The carbohydrates in those legumes tend to have a slow hydrolysis rate.

Santé-Lhoutellier (2007) investigated the effect of chemical oxidation on myofibrillar protein digestibility. Oxidized or nonoxidized myofibrillar proteins were then exposed to proteases of the digestive tract (pepsin, trypsin, and R-chymotrypsin). Results showed a direct and quantitative relationship between protein damages by hydroxyl radical and loss of protein digestibility.

To study the effects of storage and cooking process on the digestion rate of rhea meat, a static model was used (Filgueras et al., 2011). Rhea myofibrils protein was first digested with pepsin at pH 1.8, 37 °C for 1 hour. Then the non-soluble pepsin hydrolysate was hydrolysed for 30 minutes at pH 8, 37 ºC by mixing with trypsin and α-

chymotrypsin. Results showed that cooking decreased the susceptibility of myofibrillar protein to pepsin activity. After cooking, the proteolysis rate by pancreatic enzymes increased. The findings support the importance of protein aggregation in the nutritional value of meat proteins.

However, these models have over-simplified the mechanism of *in vivo* digestion. As discussed before, mechanical breakdown is one of the processes that happened simultaneously with enzyme hydrolysis, which has been neglected in those models. Static models also fail to reproduce the dynamic nature of human digestion, such as gastric emptying or continuous changes in pH and secretion flow rates (Guerra et al., 2012).

Table 2.3.2.1. A table summarising currently available static *in vitro* digestion models built to examine food digestion.

Sample	Digestion protocol	Enzymes	Digestion time (min)	Measured parameters	Reference
Phaseolus vulgaris	multienzymatic protocol[a] (Holm et al., 1986)	Pepsin Pancreatic amylase	180	Hydrolysis index and pGI*	Osorio-Díaz et al., 2005
lima bean starch	multienzymatic protocol (Holm et al., 1986)	α-amylase	20	available starch content	Bello-Pérez et al., 2007
		amyloglucosidase	30		
	Digestion/dialysis protocol	Subject chewing, pepsin, and porcine pancreatic amylase	0-180	Hydrolysis index and pGI*	
Navy bean	2-stage in vitro model simulated gastric	pepsin	0-30	Starch hydrolysis	Berg, Singh, Hardacre, & Boland, 2012
	intestinal digestion	Pancreatin, amyloglucosidase and invertase	0-120	Starch hydrolysis	
Faba bean	multienzymatic protocol (Holm et al., 1986)	Termamyl® (α-amylase)	15	Starch content	Bello-Perez et al., 2007
		amyloglucosidase	30		
		hog pancreatic α-amylase	5-60	Hydrolysis rate	
Tortilla, Black Beans, and Tortilla-Bean mixture	multienzymatic protocol (Holm et al., 1986)	Termamyl® (α-amylase)	15	available starch content	Sayago-Ayerdi et al., 2005
		amyloglucosidase	30		
	Chewing, dialysis Test	bovine pepsin	30	Hydrolysis index	
		porcine pancreatic α -amylase	180		
Almonds	Model stomach system	Simulated gastric juice (pepsin, mucin)	1-5h	disintegration rate	Kong & Singh, 2009
Probiotic bacteria in	Artificial gastric digestive system	HCl and NaCl without any enzyme); pH 2.5 and pH 2.0	0-120	The effect of gastric acidity on the viability of	Ortakci & Sert, 2012

yogurt				probiotic bacteria	
Pork meat	Simulated gastrointestinal digestion	Pepsin	120	Peptide characterisation	Escudero, Sentandreu, & Toldrá, 2010
		pancreatin	180		
Pig Myofibrils	Sequential enzyme digestion	Pepsin	0- 60	Hydrolysed peptide	Sante-Lhoutellier et al., 2007
		Trypsin and α-chymotrypsin	0-30		
Rhea meat	Sequential enzyme digestion	Pepsin	0, 10, 20, 30, 40, and 60	Aromatic amino acids	Filgueras et al., 2011
Spaghetti with common bean flour	multienzymatic protocol	Trypsin α-chymotrypsin	0, 5, 10, 20, 30	Hydrolysis index	Gallegos-Infante et al., 2010
		pancreatic α-amylase	0-100		

*pGI: predicted glycaemic index; [a] multienzymatic protocol: samples were first incubated with pepsin solution (pH 1.5, 37 ºC) for 60 minutes, then the pH was adjusted to 6.9 with NaOH and the mixture was incubated with pancreatic alpha amylase solution at 37 ºC (Holm et al., 1985)

2.3.2.2 Dynamic models used in *in vitro* digestion studies

In order to simulate the *in vivo* testing of drugs on animals, some physicochemical models have been developed, Table 2.3.2.2. Two well-known models, SHIME (the simulator of the human intestinal microbial ecosystem) and TIM (TNO's gastrointestinal model), have been reviewed by Yoo and Chen (2006).

Briefly, SHIME is a multistage simulator, which has six computer controlled multi-chamber reactors simulating the conditions of the GI tract including duodenum/jejunum, ileum, caecum/ascending colon, transverse colon and descending colon. The SHIME has been applied for various studies such as nutritional studies (Alander et al., 1999), micro-ecological studies (De Boever, Deplancke, & Verstraete, 2000) and the studies of bioavailability and bioaccessibility of environmental contaminants (Van de Wiele et al., 2004) and antimicrobial agents (Possemiers et al., 2005). SHIME over-simplified the dynamic feature of human GI tract where absorption mechanisms and buffering capacity are absent (Yoo, 2009).

The Modified in vitro stomach stir tank (MISST) developed by Yoo (2009) is modified from the SHIME. They closely resemble one another in terms of how the individual compartments are built. The MISST has a mouth compartment where the food is hydrolysed with α-amylase, while in the SHIME, the mouth compartment is missing. The MISST does not contain an intestinal compartment, while the SHIME does. Other than that, composition of secretions used, flow rate, building material, fill- and draw mechanism, and temperature are all the same between the MISST and the SHIME.

TNO's gastrointestinal model (TIM) is another widely used *in vitro* digestion model, created by Havenaar and Minekus (1995) in Netherlands. This model is composed of the stomach and three parts of the small intestine, the duodenum, jejunum, and ileum. It integrates key parameters of human digestion: temperature, kinetics of gastric and intestinal pH, gastric and ileal deliveries, transit time, peristaltic mixing and transport, sequential addition of digestive secretions, and passive absorption of water and small molecules through a dialysis system (Guerra et al., 2012).

Some of the dynamic gastric models are focused on the digestion occurring in the upper gastrointestinal tract. The dynamic gastric model (DGM) uses a fixed outer cylinder with a movable inner cylinder to crush foods in between, creating mechanical breakdown of foods (Wickham & Faulks, 2008). The types of forces applied on foods in the DGM are different from the forces that foods receive during peristaltic movement. A human gastric simulator (HGS) designed by Kong and Singh (2010) mimics in such a way as to simulate the continuous peristaltic movement of stomach walls, with similar amplitude and frequency of contraction forces as reported *in vivo*. The HGS mainly consists of a latex vessel, simulating the stomach chamber, and a series of rollers secured on belts that are driven by motors and pulleys to create a continuous contraction of the latex wall. It also incorporates gastric secretion, emptying systems, and temperature control that enable accurate simulation of dynamic digestion process for detailed investigation of the changes in physical and chemical properties of ingested foods. The size of the gastric compartment of HGS is larger, 5.7L, than the size of a human stomach which is 1 - 1.6 L (Kararli, 1995). The *in vitro* Physicochemical Upper Gastrointestinal System (IPUGS) developed by Yoo (2009) contains mouth, oesophagus and stomach compartments. The stomach compartment is J-shaped, reflecting the geometrical shape and the size of a human stomach. The IPUGS uses silicon-based material which can withstand repetitive motions without tearing apart. The peristaltic movement in the IPUGS is achieved by squeezing the outer side of the stomach wall, 3 cycles per min. Transit times, mucosal secretions and simplified gastrointestinal secretions have also been taken into account to mimic the dynamic human digestion process.

Even though the GI systems have many advantages and applicability, they failed to mimic the full process occurring *in vivo*. Hormonal and nervous control, mucosal cell activity and involvement of the local immune systems are missing in those GI systems (Guerra et al., 2012). A good *in vitro* model should provide an accurate estimation of the *in vivo* situation. However, human GI tract has great complexity, which is hard to replace. Therefore, careful interpretation of results is always required (Guerra et al., 2012). *In Vitro*/*In Vivo* Correlation (IVIVC), which reliably associates *in vitro* and *in vivo* data, remains a high priority to validate *in vitro* results (Souliman et al., 2006).

Table 2.3.2.2. Key features of dynamic multi-compartmental *in vitro* digestion models in the literature.

		SHIME Molly, Woestyne, & Verstraete, 1993	**TIM** Minekus et al., 1995	**DGM** Wickham & Faulks, 2008	**HGS** Kong & Sign, 2010	**IPUGS** Yoo, 2009
General	Model type	Static	Dynamic	Dynamic	Dynamic	Dynamic
	Temperature	37°C	37°C	37°C	37°C	37°C
	Motility	Mechanical stirring, 150rpm	Peristaltic movement	Peristaltic movement	Peristaltic movement	Peristaltic movement
	Anaerobic	Yes	Yes	No	No	Yes
Mouth	saliva	No	No	No	Yes	Yes
	Incubation time				2min	5min
Stomach	Gastric compartment	Yes	Yes	Yes	Yes	Yes
	Shape and Material	Glass beaker	Glass jacket with flexible inner wall	Glass jacket with flexible main body	round cylindrical	J-shaped, Silicone
	Size	N/S[a]	N/S	N/S	5.7L	20cm×15cm×8 cm
	Flexible	No	Yes	Yes	Yes	Yes
	Gastric Emptying	Yes	Yes	Yes	Yes	Yes
	Retropulsion	No	No	Yes	Yes	Yes
	Peristaltic Contraction	No	Water pressure	Cyclically pressure	Mechanical driving device	Hand squeezed actions
	Sequential use of digestive enzymes	Yes	Yes	Yes	Yes	Yes
	Sequential control of pH	No	Yes	Yes	Yes	Yes
	Feedback control of GI	No	No	No	Yes	Yes

	secretion rate					
	Delivery of the GI secretions	Pouring	Pumping	Pumping	Pumping	complex
	Incubation time	2-3hours	1-2 hours		3hours	2-4hours
Intestine	Intestinal compartment	Yes	Yes	No	No	No
	pH	6.5-7.0	Duodenum:6.5 Jejunm:6.8 Ileum:7.2			

[a] N/S: non-specific;

2.4 Sensory evaluation

The primary reason for conducting sensory analysis is to perform tests that are valid and reliable to produce data based on which sound decisions can be made. The main concern of the food industry is to fulfill consumer needs. It is thus crucial for the food industry to explore and understand consumer preferences. Sensory evaluation by consumers is important in product development for new product development guidance, product improvement, and optimisation (Choi, Phillips, & Resurreccion, 2007).

Sensory quality of a food or food products is defined as the acceptance for the sensory attributes of a product by consumers who are the regular users of the product category, or who comprise the target market for the product (Galvez & Resurreccion, 1992). The consumer plays a very important role in the success of a product in the market. Product developers and manufacturers are rapidly realising that a high level of acceptance by the target consumers is an essential prerequisite for a successful product (Land, 1988).

2.4.1 Consumer Testing

Consumer acceptability evaluation can provide the most important and reliable information because only consumers can accurately indicate the degree of liking or preference for a product. Moreover, consumer perceptions and acceptance of a product are critical elements in defining quality (Choi et al., 2007). Consumer-oriented product optimisation brings the consumer into the product development or product improvement process at an early stage. Optimisation very often involves finding the values of factors affecting a process that maximises or minimizes the response, thus determining the best possible or most favorable formulation and process for producing a successful product. In an optimal formulation, consumer acceptance by the target group can be maximised.

Sensory analysis in product development is becoming more important as the value of sensory techniques becomes recognized and the consumer industry increases its use of sensory evaluation. The hedonic tests carried out by sensory analysts attempt to assess product sensory acceptability (Boutrolle et al., 2005). Hedonic scales and preference tests are common instruments used when decisions on market introductions

are made (Hersleth et al., 2005). Information about consumers' preferences is relevant for the food industry, both for modifying and/or improving the product according to these preferences and for developing new products. In addition, knowledge on customers' age, gender, demographics and so on, is itself relevant. These can add to developing a product more strategically or specifically targeted for certain population.

2.4.2 Projective mapping

Perceptual mapping technique shows the relationship among multiple products in a visual manner (Lawless & Heymann, 1998). It is used primarily when information about product relationships is desired, and in some circumstances the linking of attributes to those relationships (Nestrud & Lawless, 2008). In these maps, complex multivariate information is simplified down to important dimensions for easier interpretation, in a manner analogous to principle components analysis. Products that are similar on a given dimension are positioned close to each other, and products that are dissimilar are pictured further apart. One of the primary uses of perceptual mapping is in strategic research and competitive analysis, where some or all products in a similar category are compared (Nestrud & Lawless, 2010).

One way to generate perceptual maps is to perform a series of paired comparisons on the perceived similarity/dissimilarity of products (Nestrud & Lawless, 2010). However, the use of paired comparisons is very time consuming, as each possible pair of products needs to be presented to assessors. As the number of products to be judged increases, the number of paired comparison tests increases rapidly. Sorting may generate the similar data but may not provide information on the degree of difference between products (Nestrud & Lawless, 2010).

A more streamlined procedure, projective mapping, was put forth by Risvik et al. (1994; 1997). Risvik et al. (1994) explored projective mapping in comparison with conventional profiling and dissimilarity scaling techniques using a set of five chocolates. Panellists were asked to structure maps on a sheet of paper based on the perceived product relationships. Quantitative data were then collected from the maps as the x- and y- coordinates of each product. Results from nine trained panellists indicated that projective mapping yielded greater similarity over replications than conventional

profiling or dissimilarity scaling. The RV coefficients were used to determine how well a panellist's map fitted with the consensus maps.

Risvik and others then explored the relationship between consumer-derived projective maps and conventional profiling by trained panellists (Risvik et al., 1997). Results showed that consumers and trained panel agreed on the most obvious differences between products. However, a high degree of variation was noted among the individual consumer maps, indicating consumers were not consistent in which product characteristics they used to construct their maps. Comparison of projective maps from consumers and trained panellists indicated that sample configurations from both consumer and trained panel groups followed similar trends (Barcenas, Elortondo, & Albisu, 2004). However trained panellists provided a better separation of the products.

Projective mapping of food products remained infrequently used and was later reintroduced under the name "Napping", from the French word for tablecloth (Pagès, 2005). The nappe method was used for perceptual mapping of a group of wines from the Loire valley and individual configurations were obtained via the statistical analysis by multifactor analysis (MFA). MFA helped uncover more than two dimensions in the data based on how panellists considered the different attributes. For example, if half of the panellists grouped the products according to taste and texture, and the other half based the groupings on taste and appearance, then the MFA would come up with a group configuration with three dimensions, with 50% of the variance coming from taste, 25% comes from texture, and 25% from appearance (Lawless & Heymann, 1998).

This method has been applied for the sensory characterization of various food products as shown in Table 2.4.2.

Table 2.4.2. Studies carried out using sensory projective mapping.

Sample	Objective	Number of panellists	Findings	References
Chocolate (5)	To compare maps obtained from projective mapping, conventional profiling and dissimilarity scaling techniques	9 trained	Higher consistency was achieved over repeated trials from projective mapping compared with the other two methods.	Risvik et al., 1994
Blue berry soup (7)	To compare maps obtained from projective mapping using naive consumers and descriptive analysis that utilised a trained panel.		Mapping replicates showed visually similar maps although RV coefficients indicated that panellists perceived products differently which highlighted the dimensionality of consumer perception compared with trained panellists	Risvik et al., 1997
Ewes milk cheese (8)	To compare maps obtained from projective mapping by trained panel and naïve consumers	8 trained and 12 untrained	Trained panel in sensory profiling got better performance quality index than consumer panel, but overall both panel sample configurations followed similar trends	Barcenas et al., 2004
White wine (10)	To determine the dimensions of perception of a panel about a set of Touraine wines			Pagès, 2005
Citrus juices (11)	to explore the projective mapping using chef and consumer groups and to examine patterns of response among chefs compared to consumer using napping	14 chefs and 16 consumers	Nappe configurations were similar within a group, while configuration between groups were less similar; a good correspondence of scaled attribute results to nappe results for the consumers, but less so for the chefs	Nestrud & Lawless, 2008
Milk and dark chocolates (14)	To compare results from projective mapping and descriptive analysis data; An untrained panel was used for projective mapping.	3 groups panellists (9, 9 and 8)	Untrained judges for the projective mapping provided equivalent product maps as obtained by descriptive analysis for this set of products; Similarity among panels indicates that overall the	Kennedy & Heymann, 2009

	Once completed, the same panel was trained for descriptive analysis and results were compared.		panellists perceived the products in a similar manner	
Apples (10) and cheese (10)	To compare the results obtained from projective mapping and sorting	19 and 21 untrained panellist doing apple and cheese respectively	Product maps were similar for both the sorting and projective mapping procedures. Subjects had more difficulty with the apples than the cheeses. Cluster analysis was easier to interpret for the napping configurations	Nestrud & Lawless, 2010
Granola bars (8)	To obtain maps and descriptions (terms) of berry flavoured granola bars using projective mapping and evaluate the consistency of results obtained from three different sessions	15 consumers	The repeat maps for each consumer did not show a high degree of similarity in all consumers. However, maps showed that the products were perceived similarly in terms of how the products were grouped	Jessica Kennedy, 2010
Italian red wine (11)	To compare the perceptions of differences in the aroma of high quality Italian red wines in experts and consumers by Projective Mapping		Product separation by experts was mainly based on the perceived overall quality rather than on specific sensory differences; Product differentiation by consumers was poor and worse than that of experts and trained subjects;	Torri et al., 2013

Chapter 3. Materials and methods

3.1. Gnocchi sample

3.1.1 Raw Gnocchi Preparation

Agria potatoes and semolina flour (Sun Valley Foods) were bought from a New Zealand supermarket. In our pilot study, gnocchi made with semolina flour was better than gnocchi made with buckwheat flour in terms of texture. Therefore, semolina flour was used instead of buckwheat flour. AgResearch Ltd. New Zealand, provided the minced beef obtained from New Zealand dairy bulls (18 - 24 months old,). Haricot (navy) beans (*Phaseolus vulgaris*) were purchased in a New Zealand supermarket in dried form. Delmaine fresh filled pasta potato gnocchi (Delmaine, New Zealand) was chosen as a commercial sample to be used for comparison with laboratory prepared samples.

Potatoes were boiled for 30 minutes until soft. The skin was removed and the potatoes were mashed using a potato-ricer. A beef emulsion containing 57.15% meat and 0.57% salt was then prepared. Minced beef (200 g), salt (2 g) and water (150g) were homogenised at 7000 rpm for 20 minutes using a homogeniser (L5M-A Laboratory Mixer, Silverson®) to make a meat emulsion. The meat emulsion was then stored at 4°C and used within the same day of the preparation.

Navy beans were soaked overnight and boiled for 2 hours to deactivate the trypsin inhibitor (Wagner & Riehm, 1967). The cooked beans were drained, cooled, and then ground using a food processor (FP734, Kenwood) for 20 minutes at high speed. Mashed potatoes, semolina, beef meat emulsion and navy beans were combined according to the formulation in Table 3.2.1 to make gnocchi dough using a dough maker (KM210, Kenwood). A lemon-size piece of dough was rolled into a rope (approximately 2 cm diameter). A knife was then used to cut the long pieces of dough into square bites. Gnocchi was either cooked within 2 - 3 hours of being made (if kept at

room temperature, 25ºC) using a steamer for 20 minutes or stored frozen at -15 ºC before further use.

3.2 Experimental design

Based on a preliminary study, gnocchi made with 70% mashed potatoes and 30% semolina flour were the most similar in terms of texture with commercial gnocchi (Delmaine fresh pasta potato gnocchi). A full factorial experimental design was generated with meat (10, 20, 30 and 40%) and navy bean (0, 10, 20 and 30%) at 4 levels using Minitab (v16, Minitab Inc). Semolina flour (30%), mashed potatoes and varying amounts of meat and navy bean were used in the gnocchi formulations. A formulation containing 70% mashed potatoes and 30% semolina was used as control. Samples are listed in Table 3.2.1.

Table 3.2.1. Gnocchi sample formulated with varying amount of meat and navy bean using a full factorial design.

Sample number		Meat Emulsion (%)	Navy Bean (%)	Mashed Potatoes (%)	Semolina Flour (%)
1	(10M)	10	0	60	30
2	(10M10N)	10	10	50	30
3	(10M20N)	10	20	40	30
4	(10M30N)	10	30	30	30
5	(20M)	20	0	50	30
6	(20M10N)	20	10	40	30
7	(20M20N)	20	20	30	30
8	(20M30N)	20	30	20	30
9	(30M)	30	0	40	30
10	(30M10N)	30	10	30	30
11	(30M20N)	30	20	20	30
12	(30M30N)	30	30	10	30
13	(40M)	40	0	30	30
14	(40M10N)	40	10	20	30
15	(40M20N)	40	20	10	30
16	(40M30N)	40	30	0	30

3.3 Physicochemical analysis

All trials for physicochemical analysis were carried out on the different gnocchi balls made from two different batches for each of the sixteen formulations (Table 3.2.1).

3.3.1 Moisture content and water activity

Moisture content was analysed using the oven drying method according to AOAC 945.15 (AOAC, 2000). Mashed cooked gnocchi samples (5g) were dried in an oven at 105ºC until constant weight (dry weight) was achieved. Each analysis was replicated three times.

A water activity meter (Novasina) was used to measure the water activity of raw gnocchi sample according to AOAC 978.18 (AOAC, 2000).

3.3.2 Texture analysis

Texture analyses were performed using a Stable Micro Systems TA.XTplus Texture Analyser equipped with a Film Support Rig (HDP/FSR) on a Heavy Duty Platform (HDP/90). Texture profile analysis (TPA) was performed to determine hardness, springiness, cohesiveness, "chewiness," and resilience of the cooked gnocchi. TPA was carried out as described by Garcia-Segovia and others (2008). The samples were placed on the base plate of the TA-XT2 texture analyzer equipped with a 50-mm cylindrical aluminium probe (5 cm in diameter) and using a 25 kg load cell. Pre-test speed was 10 mm/s, test speed was 0.5 mm/s, and post-test speed was 10 mm/s. The crosshead speed was 0.5 mm/s, with a rest period of 5 seconds between cycles. Four textural parameters were determined: hardness (peak force of the first compression cycle in kg), springiness (ratio of the time duration of force input during the second compression to that obtained during the first compression, dimensionless), cohesiveness (ratio of positive force area during the second compression compared to that during the first compression, dimensionless), and chewiness (hardness multiplied by cohesiveness multiplied by springiness in kg).

The obtained textural data were normalized by dividing the respective numerical values by the weight of each different sample (Alessandrini et al., 2010).

3.3.3 Colour analysis of raw and cooked gnocchi

Colour was evaluated using a Hunter lab (45/0, Colorflex). Readings were taken 3 times for each sample and recorded as L* (lightness), a* (green to redness), and b* (blue to yellowness).

3.4 Nutritional composition of gnocchi samples

All trials for nutritional composition analysis were carried out on the different gnocchi balls made from two different batches for each of the sixteen formulations (Table 3.2.1).

3.4.1 Fat analysis

Total fat content of gnocchi was analysed using the Soxhlet extraction method (AOAC, 2000). Pre-dried sample (2g) was ground into fine powder and put in an extraction thimble, with porosity permitting a rapid flow of solvent. The sample was covered with glass wool. The weight of a pre-dried boiling flask was recorded. Petroleum ether (50 ml) was then added into the boiling flask. The boiling flask, Soxhlet flask, and condenser were assembled. Lipid was extracted using a Soxhlet extractor at a rate of five or six drops per second by condensation for approximately 4 h, by heating solvent in a boiling flask.

Boiling flask with extracted fat was dried in an air oven at 100ºC for 30 min, cooled in a desiccator, and then weighed.

% Fat on dry weight basis = (g of fat in sample/g of dried sample) ×100

3.4.2 Total Protein Analysis by Kjeldahl method

The ground sample (500 mg) was accurately weighed, wrapped in nitrogen-free paper, and then placed in a 250 ml digestion tube. A mixture of potassium sulfate (7g) and copper sulfate (0.5 g) was used as catalyst. Concentrated sulphuric acid (12 mL) was added and the tube contents were carefully mixed prior to digestion. The sample was digested at 420 ºC for 60 minutes using a Velp DK 20 heating block. The level of

liquid was monitored during the digestion, and if 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 3 mL of cold 30% hydrogen peroxide was added. The liquid in the test tube now should be clear and colourless. If the liquid was cloudy or retained a colour, a further 3 mL of 30% peroxide was added and the mixture digested for a further 20 minutes. This was repeated as necessary until the solution remained colourless and clear.

After digestion, the sample was distilled using a VelpUDK 139 distillation unit. The digestion tube and sample were attached, and the automatic distillation process started. The digest was made alkaline with 50 mL of 35% sodium hydroxide solution, and the released ammonia was steam distilled into a receiver filled with 20 mL of 4% boric acid. When distillation finished, 10 drops of mixed Kjeldahl indicator was added to the receiver flask, and the contents titrated with 0.1mol/L standard hydrochloric acid. Nitrogen content was calculated according to the equation below:

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

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

3.4.3 Ash analysis

The ash content of gnocchi samples was measured according to AOAC 942.5 (AOAC, 2000). Cooked gnocchi sample was dried and ground before being placed into a tared crucible that was placed in a cool muffle furnace (Model 200, McGregor Kiln Furnace). The muffle furnace was ignited 12 - 18h (or overnight) at approximately 550

ºC. At the end of the ashing period, the muffle furnace was turned off and cooled down to at least 250 ºC. The door was carefully opened to avoid loss of ash. Safety tongs were used to quickly transfer crucibles to a desiccator with a porcelain plate and desiccant. Crucibles were 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:

$$\% \text{ ash (dry basis)} = \frac{\text{wt after ashing} - \text{tare wt of crucible}}{\text{dried sample weight}} \times 100 [1]$$

3.4.4 Carbohydrate content calculation

The proximate carbohydrate content was estimated according to Fraser and Holmes (1958). Carbohydrates were calculated by subtracting the total fat, ash and protein content from 100%.

3.5 Sensory Evaluation

Samples with texture properties similar to commercial gnocchi from the full factorial design, and the control sample were chosen for further consumer testing and sensory projective mapping. Ethics application for this study was approved by the Auckland University of Technology Ethics Committee (AUTEC) on 9 May 2013 (Appendix 1).

3.5.1 Gnocchi samples for consumer testing and projective mapping

A preliminary study showed that there was no significant difference in terms of texture and colour after steaming fresh and frozen gnocchi (data not shown). Therefore seven raw gnocchi samples including the control sample were prepared the week before sensory testing and kept frozen at -15 ºC before use. Frozen gnocchi samples were steamed for 30 minutes in a steamer for one hour prior to sensory analysis. Then, a

bowl of water in an ovenproof dish was placed in the oven (Elba, Fisher & Paykel) and set using the "Warmer" function (40 ºC) to provide warm and humidified air to keep the gnocchi warm.

The gnocchi samples were served with a simple Leggo's classic tomato sauce. In a preliminary run, the tomato sauce was reported to be too sour and masked the pasta flavour. The sauce was therefore diluted in a ratio of 7:3 with water, with 4% sugar and 2% salt added to produce a sauce with mild taste. The sauce temperature was maintained at 45 ºC in the oven until use.

Approximately 10 grams of each gnocchi sample and 5 mL pasta sauce 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). Cold water was served as a palette cleanser and testing was conducted under red light to mask the colour differences between samples allowing panellists to concentrate more on flavor and texture attributes.

3.5.2 Consumer Testing

Regular gnocchi consumers were recruited. These consumers who consumed gnocchi at least once a month were invited to attend a consumer testing session at the AUT University Sensory Lab between 10 am and 3 pm. Panellists then tasted the randomised and coded samples in sensory booths, in the order presented from left to right. A mandatory break of 30 seconds between each sample was implemented. Panellists rated each sample in terms of overall liking, flavour, odour, taste and texture. A 100 mm unstructured line scale labeled extremely dislike on the left, extremely like on the right and neither like nor dislike in the middle was used by consumers to indicate their liking of the products. The questionnaire for consumer testing is attached in Appendix 2.

3.5.3 Projective mapping

Like consumer testing, panellists were recruited on the basis that they were regular consumers of gnocchi. 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 2 & 3) 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 to obtain overall product coordinates, and Principal Component Analysis to obtain product and attribute biplots using Addinsoft XLSTAT-MX version 2011.5.01. Sensory attributes that occurred a minimum of five times across panellists per product were included in the PCA biplots.

3.6 *In vitro* digestion of gnocchi

All trials for digestion were carried out on the different gnocchi balls made from two different batches for each of the sixteen formulations (Table 3.2.1). Prior to initiating the digestion experiment, salivary amylase and pepsin were tested for their enzymatic activity, in order to adjust the amount for correct use.

3.6.1 α-Amylase activity assay

The activity of the fungal (Aspergillus oryzae) α-amylase (Grindamyl™ A5000, 5000U/g, Danisco 071314) was determined according to Bernfeld (1995) (Appendix-4).

3.6.2 Pepsin Assay

The activity of pepsin from porcine stomach mucosa (Sigma-Aldrich P-7000, E.C.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-5).

3.6.3 Chemical composition of secretions used in *in vitro* digestion

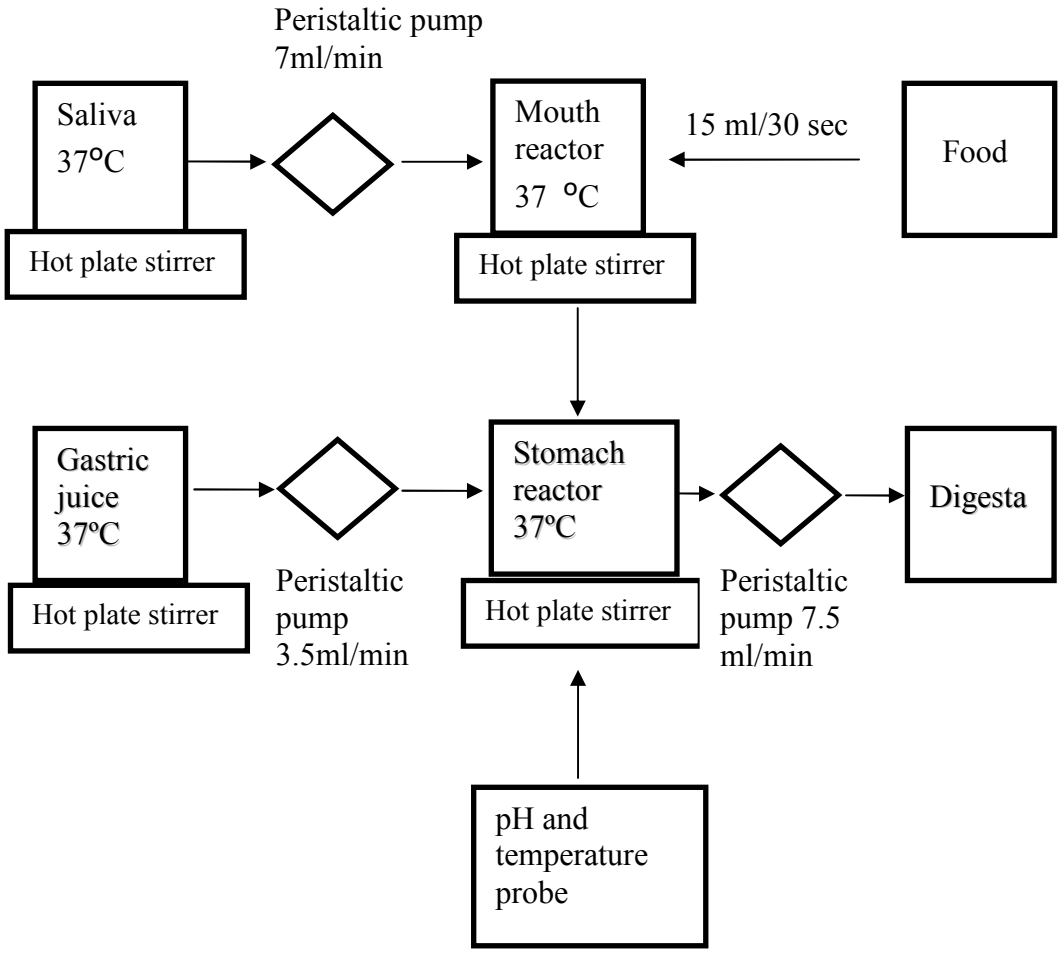
The artificial saliva was prepared according to Yoo (2009) by dissolving 2 g fungal (*Aspergillus oryzae*) α-amylase (Grindamyl™ A5000, 5000U/g, Danisco 071314) in 200 ml deionized water.

The gastric juice contained 2.2 $g.L^{-1}$ KCl, 1 g Pepsin (Sigma-Aldrich P-7000, E.C.3.4.23.1), 5 $g.L^{-1}$ NaCl, 0.22 $g.L^{-1}$ $CaCl_2$, 1.5 $g.L^{-1}$ $NaHCO_3$ and 0.15 N HCl (RCI Labscan Ltd) (Yoo, 2009).

3.6.4 *In vitro* digestion of gnocchi using Modified *In vitro* Stomach Stir Tank (MISST)

The Modified In vitro Stomach Stir Tank (MISST) used in this study is shown in Figure 3.6.4.1. The MISST is compartmentalised into mouth and stomach reactors with a digesta-collecting unit.

Figure 3.6.4.1 Schematic diagram of the MISST



3.6.4.1. Mouth reactor

A homogeniser was used as an alternative to mimic the mechanical grinding by teeth (Fraser et al., 1994) because the blended mixture allowed for easier and more continuous pattern of pumping the feed mixture via peristaltic pumps as well as minimizing heterogeneity in the feeding mixture. It also inhibits any potential blockage in the peristaltic pump tubings to operate in a more controlled manner (Yoo, 2009). A homogeniser (L5M-A Laboratory Mixer, Silverson®) was used at a speed of 400 rpm for 10 minutes to breakdown the gnocchi into very fine particles (Frazier et al., 1997; Yoo, 2009). Gnocchi was cooked following the method described in section 3.1. A smooth blend of 250 ml of drinking water (liquid) and 100 g of cooked gnocchi (solid) was produced.

The mouth compartment started with an artificial saliva beaker which was maintained at 37±1ºC on top of a hot plate magnetic stirrer (Microspin, PTFE) and stirred at 150 rpm (Yoo, 2009). Then saliva was delivered to the mouth reactor (600ml glass beaker) in the first 30 minutes at 7ml/min (Yoo, 2009) through a silicon peristalsis tubing with 0.6 mm internal diameter (RS component Ltd) by a peristalsis pump (MU-D01, Major Science). Since both the spinning speed of the pump and the tubing size affected the flow rate, the flow rates were tested in triplicate with the same tubing prior to any experiment (data not shown). The speed of 85 rpm was used to achieve a 7 ml/min flow rate. The mouth reactor contained 35 ml of artificial saliva before ingestion began, to simulate the cephalic phase of digestion. From the zero minute mark, 15ml of homogenised gnocchi (20 ºC) was injected into the mouth reactor, every 30 seconds for 8 minutes. The artificial saliva homogenised gnocchi was further mixed by magnetic stirring to mimic the chewing movement *in vivo*.

3.6.4.2. Stomach reactor

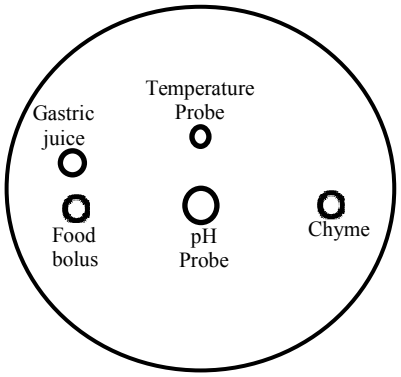
A 1L glass beaker representing a stomach reactor was accompanied with a gastric secretion container, which was maintained at 37±1ºC on a hot plate magnetic stirrer with a stirring speed of 150 rpm (Molly et al., 1993). The open top of the beaker was tightly sealed with a disc of polystyrene, with the thickness of 2.0±0.1cm, to mimic the anaerobic condition of the human stomach as well as to use it as an insulator to better control the temperature over time. The pH and temperature probes, and two peristaltic pump tubings for the delivery of the gastric secretion (0.6mm ID, RS component Ltd.) and withdrawal of the chyme (4.8 mm ID, RS component Ltd) were placed to simulate the fill-and-draw mechanism used by Molly et al (1993). The styrofoam disc was pierced to tightly fit the probes and the tubings to the stomach reactor. A small hole for pipetting food bolus into the stomach reactor was pierced besides the gastric secretion tubing in the disc. The arrangement of tubings, probes and a hole on the Styrofoam disc is shown in Figure 3.6.4.2. The pH and the temperature probes were placed in the middle of the stomach reactor. The tubing for collecting the chyme was placed on the opposite side to the tubings delivering the food and the gastric secretion. Thus the stomach reactor was anatomically coordinated, i.e. oesophagus for

the food delivery, fundus of the stomach for the gastric secretion and pylorus for the chyme collection (Easton, 1974a).

The stomach reactor was heated to and maintained at 37±1ºC on top of a hot plate magnetic stirrer, and was constantly stirred at 150 rpm with a magnetic stir bar (4cm x 0.5cm diameter) to mimic the gastric motility (Molly et al., 1993). In order to simulate the cephalic phase of digestion, the stomach reactor was filled with 17.5ml of the gastric secretion prior to addition of food (Mainville, Arcand, & Farnworth, 2005). The gastric secretion was delivered (3.5ml per min) to the stomach reactor via a peristaltic pump (505U, Watson Marlow) to simulate the gastric phase (Mainville et al., 2005). For every 15 seconds, 5ml of the food bolus from the mouth reactor was pipetted into the stomach reactor, which is equal to a feeding rate of 20ml.min^{-1}. The bolus in the mouth reactor was transferred to the stomach reactor during the first half an hour of digestion period to simulate a half an hour of meal intake (Kong & Singh, 2008a).

The chyme mixture was then pumped out of the stomach reactor by a peristaltic pump through peristaltic tubing (4.8mm ID, RS component Ltd). The chyme sample (20ml) was collected during the first 10 minutes of digestion. The sample was immediately place into a boiling water bath to halt any further enzymatic digestion. From 10 to 20 minutes, 60 ml of digesta was collected. From time 30 minutes till 120 minutes, 75 ml of chyme was collected every 10 minutes (Yoo, 2009). From a preliminary study, insufficient amount of chyme was left in the stomach reactor at the 120 min mark, which could not be pumped out for sample collection by a peristaltic pump. The stomach reactor was completely empty at the 140 min mark. Hence 120 minutes was chosen as the overall digestion period in the stomach. After the samples were immersed in a boiling water bath for 10 min to inactivate the digestive enzymes, 10 ml of each sample was transferred into a centrifuge tube (15 ml) and centrifuged (400e, Labofuge) at 2500rpm for 20 minutes. The supernatant was stored at 4^{o}C for further analyses.

Figure 3.6.4.2 The position of tubings and probes on the Styrofoam disc



3.6.4.3 pH and temperature measurements

The measurement of the pH profile is one of the simplest analyses, which directly indicate the conditions of the stomach and it is of extreme importance as it is able to detect even minor changes of the gastric conditions (Yoo, 2009). An auto-logging pH meter (HI 4212, Hanna Instrument) was used to record the pH and temperature in the stomach reactor every 30 seconds for 2 hours.

3.6.5 Total soluble carbohydrates assay

The amount of total water-soluble carbohydrates was tested using the phenol-sulphuric acid assay (Dubois et al., 1956). D-glucose was used to make a standard curve. D-glucose (100 mg) from Fisher chemicals was dissolved in 10 mL of deionised water to make the glucose stock solution (10 mg/mL). The stock solution was diluted with deionized water to achieve a working solution of 0.1 mg/mL. Then the working solution was further diluted to make glucose standards with concentrations of 0.02 mg/mL, 0.04 mg/mL, 0.06 mg/mL, 0.08 mg/mL and 0.10 mg/mL.

Concentrated sulphuric acid (3 mL) was added to 1 mL of diluted (dilution factor of 200) sample and glucose standards in a test tube followed by vigorous shaking at high speed in a vortex mixer. 50 μL of 80% (w/w) phenol (Fisher BioReagents) was pipetted into the mixed solution and placed in a water bath at 90 ºC for 10 minutes. The mixture was vortexed at high speed for 30 seconds and left at room temperature for 5

minutes to cool down before reading the absorbance using a UV spectrophotometer (Ultraspec 7000, Biochrom Ltd) at 490 nm.

3.6.6 Total maltodextrins analysis

α-amylase hydrolyses starch and produces low molecular weight maltodextrins, maltriose, maltose and glucose. Therefore, the digested starch can be determined by measuring all the substrates. The maltodextrins can be measured using the total starch assay procedure kit (K-TSTA, Megazyme). This kit is based on the use of amyloglucosidase (McCleary, Gibson, & Mugford, 1997) and has been adopted by AOAC (Official method 996.11) (AOAC, 2000). The materials were provided in the starch kit was listed in Appendix 6. The solutions or reagents were prepared according to Appendix 6.

Determination of total maltodextrins in the supernatant of digesta that was stored at 4 ºC from section 3.6.4.2, was performed the following day after digestion started. First of all, supernatant (2 mL), 1.9 mL sodium acetate buffer (Reagent 1, Appendix 6), and 0.1 mL AMG (Solution 2, Appendix 6) were added into a glass test tube, and mixed vigorously before incubation in a water bath at 50 ºC for 30 minutes. The solution was diluted 5 times prior to the assay (20 µL of the solution made up to 100 µL with Reagent 1). Then 3 mL of GOPOD reagent (Solution 4, Appendix 6) was added. The solution was incubated at 50 ºC for 20 minutes.

For the D-glucose control, 0.1 mL of D-glucose standard solution (1 mg/mL) (bottle 5) and 3 mL of GOPOD reagent were mixed. Reagent blank solutions consisted of 0.1 mL of deionized water and 3.0 mL of GOPOD reagent.

The absorbance of the supernatant and D-glucose control was read at 510 nm against the reagent blank. The total maltodextrins in the supernatant was calculated according to the following equation:

$$\text{Maltodextrins (mg/100 mL)} = \Delta A \times F \times \frac{100}{0.1} \times \frac{1}{1000} \times \frac{162}{180} \times 2 \times D$$

$$=\Delta A \times F \times D \times 1.8$$

Where:

ΔA = Absorbance (reaction) against the reagent blank;

F = $\frac{100\ (\mu g\ of\ D-glucose)}{absorbance\ for\ 100\ \mu g\ of\ glucose}$ (conversion from absorbance to µg)

100 = conversion to 100 mL sample volume

0.1 = volume of sample analyzed

$\frac{1}{1000}$ = conversion from µg to mg.

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

2 = Dilution of the sample solution in incubation with AMG

D = Further dilution of the incubation mixture (which is 5)

3.6.8 Total soluble protein analysis

The total soluble protein content was analysed using the Bradford Dye-binding method proposed by Bradford (1976). Coomassie Brilliant Blue G-250 (Thermo Scientific™) (100mg) was dissolved in 50 ml of 95 % ethanol and acidified with 100 mL of 85 % (w/v) phosphoric acid (BDH chemicals Ltd). The mixture was transferred into a one-litre volumetric flask and diluted to the graduation mark with deionised water.

Bovine serum albumin (Sigma Aldrich) was used to make a standard curve for total soluble protein analysis. It was diluted with 0.15 M NaCl to concentrations of 0 (blank), 250, 500, 750 and 1500 µg/mL.

100 µL aliquots of each sample and standards were transferred into a separate test tube. Coomassie Blue solution (5.0 mL) was added to each tube and mixed by vortex. Samples were incubated for 15 minutes at room temperature and the absorbance was measured at 595nm after zeroing the spectrophotometer (Ultrospec 2100) with 0.15 M NaCl, which was treated in the same way. The absorbance of the standards with their concentration was plotted. The soluble protein concentration was calculated according to the standard curve.

Chapter 4. Results and Discussion

4.1 Nutritional composition of gnocchi

The nutritional composition of gnocchi is summarised in Table 4.1.1. Samples containing 30 and 40% meat (30M, 30M10N, 30M20N, 30M30N, 40M, 40M10N, 40M20N, 40M30N,) had significantly higher fat and protein content ($P<0.05$) than the sample containing 10% meat. The protein content of beef (diced, fully-trimmed, raw) is 25–30 % (FSANZ, 2013). Potato (new, peeled, boiled, no added salt) has a protein content of 2.5%, and in navy bean (haricot, dried, boiled, no added salt, drained) 8.2% (FSANZ, 2013). In our study, the meat emulsion contained 57% (wet weight) of beef mince. It is the high protein content of meat emulsion that is mainly responsible for the increasing amount of protein content in gnocchi samples. This is supported by our findings (Table 4.1.1) as samples containing increasing amount of meat emulsion (10%, 20%, 30% and 40%) had increased protein content. However increasing the amount of navy bean at each of these levels only increased the protein content slightly.

The ash content of gnocchi samples was around 1%. Gnocchi samples with added meat and navy bean showed no significant differences with control and commercial gnocchi. Gallegos-Infante et al. (2010) reported that ash content of pasta increased linearly with the addition of common bean flour (0.86 - 2.09 %). In their study however, pasta was prepared with 15 % to 45 % common bean flour (dried), which was at a much higher concentration of navy bean than used in our study. In our study, the highest concentration of boiled navy bean used was 30 % (wet weight). The ash content in mashed potatoes has been reported to be 0.92 % (U.S. Department of Agriculture, 2013b). 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 in the final product.

Carbohydrates, the major solid component of cooked gnocchi (Figure 4.1.1), ranged from 39.1 % to 49.7 % and were mainly starch. This value was much lower than the value of pasta (Gallegos-Infante et al., 2010; Petitot et al., 2010). The carbohydrate content in pasta containing 45 % common bean was 60.5 %, lower than the pasta

without any bean addition (72 %) (Gallegos-Infante et al., 2010). The starch content in 35 % faba bean pasta was 66 %, compared to its control, 77 % (Petitot et al., 2010). However, pasta and gnocchi are different in terms of the main ingredient. The main ingredient in gnocchi is mashed potatoes, of which the carbohydrate content is reported to be 12.8 % (FSANZ, 2013). Compared to mashed potatoes, durum wheat flour as the main ingredient in pasta, has a much higher carbohydrate content of 71 % (U.S. Department of Agriculture, 2013a). Therefore, gnocchi samples in our study showed lower values in carbohydrate content compared to pasta.

Samples containing 30 % navy bean (10M30N, 20M30N, 30M30N, 40M30N) contained significantly higher moisture content ($p < 0.05$). This might be attributed to the high fiber content in navy bean, which was reported to be 10 – 20 % of dry mass (Kereliuk & Kozub, 1995). The presence of fiber in navy bean can increase the water binding capacity and water holding capacity of gnocchi (Chen, Piva, & Labuza, 1984). The number of hydroxyl groups in the fiber structure can increase water absorption and allow water interaction through hydrogen bonding (Belitz, Grosch, & Schieberle, 2009).

Table 4.1.1 Nutritional composition of gnocchi samples containing meat and navy bean

Sample*	Component (g/100 g dry basis)					
	Moisture	Fat	Protein	Ash	Carb	Energy[1]Kj
10M	38.52±0.13^{a}	0.81±0.21^{ab}	9.94±0.58^{bcd}	1.15±0.04^{abcd}	49.59	1024.65
10M10N	40.49±0.25^{bc}	1.00±0.07^{bc}	9.15±0.63^{abc}	1.03±0.04^{abc}	48.32	997.47
10M20N	42.45±0.21^{efg}	0.72±0.03^{ab}	10.30±0.97^{bcd}	1.07±0.01^{abc}	45.46	958.44
10M30N	44.18±0.16^{hi}	0.72±0.01^{ab}	10.44±0.13^{bcde}	0.82±0.02^{a}	43.83	933.61
20M	39.74±0.07^{ab}	1.06±0.02^{bcd}	11.11±0.38^{cdef}	1.23±0.15^{abcd}	46.86	1008.16
20M10N	41.85±0.28^{def}	1.04±0.12^{bcd}	11.59±0.52^{cdefg}	1.16±0.05^{abcd}	44.37	973.63
20M20N	42.97±0.68^{fgh}	1.17±0.01^{bcde}	12.25±0.16^{defghi}	0.995±0.04^{ab}	42.62	960.20
20M30N	44.21±0.08^{hi}	1.16±0.02^{bcde}	11.73±0.22^{cdefgh}	0.99±0.00^{ab}	41.91	939.59
30M	39.23±0.02^{a}	1.28±0.11^{cde}	14.51±0.47^{hij}	1.2±0.00^{abcd}	43.79	1021.71
30M10N	40.91±0.41^{bcd}	1.44±0.35^{cdef}	11.62±1.94^{cdefg}	1.01±0.03^{abc}	45.02	1000.22
30M20N	43.14±0.17^{gh}	1.43±0.01^{cdef}	13.19±0.91^{efghij}	0.95±0.11^{ab}	41.29	963.80
30M30N	45.28±0.30^{i}	1.59±0.03^{ef}	13.79±0.94^{fghij}	0.92±0.11^{ab}	38.43	931.92
40M	38.86±0.15^{a}	1.48±0.05^{def}	14.77±0.24^{ij}	1.45±0.25^{cd}	43.44	1027.88
40M10N	43.05±0.80^{fgh}	1.73±0.03^{f}	14.57±0.22^{hij}	1.54±0.04 ^{d}	39.12	961.81
40M20N	42.54±0.04^{fg}	1.84±0.09^{f}	15.22±0.12^{j}	1.15±0.30^{abcd}	39.26	978.92
40M30N	45.38±0.17^{i}	1.83±0.10^{f}	14.09±1.01^{ghij}	1.28±0.10^{bcd}	37.41	929.30
Control	41.22±0.08^{cde}	0.40±0.04^{a}	7.80±0.05^{ab}	0.88±0.01^{ab}	49.7	975.16
Commercial	42.13±0.20^{defg}	1.00±0.01^{bc}	6.92±0.09^{a}	1.01±0.00^{abc}	48.94	970.44

Different superscript letters in column are significantly different using one-way ANOVA and Tukey's test ($p<0.05$).
[1]Energy (kJ/g) is calculated according to Merrill & Watt (1973). Samples are expressed as percentage meat (M) and navy bean (N) content. For example, 20M10N refers to the gnocchi sample containing 20% meat and 10% navy bean.
Each point corresponds to the average value ±standard deviation of three independent determinations.

Figure 4.1.1 The proximate composition of gnocchi samples containing meat (10%, 20%, 30% and 40%) with increasing navy bean content (10%, 20% and 30%). Each column represents 100% of the sample. Samples are expressed as meat and navy bean content, e.g., 20M10N refers to the sample containing 20% meat emulsion (i.e., 11.4% beef) and 10% navy bean powder.

4.2 Physicochemical property

4.2.1. Water activity

Water activity is used in predicting the growth of bacteria, yeasts and moulds. It can be used as an indicator for the shelf life of foods. It is necessary to control either acidity level (pH) or the level of water activity, or a suitable combination of the two. This can effectively increase the stability of the product and make it possible to predict its shelf life under known ambient storage conditions (Belitz et al., 2009). Decreased water activity retards the growth of microorganisms, and slows down enzyme-catalyzed reactions.

The water activity of all raw gnocchi samples was around 0.8 (Table 4.2.2). Raw gnocchi samples (20M, 20M20N and 30M30N) had significantly higher values ($p < 0.05$) than control and commercial gnocchi (raw). However, all the values for both raw and cooked gnocchi were below the values of limit (0.85) for the growth of selected pathogens in food (FDA, 1984).

4.2.2 Texture profile analysis (TPA)

Textural parameters, especially firmness, are important attributes for gnocchi cooking quality. The optimisation of texture parameters is a critical point to ensure the acceptance of the developed products by consumers. Table 4.2.2 summarises the TPA results of cooked gnocchi samples.

Table 4.2.2. Physicochemical properties raw and cooked gnocchi samples containing meat and navy bean (Mean ± SD)

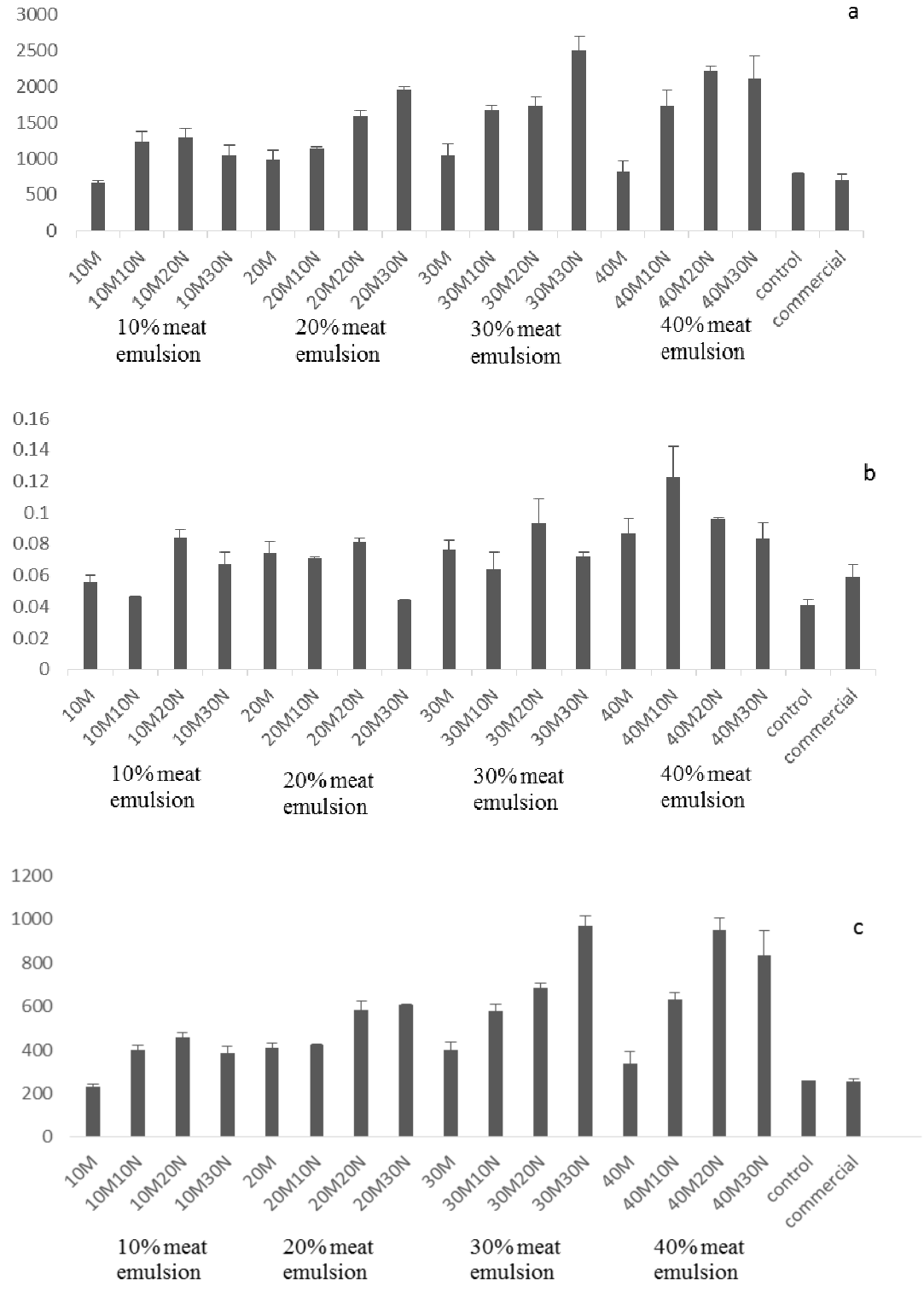
Sample	Cooked gnocchi							Raw gnocchi			
	Texture profile			Colour			Water activity	Colour			Water activity
	Hardness (g)	Springiness (dimensionless)	Chewiness (g)	L*	a*	b*		L*	a*	b*	
10M	668±35^{i}	0.06±0.01^{efg}	84±18^{hi}	61±0^{d}	4.7±0.21^{g}	25±0^{bc}	0.79±0.02^{de}	74±0^{e}	2.63±0.20^{l}	25±0^{c}	0.80±0.01^{bcde}
10M10N	1233±209^{fg}	0.05±0.00^{fg}	125±27^{fghi}	64±0^{c}	3.16±0.05^{j}	24±0^{bcd}	0.84±0.02^{a}	73±0^{e}	3.53±0.10^{i}	26±0^{b}	0.78±0.02^{de}
10M20N	1285±187^{efg}	0.08±0.01^{bc}	231±26^{cdefgh}	61±0^{d}	4.21±0.13^{i}	24±0^{bcd}	0.81±0.01^{abcde}	74±0^{d}	3.39±0.08^{i}	23±0^{fg}	0.80±0.02^{bcde}
10M30N	1052±199^{ghi}	0.07±0.01^{cdef}	139±43^{fghi}	59±0^{f}	4.35±0.07^{hi}	22±0^{cdef}	0.83±0.02^{ab}	75±0^{c}	3.13±0.04^{j}	24±0^{e}	0.79±0.01^{cde}
20M	995±186^{ghi}	0.07±0.01^{bcde}	171±33^{efghi}	61±0^{e}	4.23±0.08^{hi}	22±0^{defg}	0.81±0.01^{abcde}	69±0^{jk}	4.51±0.03^{g}	26±0^{bc}	0.85±0.01^{a}
20M10N	1148±26^{gh}	0.07±0.01^{bcde}	171±4^{efghi}	60±0^{e}	4.41±0.07 h	22±0^{defgh}	0.79±0.00^{e}	72±0^{e}	4.27±0.07^{h}	24±0^{ef}	0.80±0.01^{bcde}
20M20N	1601±90^{def}	0.08±0.01^{bcd}	273±60^{cde}	58±0^{g}	5.56±0.08^{ef}	22±0^{cdef}	0.80±0.00^{cde}	72±0^{g}	2.85±0.11^{k}	23±0^{hi}	0.83±0.01^{ab}
20M30N	1962±52^{bcd}	0.04±0.01^{fg}	182±5^{efghi}	58±0^{g}	4.87±0.07^{g}	20±0^{fghij}	0.82±0.02^{abcd}	70±0^{i}	5.74±0.14^{c}	24±0^{e}	0.77±0.00^{e}
30M	1053±216^{ghi}	0.08±0.01^{bcde}	195±54^{defgh}	58±0^{f}	5.50±0.14^{ef}	21±0^{efghi}	0.81±0.01^{abcde}	70±0^{h}	4.33±0.04^{h}	24±0^{e}	0.80±0.02^{bcde}
30M10N	1676±89de	0.06±0.02^{cdefg}	243±43^{cdef}	56±0^{i}	5.57±0.16^{ef}	25±1^{b}	0.80±0.01^{cde}	67±0^{n}	6.48±0.07^{a}	24±0^{d}	0.78±0.01^{e}
30M20N	1745±174^{cd}	0.09±0.02^{b}	303±30^{cd}	54±0^{j}	6.05±0.13^{c}	21±0^{fghi}	0.82±0.02^{abc}	70±0^{j}	4.71±0.22^{f}	22±0^{ij}	0.82±0.01^{abc}
30M30N	2512±251^{a}	0.07±0.01^{bcde}	455±63^{a}	57±0^{h}	5.67±0.02^{def}	19±0^{ij}	0.82±0.02^{abcd}	69±0^{k}	4.96±0.07^{e}	22±0^{jk}	0.83±0.02^{ab}
40M	824±198^{hi}	0.09±0.01^{bcd}	178±77^{efghi}	57±0^{h}	5.47±0.09^{f}	20±0^{fghij}	0.80±0.01^{bcde}	67±0^{n}	6.31±0.06^{b}	23±0^{h}	0.78±0.02^{de}
40M10N	1736±305^{cd}	0.12±0.03^{a}	335±44^{bc}	52±0^{k}	6.39±0.07^{b}	20±0^{ghij}	0.79±0.01^{de}	68±0^{l}	5.42±0.04^{d}	22±0^{k}	0.78±0.02^{de}

40M20N	2216±105^{ab}	0.10±0.01^{b}	538±74^{a}	53±0^{k}	5.84±0.06^{d}	18±0^{j}	0.79±0.00^{de}	68±0^{m}	4.59±0.09^{fg}	21±0^{l}	0.80±0.01^{bcde}
40M30N	2112±444^{abc}	0.08±0.01^{bcd}	440±161^{ab}	53±0^{k}	6.99±0.06^{a}	20±0^{hij}	0.80±0.00^{cde}	66±0^{o}	5.42±0.09^{d}	22±0^{jk}	0.81±0.01^{bcd}
Control	800±0^{hi}	0.04±0.01^{g}	66±0^{i}	70±0^{a}	0.65±0.14^{l}	27±0^{a}	0.80±0.01^{bcde}	80±0^{a}	0.18±0.02^{c}	29±0^{a}	0.79±0.00^{cde}
Commercial	699±127^{i}	0.06±0.01^{defg}	120±17^{ghi}	69±0^{b}	2.03±0.13^{k}	23±1^{bcde}	0.80±0.01^{bcde}	77±0^{b}	2.21±0.01^{m}	23±0^{g}	0.79±0.00^{cde}

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 (N) content. For example, 20M10N refers to the gnocchi sample containing 20% meat and 10% navy bean.
Each point corresponds to the average value ±standard deviation of three independent determinations.

The hardness, springiness and cohesiveness of gnocchi samples are further summarised in Figure 4.2.2 for easier interpretation.

Figure 4.2.2 Texture of cooked gnocchi samples with varying meat and navy bean content: (a) hardness, (b) springiness, (c) chewiness



Addition of a combination of navy bean and meat emulsion into gnocchi formulation significantly increased the hardness, springiness and chewiness of cooked gnocchi except for six samples (10M, 10M30N, 20M, 20M10N, 30M, 40M). This was similar to a study on the fortification of pasta with split pea and faba bean flour (Petitot et al., 2010). Fortification of durum wheat pasta with 35 % of split pea or faba bean flour significantly increased pasta hardness by 38 % and 43 %, respectively (Petitot et al., 2010). Fortification of pasta with legume flour (navy bean, pinto bean, lentil, green pea) or protein concentrates was reported to increase pasta firmness (Alireza & Bhagya, 2008; Zhao et al., 2005). In Figures 4.2.2 a, b and c, when the meat content remained constant, the hardness and chewiness of cooked gnocchi sample increased with increased navy bean content. Samples containing navy bean had however significantly higher hardness value than sample without navy bean.

According to Malcolmson (1991), spaghetti firmness improved with increasing protein content. This has been attributed to the higher number of polypeptide chains associated with higher protein levels that increase the chance for proteins to interact to form an insoluble network. This insoluble protein network can entrap swollen and gelatinized starch granule, which prevents pasta or in this case gnocchi from surface disruption (Chillo et al., 2010; Malcolmson, 1991). The protein network may also limit the access of water to the raw flours when submitted to gelatinization during cooking (Fradique et al., 2010). Consequently, the hardness of gnocchi in our study increased with addition of both meat and navy bean. There have been no studies to date that investigated the effect of incorporating meat on pasta quality.

Samples 10M, 10M30N, 20M, 20M10N, 30M and 40M were not significantly different from commercial gnocchi in hardness, chewiness and springiness. The meat emulsion content of these gnocchi samples ranged from 10 % to 40 %. Meat helped maintain the texture of cooked gnocchi samples. Myosin and actin in meat protein contribute most to the development of desirable gel characteristics in processed meat products. The heat-induced gelation of myosin results in the formation of a 3-dimensional network structure that holds water in a less mobile state (Yasui et al., 1979). During network formation fat and water retention are enhanced and affects texture of the final product. Gelation of muscle protein involves partial denaturation followed by irreversible aggregation of myosin heads through formation of disulphide bonds and

helix-coil transitions of the tail part of the molecules, resulting in a 3-dimensional cross-linked network structure. During thermal gelation, myosin and other salt-soluble myofibrils protein exhibit complex changes in rheological characteristics depending upon specific temperature and pH exposures (Sun & Holley, 2011).

4.2.3 Colour

The appearance of a food 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, plays a major role in the acceptability of a food product. It is an indicator of quality, freshness, conservation state, flavor expectation and commercial value (Fradique et al., 2010). A little yellow color in fresh pasta is generally considered an important positive quality attribute (Alessandrini et al., 2010).

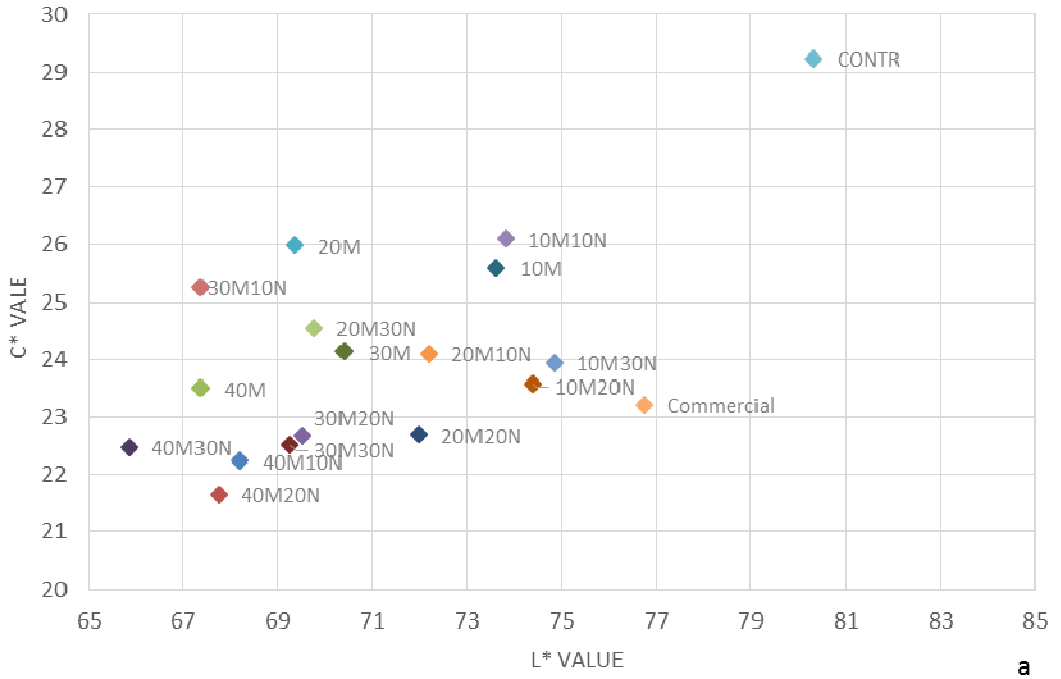
The incorporation of meat emulsion and navy bean changed the colour of cooked gnocchi samples (Figure 4.2.3.1). The control sample was yellow.

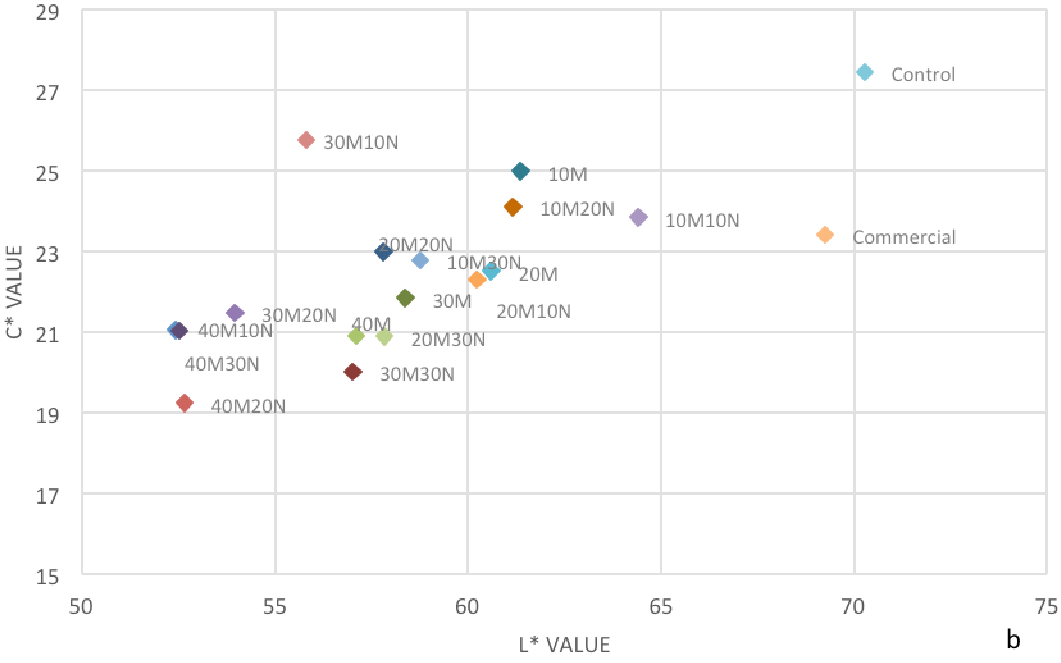
Figure 4.2.3.1. The image of several cooked gnocchi samples. Numbers besides the gnocchi are the sample code. 1 (40M10N), 2 (40M20N), 3 (40M), 4 (30M20N), 5(20M), 6 (20M10N), 7 (20M20N), 8 (30M30N), 9 (30M), 10 (40M30N), 11 (10M), 12 (10M20N), 13 (10M30N)

Samples containing meat or navy bean were darker with a redder colour. This was consistent with Hunter L*, a*, and b* values shown in Table 4.2.2.1. To simplify interpretation of these three dimensional colour values, the Hunter L*, a*, and b* values for each sample were transformed to C* ($C^*=[a^2+b^2]^{1/2}$) (Minolta, 1991). C* value is an indication of saturation. The C* value was plotted against the L* value to give two-dimensional plots of raw and cooked gnocchi samples (Figures 4.2.3.2 a and b).

In Figure 4.2.3.2a, the raw control sample had significantly higher L* and C* values, which indicated the high lightness and saturation (C* value) compared to the commercial and other gnocchi samples. According to Table 4.2.2, control sample (raw) exhibited significantly higher yellowness (29.23 ± 0.03) than commercial gnocchi (raw) (23.10 ± 0.30). The colour difference between the control and commercial sample may have resulted from the use of different potato cultivars. In our study, we used agria potatoes, which is a deep yellow flesh cultivar ("Agria," 2014) to make gnocchi. The yellow colour may be due to the considerably high amount of lutein and zeaxanthin (two kinds of carotenoid in yellow-flesh potatoes) in agria potatoes (Brown et al., 2005). Commercial gnocchi (Delmaine, NZ) sample however listed the use of a potato puree as the main ingredient. Basically, potato puree (PP) is formulated with white potato starch (Alvarez et al., 2012), which explains the lighter yellow colour of commercial pasta.

Figure 4.2.3.2: The L^* and C^* values for raw (a) and cooked (b) gnocchi containing meat and navy bean.





Raw gnocchi samples with the same meat content had similar L* values (Figure 4.2.3.2a). The higher the meat content, the lower the L*-value becomes. The saturation of raw gnocchi samples ranged from 21 (40M20N) to 26 (10M10N) except raw control sample, which was the highest (29). Both redness (a* value) and yellowness (b* value)

affected the saturation. As shown in Table 4.2.2, the redness of raw gnocchi increased significantly with increasing amount of meat content. The higher redness value in samples with meat is caused by colour of myoglobin in meat. Myoglobin is responsible for the purplish red muscle colour, observed in the depth of the muscle when meat is freshly cut. This quickly changes to the bright red oxymyoglobin, due to oxygenation when the muscle surface is exposed to air (Mancini & Hunt, 2005). With time this slowly reverts to the unattractive, dull brown, metmyoglobin associated with stale and spoiled meat.

It can be seen in Figure 4.2.3 b and Table 4.2.2 that the L* value (lightness) of most gnocchi samples after cooking decreased with increased redness. High redness is mainly caused by the addition of meat emulsion. Meat colour turned to dark brown during heat treatment due to the denaturation of myoglobin (Mancini, 2005). Therefore the lightness decreased with increasing amount of meat.

4.2.4 Correlation between Colour and Texture

As consumers may also predict other properties (e.g., flavour, texture) of food based on what they have seen, the correlation between colour and texture was investigated. The incorporation of meat and navy bean changed the colour and texture properties as discussed in sections 4.2.2 and 4.2.3. To investigate the relationship between colour and texture of cooked gnocchi, Pearson correlation was carried out between colour (lightness, redness and yellowness) and texture (hardness, springiness and chewiness) of cooked gnocchi.

The lightness of cooked gnocchi samples was negatively correlated with hardness (Pearson correlation -0.63, $p < 0.001$), springiness (Pearson correlation -0.60, $p < 0.001$) and chewiness (Pearson correlation -0.73, $p < 0.001$). From Figure 4.2.3 b, we can see that the beef addition decreased the lightness of cooked gnocchi samples. As discussed in section 4.2.2, the addition of meat emulsion and navy bean however increased the hardness, chewiness and springiness of cooked gnocchi.

The redness (a*) of the cooked gnocchi was correlated with chewiness (Pearson correlation 0.67, $p < 0.001$). High meat emulsion (40 %) samples (samples 40M, 40M10N, 40M20N and 40M30N) had significantly higher hardness ($p < 0.05$),

chewiness, and springiness, with increased redness and were correspondingly less light in colour. The myoglobin in meat is responsible for the redness of gnocchi sample and the myofibrillar protein of meat may be responsible for the increases in TPA parameters. The denaturation of myofibrillar protein during thermal processing probably results in high hardness values of the chicken myofibrillar protein gels (Smith, Alvarez, & Morgan, 1988). Myofibrillar proteins unfold and aggregate when heated to form a three-dimensional cross-linked protein network, which traps fat and macroparticulates within the gel matrix (Sun & Holley, 2011). This formation of a gel matrix might attribute to the increased hardness, chewiness and springiness of gnocchi with high meat content.

4.2.5 Correlation between TPA and Nutritional composition

Food texture is affected by composition (proteins, fat, moisture and structural carbohydrates, e.g. cellulose, starch and pectic). Alterations in texture are caused by changes in moisture, fats, hydrolysis of polymeric carbohydrates and coagulation or hydrolysis of proteins (Zhou et al., 2007).

The hardness of cooked gnocchi was positively correlated (Pearson Correlation 0.692, $p < 0.00$) with moisture content. High moisture content probably results from the higher water holding capacity of protein. As discussed in section 4.2.2, protein in the food matrix can form an insoluble network, which will entrap water and limit complete gelatinization of starch, resulting high hardness. This has been reported in several studies on protein containing food systems. Kuhn (2010) studied the cold-set whey protein gels induced by calcium or sodium salt addition. The increase of whey protein isolates concentration led to an increase of hardness, elasticity and water-holding capacity for both systems ($CaCl_2$ and NaCl).

Fat content was positively correlated with hardness (Pearson correlation 0.689, $p < 0.00$) and chewiness (Pearson correlation 0.779, $p < 0.00$). This is an unexpected finding. Bryant et al. (1995) studied the texture profiles of Cheddar cheeses at five different fat levels (34, 32, 27, 21, and 13 %). They reported that the reduction in fat content increased the hardness of cheese. Another study on the low fat frankfurter found that the fat reduction significantly increased the hardness (Barbut & Mittal, 1996). The hardness of frankfurter with 26 % fat was 16.2 N/cm^2 and the hardness increased to 19.6

N/cm^2 when the fat content decreased to 13% (Barbut & Mittal, 1996). However, these two products are fat- rich product (>10% of fat). In our gnocchi sample, the fat content of gnocchi containing meat and navy bean was only around 1 % (Table 4.1.1) compared to control (0.4 % fat). In this case, fat was useful in providing structure to the gnocchi through the formation of a fat crystal network.

The microscopic studies of meat emulsions distinguished two groups of fat particles on the basis of their size (Lee, 1985). Fat particles ranging in diameter from 1 to 20 μm formed the first group, and those having diameters of over 20 μm represented the second group. The first group formed spherical globules that remain in a suspension in the so-called "true emulsions." The latter group formed angular structures physically entrapped in the protein matrix (Lee, 1985). The majority of fat particles appeared in an angular form and reached more than 20 μm in diameter. These angular fat particles were found to be most responsible for the stability of the emulsion (Wioletta & Józef, 2010).

In addition, animal fat chopped into small globules of roughly 50 μm in diameter are stabilized by a membrane coating made of salt-soluble myofibrillar proteins. These discontinuous fat particles act as fillers or co-polymers and thereby stabilizing the myofibrillar network (Miklo et al., 2013). Interactions of emulsified fat-globules with proteins may increase the hardness and chewiness of gnocchi.

4.3 Sensory Evaluation of Gnocchi

From the physiochemical property study, six samples from the full factorial design that had similar textural properties to commercial gnocchi and a control sample were chosen and subjected to consumer testing and project mapping. The combination of the 6 gnocchi sample and control are listed in Table 4.3.1.

Table 4.3.1 Gnocchi Samples used for consumer testing and projective mapping.

Sample*	Meat Emulsion %(w/w)	Navy Bean powder %(w/w)	Mashed Potatoes %(w/w)	Semolina flour %(w/w)
10M	10	0	60	30
10M30N	10	30	30	30
20M	20	0	50	30
20M10N	20	10	40	30
30M	30	0	40	30
40M	40	0	30	30
Control	0	0	70	30

* Samples are expressed as meat and navy bean content, e.g., 20M10N refers to the sample contains 20% meat emulsion (i.e., 11.4% beef) and 10% navy bean powder

4.3.1 Consumer testing

The consumer testing data of 53 panellists were collected over two weeks. The data were analyzed using analysis of variance. There were no significant differences in overall liking and all other attributes (odour, taste, texture and flavour) among all the gnocchi samples ($p > 0.05$) between the seven gnocchi samples shown in Table 4.3.1.1. Most samples have a score of around 5, which means they are all acceptable by the consumers. Gnocchi samples were served with diluted pasta sauce, which may have masked the flavor, odour and taste of gnocchi. There was also no significant difference in terms of texture ($p > 0.05$), which was consistent with our TPA data.

Gnocchi is not a popular food in New Zealand. The gnocchi sold in the New Zealand supermarket (Delmaine, New Zealand) are not the same in terms of texture and flavour as traditional Italian homemade gnocchi served in some Italian restaurants. The commercial gnocchi was salty and chewy, while the homemade gnocchi was usually soft. The gnocchi consumers (panellists) in this study are probably familiar with commercial gnocchi. Since our gnocchi sample was similar in texture to the commercial gnocchi (Delmaine, New Zealand), there were no significant differences in terms of liking.

Table 4.3.1.1. Consumer test of cooked gnocchi products containing meat and navy bean*

Gnocchi Sample	Overall liking	Odor	Taste	Texture	Flavor
10M	5.24±2.06a	5.24±2.22a	4.91±2.43a	5.05±2.33a	5.06±2.39a
10M30N	4.75±2.36a	4.79±2.46a	4.27±2.54a	4.31±2.55a	4.34±2.56a
20M	5.07±2.09a	4.94±2.10a	4.71±2.10a	5.07±2.31a	4.94±2.33a
20M10N	4.74±2.46a	4.99±2.22a	4.54±2.52a	5.11±2.53a	4.64±2.61a
30M	5.44±2.30a	5.23±2.27a	5.11±2.41a	5.53±2.50a	5.27±2.45a
40M	5.10±2.32a	4.82±2.26a	4.91±2.34a	5.16±2.35a	4.88±2.42a
Control	4.77±2.24a	4.99±2.27a	4.52±2.44a	4.62±2.55a	4.84±2.44a
p-value	0.313	0.925	0.624	0.226	0.603

*Hedonic line scale with left end represents 0 (extremely dislike), right end represents 10 extremely like, and middle represents 5 (neither like nor dislike);

4.3.2 Projective mapping

A total of 12 panellists took part and 11 completed the sensory projective mapping of the seven gnocchi products over three weeks. The RV coefficients were used to determine how well a panellist's map fitted with the consensus maps, as shown in Appendix 7. All the panellists scored well in terms of fit with the rest (as assessed by MFA) (RV > 0.500). Three panellists scored poorly for only one out of the three sensory trials (RV < 0.500).

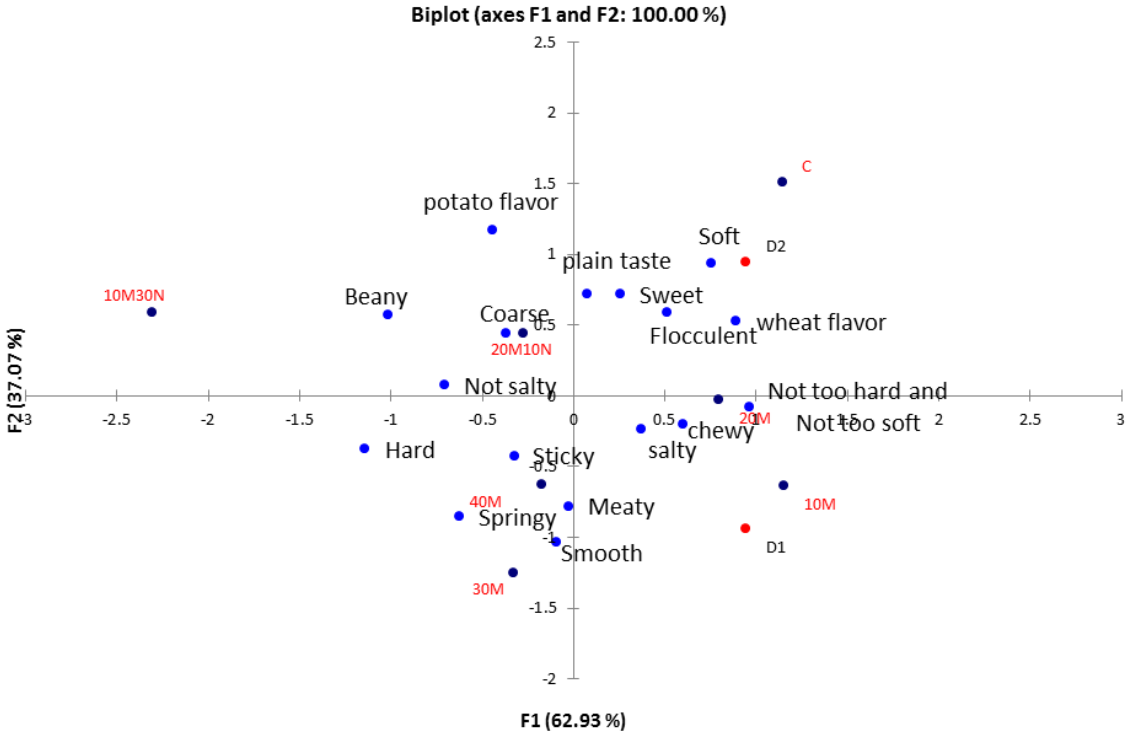
RV coefficients obtained during the three trials indicated an improvement in the consensus maps of the panellists with time. In week 1, 81 % of the 15 panellists scored RVs of > 0.500, while in the second and third trials showed more than 90 % agreement among the panellists.

Product and attribute maps were plotted, as shown in Figure 4.3.2. Control sample was separated from the rest samples due to its soft texture, which is consistent with the TPA results. 40M, 30M and 20M10N were associated with hard texture, while 20M and 10M were associated with chewy, and neither hard nor soft texture. These samples were not significantly different ($p > 0.05$) from each other in terms of TPA values (hardness, chewiness and springiness). However, the panellists were able to separate them in terms of texture. Hence sensory analysis can complement instrumental texture analysis. In the Figure these samples are highlighted using red text.

Samples 30M and 40M were associated with smooth mouth feel and meaty flavor. The smooth mouth feel of high meat sample may result from the interaction of proteins and fat to form a gel network (Chillo et al., 2010; Malcolmson, 1991). Samples

with navy bean (10M30N and 20M10N) were separated due to their beany flavour and coarse mouth feel. Navy bean has higher fiber content (10 - 20% of dry mass) (Kereliuk & Kozub, 1995), which might be responsible for the coarse mouth feel. In the Figure these samples are highlighted using red text.

Figure 4.3.2 Principal component analysis of seven gnocchi samples over the combined three sensory trials.



4.4 Digestibility of Gnocchi

All of the samples subjected to consumer testing (Table 4.3.1) had acceptable overall likings. Therefore, the samples with meat emulsion and navy bean powder levels between 10 % and 30 % were selected for *in vitro* digestibility test. i.e. samples 10M, 10M30N, 20M10N, 30M and 40M were tested for their digestibility. Digestion was conducted once for each sample, due to insufficient time. We acknowledge the limitations of single runs carried out in this study that lack statistical power.

4.4.1 pH profile over the course of digestion

pH profiles in the stomach reactor of the MISST are shown in Figure 4.4.1. The pH values of potatoes, meat and bean, in their natural forms are 5.7 - 6.1, 5.1 - 6.2, and 5.6 - 6.5, respectively (FDA, 2012). The initial pH values of the homogenized cooked gnocchi samples were approximately 6 (Table 4.4.1). The initial pH in the stomach reactor was 1.3 for all experiments, which reflects the acidic pH of the artificial gastric juice used. Upon feeding of gnocchi samples into the MISST, the pH of the stomach reactor increased. This was caused by addition of gnocchi with higher pH values, as shown on Table 4.4.1. The initial rapid increase in pH suggests quick consumption of acid in the digestion process (Yoo, 2009). Kalantzi et al. (2006) studied the changes of human upper GI contents after ingestion of food. They observed a temporary raise in the gastric pH in the first 20 minutes, in healthy subjects. According to Kalantzi et al (2006), the temporary increase in the gastric pH reflects the dilution of gastric fluids with saliva or nasal secretions (Kalantzi et al., 2006). The results shown in Figure 4.4.1 agree with *in vivo* human studies conducted by Kalantzi et al (2006) and Dressman et al (1990), where the gastric pH ranged from 1.0 to 3.5 during the meal ingestion period.

Table 4.4.1. The pH value of gnocchi samples.

Sample emulsion[1]	Control	40M*	10M30N	10M	30M	20M10N
pH value	5.87	6.13	6.01	5.92	6.11	6.02

[1]Sample emulsion was made by mixing 100 g sample with 250 ml drinking water.
*sample was expressed as percentage of meat and navy bean, e.g., 20M10N refers to the sample containing 20 % (w/w) meat emulsion (i.e., 11.4% beef) and 10 % (w/w) navy bean powder.

The food bolus was totally transferred to the stomach reactor in the first 20 minutes. Then, no food bolus was fed into the MISST. Therefore, the highest pH was achieved at around 20 minutes as shown in Figure 4.4.1.

In the first 20 minutes of the digestion period, samples containing meat and navy bean increased in pH more quickly than that of the control sample. The maximum pH in the stomach reactor for different samples was: 3.04 (40M), 3.0 (30M), 2.9 (20M10N), 2.9 (10M30N), 2.84 (10M) and 2.66 (Control). The difference may be results of different protein content in samples. High protein samples (40M, 30M, 20M10N) showed a higher buffering capacity (high maximum pH) in the first 20 minutes. The

buffering capacity is an indicator for the ability of food to resist changes in pH (Kylä-Puhju et al., 2004). In meat, inorganic phosphate, protein-bound L-histidine residues, free L-histidine or histidine-relate dipeptides are the proposed constituents, which play a key role in buffering effect. However, sample 10M30N showed a higher buffering capacity than 10M, even though there is no significant difference ($P > 0.05$) between those two samples in terms of protein content (9.94 % for 10M and 10.44 % for 10M30N). The high buffering capacity in 10M30N may have resulted from the fiber from the navy bean. Navy bean contains a considerable amount of fiber, 10 – 20 % of dry mass (Kereliuk & Kozub, 1995). Fiber has been shown to be positively correlated with the buffering capacity in several studies (Al-Dabbas, 2010; McBurney, van Soest, & Chase, 1983).

The pH of all samples decreased between 20 and 30-minute mark due to the stomach emptying and constant gastric juice delivery into the stomach reactor. The volume of gastric contents decreased from 30 minute. Thereafter, a higher proportion of acid being present compared to the gnocchi bolus, resulting in a decrease in pH as a general pattern.

Towards the end of the 2 hour digestion period, the pH of all samples decreased to 1.30 ± 0.05. During the digestion, the chyme in the stomach reactor has been pumped out, and gastric juice was pumped into the stomach reactor at a constant rate. The proportion of acid to the gastric contents increased gradually. By the end of the 2hour digestion period, most of chyme has been pumped out of the stomach reactor, where the percentage gastric acid was approaching to 100 % in the stomach reactor. Therefore, the end pH was similar to the pH of gastric juice.

The pH profile of control sample was different from the others. The pH increased slowly to its peak (pH 2.66), which is lowest peak among all the samples, and decreased slowly. The pH of control gnocchi decreased to 2 at 80 minute, while most other samples decreased to 2 at 50 minute except 10M30N. This may be caused the fluctuation of pH readings.

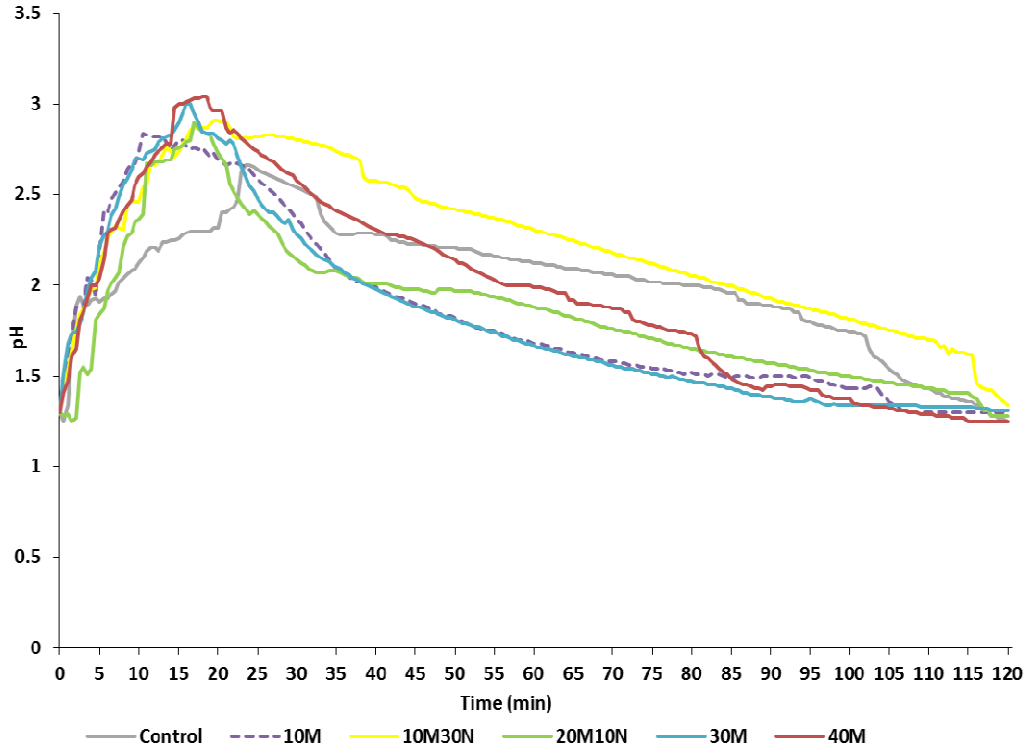


Figure 4.4.1. pH profile of gnocchi samples in the stomach reactor of the MISST (n=1).

4.4.2 Total water-soluble starch in collected chyme

As shown in Figure 4.4.2.1, the concentration of water-soluble carbohydrates decreased over the digestion period. The concentration indicates the total amount of available carbohydrates in the stomach reactor at given times. In other words, it provides information on the transit of the bolus and the chyme through the digestive system. The initial concentration of total soluble carbohydrates was the highest for all the samples, ranged from 34 % (10M) to 44 % (control). At the beginning, the ratio of food bolus to gastric content was higher. Thus, the concentrations of total soluble starch were the highest. The changes could be easily seen when comparing the collected digesta over the 2 hour digestion period. The collected samples from the stomach reactor are more turbid in the beginning, which indicate more starch being present, where the turbidity of the collected samples became clearer over the digestion period.

The 40M sample showed the lowest concentration of water soluble carbohydrates than other samples throughout the entire 2 hour digestion period. This may be a result of the lower total carbohydrates (43 %) in the 40M sample compared to

other gnocchi samples (Table 4.1.1). However the 30M sample, which was only 25 % lower in carbohydrates, showed a markedly higher carbohydrate concentration than 40M at all given times. The collected chyme was centrifuged to get the clear supernatant for futher analysis. The soluble carbohydrates may have been trapped in the particles, which was centrifuged to the bottom. Thus the errors may have occurred, which was responsible for the unexpected results.

Figure 4.4.2.2 shows the mass of total water-soluble starch in the stomach reactor during the 2 hour digestion period. The mass of soluble starch indicates the amount of carbohydrates in the stomach compartment, at given times. The volumes in the stomach reactor increased to 455 mL at time 30 minute, and then decreased to 95 mL in the end due to secretions and bolus delivery and gastric emptying. Therefore, the available starch for all samples in the stomach reactor reached the highest amount at the 30-minute mark. Control, 20M10N and 10M30N had the highest mass of water-soluble starch at 30 minutes, followed by 30M, 10M and 40M. According to Table 4.1.1, the total carbohydrates contents in those samples are 49 % (10M), 43 % (10M30N), 44 % (20M10N), 43 % (30M), 43 % (40M), and 49 % (control). Control and 10M were expected to have the highest mass of water-soluble starch since their carbohydrate was the highest. However, the 20M10N and 10M30N had higher mass of water-soluble starch than 10M. The total water-soluble carbohydrates in these samples varied, causing differences in mass of water soluble carbohydrates in the collected digesta.

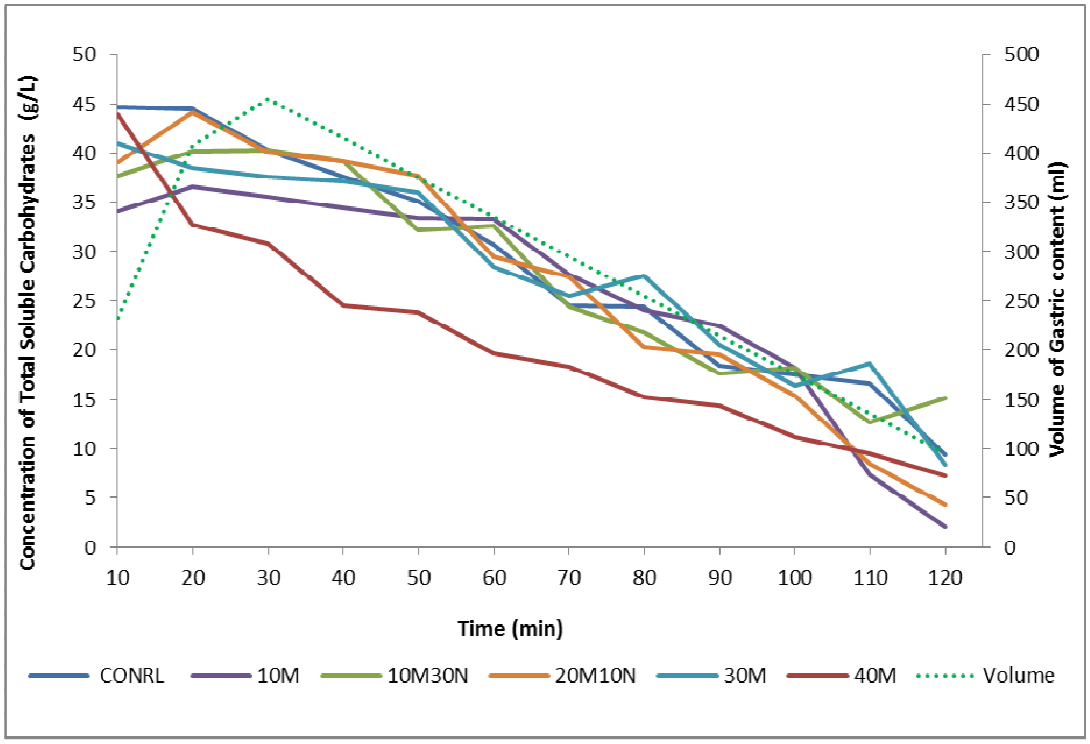


Figure 4.4.2.1. A graph of the concentration of total water-soluble carbohydrates (g/L) over the 2 hours of digestion of each gnocchi sample in the MISST. The volume of the gastric content (mL) is shown with the axis on the right hand side (n=1).

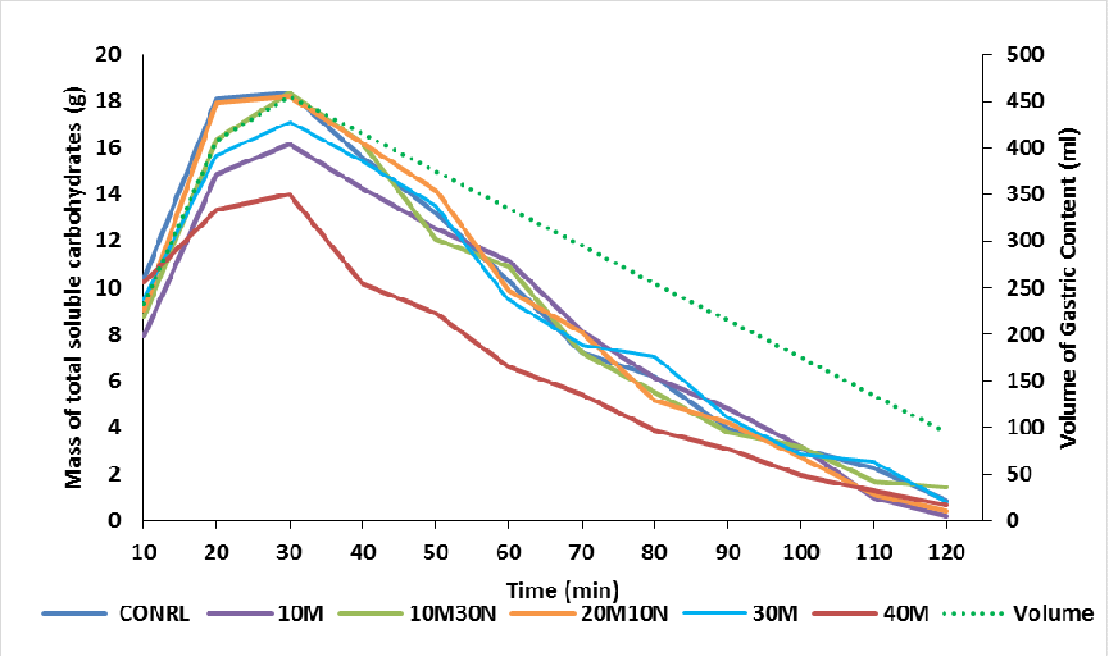


Figure 4.4.2.2 A graph showing the mass of total water soluble carbohydrates (g) over the 2 hours of digestion of each gnocchi sample in the MISST. The volume of the gastric content (mL) is shown on the y-axis on the right hand side (n=1).

4.4.3 Total Digested Starch (Maltodextrins, maltotriose, and maltose and glucose) in collected chyme

Figures 4.4.3.1 and 4.4.3.2 show total concentration and mass of digested starch, respectively. Hydrolysis of starch by alpha amylase is a complex process. Starch in the gnocchi samples were hydrolyzed by alpha amylase in saliva, producing mainly low molecular weight molecules such as maltodextrins, maltotriose, and small portions of maltose and glucose (Robyt, 2008). As described in Section 3.6.6, the digested starch includes all the products produced by the interaction between alpha amylase and starch. The higher the concentration of digested starch; the more the interactions between starch and alpha amylase have occurred.

From Figures 4.4.3.1 and 4.4.3.2, it is evident that the control sample had higher mass of total digested starch than the others from 40 minute till 120 minute. Several factors could have influenced the mass of digested starch in the collected samples. First of all, the amount of total available starch in different samples could release different amounts of digested starch. Secondly, other substances in the samples could have affected the interaction between starch and alpha amylase. For example, protein or lipid outside the starch granule may hinder the starch-amylase reaction (Butterworth et al., 2011). The mechanical homogenization may have assisted release of starch granules to react with alpha amylase.

As shown on Figure 4.4.3.1, the highest initial (10 minutes) concentration of digested starch was 15 g/L for control, while the lowest was 12 g/L for 20M10N. In the first 40 minutes of digestion, the concentration of digested starch remained above 10 g/L. The concentration curves of digested starch for these samples were crossed with one another in the first 40 minutes. This could be due to poor mixing in the stomach reactor. To mimic the movement of human stomach, the stomach compartments was stirred at 150 rpm (Yoo, 2009), which was a poor mixing rate. The chyme collected may not fully represent the concentration of digested in the stomach reactor.

After the first 40 minutes, the control sample released the highest digested starch at all given times. This is likely to be due to the composition of the control sample, which had the highest carbohydrates content (49 %). In contrast, 10 M had the lowest amount of digested starch during the 2 hour digestion period, especially from 30

minutes to 60 minutes. This was unexpected since 10M had the second highest carbohydrates content (49 %) amongst all samples. The rest of samples produced similar concentrations of digested starch from 60 minutes till the end of the digestion period. This pattern is clearer when the concentration is converted to the mass of digested starch (Figure 4.4.3.2). This could be caused by the samples having similar total carbohydrates contents, which are from 43 % to 44 %.

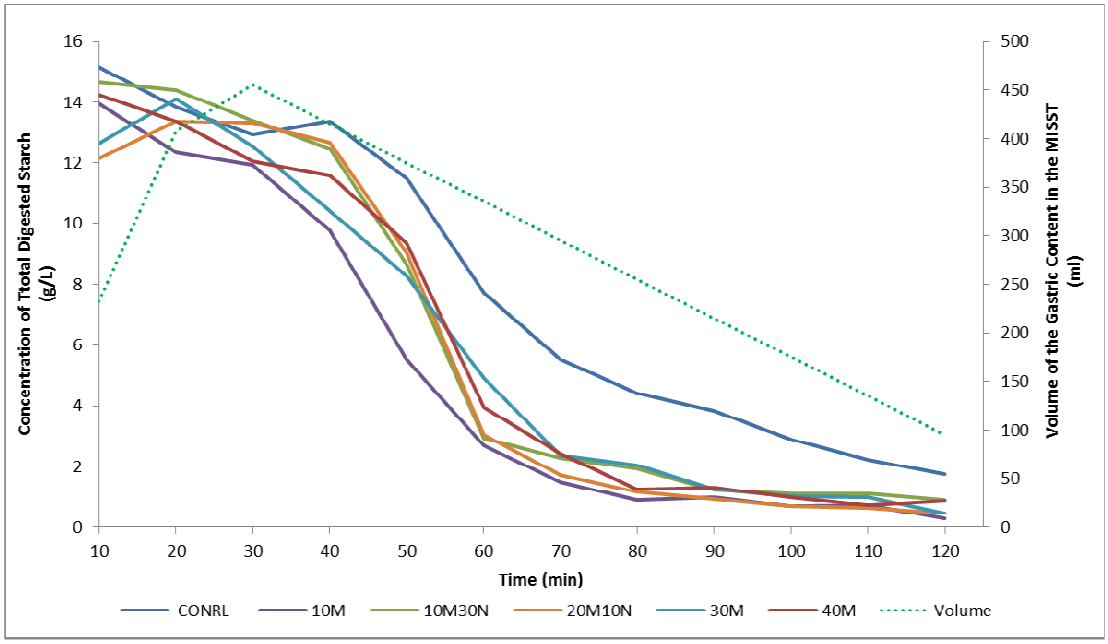


Figure 4.4.3.1. A graph showing the concentration (g/L) of digested starch of different samples over the 2 hours of digestion of each gnocchi sample in the MISST. The volume of the gastric content in the MISST is shown in y-axis on the right side (n=1).

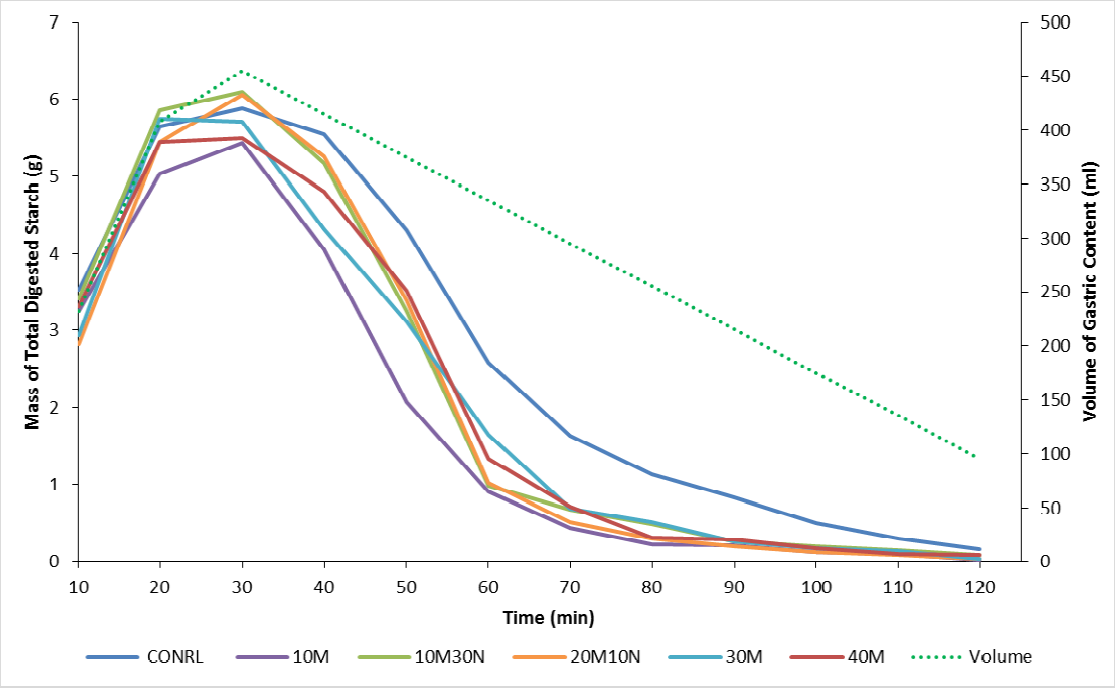


Figure 4.4.3.2. A graph showing the mass of digested starch (g) over the 2 hours of digestion of each gnocchi sample in the MISST. The volume of the gastric content (mL) is shown with the axis on the right hand side (n=1).

4.4.4 Hydrolysis of starch in the collected chyme

Figure 4.4.4 shows the percentage that products from the hydrolysis of starch contribute to total carbohydrates in collected chyme from the MISST. The values were calculated by dividing the mass of digested starch by the mass of total water-soluble carbohydrates, at the same time point. The highest proportion hydrolysed was 47 %, which was achieved by 40M at 40 minute. The hydrolysis of starch should have ceased before 30 minutes due to the inactivation of alpha amylase by the acidic condition in the stomach reactor (Yoo, 2009). In theory, the starch hydrolysis curve should have maximum value at the 30 minute mark and decrease from then on. However, from the results shown in Figure 4.4.4, percentage of hydrolysis started to decrease from 40 or 50 minutes onwards. The feeding period ended before 30 minutes, while the hydrolysis of starch continued to increase up to 50 minutes. When salivary amylase reaches the stomach, the enzyme is deactivated due to the acidic condition of the stomach. However when sufficient amount of starch and oligosaccharides are ingested, triggering buffering effect from the ingested foods, and resulting in a higher pH in the stomach, the salivary amylase can be protected from deactivation (Fried, Abramson, & Meyer, 1987).

From 20 to 50 minutes, the starch hydrolysis proportion of 40M was higher than the other samples. It reached 47 % at 40 minutes. As discussed in section 4.4.1, 40 M achieved the highest pH 3.04. The high buffering capacity of 40M might have protected α-amylase from deactivation (Fried, Abramson & Meyer, 1987).

The starch hydrolysis proportion of control sample had a different pattern from the other samples. It fluctuated between 30 % and 35 % in the first 40 minutes (lower than the other samples), and slowly decreased to 18 % at 80 minutes. The other samples dropped below 9 % at 80 minutes. This is consequence of the slower release of maltodextrins from control compared to other sample. The pH profile of control sample was different from the others as discussed in section 4.4.1. The pH increased slowly to its peak (pH 2.66), which is the lowest peak among all the samples, and decreased slowly. The pH of control gnocchi decreased to 2 at 80 minutes, while for most other samples pH decreased to 2 at 50 minutes. The control sample maintained relatively higher pH than other samples from 40 to 90 minutes, which may partially protect the α-amylase from deactivation (Fried, Abramson & Meyer, 1987). Therefore, the proportion of hydrolysed starch of control sample was higher than the rest of the samples from 40 to 90 minutes. The chyme collected after 100 minutes had a higher digested starch to total soluble starch ratio, which caused an increase in the hydrolysis rate from 100 minutes to 120 minutes.

From the experiments, some of the irregularities in the curve may have resulted from inappropriate mixing and sample collecting methods. The chyme in the stomach reactor was not mixed vigorously or perfectly. Stirring using a magnetic stirrer inside the stomach reactor was maintained at 150 rpm at all times following a protocol by Molly et al (1995). This may reflect the non-homogeneous gastric motility in humans, and hence resulting in non-homogeneous mixing pattern of particles in the stomach. It was difficult to mimic the complex gastric motility with peristaltic pumps and glass based reactors, where everything is assumed to be in a perfect homogenous condition. It is possible that the fluctuations in the collected data may represent a better simulation compared to high speed thorough mixing to produce an ideal setting. In addition to the mixing method, sample collection was also non-ideal. Due to the position of the peristaltic pump tubing to remove chyme out of the stomach reactor, which was near the wall of the glass beaker, it was difficult to achieve all of particles being removed from

the stomach reactor completely. With the low stirring speed, this has caused some gritty particles to be left in the centre of the stomach reactor at the end of the digestion period. These could have contributed to the extra spike in % hydrolysis shown in Figure 4.4.4.

The initial hydrolysis proportion of our gnocchi sample using this model ranged from 30 % to 41 %. The highest hydrolysis proportion of 47 % was achieved by the 40M sample. In past literature, the *in vitro* hydrolysis rate of faba bean (Bello-Pérez et al., 2007), fermented soybean (Kiers et al., 2000) and black beans (Sayago-Ayerdi et al., 2005) have been studied. The *in vitro* hydrolysis rates of fermented soybean ranged from 29 % to 43 % after 15 hours of digestion. However, it was hard to compare our results with those from previous literature as digestion in our model ended in the stomach. Hence the hydrolysis proportion measured in our study was the digested starch (maltodextrins are the major component) vs total starch ratio. Most of the previous research determined the hydrolysis rate by measuring the ratio of glucose vs total carbohydrate (Bello-Pérez et al., 2007; Kiers et al., 2000).

In our model, the chyme in the MISST was stirred at 150 rpm to mimic the gastric motility. The rheological properties of the chyme vary due to different compositions used in making gnocchi samples. Overall, rates of starch hydrolysis were hard to distinguish between these samples. There was no observable effect of meat and navy bean on the starch hydrolysis rate.

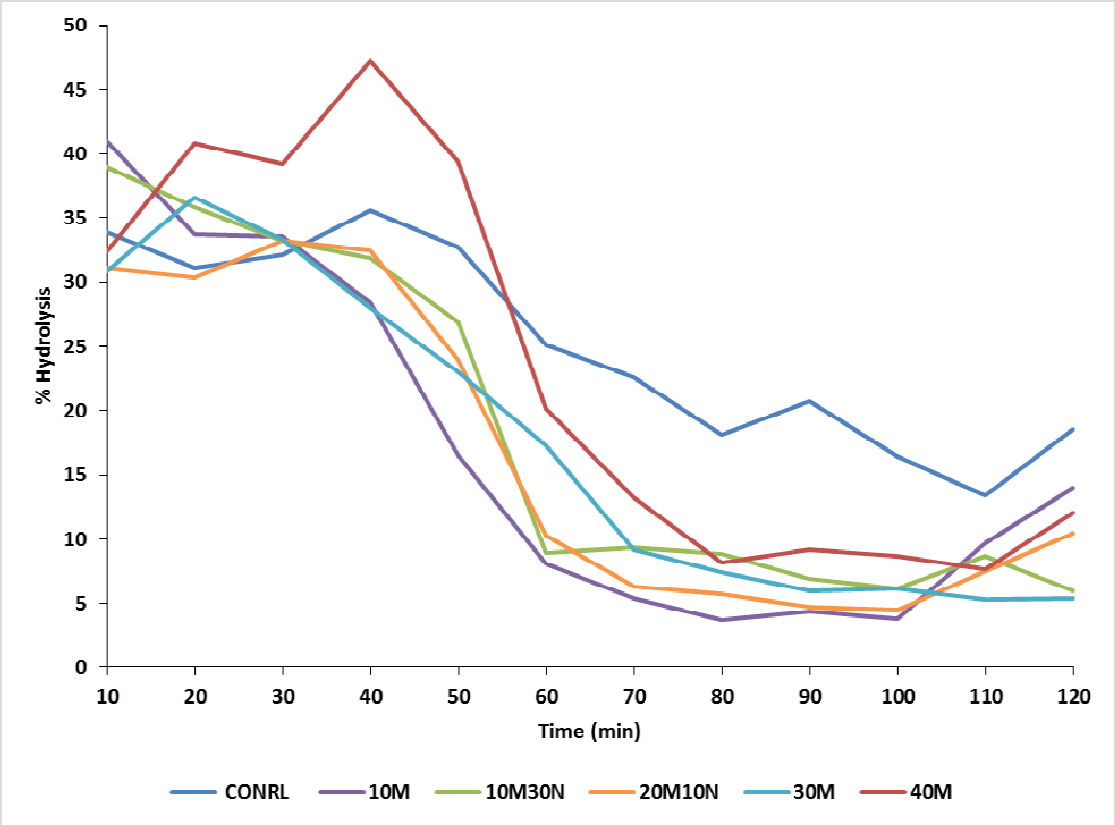


Figure 4.4.4 Hydrolysis rates of starch of gnocchi samples over the 2 hours of digestion of each gnocchi sample in the MISST.

4.4.5 Total water-soluble protein content in collected chyme

The first phase of protein digestion takes place in the stomach. Chief cells, found in the stomach, secrete pepsinogen, the inactive form of pepsin. This enzyme is activated by hydrogen ions, H+. These ions are from the hydrochloric acid secreted by parietal cells that also line the gastric glands. Pepsin (optimum pH 2.5) hydrolyzes at sites involving aromatic amino acids, leucine and acidic amino acids (Briggs & Chandler, 1995).

Meat proteins consist of three types of protein: myofibrillar (structural and contractile, 55%), stromal proteins (10-15%) and sarcoplasmic proteins (30%). Sarcoplasmic proteins are water soluble (Kerry, Kerry, & Ledward, 2002). The homogenization of meat could increase the solubility of myofibrillars protein (Kerry et al., 2002).

The concentration and the mass of total water-soluble protein are shown in Figures 4.4.5.1 and 4.4.5.2. The protein content for these gnocchi samples are 7.8 % (control), 9.9 % (10M), 10.4 % (10M30N), 11.5 % (20M10N), 14.5 % (30M), and 14.7 % (40M). 40M and 30M gnocchi samples contained the highest amount of total water-soluble protein, based on the ingredients used to make gnocchi (Table 3.2.1). Therefore, 40M had the highest values in the concentration and the mass of total water soluble protein at all times, followed by 30M, as shown in Figures 4.4.5.1 and 4.4.5.2. 10M, 20M10N, and 10M30N are not significantly different in terms of protein content ($p > 0.05$) even though they have different protein content values (Table 4.1.1). The protein content in collected chyme for control, 10M and 20M10N shared similar lower values at all given times, which corresponded to the gnocchi protein content. However, 10M30N had higher soluble content at all given times even though the protein content was not significantly different from the previous three samples. This may be attributed to the different proteins in navy bean and meat. After heat treatment, soluble proteins form insoluble aggregates. But aggregation is inhibited in the presence of β-conglycinin in legumes (Carbonaro et al., 1997). This may explain why the cooked gnocchi sample (10M30N) released more soluble proteins.

The pH in the stomach reached 2.5 within 10 minutes and dropped under 2.5 around 30 minutes (Figure 4.4.1). In this period, the pepsin activity is low due to the high pH that its optimum working pH. Pepsin is an endopeptidase. It has a high specificity for cleaving peptide bonds, in which the carboxyl groups are provided by aromatic amino acids such as Phenylalanine, Tyrosine and Tryptophan and Leucine. Pepsin can partially digest 10 - 15 % of dietary protein (Zheng et al, 2013). Zheng et al (2013) found that 9 - 10 % protein in the peanut meal was hydrolyzed by pepsin. Therefore, the protein content we tested in the collected sample likely represents approximately 90 % of the original total protein in the gnocchi. Pancreatic enzymes in the small intestine are required to fully digest proteins.

The concentrations of total soluble protein slightly increased in the first 30 minutes, then decreased gradually till the end except for 30M. At the beginning, the higher total soluble protein concentration is due to the high food bolus to gastric ratio. As mentioned in section 4.1, meat, potato, navy bean and semolina flour contained 25–30 %, 2.5 %, 8.2 % and 13 % protein respectively (FSANZ, 2013). According to Fox

and Condon (1982), 30 % of muscle protein is water-soluble (e.g., myoglobin). Hence myoglobin is one of the dominating water-soluble proteins in the high meat 30M gnocchi sample. The soluble protein content might have increased due to pepsin hydrolyzing insoluble proteins (myofibrillar). The insoluble protein was hydrolyzed to polypeptides and amino acids, which can be detected using the Bradford method. A further study on the characterization of pepsin substrate (e.g., peptides or pigments) can help identify the pattern of protein digestion in the current model.

From 30 minutes till 120 minutes, the concentration and mass of protein decreased gradually for all the samples. The pattern is in line with the total carbohydrate content. The feeding period ceased within 30 minutes, and the food bolus to gastric ratio increased thereafter. Therefore, the diluting effect on the soluble protein content influenced the changes.

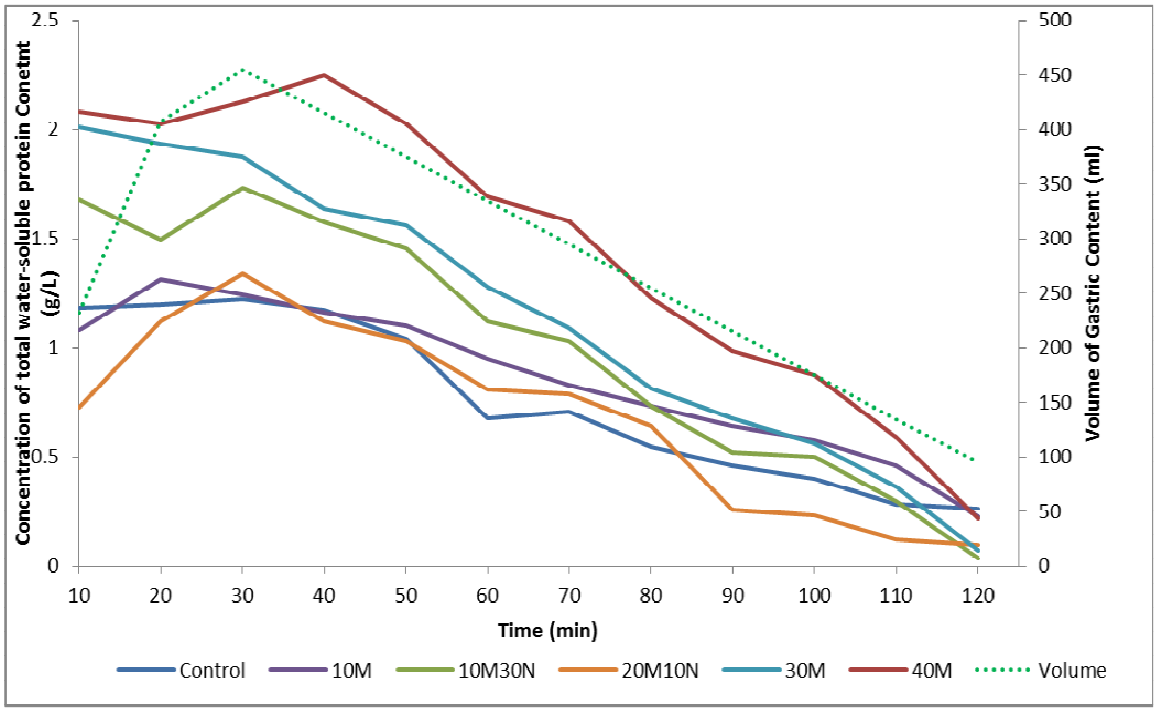


Figure 4.4.5.1. A graph showing the concentration of protein ($g.L^{-1}$) over the 2 hours of digestion of each gnocchi sample in the MISST. The volume of the gastric content (mL) is shown with the axis on the right hand side (n=1).

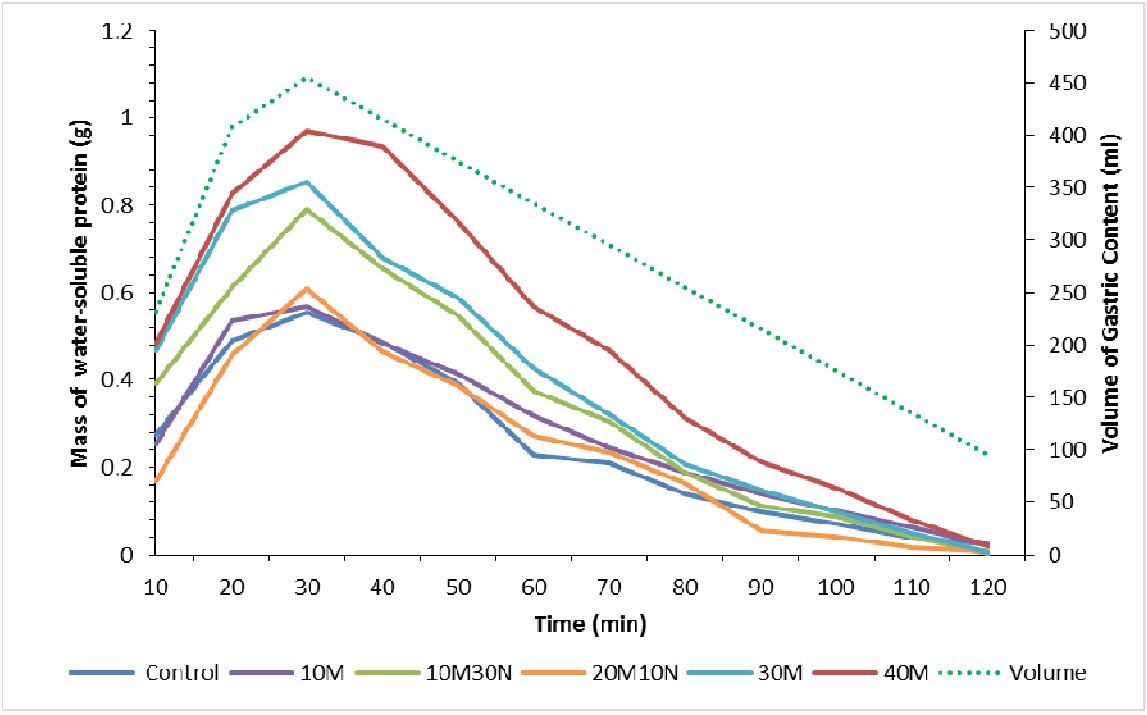


Figure 4.4.5.2. A graph showing the mass of total soluble protein (g) over the 2 hours of digestion of each gnocchi sample in the MISST. The volume of the gastric content (mL) is shown with the axis on the right hand side (n=1).

Chapter 5. Conclusions

In our study, we used gnocchi as a vehicle to deliver meat nutrition, and to explore how meat proteins interact with the macromolecules of other ingredients (e.g., starches and fibre) to affect the physicochemical, sensory and digestibility characteristics. Meat emulsion at four levels (10 %, 20 %, 30 % and 40 % of wet weight, providing 5.7, 11.4, 17.1 and 22.8 % beef, respectively) and ground navy bean at four levels (0 %, 10 %, 20 %, and 30 % of wet weight) were added into gnocchi samples according to a full factorial design. The addition of meat and navy bean increased the protein and fat content. Samples containing increased amount of meat had increased protein content. The addition of navy bean significantly increased the moisture content of cooked gnocchi, which may be related to the higher fiber content of navy bean. Addition of red meat significantly increased the redness of both raw and cooked gnocchi, and decreased their lightness. The hardness, springiness and chewiness of gnocchi increased with the addition of meat and navy bean due to the high protein content in beef and navy bean. Addition of meat and navy bean however did not affect the consumer acceptability of gnocchi.

The pH profiles in the stomach reactor varied amongst the different gnocchi samples. This might be accounted by the different buffering capacity of gnocchi arising from the added ingredients. Apart from the pH, amount of total carbohydrates and the ratio of starch hydrolysis were also measured. Although the collected data have provided insights to the breakdown pattern of carbohydrates in the upper gastrointestinal tract, it should be noted that the process of digestion is only complete when foods pass through the small and the large intestines. Overall, there only minor effects of meat and navy bean on the ratio of starch hydrolysed. There were no differences between the novel formulations of gnocchi and the traditional control formulation in terms of their digestibility, yet their nutrition value was increased. In future, the intestine component, including bile salt and intestinal secretions with pancreatic juice, should be included in our *in vitro* digestion model in order to gain a full picture of digestibility.

The rates of soluble protein released from the stomach reactor reflected the protein content of gnocchi samples. However, a further determination of the polypeptide composition of collected chyme will help to further understand the digestibility of protein in the upper gastrointestinal tract. In addition, it would have been valuable to determine the Protein Digestibility Corrected Amino Acid Score (PDCAAS) of the gnocchi produced as meat protein has a higher score than plant sourced protein (Schaafsma, 2000).

A limitation in this study was not correlating the textural changes associated with adding new ingredients to their effect on the gelatinization of starch. This could be determined by investigating the microstructure of cooked gnocchi using a scanning electron microscope. The degree of starch gelatinization can be visualized, which can better support our findings. Our study used both animal and plant source proteins to enrich a starch food. It is the first investigation of a gnocchi-type food made from combinations of potatoes, navy bean flour, and the proteins and concomitant constituents of red meat. The nutritional values were increased by the addition of new ingredient. In future, with full *in vitro* digestibility test of the gnocchi samples, glucose release and absorption can be determined to determine the GI of our product. There exist a potential that the gnocchi developed in our study could be a low GI food that can be further developed to a functional food.

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Appendices

1. Letter of Ethics Approval from AUTEC

AUTEC
SECRETARIAT

9 May 2013

Nazimah Hamid
Faculty of Health and Environmental Sciences

Dear Nazimah

Re Ethics Application: **13/21 Characterization of sensory properties of a Gnocchi-type food.**

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 8 May 2016.

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 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 8 May 2016;
- 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 8 May 2016 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. If your research is undertaken within a jurisdiction outside New Zealand, you will need to make the arrangements necessary to meet the legal and ethical requirements that apply there.

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,

Madeline Banda
Acting Executive Secretary
Auckland University of Technology Ethics Committee

Cc: Tingting Liu Ifif_love@hotmail.com

2. Instruction and questionnaire for consumer testing

Welcome screen 1

Sensory Evaluation of gnocchi-type food

Next screen

Demographs screen 1

Sensory Evaluation of gnocchi-type food

Please indicate your gender

- ☐ Male
- ☐ Female

Next screen

Demographs screen 2

Sensory Evaluation of gnocchi-type food

Please indicate your age

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

Next screen

Demographs screen 3

Sensory Evaluation of gnocchi-type food

Please indicate if you are allergic or unable to consume the ingredients listed below

- ☐ Gluten
- ☐ Navy bean

* If you have any allergies associated with above mentioned food ingredient, please do not proceed with this test

Next screen

Sensory Evaluation of gnocchi-type food

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

- ☐ Yes
- ☐ No

*If you are a vegetarian or culturally sensitive to the presence of meat in gnocchi, please do not proceed with this test

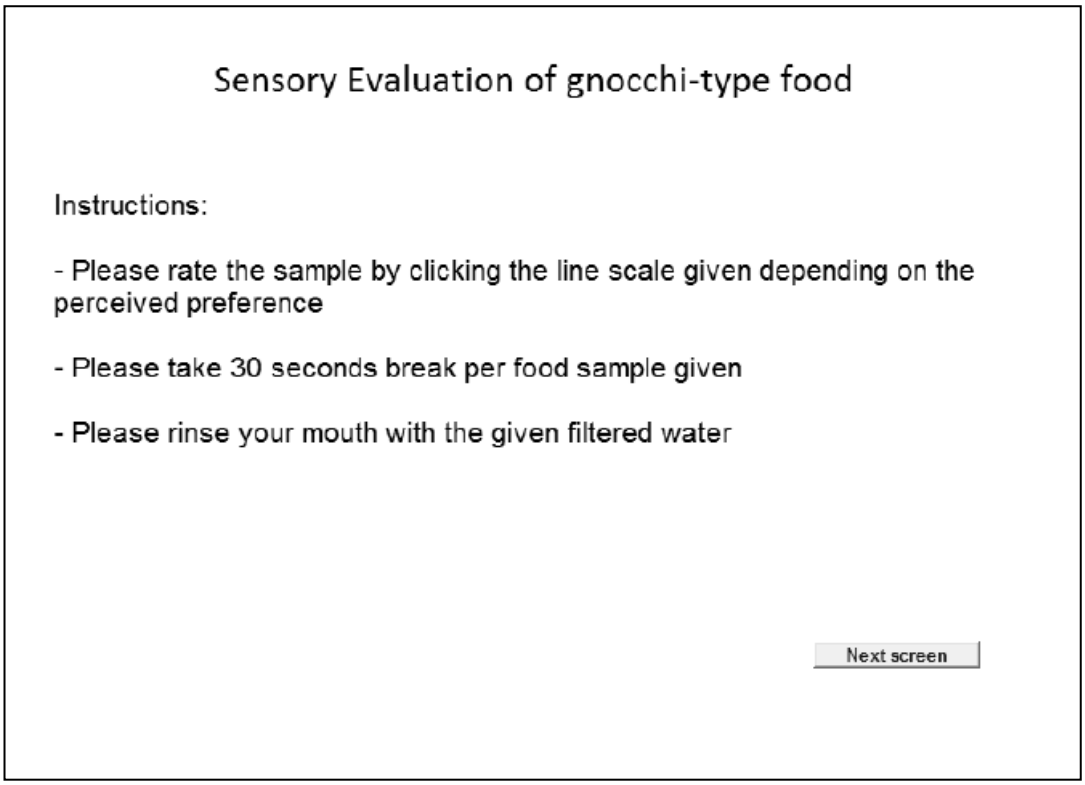
Next screen

Sensory Evaluation of gnocchi-type food

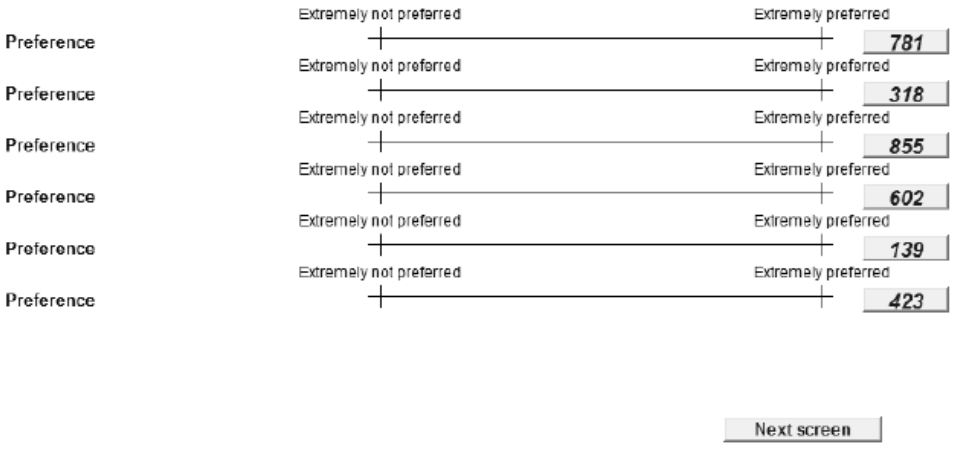
How often do you consume gnocchi?

- ☐ Never
- ☐ Once a month
- ☐ Twice a month
- ☐ Once a week
- ☐ More than once a week

Next screen



Consumer testing of Gnocchi



3. Projective mapping instruction and questionnaire

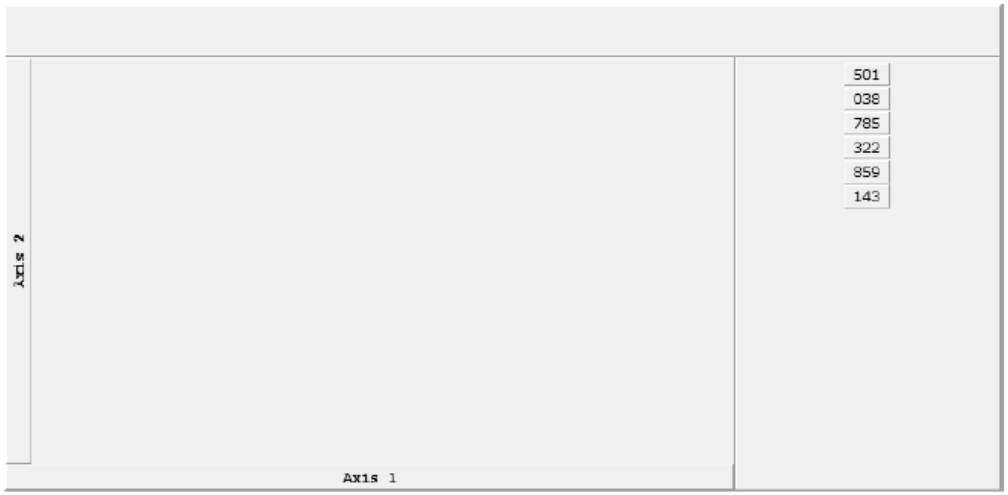
Sensory Evaluation of gnocchi-type food

Instructions:

- Please position those samples in the map according to their similarities
- Please take 30 seconds break per food sample given
- Please rinse your mouth with the given filtered water

Next screen

Sensory Evaluation of gnocchi-type food



Next screen

4. Alpha-amylase activity assay

SIGMA QUALITY CONTROL TEST PROCEDURE

Product

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

PRINCIPLE:

$$\text{Starch} + H_2O \xrightarrow{\alpha\text{-Amylase}} \text{Reducing Groups (Maltose)}$$

CONDITIONS: T = 20 °C, pH = 6.9, A_{540nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 20 mM Sodium Phosphate Buffer with 6.7 mM Sodium Chloride, pH 6.9 at 20 °C
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751, and Sodium Chloride, Sigma Prod. No. S-9625. Adjust to pH 6.9 at 20 °C with 1 M NaOH.)

B. 1.0% (w/v) Soluble Starch Solution (Starch)
(Prepare 25 ml in Reagent A using Starch Potato Soluble, Sigma Prod. No. S-2630. Facilitate solubilization by heating the starch solution in a glass beaker directly on a heating/stir plate using constant stirring. Bring to boil and maintain the solution at this temperature for 15 minutes. Allow the starch solution to cool to room temperature with stirring. Return the starch solution to its original volume (25 ml) by the addition of water and dispense samples for assay while stirring.)

C. Sodium Potassium Tartrate Solution
(Dissolve 12.0 grams of Sodium Potassium Tartrate, Tetrahydrate, Sigma Prod. No. S-2377, in 8.0 ml of 2 M NaOH. Heat directly on a heating/stir plate using constant stirring to dissolve. DO NOT BOIL.)

D. 96 mM 3,5-Dinitrosalicylic Acid Solution
(Prepare 20 ml in deionized water using 3,5-Dinitrosalicylic Acid, Sigma Prod. No. D-0550. Heat directly on a heating/stir plate using constant stirring to dissolve. DO NOT BOIL.)

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.

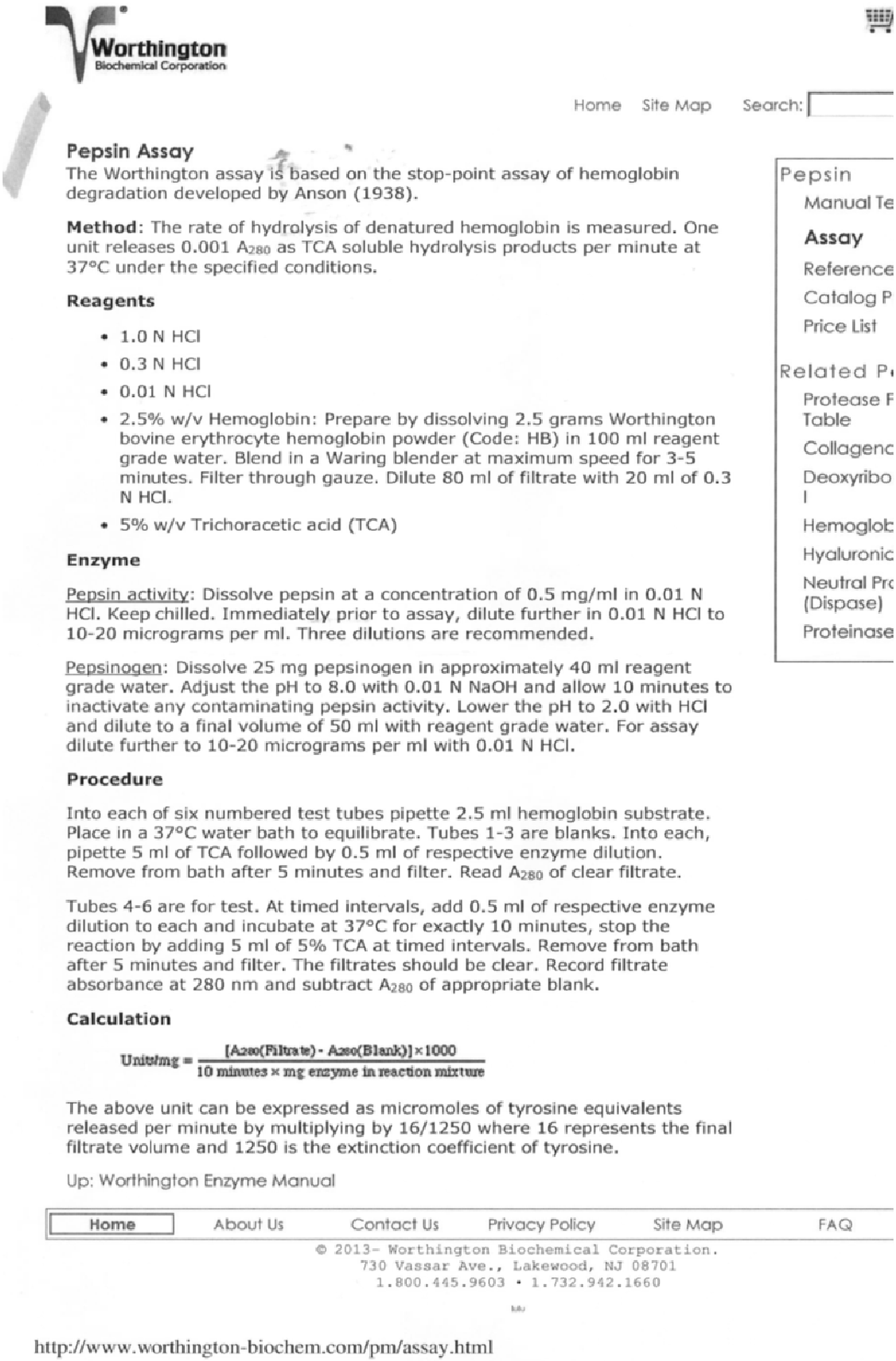
REFERENCE:

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.

5. Pepsin assay (Worthington assay: http://www.worthington-biochem.com/pm/assay.html)



Worthington Biochemical Corporation

Home Site Map Search:

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
Reference
Catalog P
Price List
Related P
Protease F Table
Collagenc
Deoxyribo I
Hemoglob
Hyaluronic
Neutral Pr (Dispase)
Proteinase

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730 Vassar Ave., Lakewood, NJ 08701
1.800.445.9603 • 1.732.942.1660

http://www.worthington-biochem.com/pm/assay.html

6. Total Maltodextrin assay procedure Kit

6.1Principle (AOAC, 2000):

(1) Maltodextrins $\xrightarrow{\text{AMG (Amyloglucosidase)}}$ D-glucose;

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

(2) D-Glucose + O_2 + H_2O $\xrightarrow{\text{glucose oxidase}}$ D-gluconate + H_2O;

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.

(3) 2 H_2O_2 + p-hydroxybenzoic acid + 4 aminonatipyrine
$\xrightarrow{\text{peroxidase}}$ quinoneimine dye + 4 H_2O;

6.2 Kit

The kits contain the full assay method plus:

Bottle 1: Thermostable α-amylase (10 mL, 3,000 U/mL on Ceralpha reagent* at pH 6.5 and 40°C or 1600 U/mL on Ceralpha reagent at pH 5.0 and 40°C). Stable for > 4 years at 4°C.

Bottle 2: Amyloglucosidase (10 mL, 3300 U/mL on soluble starch (or 200 U/mL on p-nitrophenyl β-maltoside*) at pH 4.5 and 40°C. Stable for > 4 years at 4°C.

Bottle 3: 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 4: GOPOD Reagent Enzymes. Glucose oxidase plus peroxidase and 4-aminoantipyrine. Freeze-dried powder. Stable for > 5 years at -20°C. Bottle 5: D-Glucose standard solution (5 mL, 1.0 mg/mL) in 0.2 % (w/v) benzoic acid. Stable for > 4 years at room temperature.

Bottle 6: Standardised regular maize starch control. Starch content shown on vial label. Stable for > 4 years at room temperature.

6.3 Preparation of reagent solutions/suspensions

Reagents 1 (NOT SUPPLIED): Sodium acetate buffer (100 mM, pH 5.0) plus calcium chloride (5 mM).

Add 5.8 mL of glacial acetic acid (1.05 g/mL) to 900 mL of distilled water. Adjust the pH to 5.0 by the addition of 1 M (4 g/100 mL) sodium hydroxide solution (approx. 30 mL is required). Stable for approximately 2 months at 4°C. Add 0.74 g of calcium chloride dihydrate and dissolve. Adjust the volume to 1 litre and store the buffer at 4°C. Stable for > 6 months at 4°C. The stability of this buffer can be increased by adding sodium azide (0.2 g of sodium azide/L buffer). Stable for approximately 2 years at room temperature.

Solution 1. Dilute 1.0 mL of the contents of bottle 1 to 30 mL with Reagent 1 (100 mM sodium acetate buffer, pH 5.0; not supplied). Store the diluted enzyme frozen between use. Divide into appropriately sized aliquots and store in polypropylene tubes at -20°C between use and keep cool during use if possible. Stable for > 3 years at -20°C.

Solution 2. Dilute AMG (bottle 2) 50 fold in Reagent 1 . This solution is viscous and thus should be dispensed with a positive displacement dispenser e.g. Eppendorf Multipette® with 5.0 mL Combitip® (to dispense 0.1 mL aliquots). Stable for > 3 years at 4°C

Solution 3. Dilute the contents of bottle 3 (GOPOD Reagent Buffer) to 1 L with distilled water. Use immediately.

Solution 4. Dissolve the contents of bottle 4 in 20 mL of solution 3 and quantitatively transfer this to the bottle containing the remainder of solution 3. Cover this bottle with aluminium foil to protect the enclosed reagent from light. This is Glucose Determination Reagent (GOPOD Reagent). Stable for ~ 3 months at 2-5°C or > 12 months at - 20°C. If this reagent is to be stored in the frozen state, preferably it should be divided into aliquots that should be freeze/thawed only once during use. When the

reagent is freshly prepared it may be light yellow or light pink in colour. It will develop a stronger pink colour over 2-3 months at 4°C. The absorbance of this solution should be less than 0.05 when read against distilled water. Solutions Use the contents of bottles 5 and 6 as supplied.

Solutions 5 & 6. Use the contents of bottles 5 and 6 as supplied. Stable for > 5 years at room temperature.

7. The RV coefficient between projective maps and multifactor analysis

Table 3.2.1 RV coefficient between projective maps and multifactor analysis (MFA) for week one where 81% of panellists scored >0.5. Panellists are identified as N1 to N11. Values shown in red indicate poor fit with MFA.

	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11
N1	1.000	0.541	0.306	0.348	0.718	0.212	0.417	0.615	0.312	0.433	0.398
N2	0.541	1.000	0.368	0.084	0.408	0.174	0.236	0.280	0.360	0.327	0.563
N3	0.306	0.368	1.000	0.146	0.183	0.025	0.029	0.335	0.371	0.084	0.394
N4	0.348	0.084	0.146	1.000	0.249	0.379	0.297	0.035	0.222	0.139	0.141
N5	0.718	0.408	0.183	0.249	1.000	0.162	0.302	0.511	0.364	0.550	0.613
N6	0.212	0.174	0.025	0.379	0.162	1.000	0.752	0.031	0.130	0.327	0.426
N7	0.417	0.236	0.029	0.297	0.302	0.752	1.000	0.012	0.487	0.245	0.307
N8	0.615	0.280	0.335	0.035	0.511	0.031	0.012	1.000	0.063	0.206	0.407
N9	0.312	0.360	0.371	0.222	0.364	0.130	0.487	0.063	1.000	0.111	0.351
N10	0.433	0.327	0.084	0.139	0.550	0.327	0.245	0.206	0.111	1.000	0.559
N11	0.398	0.563	0.394	0.141	0.613	0.426	0.307	0.407	0.351	0.559	1.000
MFA	0.798	0.652	0.475	0.441	0.772	0.516	0.594	0.526	0.563	0.596	0.776

Table 3.2.2 RV coefficient between projective maps and multifactor analysis (MFA) for week two where all the panellists scored >0.5.

	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11
N1	1.000	0.354	0.609	0.068	0.610	0.237	0.042	0.359	0.229	0.538	0.365

N2	0.354	1.000	0.479	0.455	0.518	0.336	0.559	0.256	0.694	0.201	0.540
N3	0.609	0.479	1.000	0.183	0.475	0.336	0.295	0.482	0.383	0.445	0.754
N4	0.068	0.455	0.183	1.000	0.259	0.398	0.317	0.293	0.504	0.172	0.325
N5	0.610	0.518	0.475	0.259	1.000	0.187	0.348	0.407	0.450	0.625	0.470
N6	0.237	0.336	0.336	0.398	0.187	1.000	0.017	0.379	0.476	0.144	0.367
N7	0.042	0.559	0.295	0.317	0.348	0.017	1.000	0.293	0.461	0.184	0.468
N8	0.359	0.256	0.482	0.293	0.407	0.379	0.293	1.000	0.392	0.701	0.579
N9	0.229	0.694	0.383	0.504	0.450	0.476	0.461	0.392	1.000	0.271	0.568
N10	0.538	0.201	0.445	0.172	0.625	0.144	0.184	0.701	0.271	1.000	0.406
N11	0.365	0.540	0.754	0.325	0.470	0.367	0.468	0.579	0.568	0.406	1.000
MFA	0.606	0.748	0.739	0.539	0.732	0.550	0.532	0.689	0.752	0.626	0.796

Table 3.2.3 RV coefficient between projective maps and multifactor analysis (MFA) for week three where 90% of panellists scored >0.5.

	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11
N1	1.000	0.360	0.072	0.499	0.422	0.596	0.067	0.281	0.309	0.321	0.182
N2	0.360	1.000	0.038	0.261	0.535	0.195	0.080	0.123	0.263	0.478	0.271
N3	0.072	0.038	1.000	0.028	0.505	0.046	0.687	0.751	0.290	0.009	0.588
N4	0.499	0.261	0.028	1.000	0.287	0.589	0.018	0.241	0.586	0.131	0.293
N5	0.422	0.535	0.505	0.287	1.000	0.290	0.508	0.504	0.323	0.589	0.374
N6	0.596	0.195	0.046	0.589	0.290	1.000	0.107	0.196	0.319	0.226	0.192
N7	0.067	0.080	0.687	0.018	0.508	0.107	1.000	0.754	0.364	0.154	0.431
N8	0.281	0.123	0.751	0.241	0.504	0.196	0.754	1.000	0.470	0.064	0.391
N9	0.309	0.263	0.290	0.586	0.323	0.319	0.364	0.470	1.000	0.008	0.432
N10	0.321	0.478	0.009	0.131	0.589	0.226	0.154	0.064	0.008	1.000	0.017

N11	0.182	0.271	0.588	0.293	0.374	0.192	0.431	0.391	0.432	0.017	1.000
MFA	0.623	0.553	0.579	0.593	0.786	0.558	0.598	0.690	0.667	0.433	0.635