## Physicochemical and Sensory Characterization of Spaghetti with Added Meat and Navy Bean

By

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A Thesis

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## Physicochemical and Sensory Characterization of Spaghetti with Added Meat and Navy Bean

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## **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 spaghetti with added meat and navy bean', 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

Spaghetti is a high carbohydrate food with little protein. There is an increasing demand by consumers to have more protein in their diets. Protein is generally viewed positively in promoting general health, weight management and maintaining lean muscle mass. In fact nowadays, high protein claims are appearing on everything from breakfast cereals to chilled food products. In this study, we developed spaghetti with added proteins from lamb meat and fiber rich navy beans to produce a product with higher nutritional value. The study aims to characterize and evaluate physicochemical and sensory properties of the reformulated spaghetti containing lamb meat and navy bean. Our hypothesis is that addition of meat and navy bean will improve the nutritional value of pasta with improved physicochemical and sensory properties as well. In our study, spaghetti formulations with added meat emulsion (40g, 50g, 60g and 70g) containing (28%, 36%, 43% and 50%) actual meat and navy bean powder (7%, 14% and 21%) were developed. All samples were then evaluated for their physical, nutritional and sensory properties.

Spaghetti samples with 36%, 43% and 50% meat had significantly higher fat and protein content. Spaghetti containing meat and bean had a noticeable impact on the cooking quality of pasta with increased cooked weight, lower cooking time, higher swelling index and higher cooking loss. Increased incorporation of meat significantly increased the redness and decreased the lightness of cooked spaghetti samples. Addition of meat significantly increased the tensile strength, extensibility and elasticity of the spaghetti samples as compared to addition of bean. Samples were then subjected to consumer testing and sensory projective mapping. All samples were significantly preferred in terms of overall liking, odor, texture and flavor except for samples containing high bean content (14% and 21%) and the highest meat sample (50% M). The product and attribute maps obtained from projective mapping separated the commercial samples from the other samples in terms of texture and taste. Spaghetti prepared with 36% meat, 50% meat, 43% meat 7% bean, and 50% meat 7% bean were associated with meaty taste, smooth mouth feel, soft, chewy and good texture.

Our results showed that spaghetti with added meat and bean was an ideal vehicle to deliver good nutrition. In fact some of the reformulated spaghetti containing a combination of meat and bean had improved physical, nutritional and sensory properties. This high protein spaghetti can be used as go-to meal for health conscious people. The reformulated spaghetti may also address the nutrition concerns for the aging populations as well as children with positive implications on human health.

### **Chapter 1: Introduction**

#### **1.1 Motivation**

Pasta, a traditional food product with origins dating back to the first century BC (Agnesi, 1996), is a staple food in many countries. Consumption of pasta has increased because of their sensory attributes, low cost, as well as ease of preparation and transportation (Fradique et al., 2010). It also has a relatively long shelf life if stored properly, is easy to cook and can be used in the preparation of a wide variety of meals (Marchylo & Dexter, 2001).

Pasta is made from unleavened dough, mostly of durum wheat semolina (Feillet & Dexter, 1996), water and sometimes eggs (Bashir, Aeri, & Masoodi, 2012). Hence it is a multicomponent system consisting of macromolecules such as proteins, carbohydrates and lipids (Kill & Turnbull, 2001). It contains proteins (11-15%) but is deficient in some of the essential amino acids such as lysine and threonine (Bashir et al., 2012). There have been numbers of studies conducted on pasta products aimed at increasing their nutritional value (Fuad & Prabhasankar, 2010). Some of the studies used proteins rich in lysine, such as soy protein (J. Collins & Pangloli, 1997; Ugarcic-Hardi, Hackenberger, Subaric, & Hardi, 2003), fish protein concentrate (Kwee, Sidwell, Wiley, & Hammerle, 1967; Sidwell, Stillings, & Knobl, 1970), legumes (Bahnassey & Khan, 1986; Bahnassey, Khan, & Harrold, 1986), corn distillers' dried grains (Y. Wu, Youngs, Warner, & Bookwalter, 1987), and corn gluten meal (Y. V. Wu, Hareland, & Warner, 2001). So far, there has been no report of using red meat to increase the nutritional value of pasta although pasta sauce that accompanies it often contains meat. For this study, red meat and navy beans will be used to produce high protein pasta.

Proteins from meat provide all the essential amino acids (lysine, threonine, tryptophan, leucine, isoleucine, valine) and have no limiting amino acids (Williams, 2007). It also acts as a rich source of micronutrients such as iron, selenium, vitamins A, B12 and folic acid (Biesalski, 2005). Navy bean also known as haricot bean is a variety of common bean (*Phaseolus vulgaris*). It is a rich and inexpensive source of proteins (20-25%) and carbohydrates (50-60%). In addition, consumption of navy bean may increase blood sugar gradually resulting in reduced glycaemic postprandial responses (Gallegos-Infante, Bello-Perez, Rocha-Guzman, Gonzalez-Laredo, & Avila-Ontiveros, 2010). Incorporation of both meat and navy bean will not only increase the nutritional value of spaghetti but also potentially result in a lower GI product. Hence the main objective of this research is to increase the

nutritional value of spaghetti by adding meat and navy bean as additional protein sources. Physical and chemical characteristics of the enriched spaghetti containing meat and navy bean was determined followed by sensory analysis of the reformulated pasta. The hypothesis is that addition of red meat and navy bean will improve the nutritional value of pasta with improved physicochemical and sensory properties.

#### **1.2 Structure of the thesis**

This thesis is arranged in 5 chapters. After the introductory chapter, chapter 2 presents a detailed literature review for this thesis detailing physical, chemical, and sensory studies on fortified pasta. The research approach used in this thesis is explained in chapter three, together with the experimental design and data analysis methods. The justification and implementation of this research is also detailed in this chapter. The main contribution of the thesis is presented in chapter four, where the findings are presented and discussed. The final chapter of this thesis, chapter five, summarizes the main results, answering the research questions, and suggests some of avenues of future related research.

### **Chapter 2: Literature Review**

#### 2.1 Pasta

Pasta is the most commonly consumed product made from durum wheat. The term 'pasta' is generally used to describe paste products fitting the Italian style of extruded foods such as spaghetti or lasagne, and is usually distinguished from the oriental style of sheeted and cut foods called 'noodles', commonly made from wheat other than durum (Dick & Matsuo, 1988). Pasta is traditionally made from extruded dough, durum wheat semolina flour (Feillet & Dexter, 1996), water and sometimes eggs (Bashir et al., 2012). Pasta made from semolina is a good source of complex carbohydrates and contains relatively high levels of resistant starch and is low in fat (Seibel, 1996). Pasta with ideal physical and sensory quality is characterized by strength and elasticity in the dough form, high tensile strength in the dried form and cooking quality. Cooking quality is determined by two parameters: Viscoelastic behavior (firmness after cooking), minimal cooking losses, minimal stickiness and reasonable firmness after cooking (Dexter, Matsuo, & Morgan, 1983; Feillet, 1984; Raina, Singh, Bawa, & Saxena, 2005; Sissons, Soh, & Turner, 2007).

Traditional pasta made from durum wheat semolina flour has the best quality because of its high gluten content. According to the (Council, 2011), durum wheat has a higher protein and gluten content than other types of wheat. The endosperm of the milled durum wheat is ground into a product called semolina, which is then mixed with water to form thick dough that is forced through holes of different shapes to make different types of pasta. The natural rich yellow color of the durum endosperm gives pasta its golden color (WHEAT & GLUTENS, 1996). Wheat gluten is made of glutenin and gliadin proteins, which together have extremely good binding properties and are capable of forming a firm and elastic network with starch and water during dough formation (Sissons et al., 2007).

Although durum wheat is ideal for making pasta, its protein content is substandard as it is deficient in lysine, an essential amino acid required in the diet for normal body function (Howard, Hung, & McWatters, 2011). Since pasta is widely consumed in many parts of the world, there are several research studies have been carried out to improve the protein quality in pasta and pasta products. Over the past few decades, wheat pasta has been successfully fortified with different supplements. Some of them are rapeseed (Matsuo, Bradley, & Irvine, 1972), fish protein concentrate (Matsuo et al., 1972), soy flour (Matsuo et al., 1972; Shogren, Hareland, & Wu, 2006), pea flour (Nielsen, Sumner, & Whalley, 1980), isolated L-

lysine(Molina, Gudiel, Baten, & Bressani, 1982), navy bean flour (Bahnassey & Khan, 1986), pigeon pea flour (Torres, Frias, Granito, & Vidal-Valverde, 2007), seaweed (Prabhasankar, Ganesan, & Bhaskar, 2009a; Prabhasankar et al., 2009b), lupin flour (Torres et al., 2007), whey protein concentrate (Prabhasankar, Rajiv, Indrani, & Rao, 2007) and tubers (Jyothi, Renjusha, Gourikutty, Moothandassery Sankarankutty, & Subramoney Narayana, 2011).

#### 2.1.1 Value added pasta

The addition of ingredients containing protein in pasta is to cater for health- conscious consumers who prefer having a product rich in protein, healthy lipids and other health benefits (S. U. Kadam & Prabhasankar, 2012). According to nutritionists, pasta is highly digestible, providing significant amounts of complex carbohydrates, low sodium and total fat (Douglass & Matthews, 1982). However, it is low in fiber, minerals and essential fatty acids (Prabhasankar et al., 2009b). Pasta is considered as an energy source due to its complex carbohydrate content with low GI due to the structure of the starch granules in durum wheat and the effects of extrusion processing. Due to these nutritional advantages along with the appeal of pasta among consumers, pasta is an ideal vehicle for nutraceuticals such as vitamins or polyunsaturated fatty acids. In fact, pasta was one of the first foods permitted by the US FDA in the 1940s for vitamin and iron enrichment (Fradique et al., 2010). There are many studies aimed at increasing its nutritional value (Fuad & Prabhasankar, 2010).

Many studies have incorporated ingredients such as legumes, cereals, marine foods and supplements such as inulin to boost the nutritional value of pasta. Table 2.1.1 below summarizes the studies that have used these different ingredients. Commercial pasta is usually made using durum wheat semolina, flour, water, egg, and oil. Enriched pasta is made the same way with the addition of extra supplements used as enrichment agents. Some of the ingredients used in improving pasta quality will be discussed in detail.

Predominant characteristics that define the quality of pasta products are related to appearance and textural factors such as color, uniformity of appearance, structural strength and integrity, absence of a sticky surface and '*al dente*' eating properties as characterized by high degrees of firmness and elasticity (Antognelli, 1980). Accordingly, the table below summarizes physicochemical properties (such as color, texture, and cooking quality) and sensory characteristics of enriched pasta.

Legumes									
Pasta type	Additional supplement	Level % (w/w)	Brightness	Texture	Cooking quality	Nutritional properties	Significant results	Sensory properties	References
Spaghetti	Chickpea flour	0,10,15,20,25,3 0	Brightness decreased	Improved stickiness and retained firmness	Optimum cooking time 10.5mins. Cooking loss decreased	High protein and amino acid content	Gluten content important- firmness, protein- polysaccharide- cooking loss	Acceptable to consumers	Wood, 2009
Spaghetti	Corn-broad beans	70% corn flour 30% broad bean	NA	Stickiness and firmness decreased	Optimum cooking time between 8-13 mins, cooking loss increased, less water absorption	Increased protein and dietary fiber content	Gelatinization point was obtained, beyond which product quality declines	Acceptable scores	Gimenez et al., 2012
Pasta or spaghetti	Chickpea and defatted soy flour	(0,0), (10,16), (14,10), (18,14)	NA	Increased stiffness	Cooking time increased, water absorption increased	High protein, ash, and dietary fiber content keeping fat at optimum level	Better quality pasta with increasing nutritional value	10%CPF and 6% DSF was highly acceptable	Bashir et al., 2012
Spaghetti	Green, yellow pea, lentil and chickpea flour	5,10,15,20,30	Brightness decreased, color intensity increased	Firmness increased, elasticity decreased	Cooking time increased not significantly, cooked weight changed, cooking loss increased TIA/g reduced	High protein	Enhanced protein and nutritional value due to cereal and legume amino acid pattern	Control was preferred but there were a few preference for pasta with 15% lentil and 20% chickpea	Zhao et al., 2005
Pasta or spaghetti	Chickpea flour	80:20, 60:40	NA	NA	NA	Increased protein, ash, lipid, and dietary fibre. Increased RS	Chickpea flour, an alternative for people with special metabolic requirements.	NA	Osorio-Diaz et al., 2008
Spaghetti	Defatted soy flour	025,35,50	NA	NS	NA	Increased protein, lysine and ash content and other essential amino acids	Improved nutritional value	35% enriched pasta indicated no significant difference in flavour and texture compared to control	Shogren, et al., 2006

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

Pasta or spaghetti	Plant proteins (mushroom powder, Bengal gram flour, defatted soy flour)	0,6,9,12,15,18,2 1	NA	Increased volume expansion	Increased cooking time, water absorption, cooking loss	High protein and fiber contents, optimum levels of fat	Improved nutritional value	Highly acceptable	Kaur et al., 2013
Lasagne	Chickpea flour	5,10,20,30,50	NA	5 or 10% chickpea flour retains firmness, low breakage; Diluted gluten fraction- less firm pasta	Increased swelling index	High protein; decreased moisture content	Pasta with chickpea flour meets the specification of pasta products in terms of firmness, cooking quality	5% and 10% enriched lasgane was acceptable	Sabanis, Makri & Doxastakis, 2006
Spaghetti	Lupin protein	0-20	Brightness decreased	Diluted gluten strength- weak structure, increased firmness	Decreased cooked weight, increased cooking loss	Increased lysine content	Improved nutritional characteristics	NA	Doxastakis et al., 2007
Spaghetti	Common bean (Phaseolus vulgaris L.)	15,30,45	NA	NA	Decreased cooking time, increased cooking loss	High protein; low hydrolysis rate	Positive effect on human health	NA	Gallegos- Infante et al., 2010
Spaghetti	Legume flour ( mung bean, soya bean, red lentil or chickpea)	10	NS	Hardness and adhesiveness increased	Increased cooking time; cooking loss and swelling index did not change	No impact on glycemic index	No effect on cooking quality or increase in glycemic index	NA	Chillo et al., 2010
Pasta	Germinated pigeon pea	5,8,10	NA	NA	Decreased cooking time; increased cooking loss and swelling index	Improved protein, fiber, mineral and vitamin content	Excellent ingredient to increase the nutritional value of pasta	Similar acceptability with control	Torres et al., 2007
Spaghetti	Split bean and faba bean	35	Brightness decreased	Increased hardness	Decreased cooking time and swelling index; increased cooking loss	Increased protein, vitamins and minerals content	Nutritionally enhanced spaghetti was produced	Higher hardness, elasticity and fracturability	Petitot et al., 2009
Pasta	Peanut flour	30,40,50	Brightness decreased	NS	Increased cooking loss	NA	30% peanut flour pasta was preferred	30% received higher acceptability	Howard, Hung, & McWatters, 2011

Spaghetti	Pea flour and air-classified pea protein concentrate	33,20	Brightness increased	Reduced tenderness index; improved compressibility and recovery	Decreased cooking time and water absorption capacity; increased cooking loss	High protein content	Improved nutritional value	RPF and RPPC were given a poor flavour rating	Nielsen et al., 1980
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Cereals										
Spaghetti	Corn gluten meal	5,10	Brightness decreased	Firmness decreased, dough strength decreased	Cooked weight increased, cooking loss increased	High protein	Improved nutritional value	Overall acceptability was in terms of flavour for pasta with 5% water/ethanol – washed corn gluten meal	Wu, Hareland, & Warner 2001	
Pasta	Oat, teff, wheat flour	69.6,62.8,64.7	NS	Firmness was almost comparable	NA	high protein, fiber, ash and mineral content; reduced GI	High energy food	Spaghetti made with teff showed low sensory quality	Hager et al., 2013	
Pasta or spaghetti	Cereal brans	0,5,10,15,20,25	Brightness decreased	NA	Cooking time decreased; increased water absorbing capacity; increased cooking loss	High fiber and protein content	Increased nutritional benefits	15% enriched pasta scored highest acceptability	Kaur et al., 2012	

Marine foods	5								
Pasta or spaghetti	Wakame (U. <i>pinnatifida</i> ) and surimi	1.0, 2.5, 5.0	NA	Enhanced gluten network	NA	Increased protein, fat, ash and fiber contents	Increased fucoxanthin and fucosterol	Surimi pasta was accepted in moderate concentrations	Kadam, & Prabhasankar, 2010
Pasta or spaghetti	Shrimp meat	10,20,30	Brightness decreased	Increased shear force to break pasta	Increased cooking loss	High protein, fat and ash content	Improved nutritional value	20% SM pasta had a better overall score	Kadam & Prabhasankar, 2012
Pasta	Blue-green algae	1,2,3	Brightness increased	Firmness increased	Increased cooking loss; decreased swelling index	High protein content	Increasing antioxidant activity	Higher taste and acceptance scores	Zouari et al., 2011
Pasta products	Microalgae (Chlorella vulgaris and Spirulina maxima)	0.5, 1.0, 2.0	Brightness increased	Hardness decreased	Increased cooking time and swelling index	Higher protein, total fat and ash content	Antioxidant	Highly acceptable	Fradique et al., 2010
Pasta or spaghetti	Wakame (Japenese seaweed)	100:0, 95:5:0, 90:10, 80:20, 70:30	NA	NA	Cooked weight increased, cooking loss increased	Essential amino acid content increased	Increased nutritional value in terms of amino acids	10% enriched pasta had a better quality score	Prabhasankar et al., 2009b
Pasta or spaghetti	Sweet potato	40,50,60,70	NA	NA	Optimum cooking time (2-4mins); cooking loss and swelling index increased	High protein, lysine and threonine contents, high essential amino acid index, high hydrolysis, decreased NSI	WPC-pasta may be suitable for type 2 diabetic patients due to low glycemic index	NA	Gopalakrishna n et al., 2011
Pasta	Fiber-enriched Sweet potato flour	50,60	NA	Firmness increased	Decreased cooking time, cooking loss and swelling index	High crude protein content	Ideal foods for diabetic and obese people	NA	Krishnan et al., 2012
Pasta (Tagliatelle)	Tiger nut flour	30	Brightness decreased	Faster and greater loss of firmness, elasticity; decreased hardness	Increased water absorbing capacity	High dietary fibre content	Good option to obtain high – fiber product	NA	Martin- Esparza et al., 2013

Noodles	Sweet potato and soy flour	0%, 5%, 10% Defatted soy flour with 10% and 15% Sweet potato flour and puree	Lightness decreases, redness and yellowness increased`	Stickiness reduced except for 10% SPF at 0% DSF	Cooking loss increased	Increased protein, ash and total dietary fiber; decreased fat and carbohydrates	Sweet potato provided β- carotene for vitamin A and protein	Noodles with 10%SPF received higher overall acceptability score	Collins & Pangloli, 1997
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Fruits									
Spaghetti	Banana starch	5,10,15,20	Brightness decreased	Firmness decreased	Cooking loss increased	Increased resistant starch	Acceptable as a functional food with higher levels of RS	15% enriched pasta received highest score of acceptability	Hernandez- Nava et al., 2009
Pasta or spaghetti	Unripe banana flour	85:15, 70:30, 55:45	NA	NA	Increased cooking loss	Decreased protein content, increased ash and TS content, decreased DS, increased RS	Banana flour spaghetti possessed increased antioxidant capacity	NA	Ovando- Martinez et al., 2009

Other ingredients									
Spaghetti	Insulin	2.5,5,7.5,10	NA	Firmness decreased	Decreased swelling index and water absorption capacity	Reduced GI up to 15%	Non- starch polysaccharides are able to enhance nutritional value of pasta	NA	Brennan, Kuri & Tudorica, 2004
Pasta	Pea, guar, inulin	7.5,10,12.5,15	NA	Firmness decreased (pea, guar); increased elasticity, adhesiveness and stickiness (insulin)	Increased swelling index (guar); increased cooking loss (pea and inulin)	High fiber content	Incorporation of fiber alters the quality attributes of pasta products	NA	Tudorica, Kuri, & Brennan, 2002
Spaghetti	Resistant starch	10		Decreased chewiness and stickiness	Increased water absorption, cooking time; decreased cooking loss	High dietary fiber	Good alternative to bran fibers	Sensory results correlated with TPA results	Sozer, Dalgic, & Kaya, 2007
Pasta	Mustard protein isolate	2.5,5,10	Brightness decreased	Increased firmness; decreased adhesiveness	Decreased cooking loss and swelling index	High protein and amino acid content	Improved nutritional value	Higher score in color and texture	Alireza & Bhagya, 2008

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

#### **2.1.1.1 Legumes**

Legumes include peas, beans, lentils, peanuts and other podded plants that are used as food. Legumes are rich source of proteins, starch, oil, minerals, vitamins and health protecting compounds (phenolics, inositol phosphates and oligosaccharides) making them important in diets of many regions throughout the world (Messina, 1999). Due to their nutritional composition, legume seeds can be used as meat replacers as well as components of rational nourishment and food for vegetarians. Legume seeds contain 15-25% protein. The protein is rich in lysine and poorer in sulphur-containing amino acids (methionine and cysteine) compared to cereals. Lysine is the first limiting amino acid, so it is important that legumes complement cereals in lysine balance.

The protein component in legumes is summarized below in the table:

Legume	Protein content (%)					
Chickpea	15.5-28.2					
Lentils	24.7					
Lupin	34.8-62.5					
Beans	19.4-24.8					
Peas	23.9-25.1					

Table 2.1.1.1 Protein content of raw legumes (as % dry matter) (Messina, 1999)

The isolated proteins, starch and fibers from legume seeds have good physicochemical and health protecting properties making it a promising material for food (SCHUSZTER-GAJZÁGÓ, 2004). Commercially available pasta is low in proteins (< 15%) and have comparatively low amounts of lysine, a crucial amino acid (Shogren et al., 2006). Enrichment of pasta with richer protein sources such as soy flour or soy protein (Shogren et al., 2006), and legumes such as split pea or faba bean (Petitot, Boyer, Minier, & Micard, 2010) have been studied previously. Pasta made from soy proteins is rich in lysine (Shogren et al., 2006). And legumes used in making of pasta provide good source of proteins, with high amounts of lysine, fibers, vitamins and minerals. Legume proteins contain low amounts of sulphur-containing amino acids such as methionine, tryptophan, and cysteine but contain high amounts of lysine (Petitot et al., 2010).

Different types of legumes (such as chickpea, beans, and soy), plant proteins and lupin proteins (Table 2.1.1) have been used in pasta fortification. Legume flours could effectively replace durum wheat semolina in pasta. However, depending on the replacement amount, pasta preparation process could be more or less challenging (Petitot et al., 2010). According to nutritionists, pulses (grain legumes) such as chickpea in diets have many nutritional benefits. Chickpea seed has a high protein digestibility, contains high levels of complex carbohydrates (low GI), is rich in vitamins and minerals and is relatively free from anti-nutritional factors (Muzquiz & Wood, 2007).

#### 2.1.1.1.1 Texture

One of the predominant characteristics that define the quality of pasta products is related to textural factors such as firmness, resilience, stickiness, hardness and elasticity (Cole, 1991). Pasta texture is mostly considered in its cooked form than raw in the case of fresh pasta. The texture of pasta fortified with different ingredients (as represented in Table 2.1.1) was determined in cooked form. Fortification of durum wheat pasta with chickpea flour retained firmness better than durum after refrigeration (J. A. Wood, 2009). This seems to be the case with spaghetti containing 15% chickpea flour (J. A. Wood, 2009). However, chickpea flour contains no gluten and with increasing chickpea flour added, the semolina gluten content is lowered, leading to weakening of the gluten matrix and a decrease in the spaghetti firmness. Hence it is the gluten content and composition that may be more important in determining spaghetti firmness. However, the chickpea fortified pasta was found to be less sticky.

Durum wheat pasta containing 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), Doxastakis et al. (2007) and Zhao, Manthey, Chang, Hou, and Yuan (2005) reported that fortification of pasta with legume flour (navy bean, pinto bean, lentil, green pea) or lupin protein or protein concentrates increased pasta firmness. Protein-fortified pasta tended to have higher firmness than conventional pasta. This is probably because of the higher number of polypeptide chains associated with higher protein contents which increase the ability of proteins to form an insoluble network (Chillo, Monro, Mishra, & Henry, 2010).

On the contrary, 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). However,

other studies have reported increased pasta firmness measured mechanically with increasing level of soy flour (Chillo et al., 2010; Petitot et al., 2010).

#### 2.1.1.1.2 Cooking quality

Cooking quality is the characteristic of greatest importance to consumers and therefore of great importance to wheat producers, breeders and processors (Sabanis, Makri, & Doxastakis, 2006). Cooking quality includes cooking loss, cooking time, swelling index, water absorption capacity and surface stickiness. During cooking, the product should maintain its form without disintegration, substantial increase in volume; exude minimal material into the cooking water and exhibit tolerance to over-cooking (Cole, 1991). Loss or gain of weight during cooking has an effect on the nutritive value of a given weight of cooked food (Chappell, 1954).

Cooking loss is considered to be an important technological quality attribute in product development (Bertram et al., 2003). Legume-fortified pasta has been reported to increase cooking loss. As summarized in Table 2.1.1, pasta enriched with corn- broad beans (Gimenez et al., 2013), green and yellow pea, lentil and chickpea flour (Zhao et al., 2005), plant proteins (Gurpreet Kaur, Sharma, Nagi, & Ranote, 2013), lupin protein (Doxastakis et al., 2007), split pea, faba bean (Petitot et al., 2010), germinated pigeon pea (Torres et al., 2007) and common bean flour (Gallegos-Infante et al., 2010) had a higher cooking loss compared to durum flour pasta. Higher cooking loss is associated with the formation of a weaker gluten network as a result of the dilution effect of the wheat gluten (Martín-Esparza, González-Martínez, & Albors, 2013). As a consequence, the starch leaches more easily into the cooking water.

Apart from cooking loss, there were changes in the optimal cooking time of legumefortified pasta as well (Table 2.1.1). Pasta enriched with chickpea and defatted soy flour (Bashir et al., 2012), green and yellow pea (Zhao et al., 2005), plant proteins (Gurkirat Kaur, Sharma, Nagi, & Dar, 2012), and legume flour like mung bean, soya bean, red lentil or chickpea (Chillo et al., 2010) had higher optimal cooking times compared to control. On the contrary pasta enriched with common bean (Gallegos-Infante et al., 2010), germinated pigeon pea (Torres et al., 2007), split bean and faba bean (Petitot et al., 2010) and pea protein concentrate (Nielsen et al., 1980) showed a decrease in the optimal cooking time. This again was associated to the formation of weaker gluten network as a result of a dilution effect of the gluten and the differences between semolina batches or the mixing conditions used (Gallegos-Infante et al., 2010). Besides cooking loss and cooking time, authors also reported changes in swelling index (grams of water per gram of dry spaghetti) and water absorption capacity in legume-fortified pasta (Table 2.1.1). Swelling or water uptake increased in pasta enriched with chickpea and defatted soy (Bashir et al., 2012), plant proteins (Gurpreet Kaur et al., 2013), chickpea flour (Sabanis, Makri & Doxastakis, 2006) and germinated pigeon pea (Torres et al., 2007). According to Torres et al. (2006), increased cooking water absorption could be due to changes in the nature of the interaction of legume starch with fibre and/ or with proteins. In contrast, pasta enriched with corn-broad beans (Gimenez et al., 2012), split bean and faba bean (Petitot et al., 2010) and pea concentrates (Nielsen et al., 1980) absorbed less water during the cooking process. Low water absorption could possibly be due to a higher proportion of denatured proteins caused by prior heating (Nielsen et al., 1980), and the formation of new structures such as retrograded amylose and amylose-lipid complexes, which favour the structural stability of pasta like products and provide greater resistance to hydration (Gimenez et al., 2012).

#### 2.1.1.1.3 Sensory properties

Fortified pasta varied in its acceptability and sensory attributes (Table 2.1.1). Pasta incorporated with chickpea flour (Wood, 2009) and corn-broad beans (Gimenez et al., 2012) had acceptable consumer scores. Similarly, pasta incorporated with 10% chickpea flour and 6% defatted soy flour resulted in higher acceptability scores with respect to sensory attributes compared to pasta containing combinations of 14% chickpea flour and 10% defatted soy flour, and pasta with 18% chickpea flour and 14% defatted soy flour. (Bashir et al., 2012).

Another study carried out on spaghetti with green and yellow pea, lentil and chickpea flours (Zhao et al., 2005) however showed that the acceptability for overall colour, flavour, and texture between the control and spaghetti containing legume flours (green, yellow pea, lentil and chickpea flour) significantly differed. Consumers preferred the control spaghetti (without legume additives) compared to spaghetti containing legume flours. Legume spaghetti had a beany flavour due to the raw legume flour used.

Shogren et al. (2006) further reported that spaghetti enriched with 35% soy flour that was not significantly different in flavour and texture compared with control (without soy) had greater consumer acceptability. However spaghetti with 50% soy flour had slightly higher beany and bitter flavours compared to control, and was not acceptable to consumers. Descriptive analysis by a

trained panel showed that split pea and faba bean (35%) fortified pasta had higher hardness, elasticity and fracturability, which resulted in excessively firm and rubbery pasta that was not acceptable to consumers (Petitot et al., 2010).

Other ingredients like germinated pigeon pea, plant proteins (mushroom powder, Bengal gram flour, defatted soy flour) and peanut flour incorporated in pasta have been reported to have high acceptability scores (Howard et al., 2011; Gurpreet Kaur et al., 2013; Torres et al., 2007). Pasta supplemented with germinated pigeon pea (Torres et al., 2007) and plant proteins (Gurpreet Kaur et al., 2013) were highly acceptable. Pasta containing 30% peanut flour (Howard et al., 2011) scored higher acceptance due to its colour, higher moisture content and softer texture than samples 40% and 50% peanut flour. Therefore, the incorporation of added ingredients into pasta not only changes the overall nutritional profile but can also influence consumer preference and acceptance.

#### 2.1.1.2 Cereal

Cereals are grains or edible seeds of the grass family Gramineae (Bender & Bender, 1999). Due to their nutritional benefits, cereals are used as additional ingredients in pasta to increase nutritional value. Y. V. Wu et al. (2001) incorporated corn gluten meal as a high-protein ingredient. Corn gluten meal is the high-protein fraction from the wet milling of corn to yield starch, oil, protein and other fiber. Due to its unpleasant taste it has not been used in food. However processing it by supercritical carbon dioxide extraction or hexane/ethanol extraction significantly improved corn gluten meal flavour (Y. V. Wu, Rosati, Sessa, & Brown, 1995).

Cereal brans are a by-product of the milling industry. During the recent years, there has been a special reorganization of the role of fiber in the human diet and a great deal of interest was expressed in cereal fiber (Gurkirat Kaur et al., 2012). Consumers are becoming increasingly health conscious and there is a demand for natural, wholesome, health-promoting foods. Increased concerns by the public about the health effects of dietary fiber have coaxed a fast-growing market of high fiber and low calorie products. Cereals are considered to be an important source of fiber. However, little information is available concerning the quantity and composition of fiber in manufactured cereal products (Gurkirat Kaur et al., 2012).

#### 2.1.2.1 Texture

Addition of cereals to pasta can contribute to changes in texture due to high fiber content present in cereals (Table 2.1.1). The presence of bran particles physically interferes with dough development, which results in weak dough properties. Since the dough strength affects the mechanical energy required for extrusion and the rate of extrusion (Levine, 2001), addition of fiber to foods must be done in a manner appealing to consumers in terms of its texture, taste and color (Gurkirat Kaur et al., 2012). Addition of corn gluten meal decreased the firmness of the pasta (Y. V. Wu et al., 2001). The decreased in firmness was more pronounced with regular corn gluten meal than with water-washed corn gluten meal. Dough strength is important to maintain the structural integrity of the pasta. In the case of pasta fortified with teff, oat and wheat flour (Hager, Czerny, Bez, Zannini, & Arendt, 2013), the texture of pasta with oat was found to be similar to pasta made with wheat flour. However the texture of pasta with teff could be further improved. This could be attributed to the high fiber and protein content present in teff flour.

#### 2.1.1.2.2 Cooking quality

The cooking quality of pasta is also affected by the addition of cereals. The optimum cooking time for pasta fortified with cereal brans was less as compared to durum wheat semolina pasta. This could be attributed to the physical disruption of the gluten matrix by the bran and germ particles which provide a path of water absorption into the whole wheat spaghetti strand that also reduced cooking time (Gurkirat Kaur et al., 2012). Manthey and Schorno (2002) reported similar observations. Significant variation was also observed in pasta with respect to water absorption. Beyond 5%, there was significant increase in water absorption by pasta on cooking. The increase in water absorption was due to the increase in fiber content of the resultant pasta. According to Chen, Rubenthaler, Leung, and Baranowski (1988), increased water absorption was due to the strong water binding ability of fibers. Disruptions in the protein matrix by bran particles would promote water absorption and facilitate starch granule swelling and rupture (Manthey, Yalla, Dick, & Badaruddin, 2004). Similarly, incorporation of fiber (pea hull, wheat, corn and wild oat bran) ingredients into wheat flour increased water hydration values in proportion to the level of replacement (Sosulski & Wu, 1988).

#### 2.1.1.2.3 Sensory properties

In a study by Y. V. Wu et al. (2001), the overall flavour quality score of spaghetti decreased with the increasing addition of either washed (water/ethanol) or regular corn gluten meal (Table 2.1.1), and control was rated the highest in overall quality. Spaghetti with acceptable quality can be prepared with 5% washed or regular corn gluten meal. Pasta enriched with different cereal brans showed maximum acceptability score when enriched with cereal brans up to 15% level. Beyond that, acceptability score was affected due to the color of enriched pasta. On the other hand, teff spaghetti (62.8% teff flour) showed reduced sensory quality (Hager et al., 2013). Teff spaghetti was much drier than oat (64.7% oat flour) and wheat (69.6% wheat flour) spaghetti. The stickiness of teff and wheat were comparable, while oat was stickier than teff and wheat.

#### 2.1.1.3 Marine foods

Marine foods have been found to be abundant in compounds which are good for health and have nutraceutical value (S. Kadam & Prabhasankar, 2010). These include omega-3 oils, chitin and chitosan, fish protein hydrolysates, algal constituents, carotenoids, antioxidants, fish processing by- products such as fish bone, shark cartilage, taurine and bioactive compounds. Omega-3 oils are more popular and widely used than any other ingredients of marine source. Chitin and chitosan are polysaccharides, recently gaining attention in the food world. Algae (*Chlorella vulagris* and *Spirulina maxima*) and seaweed (*U. pinnatifida*) have also been known to be a good source of dietary fiber, antioxidants and carotenoids. On the other hand fish bone and shark cartilage are extensively used as source of calcium (S. Kadam & Prabhasankar, 2010). Hence for these reasons, marine foods have been incorporated into pasta to improve its nutritional properties.

Wakame (*U. pinnatifida*), one of the widely consumed brown seaweed, rich in fucoxanthin was used to develop pasta (S. Kadam & Prabhasankar, 2010). Fucoxanthin is a xanthophyll characterisitic of brown seaweed, most abundant among aquatic carotenoids accounting for more than 10% of estimated total natural production of carotenoids. Besides this shrimp meat (S. U. Kadam & Prabhasankar, 2012), blue-green algae and microalgae have also been used in reformulating pasta (Table 2.1.1) to improve its biofunctional and nutritional qualities.

#### 2.1.1.3.1 Texture

The addition of functional ingredients can either result in an increase or decrease in pasta textural properties. Microstructure studies show that when wakame (*U. pinnatifida*) (S. Kadam & Prabhasankar, 2010) was substituted at different levels (Table 2.1.1), an enhanced gluten network of pasta was obtained. Similar results were also obtained when Indian brown seaweed was incorporated in pasta (Prabhasankar et al., 2009a). Addition of microalgae on the other hand (*Chlorella vulagris* and *Spirulina maxima*) resulted in an increase in the raw pasta firmness when compared to the control sample (Fradique et al., 2010). For all the microalgae studied, an increase in the biomass concentration (0.5- 2.0%) resulted in increased pasta firmness. Similar results in terms of firmness were seen in pasta incorporated with shrimp meat (S. Kadam & Prabhasankar, 2010) and blue-green algae (Zouari et al., 2011). The higher pasta firmness could be due to the higher protein content of microalgae pastas and lower water uptake, compared to durum wheat pasta (Fradique et al., 2010).

The firmness of cooked pasta is related to the starch granules hydration, during the cooking process and consequent embedding of gelatinizing starch granules in a matrix partially denatured protein (Verardo et al., 2009). Furthermore, differences in firmness values can be due to differences in gluten fraction. Increasing the amount of gluten results in a structural reinforcement expressed by a higher resistance to cutting and extensional forces (Sozer, Dalgic, & Kaya, 2007). In contrast, Chang and Wu (2008) showed that the addition of seaweed powder (0-8%) resulted in a decrease of cooked pasta breaking energy and thereby concluded that seaweed could not function as an effective ingredient to fortify the network structure of noodles.

#### 2.1.1.3.2 Cooking quality

Optimal cooking time depends on the rates of water penetration and starch gelatinization (Edwards et al., 1993). Pasta fortified with microalgae had an increased cooking time especially in *Chlorella vulgairs* (Cv) substituted pasta (Fradique et al., 2010). This could be due to the additional starch content present in Cv pasta that requires longer cooking times in order to obtain complete gelatinization. In addition microalgae incorporated pasta also present higher swelling indexes as compared to the control sample. Similar results in terms of higher swelling index were observed in pasta fortified with blue- green algae (Zouari et al., 2011). This results from the ability of the microalgae to absorb water and retain it in the protein-starch network. Accordingly, water

absorption was also affected resulting in higher moisture content of the pasta, which may be due to higher water holding capacity in Cv during dough formation (Fradique et al., 2010).

Increased cooking loss was observed in pasta fortified with shrimp meat (S. Kadam & Prabhasankar) and in Wakame (Prabhasankar et al., 2009a; Prabhasankar et al., 2009b). This means that there was loss in weights due to rupture in the gluten network. Cooking loss in blue - green algae incorporated pasta however decreased (Zouari et al., 2011). This could be explained by the reinforced dough matrix of microalgae proteins and gluten, which was able to entrap starch in the resulting network.

#### 2.1.1.3.3 Sensory properties

As shown in Table 2.1.1, pasta incorporated with microalgae resulted in higher consumer acceptance score (Fradqiue et al., 2011; Zouari et al., 2010) and with appealing color (orange and green), which was found to be highly acceptable. Due to low levels (0.5-2%) of microalgae added to the pasta dough, changes in structure and pasta taste were hardly detectable by panellists. On the other hand, pasta with 20% shrimp meat showed better consumer acceptability score and was found to be more nutritionally enhanced and acceptable. The results have been found to similar to the studies done by Yousif, Cranston, and Deeth (2003) and Prabhasankar et al. (2009a).

#### 2.1.1.4 Tubers

Tubers belong to the class of foods that provide energy in the form of carbohydrates. Some of the tubers that have been incorporated in pasta to enhance the nutritional value include cassava, sweet potato, potato and yam. Their nutritional composition varies from place to place depending on the climate, soil, the crop variety and other factors (FAO, 1990). Carbohydrates provide dietary energy, which is the main supply of nutrient in tubers. The protein content of tubers is low (1-2%), and in almost all tuber crop proteins, sulphur-containing amino acids are the limiting amino acids (FAO, 1990). Cassava, sweet potato, potato and yam contain some vitamin C and yellow varieties of sweet potato, yam and cassava contain beta-carotene or provitamin A. Tubers however lack most other vitamins and minerals but contain significant amount of dietary fiber (FAO, 1990).

Plant carbohydrates include celluloses, gums and starches However starches are the main source of energy, as celluloses are not digested. In addition to starch and sugar, tuber crops also contain non-starch polysaccharides, including pectins, celluloses and hemicelluloses, as well as other structural proteins and lignins, collectively referred to as dietary fiber (FAO, 1990). Sweet potato has a significant amount of dietary fiber that can be as high as 5% on wet basis or 20% on dry basis (W. Collins & Walter, 1982).

Roots and tubers contain a very low amount of protein and most of the protein intake is provided by potato and sweet potato. Usually the addition of nitrogen fertilizer increases the protein content in some root crops such as potato and sweet potato but decreases the lysine content, while aspartic acid and free amino acids are increased (Hoff et al., 1971). In root crops the quality of the protein, in terms of the balance of essential amino acids present, may be compared to that of the standard animal proteins (FAO, 1990). Most root crops contain a reasonable amount of lysine, though less than in legumes, but are limited in sulphur amino acids ((FAO), 1990; Bhandari, Kasai, & Kawabata, 2003). For example, yam is rich in phenylalanine and threonine but limiting in cystine, methionine and tryptophan.

Pasta is considered as a healthy food as it is poor in sodium and fat, with no cholesterol, and rich in complex carbohydrates, thereby producing a low post-prandial response to glucose and insulin in the blood (Gallegos-Infante et al., 2010). Nonetheless, it is not recognized as a balanced product due to its poor nutritional value in terms of proteins and low content of dietary fiber (Martin-Esparza et al., 2013). Fresh egg pasta has a higher nutritional value. The addition of flours of different origin such as tiger nut, sweet potato, and combinations of sweet potato and whey protein concentrates (fish powder, defatted soy flour and whey protein concentrate) to improve the protein, mineral, vitamin and dietary fiber content of pasta and pasta products has been a goal in recent years (Gallegos-Infante et al., 2010; Gelencsér, Gál, Hódsági, & Salgó, 2008; Howard et al., 2011).

Tiger nut is rich in starch (26.54%), dietary fiber (24.13%), fat (23.56%) and oleic acid (Martín-Esparza et al., 2013). However it has poor protein content (4.15%). Many studies have evaluated the benefits of tiger nut dietary fiber and showed positive results indicating that tiger nut can help prevent colon cancer, heart attack, obesity, diabetes and gastrointestinal disorders (Ade-Omowaye, Akinwande, Bolarinwa, & Adebiyi, 2008; Anderson, Smith, & Gustafson, 1994).

Sweet potato has been recognized as a health food of great significance due to the various bioactive compounds ( $\beta$ -mcarotene, phenolic acids and anthocyanins) in the roots. Despite being

rich in carbohydrates, sweet potato has been reported to have a low glycemic index (<55) and is ideal for consumption by diabetic people (Björck, Liljeberg, & Östman, 2000).

#### 2.1.1.4.1 Texture

According to (Granito, Torres, & Guerra, 2003), replacement of wheat semolina by other flours or ingredients decreases the gluten content, leading to poorer quality pasta products from a technological point of view. High quality cooked pasta should maintain firmness ("al dente"), has to be resistant to surface disintegration, and has no excessive stickiness (Martin-Esparza et al., 2013). Addition of tiger nut flour to pasta hardly affected firmness and elasticity of cooked pasta (Table 1). In fact, an abrupt decrease of hardness after cooking was observed in tiger nut supplemented pasta as compared to other samples (control and fresh egg samples) where the hardness decrease was progressive.

In the case of sweet potato, three bran sources such as oat bran, wheat bran and rice bran were used to enhance the nutritional and physicomechanical properties of dietary fiber-enriched sweet potato of Sree Arun and Sree Kanaka varieties (Jyothi et al., 2011). It was found that the bran sources increased the firmness of cooked sweet potato pasta compared to the control sample without bran. A higher level of fortification resulted in increased firmness and hardness. Steffe (1996) reported that starch gelatinization and protein coagulation are the major structural changes during cooking that affects the texture of pasta. Higher levels of bran were found to give pasta higher protein levels, and Del Nobile, Baiano, Conte, and Mocci (2005) reported that protein level affected the firmness of cooked pasta. It was also found that increasing the gluten levels enhanced the force required to produce a given extension of cooked spaghetti (Matsuo et al., 1972). Unlike pasta firmness, which is influenced by the internal structures of cooked product, adhesiveness depends on the surface properties (Jyothi et al., 2011). Adhesiveness, a measure of the stickiness of the pasta while eating was found to be a maximum for the control. The addition of sweet potato flour along with oat bran, rice bran and wheat bran lowered the stickiness levels.

In contrast to sweet potato, tiger nut fortified pasta resulted in loss of firmness that demonstrated the weaker structure due to a less dense gluten network that does not trap the fine starch granules properly (Martin-Esparza et al., 2013). As reported by Resmini and Pagani (1983), if protein coagulation prevails during pasta cooking, starch granules will be trapped inside the protein network, leading to a firm pasta product. However, if starch gelatinization and swelling
prevails (more likely in case of tiger nut as it is rich in starch and poor in protein), protein will coagulate in discrete masses and no continuous structure will be formed. This would lead to soft and sticky pasta being produced (Maningat, Seib, Bassi, Woo, & Lasater, 2009).

#### 2.1.1.4.2 Cooking quality

Pasta quality and cooking properties are dependent on the protein-starch developed matrix (C. Brennan & Tudorica, 2007). Addition of tubers does affect the cooking properties of pasta (Table 1). Replacement of wheat semolina by tiger nut flour increased water absorption capacity. Water penetration during pasta cooking process is mainly related to protein content. As tiger nut flour is rich in fiber and starch, and poor in protein, partial replacement of semolina wheat by tiger nut flour may induce faster starch gelatinization (thus increasing water absorption capacity), although the dough may take longer to rehydrate due to the presence of significant amount of fiber (Martin-Esparza et al., 2013).

The quantity of solids going into water during cooking of pasta is a determinant of pasta quality and compact textured pasta leads to a lower cooking loss (Jyothi et al., 2011). The optimum cooking time for sweet potato flour fortified pasta was found to be between 2-4 minutes as compared to the values of 7-9 minutes for durum wheat pasta, which was less. All samples had a higher cooking loss in the case of pasta fortified with sweet potato with different formulations (whey protein concentrate, defatted soy flour and fish powder). In the case of pasta fortified with fiber–enriched sweet potato, cooking loss was reduced (13-14% cooking loss). Higher cooking loss results in dilution of gluten and weakening of starch-protein network (Jyothi et al., 2011. Besides protein content, gluten strength is an important factor in determining cooking quality. As sweet potato lacks gluten, addition of protein sources like whey protein concentrate, defatted soy flour and fish powder their impact on the starch-protein network formation.

Swelling index in case of sweet potato fortified pasta with different formulations (Jyothi et al., 2011) was found to be higher as compared to fiber-enriched sweet potato fortified pasta. Non-wheat ingredients lead to discontinuity within the gluten matrix and result in weak dough properties (Manthey et al., 2004). During cooking, starch absorbs water and swells, and the granular structure collapses leading to the leaching of amylose. Addition of non-wheat ingredients in case of sweet potato fortified with different protein concentrates (Jyothi et al., 2011) could have

led to dilution of the gluten structure. Tudorica, Kuri, and Brennan (2002) reported that the uneven distribution of water within the protein matrix due to the competitive hydration of fiber, led to prevention of swelling. Hence the increased cooking loss was due to the disruption of protein-starch network. This could explain the reason for the decreased swelling index in case of fiber-enriched sweet potato fortified pasta (Jyothi et al., 2012).

#### 2.1.1.4.3 Sensory properties of Tubers

The study by J. Collins and Pangloli (1997) with the addition of sweet potato (10% and15%) and soy flour (5% and 10%) showed that noodles with 10% sweet potato flour scored greater overall acceptability. Samples with 10% sweet potato flour was ranked above the "like moderately" category and the rest of the samples were ranked just below that category. In terms of Defatted soy flour, panellists preferred the noodles with 10% soy flour than the rest. Overall acceptability has been reported to decrease with when corn flour, corn protein isolates and soy products were incorporated into pasta (Bahnassey & Khan, 1986; Breen, Banasik, & Walsh, 1977; Molina, Mayorga, Lachance, & Bressani, 1975; Singh, Chauhan, & Bains, 1989; Y. Wu et al., 1987).

In terms of tenderness of the product with the addition of sweet potato and soy flour (Collis & Pagani, 1997), panellists scored the samples in the same way as in the case of overall acceptability from just below to just above "moderately tender". Bahnassey and Khan (1986) reported that flours of legumes or defatted soy increased firmness of pasta and so did Wu et al. (1987).

### 2.1.1.5 Fruits

Besides using beans, cereals, seaweed and tubers, fruits such as unripe banana and banana starch are also used to make pasta, low in carbohydrate digestibility, and high in resistant starch and high antioxidant phenolics content.

Bananas are mainly produced in tropical and subtropical developing countries and are a source of carbohydrates and nutritionally interesting bioactive compounds (Ovando-Martinez, Sáyago-Ayerdi, Agama-Acevedo, Goñi, & Bello-Pérez, 2009). Starch is the main component of unripe banana, corresponding to 60-80g/100g (dried weight) of the fruit, percentage similar to that of corn or potatoes (Whistler et al., 2005). Besides starch, unripe banana contains cellulose,

hemicelluloses and lignin in the pulp (Hernández-Nava, Berrios, Pan, Osorio-Diaz, & Bello-Pérez, 2009). The indigestible fraction of foods is made of dietary fiber and other compounds that are resistant to the action of digestive enzymes, such as resistant starch, resistant protein and associated bioactive compounds (polyphenols, carotenoids, phytosterols and others). The indigestible fraction of tropical fruits has high antioxidant activity (Saura-Calixto, Serrano, & Goñi, 2007). In 2009, Goni et al. showed that a significant quantity of polyphenols in fiber, which contributed to the antioxidant activity of fruits and vegetables. Banana is rich in fatty acids, phytosterols and steryl glucosides. Hernandez-Nava et al. (2009) developed spaghetti with high resistant starch content by supplementing spaghetti with banana starch and Ovando-Martinez et al. (2009) made pasta using unripe banana flour. There are not many studies that have used banana flour or banana as a supplement in pasta.

# 2.1.1.5.1 Texture

According to Table 2.1.1, addition of banana starch to spaghetti (Hernandez-Nava et al., 2009) significantly lowered the firmness of spaghetti after cooking. Firmness decreased with an increase in banana starch levels. This may be a direct effect of the possible increase in amylose loss during the cooking of spaghetti. According to Vignaux et al. (2005), the cooking loss from pasta, due to starch damage and lack of amylose–protein interaction, is a major factor in the loss of firmness.

# 2.1.1.5.2 Cooking Quality

As shown in Table 2.1.1 spaghetti fortified with banana starch (Hernandez-Nava et al., 2009) and unripe banana flour (Ovando-Martinez et al., 2009) had increased cooking loss compared to control spaghetti. The addition of non-gluten flours in the formulation of spaghetti was reported to dilute the gluten strength of the semolina and interrupted and weakened the overall structure of the spaghetti (Rayas-Duarte, Mock, & Satterlee, 1996). Spaghetti made with 100% semolina has a cooking loss of  $\leq 8\%$  and this is considered acceptable for good quality pasta (Dick & Youngs, 1988). Based on this, spaghetti containing different levels of banana starch (Hernandez-Nava et al., 2009) and unripe banana flour (Ovando-Martinez et al., 2009) were within the expected levels of cooking loss and could be considered as spaghetti of good cooking quality.

#### 2.1.1.5.3 Sensory Properties

The results of sensory evaluation test (Table 2.1.1) showed that spaghetti fortified with 15% of banana starch (Hernandez-Nava et al., 2009) received a higher acceptability score by testers, followed by spaghetti with 5 and 10% banana starch addition, which were not significantly different from each other. On the other hand, the control spaghetti (spaghetti containing bread wheat flour only) and spaghetti with 20% banana starch received the lowest acceptability sensory scores and did not differ significantly from the control sample. Spaghetti containing 15% banana starch received the highest score of acceptance followed by 5 and 10% banana starch addition. The results also showed that spaghetti with 20% banana starch had similar sensory attributes as commercial spaghetti made from 100% semolina. These results suggest that there could be a potential for commercial acceptability of spaghetti containing considerable levels of banana starch as a functional food with high levels of healthy resistant starch (RS).

### 2.1.1.6 Other ingredients

Inulin, mustard protein isolate and resistant starch have also been used to make spaghetti (Table 2.1.1). Pasta is regarded as a low glycemic index food product (Björck et al., 2000; D. J. Jenkins, Kendall, Axelsen, Augustin, & Vuksan, 2000). The glycemic index is a means of quantifying the effect of ingestion of a food product on the blood glucose level when compared with standard white bread or glucose (Liljeberg, Åkerberg, & Björck, 1996; Wolever, 1989). Digestion of carbohydrates is relatively slow within the matrix, in the case of pasta. This in turn results in a slow and progressive starch breakdown and hence sugar production in the body, leading to low postprandial blood glucose and insulin responses (Granfeldt, Liljeberg, Drews, Newman, & Björck, 1994). Incorporation of hydrocolloids into foods has shown beneficial regulation effects on post-prandial blood glucose, insulin and fasting plasma cholesterol (Blake, Hamblett, Frost, Judd, & Ellis, 1997; C. Brennan, Blake, Ellis, & Schofield, 1996; P. J. Wood, 2008). One of these ingredients used by the food industry is inulin (C. S. Brennan, Kuri, & Tudorica, 2004). Inulin is a non-digestible fructo-oligosaccharide (Tungland, 2000). It has traditionally been used as a fat replacer in dairy foods and has been shown to have positive effects on the rheology and stability of products (El-Nagar, Clowes, Tudorică, Kuri, & Brennan, 2002). However there are not many studies done showing the effects of inulin incorporated in cereal products, and pasta in particular.

Resistant starch is another ingredient that is used in pasta (Sozer et al., 2007). Starch, which is the major dietary source of carbohydrates, is the most abundant storage polysaccharide in plants. The recent recognition of incomplete digestion and absorption of starch in the small intestine as a normal phenomenon has raised an interest in non-digestible starch fractions and these are called "resistant starches". Several studies have shown them to have physiological functions similar to those of dietary fiber (McCleary & Monaghan, 2002; Sajilata, Singhal, & Kulkarni, 2006). Resistant starch, a natural component that is present in many foods, has a role to play with regard to the nutritional benefits of fiber fortification. It is starch that is resistant to digestion in the stomach and small intestine (Sozer et al., 2007). Resistant starch offers advantages over cellulosic sources of fiber such as bran. It provides low water holding capacity thereby aiding processing; it enhances organoleptic qualities of food as a replacement for, or complement to natural fiber, and can be labelled as 'dietary fiber' (Sozer et al., 2007). Nowadays most of the diseases result from inadequate feeding and some of them may be related to insufficient fiber intake. Therefore, it is reasonable to assume that an increased consumption of indigestible components would be important (Walter, da Silva, & Denardin, 2005). As a result RS sources can be included in the diet, as they do not result in pronounced organoleptic changes as traditional fiber sources like bran.

Mustard protein isolate has also been used to enrich pasta (Alireza Sadeghi & Bhagya, 2008). The food industry is looking for less expensive protein for use in the manufacture of modern convenience foods. Proteins, as isolates or concentrates, are necessary ingredients in many food processes, where they perform specific function. Mustard meal has a high protein content of about 38% that makes it a good potential source of food-grade vegetable protein and has a reasonable well balanced amino acid composition (Alireza Sadeghi & Bhagya, 2008). The protein is of excellent nutritional quality being rich in lysine with sufficient amount of sulphur containing amino acids- limiting amino acids is most of the cereals and oilseed proteins (Tzeng, Diosady, & Rubin, 1988). The presence of toxic and anti-nutritional constituents such as glucosinolates, phytates, phenolics and hulls limit the use of mustard as a source of protein in food products (Thompson, 1993; Tzeng et al., 1988). Recently mustard protein isolate with reduced toxic and anti-nutritional constituents has been produced so that it can be used in food products (Sadeghi, Rao, & Bhagya, 2006).

# 2.1.1.6.1 Texture

Addition of inulin (Brennan et al., 2004) to the pasta formulation from Table 2.1.1 resulted in decreased firmness of pasta compared to control and this increased with increasing inulin levels. These results are similar to those reported by Tudorica et al. (2002) with spaghetti containing pea, inulin and guar. Pasta firmness can be related to the hydration of starch granules during the cooking process, and the subsequent embedding of gelatinizing starch granules in a matrix of partially denatured protein. As a result, the decrease in firmness and swelling index could be associated with a reduction in starch gelatinization in the pasta.

Spaghetti enriched with resistant starch (RS) (Sozer et al., 2007) was found to have better texture properties than bran containing spaghetti. RS enriched spaghetti was found to be less sticky and the firmness values were found to be close to the control spaghetti.

Spaghetti enriched with mustard protein isolate (MPI) had increased firmness. The stickiness values of spaghetti with MPI were lower. The reduction in stickiness could be due to the reduction in starch proportion in the enriched spaghetti, or physical entrapment of starch in protein network with increased replacement level.

# 2.1.1.6.2 Cooking quality

Pasta enriched with inulin (Brennan et al., 2004) showed a significant decrease in swelling index as compared to the control. Water absorption capacity of pasta significantly decreased as the amount of inulin increased in the formulation. Both the decrease in swelling index and water absorption capacity could be due to the characteristics of inulin. Being highly hydrophilic, it is likely that inulin absorbs water, inhibiting starch swelling, and absorption of water, which in turn may alter the structure of the pasta produced (Tudorica et al., 2002). Similar results were obtained for pasta enriched with guar gum in terms of swelling index (Tudorica et al., 2002). In contrast, spaghetti enriched with RS (Sozer et al., 2007) had higher water absorbing capacity due to high amylose content. Amylose is known to have higher water binding capacity than native starch (starch isolated from plant sources such as corn, wheat, rice) (Zhiqiang, Xiao-su, & Yi, 1999). Spaghetti enriched with RS also had a higher cooking time, which could increase water absorption since more water can diffuse and interact with both gluten and starch.

Pasta enriched with pea fiber and inulin had a higher cooking loss than control pasta (Tudorica et al., 2002). Similar results were reported in spaghetti fortified with bran. The increase could be due to a disruption of the protein-starch matrix. However spaghetti enriched with MPI (Alireza & Bhagya, 2008) showed decreased cooking loss and protein loss as the level of enrichment increased. This decrease may be due to the low solubility of MPI that resulted in lower cooking loss and protein loss of the enriched pasta.

#### 2.1.1.6.3 Sensory properties

Spaghetti enriched with mustard protein isolate (MPI) (Alireza & Bhagya, 2008) had a higher score in terms of texture than the control (spaghetti containing only semolina). This could be due to higher firmness and lower stickiness of the enriched samples. There was a significant difference between 5 % and 10% enriched spaghetti as compared to control, and 2.5% enriched spaghetti that showed no significant difference. The higher acceptability of 5% and 10% enriched spaghetti was mainly attributed to the color but scored lower in terms of taste and flavour. Generally, the higher yellow color in pasta products is highly acceptable to consumers.

In the case of spaghetti enriched with resistant starch (RS) sensory testing was done to check if the instrumental texture measurement of cooked spaghetti texture was reliable (Sozer et al., 2007). Results from texture profile analysis showed that hardness values were not significantly different between the control (commercial spaghetti containing bran was used as control) and RS spaghetti. Adhesiveness was high during the early cooking stages but ultimately decreased as cooking time progressed. There were no significant differences in the cohesiveness and chewiness of the spaghetti enriched with RS. There was a strong correlation between instrumental chewiness-sensory hardness, and a strong negative correlation between instrumental cohesiveness sensory adhesiveness and hardness. The increase in adhesiveness was due to the bran particles preventing formation of strong gluten network.

Ideally when manufacturing a highly nutritious pasta, high protein, low carbohydrate and low fat levels in the pasta should be taken into consideration with respect to chemical composition. Besides the chemical composition, physical attributes such as texture quality (stickiness, firmness), cooking quality (cooking loss, swelling index, water absorption) are also important. The physical and chemical attributes together will influence consumer acceptability of pasta product in terms of overall liking, and liking of taste, appearance, texture and odour. Pasta fortified with different ingredients such as legumes, cereals, fruits, nuts and marine food sources at different levels have been successfully prepared with a definite increase mainly in protein content (Table 2.1.1). Changes in the physical attributes of pasta influenced consumer acceptance. This is the first study that incorporates meat and navy bean into pasta to increase its nutritional content. The following sections will further explain the nutritional value of meat and bean along with their physicochemical and functional properties.

# 2.2 Pasta reformulation with meat and navy bean

The reformulation studies summarized above have been carried out on different pasta types with spaghetti being the dominant type of pasta used. The purpose of this research is to explore the effects of navy bean and red meat addition in spaghetti formulation to improve it nutritional value. This section reviews the two protein sources used in this research.

# 2.2.1 Navy Bean

# 2.2.1.1 Nutritive value

Navy bean (*Phaseolus vulgaris*) is a pulse that is rich in starch, protein and dietary fiber with significant amounts of vitamins and minerals (Chung, Liu, Pauls, Fan, & Yada, 2008). Navy bean has anticancer properties that include the presence of bioactive microconstituents and the physicochemical properties of bean starch. Protease inhibitors, saponins, phytosterols, and phytate are putative carcinogens that are present in significant quantities in beans (Schweizer, Andersson, Langkilde, Reimann, & Torsdottir, 1990). Navy beans contain a considerable amount of resistant starch (Englyst, Kingman, & Cummings, 1992; Schweizer et al., 1990). The poor starch digestibility of pulses is because of its intact cell-wall structures enclosing starch granules, the presence of various antinutrients such as amylase inhibitors, phytates and polyphenolics, significant levels of amylose and high content of viscous soluble dietary fiber components (Hoover & Zhou, 2003).

Legumes have been shown to contain significant amounts of resistant starch in comparison with cereals, tubers and unripe foods. Therefore, the rate of starch digestion and release of glucose in to the blood stream is slower after the ingestion of legumes, resulting in reduced glycemic and postprandial insulin response in comparison with tubers and unripe fruits (Tovar, Granfeldt, & Bjoerck, 1992). Navy bean (*Phaseolus vulga*ris) is a valuable source of protein and carbohydrates with starch and dietary fiber as predominant fractions and significant amounts of oligosaccharides (Bravo, Siddhuraju, & Saura-Calixto, 1998). Navy bean is shown to contain significant amounts of slowly digestible starch (SDS) and resistant starch (RS) (Chung et al., 2008; Sandhu & Lim, 2008). Navy bean starch is slowly digested, produces a low glycemic index (D. Jenkins et al., 1981) and attenuates the postprandial insulin response (Schweizer et al., 1990).

#### **2.2.1.1.1 Physicochemical characteristics of navy bean**

The importance of legume seeds as food and functional ingredients has stimulated much attention to their utilization. The have been used as ingredients and supplements successfully by the food industry. For successful use in food applications, they should possess several desirable properties, known as functional properties (Sai-Ut et al., 2009). These functional properties affect processing, manufacturing, storage and preparation (Du et al., 2014). Legumes are mainly used in the flour form as a food ingredient because of its high protein content (Kaur et al., 2007; 2009). The functionality of proteins is closely related to their physical and chemical properties, such as molecular weight, amino acid composition and sequence, structure, surface electrostatic charge, and effective hydrophobicity (Damodaran, 1990). In addition to proteins, the complex carbohydrates of legumes, such as starch, fibres and other components (Pectins and mucilages), contribute to their functionality (Kaur & Singh, 2005).

A study of legume seed flour functionality is important for efficient utilization and consumer acceptance (Adebowale & Lawal, 2004). For legume seeds to function as successful food ingredients, it depends on their functional characteristics, such as foaming, water and oil absorption capacities, emulsification, gelation and swelling power. There are several studies on the functional properties of legume seeds. Some of the studies focused on the functional properties of lima bean, mung bean (Chel-Guerrero et al., 2002), chickpea (Kaur & Singh, 2005) and field pea flours (Kaur et al., 2012). Adebowale and Lawal, (2004) further reported a comparative study on the functional properties of bambarra groundnut, jack bean and mucuna bean flour. Onimawo and Asugo (2004) studied the nutrient and functional properties of pigeon pea flour.

In the present study, navy bean was used as an additional ingredient in pasta along with meat. Navy bean is a legume and an important food source that plays a significant role in the human diet. Due to its nutritional qualities navy bean can use as a functional ingredient/food to

improve nutritional quality of a variety of food products. Some of the physicochemical properties relevant to navy bean that will be discussed in this chapter of the thesis are water absorbing capacity, swelling power, foaming capacity, emulsion activity and stability and amylose leaching. Water absorption index, water absorption capacity and swelling factor of navy bean will be taken into consideration in this review. However emulsion properties and foaming properties of navy bean have not been taken into consideration in the present study.

#### a) Water absorption index (WAI) and water soluble index (WSI)

The WAI determines the absorption and retention of water is the volume occupied by the starch after it swells in excess water and indicates the integrity of starch released (Du et al., 2014). In the study by Du et al. (2014), navy bean showed a moderate amount of WAI as compared to small red beans being the highest and black bean being the lowest. WAI is related to the hydrophobicity and gelation capacity of chemical components, such as starch and protein (Kaur & Singh, 2005). However, components of different kinds of legumes are diverse, which may induce different interactions with water. According to Du et al. (2014), WAI of legume flours may not entirely depend on water absorption and swelling of the starches. In another study by Siddiq et al. (2013), navy bean extruded flour had a high WAI as compared to raw and steamed cooked flour. This shows that heat and other treatments during extrusion breaks down the amylopectin and gelatinization takes place, therefore greater water absorption (Whalen et al., 1997).

The water solubility index indicates the solubility of molecules. The WSI increases with an increase in starch depolymerisation, and the ultimate reduction in the length of amylose and amylopectin chains (Balandran- Quintana et al., 1998). In a study by Du et al. (2014), the WSI of legume flours differed significantly, where the highest value was obtained for lima bean and the lowest was obtained for pinto bean. In this case, amylose- lipid and protein-starch complexes formed in the process of heating could affect the WSI (Sathe et al., 1982). In another study by Siddiq et al. (2013), extruded bean showed a higher WSI than the raw and cooked beans. This could be due to the shear effect from extrusion that may have degraded the starch producing more soluble molecules (Batista et al., 2010), and thus increasing the solubility of extruded bean flour.

#### b) Water absorbing capacity

The water absorbing capacity of legume flours plays an important role in the food preparation process because it influences other functional and sensory properties (Du et al., 2014) and is a crucial factor in protein functionality (Sai-Ut et al., 2009). Water absorption capacity is important for certain product characteristics, such as moistness of the product, starch retrogradation and subsequent product staling (Siddiq et al., 2010). The water absorbing capacity of navy bean according to a study by Du et al.(2010) was found to be 1.39g/g, moderate in comparison to small red bean (1.89g/g), which was the highest, and the lowest water absorbing capacity was in black bean (1.12g/g). In a study carried by Sai-Ut et al. (2009) navy bean had the highest water absorbing capacity (3 times higher) in comparison to red kidney bean and adzuki bean. Kaur and Singh (2005) reported that legume flour containing several hydrophilic components, such as polysaccharides; generally have high water absorbing capacity. In addition, the protein quality of legumes or legume flours also affects their water absorbing capacity.

# c) Swelling factor

Swelling factor among starches of legumes can influence (1) bound lipid content (Sasaki & Matsuki, 1998), (2) Amylose content (Sasaki & Matsuki, 1998), (3) amylopectin structure (Sasaki & Matsuki, 1998), (4) extent of interaction between starch chains in the native granule (Ambigaipalan et al., 2011), and (5) granule crystallinity (Ambigaipalan et al., 2011). Du et al. observed swelling factor of different legumes between a temperature range of 60-85°C. Swelling factor differences were significant only between 60-80°C in the following order (Tepary bean> lablab bean> rice bean > navy bean> velvet bean). Swelling factor differences were seen in the range of 60-80°C between rice bean and navy bean (RB> NB) reflecting the higher amylopectin content and the presence of larger number of cracked granules in rice bean. Similarly, another study by Lee et al. (1995) showed swelling responses of navy bean at 85°C, thereby increasing the viscosity.

Gujska et al.(1994) observed increase in swelling factor for navy bean and pinto bean at temperatures above 70°C. According to Stone and Lorenz (1984), the strength and character of the micellar network within the granule is the major controlling swelling behavior of starch. Therefore, starch with extensive, strongly- bonded micellar structure should be relatively resistant to swelling.

In this case navy and pinto bean were resistant to swelling at 60°C as compared to field pea starch indicating a more strongly bonded micellar network.

# d) Emulsion capacity and emulsion stability

The emulsion activity reflects the ability and capacity of a protein to aid in the formation of an emulsion and is related to the protein's ability to absorb the interfacial area of water or oil in an emulsion (Singh et al., 2010). Sai- Ut et al. (2009) observed that navy beans showed the highest emulsifying capacity and stability as compared to azuki beans and red kidney. Similarly Du et al. (2014) reported that navy beans possessed the highest emulsion activity and stability in comparison to pinto bean, black bean, red kidney bean, chickpea, mung bean, lentil, black eye bean and lima bean.

Emulsification of proteins is influenced by solubility and surface hydrophobicity (Voutsinas et al., 1983). The differences observed are due to the presence of protein contents (soluble and insoluble) and other components, such as starch, fat and sterol contents of the legume flours. Protein-water interactions occur in the polar amino acid regions of protein molecules, and most proteins contain several polar side chains with peptides on the parent chains, making them hydrophobic and therefore, affecting their solubility and emulsification properties (Okaka & Potter, 1979). Emulsification activity differs among legumes due to the presence of other components (such as fat, starch and sterols) in the flour (Du et al., 2014).

# 2.2.2 Red meat

According to the Food Standards Australia New Zealand (FSANZ) Food Standard Code, meat is defined as ' the whole or part of the carcass of any buffalo, camel, deer, goat, hare, pig, poultry, rabbit or sheep, slaughtered other than in a wild state, but does not include eggs or foetuses' (FSANZ, 2002). The term 'red meat' in Australia is used by the meat industry to refer to meat from cattle, sheep and goat (i.e. beef, veal, lamb, mutton and goat meat). It does not include meat from pigs (pork, bacon and ham) or kangaroo, nor buffalo or camel meat (Beilken et al., 2007).

#### 2.2.2.1 Nutritional composition

Very often meat is associated with a "negative" health image due to its "high" fat content. Red meat in particular is seen as a cancer-promoting food. Therefore, low meat intake diet especially red meat is recommended to avoid the risk of cancer, obesity and metabolic syndrome (Biesalski, 2005). However, red meat contains high biological value protein and is an important source for some micronutrients such as iron, selenium, vitamins A, B12 and folic acid that are needed for good health (Biesalski, 2005; Williams, 2007). These micronutrients are either absent in plant-derived foods or have a poor bioavailability. It also contains a range of fats, including essential omega-3 polyunsaturated fats (Williams, 2007). In addition, meat is a protein rich and carbohydrate "low" product, which contributes to a low glycemic index, which is assumed to be beneficial with respect to obesity, diabetes development and cancer (Biesalski, 2005).

### 2.2.2.2 Functional properties of meat

Besides understanding the nutritional value of meat, it is also important to understand the functional or chemical properties of meat as it influences the cooking quality and the overall quality of the product. Functional properties of proteins in meat are those physicochemical properties, which affect their behavior in food products during preparation, processing, storage and consumption and contribute to the quality and sensory attributes of the food product. The important functional property relevant to this study is the water holding capacity of meat.

# 2.2.2.3 Water holding capacity of proteins

Globular proteins can influence the water binding capacity of food. More polar charged amino acids present towards the surface of globular proteins accelerate the solubility, swelling and hydration. High water retention is observed when globular protein surfaces have large amounts of hydrophilic residues on their surfaces (Zayas, 1997). The terms water holding capacity, water binding, water absorption and water hydration of proteins are used interchangeably to specify the ability of protein to retain or take up maximum amount of water under food formulation conditions (Quinn & Paton, 1979). In the case of meat and navy bean addition into spaghetti as in this study, incorporation of these proteins will influence water binding and swelling capacities.

Swelling of proteins is the primary step in their solvation and can be defined as the spontaneous uptake of water by a protein matrix. Protein ingredients with high water holding

capacity may dehydrate other components in the product. Water retention is also an important factor in protein functionality since it affects the texture, colour and sensory properties. Water retention can be used to determine if the ingredient should be added in powder or rehydrated form in the mixture.

# 2.2.2.4 Water holding capacity of meat

Meat structure is very complex. Water is in the muscle fiber as a lubricant, as well as a medium to transport metabolites in the fiber. According to Offer and Knight (1988) most water is held by the physical structure of the muscle. Most of the water in the muscle cell is present in the myofibrils in the spaces between the thick and thin filaments (Hertog-Meischke et al., 1997) in the muscle cell post- mortem (Cheng & Sun, 2008), while only a small amount of water (0.5g water/g protein) is bound to the charged and polar groups by the sarcoplasmic proteins and connective tissue (Wismer-Pedersen, 1987) by electrostatic attraction (Cheng & Sun, 2008).

Since water is a dipolar molecule it is attracted to charged species like proteins. Water in muscle cells is very closely bound to protein. Since the total concentration of protein in muscle is approximately 200mg/g, bound water only makes up less than a tenth of the total water in muscle (Lonergan & Lonergan, 2005). The amount of bound water changes very little if at all in post-rigor muscle (Offer & Knight, 1988). Another fraction of water that can be found in muscle and in meat is entrapped water (Fennema, 1985). The water molecules in this fraction may be held by steric (space) effects and/or by attraction to the bound water. In post mortem tissue, this water does not flow freely from the tissue. The rigor process and conversion of muscle to meat has an effect on the entrapped water. Weak surface water holds this fraction of water in meat. Free water is not seen in pre-rigor meat, but can develop as conditions change that allows entrapped water to move from structures where it is found (Fennema, 1985).

The majority of the water that is affected by the conversion of muscle to meat is the entrapped water. The ability of post- mortem muscle (meat) to retain its natural water content and added water is known as water holding capacity (WHC) or water binding capacity (Toldra, 2003; Grau & Hamm, 1956). The water content of meat products is an important quality parameter as it relates to the final yield (Bertram et al., 2003) of the end product. Quality parameters such as tenderness, juiciness, color, taste, shrinkage on cooking, and drip on freezing and thawing are directly related to the water holding capacity of meat (Wierbicki & Deatherage, 1955). Higher loss of water gives

an expectation of less optimal quality, due to shrinkage (if excessive can have adverse effect of product appearance) of products. It also impacts other quality attributes such as juiciness and tenderness (Bertram et al., 2002; Lawrie, 1998).

#### 2.2.2.5 Factors affecting water-holding capacity of meat

There are a number of intrinsic and extrinsic factors that affect the water holding capacity of meat. Among the intrinsic factors, genotype and feeding of animals are the most important, which affect muscle characteristics directly (Cheng & Sun, 2008). Extrinsic factors prior to slaughter such as fasting, epinephrine injection, and stunning, may also affect WHC of meat. Such treatments affect the WHC through stress, which decreases muscular glycogen reserves, which may lead to high ultimate pH and low water content of meat. Moreover post-slaughter treatments like chilling, ageing, injecting non-meat ingredients as well as tumbling has shown to affect the WHC of meat. In addition, several other processes related to cooking (cooking techniques, cooking temperature, etc.) and cooling processes (cooling methods, cooling rates, etc.) of the final meat products also greatly influence the final product (Cheng & Sun, 2008).

Amount of fat in meat also affects WHC. In meat, fat is a variable component (approximately 5% in lean meat) (Lonergan & Lonergan, 2005). As the amount of fat increases, water decreases thereby decreasing the amount of protein available for attracting and holding water (Warriss, 2010). In the subsequent section, the effect of pH on water holding capacity of meat (a) will be discussed, as it is the most important and relevant factor in this study.

#### a) Effect of pH on the water holding capacity of meat

pH is an important factor that has an intense effect as it influences the net charge of proteins, being more negative at basic pH and thus retaining larger amounts of water (Toldra, 2003). During the conversion of muscle to meat, the pH of muscle changes from neutral (7) to about 5.5 to 5.7 (Cheng & Sun, 2008; Toldra, 2003). The normal pH of post-rigor muscle and lactic acid build up in the tissue will lead to a reduction in pH of the meat (Lonergan & Lonergan, 2005). All proteins have a typical pH where the net electronic charge on the protein is zero (the number of positive and negative charges are equal). pH of meat proteins reach an isoelectric charge at pH is 5.0. At the isoelectric pH, the WHC is the lowest because there is minimal attraction between proteins and water. There is also tightening of the structure and partial denaturation of myofibrillar proteins.

# 2.2.2.6 Functional modifications in meat and meat products

A number of non-meat ingredients have used to improve the water holding capacity of meat based on their functional properties. Additionally, the use of additives for meat processing is one of the most effective approaches to utilize inferior meat such as dark, firm and dry (DFD) and pale, soft and exudative (PSE) meat, which appears to be a challenge to the meat industry. There are many different additives such as addition of salts, functional animal proteins, functional plant proteins, fish oils, vegetable oils and vegetable products that are added to meat to improve its water holding capacity and other qualities. In the following section, the commonly used additives for improving the WHC of meat products will be discussed. Since the present study has incorporated spaghetti with a meat emulsion containing salt, and navy bean, the effects of addition of salts and functional plant proteins on WHC will be discussed in detail.

### a) Addition of salts

Normally, the WHC of meat is minimal when the pH is just at the isoelectric point (5.0 to 5.3 in red meat) of meat proteins. On either side of the isolectric point, the ionic strength could be improved by adjusting the pH, leading to increased WHC of meat products. Several additives that have the ability to adjust the ionic strength of meat products include sodium chloride/salt, phosphates, lactic acid and sodium lactate. The most commonly used additive is sodium chloride.

Sodium chloride plays an important role in the solublization of myofibrillar proteins for subsequent denaturing/ aggregation to give good water retention and acceptable rigidity/elasticity of the meat gels (Gordon & Barbut, 1992). Cooking loss and weakening the texture (Ruusunen & Puolame, 2005). One hypothesis that explains the effect of sodium chloride in improving the WHC is the swelling of myofibrillar proteins that depend on the concentration of sodium chloride. Chloride ions tend to bind to the meat protein filaments and increase the electrostatic repulsive force between them. With increasing the repulsive forces, the protein structure matrix unfolds and then swelling occurs. The sodium ion "cloud" around the filaments, which results in local ion concentration differences leads to an increased osmotic pressure within the myofibrils causing the filament lattice to swell. The swelling provides a higher number of protein side chains to bind water, which in turn improves WHC of meat (Hamm, 1961).

In the present study meat and navy bean was added to spaghetti. Meat added into the spaghetti was in the form of an emulsion in order to get a consistent dough mixture. The meat emulsion was prepared by adding water and salt to it. In this study, sodium chloride was added to increase the water binding capacity of meat as NaCl due to its charge results in increased swelling (Toldra, 2003; Offer and Trinick, 1983).

#### 2.3 Sensory evaluation

One of the most important goals of the food industry is to determine how food products affect consumer's senses. Since our five senses (sight, smell, taste, touch and hearing) act as the gatekeeper of our bodies, the benefits of healthy food will be reaped only if our senses accept it. Therefore, consumer reaction, as perceived by the five senses, is considered an important measure of food quality. Consumer acceptability evaluation can provide the most important and dependable information as only consumers can accurately indicate the degree of liking or preference for a product.

### 2.3.1 Projective mapping

The important role of consumer input for product development, advertisement, marketing positioning and communication led to the development of a number of methods to gather information about consumers' perceptions of the sensory characteristics of food products (Torri et al., 2013). Lately, alternatives to conventional and traditional descriptive analysis methods (generic descriptive analysis (DA)) have gained more interest. All these alternate methods try to overcome the drawbacks from DA, which are (i) longer time to obtain results due to the need of panellist training, and (ii) obtain consensus on particular attributes, which at times induces a difficult task when working with expert judges like chefs and wine professionals. One of the alternatives to DA that was developed in the early nineties by Risvik et al. (1994, 1997) was 'projective mapping' (PM). Projective mapping is a comparative sensory technique that allows consumers to evaluate products in an overall and simple way by expressing perceptual similarities/ dissimilarities in a two dimensional projection (Torri et al., 2013).

Perceptual mapping is a technique that shows the relationship among multiple products in a visual way (Lawless & Heymann, 1998). It is used mainly when information about product relationships is needed, and in some cases the linking of attributes to those relationships (Kennedy & Heymann, 2009). In these maps, complex multivariate information is broken down to important dimensions for easier interpretation, usually only two or three, which can then be easily graphed. Products that are similar on a given dimension are pictured close to each other and products that are dissimilar are pictured further apart. One of the important uses of perceptual mapping is in strategic research and competitive analysis, where some or all products in a similar group are compared (Nestrud & Lawless, 2010).

Risvik et al. (1994, 1997) developed projective mapping that involves subjects placing products on a two-dimensional space according to similarity. The more similar two objects are perceived, the closer they are placed on the map and the product coordinates on the two-dimensional space quantify their separation (Risvik et al., 1994). Projective mapping is considered to be a simpler and faster way to obtain product inter-distances than similarity scaling (Risvik et al., 1997) and provides better product differentiation than sorting (King et al., 1998). Configurations from projective mapping can provide similar product maps as those from descriptive data and similarity scaling (Risvik et al., 1994). In addition, projective mapping configurations are more consistent over replications than descriptive analysis and similarity scaling (Risvik et al., 1994, 1997). An advantage of projective mapping over descriptive analysis is that it can be used to evaluate the importance of product attributes to consumers (Kennedy & Heymann, 2009).

Perceptual maps are generated from projective mapping data by multidimensional analysis methods (Multidimensional Scaling (MDS), Generalized Procrustes Analysis (GPA) and Principal Component Analysis (PCA)) (Risvik et al., 1994). Recently projective mapping was re-introduced method under the name "Napping" by Pagès (2005) along with a new way to analyse the obtained maps by Multi-Factor Analysis (MFA). MFA is a version of PCA on different datasets that are horizontally merged and standardized. MFA helped uncover more than two dimensions in the data based on how panellists considered the different attributes. For instance, if half of the panellists grouped the products according to taste and texture, and the other half grouped based on taste and appearance, then the MFA would come up with a group configuration with three dimensions, with 50% of variance coming from taste, 25% from texture and 25% from appearance (Lawless & Heymann, 1998).

Table 2.3.1 shows how different food products have been characterized based on their sensory properties using projective mapping. Several studies were carried out to compare projective mapping either with descriptive analysis (DA) method, sorting or other sensory methods (conventional profiling, flash profiling) as shown the Table 2.3.1. From the different studies summarize, it can be seen that projective mapping has been used either in combination with other sensory methods. In all cases, projective mapping has been shown to produce accurate results/ similar results with the other methods, such as sorting, descriptive analysis and in some cases conventional profiling and flash profiling. Results from projective mapping also correlated well with the results obtained from other sensory methods. Furthermore, projective mapping can be used on its own to obtain accurate results and attributes to describe products. This can be seen in the case of studies using granola bars (Kennedy, 2010), Italian red wine (Torri et al., 2013) and Ewes milk cheese (Barcenas et al., 2004), in which maps showed similarity in all consumers (whether it was a trained panel, panel of experts or untrained panel). Hence this method has been employed in the present study where consumers were required to separate/ group spaghetti enriched samples containing meat and navy bean according to their similarity and differences.

Products	Number of samples	Objective	Number of panellists	Findings	Reference
Milk and dark chocolates	14	To compare results using PM and DA Untrained panel was used for PM. Once completed, the same panel was trained for DA and results were compared	3 groups (9, 9 and 8 participants)	Untrained judges for PM provided equivalent maps as data obtained by DA Similarity among panels show that overall the panellists perceived the product in a similar manner	Kennedy & Heymann, 2009
Apples and cheeses	10,10	To compare results obtained from PM and sorting	19 & 21 untrained panellists	PM was better suited than sorting. Maps were similar for both PM and sorting. 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 PM and evaluate the consistency of results obtained from 3 different sessions	1 untrained panel (15 participants)	Maps showed similarity in all consumers. However, maps showed that the products were perceived similarly in terms of how the products were grouped.	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 PM	1 trained panel (9 subjects), 1 expert panel (13 subjects) and 81 consumers	<ul> <li>Product separation by experts was mainly based on overall quality rather than specific sensory differences</li> <li>Product differences by consumers was poor and worse than that of experts and trained subjects.</li> <li>Consumer's maps showed good sample separation based on liking data and allowed identification of the aroma attributes.</li> </ul>	Torri et al., 2013
Ewes milk cheese	8	To compare maps obtained from PM by trained panel and naïve consumers	8 trained and 12 untrained panellists	<ul> <li>Trained panel got better performance quality index than consumer panel.</li> <li>Overall both panel sample configurations followed similar trends</li> </ul>	Barcenas et al., 2004
Citrus juices	11	To obtain maps using PM from chefs and consumer groups and to examine the patterns of response among chefs and consumers using napping	14 chefs and 16 consumers	<ul> <li>Nappe configurations were similar with a group, but between groups was less similar</li> <li>A good correspondence of scaled attribute results to nappe results for consumers, but less for the chefs</li> </ul>	Nestrud & Lawless, 2008

Table 2.3.1 Studies carried out using sensory projective mapping.

Smoothies	8	To obtain dating by combining PM with sorting (sorted napping)	1 panel (24 subjects)	<ul> <li>Combing PM with categorization using sorting task to group similar samples showed similar results from the subjects</li> </ul>	Pagès et al., 2010
Blueberry soups	7	To compare maps obtained from PM using naïve consumers and DA from a trained panel	DA was carried out 12 trained participants)& PM by 8 consumers	• Mapping replicates showed visually similar maps although RV coefficients indicated that panellists perceived products differently which recommended the dimensionality of consumer perception compared with trained panelists	Risvik et al., 1997
Chocolates	5	To compare maps obtained from PM, conventional profiling and dissimilarity scaling techniques9 untrained subject		Higher consistency was obtained over repeated trials from PM compared with the other two methods	Risvik et al., 1994
White wine	10	To determine the dimensions of perception of a panel about a set of Touraine wines	PM carried out by 11 professionals & DA by 8 experts	<ul> <li>Possible to show how the importance given to dimensions of perception differ from one subject to another</li> <li>Recommends DA to be carried out to obtain attributes</li> </ul>	Pagès, 2005
Fish nuggets	9	To compare and study the three sensory methods in case of hot served foods with contrasting textural layers	Conventional profiling- 10 subjects, flash profiling- 10 semi-trained participants) & PM- 20 untrained participants)	<ul> <li>Maps obtained by the three methods were well correlated</li> <li>These methods could be used as a tool in consumer research with the use of an untrained panel</li> </ul>	Albert et al., 2011

# **Chapter 3: Materials and Methods**

# 3.1 Spaghetti sample

# **3.1.1 Raw Spaghetti Preparation**

High-grade flour (Homebrand), semolina fine (Sun Valley Foods) and eggs were the main ingredients used to make spaghetti. In addition, olive oil and salt were used. These ingredients were purchased from a New Zealand supermarket. AgResearch Ltd. New Zealand, provided lamb meat obtained from a New Zealand farm (6-9 months old). Dried haricot (navy) beans (*Phaseolus vulgaris*) were purchased from a New Zealand supermarket. Signature Range fresh egg spaghetti (Signature, New Zealand) was chosen as a commercial sample that was compared with laboratory prepared samples.

Lamb meat (leg of lamb with bone- hind limb) was first cut and then minced in order to make a lamb emulsion. A lamb meat emulsion containing 56.82% meat and 0.57% salt was prepared. This meat emulsion was made up of minced lamb (200g), water (150g) and salt (2g). The mixture was homogenized at 7000 rpm for 20 minutes using a homogenizer (L5M-A Laboratory Mixer, Silverson®). The meat emulsion was stored at 4°C and used within the same day of preparation.



Fig 3.1: Ingredients used to make spaghetti



Fig 3.2: Lean Lamb meat used to make an emulsion using a homogenizer for incorporation in to the spaghetti dough mixture

Navy beans were soaked overnight and boiled for 2 hours in order to deactivate the trypsin inhibitor (Wagner & Riehm, 1967). The cooked beans were then drained, cooled and put in a dryer overnight. The dried beans were then finely ground using a food processor (900 Watts motor FP734, Kenwood) for 20 minutes at high speed. High grade flour, semolina, lamb meat emulsion, navy bean powder and were combined according to the formulation in Table 3.2.1 to make spaghetti dough using a dough maker (Breville, BBM400). In addition to these ingredients 0.2g of oil and 0.5g of salt were also added. The dough was then cut into two halves and passed through the rollers of the pasta machine where it was pressed into sheets. The knob on the pasta machine was turned to widen or narrow the opening between the rollers, which were set at different positions (starting from position 7 and ending at position 4). The pressed sheet was then fed through the cutting rollers of the pasta machine to slice the sheet into ribbons or strings to obtain spaghetti strands. Spaghetti was cooked within 30 minutes to an hour of preparation. The spaghetti samples were cooked by adding it to a pot containing boiling water with a little bit of olive oil and salt added.



Fig 3.3: From the left top to the right bottom- pasta dough, flattening of dough followed by passing the flattened dough through the spaghetti strand maker.

# **3.2 Experimental design**

This study is the first study that has incorporated red meat in spaghetti. After a few preliminary trials with different amounts of meat emulsions added in spaghetti, a full factorial experimental design was generated. The experimental design with meat emulsion (40, 50, 60 and 70 g) and navy bean powder (0, 10, 20 and 30 g) at 4 levels using Minitab (v16, Minitab Inc.) was produced. Semolina flour (10 g), an egg, and varying amounts of high grade flour, meat and navy bean were used in the spaghetti formulations. A formulation containing 70 g high grade flour and 30 g semolina flour was used as control (that is containing no meat or bean). In the full factorial design used in this study four meat levels (28g, 36g, 43g and 50g) and four bean levels (0g, 7g, 14g and 21g) used. However for the spaghetti formulation containing 43g of meat only three bean levels (0g, 7g and 14g navy bean) were used as shown in Table 3.2.1. This is because the pasta dough obtained during the preparation of spaghetti containing a mixture of 43g meat emulsion with 21g navy bean could not form spaghetti strands when passing the dough between the rollers of the pasta machine and became lumpy instead. The actual amount of meat and navy bean used in the formulation has been summarized in the following table (Table 3.2.1).

Serial	Samples	Meat	Navy	High	Semolina	Egg	Oil	Salt	Actual	Actual
No.		Emulsion	Bean	Grade	<b>(g)</b>	<b>(g)</b>	<b>(g</b> )	<b>(g)</b>	percentage	percentage
		<b>(g)</b>	<b>(g)</b>	flour					of meat	of navy
				<b>(g)</b>					(%)	bean (%)
1	28M	40	0	50	10	40	0.2	0.5	28	0
2	28M7B	40	10	40	10	40	0.2	0.5	28	7
3	28M14B	40	20	30	10	40	0.2	0.5	28	14
4	28M21B	40	30	20	10	40	0.2	0.5	28	21
5	36M	50	0	40	10	40	0.2	0.5	36	0
6	36M7B	50	10	30	10	40	0.2	0.5	36	7
7	36M14B	50	20	20	10	40	0.2	0.5	36	14
8	36M21B	50	30	10	10	40	0.2	0.5	36	21
9	43M	60	0	30	10	40	0.2	0.5	43	0
10	43M17B	60	10	20	10	40	0.2	0.5	43	7
11	43M14B	60	20	10	10	40	0.2	0.5	43	14
12	43M21B	60	30	10	0	40	0.2	0.5	43	21
13	50M	70	0	20	10	40	0.2	0.5	50	0
14	50M7B	70	10	10	10	40	0.2	0.5	50	7
15	50M14B	70	20	10	0	40	0.2	0.5	50	14

Table3.2.1. Formulation of spaghetti samples with varying amount of meat emulsion and navy<br/>bean using a full factorial design.

# **3.3 Physicochemical Analysis**

All the physicochemical test trials were carried out on different spaghetti samples made from two different batches each consisting of 15 different formulations.

#### **3.3.1** Moisture content

Moisture content was analyzed using the oven drying method according to AOAC 945.15 (AOAC, 2000). Cooked spaghetti samples (25g) were dried in an oven at 105°C until constant weight (dry weight) was achieved.

Percentage moisture was calculated using the formula:

% Moisture= ((moisture loss in grams)/original weight of sample) x 100

Each analysis was done in triplicates and the mean was taken.

# **3.3.2** Cooking quality

# **3.3.2.1** Cooking time

Cooking time for fresh egg spaghetti samples was estimated according to AACC method 66-50.01 (AACC, 2005). The cooking time for spaghetti was determined by adding 25g of sample into a 500mL beaker containing boiling water. Strands of pasta were stirred to separate while boiling. The cooking water was maintained to at least 90% of its original volume. A strand of pasta was removed from the cooking water at 30-second interval and squeezed to check if it is cooked. The time when the white center of the sample just disappeared was designated as "cooking time". Cooking time was carried out in triplicates and the mean values were reported.

### 3.3.2.2 Cooked weight

The spaghetti samples were cooked and drained (Fig 3.4). Cooked weight was determined by weighing the drained pasta. The average means of triplicates were reported in g.

#### **3.3.2.3** Cooking Loss

Cooking loss was measured according to the AACC method 66-50.01 (AACC, 2005). Twenty-five grams of spaghetti were broken down into a length of approximately 5cm and cooked in 300mL of boiling distilled water. Spaghetti was cooked to its optimal cooking time with occasional stirring. After cooking, the sample was rinsed with a stream of distilled water (around 50mL) for about 30 seconds and then allowed to drain for 2 minutes. The total volume of gruel and rinsed water was collected in a beaker and dried in an oven at 100°C to achieve constant weight for approximately 20 hours. The beaker was cooled, weighed, and the cooking loss value calculated.



Fig 3.4: Determining cooking quality of pasta

# 3.3.3.3 Swelling Index

Swelling index of cooked spaghetti (grams of water per gram of dry spaghetti) was determined according Cleary and Brennan (2006). The spaghetti samples were cooked at the optimum cooking time and dried at 105°C until a constant weight was reached. The swelling index was expressed as:

SI= (Weight of cooked pasta- Weight of pasta after drying)/ Weight of pasta after drying

Three measurements were performed to obtain mean values.

# 3.3.3 Texture analysis

Texture analysis was performed using a Stable Micro Systems TA.XT plus Texture analyzer (Stable Micro Systems, Surrey, UK) equipped with tensile rigs (A/SPR) on a heavy duty platform (HDP/90). Prior to texture analysis, the samples were cooked at their optimal cooking time. After that, they were rinsed, drained and analyzed within 20 minutes, being at room temperature at the time of analysis. Texture parameters were measured under tension using the tensile grip A/GT and a 5 kg load cell (Table 3.1).



Fig3.5: Texture Analyser instrument using tensile rigs for determination of tensile strength of spaghetti. The instrument was connected to the computer for data collection.

Parameter	Setting				
Test Mode	Tension				
Pre-Test Speed	1.00 mm/sec				
Test Speed	3.00 mm/sec				
Post-Test Speed	10.00 mm/sec				
Target Mode	Distance				
Distance	100.0 mm				
Trigger Type	Auto (Force)				
Trigger Force	5.0g (Meat samples)				
Trigger Force	1.0g (Meat and Bean sample)				

Table 3.2.2 Instrumental settings on TA

The grips were tightened with the same distance (return distance of 15mm) between them which was the same distance before and after the running of the texture analyzer. Texture profile data was collected from the texture analyser using the Texture Exponent software (Stable Micro Systems, Surrey, UK), version 6 supplied with the texture analyser as shown in Fig 3.2. The apparent fracture stress/tensile strength (T= F/A), Hencky strain ( $\epsilon_h$ = ln L/ L<sub>o</sub>) and the Young's modulus (E<sub>u</sub>= Stress/Strain) were determined.

Where,

F= the extension force,

A= cross sectional area of the spaghetti sample,

L<sub>0</sub>= original length,

L= the current length

The fracture stress (Pa), Hencky strain ( $\epsilon$ ) and Young's modulus (Pa) are related to the strength, extensibility and elasticity of the noodles respectively (Walstra, 2003). Reported values are an average of the samples measured in five replicates.

# 3.3.4 Color analysis of cooked spaghetti

In food industries, the most commonly used measurement instruments are based on the color- space system L\*, a\*, b\* as defined by the Commission Internationale de l'Eclairage (CIE, 1986). Within this system, L\* measures the lightness of the color of the sample, a\* measures the red and green characteristics and b\* measures the yellow and blue characteristics (McCaig, 2002). Hunter L, a, B and CIE L\*, a\*, b\* are both color scales based on the Opponent- Color Theory (Hunter & Harold, 1989; HunterLab, 2012). According to this theory, the receptors in the human eye perceive color as the following pairs of opposites.

- L scale: Light vs. dark, low number (0-50) indicates dark and high number (51-100) indicates light.
- a scale: Red vs. green, a positive number indicates red and a negative number indicates green.
- b scale: Yellow vs. blue, a positive number indicates yellow and a negative number indicates blue.

Color was determined using a Hunter Lab (45/0, Colorflex) color analyzer. Cooked spaghetti strands were placed in a small disposable petridish over the 2cm wide sample port with black background. Readings were taken in triplicates for each sample and recorded as  $L^*$  (lightness), a<sup>\*</sup> (green to redness) and b<sup>\*</sup> (blue to yellowness) (Fig 3.6).



Fig 3.6: HunterLab color analyzer used for determining enriched spaghetti color

# 3.4 Nutritional composition of spaghetti samples

Tests for determining the nutritional composition of spaghetti were carried out on all the fifteen spaghetti formulations (Table 3.2.1).

# 3.4.1 Fat analysis

Total fat content of spaghetti was analyzed using the Soxhlet extraction method (AOAC, 2000). A pre-dried sample (5g) was ground into a fine powder and put in an extraction thimble that was porous enough to permit a rapid flow of solvent. The sample was covered with glass wool. The weight of a pre-dried round bottom flask was recorded. Petroleum ether (100mL) was then added into the flask. The round bottom flask, Soxhlet flask and condenser were assembled as shown in Fig 3.7. Lipid was extracted using a Soxhlet extractor at a rate of five or six drops per second by condensation for approximately 5 hours, by heating the solvent in the round bottom flask.

The bottom flask with the extracted fat was dried in an air oven at 100°C for 30 minutes, cooled in a desiccator and then weighed.

%Fat (dry basis) = ((grams of fat in sample)/ grams of dried sample) X 100



Fig3.7: Fat extraction by the Soxhlet method

# 3.4.2 Total Protein Analysis by the Kjeldahl method

A ground sample (500mg) was accurately weighed, wrapped in a nitrogen-free paper and then placed in a 250mL digestion tube. A mixture of potassium sulphate (7g) and copper sulphate (0.5g) was used as catalyst. Concentrated sulphuric acid (12mL) was added and the tube contents were carefully mixed prior to digestion. The sample was digested at 420°C for 60 minutes using a Velp DK 20 heating block. The level of liquid was monitored during the digestion process, and in case there was a significant drop in the level, an extra 5-10mL of concentrated sulphuric acid was added. The tube was allowed to stand after 60 minutes to cool for 5 minutes and 3mL of cold 30% hydrogen peroxide was added. The liquid in the test tube should be clear and colorless. If the liquid was cloudy or had retained a color, a further 3mL of 30% peroxide was added and the mixture was digested for 20 minutes. This was repeated until the solution remained colorless or clear.



Fig 3.8: VelpUDK 139 distillation unit used for protein analysis using the Kjeldahl method

After digestion, the sample was distilled using a VelpUDK 139 distillation unit (Fig 3.8). The digestion tube containing the sample was attached, and the automatic distillation process started. The digest was made alkaline with 50mL of 35% sodium hydroxide solution, and the released ammonia was steam distilled into a receiver filled with 20mL of 4% boric acid. When the distillation process was completed, 10 drops of mixed Kjeldahl indicator was added to the receiver flask and the contents were titrated with 0.1mol/L standard hydrochloric acid. Nitrogen content was calculated according to the equation below:

Nitrogen content (mg/g) = ((v1-v2) x c x 14) w

Where,

v1= titrated volume of standard acid for sample in mL,

v2= titrated volume of standard acid for reagent blank in mL,

c= concentration of standard hydrochloric acid in mmol/mL,

14= Molar mass of N in mg/mmol,

w= sample weight in g

# 3.4.3 Ash analysis

The ash content of the spaghetti samples was determined according to AOAC method, 942.5 (AOAC, 2000). Cooked spaghetti samples were dried and ground before placing the samples into a crucible that was placed in a cool muffle furnace (Model 200, McGregor Kiln Furnace). The sample was ignited for 8 - 10 hours at 550°C. After the ashing was completed, the muffle furnace was turned off and cooled down before removing the samples. The door was carefully opened to avoid loss of ash. Safety tongs were used to transfer crucibles to a desiccator with a porcelain plate and desiccant. Crucibles were cooled down in the desiccator prior to weighing. The cool crucible containing ash was weighed. The ash content was calculated as follows:

% ash (dry basis) = ((Weight after ashing-tare weight of crucible)/ dried weight of sample) x 100



Fig3.9: Muffle furnace set at 550°C for ashing of spaghetti samples

# 3.4.4 Carbohydrate content calculation

The proximate carbohydrate content was estimated according to Fraser and Holmes (1958). The carbohydrate content in samples was determined by subtracting the total fat, ash and protein content from 100% as shown below.

% Carbohydrates= 100- (% moisture+% ash+% fat+% protein)

# **3.5 Sensory Evaluation**

Sensory analysis was carried out on all the 15 different spaghetti samples including the control and commercial samples as well. Sensory analysis involved consumer testing and sensory projective mapping. The Auckland University of Technology Ethics Committee approved an ethics application for this study (Application number - 13250\_30072014 on 16 September 2013 as shown in the Appendix 3.

# 3.5.1 Consumer testing and projective mapping of spaghetti samples

All fifteen samples were used for consumer sensory testing along with the control and commercial sample. All the samples were prepared fresh and cooked one day before sensory testing. Likewise the commercial sample purchased from the supermarket followed by cooking it and refrigeration. The spaghetti samples were heated an hour prior to testing in the oven (Elba, Fisher & Paykel) and set at 30°C using the "warmer" knob function to provide warm and humidified air to keep the samples warm.

The spaghetti samples were served with Leggo's tomato pasta sauce with Italian herbs and basil flavor. In the pilot run the sauce was reported to be quite thick and masked the flavor of spaghetti. Therefore, the sauce (700g) was diluted with water (300g). The sauce was heated in a slow cooker (Goldair, 5Litre) with the knob set on the "warm" function of the cooker.

Approximately 6 g of each spaghetti sample together with 5mL pasta sauce was served individually in 25mL paper cups coded with three-digit random numbers. The order of samples was randomized to avoid sample order and carry-over effects (Macfie et al., 1989). Regular room temperature water was served as a palette cleanser and the sensory test was carried out under red light to mask the color differences among samples allowing panelists to concentrate more on taste, flavor and texture attributes.

#### **3.5.2** Consumer Testing

Consumer sensory evaluation of the fifteen different spaghetti samples along with the control and commercial pasta was carried out in order to evaluate the acceptability of the enriched products. The sensory consumer test was performed twice a week over a period of 3 weeks. Consumer preference testing was performed as follows. Consumers were mainly students and staff members from the University campus who consumed spaghetti at least three times a month. The sensory test sessions were carried out at the AUT Sensory Lab. Panelists were given verbal instructions before entering the sensory booths on how about the tests should be carried out. The same set of instructions was also displayed on the computer terminals using the FIZZ software (FIZZ Network v2. 46C, Biosystemes, France). Panelists were asked to taste 6 different samples each time a day. The samples were randomized and coded and the test was carried out in a sensory room (Meilgaard et al., 2006). A 15 minute break between each sample was imperative. The samples were rated in terms of overall liking, flavor, odor, taste and texture. A 100mm unstructured line scale was used to rate the samples where extremely dislike was labelled on the left and extremely like was labelled on the right and neither like nor dislike was in the middle to indicate the liking of products by consumers. The questionnaire for consumer testing is attached in Appendix 1.



Fig 3.10: Consumer testing of spaghetti samples

# **3.5.3 Projective Mapping**

Like consumer testing, projective mapping was carried out on eight different spaghetti samples. The samples chosen for projective mapping were decided after analyzing the consumer testing results using one-way ANOVA. Consumer test results obtained by ANOVA showed that meat with low bean content (28%M 7%B, 36%M 7%B and 43%M 7%B) was highly preferred by consumers followed by commercial, 50%M and control. Samples containing high amounts of bean with meat were not highly preferred by consumers. Based on these results, eight samples that were acceptable and unacceptable were chosen for further sensory projective mapping to understand why consumers liked or disliked these spaghetti samples. The sensory projective mapping sessions were carried out at the AUT Sensory Lab on three consecutive days over a period of 3 weeks. Verbal instructions were given to panelists before entering the sensory booths. The same set of instructions (Appendices 1 and 2) was also displayed on the computer terminals using a FIZZ programmed sensory projective mapping test (FIZZ Network v2.46C, Biosystemes, France). Panelists then tasted the randomized and coded samples in the sensory booths (Fig 3.10).

Panelists grouped the samples according to their similarities and differences with those grouped close together being 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 panelists and recorded using the FIZZ Network v2.46C system.

#### **3.6 Statistical analysis**

#### **3.6.1** Physicochemical analysis

The experiment was carried out in two different batches and each batch had triplicate runs. All univariate analysis in this study was analyzed using XLSTAT-MX version 2012.4.02 (Addinsoft, U.S.A). One way analysis of variance (ANOVA) was examined for significance and in case of significance, mean separation was accomplished by Tukey's post-hoc comparisons.

Two-way unbalanced ANOVA using XLSTAT was used to determine the interactions of meat, and navy bean addition on the physicochemical characteristics of spaghetti. As one of the samples (50M 21B) obtained from the full factorial design was not included in the analysis as the dough formed was lumpy and could not form spaghetti strands, the experiment is a typical example of an unbalanced ANOVA. The results shown are as average  $\pm$  standard deviation along with Tukey's letters of significance for triplicate measurements.

### **3.6.2** Consumer testing analysis

The consumer test data of seventeen enriched spaghetti samples including the control and commercial samples collected over a period of three weeks was analysed by one-way analysis of variance (ANOVA) using Addinsoft XLSTAT-MX version 2012.4.02 (Addinsoft, U.S.A). The results presented in Chapter 4 are presented as average  $\pm$  standard deviation along with Tukey's letters of significance.

### 3.6.3 Projective Mapping analysis

Analysis of results was performed using Multifactorial Analysis (MFA) to obtain overall product maps. General Procrustes Analysis (GPA) was carried out to obtain overall product coordinates and Principal Component Analysis (PCA) was used to obtain product and attribute biplots using Addinsoft XLSTAT-MX version 2012.4.02. Sensory attributes that occurred a minimum of five times across panelists per product were included in the PCA biplots.

## **Chapter 4: Results and Discussion**

#### 4.1 Physicochemical evaluation of spaghetti

## **4.1.1** Cooking quality

Pasta quality is influenced by a range of characteristics: physical, chemical, textural and nutritional. The quality of cooked pasta and its texture is considered to be of great importance to the overall quality of pasta in determining consumer acceptability (Tudorica et al., 2002; Sissons & Hare, 2002). This includes optimal cooking time, swelling index or water uptake during cooking, stickiness, aroma and taste.

## 4.1.1.1 Optimal cooking time

The cooking attributes of pasta are related to gelatinization rates and chemical composition of the pasta used (Tudorica et al., 2002). Cooking time is an important factor for pasta quality. Optimum cooking time is the actual time at which the ungelatinised core of the spaghetti disappears (Gregor, 2005). This state is also referred to as 'al dente'. The optimum cooking time depends primarily on two phenomena: water absorption and starch gelatinization (Edwards et al., 1993).

Cooking times of spaghetti samples based on different percentages of meat, navy bean and combination of meat and navy bean are reported in Table 4.1.1. The obtained results showed that pasta enriched with meat, navy bean, and combinations of meat and navy bean were significantly (P<0.0001) different than commercial pasta. The enriched spaghetti samples had an optimal cooking time between 2.5 and 4 minutes.

Samples containing a combination of meat and higher bean content (14 % and 21%) had slightly shorter cooking time than the control. This could be related to the formation of a weaker gluten network as a result of a dilution effect on gluten (Gallegos-Infante et al., 2010). Similarly Gallegos- Infant et al. (2010) reported that spaghetti containing common bean had different optimal cooking time, and a decreased cooking time compared to the control. This could be related to the differences between semolina batches or the mixing conditions used. Ferreira et al. (2004) reported a diminution of cooking time in pasta products made from wheat and soya. The higher the substitution level of soya bean (10% to 40%), the shorter cooking time became. However, Zhao et al. (2005) reported that spaghetti made with green and yellow pea, lentil and chickpea flours showed an increase in cooking time. This could be due to the presence of fiber in peas that took more time for water penetration to gelatinize the core thus resulting in increase in the cooking time. Similarly increased cooking time was observed in pasta enriched with white chickpea flour and defatted soy flour (Bashir et al., 2012). Besides being high in protein, chickpea and soybean both contained high amounts of fiber and this resulted in an increased cooking time. Bahnassey et al. (1986) found that supplementation of durum wheat with a variety of legume flours and concentrate increased the cooking time of pasta from 1 to 10 minutes longer than the control. Such an increase in cooking time may be due to the slow water penetration into pasta with greater quantities of protein and therefore more time required to gelatinize the core (Marshall & Wasik, 1974; Resmini & Pagani, 1983).

Spaghetti samples with meat on the other hand had a slightly higher cooking time (Figure 4.1.2) than the control but this was not significant. In contrast, Kadam & Prabhasankar (2012) reported that with the addition of shrimp meat, cooking time of spaghetti was however significantly higher than control.

## 4.1.1.2 Cooked weight

Cooked weight of pasta is basically the amount of water spaghetti absorbs during the cooking process. It is expressed as the cooked spaghetti weight in relation to the dry spaghetti weight (Faure & Feillet, 1989). This measurement corresponds to the mass of the meal available to the consumer (yield). Generally, a cooked pasta sample will weigh three times its precooked weight. A low cooked weight indicates a higher volume of gruel and high swelling ability of starch (Kadam & Prabhasankar, 2012). Cooked weight of the spaghetti samples enriched with meat, navy bean, and a combination of the two are shown in Table 4.1.1. The enriched spaghetti samples had a significantly higher (P<0.0001) cooked weight or water absorption capacity as compared to the control and commercial pasta.

The higher cooked weight of the enriched spaghetti samples in our study was also associated with high moisture content of samples (Table 4.1.1). This was probably due to the higher water holding capacity of meat during dough formation. Since the meat used is lean cut lamb meat, it contains higher water content than a fattier cut meat (USDA, 2011). On the other hand as the percentage of bean in the spaghetti samples increased, cooked weight decreased in samples containing a combination of meat and bean. These results are in agreement with findings from Gallegos-Infante et al. (2010) that reported a decrease in water absorption along with cooking time when spaghetti was made with common bean. Similarly Nielsen et al. (1980), reported lower water absorption/cooked weight for spaghetti containing 33% pea flour or 20% air- classified pea protein concentrates. Breen et al. (1977) also showed that the cooked weight of spaghetti made from a bean formula was lower than that of the control. Cooked weight also decreased in spaghetti containing different legumes such as navy bean, pinto and lentils (Bahnassey and Khan, 1986).

#### 4.1.1.3 Cooking Loss

Cooking loss is the material released from spaghetti during the cooking process. It is a reflection of spaghetti breakdown during cooking and is greatly correlated to overall spaghetti quality (D'Egidio & Nardi, 1996), protein content and starch damage (Resmini & Pagani, 1983; Matsuo, 1988). Cooking loss is an important indicator of the overall spaghetti cooking performance by both consumers and industry (Brennan et al., 2004). Cooking loss occurs when gelatinized starches are dissolved and released from the surface of pasta into the cooking water. The inclusion of meat and bean increased cooking losses of spaghetti significantly (P<0.0001), presenting values between 6-10%. Babiker et al. (1990) reported that lamb meat has a high water holding capacity (2.84%) and high cooking loss (36.6%). Similarly Kadam and Prabhasankar (2012) reported that the addition of shrimp meat in spaghetti increased cooking loss from 6% in control to 8% in the sample containing highest substitution of shrimp meat (30% shrimp meat).

A significant increase in cooking loss was also observed when bean was added, especially in high bean content samples (14% and 21%). Zhao et al. (2005) who produced spaghetti made with green and yellow pea, lentil and chickpea flours reported similar results. The use of ingredients other than semolina produces an increase in cooking loss, because the gluten network becomes weaker and the starch leaches more easily into the cooking water as reported by Ovando-Martinez et al. (2009) who researched on pasta made with unripe banana flour. Increase in cooking loss was also observed by Tudorica et al. (2002) when pea fiber was added to pasta. This was due to the disruptive effect of pea fiber inclusion had on the protein matrix which allowed starch granules to rupture during cooking, hence releasing high levels of amylose into the cooking water. Therefore, addition of both meat and bean contributed to cooking losses in this study.



Figure 4.1.1 Cooking loss of spaghetti samples containing meat and bean. From left to right 28M 7B, 49M 14B and 42M 21B

## 4.1.1.4 Swelling Index

Swelling index (SI) of pasta is an indicator of the water absorbed by the starch and proteins during cooking, which is utilized for the gelatinization of starch and hydration of proteins. Swelling index is expressed as grams of water per gram of dry spaghetti (Chillo et al., 2010). In this study, a higher swelling index was observed in the samples containing a combination of high bean content (14% and 21%) and meat. As the bean content increased, the swelling index increased significantly (Figure 4.1.5). The presence of fiber in bean can increase water-binding capacity and result in a high swelling index as compared to meat. Samples containing only meat at different substitution levels showed no change in swelling index. Similarly, an increase of 2.9 to 3.0 times in swelling index was reported in spaghetti fortified with buckwheat, amaranth and lupin flours (Rayas-Duarte et al., 1996), and pasta fortified with pea fibers (Tudorica et al., 2002).



Figure 4.1.2 Cooking time of the spaghetti samples containing meat (28%, 36%, 43% and 50%) with increasing navy bean content (7%, 14% and 21%) along with control and commercial pasta. Each column represents 100% of the sample. Samples are expressed as meat and navy bean content, e.g., 283M 7B refers to the sample containing 28% actual meat and 7% actual bean powder.

\*Different letters above bars are significantly different (p < 0.05).



Figure 4.1.3 Cooked weight of the spaghetti samples containing meat (28%, 36%, 43% and 50%) with increasing navy bean content (7%, 14% and 21%) along with control and commercial pasta. Each column represents cooked weight of 25g of the sample.

\*Different letters above bars are significantly different (p < 0.05).



Figure 4.1.4 Cooking loss of spaghetti samples containing meat (28%, 36%, 43% and 50%) with increasing navy bean content (7%, 14% and 21%) along with control and commercial pasta. Each column represents 100% of the sample.

\*Different letters above bars are significantly different (p < 0.05)



Figure 4.1.5 Swelling index of the spaghetti samples containing meat (28%, 36%, 43% and 50%) with increasing navy bean content (7%, 14% and 21%) along with control and commercial. Each column represents 100% of the sample.

\*Different letters above bars are significantly different (p < 0.05).

Interaction	Sample	Cooking time (min)	Cooked weight (g/25g)	Cooking loss (%)	Swelling index (g water/g dry pasta)
	Control	3.00±0.00 <sup>cdef</sup>	$52.28{\pm}0.88^{\mathrm{i}}$	4.22±0.13 <sup>h</sup>	2.01±0.05 <sup>def</sup>
	Commercial	7.75±0.65ª	$57.25 \pm 0.72^{efg}$	3.21±0.13 <sup>i</sup>	2.63±0.09ª
	28M	3.00±0.00 <sup>cdef</sup>	59.28±0.94 <sup>de</sup>	6.10±0.09 <sup>fg</sup>	1.79±0.04 <sup>fg</sup>
	28M 7B	2.50±0.58 <sup>ef</sup>	58.05±0.46 <sup>e</sup>	6.71±0.32 <sup>ef</sup>	2.35±0.08 <sup>bc</sup>
	28M 14B	2.50±0.58 <sup>ef</sup>	$55.25 \pm 0.71^{gh}$	7.81±0.22 <sup>d</sup>	2.30±0.13 <sup>bc</sup>
	28M 21B	2.50±0.58 <sup>ef</sup>	$54.16 \pm 1.61^{hi}$	9.95±0.06°	2.61±0.08 <sup>a</sup>
	36M	3.25±0.50 <sup>bcde</sup>	$60.42 \pm 0.43^{d}$	6.79±0.23°	1.96±0.09 <sup>efg</sup>
	36M 7B	3.00±0.00 <sup>bcdef</sup>	57.62±0.19 <sup>ef</sup>	7.88±0.24 <sup>d</sup>	2.22±0.12 <sup>cd</sup>
	36M 14B	2.50±0.58 <sup>def</sup>	$54.29 \pm 1.23^{hi}$	8.21±0.04 <sup>d</sup>	2.33±0.04 <sup>bc</sup>
	36M 21B	2.50±0.58 <sup>f</sup>	$52.53{\pm}0.40^{i}$	10.20±0.61 <sup>bc</sup>	2.47±0.08 <sup>ab</sup>
	43M	4.00±0.00 <sup>bc</sup>	64.30±0.35°	$6.05 \pm 0.06^{g}$	1.76±0.08 <sup>g</sup>
	43M 7B	3.25±0.50 <sup>bcde</sup>	59.30±0.60 <sup>de</sup>	6.48±0.09 <sup>efg</sup>	$1.96 \pm 0.04^{efg}$
	43M 14B	2.75±0.50 <sup>cdef</sup>	$55.49{\pm}0.54^{fgh}$	7.02±0.13 <sup>e</sup>	2.33±0.15 <sup>bc</sup>
	43M 21B	2.50±0.58 <sup>ef</sup>	$52.34{\pm}0.43^{i}$	10.84±0.16 <sup>a</sup>	2.50±0.13 <sup>ab</sup>
	50M	4.00±0.00 <sup>b</sup>	71.95±0.22ª	6.85±0.21 <sup>e</sup>	1.97±0.11 <sup>efg</sup>
	50M 7B	4.00±0.00 <sup>bcd</sup>	70.54±0.35ª	8.16±0.46 <sup>d</sup>	2.17±0.05 <sup>cde</sup>
	50M 14B	2.25±0.50 <sup>ef</sup>	68.06±0.29 <sup>b</sup>	10.73±0.12 <sup>ab</sup>	2.32±0.13 <sup>bc</sup>
Meat			F= 213.675****	F=172.460****	
Bean			F= 81.811****	F= 453.475****	
Meat*Bean			F= 3.570	F= 21.080****	

Table 4.1.1 Cooking qualities of spaghetti samples containing meat and navy bean (Mean±SD)

Level of significance: \*, p < 0.05; \*\*, p < 0.01; \*\*\*, p < 0.001; \*\*\*\*, p < 0.001 expressed in F value. Mean  $\pm$  SD of three repetitions on pasta produced at three different occasions; Mean values within a column with different superscripts differ significantly (p < 0.05) using one-way ANOVA and Tukey's test.

#### **4.1.2 Color measurements**

The appearance of food is one of the factors that define its quality and the first impression the consumer gets directly from foods (Fradique et al., 2010). Color is an important component of quality throughout the agricultural and food industries. Pasta color is a property that is well known and accepted by consumers. For this reason, grain color is an important characteristic paid attention to, by constitutors and grain dealers in general. Semolina color is due to the natural dyeing pigments, which are xanthophyll, carotene, flavones and cryptoxanthin, although carotenoids in flour are mainly represented by xanthophyll (Lepage & Sims, 1968). A light yellow color in fresh pasta is considered as important positive qualities attribute (Alessandrini et al., 2010).

The incorporation of meat emulsion and navy bean changed the color of cooked spaghetti samples (Fig. 4.1.6). The commercial and control samples were yellow. The spaghetti samples containing meat and navy bean were slightly dark with a red color. This was consistent with the Hunter L\*, a\* and b\* values shown in Table 4.1.2.

It can be seen from Table 4.1.2 that as the meat content increases, the L\* value becomes lower. The L\* values ranged from 68 (28% meat) to 53 (50% meat). Both redness (a\* value) and yellowness (b\* value) affected the saturation of spaghetti samples containing meat and bean. As shown in Table 4.1.2, the redness of spaghetti samples increased significantly with the addition of meat. The higher value of a\* in samples with meat is caused by the color of myoglobin in meat. Myoglobin in meat is responsible for the purplish red muscle color, observed in the depth of the muscle when the meat is freshly cut. This quickly changes to bright red oxymyoglobin, due to oxygenation when the muscle surface is exposed to air (Mancini & Hunt, 2005). With heat treatment the meat color will change to dark brown due to denaturation of myoglobin (Mancini & Hunt, 2005). Hence lightness decreased and redness increased with increasing amount of meat in the spaghetti samples.



Figure 4.1.6a Images of several cooked spaghetti samples starting from the commercial sample all the way to samples containing meat and navy bean in varying amounts.

## 4.1.3 Meat Bean Interactions on colour

In the case of lightness (L\* values) and redness (a\* values) of the enriched spaghetti samples, increased meat addition decreased lightness with corresponding increase in redness as shown in Fig. 4.1.6 b and c. This increase in redness (a\*) is due to the presence of myoglobin in meat, which is responsible for the red color.



Figure 4.1.6b Interaction of meat and bean on lightess (L\*) of spaghetti containing meat (28%, 36%, 43% and 50%) with increasing amount of navy bean (0%, 7%, 14% and 21%).



Figure 4.1.6c Interaction of meat and bean on redness (a\*) of spaghetti containing meat (28%, 36%, 43% and 50%) with increasing amount of navy bean (0%, 7%, 14% and 21%).

Interaction	Sample	Colour			Texture		
		L*	a*	b*	Tensile strength (N/mm <sup>2</sup> )	Extensibility	Elasticity (N/mm <sup>2</sup> )
	Control	66.15±2.07 <sup>bc</sup>	0.82±0.19 <sup>h</sup>	18.24±0.06 <sup>c</sup>	0.15±0.01 <sup>d</sup>	86.53±2.47 <sup>a</sup>	0.17±0.01 <sup>d</sup>
	Commercial	66.86±0.64 <sup>abc</sup>	0.16±0.01 <sup>h</sup>	21.37±0.81 <sup>b</sup>	0.07±0.003 <sup>e</sup>	71.43±5.16 <sup>b</sup>	0.09±0.01 <sup>d</sup>
	28M	68.56±0.25 <sup>a</sup>	$4.06 \pm 0.05^{f}$	15.57±1.35 <sup>e</sup>	0.19±0.01 <sup>abc</sup>	65.06±2.81 <sup>bc</sup>	0.29±0.01°
	28M 7B	67.26±0.24 <sup>ab</sup>	2.43±0.14 <sup>g</sup>	21.00±0.34 <sup>b</sup>	$0.18 \pm 0.01^{abcd}$	58.43±0.3 <sup>cde</sup>	$0.32 \pm 0.02^{abc}$
	28M 14B	66.58±0.53 <sup>abc</sup>	$3.94{\pm}0.08^{f}$	23.45±0.27 <sup>a</sup>	0.16±0.01 <sup>cd</sup>	57.15±3.36 <sup>cde</sup>	$0.32 \pm 0.02^{c}$
	28M 21B	66.62±0.40 <sup>abc</sup>	2.14±0.08 <sup>g</sup>	18.73±0.21 <sup>c</sup>	$0.15 \pm 0.01^{d}$	53.44±11.46 <sup>de</sup>	0.3±0.01 <sup>c</sup>
	36M	65.91±0.31 <sup>bc</sup>	5.42±0.03 <sup>e</sup>	18.34±0.25 <sup>c</sup>	0.19±0.03 <sup>abc</sup>	53.13±8.64 <sup>e</sup>	0.37±0.01 <sup>abc</sup>
	36M 7B	64.95±0.80°	5.78±0.59 <sup>de</sup>	16.32±0.17 <sup>de</sup>	0.20±0.01 <sup>abc</sup>	53.83±1.35 <sup>de</sup>	0.36±0.02 <sup>abc</sup>
	36M 14B	60.84±0.68 <sup>de</sup>	6.76±0.18 <sup>bcd</sup>	15.15±0.56 <sup>ef</sup>	$0.17 \pm 0.02^{bcd}$	53.81±1.19 <sup>de</sup>	0.32±0.03 <sup>abc</sup>
	36M 21B	62.05±0.59 <sup>d</sup>	6.13±0.62 <sup>cde</sup>	12.77±0.27 <sup>g</sup>	0.16±0.02 <sup>cd</sup>	50.62±2.16 <sup>e</sup>	0.32±0.05 <sup>abc</sup>
	43M	59.63±0.11 <sup>ef</sup>	7.22±0.24 <sup>abc</sup>	22.38±0.25 <sup>ab</sup>	0.19±0.01 <sup>abc</sup>	63.81±1.54 <sup>bcd</sup>	0.3±0.01 <sup>bc</sup>
	43M 7B	58.72±0.49 <sup>ef</sup>	$7.45 \pm 0.68^{ab}$	15.52±0.40 <sup>e</sup>	0.19±0.01 <sup>abc</sup>	59.73±2.48 <sup>cde</sup>	$0.32 \pm 0.002^{abc}$
	43M 14B	58.61±0.09 <sup>f</sup>	7.58±0.53 <sup>ab</sup>	12.12±0.51 <sup>g</sup>	$0.18 \pm 0.01^{abcd}$	59.43±0.64 <sup>cde</sup>	0.31±0.02 <sup>bc</sup>
	43M 21B	57.76±0.74 <sup>f</sup>	7.20±0.25 <sup>abc</sup>	13.00±0.66 <sup>g</sup>	0.16±0.01 <sup>cd</sup>	55.23±0.86 <sup>cde</sup>	0.3±0.01°
	50M	55.28±0.32 <sup>g</sup>	8.39±0.63ª	17.95±1.01 <sup>cd</sup>	0.21±0.02 <sup>a</sup>	52.49±0.62 <sup>e</sup>	$0.41 \pm 0.05^{a}$
	50M 7B	54.59±0.56 <sup>g</sup>	8.22±0.54ª	16.44±0.42 <sup>de</sup>	0.20±0.01 <sup>ab</sup>	52.16±1.3 <sup>e</sup>	$0.4{\pm}0.02^{ab}$
	50M 14B	53.45±0.53 <sup>g</sup>	$7.86 \pm 0.47^{ab}$	$13.44 \pm 0.50^{fg}$	$0.18 \pm 0.01^{abcd}$	52.47±1.68 <sup>e</sup>	0.35±0.01 <sup>abc</sup>
Meat		F=532.845****	F= 429.360****	F= 52.524****	F= 23.998****	F= 29.453****	F= 45.338****
Bean		F= 32.759****	F= 6.753***	F= 43.056****	F= 7.291***	F= 4.289	F= 2.325
Meat*Bean		F= 5.117***	F= 6.892****	F= 47.166****	F=0.162	F= 0.680	F= 0.560

Table 4.1.2 Color and texture of cooked spaghetti samples containing meat and navy bean

Level of significance: \*, p < 0.05; \*\*, p < 0.01; \*\*\*, p < 0.001; \*\*\*\*, p < 0.001 expressed in F value. Mean ± SD of three repetitions on pasta produced at three different occasions; Mean values within a column with different superscripts differ significantly (p < 0.05) using one-way ANOVA and Tukey's test.

## 4.1.4 Texture analysis

The textural characteristics of pasta play an essential role in determining the final acceptance by consumers (Bhattacharya et al., 1999), who have shown a preference for pasta that retains texture characteristics not only with normal cooking time but also with overcooking. The optimization of texture parameters is important to ensure the acceptance of the developed products by the consumers. Spaghetti is considered as good quality when it is firm and elastic (Gianibelli et al., 2005).



Figure 4.1.7 Texture analysis of spaghetti using tensile rigs

Tensile strength, extensibility and elasticity of enriched spaghetti samples were determined using tensile rigs (Figure 4.17). Statistical analysis showed that the values for each textural attribute measured (strength, extensibility and elasticity) were significantly different (P<0.0001) between the samples studied. Tukey's test performed after ANOVA revealed the specific origins of differences (Table 4.1.2). The addition of meat to spaghetti showed a significant increase (P<0.0001) in tensile strength of the spaghetti. Samples that were fortified with meat (28%M,

36% M, 43% M and 50% M) had higher tensile strength (higher energy for the spaghetti strands to snap) compared to samples fortified with high bean (14%B and 21%B). The maximum tensile force at break for meat containing samples ranged from 0.14 N (28%M) to 0.37 N (50%M). The corresponding tensile strength values account for 0.19 N/mm<sup>2</sup> (28%M) and 0.21 N/mm<sup>2</sup> (50%M) respectively. These values are significantly higher than that of the control (0.15 N/mm<sup>2</sup>) and commercial (0.07 N/mm<sup>2</sup>) pasta samples.

On the other hand there was a slight non- significant decrease in tensile strength observed as the amount of bean in the samples increased. Less force was required to break samples containing high amounts of bean and meat. The force ranged from 0.10 N (28%M 21% B) to 0.19 N (50%M 14% B), and tensile strength from 0.15 N/mm<sup>2</sup> (28%M 21%B) and 0.18 N/mm<sup>2</sup> (50%M 14%B) similar to the control sample. The inclusion of fiber fractions from navy bean probably promoted formations of discontinuities or cracks inside the pasta strand, which weakened the pasta structure leading to a less tensile strength (faster and early breaks of strands) (Petitot et al., 2010).



Figure 4.1.8a Tensile strength, texture parameter of the spaghetti samples containing meat (28%, 36%, 43% and 50%) with low navy bean content (7%) along with control and commercial pasta.

\*Different letters above bars are significantly different (p < 0.05).





\*Different letters above bars are significantly different (p < 0.05).

One possible measure for extensibility is the strain at break. This strain value is obtained from the initial and the maximum length of a sample subjected to an external tensile force. Extensibility was examined as the maximum tensile strength of the spaghetti before failure (Chang & Wu, 2008). Spaghetti samples containing 28%M and 43%M have higher extensibility, with strain values of 65.06% and 63.81% respectively than other spaghetti samples (Table 4.1.2). The values are however lower than control (86.53%) and commercial (71.43%) pasta samples.



Figure 4.1.9a Extensibility of the spaghetti samples containing meat (28%, 36%, 43% and 50%) with low navy bean content (7%) along with control and commercial pasta.

\*Different letters above bars are significantly different (p < 0.05).



Figure 4.1.9b Extensibility of the spaghetti samples containing meat (28%, 36%, 43% and 50%) with high navy bean content (14% and 21%) along with control and commercial pasta.

\*Different letters above bars are significantly different (p < 0.05).

Young's Modulus is a measure for elasticity. Since the spaghetti samples varied in swelling sizes after cooking, Young's module, which is defined as the ratio of stress and strain in a tensile test might be another good parameter to describe the textural property of spaghetti. As seen in Table 4.1.2, the increasing amount of bean added to the sample resulted in increases the in Young's Modulus value. Most of the studies reported to date only investigated the firmness of pasta with the addition of legumes (Bahnaseey et al., 1986; Zhao et al., 2005; Edwards et al., 1995; Manthey et al., 2004).

The matrix structural network of starches, glutens, additional proteins and other ingredients mainly affect textural properties of spaghetti. These may either weaken or strengthen formation of hydrogen bonds within the spaghetti structure network (Chang & Wu, 2008). In this study, meat proteins interacted with the insoluble networks of spaghetti, forming stable matrix structures and leading to higher tensile strength as observed from texture analysis results. Lower tensile strength in spaghetti containing navy bean indicates that navy bean could not function as an effective ingredient to fortify network structures of spaghetti. Moreover, this lower tensile strength of spaghetti might result from disintegration of pasta structure from high water absorption and/ or over- swelling effects, due to fibers and polysaccharides in navy bean. Tudorica et al. (2002) reported an overall reduction of pasta elasticity when pea was added due to presence of fiber in pea, which resulted in a disruptive behavior of fiber on the protein- starch binding during pasta matrix formation.



Figure 4.1.10a Elasticity (Young's module) of the spaghetti samples containing meat (28%, 36%, 43% and 50%) with low navy bean content (7%) along with control and commercial pasta.

\*Different letters above bars are significantly different (p < 0.05).



Figure 4.1.10b Elasticity of the spaghetti samples containing meat (28%, 36%, 43% and 50%) with high navy bean content (14% and 21%) along with control and commercial pasta.

\*Different letters above bars are significantly different (p < 0.05).

## 4.2 Nutritional composition of spaghetti

#### **4.2.1 Moisture content**

The nutritional composition of spaghetti is summarized in Table 4.2.3. All cooked spaghetti samples had significantly higher moisture content (P<0.0001, F-42.027). Moisture values ranged from 63-73%. The commercial and control pasta had high moisture values of 72% and 66% respectively. Commercial fresh pasta usually has high moisture values according to Petitot et al (2010). Spaghetti samples containing meat with higher amounts of bean (28M 21B, 36M 21B, 43M 21B and 50M 21B) showed a higher moisture content as compared to the other spaghetti enriched samples. Bean has high fiber content, which was reported to be 10-20% of dry mass (Kereliuk & Kozub, 1995). The presence of fiber in navy bean can also increase the water binding and water holding capacity of spaghetti (Chen et al., 1984). The number of hydroxyl groups in the fiber structure can increase water absorption and allow water interaction through hydrogen bonding (Belitz et al., 2009). Hence, moisture content of spaghetti samples containing meat with higher amounts of bean was similar to the commercial spaghetti sample. Commercial spaghetti has high moisture content due to the various industrial processes involved in spaghetti manufacturing.

## 4.2.2 Meat Bean Interactions on moisture content

In our study, spaghetti samples had high moisture content. It was increasing navy bean content that drove the increase in moisture content (Fig. 4.3.4.1). Navy bean contains high fiber content (10-20% of dry mass) (Kereliuk & Kozub, 1995) that can increase the water binding and water holding capacity of pasta (Chen et al., 1984) as mentioned in section 4.2. The number of hydroxyl groups in the fiber structure can increase the water absorption and allow water interaction through hydrogen bonding (Belitz et al., 2009). In addition, the high water holding capacity of protein can result in high moisture content as well.



Figure 4.1.11 Interaction of meat and bean on moisture of spaghetti containing meat (28%, 36%, 43% and 50%) with increasing amount of navy bean (0%, 7%, 14% and 21%).

#### **4.2.3 Protein content**

As expected, there was an increase in protein content of spaghetti with increasing meat and navy bean addition. The protein content of the enriched samples was between 8% and 14% higher than the control and commercial spaghetti respectively. The protein content of lamb (diced, fully-trimmed, raw) is 21.2% and that of navy bean (haricot, dried, boiled, no added salt) is 8.2% (FSANZ, 2013). In our study, the meat emulsion contained 57% lamb mince and 43% of water. It is the high protein content present in the meat emulsion that primarily increased the protein in the spaghetti samples. These results (Table 4.2.1) showed that as the amount of meat (28%, 36%, 43% and 50%) added to the spaghetti dough mixture increased, the protein content also increased. However, increasing the amount of navy bean contributed very little to changes in protein content.

## 4.2.4 Carbohydrate content

With increasing protein, there was a corresponding decrease in carbohydrates. Carbohydrates are the major component of pasta (Fig. 4.2.1), representing 57% (w/w) of raw control, which is mainly starch (Fradique et al., 2010). Carbohydrate content in the spaghetti enriched samples containing meat and bean ranged from 11% to 24% (Table 4.2.3). This value was much lower than the value of pasta containing shrimp meat (Kadam & Prabhasankar, 2012) and pasta containing common bean (Gallegos-Infante et al., 2010; Petitot et al., 2010).

## 4.2.5 Fat content

The total fat content of cooked spaghetti samples containing meat, navy bean or a combination of the two that ranged from 1.58% to 3.32% were significantly higher than the control (0.71%) and commercial (1.26%) spaghetti (P<0.0001). The lamb meat used in this study is lean meat which contains a total fat of 5.6% and the navy bean contains a total fat of 0.7% (FSANZ, 2013). Since the amount of fat present in the lean lamb meat is low, the fat content in the spaghetti samples was not as high as expected. This is supported by our findings (Table 4.2.1), as samples containing increasing amount of meat emulsion (28%, 36%, 43% and 50%) significantly increased fat content. Increasing the amount of navy bean (7%, 14% and 21%) at each level also significantly increased fat content in the spaghetti samples.

## 4.2.6 Meat Bean Interactions on fat content

In the case of fat content, the addition of meat showed a higher difference (F- 482.152) as compared to bean (F- 27.056). As shown in Figure 4.3.4.2, fat content increased with increasing of meat content. Lean meat contains about 5% fat (Jimenez- Colmenero & Cofrades, 2001) besides the high water (75%) and protein content (20%) found in it. The fat content in navy bean is about 0.1% (FSANZ, 2013), way lower than meat. Williams (2007) reported that fat content in lean lamb is about 4.7%.



Figure 4.3.4.2 Interaction of meat and bean on fat of spaghetti containing meat (28%, 36%, 43% and 50%) with increasing amount of navy bean (0%, 7%, 14% and 21%).

#### 4.2.7 Ash content

The ash content of the spaghetti enriched samples ranged from 0.5%-1%, and was not much different compared to the control (0.75%) and commercial (1%) spaghetti. The ash content of raw lean lamb meat ranged between 0.9% and 1.20% (Hoke et al., 1999; Badiani et al., 1998; Maranesi et al., 2005), and on cooked basis between 0.83% and 1.09% (Hoke et al., 1999; Badiani et al., 1998). Gallegos- Infante et al. (2010) however reported that ash content gradually increased with the addition of common bean flour from around 0.86% to 2.09%. In their study, pasta was prepared with 15% and 45% common bean flour (dried), which was at a much higher concentration of navy bean compared to our study. In our study, the highest concentration of navy bean used was only 21% (wet basis). Navy bean (boiled, powdered) and raw lamb have similar ash content of around 0.80%.

1% (U.S. Department of Agriculture. 2013b; Badiani et al., 1998; Hoke et al., 1999). Therefore, the addition of meat and navy bean did not have a significant effect on the ash in spaghetti samples.



Figure 4.2.1 Proximate composition of spaghetti samples containing meat (28%, 36%, 43% and 50%) with increasing navy bean content (7%, 14% and 21%). Each column represents 100% of the sample. Samples are expressed as meat and navy bean content, e.g., 28M 7B refers to the sample containing 28% meat and 7% navy bean powder.

Interaction	Sample	Component (g/100g wet basis)					
		Moisture	Fat	Protein	Ash	Carbohydrates	
	Control	66.76±0.55 <sup>fg</sup>	0.71±0.05 <sup>m</sup>	6.46±0.01 <sup>k</sup>	0.75±0.001 <sup>b</sup>	24.91±0.50ª	
	Commercial	72.41±0.66ª	1.26±0.01 <sup>1</sup>	5.69±0.10 <sup>1</sup>	1.00±0.001ª	19.86±0.84 <sup>bcd</sup>	
	28M	64.21±0.48 <sup>hi</sup>	1.58±0.07 <sup>k</sup>	8.61±0.10 <sup>j</sup>	0.66±0.004 <sup>bc</sup>	24.64±0.52ª	
	28M 7B	70.20±0.67 <sup>bcde</sup>	1.63±0.04 <sup>k</sup>	8.76±0.06 <sup>j</sup>	0.50±0.004°	18.96±0.82 <sup>cde</sup>	
	28M 14B	69.15±0.15 <sup>de</sup>	1.78±0.05 <sup>j</sup>	9.04±0.003 <sup>i</sup>	0.74±0.001 <sup>b</sup>	19.29±0.04 <sup>bcd</sup>	
	28M 21B	73.32±0.66 <sup>ab</sup>	2.00±0.06 <sup>i</sup>	9.47±0.00 <sup>h</sup>	1.07±0.15 <sup>a</sup>	14.92±0.25 <sup>gh</sup>	
	36M	66.23±1.08 <sup>gh</sup>	2.15±0.01 <sup>h</sup>	10.13±0.04 <sup>g</sup>	0.74±0.003 <sup>b</sup>	21.64±0.57 <sup>b</sup>	
	36M 7B	68.80±1.25 <sup>ef</sup>	2.34±0.05 <sup>g</sup>	10.50±0.07 <sup>f</sup>	0.74±0.002 <sup>b</sup>	17.40±1.03 <sup>defg</sup>	
	36M 14B	69.96±0.35 <sup>cde</sup>	2.65±0.02 <sup>f</sup>	10.85±0.03e	1.09±0.17 <sup>a</sup>	15.21±0.27 <sup>fgh</sup>	
	36M 21B	71.26±0.61 <sup>abcd</sup>	2.72±0.02 <sup>ef</sup>	11.00±0.08 <sup>e</sup>	0.99±0.010ª	14.06±0.07 <sup>h</sup>	
	43M	63.74±1.00 <sup>i</sup>	2.81±0.04 <sup>e</sup>	12.19±0.05 <sup>d</sup>	0.50±0.002°	20.88±1.16 <sup>bc</sup>	
	43M 7B	66.26±0.44 <sup>gh</sup>	2.82±0.03 <sup>de</sup>	12.46±0.06 <sup>d</sup>	0.99±0.010ª	17.52±0.19 <sup>def</sup>	
	43M 14B	69.85±1.34 <sup>cde</sup>	2.92±0.03 <sup>cd</sup>	12.84±0.06°	0.74±0.003 <sup>b</sup>	13.64±0.65 <sup>hi</sup>	
	43M 21B	71.77±0.62 <sup>abc</sup>	2.99±0.05°	12.94±0.02°	1.11±0.190ª	10.81±0.78 <sup>j</sup>	
	50M	67.27±1.29 <sup>gh</sup>	3.14±0.04 <sup>b</sup>	13.62±0.19 <sup>b</sup>	0.50±0.003°	14.72±0.81 <sup>efg</sup>	
	50M 7B	68.60±0.41 <sup>ef</sup>	3.24±0.03 <sup>ab</sup>	13.98±0.02ª	0.74±0.010 <sup>b</sup>	13.28±0.39 <sup>hij</sup>	
	50M 14B	69.83±1.20 <sup>cde</sup>	3.32±0.03ª	14.04±0.03ª	0.99±0.010ª	11.30±0.42 <sup>ij</sup>	
Meat		F= 16.204****	F= 539.607****	F= 1138.666****	F= 13.901****	F= 24.516****	
Bean		F= 59.065****	F= 29.050****	F= 27.780****	F= 50.414****	F= 54.425****	
Meat*Bean		F= 2.640*	F= 2.456*	F= 0.500	F= 10.951****	F= 1.064	

Table 4.2.1. Nutritional composition of spaghetti sample enriched with meat and navy bean

Level of significance-\*, p < 0.05; \*\*, p < 0.01; \*\*\*, p < 0.001; \*\*\*\*, p < 0.001 in F value. Mean  $\pm$  SD of three repetitions on pasta produced at three different occasions; Mean values within a column with different superscripts differ significantly (p < 0.05) using one-way ANOVA and Tukey's test.

## 4.4 Sensory Analysis

Although there are objective methods that are developed to evaluate the cooking quality of pasta, sensory evaluation still remains the most reliable test (Cubadda, 1988), provided certain critical points are considered in the sensory evaluation (Menger, 1979). Appearance, flavor and texture are sensory factors that give people a sensory pleasure while eating which the nutrition factor does not give. Sensory evaluation is nearest to a consumer's estimation and remains the most reliable and dependable test because it gives an overall idea of the characteristics of cooked pasta.

#### **4.4.1 Consumer Testing**

The sensory characteristics of the cooked enriched spaghetti samples were evaluated for different parameters such as, overall liking, odor, texture and flavor and the results are presented in Table 4.3.1. Consumer testing data of 70 spaghetti consumers were collected over a period of three weeks. The data was analyzed using one-way analysis of variance. In general, in terms of overall liking, odour, texture and flavor, almost all spaghetti samples except for high bean content samples (14%B and 21%B)and the highest meat content (50%M) samples were significantly liked.

Product	Overall liking	Odor	Texture	Flavour
Control	4.13±2.23 <sup>ab</sup>	4.41±2.35 <sup>abcd</sup>	4.22±2.53 <sup>abc</sup>	4.12±2.30 <sup>abcd</sup>
Commercial	$4.84{\pm}2.28^{a}$	5.14±2.09 <sup>a</sup>	$4.98 \pm 2.28^{a}$	4.60±2.26 <sup>ab</sup>
28M	3.89±2.13 <sup>abc</sup>	4.19±2.13 <sup>abcde</sup>	3.81±2.17 <sup>bcde</sup>	3.72±2.29 <sup>bcd</sup>
36M	4.71±2.06 <sup>a</sup>	5.12±1.82 <sup>a</sup>	4.44±2.10 <sup>abc</sup>	4.47±2.12 <sup>abc</sup>
43M	4.54±2.34 <sup>a</sup>	4.74±2.09 <sup>abc</sup>	4.43±2.15 <sup>abc</sup>	4.36±2.24 <sup>abc</sup>
50M	4.14±2.19 <sup>ab</sup>	4.53±2.17 <sup>abc</sup>	4.05±2.07 <sup>abcd</sup>	4.11±2.40 <sup>abcd</sup>
28M 7B	5.06±2.08 <sup>a</sup>	5.20±1.90 <sup>a</sup>	5.02±2.27 <sup>ab</sup>	5.24±2.36 <sup>a</sup>
28M 14B	4.20±1.93 <sup>ab</sup>	4.70±2.18 <sup>abc</sup>	3.91±2.06 <sup>abcde</sup>	4.69±1.95 <sup>ab</sup>
28M 21B	2.76±2.42 <sup>bcd</sup>	3.18±2.37 <sup>cdef</sup>	2.50±2.25 <sup>de</sup>	2.61±2.17 <sup>de</sup>
36M 7B	5.09±2.16 <sup>a</sup>	5.31±2.21 <sup>a</sup>	5.03±2.19 <sup>ab</sup>	5.05±1.99 <sup>a</sup>
36M 14B	4.50±2.60 <sup>ab</sup>	4.91±2.41 <sup>ab</sup>	4.32±2.72 <sup>abc</sup>	4.34±2.79 <sup>abcd</sup>
36M 21B	$2.93 \pm 2.46^{bcd}$	3.09±2.71 <sup>def</sup>	$2.65 \pm 2.44^{de}$	$2.82 \pm 2.68^{de}$
43M 7B	5.03±2.50 <sup>a</sup>	5.06±2.54 <sup>a</sup>	5.03±2.41 <sup>ab</sup>	5.10±2.55 <sup>a</sup>
43M 14B	$5.02 \pm 2.47^{a}$	5.18±2.15 <sup>a</sup>	4.60±2.53 <sup>abc</sup>	$4.92 \pm 2.64^{ab}$
43M 21B	2.60±2.41 <sup>cd</sup>	2.74±2.22 <sup>ef</sup>	2.68±2.47 <sup>de</sup>	$2.67 \pm 2.55^{de}$
50M 7B	2.91±2.17 <sup>bcd</sup>	3.39±2.26 <sup>bcdef</sup>	3.24±2.60 <sup>cde</sup>	3.00±2.33 <sup>cde</sup>
50M 14B	2.05±1.93 <sup>d</sup>	2.16±2.18 <sup>f</sup>	2.36±2.21 <sup>de</sup>	2.30±2.24 <sup>de</sup>
F value	6.480****	7.207****	5.686****	5.813****

Table 4.3.1 Consumer test of cooked spaghetti products containing meat and navy bean

Level of significance- \*, p < 0.05; \*\*, p < 0.01; \*\*\*, p < 0.001; \*\*\*\*, p < 0.001 expressed in F value. Mean  $\pm$  SD of three repetitions on pasta produced at three different occasions; Mean values within a column with different superscripts differ significantly (p < 0.05) using one-way ANOVA and Tukey's test. Based on 10- point hedonic line scale with left end represents 0 (extremely dislike), right end represents 10 extremely like, and middle represents 5 (neither like nor dislike).

From Table 4.3.1 it can be seen that there is a significant difference (p<0.0001) in texture of spaghetti samples. As the amount of bean increases, the texture score decreases which is consistent with the instrumental texture analyzer results. As the content of bean increases, the spaghetti samples break easily, therefore requiring less force at break. Samples containing meat (28%M, 36%M, 43%M and 50%M) and combinations of meat and low bean (28%M 7%B, 36%M 7%B, 43%M 7%B and 50%M 7%B) have higher texture scores, which means that more force is required for the spaghetti strands to break, therefore resulting in higher tensile strength. That the tensile strength in meat is higher than navy bean could be due to the protein structure of meat. The complex mixture of proteins present in muscle consists predominantly of fibrous proteins and these proteins that give it its structure (Bailey, 1972) can contribute to the higher tensile strength in meat containing spaghetti. Proteins in beans on the other hand are mainly composed of salt-soluble

globulins (soluble in dilute salt solutions) and water-soluble albumins (Gueguen & Barbot, 1988). Spaghetti is made of durum wheat, which is composed of glutenins, and gliadins that form intraand intermolecular disulphide bonds. This leads to the formation of a gluten network responsible for the texture of pasta. The addition of non-gluten material to pasta dilutes the gluten strength and probably weakenes the overall structure of spaghetti (Raya-Duarte et al., 1996). Hence, the tensile strength of spaghetti containing high amounts of bean was weak.



Figure 4.3.1a: Liking scores of spaghetti formulations containing meat (M) plus low content navy bean (B). Assistive lines were added to assist interpretation of the Tukey's significance letters. \*Different letters above bars are significantly different (p < 0.05).



Figure 4.3.1b: Liking scores of spaghetti formulations containing meat (M) plus high content of navy bean (B). Assistive lines were added to assist interpretation of the Tukey's significance letters. \*Different letters above bars are significantly different (p < 0.05).

## 4.4.2 Projective mapping

Projective mapping was carried out to gain an overall perspective on product differences according to consumer's perceived similarities and differences between the products of interest. This is very useful when finding out how a new product compares with ones that are already available in the market. A total of 18 panellists participated in sensory projective mapping out of which 16 panellists completed the mapping of the eight spaghetti products over a period of three weeks. The RV coefficients were used to determine how well a panellist's map fitted with the consensus maps as shown in Appendix 3. All the panelists scored well in terms of fit with the rest as assessed by MFA (Multi Factor Analysis) with RV (Random Variable) coefficients >0.500. Out of the 16 panellists, six panelists scored poorly for the overall sensory projective mapping with RV coefficients < 0.500. RV coefficients obtained during the three trials varied indicating an improvement in the consensus maps of the panelists with time. In week1, 56% of the 16 panellists scored RVs > 0.500, while in the second trial 75% and in the third trial 94% agreement were seen among panelists. Product and attribute maps were plotted as shown in Figure 4.3.2.

Spaghetti samples prepared with high amounts of meat (36%M and 50%M) and high amounts of meat with low bean content (43%M 7%B and 50%M 7%B), were associated with meaty taste, along with floury taste, pale look, smooth mouth feel, hard, tasty, chewy and good texture. The smoothness and good texture of samples containing high meat emulsion may be attributed to the interactions of protein and fat to form a gel network (Chillo et al., 2010). The water holding capacity of meat may also affect the appearance and consumer appeal of the sample (Van Laack, 1999). The floury taste is probably due to the extra addition of flour to the pasta mixture due to the presence of meat in order to achieve dough of homogenous texture. The denaturation of myofibrillar protein during thermal processing has been reported to result in high hardness values of the chicken myofibrillar protein gels (Smith et al., 1988). Myofibrillar proteins unfold and aggregate when heated to form three- dimensional cross- linked protein network, which traps fat and macroparticulates within the gel matrix (Sun & Holley, 2011). This gel matrix formation might account for the hardness and chewiness of the spaghetti samples with high meat content in this study.

Samples with higher bean content and low meat content (28%M 7%B and 28%M 21%B), were described as being soft, gritty, soggy and rough. This could be due to the high fiber content in navy bean (10-20% of dry mass) (Kereliuk & Kozub, 1995). Navy bean also contains no gluten. Hence as bean content increased, the semolina gluten was diluted leading to a weakening of the gluten matrix and resulted in rough and gritty texture (Wood, 2009).



Figure 4.3.2 Principal Component Analysis of eight spaghetti samples over the combined three sensory trials.

MFA of the projective mapping results confirmed that panel judgement improved over a period of 3 weeks producing RV coefficients greater than 0.5, which indicates a degree of similarity between the individual maps and in the way spaghetti was perceived by panellists. In addition, this study also confirmed that sensory projective mapping using consumers can be used successfully in differentiating products which may be useful in profiling newly- developed products and comparing them with products that are already available in the market.

# **Chapter 5: Conclusion**

The intention behind this research was to investigate the impact of meat and navy bean on the physical, chemical and sensory characteristics of pasta, and to explore how meat proteins interacted with macromolecules (e.g., starch and fiber) present in the other ingredients (navy bean, flour, semolina) used to make spaghetti. Consumers often associate food products that are rich in protein, healthful lipids and fiber as providing health benefits that exceed a regular diet. Meat contains high biological value protein and other important micronutrients, essential omega-3 polyunsaturated fats. Navy bean is an excellent source of protein and carbohydrates with starch and non- starch polysaccharides (dietary fiber) as predominant fractions and also contains a significant amount of oligosaccharides and other nutrients (e.g., phytochemicals and minerals). Hence, the incorporation of these ingredients into spaghetti can increase its nutritional value.

Spaghetti samples were formulated with meat (28%, 36%, 43% and 50%) and bean (7%, 14% and 21%). The moisture, protein and fat content of the samples increased significantly with the addition of meat and bean. In addition, fortification of pasta with meat and bean had a noticeable impact on the cooking quality of pasta. An increase in the cooked weight was observed in samples containing only meat. As navy bean content in the samples increased, the cooked weight decreased. A higher cooking loss and swelling index was observed in samples containing increasing amounts of bean along with meat. The texture characteristics of the pasta namely tensile strength, extensibility and elasticity, are positively affected by the inclusion of meat and negatively affected by bean. Additionally, increased red meat addition increased the redness and decreased lightness of cooked spaghetti samples.

Finally, incorporation of meat and bean also affected the overall consumer acceptability of spaghetti. Significant differences were observed in terms of overall liking, odour, texture and flavor for almost all spaghetti samples except for samples containing high bean content (14%B and 21%B) and the highest meat content (50%M). From sensory projective mapping we found that panelists were able to differentiate the enriched spaghetti samples resulted in terms of 17 taste and texture attributes. Analysis of the projective mapping data helped explained the consumer liking of reformulated spaghetti samples.

## **Future investigation**

It would be interesting to observe the gluten network of the spaghetti samples enriched with new ingredients to study the effect of gelatinization of starch. This could be carried out using a scanning electron microscope. The degree of starch gelatinization if visualized, could further support our findings on texture. Our study is the first study that has incorporated combinations of meat and bean to increase the nutritional value of spaghetti. Since our reformulated pasta can be considered to be a low GI food, it would be good to carry out in vitro digestibility studies to confirm this. Furthermore, research can also be carried out on the drying of pasta samples containing meat and navy bean for commercial purposes. A comparison of spaghetti containing other meat would be useful too. Currently, spaghetti containing veal meat is being trialed based on the success of the current study. Further studies on in vivo digestibility studies would also be useful to determine effects of reformulated spaghetti consumption on health.

#### Significance of this study

The development of new products plays an important role in the food industry. Consumers are constantly demanding food products with high nutritional value. In this study, spaghetti was used as a vehicle to deliver high nutrition by the incorporation of meat and bean into it. The enrichment of spaghetti has been shown to be a successful way to improve its nutritional properties. In our study, we have successfully incorporated high amounts of meat and navy bean into spaghetti that have acceptable characteristics and additional health benefits. Incorporation of meat and bean in spaghetti can make it a healthier alternative to children and also to other consumers such as pregnant women and health-conscious people. The production of reformulated spaghetti will not only increase the nutritional quality of spaghetti but can also have a positive implication for human health.

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- **1.** Instruction and Questionnaire for Consumer Testing and Projective Mapping
- a) Consumer testing

Instructions and questionnaire for consumer testing

I lease multate your genuer	Please	indicate	vour	gender
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Male
Female

Please indicate your age

Under 20
20-29
30-39
Above 40

Please indicate if you are allergic to the ingredients listed below

- **Meat**
- Navy Bean

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

Yes
No

### How often do you consume spaghetti?



### **Instructions:**

- Please rate the sample by clicking the line scale given depending on the perceived preference
- Please take 30 seconds break per food sample given
- Please rinse your mouth with the given filtered water

	Extremely not preferred	Extremely preferred
Preference	-	781
	Extremely not preferred	Extremely preferred
Preference	-	318
	Extremely not preferred	Extremely preferred
Preference	-	855
	Extremely not preferred	Extremely preferred
Preference	+	602
	Extremely not preferred	Extremely preferred
Preference	-	139
	Extremely not preferred	Extremely preferred
Preference	+	423

### b) Projective mapping

### **Instructions:**

- Please rate the sample by clicking the line scale given depending on the perceived preference
- Please take 30 seconds break per food sample given
- Please rinse your mouth with the given filtered water



## THANK YOU FOR PARTICIPATING

### **2.** Letter of Ethics Approval from AUTEC



A U T E C S E C R E T A R I A T

30 July 2014

Nazimah Hamid Faculty of Health and Environmental Sciences

Dear Nazimah

Re: Ethics Application: 13/250 Sensory testing of pasta with added meat and navy bean.

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

I have approved the minor amendment to your ethics application allowing the addition of an online survey.

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

A brief annual progress report using form EA2, which is available online through <a href="http://www.aut.ac.nz/researchethics">http://www.aut.ac.nz/researchethics</a>. When necessary this form may also be used to request an extension of the approval at least one month prior to its expiry on 16 September 2016;

A brief report on the status of the project using form EA3, which is available online through <a href="http://www.aut.ac.nz/researchethics">http://www.aut.ac.nz/researchethics</a>. This report is to be submitted either when the approval expires on 16 September 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 <u>ethics@aut.ac.nz</u>.

All the very best with your research,

H Courson

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

Cc: Loveena Pereira loveena14@gmail.com

### **3.** 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 56% 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	N12	N13	N14	N15	N16	MFA
N1	1.000	0.198	0.121	0.135	0.765	0.375	0.025	0.071	0.277	0.273	0.198	0.081	0.287	0.092	0.137	0.154	0.507
N2	0.198	1.000	0.049	0.429	0.275	0.366	0.123	0.106	0.472	0.033	1.000	0.055	0.069	0.078	0.394	0.011	0.555
N3	0.121	0.049	1.000	0.301	0.071	0.037	0.093	0.270	0.288	0.541	0.049	0.165	0.223	0.112	0.019	0.458	0.446
N4	0.135	0.429	0.301	1.000	0.106	0.261	0.347	0.061	0.259	0.178	0.429	0.193	0.147	0.234	0.445	0.136	0.574
N5	0.765	0.275	0.071	0.106	1.000	0.205	0.191	0.081	0.361	0.030	0.275	0.052	0.302	0.057	0.152	0.152	0.483
N6	0.375	0.366	0.037	0.261	0.205	1.000	0.041	0.235	0.129	0.084	0.366	0.052	0.070	0.151	0.251	0.105	0.467
N7	0.025	0.123	0.093	0.347	0.191	0.041	1.000	0.128	0.487	0.250	0.123	0.224	0.065	0.230	0.061	0.139	0.422
N8	0.071	0.106	0.270	0.061	0.081	0.235	0.128	1.000	0.111	0.239	0.106	0.557	0.351	0.175	0.381	0.256	0.498
N9	0.277	0.472	0.288	0.259	0.361	0.129	0.487	0.111	1.000	0.282	0.472	0.274	0.051	0.385	0.065	0.061	0.594
N10	0.273	0.033	0.541	0.178	0.030	0.084	0.250	0.239	0.282	1.000	0.033	0.411	0.309	0.162	0.052	0.521	0.527
N11	0.198	1.000	0.049	0.429	0.275	0.366	0.123	0.106	0.472	0.033	1.000	0.055	0.069	0.078	0.394	0.011	0.555
N12	0.081	0.055	0.165	0.193	0.052	0.052	0.224	0.557	0.274	0.411	0.055	1.000	0.510	0.686	0.356	0.263	0.612
N13	0.287	0.069	0.223	0.147	0.302	0.070	0.065	0.351	0.051	0.309	0.069	0.510	1.000	0.266	0.201	0.650	0.547
N14	0.092	0.078	0.112	0.234	0.057	0.151	0.230	0.175	0.385	0.162	0.078	0.686	0.266	1.000	0.224	0.009	0.495
N15	0.137	0.394	0.019	0.445	0.152	0.251	0.061	0.381	0.065	0.052	0.394	0.356	0.201	0.224	1.000	0.078	0.513
N16	0.154	0.011	0.458	0.136	0.152	0.105	0.139	0.256	0.061	0.521	0.011	0.263	0.650	0.009	0.078	1.000	0.469
MFA	0.507	0.555	0.446	0.574	0.483	0.467	0.422	0.498	0.594	0.527	0.555	0.612	0.547	0.495	0.513	0.469	1.000

Table 3.2.2 RV coefficient between projective maps and multifactor analysis (MFA) for week two where 75% of panellists scored >0.5.

	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13	N14	N15	N16	MFA
N1	1.000	0.325	0.367	0.413	0.091	0.383	0.452	0.110	0.176	0.234	0.070	0.473	0.314	0.398	0.046	0.342	0.534
N2	0.325	1.000	0.299	0.062	0.097	0.256	0.581	0.353	0.356	0.307	0.199	0.236	0.184	0.493	0.220	0.049	0.510
N3	0.367	0.299	1.000	0.436	0.327	0.569	0.097	0.382	0.444	0.186	0.247	0.648	0.065	0.127	0.390	0.474	0.634
N4	0.413	0.062	0.436	1.000	0.448	0.550	0.079	0.478	0.249	0.253	0.455	0.712	0.149	0.056	0.374	0.602	0.706
N5	0.091	0.097	0.327	0.448	1.000	0.237	0.070	0.057	0.561	0.233	0.181	0.334	0.085	0.121	0.152	0.289	0.485
N6	0.038	0.084	0.003	0.382	0.427	0.008	0.013	0.151	0.121	0.212	0.502	0.131	0.048	0.308	0.007	0.096	0.374
N7	0.383	0.256	0.569	0.550	0.237	1.000	0.180	0.481	0.418	0.378	0.268	0.528	0.098	0.138	0.461	0.471	0.675
N8	0.452	0.581	0.097	0.079	0.070	0.180	1.000	0.120	0.358	0.520	0.106	0.141	0.344	0.266	0.364	0.214	0.487
N9	0.170	0.052	0.168	0.215	0.107	0.146	0.038	0.198	0.012	0.116	0.545	0.252	0.316	0.124	0.169	0.022	0.393
N10	0.110	0.353	0.382	0.478	0.057	0.481	0.120	1.000	0.199	0.231	0.562	0.309	0.285	0.315	0.551	0.355	0.624
N11	0.176	0.356	0.444	0.249	0.561	0.418	0.358	0.199	1.000	0.429	0.005	0.214	0.233	0.355	0.344	0.289	0.577
N12	0.234	0.307	0.186	0.253	0.233	0.378	0.520	0.231	0.429	1.000	0.001	0.253	0.305	0.077	0.278	0.535	0.558
N13	0.070	0.199	0.247	0.455	0.181	0.268	0.106	0.562	0.005	0.001	1.000	0.278	0.305	0.104	0.459	0.192	0.553
N14	0.473	0.236	0.648	0.712	0.334	0.528	0.141	0.309	0.214	0.253	0.278	1.000	0.206	0.108	0.151	0.437	0.653
N15	0.314	0.184	0.065	0.149	0.085	0.098	0.344	0.285	0.233	0.305	0.305	0.206	1.000	0.206	0.311	0.369	0.488
N16	0.398	0.493	0.127	0.056	0.121	0.138	0.266	0.315	0.355	0.077	0.104	0.108	0.206	1.000	0.064	0.049	0.411
MFA	0.534	0.510	0.634	0.706	0.485	0.675	0.487	0.624	0.577	0.558	0.553	0.653	0.488	0.411	0.611	0.656	1.000

Table 3.2.3 RV coefficient between projective maps and multifactor analysis (MFA) for week three where 94% of panellists scored >0.5.

N1         N2         N3         N4         N5         N6         N7         N8         N9         N10         N11         N12         N13         N14         N15         N16         MFA           N1         1.000         0.125         0.092         0.134         0.549         0.255         0.099         0.220         0.147         0.800         0.111         0.418         0.464         0.106         0.314         0.393         0.521         0.170         0.292         0.266         0.966         0.214         0.427         0.215         0.198         0.322         0.164         0.493         0.527         0.414           N4         0.337         0.160         0.080         0.126         0.229         0.366         0.314         0.226         0.105         0.126         0.149         0.449         0.441           N4         0.134         0.397         0.160         0.089         0.546         0.121         0.323         0.622         0.624         0.629         0.626         0.229         0.546         0.129         0.524         0.623         0.624         0.629         0.626         0.229         0.536         0.129         0.231         0.623         0.547																		
N1       1.000       0.125       0.092       0.134       0.549       0.255       0.099       0.220       0.147       0.080       0.111       0.418       0.464       0.106       0.314       0.394       0.393       0.466         N2       0.125       1.000       0.045       0.397       0.170       0.290       0.206       0.096       0.254       0.194       0.447       0.251       0.198       0.342       0.184       0.493       0.502         N3       0.092       0.045       1.000       0.160       0.808       0.126       0.299       0.366       0.314       0.242       0.454       0.212       0.454       0.212       0.454       0.212       0.454       0.212       0.459       0.170       0.889       0.644       0.129       0.224       0.327       0.922       0.644       0.79       0.29       0.858       0.121       0.363         N5       0.549       0.170       0.889       0.644       1.000       0.179       0.224       0.327       0.922       0.264       0.799       0.208       0.608       0.494       0.398       0.464       0.798       0.688       0.618       0.488       0.314       0.266       0.505 <t< th=""><th></th><th>N1</th><th>N2</th><th>N3</th><th>N4</th><th>N5</th><th>N6</th><th>N7</th><th>N8</th><th>N9</th><th>N10</th><th>N11</th><th>N12</th><th>N13</th><th>N14</th><th>N15</th><th>N16</th><th>MFA</th></t<>		N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13	N14	N15	N16	MFA
N2       0.125       1.000       0.045       0.397       0.170       0.290       0.206       0.096       0.254       0.194       0.447       0.251       0.198       0.342       0.184       0.493       0.502         N3       0.092       0.045       1.000       0.160       0.080       0.126       0.229       0.366       0.314       0.226       0.105       0.292       0.045       0.327       0.065       0.127       0.414         N4       0.134       0.397       0.160       1.000       0.009       0.389       0.546       0.179       0.322       0.620       0.454       0.212       0.749       0.149       0.056       0.654         N5       0.549       0.170       0.880       0.009       1.000       0.179       0.224       0.825       0.178       0.547       0.598       0.396       0.608       0.448       0.308       0.734         N6       0.220       0.206       0.229       0.546       0.213       0.179       1.000       0.231       0.178       0.188       0.547       0.588       0.326       0.306       0.048       0.342       0.342       0.343       0.546       0.114       0.147       0.254       0	N1	1.000	0.125	0.092	0.134	0.549	0.255	0.099	0.220	0.147	0.080	0.111	0.418	0.464	0.106	0.314	0.398	0.466
N3       0.092       0.045       1.000       0.160       0.080       0.126       0.229       0.366       0.314       0.226       0.105       0.292       0.045       0.327       0.065       0.127       0.414         N4       0.134       0.397       0.160       1.000       0.009       0.389       0.546       0.107       0.308       0.220       0.630       0.454       0.212       0.749       0.149       0.056       0.654         N5       0.549       0.170       0.080       0.009       1.000       0.064       0.213       0.129       0.022       0.327       0.092       0.264       0.079       0.029       0.085       0.121       0.363         N6       0.255       0.290       0.126       0.389       0.064       1.000       0.179       0.234       0.835       0.178       0.547       0.598       0.396       0.608       0.048       0.308       0.734         N7       0.099       0.206       0.229       0.546       0.213       0.179       1.000       0.631       0.929       0.538       0.306       0.505       0.998       0.138       0.546         N8       0.220       0.096       0.366       0.107<	N2	0.125	1.000	0.045	0.397	0.170	0.290	0.206	0.096	0.254	0.194	0.447	0.251	0.198	0.342	0.184	0.493	0.502
N4       0.134       0.397       0.160       1.000       0.009       0.389       0.546       0.107       0.308       0.220       0.630       0.454       0.212       0.749       0.149       0.056       0.654         N5       0.549       0.170       0.080       0.009       1.000       0.064       0.213       0.129       0.022       0.327       0.092       0.264       0.079       0.029       0.858       0.121       0.363         N6       0.255       0.290       0.126       0.389       0.064       1.000       0.179       0.234       0.835       0.178       0.547       0.598       0.396       0.608       0.048       0.308       0.734         N7       0.099       0.266       0.229       0.546       0.213       0.179       1.000       0.63       0.239       0.787       0.188       0.252       0.306       0.505       0.98       0.138       0.584         N8       0.220       0.966       0.366       0.107       0.129       0.234       0.063       1.000       0.312       0.388       0.436       0.372       0.534       0.316       0.124       0.692         N10       0.080       0.194       0.226 </td <td>N3</td> <td>0.092</td> <td>0.045</td> <td>1.000</td> <td>0.160</td> <td>0.080</td> <td>0.126</td> <td>0.229</td> <td>0.366</td> <td>0.314</td> <td>0.226</td> <td>0.105</td> <td>0.292</td> <td>0.045</td> <td>0.327</td> <td>0.065</td> <td>0.127</td> <td>0.414</td>	N3	0.092	0.045	1.000	0.160	0.080	0.126	0.229	0.366	0.314	0.226	0.105	0.292	0.045	0.327	0.065	0.127	0.414
N5       0.549       0.170       0.080       0.009       1.000       0.064       0.213       0.129       0.022       0.327       0.092       0.264       0.079       0.029       0.085       0.121       0.363         N6       0.255       0.290       0.126       0.389       0.064       1.000       0.179       0.234       0.835       0.178       0.547       0.598       0.396       0.608       0.048       0.308       0.734         N7       0.099       0.206       0.229       0.546       0.213       0.179       1.000       0.63       0.239       0.787       0.188       0.252       0.306       0.505       0.098       0.138       0.584         N8       0.220       0.096       0.366       0.107       0.129       0.234       0.063       1.000       0.312       0.388       0.436       0.372       0.534       0.316       0.124       0.692         N10       0.080       0.194       0.226       0.220       0.327       0.178       0.787       0.098       0.312       1.000       0.106       0.115       0.196       0.311       0.285       0.315       0.507         N11       0.111       0.447       0.105	N4	0.134	0.397	0.160	1.000	0.009	0.389	0.546	0.107	0.308	0.220	0.630	0.454	0.212	0.749	0.149	0.056	0.654
N6       0.255       0.290       0.126       0.389       0.064       1.000       0.179       0.234       0.835       0.178       0.547       0.598       0.396       0.608       0.048       0.308       0.734         N7       0.099       0.206       0.229       0.546       0.213       0.179       1.000       0.063       0.239       0.787       0.188       0.252       0.306       0.505       0.098       0.138       0.584         N8       0.220       0.096       0.366       0.107       0.129       0.234       0.063       1.000       0.231       0.098       0.120       0.259       0.538       0.004       0.344       0.266       0.434         N9       0.147       0.254       0.314       0.308       0.022       0.835       0.239       0.231       1.000       0.312       0.388       0.436       0.372       0.534       0.316       0.124       0.692         N10       0.080       0.194       0.226       0.220       0.327       0.178       0.787       0.988       0.312       1.000       0.115       0.196       0.311       0.285       0.315       0.507         N11       0.111       0.447       0.10	N5	0.549	0.170	0.080	0.009	1.000	0.064	0.213	0.129	0.022	0.327	0.092	0.264	0.079	0.029	0.085	0.121	0.363
N70.0990.2060.2290.5460.2130.1791.0000.0630.2390.7870.1880.2520.3060.5050.0980.1380.584N80.2200.0960.3660.1070.1290.2340.0631.0000.2310.0980.1200.2590.5380.0040.3440.2660.434N90.1470.2540.3140.3080.0220.8350.2390.2311.0000.3120.3880.4360.3720.5340.3160.1240.692N100.0800.1940.2260.2200.3270.1780.7870.0980.3121.0000.1060.1150.1960.3110.2850.3150.507N110.1110.4470.1050.6300.0920.5470.1880.1200.3880.1061.0000.5890.0840.6190.2330.3550.631N120.4180.2510.2920.4540.2640.5980.2520.2590.4360.1150.5891.0000.4290.5710.3050.0770.741N130.4640.1980.0450.2120.0790.3960.2360.5110.1410.1120.5160.0290.2651.0000.2060.1810.3050.1040.566N140.1060.3420.3270.7490.0290.6680.5560.0040.5340.3110.6190.5710.181 <t< td=""><td>N6</td><td>0.255</td><td>0.290</td><td>0.126</td><td>0.389</td><td>0.064</td><td>1.000</td><td>0.179</td><td>0.234</td><td>0.835</td><td>0.178</td><td>0.547</td><td>0.598</td><td>0.396</td><td>0.608</td><td>0.048</td><td>0.308</td><td>0.734</td></t<>	N6	0.255	0.290	0.126	0.389	0.064	1.000	0.179	0.234	0.835	0.178	0.547	0.598	0.396	0.608	0.048	0.308	0.734
N8       0.220       0.096       0.366       0.107       0.129       0.234       0.063       1.000       0.231       0.098       0.120       0.259       0.538       0.004       0.344       0.266       0.434         N9       0.147       0.254       0.314       0.308       0.022       0.835       0.239       0.231       1.000       0.312       0.388       0.436       0.372       0.534       0.316       0.124       0.692         N10       0.080       0.194       0.226       0.220       0.327       0.178       0.787       0.098       0.312       1.000       0.106       0.115       0.196       0.311       0.285       0.315       0.507         N11       0.111       0.447       0.105       0.630       0.092       0.547       0.188       0.120       0.388       0.106       1.000       0.589       0.084       0.619       0.233       0.355       0.631         N12       0.418       0.251       0.292       0.454       0.598       0.252       0.259       0.436       0.115       0.589       1.000       0.429       0.571       0.305       0.077       0.741         N13       0.464       0.198       0.	N7	0.099	0.206	0.229	0.546	0.213	0.179	1.000	0.063	0.239	0.787	0.188	0.252	0.306	0.505	0.098	0.138	0.584
N9       0.147       0.254       0.314       0.308       0.022       0.835       0.239       0.231       1.000       0.312       0.388       0.436       0.372       0.534       0.316       0.124       0.692         N10       0.080       0.194       0.226       0.220       0.327       0.178       0.787       0.098       0.312       1.000       0.106       0.115       0.196       0.311       0.285       0.315       0.507         N11       0.111       0.447       0.105       0.630       0.092       0.547       0.188       0.120       0.388       0.106       1.000       0.589       0.084       0.619       0.233       0.355       0.631         N12       0.418       0.251       0.292       0.454       0.264       0.598       0.252       0.259       0.436       0.115       0.589       1.000       0.429       0.571       0.305       0.077       0.741         N13       0.464       0.198       0.045       0.212       0.079       0.396       0.505       0.004       0.534       0.311       0.619       0.571       0.181       1.000       0.206       0.108       0.732         N14       0.106       0	N8	0.220	0.096	0.366	0.107	0.129	0.234	0.063	1.000	0.231	0.098	0.120	0.259	0.538	0.004	0.344	0.266	0.434
N100.0800.1940.2260.2200.3270.1780.7870.0980.3121.0000.1060.1150.1960.3110.2850.3150.507N110.1110.4470.1050.6300.0920.5470.1880.1200.3880.1061.0000.5890.0840.6190.2330.3550.631N120.4180.2510.2920.4540.2640.5980.2520.2590.4360.1150.5891.0000.4290.5710.3050.0770.741N130.4640.1980.0450.2120.0790.3960.3060.5380.3720.1960.0840.4291.0000.1810.3050.1040.566N140.1060.3420.3270.7490.0290.6080.5050.0040.5340.3110.6190.5710.1811.0000.2060.1080.732N151.0000.2450.4580.1210.1090.2650.2360.5110.1410.1120.2150.1650.0290.2651.0000.2060.785N160.5690.1250.1560.3120.2250.5620.1210.3280.0030.3260.3650.3260.4030.5440.2061.0000.596MFA0.6590.5020.4140.6540.5260.7340.5840.6210.6920.5070.6310.7410.5660.7320.511 <td>N9</td> <td>0.147</td> <td>0.254</td> <td>0.314</td> <td>0.308</td> <td>0.022</td> <td>0.835</td> <td>0.239</td> <td>0.231</td> <td>1.000</td> <td>0.312</td> <td>0.388</td> <td>0.436</td> <td>0.372</td> <td>0.534</td> <td>0.316</td> <td>0.124</td> <td>0.692</td>	N9	0.147	0.254	0.314	0.308	0.022	0.835	0.239	0.231	1.000	0.312	0.388	0.436	0.372	0.534	0.316	0.124	0.692
N11       0.111       0.447       0.105       0.630       0.092       0.547       0.188       0.120       0.388       0.106       1.000       0.589       0.084       0.619       0.233       0.355       0.631         N12       0.418       0.251       0.292       0.454       0.264       0.598       0.252       0.259       0.436       0.115       0.589       1.000       0.429       0.571       0.305       0.077       0.741         N13       0.464       0.198       0.045       0.212       0.079       0.396       0.306       0.538       0.372       0.196       0.084       0.429       1.000       0.181       0.305       0.104       0.566         N14       0.106       0.342       0.327       0.749       0.029       0.608       0.505       0.004       0.534       0.311       0.619       0.571       0.181       1.000       0.206       0.108       0.732         N15       1.000       0.245       0.458       0.121       0.109       0.265       0.236       0.511       0.141       0.112       0.215       0.165       0.206       1.000       0.206       0.732       0.736       0.732         N16	N10	0.080	0.194	0.226	0.220	0.327	0.178	0.787	0.098	0.312	1.000	0.106	0.115	0.196	0.311	0.285	0.315	0.507
N12       0.418       0.251       0.292       0.454       0.264       0.598       0.252       0.259       0.436       0.115       0.589       1.000       0.429       0.571       0.305       0.077       0.741         N13       0.464       0.198       0.045       0.212       0.079       0.396       0.306       0.538       0.372       0.196       0.084       0.429       1.000       0.181       0.305       0.104       0.566         N14       0.106       0.342       0.327       0.749       0.029       0.608       0.505       0.004       0.534       0.311       0.619       0.571       0.181       0.305       0.104       0.566         N15       1.000       0.245       0.458       0.121       0.109       0.265       0.236       0.511       0.141       0.112       0.215       0.165       0.029       0.265       1.000       0.206       0.785         N16       0.569       0.125       0.454       0.526       0.734       0.584       0.621       0.692       0.507       0.631       0.403       0.544       0.206       1.000       0.596         MFA       0.659       0.502       0.414       0.654	N11	0.111	0.447	0.105	0.630	0.092	0.547	0.188	0.120	0.388	0.106	1.000	0.589	0.084	0.619	0.233	0.355	0.631
N13       0.464       0.198       0.045       0.212       0.079       0.396       0.306       0.538       0.372       0.196       0.084       0.429       1.000       0.181       0.305       0.104       0.566         N14       0.106       0.342       0.327       0.749       0.029       0.608       0.505       0.004       0.534       0.311       0.619       0.571       0.181       1.000       0.206       0.108       0.732         N15       1.000       0.245       0.458       0.121       0.109       0.265       0.236       0.511       0.141       0.112       0.215       0.165       0.029       0.265       1.000       0.206       0.785         N16       0.569       0.125       0.156       0.312       0.225       0.562       0.121       0.328       0.003       0.326       0.365       0.326       0.403       0.544       0.206       1.000       0.596         MFA       0.659       0.502       0.414       0.654       0.526       0.734       0.584       0.621       0.692       0.507       0.631       0.741       0.566       0.732       0.511       0.529       1.000	N12	0.418	0.251	0.292	0.454	0.264	0.598	0.252	0.259	0.436	0.115	0.589	1.000	0.429	0.571	0.305	0.077	0.741
N14       0.106       0.342       0.327       0.749       0.029       0.608       0.505       0.004       0.534       0.311       0.619       0.571       0.181       1.000       0.206       0.108       0.732         N15       1.000       0.245       0.458       0.121       0.109       0.265       0.236       0.511       0.141       0.112       0.215       0.165       0.029       0.265       1.000       0.206       0.785         N16       0.569       0.125       0.156       0.312       0.225       0.562       0.121       0.328       0.003       0.326       0.365       0.326       0.403       0.544       0.206       1.000       0.596         MFA       0.659       0.502       0.414       0.654       0.526       0.734       0.584       0.621       0.692       0.507       0.631       0.741       0.566       0.732       0.511       0.529       1.000	N13	0.464	0.198	0.045	0.212	0.079	0.396	0.306	0.538	0.372	0.196	0.084	0.429	1.000	0.181	0.305	0.104	0.566
N15       1.000       0.245       0.458       0.121       0.109       0.265       0.236       0.511       0.141       0.112       0.215       0.165       0.029       0.265       1.000       0.206       0.785         N16       0.569       0.125       0.156       0.312       0.225       0.562       0.121       0.328       0.003       0.326       0.365       0.326       0.403       0.544       0.206       1.000       0.596         MFA       0.659       0.502       0.414       0.654       0.526       0.734       0.584       0.621       0.692       0.507       0.631       0.741       0.566       0.732       0.511       0.529       1.000	N14	0.106	0.342	0.327	0.749	0.029	0.608	0.505	0.004	0.534	0.311	0.619	0.571	0.181	1.000	0.206	0.108	0.732
N16         0.569         0.125         0.156         0.312         0.225         0.562         0.121         0.328         0.003         0.326         0.365         0.326         0.403         0.544         0.206         1.000         0.596           MFA         0.659         0.502         0.414         0.654         0.526         0.734         0.584         0.621         0.692         0.507         0.631         0.741         0.566         0.732         0.511         0.529         1.000	N15	1.000	0.245	0.458	0.121	0.109	0.265	0.236	0.511	0.141	0.112	0.215	0.165	0.029	0.265	1.000	0.206	0.785
MFA 0.659 0.502 0.414 0.654 0.526 0.734 0.584 0.621 0.692 0.507 0.631 0.741 0.566 0.732 0.511 0.529 1.000	N16	0.569	0.125	0.156	0.312	0.225	0.562	0.121	0.328	0.003	0.326	0.365	0.326	0.403	0.544	0.206	1.000	0.596
	MFA	0.659	0.502	0.414	0.654	0.526	0.734	0.584	0.621	0.692	0.507	0.631	0.741	0.566	0.732	0.511	0.529	1.000

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