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The role of protein and starch of legumes and cereals on the formation of
healthy snack products

A thesis
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of the requirements for the Degree of
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Swapnil Shamrao Patil

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Supervisory team

Professor Charles Brennan was the principal supervisor and Doctor Sue Mason and Doctor Margaret Brennan was the associate supervisor for this PhD research mentioned in the above publications.

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Food Science.

The role of protein and starch of legumes and cereals on the formation of healthy snack products

by

Swapnil Shamrao Patil

Proteins, vitamins, minerals and carbohydrates are important nutrients that play vital role in human metabolism. Cereals and Legumes are significant source of protein, dietary fiber, carbohydrates and dietary minerals. They are also an excellent source of essential amino acid lysine. Unfortunately, legume seeds that grow in New Zealand are considered as a low value crop and mainly used in animal feed production. However, we believe that combinations of legumes and cereals grown in New Zealand could be used as a value added food ingredient for products. On the other hand there has been increased attention in the utilization of non-meat protein rich food materials for food products. This may be due to their potential to control postprandial protein digestibility and glycaemic response of individuals. However, there is limited data available on the efficacy of legume and cereal that grown in New Zealand on the protein and carbohydrate digestibility.

The main aim of this research project is to evaluate the potential of using blends of legume and cereal materials as a snack product on the manipulation of protein and carbohydrate digestibility in human. This project will advance knowledge of the transfer of processing and biochemical research to the food industry and thus the creation of a value added food chain for New Zealand grown legumes and cereals as processed snack products.

Keywords: Legumes, cereals, extrusion, pasta, protein digestibility, physico-chemical properties and glycaemic response.

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List of Symbols and Abbreviations

%	percent
µg	microgram
µl	microliters
°C	degree Celsius
CVD	Cardiovascular disease
FAO	Food and Agricultural Organization
g	grams
h	Hour
min	minutes
ml	milliliters
M	molar
mg	milligrams
n	normality
pH	potential hydrogen
rpm	rotations per minutes
UK	United Kingdom
w/v	weight by volume
w/w	weight by weight

Chapter 1

Introduction

Currently, due to convenience and the ease of preparation, processed foods are becoming popular over a broad population (Brennan *et al.*, 2013). Rapid industrialisation, fast changing lifestyles and socio-economic changes to an urban society have changed consumers dietary habits and, hence, the demand for processed food (Jemal *et al.*, 2005). Ready-to-eat (RTE) snacks are the most popular products among processed foods (Brennan *et al.*, 2013). The abundance and availability of such products (extrudates, muffins, etc.), as well as their economic affordability, are responsible for the increasing popularity of RTE snacks. At present, refined cereal flours, sugar, saturated fats and salts are the main ingredients used to produce RTE snacks (Brennan *et al.*, 2013, Struck *et al.*, 2014). Excess consumption of these products might increase the glucose response and this may increase the risk of health problems, such as obesity, cardiovascular disease and Type 2 diabetes (Brennan *et al.*, 2013).

Legumes and cereals are rich in carbohydrates, protein and fibre (Kumar *et al.*, 2010). Some studies. Anton *et al.* (2009), have showed the importance of legumes (beans) as plant based sources of proteins and fibre components. Similarly, Siddhuraju *et al.*, (2002) suggested the potential of use of beans as a source of protein (30%) and starch (28%) for the development of conventional food products, particularly in the developing world. Plant based foods; cereals and legumes are rich in carbohydrates, protein and fibre (Kumar *et al.*, 2010). Therefore legumes and cereals can be promoted as a supplementary source of food for humans. Legumes, especially beans are sources

of proteins and fibre components having protein (30%) and starch (28%). Further, they have potential advantages over animal products due to having easily digestible protein/starch, acceptance on ethical aspects and low cost (Silva-Cristobal et al., 2010). Many nutrients (vitamins, minerals, amino acids to name a few) are required by the human body; however, carbohydrates and proteins are significant in terms of nutrition. Protein is a vital source of amino acids in human diets and these are important as building blocks for muscle structure (Butts *et al.*, 2012). In addition, dietary fibre and starchy foods make a major contribution to a balanced diet in humans. In recent years, there has been much attention given to the role of the glycaemic impact from foods on obesity and diabetes, with the impact of high glycaemic foods being regarded as responsible for excess energy consumption and, hence, weight gain, obesity and the prevalence of diabetes (Brennan, 2005). However, foods that have a low glycemic impact may play vital roles in decreasing blood glucose and helping in maintaining the insulin response for people suffering from diabetes (Wang *et al.*, 2003). Brennan *et al.* (2011), have shown that the use of dietary fibre in the development of RTE snacks can be beneficial for human health, that consumption of fibre rich products reduces starch breakdown and glucose absorption. Which further leads to reduction in postprandial glucose response.

Some studies have shown that the development of healthy snacks can be achieved by altering the composition of traditional products (Boye *et al.*, 2010b; Osen *et al.* 2015). There are several processing methods used by food industries to develop RTE snacks; for instance, fermentation, germination and extrusion. However, extrusion is one of the most popular methods among food producers. This has become commercially accepted

over the past thirty years. This interest might increase in the near future due to consumers' growing interest in extruded products.

The advent of extrusion technology has allowed food manufacturers to provide a wide range of shaped, textured and precooked foods. Depending on the raw materials, the extruders can operate with low, medium or high shear rates with little or no modification of the basic equipment and, with appropriate process control, the production of a great variety of food and feed products can be achieved in the presence of heat and moisture (Steel *et al.*, 2012). The importance of extrusion cooking over conventional cooking methods is because of their versatility, efficiency and economy of space and labour (Brennan *et al.*, 2013; Chauhan and Bains, 1988).

The present study focused on the investigation of the addition of different levels \ of legumes on extrudate products and the effect of extrusion temperature on their physico-chemical properties as well as starch and protein digestibilities.

1.1 Aim and objectives:

The importance of the choice of this topic was to help in the reduction of health problems, and to provide a nutritious and economically affordable product for the growing number of consumers, especially in developing countries where plant food sources are abundantly available. Food quality is also important when considering foods consumed as much previous research has focused on illustrating the nutritional value of foods derived from legumes and cereal blends; however, more study is needed to assess the quality of the products produced using hot and cold extrusion techniques. Hence, to assess the quality of the product the physico-chemical and structural properties of the products will be studied.

1.2 Specific objectives are to:

- Evaluate the protein contents of legume and cereal flours.
- Study the protein and starch contents of legumes and cereal based food products to evaluate the effect hot and cold extrusion techniques have on the chemical composition of the foods.
- Evaluate *in vitro* protein and starch digestibility of legume and cereal based food products prepared using hot and cold extrusion.
- Determine the cooking properties, water activity and textural properties of the food products developed in relation to legume addition to cereal based food products.

1.3 Hypotheses:

- Processing will improve the protein and starch digestibility of products.
- Adding legume flours to traditional cereal based food products will improve the physico-chemical properties of the product.
- Inclusion of legume flours into cereal based foods will reduce the glycaemic response of cereal based snack foods.

Chapter 2

Literature review

This chapter critically evaluates the current literature in relation to the use of legumes to improve the nutritional quality of cereal food products. The chapter includes observations about previous studies and results from using legumes to manipulate the glycaemic impact and protein content of processed foods using extrusion techniques. The chapter also explains the reason and mechanism behind the outcomes from previous studies. The details of the role of cereals and legumes in human nutrition and the effect of extrusion processing on protein and starch digestibility on ready-to-eat (RTE) snack food products is discussed. The effects of adding legumes to traditional cereal based products were also reviewed.

The population of the world is expanding exponentially and this has resulted in problems, such as malnourishment and lack of quality food to consume in some parts of the world. In order to provide nutritionally good quality food at an affordable price to the growing numbers of people around the world research is needed in terms of improving food processing techniques. Processing foods with a combination of legumes and cereals can be viewed as a solution for such a need. Cereals and legumes contain significant amounts of nutrients, including starches, proteins, dietary fibre, vitamins and minerals and the nutrients derived from cereals and legumes are much lower in price compared to nutrients from animal origins (Ndidi *et al.*, 2014). However, when legume seeds are less bioavailable when consumed in uncooked form due to presence of anti-nutritional compounds (Kalpnadevi *et al.* 2012). The presence of anti-nutritional factors

(tannins, trypsin inhibitors and polyphenols) is responsible for the reduction in bioavailability of these nutrients (Nikmaram *et al.*, 2017). Also, the consumption of anti-nutrients might affect the absorption of nutrients in the human body during hydrolysis, and an excessive consumption of such compounds may lead to food poisoning and renal diseases (Oyewole and Soetan, 2009).

There are many processing techniques such as milling, cooking, soaking, fermentation and extrusion, which, when implemented help improve the nutritive value of food product. Khattab *et al.*, (2009) reported an increase in protein quality and the digestibility of peas, cowpeas and kidney beans after cooking. El-Adawy (2002) reported the nutritional composition and anti-nutritional factors of chickpeas (*Cicer arietinum L.*) have changed after implementing boiling, autoclaving and microwaving methods. This study showed the effects of boiling, autoclaving and microwave cooking and germination on the nutritional composition and anti-nutritional factors in chickpeas. The results showed that anti-nutritional factors, such as phytic acid, stachyose and raffinose were significantly reduced (around 80%) after cooking the chickpeas. Ndidi *et al.* (2014) concluded that anti-nutrients were more susceptible to moist heat rather than dry heat. As boiling reduces the trypsin inhibitors activity below critical level compared to roasting. In their study it was also observed that boiling groundnuts significantly reduced the levels anti-nutritional compounds such as oxalate, tannins, phytate, trypsin inhibitor, and hydrogen cyanide contents and made the nutrients more bioavailable compared to raw ground nut seeds.

Thermal treatments, enzyme applications, soaking, sprouting, irradiation and fermentation are commonly applied to improve the nutritional quality of legumes and

cereals (Nadeem *et al.*, 2010). However, cooking at a high temperature and for a long time may induce irreversible changes in the structure of the proteins and starch in cereals and legumes. Treatments may be responsible for removing other beneficial nutrients, such as vitamins and minerals (Li *et al.*, 2014). These methods are considered as traditional and economical in reducing the amounts of anti-nutritional compounds. Applying a combination of different processing methods in developing a product results in more reductions in the anti-nutritional compounds (Siddhuraju *et al.*, 2002). In recent years, several other techniques, such as high pressure processing, microwaving and extrusion, have been taken together to reduce anti-nutritional levels (Zarei, 2013). The current work, therefore, evaluates the use of cereal and legume combinations and the effect of extrusion on improving the nutritional quality of foods.

2.1 Importance of snack food products:

Research by Lloyd-Williams *et al.* (2009) has indicated that snacks are most often consumed in the afternoon. Research has identified that 90% of the U.K. and 97% of the U.S. populations consume snacks on a regular basis. Snack consumption can make a significant contribution to energy intake and positively associated with obesity (Lloyd-Williams *et al.*, 2009). Rapid industrialisation, fast changing lifestyles and socio-economic changes in urban societies are the some of the reason behind change in consumers' dietary habits and the demand for processed food. Ready-to-eat snacks are now one of the most popular products among other processed foods (Singh *et al.*, 2007). According to the Food Standards Agency (FSA, U.K.) ready-to-eat products are products that do not need further processing, such as heating, before consumption. Several other factors, such as social context and individualisation, may also be

responsible for the increasing popularity of snack foods (Shaviklo *et al.*, 2011, Norgaard *et al.*, 2013). Most of the available snacks contain significant amounts of saturated fats and salts (saturated fat average 5.1 g/serve and salt average 0.56 g/serve) compared to healthy snacks (saturated fat average 0.69 g/serve and salt average 0.05 g/serve) (Lloyd-Williams *et al.*, 2009). It's been reported previously that the increased consumption of such foods (high in fat and calories) has been associated with an increasing health problems, such as diabetes, cardiovascular disease, obesity, high blood pressure and breast cancer (Dingemans *et al.*, 2009, Granner *et al.*, 2004, Shaviklo *et al.*, 2011).

Regular consumption of energy rich foods can also decrease satiety and be related to numerous health problems (Bilman *et al.*, 2010). It has been reported that a large proportion of the American population (around 66%) are overweight or suffering from obesity and diabetes, and it has been predicted that this will increase over the next few years due to increases in high energy low nutrient quality foods (Hu, 2005). Products such as extruded snacks, salted chips, chocolates and cookies contain high amounts of saturated fat. Also, these products are contribute to increasing blood pressure, cholesterol and body weight; for instance, around 170,000 deaths in the U.K. are caused every year due to CVD and diabetes, and these are related to poor nutritional choices. (Lloyd-Williams *et al.*, 2009). Satiety is a key factor in snack consumption. A study carried out by Bilman *et al.* (2010) showed that the satiety level of an individual depends on the composition of the food. As mentioned before, products like salted chips, chocolate and sweetened beverages tend to be consumed frequently due to their low satiety effects (Bilman *et al.*, 2010). Other researchers have shown that foods rich in dietary fibres exhibit a modified food structure and functionality which can in turn affect nutrient digestibility and satiety ratings (Oliveira *et al.*, 2015, Rashid *et al.*, 2015). Results from

Clark and Slavin (2013) have shown a relationship between fibre intake and satiety; thus, it has been observed that fibre rich food reduces appetite, and that increasing satiety helps in the reduction of energy rich food intakes. It is assumed that fibre intakes can increase satiety by various mechanisms, such as slowing digestion and delaying gastric emptying (Howrath *et al.*, 2001). Results published by Mathern *et al.* (2009) indicated that the addition of fibre powder obtained from fenugreek to breakfast cereals tended to increase satiety and the increased levels of fibre powder in the samples showed a reduction in energy intake. It was observed consumption of fenugreek powder added breakfast enhanced satiety and fullness compared to no or less fenugreek powder added breakfast. Also, a decreasing trend towards energy intake was observed at lunch by the panellists. The effect on the reduction of hunger of the panellists suggests that fenugreek fibre powder may have control on food intake of individuals. Snack consumption in youth has increased over recent years and products, like cakes, cookies, chips and popcorn, are of the main foods of interest (Bleich and Wolfson, 2015a). Bleich and Wolfson (2015b) have also suggested that half of children, and one third of adults, in the U.S. are affected by obesity due to the over consumption of calories. These observations indicate a need to produce healthy alternatives to ready-to-eat snack products. It can be concluded that food consumed by humans needs to be rich in proteins, fibres and have a low in glycaemic response, as well as having good functional properties (Hu, 2005, Marsh *et al.*, 2014). So, the present study focuses on developing a nutritious extruded snack product using different levels and combinations of cereals and legumes.

2.2 Importance of grains in the human diet:

In New Zealand, more than 80% of the land is under food production. Most of the agricultural land is under maize, wheat and barley, peas and lentil cultivation and it is centred on the Canterbury region. In 2012, more than 1 million tonnes of cereals were harvested from over 140,000 hectares of land and legume (peas, lentils and beans) production was around three tonnes/hectare per seasons (Millner and Roskruge, 2013). Among all types of edible plant seeds, legumes and cereals are the major sources of dietary proteins in many parts of the world (Ndidi *et al.*, 2014). Table 2.1 illustrates the nutritional composition of the cereals and legumes selected for the present study.

Table 2.1 Nutritional composition of selected cereals and legumes (g/100g)

Seed	Peas	Chick-pea	Lentil	Wheat	Barley	Maize	Rice
Protein	21.9	18.5	20.6	11.98	14.83	5.59	7.4
Carbohydrates	52.5	54.0	56.4	26.8	81.8	82.75	79.8
Dietary fibre	4.7	10.7	4.9	36.4	20.3	1.9	0.6

(Adhikari *et al.*, 2016, Adoracion *et al.*, 1979, de Almeida Costa *et al.*, 2006, Djurle *et al.*, 2016, Henry, 1985, Ibukun, 2008, Iqbal *et al.*, 2006).

2.2.1 Cereals:

Cereals are considered the main source of carbohydrates in the human diet with wheat being one of the most consumed cereals worldwide (Matsuo, 1994). More than 30% of wheat produced in 2009 was consumed in Europe, due to the dietary requirements of Europe being driven by wheat based foods. In 2009, 33% of the wheat produced within the Europe was used as feed and, in addition, several by-products of wheat processing were used for livestock feeding (Rosenfelder *et al.*, 2013). Wheat contains 8-15% of storage proteins, mainly gluten. Gluten possesses some unique physical properties, for instance it forms a cohesive, visco-elastic protein network after heating that enables the production of various conventional products (Day, 2013). After wheat, rice is a widely accepted food cereal. Although rice is a low protein cereal (7-9%) it is widely accepted by consumers in Asian countries due to its high production in the Asian countries. The high carbohydrate composition and high digestibility rate makes it popular among snack food producers. Also, it has the potential to produce high protein fortified snack foods (Fabian and Ju, 2011). Other cereals, such as barley and maize also contribute considerable amounts of protein, dietary fibres and lipids. Maize is the grain crop preferred by the gluten intolerant (coeliac disease) population (Wojtowicz *et al.*, 2013). As coeliac disease is life-long, cereals like maize can be used as nutrient sources for the affected population due to the absence of gluten. The exclusive characteristics of maize, such as no gluten, low lipids makes it popular among specific population in the community (Wronkowska *et al.*, 2012). Barley and oats contain soluble and insoluble dietary fibres (β -glucan), which are linked with restricting the glycaemic response and developing better bowel health by lowering the absorption and re-absorption of lipids,

bile acids and cholesterol. The most widely documented nutritional benefit of β -glucan in foods is the flattening of the postprandial blood glucose and insulin rises (Brennan and Cleary, 2005).

Cereals are also high in antioxidants, such as phenolic acids, that are considered beneficial for human health. The outer layer of cereal grains mainly comprises compounds that are beneficial to human health, such as lignin, minerals, vitamins and phenolic compounds (Ragaei *et al.*, 2011).

2.2.2 Legumes:

Legumes rank second after cereals in human consumption and Leguminosae is the most important family in the di-cotyledons. It is a cold season crop grown annually in many parts of the world, such as North America and Asia. Legumes are relatively easy to cultivate using a range of agricultural practices and hence, they are popular throughout the world. Their use is expected to increase with the growing world population (Duranti, 2006, Roy *et al.*, 2010). Legumes include peas, beans and lentils, all of which are important sources of protein (about 20% dry weight in peas and beans and 38–40% of dry weight in soybeans and lupins). Essentially, they are regarded as a necessary supplement to other protein sources due to their high digestibility and bioavailability (Hu, 2005). The seed proteins in legumes are considered to be storage proteins, and globulin (7S, 11S) is the most abundantly found storage protein in legumes. Legume protein has several advantages over cereal proteins; for example, they are abundant in essential amino acids such as lysine (Table 2.2). However, all legume storage proteins are relatively low in sulphur-containing amino acids -

methionine, cysteine and tryptophan. The various functions of amino acids are illustrated in Table 2.3. It has also been reported that legume proteins may play bioactive roles in various physiological functions in the human body (Duranti, 2006).

Table 2.2 Content of essential amino acid in legumes (g/100g)

Amino acid	Chickpea	Cowpea	Lentil	Green pea
Arginine	8.3a ± 0.21	7.5c ± 0.04	7.8b ± 0.03	7.2d ± 0.04
Histidine	3.0a ± 0.03	3.1a ± 0.03	2.2c ± 0.05	2.4d ± 0.05
Isoleucine	4.8a ± 0.03	4.5b ± 0.03	4.1b ± 0.05	4.5a ± 0.06
Leucine	8.7a ± 0.03	7.7b ± 0.08	7.8b ± 0.05	7.4b ± 0.05
Lysine	7.2b ± 0.03	7.5b ± 0.04	7.0b ± 0.03	8.1a ± 0.07
Methionine	1.1b ± 0.01	2.2a ± 0.04	0.8c ± 0.02	1.1b ± 0.03
Phenylalanine	5.5b ± 0.04	7.5a ± 0.06	5.0b ± 0.12	5.2b ± 0.04
Threonine	3.1b ± 0.04	3.8a ± 0.05	3.5a ± 0.04	3.8a ± 0.05
Tryptophan	0.9a ± 0.03	0.7a ± 0.02	0.7a ± 0.03	0.8a ± 0.02
Valine	4.6a ± 0.03	5.0a ± 0.06	5.0a ± 0.05	5.0a ± 0.09

(Iqbal *et al.*, 2006)

Means in each column for each crop followed by the same letter are not significantly different ($p = 0.05$)

Legumes are considered as a good source of complex carbohydrates as they contain high molecular weight polysaccharides such as starch and cellulose (Harding, 2012). These complex carbohydrates are useful in human nutrition terms as they take a long time to digest and do not suddenly increase blood glucose levels. Previously, the importance of legumes has been studied as a source of carbohydrates as they provide some dietary fibre (Tosh and Yada, 2010) and significant amounts of both slowly

digestible and resistant starch (Chung *et al.*, 2010). A fibre rich diet is very important from a nutritional point of view because dietary fibre plays a vital role in improving gastrointestinal health and glucose tolerance, lowering serum cholesterol levels, weight management and reducing the risk of heart diseases (Kendall *et al.*, 2010).

Table 2.3 List of amino acids and their functions in and metabolism

Amino acids	Major functions
Alanine	Gluconeogenesis, transamination
Arginine	Antioxidant, regulation of hormone secretion
Asparagine	Cell metabolism and physiology
Aspartate	Urea cycle, transamination
Cysteine	Disulphide linkage in protein, transport of sulphur
Glutamate	Transamination, ammonia assimilation
Glycine	Calcium influx through a glycine gated channel in the cell membrane
Histidine	Protein methylation, haemoglobin structure and function
Isoleucine	Synthesis of glutamine and alanine
Leucine	Regulation protein turnover through cellular mTOR signalling and gene expression
Lysine	Antiviral activity, protein methylation
Methionine (Homocysteine)	Oxidant, independent risk factor for CVD
Proline	Collagen structure and function, osmoprotectant
Serine	One carbon unit metabolism, protein phosphorylation
Tryptophan (Serotonin)	Neurotransmitter
Tyrosine	Protein phosphorylation, nitrosation and sulphation
Valine	Synthesis of glutamine and alanine
Threonine	Immune function, glycine synthesis

(Li *et al.* 2007; Wu, 2009; Newsholme and Newsholme 1989; Wu and Morris, 1989)

For instance, the pea is one of the oldest crops and is a relatively low-cost and nutritious legume. Peas are rich in essential amino acids, such as tryptophan and lysine, low in sodium and fats. Chickpeas and lentils are also preferred as protein sources in many parts of the world. Chickpeas have a balanced amino acid composition with higher protein bioavailability compared to cereals (Roy *et al.*, 2010), while lentils are rich source of lysine and leucine. Lentils also contain significant amounts of minerals and vitamins (Roy *et al.*, 2010) as well as being used to increase soluble and insoluble dietary fibre contents of food (Tosh and Yada, 2010).

2.3 Effect of combining starch and protein sources:

Using starch sources, such as maize, oat, barley and wheat combined with protein sources, including peas and beans, can be a way to increase the nutritional content of foods. Tiwari *et al.* (2011) studied the addition of pigeon pea flour to wheat flour at different levels in the preparation of biscuits. The biscuits were analysed for their protein and fibre contents as well as the physical characteristics after addition of pigeon pea. Similarly, de la Hera *et al.* (2012) studied the effect of inclusion of lentil flour on the characteristics of the batter of layer and sponge cakes. This study concluded that it was possible to incorporate lentil flour into cake formulations; thus, improving the amino acid balance. Anton *et al.* (2009) studied the addition of navy and red bean flours to corn starch based snacks and observed an increase in the crude protein content of the samples. The study suggested that bean flour can be incorporated at high levels in corn starch based extruded snacks, without a great impact on their physical properties, by manipulating the processing and formulation with the use of adequate food additives. Madhumitha and Prabhasankar (2011) improved the functional and

nutritional value of pasta by the addition of black gram flour to *Triticum durum* semolina pasta and the resulting pasta samples were analysed for their physical and nutritional properties. In that study, 30% of black gram dhal flour in durum pasta showed a lower glucose release and a lower glycaemic index than the durum pasta. Semolina flour with of black gram dhal flour was found to be optimum in terms of all the characteristics required for good quality pasta. However, fortification of 30% black gram flour in semolina still affected some of the quality parameters, such as texture, pasting properties and the sensory score of the pasta and this suggested that the desirable characteristics of the legume added samples can be retained by using additives, such as hydroxypropyl methylcellulose or gluten. Balasubramanian *et al.* (2012) also investigated the effect of legume incorporation (5%, 10% and 15%) on the functional and nutritional properties of sorghum and wheat extrudates. Incorporation of legumes resulted in a slight increase in protein content and the fat content of the extrudates reduced. The extrudates incorporating legumes showed a higher water absorption index and water solubility index with better pasting properties and a decreasing trend in the degree of gelatinisation than control extrudates.

Most research mentioned here has been based on increasing the nutritive value of the products. These studies suggest the potential use of legumes to increase the amino acid content (lysine and threonine) and reduce the glycaemic response of the samples. However, these studies also suggest that more research needs to be done on the *in vitro* digestibility of the combined blends.

2.4 Importance of protein:

There are many nutrients (vitamins, minerals, amino acids, to name a few) required by the human body and proteins are the second largest store of energy in the human body. Protein is a vital source of amino acids in the diet and is essential in the building blocks of muscle structure (Butts *et al.*, 2012). It is important to consume sufficient dietary protein (0.8g/kg/day for an average adult human) for the synthesis and maintenance of body mass and muscle function (Table 2.3 as protein provides the amino acids required to synthesise all proteins in the body, as well as for tissue growth, and maintenance of lean muscle mass and function (Paddon-Jones *et al.*, 2008). Table 2.4 illustrates recommended intake of protein for different age groups that is important to regulate human physiology (Millward *et al.*, 2008). Studies conducted by Hu (2005) and Paddon-Jones *et al.*, (2008) suggest that the relevant levels of protein intake in the diet help in reducing bodyweight and avoids progressive muscle loss with age.

Table 2.4 Recommended daily intake of high-quality protein for children, teenagers and adults (g/kg body weight)

Age (years)	Body Weight (kg)	Recommended dietary allowance	
1 - 3	13	1.2	
4 - 6	20	1.1	
7 - 10	36	1.0	
		Male	Female
11 - 14	-	1.0	1.0
15 - 18	-	0.9	0.8
19 +	-	0.8	0.8

(Newsholme and Leech, 2011)

Currently, health problems arising due to lack of nutrition in the diet are undeniable. It has been reported that around 35% of adult men and 40% of adult women have dietary protein intakes below recommendations (Campbell *et al.*, 2002). However, higher intakes of animal protein (egg, meat) might be associated with higher risks of heart disease. For instance, some studies have reported that excess consumption of beef significantly increased the risk of heart diseases in men (Fraser, 1994). Whereas, consumption of food products which are derived from plant based protein sources have a significant, positive effect on the reduction of blood cholesterol levels and heart diseases (Hu, 2005). Legumes, such as lentils, peas and beans, are a rich source of protein and can replace cereal components in cereal based snack foods. The use of legumes as protein sources in the diet is inexpensive and contributes to the fraction of easily digestible proteins (Shevkani and Singh, 2015, Tharanathan and Mahadevamma, 2003). Moreover, Jenkins *et al.* (2001) showed that a diet high in wheat protein (27% energy) significantly decreased the serum concentration of triglycerol, uric acid and the proportion of oxidised LDL cholesterol compared to a control diet (16% energy) in humans. Table 2.5 illustrates requirements for essential amino acids by the human body.

Sources of amino acids: Proteins consumed as foods, such as meat, dairy and vegetables are the main sources of protein. About 90 g/day protein is consumed by an average person in developed countries and this protein is hydrolysed in the digestive track along with other nitrogenous compounds (Newsholme and Leech, 2011). Proteins in the small intestine and pancreas release amino acids in the form of digestive enzymes and mucus, and the process of protein turnover involves the breakdown of cellular

proteins with the release of free amino acids. Microorganisms from the intestines synthesise some proteins that also release amino acids for the body (Bender, 2012).

Table 2.5 Recommended requirements of essential amino acids by the human body

Essential amino acid	Requirement mg/kg/day
Isoleucine	23
Leucine	39
Lysine	30
Methionine and cysteine	15
Phenylalanine and tyrosine	39
Threonine	15
Tryptophan	6
Valine	20

(El-Khoury and Young, 1994)

2.5 Protein digestibility:

Food containing 25-30% of energy as protein are considered as healthy diets as they do not show any negative health effects, whereas higher intakes of protein (above 45% on an energy basis) may create adverse effects on human health, such as diarrhoea and nausea (Hayashi, 2003). However, the quality of protein is the most important factor in human nutrition. The content of essential amino acids and the digestibility of the proteins determines the quality of the protein (which maybe crucial for muscle synthesis in the human body) (Paddon-Jones *et al.*, 2008).

Protein digestibility is a measure derived from the protein hydrolysed and the absorption rate of the tissues. During digestion proteins are denatured by enzymes and stomach acid, and are then broken into smaller peptides and amino acids. The pancreatic and intestinal enzymes digest them into oligo-, di- and tri-peptides and single amino acids. These amino acids are transported through the membranes of the intestinal cells and released to the bloodstream (Whitney and Rolfes, 2007). The simultaneous processes in the utilisation of proteins, i.e. the digestion of protein, absorption, and the distribution of amino acids over various body parts, is known as the protein turnover. About 4 to 5 g/kg of protein (Table 2.6) is hydrolysed and re-synthesised every day in a human adult (Newsholme and Leech, 2011). However, the rate of protein turnover varies according to the nature of the proteins. The human body has no protein storage capacity (except collagen) (Hu, 2005) and proteins in the liver are replaced after a few hours, while contractile proteins can be rapidly degraded under some conditions. Protein turnover also accounts for around 20% of resting energy expenditure. Thus, protein turnover is required to maintain amino acid concentrations in blood and as well as within the cells; hence, it is essential to confirm the need for proteins and peptides that play vital role in metabolism (Newsholme and Leech, 2011). In children, poor nutrition leads to a nutritional disorder known as protein-energy malnutrition and, in the elderly, it can cause trauma after major surgery or loss of skeletal muscle to regulate daily activities, such as dressing and walking (Newsholme and Leech, 2011). Table 2.6 illustrates a various ammouns of protein absorbed and lost during metabolism.

Table 2.6 Approximate values for protein intake, protein loss and protein turnover, each day in normal adult humans

Protein intake	
Process	Amount (g)
Dietary intake	90
Proteins secreted into GI track	70
Total absorbed	150
Protein loss	
Process	Amount (g)
In faeces	10
In urine	75
Sloughing of skin	5
Total lost	90
Protein turnover	
Tissue	Amount (g)
Muscle	75
Liver, gut lung	130
White blood cells	20
Red blood cells	8
Albumin	12
Other tissues	8

(Newsholme and Leech, 2011)

A good source of protein needs to be rich in essential amino acids. However, on the one hand, products which have high quality protein may be the main source of cholesterol and saturated fats, which are responsible for increasing risk of coronary heart disease (Hu, 2005). On the other hand combination of legume and cereals give good quality proteins as they are rich in essential amino acids (Aremu *et al.*, 2006, Jezierny *et al.*, 2010) as well as being low in salt, saturated fats and cholesterol (Aremu *et al.*, 2006,

Flight and Clifton, 2006). Table 2.7 describes effect of various food components on protein digestibility.

Table 2.7 Effect of food components on protein digestibility

Author	Title	Finding
Duodu <i>et al.</i> , 2003	Factors affecting sorghum protein digestibility	Due to the close association of starch and protein in seeds, when is starch gelatinised after cooking it might reduce the accessibility of proteolytic enzymes to protein bodies and reduce protein digestion
Brownlee, 2011	The physiological roles of dietary fibre	Presence of dietary fibre in the upper GI track reduces the rate of the intestinal uptake of energy.
Kristiansen and Jensen, 2011	Dietary fibre in the regulation of appetite and food intake. Importance of viscosity	Increase in viscosity prolongs transit time and nutrient absorption, which alters gastric emptying by affecting the release of peptides.
Sumargo <i>et al.</i> , 2016	Effects of processing moisture on the physical properties and <i>in vitro</i> digestibility of starch and protein in extruded rice and bean flours	Decrease in moisture increases the shear during processing and leads to increases in protein digestibility

2.6 *In vitro* methods to determine protein digestibility:

Since 1972 there have been an array of methods used to record protein digestibility (Table 2.8). Butts *et al.* (2012) and Hur *et al.* (2011) have reported that many techniques are available to estimate protein digestibility, but every method differs from the other. Generally, the variation in methods depends on the time of digestion, enzymes used, and the concentration of the enzymes and samples used. However, *in vitro* methods are

reliable and deliver quick results compared to *in vivo* experimentation. However, a difference exists between *in vivo* and *in vitro* methods to evaluate protein digestibility. This difference in data illustrates a general need to conduct more research on the protein digestibility of food to improve the correlation between *in vitro* and *in vivo* methods, as mimicking the physiological digestion process is important.

Hsu *et al.* (1977) developed a multi-enzyme technique to evaluate *in vitro* protein digestibility of various samples, such as meat, eggs, dairy and plant based products, with the help of various pancreatic enzymes, including trypsin (1.6 mg), chymotrypsin (3.1 mg) and peptidase (1.3 mg). Protein digestibility was estimated by the pH drop over 10 min. He showed that an enzyme degrades the protein chain to release an amino acid carboxyl group causing a drop in the pH of the product. A multi-enzyme solution was used to avoid potentially inaccurate results being obtained due to trypsin inhibitors present in some products and to reduce limitations, such as the prolonged digestion time of a single enzyme system. The resulting data were correlated with *in vivo* results obtained when the same samples were fed to rats over a month. The nitrogen contents of the food products before consumption and the faeces were analysed by a macro Kjeldahl method. More recently, Kalpanadevi and Mohan (2013) reported that this method could be used successfully for processed legume samples to determine the *in vitro* protein digestibility of the products.

Another process of estimation was given by Mertz (1984). This method was used to test the protein digestibility of cereals using pepsin and this showed a high digestion value can be achieved in processed sorghum products (traditional African products). Anyango *et al.* (2011) used this method to study the effect of legume additions on the *in vitro*

protein digestibility of cereal food products, and showed that cowpea additions increased protein digestibility because of an increase in digestible globulin content and a decrease in non-digestible sorghum protein content, i.e. kafirin, and the combined effect of increases in lysine and protein digestibility increased the bioavailability of protein in the sorghum based products.

Table 2.8 Methods of *in vitro* protein digestibility

Method Name	Year	Enzymes Used	Time for digestion
Saunders <i>et al.</i>	1973	Pepsin 1.5 mg, pancreatic 4 mg	24 h
Hsu <i>et al.</i>	1977	Multi-enzyme solution: Trypsin 1.6 mg, chymotrypsin 3.1 mg and peptidase 1.3 mg	1 h
Mertz	1984	Pepsin 1.5 mg	7.5 h
Gbadamosi <i>et al.</i>	2012	Pepsin 1.5 mg and pancreatin 4 mg	24 h
Chen <i>et al.</i>	2013	Pepsin (4 U/mg on a protein basis) and pancreatin (4 U/mg on a protein basis)	4 hrs.

2.7 Starch digestibility:

Starch is the most common carbohydrate stored in plants and used in human diets. Readily digestible starch is broken down into its simpler glucose units by several enzymes, such as salivary α -amylase, but most of the hydrolysis is achieved by

pancreatic amylase (Whitney and Rolfes, 2007). The predictive glycaemic response is used in many studies to estimate the response of blood glucose after the consumption of food (Brennan *et al.*, 2012a). The precise determination of the rate of starch digestion helps in determining the glycaemic response of the serving of food (Singh *et al.*, 2007). In this study, an *in vitro* starch digestibility method was used. This method has been validated as well as standardised more than any of the other methods mentioned in Table 2.9. It has been reported that *in vivo* methods were neither time nor cost efficient in the determination of the glycaemic response. The glycaemic response is a tool that helps to detect glucose release and the absorption of digested food. The method used in this study was capable of accurately predicting the glycaemic response of a variety of foods (Woolnough *et al.*, 2008, Woolnough *et al.*, 2010).

As mentioned previously, it has been suggested that foods having a low glycaemic index are good for health. Some results have shown a relationship between the glycaemic index and obesity and high blood pressure (Thorsen *et al.*, 2011). Dietary fibre is a mixture of complex carbohydrate based polymers that resists digestion (Table 2.9) in the small intestine of the human body and, thus, do not contribute directly to increased blood glucose levels or glycaemic effects (Harding, 2012).

2.8 *In vitro* methods of starch digestibility:

Since 1990, numerous methods for carbohydrate digestibility have been reported by researchers. Many of the methods differ from each other in terms of the enzyme used, time and the temperature of digestion. Granfeldt and Björck (1991) showed that, by exposing food products to salivary α -amylase prior to digestion, the digestibility of carbohydrate can be improved. However, Muir *et al.* (1995) evaluated two different

resistant starch containing foods made from maize and their digestion, the addition of α -amylase, pepsin and amyloglucosidase. The effect of the addition of α -amylase on the hydrolysis of starch in wheat bread was studied by Brennan *et al.* (1996). Akerberg *et al.*, (1998) used a procedure for carbohydrate digestibility that mimicked physiological conditions, where samples were chewed before digestion, the samples were incubated with pepsin, pancreatin and amyloglucosidase. Table 2.9 shows how these methods were developed.

Table 2.9 Methods of *in vitro* starch digestibility

Author name	Year	Enzyme used	Time for digestion
Granfeldt	1991	α -amylase, pepsin	Pepsin 1 h, α -amylase 3 h
Muir	1995	Pepsin, α -amylase, amyloglucosidase	Pepsin 30 min, α -amylase and amyloglucosidase 15 h
Brennan	1996	Pepsin and α -amylase	Pepsin 10 min, α -amylase 5 h
Akerberg	1998	Pepsin, pancreatin and amyloglucosidase	Pepsin 30 min, pancreatin and amyloglucosidase 16 h
Monro	2008	α -amylase, pepsin, pancreatin and amyloglucosidase	α -amylase and pepsin 30 min, pancreatin and amyloglucosidase 2 h
Woolnough	2010	α -amylase, amyloglucosidase and pancreatin	Pepsin 30 min, amyloglucosidase and pancreatin 120 min

Table 2.10 Effect of various food components on starch digestibility and glycaemic index.

Author	Year	Findings
Sumargo <i>et al.</i>	2016	An increase in product moisture increases readily digestible starch due to high mechanical rupture. Also, may increase starch gelatinisation as well as create a larger surface area for starch digestion.
Fabek <i>et al.</i>	2014	Dietary fibre reduces glucose release from starch digestion. The high water absorption capacity of dietary fibre competing with starch granules which results in a low degree of gelatinisation and low postprandial glycaemic index.
Brownlee <i>et al.</i>	2011	Dietary fibre increases digesta viscosity and the presence of dietary fibre in upper gastro-intestinal track which further reduces rate of intestinal uptake of nutrients.
Brennan <i>et al.</i>	2016	Due to the compact nature of extrudates, the protein matrix could surround starch granules and reduce the effectiveness of starch digestive enzymes.
Brennan <i>et al.</i>	1996	The effect of the addition guar gum on digesta viscosity of wheat bread during starch digestion may be significant in reducing the rise in postprandial glycaemia caused by guar gum in humans.
Rasmeussen <i>et al.</i>	1996	High amounts of saturated fat in food increases the insulin concentration but with little or no change in blood glucose response
Lichtenstein and Schwab	2000	High fat intakes might alter the hepatic extraction of blood glucose resulting in hyperglycaemia by the increased fatty acids and triglycerol concentrations and a lowering of long term insulin activity

Starch consists of two fibres: insoluble (lignin) and soluble (arabinoxylan, β -glucan). During digestion, insoluble fibres form gelatinous substances that may delay gastric emptying and enhance the absorption of nutrients from the small intestine and digestion. Soluble fibres produce short chain fatty acids, such as propionic acids, that are known for their ability to manipulate cholesterol synthesis (Ragaei *et al.*, 2011 and Wood, 2007).

The presence of dietary fibre in the diet may reduce health problems such as Type-2 diabetes by slowing the digestion process of the nutrient ingested and this reduction helps to decrease (Table 2.10) the postprandial reaction from glucose and insulin (Tosh and Yada, 2010). Good maintenance of insulin-glucose plays a vital role in lowering the risk of Type-2 diabetes (Kendall *et al.*, 2010).

Delays in starch hydrolysis may be responsible for the slow glucose release, which can lower insulin levels in the bloodstream. Blood levels of glucose and insulin after consumption of food vary and are in proportion to the amount of glucose consumed. Glucose produced after starch digestion is absorbed and transported to the liver and excess production of glucose may be converted into glycogen. A lower glucose response is considered beneficial for human health (Brennan, 2005).

Cereals contain mixed linkage β -glucan molecules, dietary fibres that counteract blood glucose levels after the consumption of food. The amount of β -glucan consumed and its viscosity varies the glycaemic response. It has been previously documented that it is possible to manipulate a product's structure by adding fibre rich blends to traditional cereal based foods and to achieve a reduction in starch hydrolysis and, hence, the glycaemic response (Brennan *et al.*, 2008).

Table 2.11 Relationship between GI and human nutrition

Author	Title	Findings
EK <i>et al.</i> , 2014	Discovery of a low-glycaemic index potato and relationship with starch digestion <i>in vitro</i>	Finding a low-GI potato and developing a screening method for finding low-GI cultivars are both health and agricultural priorities. The present study identified the first commercially grown low-GI cultivar of potato (Carisma, GI ¼ 53) among a group of commonly used and newly introduced cultivars.
Schwingshacl and Hoffmann, 2013	Long-term effects of low glycaemic index/load vs. high glycaemic index/load diets on parameters of obesity and obesity-associated risks: A systematic review and meta-analysis	The present meta-analysis provides evidence for a beneficial effect on long-term diets in adopting a low glycaemic index protocol with respect to pro-inflammatory Markers.
Goff <i>et al.</i> , 2013	Low glycaemic index diets and blood lipids: A systematic review and meta-analysis of randomised controlled trials.	Meta-analysis of low GI diets on blood lipids shows that there is consistent evidence that low GI diets significantly reduce total and LDL-C without affecting HDL-C or triglycerides
Rouhani <i>et al.</i> , 2013	Effect of glycaemic index and glycaemic load on energy intake in children	The overall effect of consuming LGI and LGL meals on energy intake was not significant but consuming an LGI diet (but not an LGL diet) has a favourable effect on reducing energy intake and obesity, subsequently.

Bornet <i>et al.</i> , 2007	Glycaemic response to foods: Impact on satiety and long-term weight regulation	GI of food depends on the composition of the food. The quality and quantity of carbohydrates also regulates the GI of food, e.g. fructose or lactose containing foods tend to have low GI
Rasmussen <i>et al.</i> , 1996	Differential effects of saturated and monounsaturated fat on blood glucose and insulin responses in subjects with non-insulin-dependent diabetes mellitus 1	High GI of food is responsible for the elevation of glucose and a high insulin response, leading to obesity and hypertension

2.9 Importance of extrusion and effect on *in vitro* starch and protein digestibility:

Extrusion is one of the most commonly-adopted processing techniques used by food industries, where the involvement of an extreme mechanical shear during the process forces the material to breakdown their biopolymer bonds and breakdown the structure (Singh *et al.*, 2007). This enables the delivery of aerated cereal based products with a wide range of shapes and textures. Most extruded products today (e.g. breakfast cereals or savoury snacks) are based on refined flours (Robin *et al.*, 2012). The replacement of refined flour by whole grain flour raises several challenges that need to be addressed, including changes in organoleptic properties, reduced impression of the size due to lower expansion at the die exit, a darker colour due to the presence of bran,

and enzymatic browning and lipid oxidation after extrusion (Schaffer-Lequart *et al.*, 2017).

The increasing popularity of ready-to-eat products and the accessibility of this method have captured manufacturers' attention. For example, the extrusion method has a wide scope for developing various food products, including cereal based snacks and ready-to-eat breakfast cereals (Brennan *et al.*, 2013). Nowadays, the effects of extrusion on food components are much discussed (Ahamed *et al.*, 1997, Oliveira *et al.*, 2015, Singh *et al.*, 2007). This motivates food institutions and companies to investigate the effects of extrusion on the physico-chemical and nutritional properties of a variety of foods. In addition, the effect of extrusion temperatures on starch and protein digestibility of foods is of much interest. The nutritional importance of extruded foods has been recently raised due to the high levels of production of various foods, such as breakfast cereals and ready-to-eat snacks (Brennan *et al.*, 2011). Extrusion parameters, such as temperature, moisture and shear force, tend to increase the protein digestibility of the extrudates; and the anti-nutritional compounds present in seeds form complexes with proteins present in the seed and make it less susceptible to proteases by reducing its solubility.

Anti-nutritional factors, e.g. tannins, phytates and trypsin inhibitors, may be naturally present in grains and they can affect the nutritional quality of a food. Trypsin inhibitors are present in grains and lower the protein availability of the product, while tannins are water soluble compounds that occur in seeds and their presence results in poor digestive enzyme activity (Alonso *et al.*, 2000). The presence of such compounds may cause a 50% reduction in the digestibility of a food; however, processing techniques,

such as cooking, extrusion and soaking, help to reduce the activity of such compounds (Rathod and Annapure, 2016). Previous studies have reported that extrusion cooking plays an important role in restricting the presence of anti-nutritional compounds, such as tannins, which can reduce protein and starch digestibility. In addition, the extrusion process can be useful for lysine retention in cereal based snacks, and increasing screw speeds and decreasing die diameters help to maintain lysine (Singh *et al.*, 2007). Robin *et al.*, (2015) studied the physico-chemical properties of extruded whole grain cereals and pseudo cereals and observed an increase in the solubility of wheat and sorghum extrudates. It was also observed that the lipid content in cereals may act as a lubricant in the melt during extrusion and, possibly, affect starch gelatinisation.

However, temperature may also be responsible for the complete or partial elimination of such anti-nutrients and alterations in the structures of storage proteins and increases in the protein digestibility of proteins (Ghumman *et al.*, 2016, Sumargo *et al.*, 2016). Navale *et al.*, (2015) reported that thermal unfolding of the major globulins, thermal inactivation of trypsin inhibitors and other growth-retarding factors, such as lectins, occurred due to the extrusion process.

Furthermore, the interaction of heat during extrusion processing facilitates some physical and chemical changes; for example, it may alter the bio-accessibility of bioactive compounds by forming protein complexes that can be easily broken down in the human body (Brennan *et al.*, 2011). Kosinska-Cagnazzo *et al.*, (2017) reports that addition of goji berries in extruded products increases the content of bioactive compounds and the antioxidant activity of the products and might be responsible for the enrichment of bio actives such as 2-O- β -D-glucopyranosyl-L-ascorbic acid in the

extrudates. A recent study by Ai *et al.*, (2016) reported that extrusion techniques can be used to develop legume-based foods without reducing their protein and starch contents; changes in pasting properties of bean based extrudates were also observed, which caused a rise in resistant starch (RS) and protein denaturation of the extrudates. Figure 2.1 is single screw extruder that was used to develop hot extrudates during this study.

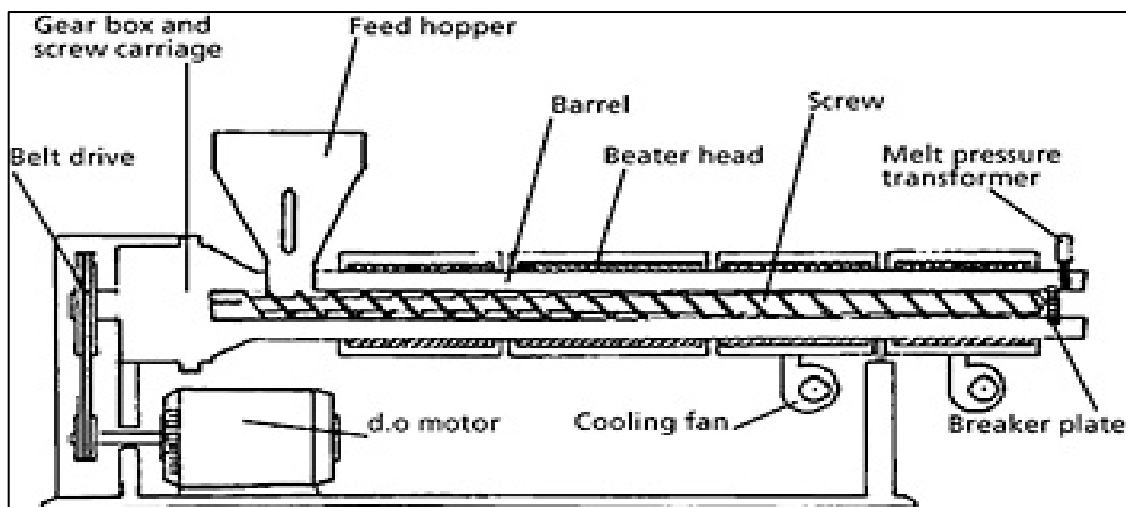


Fig 2.1 Single screw extruder (Image taken from practical guide to blow moulding, Chapter

4. The extrusion blow moulding systems by Norman C. Lee)

The human digestive system is designed to digest cooked starch; it could be argued that the extrusion process cooks starch within the extruder (although maintaining moisture levels may prevent the complete gelatinisation of the starch) (Brennan *et al.*, 2013). Starches are mainly classified into three main types. Resistant starch (RS) is the portion of starch not digested by human digestive enzymes in the small intestine; slowly digestible starch (SDS) is an intermediate fraction; and rapidly digestible starch (RDS) is fully and rapidly digested in small intestine (Robin *et al.*, 2016). The gelatinisation of starch is an important factor in starch digestion. During extrusion the barrel temperature, pressure and shear force are responsible for the partial or full starch

gelatinisation (Ai *et al.*, 2016). However, the report by Robin *et al.* (2016) contradicted this finding. They observed that the nature of RS in native starch (high amylose) could increase or retain SDS during extrusion. Redgwell *et al.*, (2011) reported that the extrusion process can increase the technological versatility of dietary fibre and, perhaps, its health benefits profile, and this could make a significant impact on the use of fibre in the food industry. Extrusion cooking of wheat flour enriched with dietary fibre (wheat bran) significantly modified the physico-chemical properties of the starch. The bran content influenced the glass transition temperature, melting temperature and sorption isotherm of the unprocessed wheat flour. This showed that higher bran levels led to an increase in water holding and a decrease in the starch glass and melt temperatures. This might affect the expansion properties of extruded starch containing wheat bran (Robin *et al.*, 2011b). Robin *et al.*, (2011a) observed an increase in the dietary fibre (wheat bran) concentration in wheat flour extrudates forced a significant reduction in volumetric and sectional expansions due to changes in the dough properties, such as high moisture.

The slow digestibility of starch could be due to the granular structure of the starch that survives through extrusion. Furthermore, secondary structures, such as amylose lipid complex formation and retrogradation during cooling and storage, help in strengthening the product's structures to some extent. Retrogradation of amylose or short amylose chains generated by extrusion may occur during cooling and storage, thereby strengthening the ordered structure of the extruded product to some extent (Robin *et al.*, 2016). It is evident that extrusion parameters, such as barrel temperature and moisture screw speed, are responsible for affecting the nutritional and functional

properties of foods (Oliveira *et al.*, 2015, Rashid *et al.*, 2015). In this study, the effects of various extrusion temperatures (hot and cold extrusion) and the combination of cereals and legumes on protein and starch digestibility were investigated.

Currently health and nutrition is a subject which is attracting much attention in both the food science and human nutrition scientific literature. The functionality of starch and proteins is important in relation to technological and dietary understandings in developing novel functional foods. There exists a possibility of utilizing the starch components derived from cereal grains and the protein and dietary fibre components from legumes to create a healthy, nutritious food for consumers. The extrusion process has a potential in generating these quality snack products by combining legume and cereals blends into food items aimed at the discerning consumer.

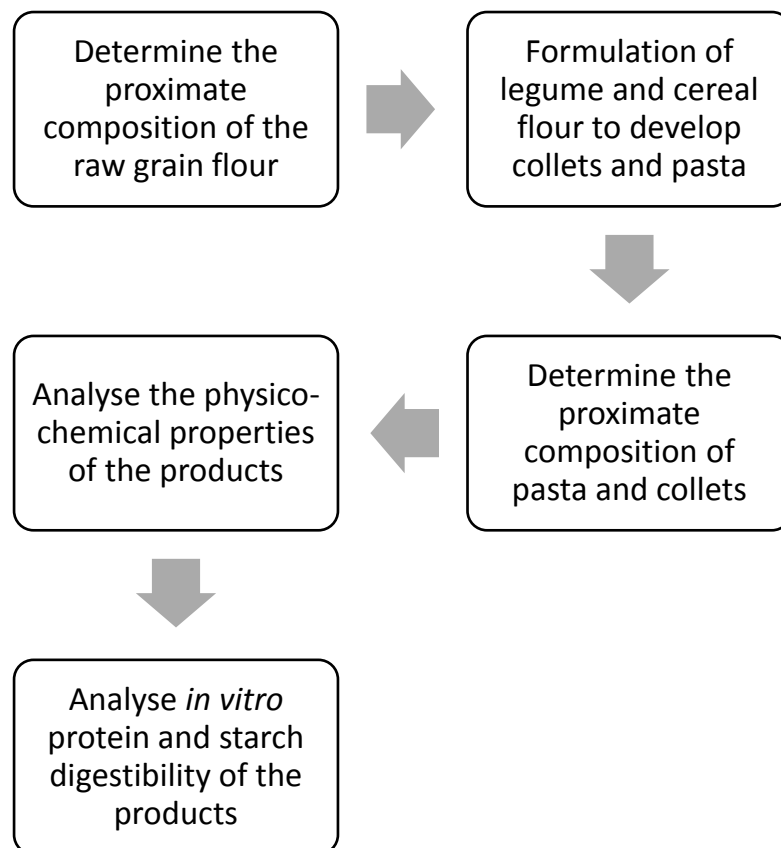
Chapter 3

Materials and methods

Overview

In this chapter a detailed explanation and information about the materials used, and suppliers for all experimental procedures, are given. The current study is focused on *in vitro* digestibility of hot extrudates (collets) and pasta developed from cereals and legumes at different levels. The research method is elaborated in a flow chart as well as in the text. The importance of the *in vitro* method is explained and the reason behind choosing this method is stated the *in vitro* method section.

Fig 3.1 Experimental design



The *in vitro* methods chosen for the estimation of protein and starch digestibility are robust, validated and reliable, as discussed in Chapter 2. The steps involved in digestion methods are very close to the human digestive systems. For instance, using α -amylase in starch digestion mimics the saliva in the mouth.

Methods used for the physico-chemical analysis of food products are described, many of these have been developed and validated by different laboratories around the world and proven to be accurate in the evaluation of the various characteristics of samples analysed.

The apparatus and equipment used for the experiments are shown in images and the formulation of collets and pasta is given in Tables 3.1 and Table 3. 3, respectively, and Table 3.2 illustrate the extrusion parameters for the collets. The required buffers were made in the laboratory and stored in at 4°C. The recipes for the buffers are given in the chemical list.

3.1 Materials:

3.1.1 Grains:

Whole grains were used to prepare hot extruded products, i.e. collets. Cereals; wheat and barley, were obtained as grain from Champion Flour Mills, Christchurch, New Zealand. Maize was purchased from Sun Valley Foods, Auckland, New Zealand. Budget brand rice was purchased from Safeway Traders Auckland, New Zealand. The legumes, green peas and yellow peas and lentils were purchased from Pams Foods, Auckland, New Zealand, and chickpeas were purchased from Sun Valley foods Auckland, New Zealand.

Table 3.1 Combination of cereal and legume grains used to prepare the collets

Ingredients Sample name	Wheat (g)	Chickpea (g)	Green pea (g)	Yellow pea (g)	Lentils (g)
Wheat Control	100	0	0	0	0
Wheat + 5% Chickpea	95	5	0	0	0
Wheat + 10% Chickpea	90	10	0	0	0
Wheat +15% Chickpea	85	15	0	0	0
Wheat + 5% Green pea	95	0	5	0	0
Wheat + 10% Green pea	90	0	10	0	0
Wheat + 15% Green pea	85	0	15	0	0
Wheat + 5% Yellow pea	95	0	0	5	0
Wheat + 10% Yellow pea	90	0	0	10	0
Wheat + 15% Yellow pea	85	0	0	15	0
Wheat + 5% Lentil	95	0	0	0	5
Wheat + 10% Lentil	90	0	0	0	10
Wheat + 15% Lentil	85	0	0	0	15
	Rice (g)	Chickpea (g)	Green pea (g)	Yellow pea (g)	Lentils (g)
Rice Control	100	0	0	0	0
Rice + 5% Chickpea	95	5	0	0	0
Rice + 10% Chickpea	90	10	0	0	0
Rice + 5% Green pea	95	0	5	0	0
Rice + 10% Green pea	90	0	10	0	0
Rice + 15% Green pea	85	0	15	0	0
Rice + 5% Yellow pea	95	0	0	5	0
Rice + 10% Yellow pea	90	0	0	10	0
Rice + 15% Yellow pea	85	0	0	15	0
Rice + 5% Lentil	95	0	0	0	5
Rice + 10% Lentil	90	0	0	0	10

Rice + 15% Lentil	85	0	0	0	15
	Barley (g)	Chickpea (g)	Green pea (g)	Yellow pea (g)	Lentils (g)
Barley Control	100	0	0	0	0
Barley + 5% Chickpea	95	5	0	0	0
Barley + 10% Chickpea	90	10	0	0	0
Barley + 5% Green pea	95	0	5	0	0
Barley + 10% Green pea	90	0	10	0	0
Barley + 15% Green pea	85	0	15	0	0
Barley + 5% Yellow pea	95	0	0	5	0
Barley + 10% Yellow pea	90	0	0	10	0
Barley + 15% Yellow pea	85	0	0	15	0
Barley + 5% Lentil	95	0	0	0	5
Barley + 10% Lentil	90	0	0	0	10
Barley + 15% Lentil	85	0	0	0	15
	Maize (g)	Chickpea (g)	Green pea (g)	Yellow pea (g)	Lentils (g)
Maize Control	100	0	0	0	0
Maize + 5% Chickpea	95	5	0	0	0
Maize + 10% Chickpea	90	10	0	0	0
Maize +15% Chickpea	85	15	0	0	0
Maize + 5% Green pea	95	0	5	0	0
Maize + 10% Green pea	90	0	10	0	0
Maize + 15% Green pea	85	0	15	0	0
Maize + 5% Yellow pea	95	0	0	5	0
Maize + 10% Yellow pea	90	0	0	10	0
Maize + 15% Yellow pea	85	0	0	15	0
Maize + 5% Lentil	95	0	0	0	5
Maize + 10% Lentil	90	0	0	0	10
Maize + 15% Lentil	85	0	0	0	15

*All values are given on the dry matter basis of the seeds

3.2 Preparation of the hot extruded product (collets):

A Millbank designed single screw extruder (Fig. 3.2) was used to develop the extruded products. The machine was operated on a single phase power supply with a 3 mm die. The extruder was equipped with a control panel. There was an adjustable feed hopper to control feed entering the barrel. The system has the option of adding water, but this was seldom used. The Millbank extruder was designed to run with an inverter. The purpose of the inverter was to control the power supply to ensure that the machine itself produces AC current from a DC power supply. This provide a constant and uninterrupted voltage to the motor as large spikes in power due to the large stresses placed on the machine can cause damage to the screw and machine structures. The overall structure of the extruder was designed to withstand the pressure placed on it and, if this pressure was exceeded, catastrophic failures might occur. If power spikes occur exceeding 12 A, the inverter will shut off the power supply to the extruder to prevent damage.

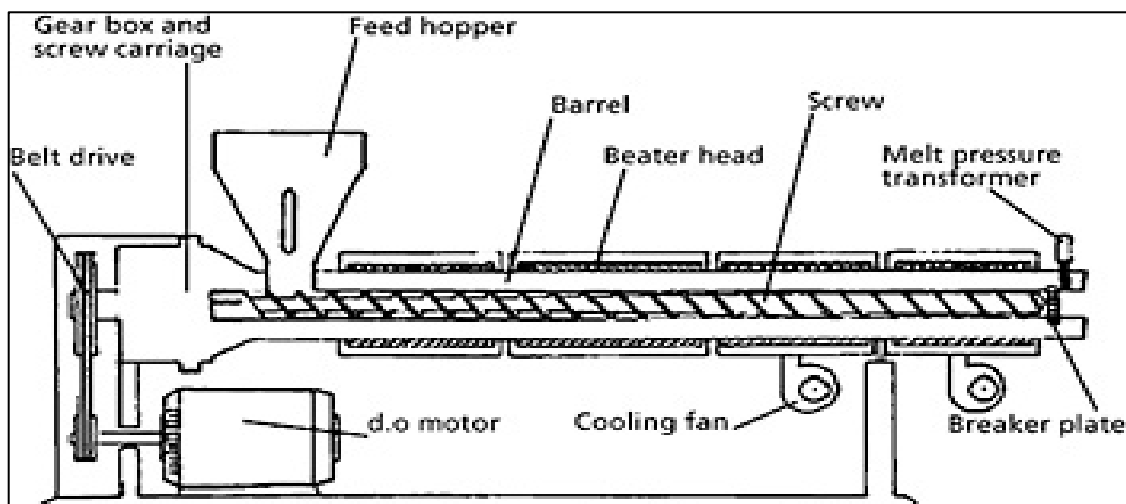


Fig. 3.2 Single screw extruder

(Image taken from practical guide to blow moulding, Chapter 4. The extrusion blow moulding systems by Norman C. Lee)

Due to a lower torque being provided by the motor running on a single phase power supply, Millbank has designed a smaller screw that required less power. The shorter screw length still created the temperature and pressure required to produce ready-to-eat extrudates; however, as a consequence of the shorter proportions, the production rate is lower than that of a three phase extruder. Secondly, the single phase extruder has a carefully controlled feed rate. If the feed rate becomes too high a backlog of product can occur. This can cause the motor to cut out due to the high torque required to overcome the blockage. Not only must the flow rate be controlled but the consistency of the feed should be of uniform size and shape. This reduces the fluctuations of torque that can occur while processing.

The grains and mixtures of grains were added to the hopper of the extruder. The process inside the heated barrel was determined by a screw driven by a motor. As the screw rotated, the material inside the barrel was conveyed towards the die. The small die diameter developed pressure on the material inside the barrel as well as increasing the temperature of the melt. A soft strand of heated melt, with a constant thickness, was emitted from the die. The strand was cut by automated cutter to the uniform lengths to form collets. The high temperature and pressure during extrusion were responsible for the expansion of the collets. The collets were cooled and then placed in zip lock bags. The bags were also sealed with the help of a sealing machine and stored at room temperature. Photos of the control extruded samples are shown in Figures 4a, 4b, 4c and 4d.

Table 3.2. Extrusion parameters of the collets

Sample	Torque (Nm)	Shaft speed (rpm)	Feed rate (mm/sec)	Current (Amps)
Wheat Control	64	210	10	5.9
Wheat + 5% Chickpea	46	210	10	5.4
Wheat + 10% Chickpea	48	210	10	5.3
Wheat +15% Chickpea	44	210	10	5.1
Wheat + 5% Green pea	72	210	10	6.4
Wheat + 10% Green pea	70	210	10	6.2
Wheat + 15 % Green pea	61	210	10	5.8
Wheat + 5% Yellow pea	57	200	10	5.9
Wheat + 10% Yellow pea	53	200	10	5.7
Wheat + 15% Yellow pea	62	200	10	5.7
Wheat + 5% Lentil	48	210	10	5.6
Wheat + 10% Lentil	48	210	10	5.4
Wheat + 15% Lentil	46	210	10	5.3
Rice Control	50	200	10	5.4
Rice + 5% Chickpea	40	200	10	4.8
Rice + 10% Chickpea	43	200	10	4.9
Rice + 5% Green pea	46.9	200	10	5.2
Rice + 10% Green pea	46.9	200	10	4.5
Rice + 15% Green pea	42	200	10	4.9
Rice + 5% Yellow pea	46	200	10	5.5
Rice + 10% Yellow pea	49	200	10	5.8
Rice + 15% Yellow pea	48.5	200	10	5.3
Rice + 5% Lentil	45.4	200	10	4.9
Rice + 10% Lentil	44.2	200	10	4.9
Rice + 15% Lentil	46	200	10	5.1
Barley Control	47	200	10	4.9
Barley + 5% Chickpea	42	210	10	4.9
Barley + 10% Chickpea	42	210	10	5

Barley + 5 % Green pea	39	210	10	4.8
Barley + 10% Green pea	41	210	10	4.8
Barley + 15% Green pea	38	210	10	4.9
Barley + 5% Yellow pea	45	210	10	4.9
Barley + 10% Yellow pea	40	210	10	4.7
Barley + 15% Yellow pea	40	210	10	4.8
Barley + 5% Lentil	41	210	10	5
Barley + 10% Lentil	44	210	10	4.9
Barley + 15% Lentil	43	210	10	4.8
Maize Control	70.2	189	10	6.3
Maize + 5% Chickpea	49.1	189	10	5.3
Maize + 10% Chickpea	55.7	189	10	5.8
Maize +15% Chickpea	53.7	189	10	5.6
Maize + 5% Green pea	53.4	189	10	5.68
Maize + 10% Green pea	59.1	189	10	5.7
Maize + 15% Green pea	64	189	10	5.8
Maize + 5% Yellow pea	65.2	189	10	5.9
Maize + 10% Yellow pea	59.1	189	10	5.8
Maize + 15% Yellow pea	59.7	189	10	6.1
Maize + 5% Lentil	56	189	10	5.5
Maize + 10% Lentil	63.4	189	10	5.71
Maize + 15% Lentil	57.4	189	10	5.61



Fig. 3.3 a) Wheat based extrudates



Fig. 3.3 b) Maize based extrudate



Fig. 3.3 c) Rice based extrudates

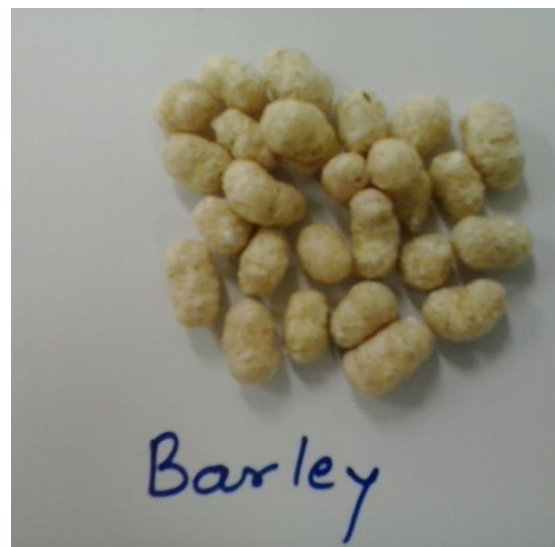


Fig. 3.3 d) Barley based extrudates

3.3 Flours used for pasta manufacture:

Whole wheat (Zentrofan), organic barley, green pea and chickpea were used for pasta making. All the flours were obtained from a local retail shop, Piko Foods, Christchurch, New Zealand.

3.3.1 Pasta making procedure:

The pasta extrusion was conducted using a Fimar villa verucchio pasta machine Model Number MPF15N235M, made in Rimini, Italy (Fig. 5). The pasta making machine consisted of a stainless steel basin, with an agitator (kneader) (Fig. 6) to facilitate the appropriate mixing of material. A bronze alloy shaft (screw Fig. 7) was fitted at the bottom of the sink for conveying material towards the die. A micro switch was located at the top of the basin lid to control the motor. Chickpea and green pea flours were used at 0%, 10%, and 20% (Table 3.3) replacement levels in wheat and barley flour in the production of pasta. Before making each sample the machine and the die (Fig. 8) were cleaned and dried. The flour, or the combination of flours, was added to machine and the machine was set on Function 1, then water added. The material was mixed for 20 min and then the switch was turned to Function 2 to start the extrusion process. The long strands of uniform thickness (3.5 mm) were collected in a plastic food tray and cut into lengths of 10- 12 cm using a stainless steel knife. For each batch in pasta making, 500 g of flour was used 165 mL of water (room temperature) was added to the extruder. The pasta samples were kept in zip lock bags (Fig. 9 and 10) and stored in a freezer until use. Various ratios of flour combinations were used to produce pasta and to evaluate the nutritional and physico-chemical properties of the pasta.

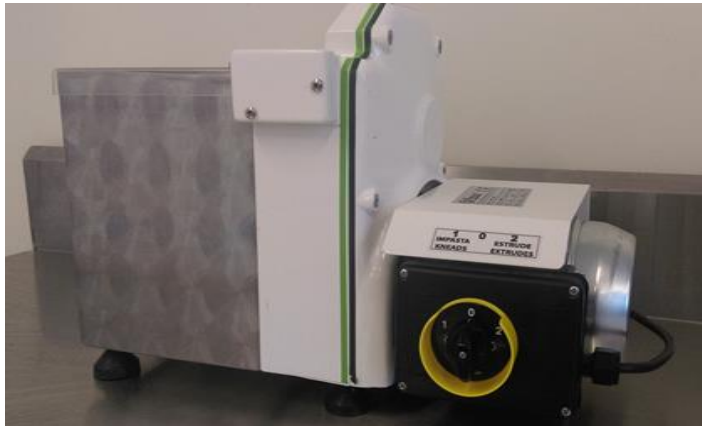


Fig. 3.4 Pasta extruder



Fig. 3.5 Pasta extruder top view

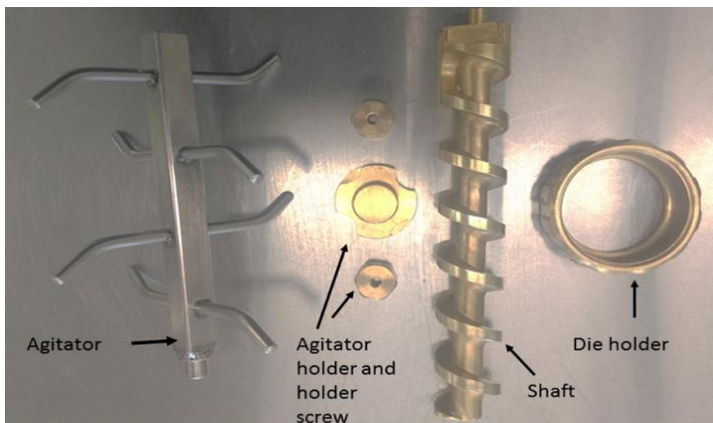


Fig. 3.6 Pasta extruder parts



Fig 3.7 Pasta extruder die



Fig. 3.8 Barley based pasta

1. Barley Control
2. Barley + 10% Chickpea
3. Barley + 20% Chickpea
4. Barley +10% Green pea
5. Barley + 20% Green pea



Fig. 3.9 Wheat based pasta

1. Wheat Control
2. Wheat + 10% Chickpea
3. Wheat + 20% Chickpea
4. Wheat + 10% Green pea
5. Wheat + 20% Green pea

Table 3.3. Combination of cereal and legume flour used to prepare pasta the values are given per 100 g

Quantity Sample	Wheat flour (g)	Chickpea flour (g)	Green pea flour (g)
Wheat Control	100	0	0
Wheat + 10% Chickpea	90	10	0
Wheat + 20% Chickpea	80	20	0
Wheat + 10% Green pea	90	0	10
Wheat + 20% Green pea	80	0	20
	Barley Flour (g)	Chickpea flour (g)	Green pea flour (g)
Barley Control	100	0	0
Barley + 10% Chickpea	90	10	0
Barley + 20% Chickpea	80	20	0
Barley + 10% Green pea	90	0	10
Barley + 20% Green pea	80	0	20

3.4 Chemicals, enzymes and buffers used:

3.4.1 Chemicals and enzymes required for the protein assay and digestibility analysis:

Pepsin (1031 U/mg) from porcine gastric mucosa, albumin bovine serum minimum (98%) for the electrophoresis was purchased from Sigma Aldrich, St Louis USA. Pancreatin (350 U/mg) for porcine pancreas was purchased from AppliChem Chemica Synthesis, Germany. The Bio-Rad kit (Cat#500-0006) for the Bradford assay was obtained from BioRad Laboratories Inc. U.S.

3.4.2 Chemicals, buffers and enzymes required for starch digestibility

analysis:

A total starch kit (CAT. # K-TSTA) was used to detect the starch contents of the pasta samples. The kit was purchased from Megazyme International Ireland Limited Wicklow, Ireland. The total starch assay measures total starch in a wide range of foods, plants and cereal products (natural and processed). For most samples, the starch was completely solubilised when the sample was incubated at approximately 100°C in the presence of thermostable α -amylase. The other chemicals required, such as glucose standard 1.0 mg/ml in 0.2 % benzoic acid, were obtained from Megazyme International, and the Megazyme starch standard (maize starch 93% starch d/w; 8.3% moisture) was also purchased. Enzymes, such as glucose oxidase peroxidase (GOPOD) were of analytical grade. Invertase (100 mL of 300U/mg in 50% glycerol - stored at -20°C) and amyloglucosidase (100 mL of 3260 u/mg (soluble starch) were also purchased from Megazyme Inc. Wicklow, Ireland and stored at 4°C.

All other chemicals, such as ethanol, HCl, NaOH, dinitrosalicylic acid (DNS) mixture and buffers were prepared in the laboratory before starting the experiment, and then stored.

3.4.3 Recipe for buffers and DNS mixture:

a) Sodium maleate buffer 0.1 M pH 6:

Maleic acid (11.6 g) was dissolved in 800 ml water then the pH was adjusted to pH 6 using 4 M NaOH. Hydrated calcium chloride, $\text{CaCl}_2\text{H}_2\text{O}$ (0.3 g) was added to the solution

followed by 0.23 g of sodium azide. The volume of the solution adjusted to 1 L with RO water in a volumetric flask.

b) Acetate buffer 0.1 M pH 5.2:

Sodium acetate (13.6 g) trihydrate was added to 900 mL water, the pH was adjusted to pH 5.2 using 0.1 M acetic acid, then 4 mL of 1 M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was added and the volume made up to 1 L with RO water.

C) Dinitrosalicylate (DNS) mixture:

DNS (10 g) was dissolved in 400 mL of 2 M NaOH at room temperature with vigorous stirring. Then 300 g sodium potassium tartrate tetrahydrate was dissolved in 500 mL of distilled H_2O , then these two solutions were mixed then the volume made 1 L using RO water, and the absorbance was read with help of spectrophotometer (VWR, V-1200) at 530 nm. As the reducing sugars have free aldehyde or keto groups, under alkaline and heating conditions, they can react with DNS to produce 3-amino-5-nitrosalicylate and show absorbance at 530 nm.

3.5 Methods:

3.5.1 Moisture content:

Aluminium cups were used to determine the moisture content of the samples. A clean, coded cup was first dried in an oven for at least 30 minutes, cooled in a desiccator and weighed. A ground sample (about 1 g) was placed in the cup that was put in an oven at 101-105°C overnight. The cup was then cooled in a desiccator. The weight of the cup plus contents after drying was recorded. Triplicate samples were weighed to perform

the analysis. The standard moisture was determined using the method given by Approved Methods of the AACC (1994).

$$\text{Moisture \%} = \frac{w_2 - w_3}{w_2 - w_1} \times 100$$

Where, w₁= initial weight of cup, w₂= weight of cup plus sample before drying, w₃= weight of cup plus sample after drying.

3.5.2 Fat:

Crude fat was determined using a BUCHI Soxhlet Extraction Unit E-816HE (Luque-Garcia and Luque de Castro, 2004). The samples (1 g in triplicate) were weighed into separate thimbles to perform the extraction. Petroleum ether was then added to the glass tubes and the thimbles were suspended in the glass tube with a holder. The principle is that a dried ground sample is extracted with petroleum spirit; this dissolves fats, oils, pigments and other fat soluble substances. The petroleum spirit was then evaporated from the fat solution by boiling the solution. After the completion of one hour cycle the glass tubes were kept in a hot air oven (105 °C) for 20 mins. The samples were then cooled for 10 min before weighing. The resulting residue was weighed and referred to as either extract or crude fat.

3.5.3 Protein content:

The protein content was determined by two different methods. The protein contents of the extrudates and raw flour samples before digestion were determined using the Dumas method (A).

A. Dumas method:

The Dumas method was used to determine the protein content of the samples. Samples (200 mg) were weighed in triplicate and loaded individually into the Dumas machine to measure total nitrogen. The nitrogen content was determined using an Elemental analyser Model Vario MAX CN Hanau, Germany. The instrument works according to the principle of catalytic tube combustion under oxygen and at a high temperature. The combustion gases are separated from the foreign gases. Carbon and N were then separated from each other by specific adsorption columns and then detected in succession using a thermal conductivity detector. The carrier gas was helium. The % protein was calculated by the following formula:

$$\% \text{ Protein} = n \times 6.25$$

Where, N = nitrogen found in sample and 6.25 is the correction factor used to convert the nitrogen content of grains into protein content (F. A. O., 2011).

3.5.4 Method for protein digestion:

Each sample (pasta and extrudate) was analysed for protein digestibility in triplicate following the method reported previously by Chen *et al.*, (2013) (Fig. 11). A 1 gm sample was weighed in triplicate into three separate digestion containers. Then 30 mL of RO water was added to the containers. The pH was measured and reduced to pH 2 using 1 M HCl and the containers were loaded onto a magnetic stirrer. A Time 0 aliquot was taken and transferred to a small Eppendorf tube and immediately placed in an ice bath to stop any further reaction. Freshly prepared pepsin (4 U/mg on a protein basis) was then added to the container to complete the gastric phase. The solution was mixed on

a magnetic stirrer for 10 min and kept for incubation at 37°C for 1 h. The samples were stirred manually at 15 - 20 min intervals during the incubation. The pH was checked and increased to pH 7 using 1 M NaOH. A freshly prepared pancreatin solution (4 U/mg on a protein basis) was then added to the containers and the volume of the digest was made up to 50 mL with RO water. The solutions were mixed for 10 min on magnetic stirrer at 10000 g and then incubated at 37°C for 2 h. The samples were stirred manually at 15 - 20 min intervals during the incubation. Aliquots were also taken at 60, 120 and 180 min (Fig. 11) and transferred to small Eppendorf tube (1 mL) kept in an ice bath to stop further reaction.

Enzymes and solutions prepared prior to the experiment:

Pepsin 1034 U/mL stock - 10 mg pepsin in 10 mL water

Pancreatin 1000 U/ml stock - 460 mg pancreatin in 10 mL water

1M HCl to adjust the pH, 1M NaOH to adjust pH

Samples 2% w/v = 1 gram of sample in 50 mL water

Materials used:

Containers with a 55 mL capacity; micropipettes - 100-1000 µL; microtiter plates.

Fig 3.10 Procedure: (for protein digestion)

Sample taken - 1 g in 30 mL water (mix well)



Record pH and adjust to 2.0 using 1M HCl



Add 4 U/mg pepsin from stock to the container, mix well and make up to a 50 mL volume



Incubate for 1 h at 37°C



Record the pH drop in the solution; remove 1 mL aliquot and adjust pH to 7.0 using 1 M NaOH



Add pancreatin solution 4U/mg from stock



Incubate for 2 h at 37°C



Take all tubes out and then centrifuge for 10 min at 10000 g at 4°C.



Collect supernatant to analyse the protein

B) Bradford method:

Protein estimation of each digested sample was carried out by the Bradford method (Fig. 11) (Kruger, 2009)

Fig 3.11 Procedure for protein detection

Prepare three to five dilutions of a protein standard



Pipette 160 μL of each standard and sample solution (triplicates) to separate microtiter plate wells



Add 40 μL of Bradford dye reagent concentrate to each well and mix thoroughly



Incubate at room temperature for at least 5 min and not more than 1 h



Measure absorbance at 596 nm



Plot a standard curve



Estimate protein concentration by using the standard curve values

3.5.5 Total Starch:

Total starch analysis was carried out in triplicate according to the official AACC method (AACC, 1994). A dried sample (100 mg) was measured into a glass tube using a plastic spoon, then the glass tube was shaken to make sure the sample accumulated at the bottom. Five mL of aqueous ethanol (80%) were added and samples were incubated at 80 °C for 5 min. All tubes then were centrifuged at 3000 RCF for 10 min and the supernatant was discarded. The pellets were re-suspended in 10 mL of 80% aqueous ethanol and then centrifuged again at 3000 RCF and the supernatant poured off. Thermostable α -amylase (3 mL) was added and all tubes were incubated in boiling water. The tubes were stirred the tubes while they were incubated. The test tubes were then placed in a water bath at 50 °C and 0.1 mL of amyloglucosidase (330 U of starch) was added. The contents of the test tubes were then transferred into 100 mL volumetric flasks, mixed thoroughly and then an aliquot of each sample was centrifuged at 3000 RCF for 10 min. The clear filtrates from the test tubes were used for the assay after the addition of 3 mL of GOPOD reagent. The absorbance was read at 510 nm. RO water was used as a blank.

3.5.6 *In vitro* starch digestion:

There are numerous *in vitro* methods available that can be applied to conduct starch digestion and to estimate the glycaemic response of a particular product. However, the methods used are sometimes different from what occurs in human physiology. In human physiology other activities such as oxidation and chewing are involved during the digestion of food. Woolnough *et al.* (2008) studied some methods and developed a suitable *in vitro* method to measure the predictive glycaemic response. The present

method is relatively quick, less expensive and easily acceptable in terms of accuracy compared to other *in vivo* and *in vitro* methods. Cutting the samples mimics the chewing process and this enables the samples to mix evenly with the solution and to accurately measure the reducing sugars. This method can also be applied to a wide range of starchy foods, is advocated by international health boards and has been extensively applied in the investigation of *in vitro* glycaemic responses of a range of starchy foods (Woolnough *et al.*, 2008)

The actual process followed is elaborated below:

Each sample (pasta and collets) was analysed for its potential glycaemic response in triplicate following the method reported recently by Gao *et al.* (2016). Triplicate samples were weighed into polyvinyl containers. The weight of each sample was calculated according to the total starch (2.5 g starch) content of the sample. RO water (30 mL) added to the containers and the containers were kept on a heating stirrer (37 °C) before 0.8 mL of 1 M HCl was added and the containers were placed on a magnetic stirrer and the temperature was brought to 37 °C. A freshly prepared 1 mL of 10% pepsin solution was added to mimic the gastric phase of digestion. The samples were digested for 30 min with steady, constant mixing. Then, 2 mL of 1 M NaHCO₃ and 5 mL of 0.1 M Na maleate buffer (pH 6) were added together before taking an aliquot at Time 0. A 1 mL aliquot was taken from each digestion container and transferred to test tubes containing 4 mL 99% ethanol. Then 0.1 mL of amyloglucosidase was added to prevent end product inhibition of pancreatic α -amylase. Freshly prepared 2.5% pancreatin (5 mL) was added to mimic the intestinal phase and the timer was started for the 120 min digestion. The volume of the digests were then accurately made up to 53 mL with RO

water. A 1 mL aliquot was taken at 20, 60 and 120 min from each container and transferred to test tubes containing 4 mL of 99% ethanol.

For the measurement of the reducing sugars, all test tubes containing the sample aliquots were centrifuged for 10 min at 1000 RCF. Clean, dry glass test tubes were placed in a stainless steel test tube stand, then 0.05 mL of a sample aliquot from each replicate was placed in individual glass test tubes. A 0.05 mL reagent blank (RO water), 0.05 mL of 5 mg/mL glucose standard and 0.05 mL 10 mg/mL were placed in separate tubes. Then, 0.25 mL of enzyme solution A was added to each glass tube and all the tubes were kept for 20 min at room temperature before 0.75 mL of the DNS (reagent) was added to each tube, the tubes were covered and heated for 10 min in a boiling water bath. The glass tubes were then cooled before adding 4 mL of RO water and the absorbance was read at 530 nm. The spectrophotometer was adjusted to zero using a RO water blank before measuring absorbance of the samples and standard.

Fig. 3.12 Method for starch digestion of samples

A) Starch digestion

Samples were weighed in triplicate (0.25 g starch in each digestion pot)



Add 30 mL of distilled water and placed sample on heated strirrer at 37 °C



0.8 mL of 1 M HCl was added (held at 37 °C)



1 mL of 10% pepsin solution was added in 0.05 M HCl



Digested for 30 min at 37°C with steady, constant mixing



2 mL of 1 M NaHCO₃ was added

↓

5 mL 0.1 M Na maleate buffer pH 6 was added
(1 mL aliquot taken at Time 0)

↓

0.1 mL amyloglucosidase was added

(To prevent end product inhibition of pancreatic α amylase)

↓

5 mL of 2.5 % pancreatin in 0.1 M Na maleate buffer pH 6 was added

↓

Start timing digest

↓

Accurately make volume up to 53 mL

↓

Incubate at 37 °C for 120 min with steady, constant mixing
(Aliquots were removed at 20, 60 and 120 min and placed into ethanol to halt the digestion.)

The samples were then analysed for their reducing sugar content using 3, 5-dinitrosalicylic acid (DNS).

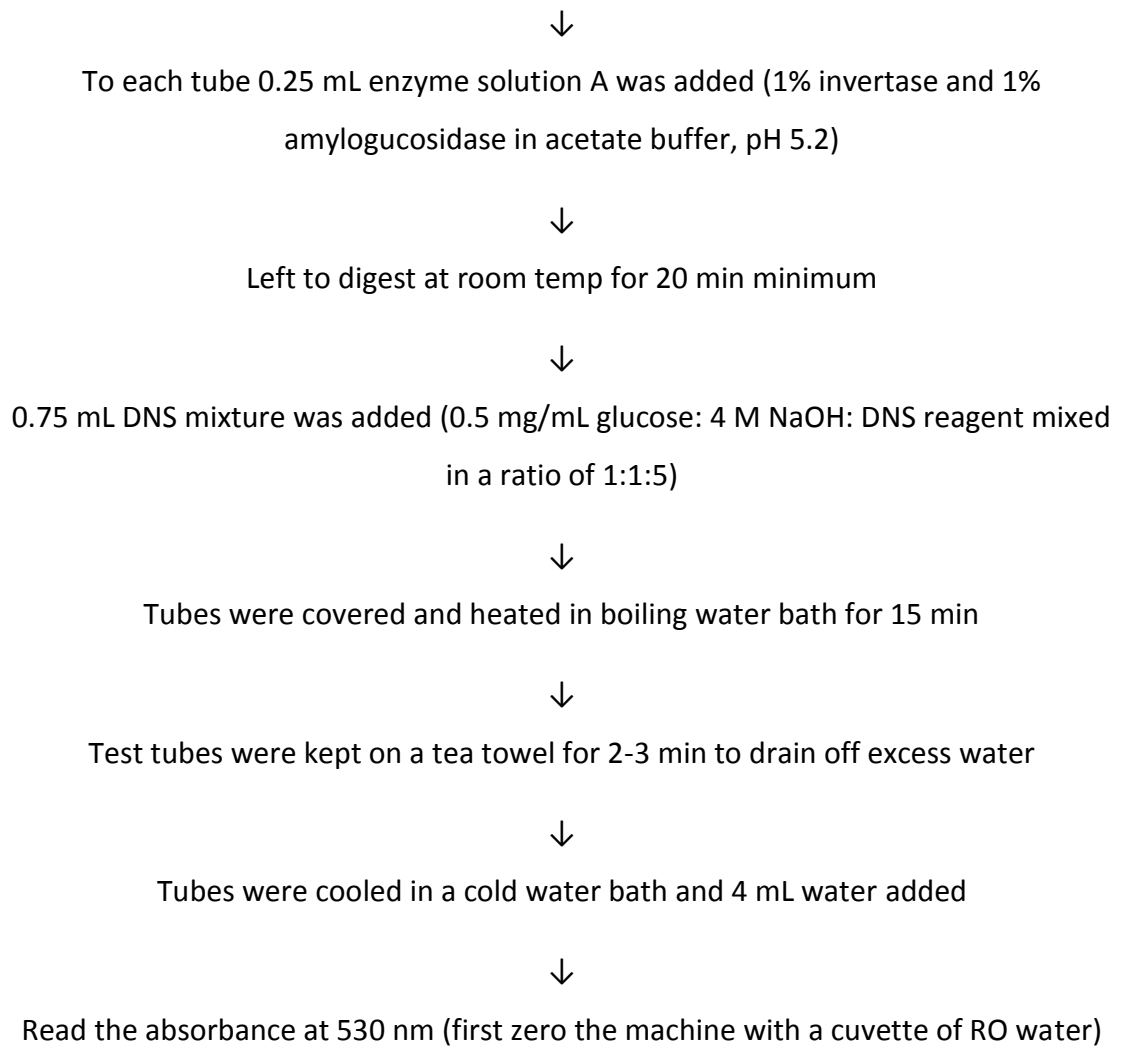
B) Measurement of reducing sugars produced during *in vitro* digestion

The test tubes containing the aliquots and alcohol were centrifuged at 1000 RCF for 5 mins

↓

In glass test tubes placed

- i) 0.05 mL of each aliquot from the *in vitro* digest
- ii) 0.05 mL of the reagent blank (RO Water) (in triplicate)
- iii) 0.05 mL of the standard 5 mg/mL glucose (in triplicate)
- iv) 0.05 mL of standard 10 mg/mL glucose (in triplicate)



3.5.7 Texture analysis:

The objective was to quantitatively determine the force required to compress the extrudates a set distance, and to determine the force required to fracture the product. The texture of the cooked pasta and hot extrudates were determined using a Texture Analyser (Fig 3.13) (TA.XT2, Stable Micro System, U.K.) equipped with a 5 kg load cell. Different probes were used for the texture of the pasta and extruded samples according to Approved Method 66-50 (AACC, 2000). The probe shown in Fig. 15 was used for pasta texture measurements and the probe attached to the machine (Fig. 14) was used for the hot extrudates. A compression test is commonly used to measure the hardness of a

food product and is defined as the force required to compress a collet to a fixed distance or the distance a fixed force will compress a collet. A collet was placed on the flat and solid platform and the probe was centred above it. Each sample was crushed by lowering the probe to calculate the force required to compress the collet. The pre-test speed was 2 mm sec^{-1} and test speed was 3 mm sec^{-1} . The count peak and area results were displayed by the analyser calculator. For the pasta texture (firmness), the measurement was shown as the maximum cutting force (N). Samples were cooked for 6 min and drained for 30 s after cooking and before testing, they were then loaded onto the platform in groups of four or six pasta strands together. The probe (blade) was fixed at the top of the samples and cutting force was calculated by lowering the blade. The distance was 4.5 mm and test speed was 0.17 mm/sec.

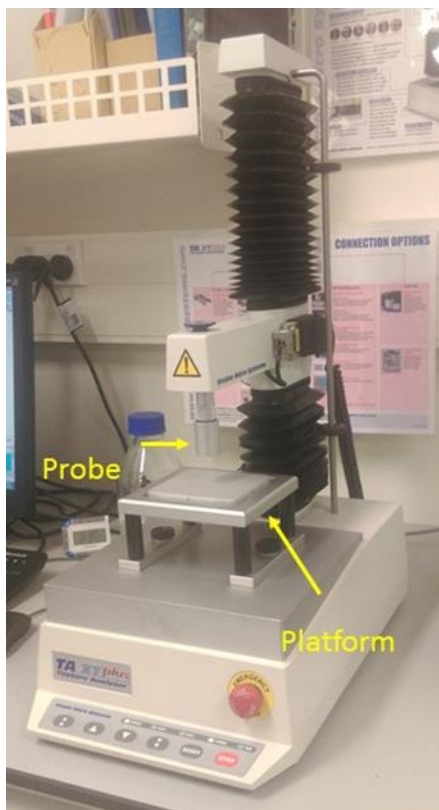


Fig. 3.13 Texture analyser analysis



Fig. 3.14 Probe for pasta texture

3.5.8 *In vitro* protein digestibility:

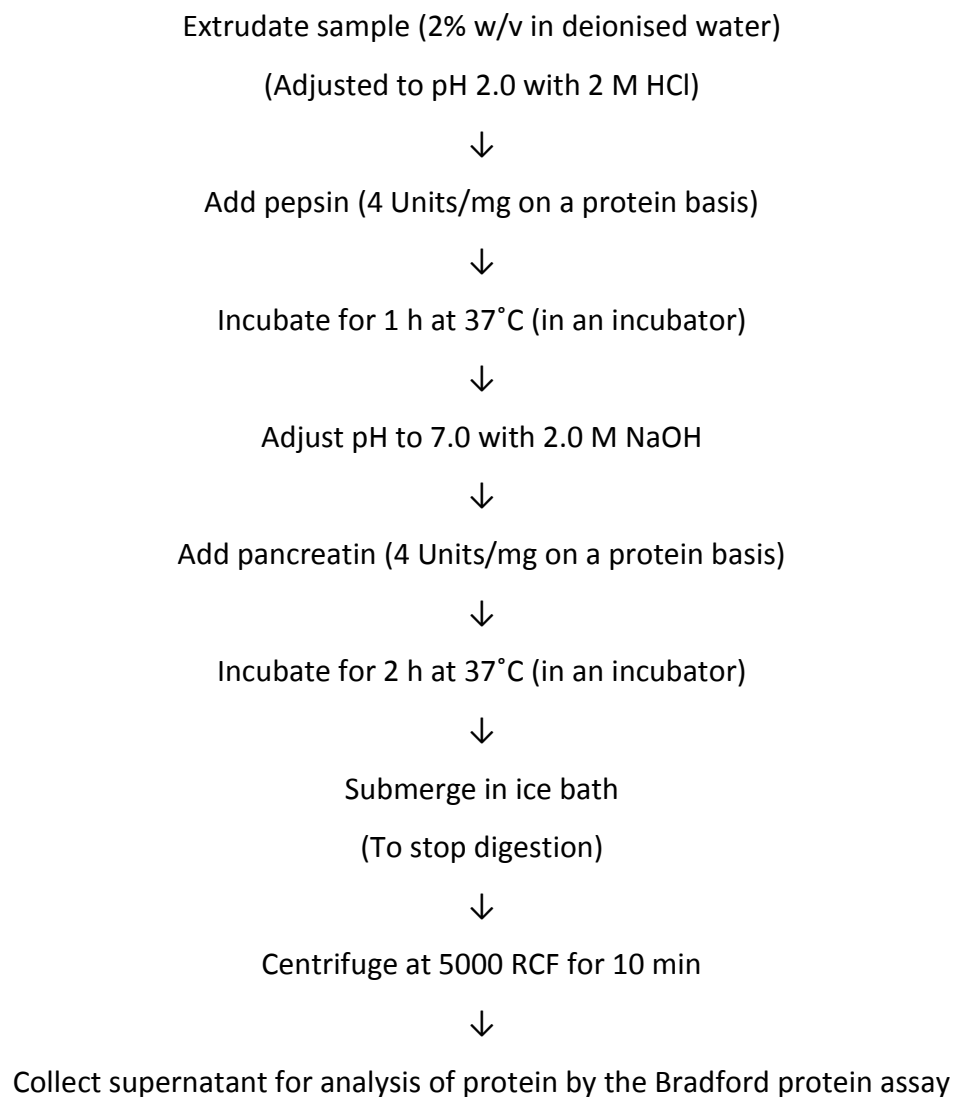
Some *in vitro* methods have the potential to give useful measures of *in vivo* amino acid and protein digestibility for humans. However, some methods rely on a pH drop to calculate protein hydrolysis by using same amount of protein digestive enzymes for all the samples (Hsu, 1977). This method does not mimic human physiology but applying *in vivo* methods is quite expensive. The *in vitro* method used simulated various physical and chemical processes of human digestion (gastric phase and intestinal phase) precisely. It also has the potential to give independent validation of the data and can be used on a wide range of foods.

Each sample (pasta and extrudate) was analysed for protein digestibility following the method of Chen *et al.* (2013) (Fig 16). A 1 g sample was weighed in triplicate into digestion containers. Then 30 mL RO water was added to the container. The pH was checked and reduced to pH 2 using 1 M HCl and the containers were loaded onto a magnetic stirrer. A Time 0 aliquot was taken and transferred to a small Eppendorf tube and then immediately placed in an ice bath to stop further reaction. Freshly prepared pepsin (4 U/mg on protein basis) was then added to the container to complete the gastric phase. The solution was mixed on a magnetic stirrer for 10 min and incubated at 37°C for 1 h. The samples were stirred manually at 15 – 20 min intervals during the incubation. In the next step, the pH was checked again and increased to pH 7 using 1 M NaOH. Then, freshly made pancreatin solution (4 U/ mg on a protein basis) was added to the containers and the volume of the digest was accurately made up to 50 mL with RO water. The solutions were mixed for 10 min on a magnetic stirrer and then incubated at 37°C for 2 h. The samples were stirred manually at 15 – 20 min

intervals during the incubation. Before starting, a 1 mL aliquot was taken and transferred to a small Eppendorf tube then kept in an ice bath to stop further reaction. Aliquots were also taken at 60, 120 and 180 min.

Fig. 3.15 Protein digestion

Pepsin (4 Unit/mg on a protein basis), Pancreatin (4 Units/mg on a protein basis)



3.5.9 Water solubility index (WSI) and water absorption index (WAI) for

collets:

Weighed ground samples (about 1 g) were added to 10 mL of distilled water in pre-weighed centrifuge tubes. The centrifuge tubes were shaken vigorously by hand and also with a vortex mixer until the sample was homogenised. Some samples required intervention by the use of a thin metal rod to break up lumps present. The samples were left to stand for at least 30 minutes and shaken once more before centrifugation. The samples were centrifuged at 3500 RCF for 30 min. The supernatants were transferred carefully into pre-weighed crucibles, dried at 65 °C overnight, then cooled and weighed. The remaining sediment in the tube was weighed. WAI (g) and WSI (%) were calculated using the equations below. Each sample was replicated three times.

$$WAI (g) = \frac{\textit{Weight of residue}}{\textit{Dry weight of residue}}$$

$$WSI (\%) = \frac{\textit{Weight of dry matter in supernatant}}{\textit{Dry weight of sample}} \times 100$$

3.5.10 Expansion ratio:

To determine the expansion ratio (ER), the cross-sectional diameter of the extrudates was measured with a digital vernier calliper (Insize electronic calliper Series 1112 Suzhou New District, 215009 China). The expansion ratio was calculated as the cross-sectional diameter of the extrudate divided by the diameter of the die opening (AACC, 2000). The ER values were obtained from 15 random samples from each extrusion condition.

$$\text{Expansion ratio} = \frac{\text{Diameter of an extrudate sample (mm)}}{\text{Diameter of the die (mm)}} \times 100$$

3.5.11 Water absorption index and swelling index for pasta:

Frozen pasta was kept at room temperature for 10 min for thawing, cooked for 6 min (as per optimum cooking time), and then used for analysis. Baking tins were used to measure WAI and SI. The swelling index (SI) of cooked pasta (g water/g dry pasta) and water absorption index (WAI) (g/100g) was determined according to the procedure described by Cleary and Brennan (2006). After cooking, the pasta was dried at 105°C to a constant weight.

The swelling index (SI) was expressed as:

$$SI = \frac{Wc - Wd}{Wd}$$

Where, Wc is weight of cooked pasta (g) and Wd is weight of pasta after drying (g).

The water absorption index (WAI, g/100 g) was determined as:

$$WAI = \frac{Wc - Wr}{Wr}$$

Where, Wc is weight of cooked pasta (g) and Wr is weight of uncooked pasta (g).

3.5.12 Cooking loss:

Cooking loss (CL, g/100 g) was determined according to Approved Method 66-50 (AACC, 2000). Pasta samples were weighed, cooked in a stainless steel pan then cooked for 6 mins on an electronically-operated hot plate. The cooked pasta was rested for 2 mins in a polyvinyl sieve and the drained water from pasta, which contained the starch,

was collected in a stainless steel vessel. The vessel was dried at 105 °C overnight until a constant weight.

3.5.13 Rapid visco analyser:

A collet sample (5 g) of each was weighed and added to a canister containing 25 mL of distilled water and the thermal-visco profiles of the resulting pastes were measured by a Rapid Visco Analyser (Perten Instruments, Hägersten, Sweden). Briefly, canisters containing distilled water and samples were heated to 90 °C, then held at this temperature for 3 min before cooling to 50°C. The peak viscosity (*PV*), final viscosity (*FV*) and breakdown (*BD*) of materials were recorded (Brennan *et. al.*, 2012b).

3.5.14 Statistical analysis:

All experiments were performed in triplicate. Statistical differences in pasta and collets characteristics were determined by one-way analysis of variance (ANOVA) using Minitab 16 software and Tukey's comparison test ($p > 0.05$). Amount of protein, its digestibility, other values such as starch digestibility, WAI, WSI, viscosities were the continuous variables where various samples were categorical variables in the analysis. Tukey's test was applied to show significant difference in the samples which are shown by different letters in the tables.

Chapter 4

The effect of fortification of legumes and extrusion on protein digestibility of wheat based snacks

Overview

In this study, the effect of legume incorporation on wheat based hot extrudates (collets) at various levels on its proximate composition and protein digestibility were studied. This chapter is adapted from a published article entitled, **“The Effects of Fortification of Legumes and Extrusion on the Protein Digestibility of Wheat Based Snack.”** This article has been published in Foods Journal (doi:10.3390/foods5020026). The changes in protein digestibility, due to changes in the structure of the extruded products, are explained. The importance of developing healthy snack products, potential for using plant based (various legumes and cereals) sources of nutrition in the development of snacks and the importance of extrusion is discussed in the introductory section. The types of extruder and differences between twin screw and single screw extruders are also given in the introduction. The material and methods are not given in this chapter, these may be referred to in the appropriate sections of Chapter 3 Material and Methods. The general experimental design is given instead of a Material and Methods section. The discussion and conclusions are elaborated in broader way with some recent references to support the research findings and the mechanisms responsible for affecting protein denaturation of the extrudates.

Abstract:

Cereal food products are an important part of the human diet with wheat being the most commonly consumed cereal in many parts of the world. Extruded snack products are increasing in consumer interest due to their texture and ease of use. However, wheat based foods are rich in starch and are associated with high glycaemic impact products. Although legume materials are generally rich in fibre and protein and may be of high nutritive value, there is a paucity of research regarding their use in extruded snack food products. The aim of this study was to prepare wheat-based extrudates using four different legume flours: lentil, chickpea, green pea, and yellow pea flour. The effects of adding legumes to wheat based snacks at different levels (0%, 5%, 10%, and 15%) during extrusion were investigated in terms of protein digestibility. It was observed that fortification of snacks with legumes caused a slight increase in the protein content by 1%–1.5% w/w, and the extrusion technique increased the protein digestibility by 37%–62% w/v. The product developed by extrusion was found to be low in fat and moisture content.

4.1. Introduction:

Snack products are becoming an important part of the human diet as their convenience and availability attract consumers attention (Brennan *et al.*, 2013, Nor *et al.*, 2013, Norgaard *et al.*, 2013,). Most of the available snacks are made from refined cereal flours and are rich in salts, saturated fats and easily digested carbohydrates (Brennan *et al.*, 2013, Struck *et al.*, 2014). Cereals are the main source of carbohydrates in our diet. Barley, wheat, rice and maize are now gaining importance as they are rich sources of protein, dietary fibre and lipids. Cereals are the main source of energy (56%) for humans

in some parts of the world (Flight and Clifton, 2006). It may be argued that the increase in the consumption of snack food products has led to an increase in obesity and, thus, an unhealthy population (Brennan *et al.*, 2013, Oliveira *et al.*, 2015, Osen *et al.*, 2015, Struck *et al.*, 2014, Woolnough *et al.*, 2008,). However, snacking with nutrient dense foods like fruits, whole grains and fibre between meals is recommended by many nutrition professionals as part of healthy weight management and obesity prevention programmes (Keast *et al.*, 2010, Larson and Story, 2013,). For instance, Keast *et al.*, (2010) found that 12 to 18 year old snack consumers were less likely to be obese and suffer from abdominal obesity than the non-snack consumers. Evans *et al.* (2015) and Kong *et al.* (2011) also reported that snack consumption improves overall diet quality amongst school-aged children as well as older women. Researchers are increasingly concerned about the quality of nutrients in snack products consumed; as the quality of snacks largely contributes to the impact on health (Shriver *et al.*, 2017). Snacks such as fruits and whole grains are lower in fat and richer in fibre than foods consumed at meals; therefore, regular consumption of nutritional snacks could help to stimulate satiety and reduce weight.

In terms of snack food products, it could be argued that legume grains and flours are underutilised in the extrusion process (Osen *et al.*, 2015, Yadav *et al.*, 2014). Legumes such as chickpea, lentils and soybean are an important source of protein for the human body, particularly in parts of the world where meat and milk consumption is constrained by factors such as low availability, ethical reasons or allergenicity. Some researchers suggest that legume based products are essential in our daily diet for leading a healthy life (Boye *et al.*, 2010b, Tharanathan and Mahadevamma, 2003;). Espinoza-Moreno *et al.* (2016) focused on the development of a ready-to-eat expanded snacks using

transgenic maize and black common beans (70:30). The results showed that due to the substantial content of nutrients and antioxidants this product can satisfy the current demand of healthier snack products.

From a nutritional point of view, legumes are of special interest because they are rich in dietary fibres (Yadav *et al.*, 2014) and protein (Tharanathan and Mahadevamma, 2003). Albumin and globulins are the dominant proteins found in legume seeds, and around 70% of legume protein is globulins (De Almeida Costa *et al.*, 2006, Freitas, 2000, Tosh and Yada, 2010). Legumes also contain considerable amounts of vitamins and other micronutrients. The composition of legume materials are known to play a key role in preventing metabolic diseases such as diabetes mellitus and coronary heart diseases (Boye *et al.*, 2010a, Boye *et al.*, 2010b, De Almeida Costa *et al.*, 2006, Simpson *et al.*, 1981). It may, therefore, be possible, by blending wheat and legume grains material, to manufacture extruded snack products that have lower starch contents but higher protein and fibre contents and, potentially, greater protein digestibility. Thus, using starch sources, such as maize, oat, barley and wheat, combined with sources of protein such as peas or beans can increase the nutritional quality of snack products. Addition of legume protein can produce nutrition rich products (Boye *et al.*, 2010a). Tiwari *et al.* (2011) studied the addition of pigeon pea to wheat flour based biscuits and De la Hera *et al.* (2012) studied the effect of the addition of legume flour to traditional cereal based flours and both research teams illustrated an increase in protein content and potential nutritional improvements, including an increase in protein digestibility. Another study by Madhumitha and Prabhasankar (2011) improved the nutritional value of pasta by adding black gram flour and also reported that processing food materials increased the

value and shelf life of the resulting products. Grain legumes have a high potential for improving the nutritional quality of food, being important sources of protein, starch, fibre and other health promoting components. Furthermore, a variety of technological processes are available to facilitate the inclusion of legumes into more innovative food products. For instance, Yagcı and Evcı (2015) used the instant controlled pressure drop process (DIC) to produce a nutritious snack product using wheat and chickpeas. The processing conditions involved during DIC, such as long processing time and high pressure, decreased the phytic acids content of the snacks and showed that the controlled use of DIC parameters was a convenient way to develop a nutritionally balanced snack. However, hydrothermal processes such as boiling, blanching and steaming are the key processes involved in the development of cereal–legume food products (Yagcı and Evcı, 2015). The extrusion technique is one of the most common and popular processing techniques among manufacturers due to its convenience and affordability, and its importance has been widely accepted by the scientific community (Alonso *et al.*, 2000, Brennan *et al.*, 2013, Robin *et al.*, 2015). Generally, twin screw extrusion involves high moisture; therefore, the products developed using a twin screw extruder may differ in physico-chemical properties from the product developed by a using single screw extruder (Akdogan, 1999). Alonso *et al.* (2000) studied the effect of twin screw extrusion on anti-nutritional factors, protein content and digestibility of beans and reported that extrusion processing is a prime method, compared to traditional processing (dehulling, soaking and germination) methods, to reduce trypsin, chymotrypsin and α -amylase inhibitors and haemagglutinating activity without modifying the protein content. Although single screw extrusion and twin screw extrusion are the two main types of extrusion, high temperatures, short times and high

pressure are common conditions for extrusion in both types (Alonso *et al.*, 2000, Brennan *et al.*, 2013) such that extrusion cooking changes the biochemical properties of foods. Extrusion can be used to produce innovative products, such as cereal based snacks, precooked breakfast cereals, modified starch and beverages, baby food, textured vegetable protein (mainly from soybean) and baro-thermally processed products for the pharmaceutical industry to name a few (Hagenimana *et al.*, 2006, Moscicki and van Zuilichem, 2011). A recent study by Espinoza-Moreno *et al.* (2016) on the development of extruded snacks using maize and black common beans resulted in a product with high content of quality protein and fibre as well as having a low energy density. They also reported that extrusion parameters such as moisture and temperature had increased antioxidant activity.

Recently, there has been a growing emphasis on increasing the nutritional value of products creating a need for research on different aspects, such as understanding the *in vitro* digestibility of the combined blends. A variety of protein and cereal sources have been utilised to improve the nutritional quality of extruded snack products (Caltinoglu *et al.*, 2014, De Pilli *et al.*, 2015, Rashid *et al.*, 2015, Soison *et al.*, 2014, Thachil *et al.*, 2014, Yin *et al.*, 2015). The main aim of this study was to investigate whether the processing of food materials would affect the *in vitro* protein digestibility.

4.2 Materials and methods:

4.2.1 Materials:

Whole wheat (Zentrofan), green pea and chickpea grain were obtained from Piko Foods, Christchurch (Refer 3.3).

4.2.2 Extrusion:

Whole grains of different chickpea and green pea were used at 0%, 5%, 10% and 15% (on a weight basis) replacement levels for wheat grain in the production of extrudates. Extrusion was conducted in a single-screw extruder through a 3 mm die face (Millbank, Auckland, New Zealand) and collected as collets (Refer 3.1.1 and 3.2).

4.2.3 Moisture content:

The standard moisture determination method given by Approved Methods of the AACC (1994) was used with a slight modification to measure the moisture content of the sample (Please refer 3.5.1).

4.2.4 Fat:

Crude fat was determined using a BUCHI Soxhlet Extraction Unit E-816HE (Luque-García and Luque de Castro, 2004). (Refer 3.5.2).

4.2.5 Protein:

Protein content of the extrudates and raw flour samples were determined using the Dumas method (element analyser Model Vario MAX CN, Hanau, Germany). (Refer 3.5.3A).

4.2.6 Protein digestibility:

In vitro protein digestibility was mainly adopted from Chen *et al.* (2013) with slight modifications. In this experiment we have used ice bath to stop enzymatic reaction instead on hot water bath. After digestion, the remaining protein was determined using the Bradford method with a slight modification (Hagenimana *et al.*, 2006, Kruger, 2009).

The per cent digestibility was calculated as the difference between protein content at 0 min and after 180 min as a percentage of the original protein content (Refer 3.5.8).

4.2.7 Statistical analysis:

Refer 3.5.14

4.3. Results and discussion:

Legumes are regarded as grains that contain high protein and high fibre (Freitas *et al.*, 2000, Osen *et al.*, 2015, Tiwari and Cummins, 2009). The results shown in Table 4.1 indicated that the protein content of legumes was considerably higher than that in wheat. They ranged from 20.27% to 25.33% for legumes, whereas for wheat it was 14.47%. Our results agrees with the previous studies by Boye *et al.*, (2010a), Tiwari *et al.*, (2011) on the use of legume protein for various product development to increase the total protein content of the food.

Table 4.1 Protein content of raw ingredients used

Sample	Protein (g/100 g on a dry basis)
Wheat	14.47 ± 0.11
Lentil	25.33 ± 0.17
Chickpea	22.96 ± 0.24
Yellow pea	21.73 ± 0.13
Green pea	20.47 ± 0.28

4.3.1. Proximate analysis of the extrudates:

The moisture content of the extrudates (Table 4.2) showed slight variations, the highest value was for wheat + 10% yellow pea, at 9.36%, whereas, for wheat + 15% lentil, it was

7.56%. Previous research has illustrated that variations observed in the moisture content of extruded products may be dependent on the feed moisture and extrusion temperature (Brennan *et al.*, 2013, Tiwari *et al.*, 2011, Yadav *et al.*, 2014). It could be argued that raw materials high in fibre content (such as brans or legumes) also contributed to an increase in water holding capacity and, hence, the moisture content of the final product (Brennan *et al.*, 2013, Tiwari *et al.*, 2011). When moisture is retained during the extrusion process this can have a serious effect in consumer acceptability of cereal foods containing high fibre ingredients; for instance, bulk density and overall product hardness due to changes in polymerisation behaviour of the raw material (Pietsch *et al.*, 2017, Struck *et al.*, 2014). Although high moisture extrusion used in the preparation of micro-particle product development, such as meat and crab analogues, low moisture extrusion processes have been studied more frequently than the high moisture extrusion (Wolz *et al.*, 2016). The presence of moisture during extrusion decreases the melt viscosity; furthermore, it reduces pressure along the die and this leads to a reduction in the expansion of the extrudates (Akdogan, 1999). Ghumman *et al.* (2016) have reported that increases in protein digestibility of lentil and horse gram were due to increased extrusion temperature and moisture. The results showed that the extrusion temperature and high moisture helped in solubilising pulse proteins and made the proteins susceptible to proteolytic attack. In this study the presence of legumes in the extruded formulations had no effect on the moisture of the extruded collets (Table 4.2).

Table 4.2 Proximate compositions of the wheat based extrudates (100 g on a dry basis)

Sample	Protein	Fat	Moisture
Wheat Ctrl	13.54 ± 0.04 ^e	0.58 ± 0.09 ^{cde}	9.29 ± 1.89 ^a
W+5% Yellow pea	14.30 ± 0.11 ^{bc}	0.51 ± 0.06 ^e	8.11 ± 0.21 ^a
W+5% Green pea	14.10 ± 0.04 ^{cd}	0.54 ± 0.01 ^e	8.41 ± 0.64 ^a
W+5% Lentils	14.53 ± 0.21 ^{bc}	0.55 ± 0.01 ^e	8.51 ± 0.26 ^a
W+5% Chickpea	14.25 ± 0.08 ^{bc}	0.62 ± 0.04 ^d	8.13 ± 0.40 ^a
W+10% Yellow pea	14.96 ± 0.04 ^a	0.72 ± 0.02 ^{bcd}	9.36 ± 0.76 ^a
W+10% Green pea	14.57 ± 0.05 ^{bc}	1.03 ± 0.11 ^a	9.05 ± 0.90 ^a
W+10% Lentils	14.59 ± 0.23 ^{bc}	0.83 ± 0.09 ^b	8.62 ± 0.41 ^a
W+10% Chickpea	14.28 ± 0.06 ^c	0.90 ± 0.10 ^{ab}	7.64 ± 0.35 ^a
W+15% Yellow pea	15.16 ± 0.17 ^a	0.75 ± 0.04 ^{bc}	7.75 ± 0.13 ^a
W+15% Green pea	14.79 ± 0.02 ^b	0.75 ± 0.08 ^{bc}	8.42 ± 0.76 ^a
W+15% Lentils	15.05 ± 0.03 ^a	0.72 ± 0.05 ^{bcd}	7.56 ± 0.14 ^a
W+15% Chickpea	14.47 ± 0.11 ^{bc}	0.29 ± 0.01 ^f	7.75 ± 1.53 ^a

* Values are expressed as mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other ($p > 0.05$).

The fat contents in all samples were less than 1% except for wheat + 10% green pea (1.03%). The lowest fat content was shown by wheat + 15% lentil. There was a significant ($p > 0.05$) difference observed between the samples for the concentration of fat. However, the fat content of most of the samples remain under 1% indicating that it was possible to include legumes into cereal extrudates without affecting the fat content of the foods (Table 4.2). In previous reports by Jenkins *et al.* (2001) and Kushi (1999) grain protein were considered as low source of triacylglycerols and cholesterol and intake of wheat and legume proteins tended to lower the risk of coronary heart disease. However, protein values (Table 4.2) showed that the addition of legume grains to the extruded samples increased the protein content of all the products. All combined

samples showed significantly ($p > 0.05$) higher protein content than the control samples. Wheat + 15% yellow pea and wheat + 15% green pea showed significantly higher protein content than control sample and the other combinations. Similar results were obtained by Gularte *et al.* (2011) where the addition of 50% of different legumes (chickpea, lentils, bean and pea) to rice based gluten free layer cakes increased the protein content. Pastor-Cavada *et al.* (2011) also observed increase in protein in corn and rice based extrudate samples after adding legumes. Similarly, Zucco *et al.* (2011) reported that adding wild legumes to wheat based cookies may have increased the amino acids content which further led to increase in the protein levels of the cookies. However, other factors, such as species of legume, cultivar and type of processing, might have significant effect on protein quality and quantity of the product. It has been reported that processing has been used to modify the hydrophilicity: hydrophobicity ratio and structural characteristics of proteins to control the extent of protein unfolding and the ability of proteins to form films around dispersed oil (Boye *et al.*, 2010a, Boye *et al.*, 2010b). For instance, soaking and defatting of lupin tended to increase the purity of the protein, where milling peas helped in development of protein enriched fractions (Pelgrom *et al.*, 2015, Schutyser *et al.*, 2015).

From above findings it can be observed that adding legumes to cereal based extrudates may alter the characteristics of the snacks. Results published by Anton *et al.* (2009) and Pastor-Cavada *et al.* (2011) concluded that legume protein and fibres interacted with starch present in cereal, and that fibre prevented the rupture of cell walls; this process avoided the formation of air bubbles and may also led to change in the structural properties of extrudates.

4.3.2. *In vitro* protein digestibility:

In vitro protein digestibility (IVPD) of raw flour mixes and extrudates are given in Table 4.4. The method used in this research was adapted from that previously used by Chen *et al.* (2013). Although the protocol was not supported by *in vivo* determinations in our experiment (due to facility constraints), Chen *et al.* (2013) provide a review of the method in relation to the *in vivo* analysis of protein digestibility. The results show that the IVPD of the raw flour mix was less than that of the extrudates. The IVPD of the control samples was relatively low compared to other combinations in both raw flour mixes and the extrudates (31.60% and 59.26%, respectively). In samples containing raw flour mixes, the highest level of digestibility was observed in wheat + 5% green pea. However, in the extruded samples, the wheat + 15% green pea product showed the highest level of protein digestibility. The values clearly indicated that extrusion processing had affected the protein digestibility of the samples with significant increases in protein digestibility being observed after extrusion processing. Previous research has indicated that the presence of anti-nutritional compounds, such as tannin and protease inhibitors can decrease protein digestibility (Park *et al.*, 2010); however, this was not observed in our results, possibly due to the fact that the mechanical factors from extrusion technology such as screw speed, mechanical shear played a larger role in protein digestibility than the limiting behaviour of anti-nutritional compounds (Alonso *et al.*, 2000). There may also be other factors, such as grain structure and cell wall components of the seeds, affecting the solubility and digestibility of protein in seeds. Protein could also react with non-protein components present in seeds during processing and it possibly leads to reducing digestibility (Duodu *et al.*, 2003). In this

study the different amounts and types of legumes added to wheat based extrudates may have varied the protein digestibility.

Table 4.4. Protein digestibility of raw flour mix and extrudates (given as a % of total protein)

Sample	Raw Mix	Extrudates
W Ctrl	16.70 ± 0.66 ^{bc}	59.26 ± 1.08 ^d
W+5% YP	32.70 ± 2.04 ^{bc}	63.39 ± 0.73 ^{abc}
W+5% GP	38.23 ± 2.11 ^a	62.95 ± 0.72 ^{bc}
W+5% L	29.33 ± 0.48 ^c	63.27 ± 0.20 ^{bc}
W+5% CP	29.97 ± 1.11 ^{bc}	61.44 ± 0.43 ^{bc}
W+10% YP	29.27 ± 2.86 ^{bc}	65.50 ± 1.49 ^{ab}
W+10% GP	28.92 ± 1.17 ^{bc}	64.03 ± 1.09 ^{ab}
W+10% L	32.00 ± 1.49 ^{bc}	62.46 ± 1.13 ^{bc}
W+10% CP	31.30 ± 0.64 ^{bc}	60.69 ± 1.29 ^{cd}
W+15% YP	31.59 ± 3.38 ^{bc}	65.61 ± 1.45 ^a
W+15% GP	33.02 ± 2.18 ^{bc}	65.69 ± 0.32 ^a
W+15% L	31.85 ± 1.55 ^{bc}	62.26 ± 0.74 ^{bc}
W+15% CP	35.21 ± 0.92 ^{bc}	62.46 ± 0.97 ^{bc}

* Values are expressed as mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other (p > 0.05).

For instance, Linsberger-Martin *et al.* (2013) reported that applying pressure and cooking legume seeds at a high temperature increased the protein digestibility of legume seeds from around 82 – 85%, possibly by increasing the solubility of the protein and fragmenting the long polymer chains of intact proteins.

Abd El-Hady and Habiba (2003) also observed an increase in the protein digestibility of legumes by the extrusion technique. Moreover, the rise could be related to the degradation of protein complexes within the extruded samples and denaturation of proteins due to heat and shear. The alterations in protein structure make the extruded products more susceptible to degradation and, hence, the release of the products of digestion was increased and the bioavailability of the protein may be elevated.

As mentioned previously, this was similar to the mechanism by which extrusion processing shears and denatures carbohydrate fractions leading to increased carbohydrate digestibility of pasta, extrudates and breakfast cereals (Brennan *et al.*, 2013, Kruger *et al.*, 1996, Woolnough *et al.*, 2008,).

Extrusion technology is based on versatile and flexible food processing concepts that minimise time, energy and cost by using high temperatures with fast, continuous-flow cooking that will inactivate protease and other inhibitors in raw soybeans and other legume seeds used in human and animal diets (Singh and Muthukumarappan, 2015). Dozier and Hess (2011) and Guzman (1989) have suggested that it may be the presence of heat during extrusion which is responsible for inactivation of protease inhibitors present in legumes. This further increased the *in vitro* protein digestibility of the extrudates. It was, therefore, sensible to suggest that in this set of experiments the extrusion processing parameters have led to the denaturation of protein structures leading to increased ease of digestion by inactivation of the enzyme inhibitors and exposing protein molecules to enzyme attack (Dahlin and Lorenz, 1993). The highest increase in protein digestibility observed in wheat + 15% green pea sample.

4.4. Conclusions:

Adding legumes to cereal based products was a growing trend in food industry. It was implemented as a useful tool to meet food requirements of growing populations and it may continue. Improving quality of extrudates in terms of nutritional value might help in maintaining the availability of high nutritional quality snacks. *In vitro* protein digestibility is an important factor in determining the quality of protein. This study concluded that adding legumes to wheat based snacks increased the nutritional quality of the products. The highest protein digestibility value was occurred in wheat+15% green pea. The wheat-legume combined blends showed (wheat+15% yellow pea) better nutritional composition than wheat based samples. This research established that adding legumes increased the protein content of the products. The increasing demand of nutrition rich food by rapidly growing populations has increased the pressure on the food processing and agricultural sector to produce food alternatives that provide nutrition and functional benefits to consumers and producers at an affordable price. This study also confirmed that the extrusion process increased protein digestibility. In addition, the results showed that a product prepared using extrusion with added legumes was (Wheat+15% chickpea) low in fat. Considering the protein content and its digestibility, the use of legumes has great potential for producing extrudate products of commercial value and it can be considered as an alternative for the high calorie, high energy snacks currently available.

Chapter 5

Investigation of the combination of legumes and cereals in the development of extrudate snacks and its effect on physico-chemical properties and *in vitro* starch digestion

Overview

This chapter was adapted from a published research article entitled “ **Investigation of the combination of legumes and cereals in the development of extrudate snacks and its effect on physico-chemical properties**” published in **Journal of Food and Nutritional Research** (ISSN 1336-8672). In this study, the effect of legume incorporation on cereal (wheat, rice maize and barley) based hot extrudates (collets) at various levels on its physical properties and starch digestibility were studied. The material and methods are not given in this chapter, these may be referred to in the appropriate sections of Chapter 3 Material and Methods. The changes in starch digestibility and physico-chemical properties of the extruded products due to addition of legumes are explained.

Abstract

The application of different levels of legumes in the cereal based extrudates has the potential to produce healthy snack foods. The aim of this study was to investigate the effect of the addition of legumes, such as yellow peas, green peas, lentils and chickpeas, to wheat, rice, barley and maize based extrudates on their physical and nutrition properties. Legume fortification reduced the water absorption index (WAI) of barley

based extrudates, WAI was unaffected for wheat- and maize based extrudates. In contrast, the type of legume influenced the water solubility index (WSI) of maize- and wheat based extrudates. The results showed significant variations in the final viscosity of the extrudates according to the levels of legume addition and cereal type. Particularly in the barley based extrudates, the final viscosity showed increased (Barley control 183.23 and Barley+10% Chickpea 265.60) with additional legume levels. However, the area under the curve (AUC) values for reducing sugars was reduced in the legume-added samples compared to the control samples, the strongest decrease ($p > 0.05$) being observed in the wheat + 5% chickpea sample. The observed difference in viscosities revealed possibilities for modifying the physical properties. Although, adding different amount of green pea have showed higher reduction in *AUC* of extrudates, the decreasing trend in the *AUC* of rest of the samples suggests the possibility of producing low glycaemic extrudates through legume fortification.

5.1 Introduction:

Processed foods are currently a popular choice for consumers in terms of convenience and accessibility. The abundance and availability of different varieties as well as their ease of use, preparation and storage, make processed foods particularly popular in urban societies (Brennan *et al.*, 2013). Socio-economic changes, such as increasing industrialisation and fast-changing life styles, are responsible for changing the dietary habits of consumers (Singh *et al.*, 2007). Among these processed products, ready-to-eat (RTE) products are gaining consumers' attention due to their accessibility and convenience; they are also affordable for the consumers. Cereal grains are important constituents of the RTE product market and, in many countries; these cereal-rich diets are the primary source of nutrition. Cereals themselves are rich in carbohydrates and

constitute the main source of carbohydrates in our diet, with around 50% of cereal production in the world used for human consumption (Brennan *et al.*, 2012a). Products, such as wheat, maize and barley, also contain considerable amounts of protein and dietary fibre and are the main source of energy (56%) for humans in some parts of the world (Brennan *et al.*, 2012b, Oliveira *et al.*, 2015).

Due to low availability, or for ethical reasons, some of the world's population are unable to access meat and milk for nutrients and the nutrition of these populations could be ensured through consumption of legume-fortified cereal based products (Boye *et al.*, 2010a). Researchers have identified nutritional improvements in traditional cereal based products, such as biscuits and pasta, to which legumes have been added (Boye *et al.*, 2010b, De la Hera *et al.*, 2012, Osen *et al.*, 2015). Legumes, such as lentils, peas and beans, are a rich source of protein and can replace cereal components in cereal based snack foods (De Almeida Costa *et al.*, 2006, Shevkani and Singh, 2015, Tharanathan and Mahadevamma, 2003, Tosh and Yada, 2010, Yağcı and Evcı, 2015). In recent years, there has been particular attention given to the use of dietary fibre in the reduction of glucose metabolism in Type 2 diabetes patients by reducing starch breakdown and glucose absorption rates from food; the steady breakdown of carbohydrates reduces the presence of excess glucose in human blood (Brennan, 2005, Brennan *et al.*, 2011). This has led to the investigation of the potential nutritional effects of dietary fibre in conventional food products (Cleary and Brennan, 2006, Brennan *et al.*, 2012a, Foschia *et al.*, 2015a). Intake of whole grains has previously been related to reductions in health problems, such as cardiovascular diseases, diabetes and cancer, as well as its role in regulating digestion and obesity (Oliveira *et al.*, 2015).

Currently, the consumption of snack foods is increasing exponentially. Most of the popular snacks available in the market (Muffins, biscuits) are considered as high calorie, high fat and easily digested carbohydrates with high glycaemic indexes. Excessive consumption of such foods induces/results in calorie intakes that may lead to health problems, such as high blood cholesterol, Type 2 diabetes, obesity and cardiovascular diseases (Brennan and Samyue, 2004, Brennan and Tudorica, 2008, Gao *et al.*, 2016). Maintaining health through nutrition is, therefore, becoming an extensively discussed topic. Growing consumer awareness of healthy food habits has increased the pressure on food researchers to develop novel RTE products that are nutritionally rich, easy to consume and affordable. This need may be addressed by the application of extrusion cooking to the development of products using whole grains (Singh *et al.*, 2007). Extrusion is a multi-step and multi-functional process that alters the chemical conformation of food ingredients through thermal and mechanical energy. Extrusion processing has the potential to develop innovative food products with unique structures through the use of underused whole grain based food products. In recent years, these have been used in the development of functional foods, such as breakfast cereals, baby foods and ready-to-eat snacks.

Due to their unique features, such as low operational costs and ease of application (Oliveira *et al.*, 2015, Rathod and Annapure, 2016), extrusion cooking is an economically affordable method and may even increase the nutritional digestibility and bioavailability of foods (Brennan *et al.*, 2012a, Brennan *et al.*, 2013). The extrusion process also has the ability to transform raw ingredients into puffed extrudates, which can be used in grain type analogue production. The production of rice analogues have been studied by Mishra *et al.*, (2012). Furthermore, it has been suggested that such analogues can be

used in RTE production. The flashing off of moisture and the pressure balance at the die interface with atmospheric pressure, were the core steps in extrusion processes during the development of RTE products. The rapid expansion and inflation of products post extruder die generates a puffed structure with the help of expanded gas cells in the product (Parada *et al.*, 2011). The process involves high temperatures, influencing the cooking of the protein and starch with a combined effect from moisture and pressure. Extrusion can also change chemical reactions and the structure of foods. The extrusion process cooks starch within the extruder, while reduced moisture levels may prevent the complete gelatinisation of the starch (Brennan, 2005, Brennan *et al.*, 2011). The extrusion process increases the availability of readily digestible starch. It has been suggested that adding legumes and dietary fibre to food products may manipulate the glycaemic index of foods. The use of dietary fibre in food production changes the food structure and reduces starch degradation (Brennan, 2005, Brennan and Samyue, 2004, Brennan *et al.*, 2011) and has an impact of non-starch polysaccharides, such as guar gum and wheat bran, in the preparation of extruded breakfast cereals (Brennan *et al.*, 2011).

In this chapter, cereal based (wheat, rice, maize and barley based) extrudates were developed in combination with legumes (yellow peas, green peas, chickpeas and lentils). The study aimed to determine the possibility of producing highly functional foods using legume in cereal based snacks and to investigate the physico-chemical properties of the samples and starch hydrolysis of them.

5.2 Material and methods:

5.2.1 Materials:

Refer to 3.1

5.2.2 Moisture content:

Refer to 3.5.1.

5.2.3 Extrusion:

Refer to 3.1.1 and 3.2

5.2.4 Total starch and *in vitro* starch digestion:

Total starch analysis was carried out in triplicate according to the official AACC method (AACC, 1994) as in Section 3.5.5. In vitro starch digestion of the samples was analysed by methods reported by Gao *et al.*, (2016). (Refer to 3.5.5 and 3.5.6).

5.2.5 RVA:

A 5 g collet sample was weighed and added to a canister containing 25 mL of distilled water and the thermal-visco profiles of resulting pastes were measured by Rapid Visco Analyser (Perten Instruments, Hägersten, Sweden). (Refer to 3.5.13).

5.2.6 WAI and WSI:

The water solubility index (WSI) and water absorption index (WAI) measurements were conducted on ground samples as referred in 3.5.9.

5.2.7 Statistics:

Refer to 3.5.14.

5.3 Results and discussion:

5.3.1 Moisture:

As illustrated in Table 5.1, significant differences ($p > 0.05$) were observed in moisture levels of maize based extrudates after the addition of legumes. The highest value of moisture was observed in the sample containing the 5% green pea addition ($8.78 \text{ g}\cdot\text{kg}^{-1}$). The moisture content of samples containing wheat or rice showed significant decreases in moisture levels after the addition of yellow peas. However, the addition of 5% green pea reduced the moisture content ($7.57 \text{ g}\cdot\text{kg}^{-1}$) of the rice based extrudates. While the lowest value in wheat based samples was observed in samples containing 10% of yellow peas ($5.57 \text{ g}\cdot\text{kg}^{-1}$). Our results were consistent with previously reported work (Tiwari *et al.*, 2011, Yadav *et al.*, 2014). The reduction in moisture levels of the samples was likely to be associated with the extrusion parameters, along with barrel temperature, as well as low feed moisture during processing. Also, high temperature may cause more starch gelatinization degree and lower the bulk density of the products which may responsible for retention of the lowest moisture (Sacchetti *et al.*, 2004).

Previous reports indicated that higher extrusion temperature led to higher moisture losses from the sample, and increases in feed the moistures during processing helped in decreasing moisture losses during extrusion (Brennan *et al.*, 2016, Rashid *et al.*, 2015). Moisture retention during extrusion can negatively affect consumer acceptance of snack foods by altering the products' physical properties, such as hardness and bulk

density (Struck *et al.*, 2014). Kasprzak *et al.*, (2013) reported that extrudates containing low moisture developed air cells with high diameters and thinner cell walls; the rupture of which under heat gave crunchiness to the products.

Table 5.1 Moisture content of extruded snack products

Added Legumes	Replace (%)	Moisture (g.kg ⁻¹)			
		Maize	Wheat	Rice	Barley
Control	0	8.67 ± 0.07 ^{ab}	7.43 ± 0.19 ^{abcd}	8.59 ± 0.19 ^a	6.67 ± 0.01 ^g
Lentil	5	8.09 ± 0.04 ^{fg}	7.32 ± 0.04 ^{bcde}	8.12 ± 0.10 ^{abcd}	7.96 ± 0.03 ^a
	10	8.24 ± 0.01 ^{def}	7.23 ± 0.09 ^{cde}	8.08 ± 0.11 ^{abcd}	7.89 ± 0.12 ^{ab}
	15	8.10 ± 0.10 ^{fg}	7.31 ± 0.07 ^{cde}	8.20 ± 0.19 ^{abc}	7.72 ± 0.02 ^{bcd}
Green pea	5	8.78 ± 0.06 ^a	7.21 ± 0.01 ^{cde}	7.57 ± 0.14 ^d	7.85 ± 0.01 ^{abc}
	10	8.18 ± 0.00 ^{ef}	7.60 ± 0.05 ^{abc}	8.50 ± 0.02 ^a	7.76 ± 0.01 ^{bcd}
	15	8.25 ± 0.03 ^{def}	7.53 ± 0.06 ^{abcd}	8.65 ± 0.10 ^a	7.71 ± 0.06 ^{cd}
Yellow pea	5	8.49 ± 0.03 ^{abcd}	6.93 ± 0.15 ^{de}	7.79 ± 0.16 ^{bcd}	7.06 ± 0.02 ^f
	10	8.50 ± 0.15 ^{abc}	5.57 ± 0.04 ^e	7.63 ± 0.23 ^{cd}	7.85 ± 0.01 ^{abc}
	15	8.29 ± 0.02 ^{cdef}	6.98 ± 0.18 ^{cde}	7.62 ± 0.17 ^{cd}	7.82 ± 0.08 ^{abc}
Chickpea	5	7.88 ± 0.09 ^g	7.72 ± 0.17 ^{ab}	8.18 ± 0.16 ^{abc}	7.38 ± 0.01 ^e
	10	7.51 ± 0.05 ^h	7.55 ± 0.14 ^{abcd}	8.30 ± 0.02 ^{ab}	7.61 ± 0.02 ^d
	15	7.61 ± 0.01 ^h	7.92 ± 0.12 ^a		

* Values are expressed as mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other (p > 0.05).

5.3.2 Effect on water solubility and water absorption indices:

Table 5.2 shows the WAI values and Table 5.3 illustrates the WSI values of the extrudates. Addition of legumes in maize and wheat based samples did not affect the WAI significantly ($p > 0.05$) of samples containing maize and wheat. Samples containing barley and rice showed significant variations ($p > 0.05$) in WAI after the inclusion of legumes. The rice containing samples with a 15% lentil addition had a WAI of 4.40 ml/kg⁻¹. The WSI of samples containing rice did not show significant changes after legume addition. However, there was a significant ($p > 0.05$) variation in the maize-, barley- and wheat based extrudates compared to the control products. Inclusion of 10% and 15% yellow peas significantly ($p > 0.05$) increased the WSI of samples containing wheat compared to the control sample.

Table 5.2 Water absorption index of extruded samples

Added Legumes	Replace (%)	Water absorption index (ml.kg ⁻¹)			
		Maize	Wheat	Rice	Barley
Control	0	3.90 ± 0.17 ^{ab}	4.39 ± 0.06 ^a	3.84 ± 0.09 ^c	6.03 ± 0.23 ^a
Lentil	5	4.15 ± 0.13 ^{ab}	4.39 ± 0.04 ^a	4.26 ± 0.16 ^{ab}	5.20 ± 0.20 ^b
	10	4.13 ± 0.01 ^{ab}	4.40 ± 0.08 ^a	4.20 ± 0.05 ^{ab}	5.18 ± 0.17 ^b
	15	4.14 ± 0.25 ^{ab}	4.39 ± 0.06 ^a	4.40 ± 0.08 ^a	5.16 ± 0.15 ^b
Green pea	5	3.92 ± 0.19 ^{ab}	4.40 ± 0.12 ^a	4.05 ± 0.11 ^{bc}	5.30 ± 0.06 ^b
	10	3.73 ± 0.22 ^b	4.24 ± 0.08 ^a	3.99 ± 0.14 ^{bc}	5.08 ± 0.10 ^b
	15	4.00 ± 0.04 ^{ab}	4.36 ± 0.01 ^a	4.05 ± 0.13 ^c	5.08 ± 0.48 ^b
Yellow pea	5	3.76 ± 0.21 ^b	4.42 ± 0.07 ^a	3.97 ± 0.05 ^{bc}	5.24 ± 0.10 ^b
	10	3.77 ± 0.11 ^b	4.37 ± 0.16 ^a	3.98 ± 0.18 ^{bc}	5.36 ± 0.21 ^b
	15	4.05 ± 0.15 ^{ab}	4.21 ± 0.13 ^a	4.18 ± 0.05 ^{abc}	5.04 ± 0.13 ^b
Chickpea	5	3.95 ± 0.01 ^{ab}	4.47 ± 0.06 ^a	4.10 ± 0.14 ^{abc}	5.40 ± 0.14 ^b
	10	4.35 ± 0.24 ^a	4.35 ± 0.15 ^a	4.26 ± 0.12 ^{ab}	5.05 ± 0.16 ^b
	15	4.25 ± 0.10 ^a	4.17 ± 0.14 ^a		

* Values are expressed as mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other (p > 0.05)

The WSI of barley based extrudates was significantly ($p > 0.05$) affected after adding legumes compared to the control samples. Sharma and Gujral, (2012) reported that changes in the WAI and WSI of extrudates depended on extrusion temperature, cultivar and feed moisture. A possible reason for current results could be that the feed moisture rate during extrusion might restrict starch gelatinisation during the extrusion process and this mechanism led to the low WAI of samples (Sharma *et al.*, 2011, Sharma and Gujral, 2012). The moisture (Table 5.1) content in rice based samples was higher than barley based samples, this might increase gelatinisation and further lead to higher WAI results.

5.3.3 Viscosities:

The pasting properties of cereal based extrudate samples are shown in Tables 5.4, 5.5 and 5.6. No significant variations were observed in highest peak velocity (PV) (Table 5.4) and breakdown viscosity (BD) (Table 5.5) of samples containing maize, barley or rice after legume addition. However, substitution by legumes altered the final viscosity (FV) (Table 5.6) of the extrudates. For instance, the FV of samples containing maize with a 5% green pea addition showed a significant decrease compared to the maize control sample; whereas, samples containing maize with a 15% green pea addition showed higher FV compared to the control maize sample. Substitution by legumes significantly ($p > 0.05$) increased the FV of barley based samples. The highest increase in FV was observed in samples containing barley with 10% yellow peas.

Table 5.3. Water solubility index of extruded samples based on different cereal

Added Legumes	Replace (%)	Water solubility index (ml.kg ⁻¹)			
		Maize	Wheat	Rice	Barley
Control	0	25.79 ± 3.04 ^{cd}	22.76 ± 0.43 ^{bc}	24.67 ± 2.82 ^a	21.38 ± 1.02 ^a
Lentil	5	34.30 ± 2.02 ^a	18.82 ± 0.28 ^d	28.21 ± 1.40 ^a	15.74 ± 0.49 ^b
	10	30.78 ± 1.93 ^{abc}	19.44 ± 0.13 ^{cd}	24.53 ± 1.97 ^a	17.53 ± 0.95 ^{ab}
	15	32.64 ± 2.46 ^{ab}	20.65 ± 1.60 ^{cd}	24.57 ± 1.64 ^a	16.86 ± 1.75 ^b
Green pea	5	30.39 ± 1.21 ^{abc}	22.06 ± 0.25 ^c	27.32 ± 1.72 ^a	19.57 ± 0.52 ^{ab}
	10	31.05 ± 2.92 ^{abc}	19.01 ± 0.29 ^{cd}	27.52 ± 1.50 ^a	16.26 ± 1.24 ^b
	15	22.71 ± 2.82 ^d	20.58 ± 1.21 ^{cd}	26.20 ± 1.13 ^a	16.71 ± 2.18 ^b
Yellow pea	5	29.77 ± 2.30 ^{abc}	25.04 ± 1.40 ^b	24.76 ± 0.39 ^a	19.21 ± 1.69 ^{ab}
	10	28.26 ± 3.43 ^{abcd}	29.53 ± 0.32 ^a	28.04 ± 0.62 ^a	17.59 ± 1.70 ^{ab}
	15	29.11 ± 2.02 ^{abcd}	28.60 ± 0.33 ^a	26.92 ± 1.77 ^a	18.62 ± 0.82 ^{ab}
Chickpea	5	32.24 ± 1.85 ^{ab}	20.65 ± 1.91 ^{cd}	27.09 ± 1.23 ^a	18.93 ± 1.76 ^{ab}
	10	26.46 ± 0.84 ^{bcd}	20.44 ± 1.09 ^{cd}	27.50 ± 0.78 ^a	17.55 ± 2.57 ^{ab}
	15	30.11 ± 0.90 ^{abc}	20.34 ± 0.14 ^{cd}		

* Values are expressed as mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other (p > 0.05)

A significant ($p > 0.05$) decrease was observed in the FV of rice based extrudates after adding legumes. The variations were observed in the FV of wheat based samples. Wheat samples with 0% green pea additions showed an increase in FV, whereas a reduction in FV was observed in wheat samples with yellow pea additions. The variation in the FV of wheat-based extrudates was explained by Balasubramanian *et al.*, (2012), who reported that legume-fortified cereal-based extrudate products developed a complex formation of starches that led to low viscosities. However, higher FV values were observed in wheat-based samples than in control samples, which corroborated with the present study. Our findings showed that an increase in legume addition to barley-based extrudates increased the FV of the final product. Sharma and Gujral, (2012) reported that the pasting properties of barley can also vary according to cultivar. Possibly in our case the area of cultivation of the raw material varied the pasting properties of samples. Furthermore, a high content of non-starch polysaccharides, such as β -glucan, may affect the pasting properties of barley-based extrudates. Sharma *et al.*, (2011) also observed a positive correlation between the final viscosity and total β -glucan content of barley samples. In this study, maize- and rice-based extrudates showed slight variations in viscosity after the addition of a legume. This was likely associated with starch gelatinisation, disruptions to shear and temperature (Parada *et al.*, 2011). The addition of legumes (fibre) to cereal-based products was reported to reduce the viscosity of the extrudates (Balasubramanian *et al.*, 2012, Brennan and Samyue, 2004, Brennan *et al.*, 2008, Brennan *et al.*, 2004, Brennan *et al.*, 2012a). However, this effect on viscosity did not follow for rice- and maize-based extrudates. The insufficient amounts of resistant

starch and fibre may be responsible for the variations in the BD, PV and FV of the samples (Brennan and Samyue, 2004, Parada *et al.*, 2011).

Table. 5.4. Peak viscosity values from the pasting profile of the extruded samples

Added Legumes	Replace (%)	Peak viscosity (mPa.s ¹)			
		Maize	Wheat	Rice	Barley
Control	0	92.63 ± 2.94 ^{abc}	194.17 ± 4.40 ^{abcd}	137.33 ± 4.68 ^a	204.87 ± 1.65 ^{ab}
Lentil	5	94.40 ± 4.45 ^{abc}	197.86 ± 2.20 ^{abcd}	151.56 ± 4.25 ^a	234.70 ± 1.01 ^{ab}
	10	99.43 ± 3.10 ^{abc}	198.76 ± 2.31 ^{abc}	131.83 ± 1.09 ^a	196.66 ± 1.33 ^b
	15	84.97 ± 4.45 ^{abc}	200.20 ± 6.66 ^{abc}	131.77 ± 1.13 ^a	231.97 ± 2.51 ^{ab}
Green pea	5	79.47 ± 2.15 ^{bc}	207.91 ± 3.00 ^{ab}	138.63 ± 1.08 ^a	234.30 ± 1.70 ^{ab}
	10	90.37 ± 8.09 ^{abc}	227.47 ± 1.20 ^a	136.47 ± 7.51 ^a	238.57 ± 2.92 ^{ab}
	15	104.30 ± 3.50 ^a	194.64 ± 9.92 ^{abcd}	145.43 ± 3.96 ^a	206.90 ± 9.50 ^{ab}
Yellow pea	5	88.33 ± 1.85 ^{abc}	166.29 ± 1.2 ^{cde}	141.36 ± 7.96 ^a	242.60 ± 2.51 ^a
	10	81.43 ± 4.71 ^{bc}	149.20 ± 1.8 ^e	146.20 ± 3.30 ^a	215.13 ± 2.05 ^{ab}
	15	100.00 ± 7.56 ^{ab}	158.27 ± 1.50 ^{de}	141.10 ± 4.00 ^a	217.10 ± 1.07 ^{ab}
Chickpea	5	78.90 ± 3.60 ^c	178.03 ± 1.72 ^{bcde}	133.17 ± 6.16 ^a	198.13 ± 7.47 ^b
	10	97.77 ± 5.71 ^{abc}	174.27 ± 7.82 ^{bcde}	138.50 ± 9.26 ^a	233.10 ± 7.45 ^{ab}
	15	90.13 ± 4.87 ^{abc}	171.50 ± 5.81 ^{bcde}		

* Values are expressed as mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other (p > 0.05).

Table. 5.5. Breakdown viscosity values from the pasting profile of the extruded samples

Added Legumes	Replace (%)	Breakdown viscosity (mPa.s ¹)			
		Maize	Wheat	Rice	Barley
Control	0	73.77 ± 2.89 ab	147.07 ± 5.67 abc	112.97 ± 3.69 a	154.03 ± 3.77 ab
Lentil	5	77.36 ± 4.11 ab	152.47 ± 2.13 abc	131.13 ± 3.95 a	178.73 ± 9.28 ab
	10	81.77 ± 2.70 ab	155.00 ± 2.11 abc	112.57 ± 1.04 a	139.73 ± 1.20 b
	15	70.17 ± 3.55 ab	157.27 ± 5.95 ab	112.37 ± 1.17 a	171.53 ± 2.10 ab
Green pea	5	64.97 ± 2.58 b	166.80 ± 3.30 a	115.60 ± 1.12 a	173.53 ± 1.99 ab
	10	75.07 ± 7.53 ab	166.53 ± 1.01 a	115.40 ± 8.25 a	185.40 ± 2.91 a
	15	85.53 ± 3.30 a	150.83 ± 7.51 abc	125.26 ± 4.29 a	154.07 ± 8.92 ab
Yellow pea	5	74.00 ± 1.85 ab	130.27 ± 4.93 abc	115.57 ± 8.91 a	180.50 ± 2.00 a
	10	67.83 ± 4.31 ab	120.10 ± 1.86 c	122.50 ± 2.60 a	158.03 ± 2.25 ab
	15	82.30 ± 6.07 ab	125.23 ± 1.39 bc	119.80 ± 4.30 a	165.17 ± 9.50 ab
Chickpea	5	65.70 ± 3.70 ab	139.40 ± 1.67 abc	115.80 ± 6.40 a	149.13 ± 8.94 ab
	10	81.57 ± 6.28 ab	124.73 ± 1.34 bc	122.36 ± 8.21 a	178.00 ± 6.48 ab
	15	75.20 ± 4.03 ab	132.20 ± 5.91 abc		

* Values are expressed as mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other (p > 0.05).

Table. 5.6. Final viscosity values from the pasting profile of the extruded samples

Added Legumes	Replace (%)	Final viscosity (mPa.s ¹)			
		Maize	Wheat	Rice	Barley
Control	0	161.83 ± 1.42 bcd	227.30 ± 4.91 cd	66.40 ± 2.51 a	183.23 ± 1.50 e
Lentil	5	165.60.0 ± 1.50 bc	211.13 ± 8.75 cdef	57.03 ± 3.51 de	224.00 ± 1.75 cd
	10	167.97 ± 4.66 b	215.80 ± 8.94 cde	53.46 ± 5.86 ef	20.80 ± 3.06 cde
	15	139.20 ± 7.62 e	240.90 ± 2.10 bc	52.87 ± 5.03 fg	227.43 ± 6.25 cd
Green pea	5	146.77 ± 2.51 de	235.15 ± 2.85 cd	62.60 ± 1.68 bc	231.00 ± 2.49 bcd
	10	152.57 ± 2.19 bcde	298.23 ± 2.55 a	60.13 ± 1.80 cd	231.63 ± 1.15 bcd
	15	200.80 ± 3.77 a	274.00 ± 1.25 ab	56.70 ± 3.61 de	236.20 ± 8.50 abc
Yellow pea	5	148.27 ± 2.59 de	189.23 ± 3.34 ef	68.90 ± 1.90 a	228.50 ± 3.48 bcd
	10	148.70 ± 2.08 cde	177.27 ± 7.25 f	65.20 ± 1.25 ab	273.10 ± 1.10 a
	15	187.93 ± 1.54 a	214.87 ± 3.78 cde	57.03 ± 3.51 de	243.97 ± 4.56 abc
Chickpea	5	121.35 ± 1.50 f	203.30 ± 3.41 def	50.53 ± 6.11 fg	196.63 ± 2.24 de
	10	139.63 ± 1.83 e	224.70 ± 7.67 cde	50.98 ± 8.33 g	265.60 ± 5.80 ab
	15	137.67 ± 8.11 ef	243.00 ± 3.25 bc		

* Values are expressed as mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other (p > 0.05)

5.3.4 Predicted glycaemic response:

The starch digestibility and predictive glycaemic responses of cereal-based extrudates were determined by an *in vitro* enzymatic starch digestion that explain area under the curve (AUC). The values for reducing sugar during *in vitro* digestion varied according to the cereal and the addition of different legume materials to it (Table 5.7). A clear reduction in AUC, indicating the lower release of reducing sugars in the maize- and wheat-based extrudates, was observed as a result of the various (5%, 10% and 15%) green pea, yellow pea or chick pea additions (Table 5.7). In the case of barley-based extrudates, legumes, such as yellow peas (5 - 15%) and chick peas (5%), AUC values were higher than the control. Table 5.7 illustrates the effect of the addition of various legumes to cereal based extrudates on standardised AUC values. A clear reduction in AUC reducing sugars of maize and wheat based extrudates was observed as a result of various (5%, 10% and 15%) amounts of green pea, yellow pea or chickpea additions. The strongest decrease was observed for samples containing wheat and maize with 5% green pea additions. However, the addition of lentil grains did not significantly alter the AUC values of wheat and maize based extrudates, although a lower AUC was obtained for rice based extrudates with 10% and 15% lentil fortification. It has been previously reported that legumes contained considerable amounts of resistant and slowly-digestible starch, resulting in a lower digestibility for legume starches compared to cereal starches (Hoover and Zhou, 2003, Sandhu and Lim, 2008). The presence of slowly-digestible and resistant starch in legumes may increase the total dietary fibre content of foods containing legumes (Tosh and Yada, 2010). Pastor-Cavada *et al.*, (2011) observed significant increases in fibre content after legume flour was added to whole

maize and brown ricebased extrudates. This was possibly due to the extrusion process tending to increase the starch content and depolymerise the starch in the product at high extrusion temperature (Pastor-Cavada *et al.*, 2011).

Table. 5.7. Standardized area under the curve values of extruded samples following *in vitro* digestion

Added Legumes	Replace (%)	Standardized area under the curve (mg.min ⁻¹)			
		Maize	Wheat	Rice	Barley
Control	0	402.34 ± 5.28 ^b	423.35 ± 19.16 ^a	409.75 ± 2.72 ^{ab}	348.47 ± 5.50 ^c
Lentil	5	407.24 ± 6.38 ^{abc}	431.36 ± 16.85 ^a	412.84 ± 8.40 ^{abc}	333.96 ± 2.49 ^e
	10	406.13 ± 13.79 ^{abc}	422.38 ± 5.45 ^{ab}	386.39 ± 15.88 ^{cd}	333.59 ± 9.01 ^e
	15	396.15 ± 12.60 ^{bcd}	430.98 ± 4.06 ^a	375.97 ± 9.57 ^d	332.44 ± 4.87 ^e
Green pea	5	348.17 ± 9.04 ^e	313.75 ± 8.05 ^g	435.95 ± 4.28 ^{bcd}	348.17 ± 14.57 ^a
	10	553.71 ± 48.58 ^{de}	316.51 ± 3.40 ^{ab}	431.73 ± 1.72 ^{bcd}	345.861 ± 3.26 ^a
	15	375.79 ± 3.67 ^{cde}	329.11 ± 5.11 ^{ab}	383.25 ± 7.01 ^{bc}	340.54 ± 4.33 ^{ab}
Yellow pea	5	393.49 ± 3.38 ^{de}	342.30 ± 4.44 ^{abc}	463.61 ± 5.25 ^{bcd}	360.71 ± 6.74 ^a
	10	400.15 ± 7.41 ^{de}	357.31 ± 9.24 ^{bcd}	443.53 ± 5.51 ^{bcd}	365.15 ± 7.38 ^a
	15	397.07 ± 8.58 ^{cde}	355.48 ± 6.47 ^{bc}	451.43 ± 5.47 ^e	376.73 ± 10.68 ^{ab}
Chickpea	5	373.07 ± 3.87 ^d	326.31 ± 5.75 ^{abc}	423.79 ± 6.50 ^c	362.41 ± 2.31 ^{ab}
	10	352.94 ± 1.08 ^d	317.77 ± 4.18 ^{bc}	397.21 ± 2.42 ^{bc}	326.56 ± 6.43 ^a
	15	421.43 ± 8.58 ^d	343.92 ± 6.41 ^{bc}		

* Values are expressed as mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other (p > 0.05).

The extrusion process also contributed to rapid starch digestion and increases in the glycaemic response (Brennan *et al.*, 2013). Alonso *et al.*, (2000) observed an increase in in vitro starch digestion due to a reduction in α -amylase inhibitors, such as tannins, polyphenols and phytic acid. However, Brennan *et al.*, (2004) reported that manipulating product composition can be useful for increasing levels of slowly-digestible starch in products. It was suggested that dietary fibres may coat the starch granules of a cereal-based product, inhibiting enzyme penetration during starch digestion. Furthermore, the viscous nature of fibre may affect enzyme functionality in starch degradation and, hence, reduce the AUC (Brennan, 2005, Brennan *et al.*, 2011, Brennan *et al.*, 2012a). From the results it can be observed that wheat+5% green pea was the best combination as it have showed lowest AUC values.

5.3.5 Conclusions:

Addition of various amounts of legumes to cereal-based extrudates led to significant differences in the physical and nutritional properties of the products such as final viscosity and glycaemic response. Production of combined legume-and cereal extrudates with low moisture (less than 9%) and low glucose responses (wheat+5% green pea) could be achieved. It was observed that the combined effect of legume addition, non-starch polysaccharides (β -glucan), fibre content, extrusion temperature and shear stress affected the pasting and physico-chemical properties of the product. A reduction in AUC sugar response could also be achieved by the addition of legumes. It was concluded that legume fortification in cereal products for making extruded snacks had the potential to increase the nutritional quality while, simultaneously decreasing its glycaemic nature

Chapter 6

The effect of adding chickpea (*Cicer arietinum* L.) or green pea (*Pisum sativum* L.) on physico-chemical properties and predictive glycaemic response of wheat and barley based pasta

Overview

In this chapter, a partial substitution of wheat and barley with (10% and 20%) green pea and chickpea was performed in order to develop nutritionally enriched pasta. The effects of legume additions on the physical, textural and cooking properties of pasta are reported in this chapter. The effect of legume addition on starch digestibility and the predictive glycaemic response of pasta is also discussed. The material and methods followed for the experiment are given in brief paragraphs and, for details, the section numbers from Chapter 3 are mentioned. Statistical analysis was carried out separately for wheat and barley based sample are presented in the same table.

Abstract:

In this study, substitutions of barley and wheat flour with green peas and chickpeas (at 10% and 20% levels) were undertaken to develop nutritionally enriched pasta. The physical, textural and cooking properties of pasta were also assessed. The results showed that addition of legumes significantly ($p \leq 0.05$) decreased the swelling index and increased the water absorption index as well as the firmness of the pasta. Adding legumes affected the reducing sugar released from the pasta during *in vitro* digestion.

The area under the curve (AUC) of reducing sugars during digestion of wheat and barley based pasta significantly decreased after addition of 20% green peas. This indicates that the predicted glycaemic responses of wheat and barley pasta after adding legumes were lower than the control samples.

6.1 Introduction:

Food grains are the fundamental sources of nutrition to human. Some studies (Balasubramanian *et al.*, 2012; Foschia *et al.*, 2014) have shown that products developed from food grains have a low glycaemic index due to the presence of dietary fibre and resistant starch. Reducing the glycaemic index of a food product has a positive effect on overcoming health problems, such as diabetes and cardiovascular diseases (Brennan *et al.*, 2004). The glycaemic index is a scale extensively used by nutritionists and industries to measure the postprandial glucose response of a particular food (Foschia *et al.*, 2014). Research has shown that the dietary fibre and resistant starch content of cereals has a major impact on manipulating the nutritional content of food. Diets with low GI have been suggested to reduce the risk of diseases, so the development of foods to decrease the glycaemic response is important (Brennan, 2005; Brennan *et al.*, 2008).

Cereals, such as wheat (*Triticum aestivum L.*) and barley (*Hordeum vulgare L.*), are functional ingredients that are used to develop nutritional products that have low glycaemic responses. The importance of these cereals in human nutrition has been documented previously (Rosenfelder *et al.*, 2013). Wheat contains considerable amounts of amino acids, such as lysine, leucine and arginine, as well as cellulose, pectin, non-starch polysaccharides and antioxidants (Rosenfelder *et al.*, 2013). Wheat is the

most widely grown and consumed cereal in the world and it has been suggested that wheat flour is well suited for the production of ready-to-cook or ready-to-eat products (Fleischman *et al.*, 2016). Barley is an ancient cereal grain production of which occupies around 9% of the total land area under cereal production. Most of the barley grown is used in alcohol production (Sharma *et al.*, 2011). The use of barley for human consumption needs to be encouraged, as evidence suggests that barley is rich in bioactive compounds, such as β -glucan, phenolic compounds, B-complex vitamins and minerals (Brennan *et al.*, 2016; Cleary and Brennan, 2006). The mixed-linked (1-3), (1-4) β -D-glucans, commonly known as β -glucans, are a major component of endosperm cell walls in barley (Lazaridou *et al.*, 2014). According to some recent reports by Brennan *et al.*, (2008) Brennan *et al.*, (2013); Brennan *et al.*, (2012b), and Tosh and Yada, (2010), β -glucan is a useful component in reducing the glycaemic response of food. It forms a gelatinous matrix during human digestion so changing the digesta viscosity, which may delay carbohydrase activity. This in turn, delays gastric emptying, increases nutrient absorption in the small intestine, and lowers glucose release (Brennan, 2005; Brennan and Cleary, 2005; Jenkins *et al.*, 2002; Tosh and Yada, 2010). Furthermore, ingestion of β -glucan decreases blood plasma cholesterol levels and, hence, reduces the major risk of coronary heart disease (Lazaridou *et al.*, 2014). Increasing use of such cereals in food production can be an alternative to currently available snacks that are low in nutrients and high in saturated fat and also fulfil the requirements of nutritionally rich ready-to-eat food products, such as pasta, to the fast growing world population.

Pasta is one of the most popular convenience extruded products available now. There is increasing literature Foschia *et al.*, (2013); Foschia *et al.*, (2014); Foschia *et al.*, (2015a,

2015b) showing the importance of pasta in terms of human nutrition. The ease of pasta preparation and its nutritional properties, such as its low glycaemic index, makes it popular around the world. Pasta ranks second in worldwide consumption after bread (Tazart *et al.*, 2016; Torres *et al.*, 2007). The addition of legume flours such as chickpeas (*Cicer arietinum L.*) and green peas (*Pisum sativum L.*) are convenient and affordable sources to develop protein-rich pasta. According to previous reports by Mohammed *et al.* (2012) and Petitot *et al.* (2010), these legumes are rich in lysine and a good source of slowly digestible starch and resistant starch, and have the potential to change the functional and nutritional properties of food products (Chung *et al.*, 2010; Petitot *et al.*, 2009). Sun *et al.*, (2006) reported that pea and chick pea starches have lower digestibility than cereal starch. This might be due to their higher contents of slowly digestible starch and resistant starch (Chung *et al.*, 2010).

Studies by Padalino *et al.* (2014) and Osen *et al.* (2015) have clearly indicated that pasta enriched with legumes, i.e. green pea flour, reduced starch digestibility. However, Brennan *et al.* (2016) reported that the addition of 10% pea flour to barley based pasta did not change the glycaemic response significantly and concluded that the optimum level of pea flour required to reduce the glycaemic response of barley based pasta needed to be found. Therefore, this study was undertaken to measure the effect of legume addition, such as chickpea and green pea flour, to wheat and barley based pasta on reducing the glycaemic response.

6.2 Materials and methods:

6.2.1 Materials:

For explanation of methods used please refer to 3.3

6.2.2 Pasta making:

For explanation of methods used please refer to 3.3.1

6.2.3 Preparation of samples for analysis:

For explanation of methods used please refer to 3.3.1

6.2.4 Moisture content:

For explanation of methods used please refer to 3.5.1

6.2.5 Fat:

For explanation of methods used please refer to 3.5.2

6.2.6 Protein:

For explanation of methods used please refer to 3.5.1A

6.2.7 Total starch:

For explanation of methods used please refer to 3.5.5

6.2.8 Texture analysis:

For explanation of methods used please refer 3.5.7

6.2.9 Water absorption index (WAI), swelling index (SI) and cooking loss:

For explanation of methods used please refer to 3.5.9 and 3.5.12

6.2.10 *In vitro* starch digestion:

For explanation of methods used please refer to 3.5.6

6.2.11 Statistical analysis:

For explanation of methods used please refer to 3.5.14

6.3 Results and discussion:

6.3.1 Proximate composition:

Table 6.1 illustrates the proximate composition of pasta made with different grains and legumes. A range of variation in the amount of water can be observed. In the wheat based pasta the addition of 10% green pea significantly reduced the moisture content; interestingly, no significant ($p > 0.05$) variation was recorded in other treatments. In contrast, for the barley based pasta (except for barley + 10 % chickpea) the moisture content in all samples significantly ($p > 0.05$) increased after adding legumes when compared to the control. The highest moisture content was found in barley + 20% chickpea, at 30.86%. In our study the presence of additional fibre due to legume inclusion or the uneven distribution of water during pasta mixing might hold or lost water during processing and varied the moisture content of pasta.

It has been previously reported that the presence of dietary fibre was responsible for water holding (Brennan *et al.*, 2016). The presence of dietary fibre might form a thicker wall around the sample to avoid water loss (Kasprzak *et al.*, 2013). The low amount of

fibre content in barley resulted in low moisture content in the barley control samples compared to the wheat control samples. In contrast the findings of Tarzart *et al.* (2016) showed a clear reduction in moisture content in wheat-semolina pasta after adding faba beans.

Table 6.1 Proximate composition of wheat and barley based pasta (g/100g)

Sample Name	Moisture	Fat	Protein
Wheat Control	30.93 ± 1.57 ^a	1.34 ± 0.24 ^a	15.00 ± 0.06 ^e
Wheat+10% Chickpea	32.17 ± 0.89 ^a	1.55 ± 0.36 ^a	16.16 ± 0.06 ^c
Wheat+20% Chickpea	32.76 ± 1.52 ^a	1.58 ± 0.39 ^a	17.24 ± 0.03 ^a
Wheat+10% Green pea	26.72 ± 1.86 ^b	1.43 ± 0.10 ^a	15.74 ± 0.03 ^d
Wheat+20% Green pea	31.33 ± 0.94 ^a	1.81 ± 0.21 ^a	16.85 ± 0.01 ^b
Barley Control	20.99 ± 1.55 ^d	0.81 ± 0.01 ^b	10.42 ± 0.02 ^d
Barley+10% Chickpea	26.20 ± 1.54 ^{cd}	1.27 ± 0.08 ^a	10.60 ± 0.01 ^c
Barley + 20% Chickpea	30.86 ± 2.91 ^a	1.45 ± 0.08 ^a	11.45 ± 0.12 ^a
Barley+10% Green pea	27.55 ± 0.57 ^{ab}	1.28 ± 0.08 ^a	10.93 ± 0.04 ^b
Barley+20% Green pea	28.24 ± 0.95 ^{bc}	1.46 ± 0.07 ^a	9.45 ± 0.04 ^e

* Values are expressed as mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other ($p > 0.05$).

The protein contents of wheat and barley pasta were 15.0% and 10.42%, respectively.

The data showed that adding chickpeas and green peas increased the protein content in both barley and wheat based pasta. A similar trend was observed by Zhao *et al.*, (2005) and Wood (2009). Generally, legumes have higher amino acids levels than cereals, which helps in increasing the protein content of cereal based products (Wood, 2009).

The fat contents of pasta (Table 6.1) samples were less than 2%. The wheat based pasta showed no significant ($p > 0.05$) variations in fat content after adding legumes when

compared to the control. In the barley based pasta samples the addition of legumes significantly increased the fat contents compared to the control samples. Results reported by Bouasala *et al.* (2016) echoed the current findings. Bouasala *et al.* (2016) reported on the development of gluten free pasta with combinations of rice and legumes and observed that adding legumes significantly ($p > 0.05$) reduced the fat content in pasta. Generally, extruded products contained lower fat than instant fried snacks, and pasta products are characterised by low fat contents (Kruger *et al.*, 1996). However, these lower values of fat content in processed pasta may be the result of amylose-lipid complex formation during pasta making, which reduced lipid extractability so the products may contain lower amounts of fat (Wojtowicz and Moscicki. 2014). This variation in fat content of pasta can also be explained by the fact that legume additions, such as chickpeas, lentils and peas, potentially contain low levels of fat, which may change the fat content of the product (Bouasla *et al.*, 2016)..

6.3.2 Cooking loss:

Cooking loss is a measure used to calculate the amount of amylose leaching into water during cooking. In the present study, there was no significant ($p > 0.05$) change in cooking loss (Table 6.2) of barley pasta recorded after adding chickpeas and green peas. However, adding 20% of chickpeas significantly reduced the cooking loss of wheat pasta. It is considered that legume starches are higher in non starch polysaccharides than cereals which may affect the physico-chemical properties of sample (Wood, 2009). In our study the reduction in cooking loss with the addition of chickpeas were in agreement with the results of Wood (2009), where addition of chickpeas reduced cooking losses in spaghetti. It was explained that the protein-polysaccharide matrix

bound amylose during cooking and this might reduce cooking losses (Wood, 2009). The disruption of the protein-starch matrix and uneven distribution of water during cooking of pasta might also have affected solid losses (Foschia *et al.*, 2014). Sharma *et al.*, (2002) also documented the reductions in cooking losses of durum wheat pasta with reducing amylose content.

Table 6.2 Cooking Loss, swelling index and water absorption index of wheat and barley based pasta (g/100g)

Sample	Cooking Loss	SI	WAI
Wheat Control	6.14 ± 1.02 ^a	2.34 ± 0.15 ^{ab}	49.52 ± 0.69 ^b
Wheat+10% Chickpea	4.30 ± 1.16 ^{ab}	1.45 ± 0.08 ^{cd}	77.90 ± 1.49 ^c
Wheat+20% Chickpea	4.02 ± 0.06 ^b	1.48 ± 0.21 ^{cd}	74.93 ± 2.55 ^a
Wheat+10% Green pea	5.06 ± 0.61 ^{ab}	2.72 ± 0.30 ^a	42.98 ± 0.66 ^c
Wheat+20% Green pea	4.93 ± 0.49 ^{ab}	1.48 ± 0.03 ^{cd}	75.64 ± 0.88 ^a
Barley Control	4.87 ± 0.36 ^a	1.21 ± 0.24 ^d	39.77 ± 1.10 ^c
Barley+10% Chickpea	4.39 ± 0.55 ^a	1.64 ± 0.11 ^{bc}	66.88 ± 1.32 ^b
Barley+20% Chickpea	4.37 ± 0.38 ^a	1.89 ± 0.18 ^{cd}	66.79 ± 1.71 ^b
Barley+10% Green pea	4.78 ± 0.26 ^a	1.57 ± 0.09 ^{cd}	57.52 ± 0.80 ^a
Barley+20% Green pea	4.55 ± 0.46 ^a	1.38 ± 0.10 ^d	56.77 ± 4.39 ^a

* Values are expressed as mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other (p > 0.05).

6.3.3 Swelling index (SI):

In the wheat based pasta samples the addition of 10%, 20% chickpea and 20% green pea reduced the swelling index of wheat pasta (Table 6.2). There appeared to be a 20% threshold for green pea fortification while, in barley based pasta, 10% chickpea additions caused significant (p>0.05) increases in the swelling index. This was in agreement with reports by Foschia *et al.* (2014, 2015a). The high fibre and protein

contents of legumes tended to absorb and retain water within the starch protein network during cooking (Lu *et al.*, 2016). In the wheat based pasta; fibre appeared to be competing with starch for water during pasta formation, thus, reducing starch swelling. The nature of starches and degree of starch gelatinisation could also be responsible for the decreases swelling index (Brennan and Samyue, 2004). The encapsulation of starch granules with protein molecules might reduce the swelling of starch during cooking and lead to reductions in the swelling index of wheat pasta (Petitot *et al.*, 2009).

6.3.4 Water absorption index (WAI):

The values for the WAI (Table 6.2) of pastas agreed with results reported in some recent studies (Foschia *et al.*, 2014, 2015a; Lu *et al.*, 2016). The WAI of pasta increased in all legume-added samples except wheat + 10 % green pea. The structural change in the protein network and the increased fibre content of pasta may be responsible for the increase in WAI. Adding 10% green pea was probably below the threshold level to affect the WAI of wheat based pasta. According to the findings of Foschia *et al.* (2014), the addition of 20%, or more, pea flour could be the optimum level that can affect the WAI of pasta. The change in WAI values can result from the impact of adding legume fibre fractions, as pasta enriched with fibre tended to absorb and retain water within a very well developed starch-protein-polysaccharide network in comparison with the control samples (Lu *et al.*, 2016). Moreover, the screw speed and presence of heat during pasta manufacture might be responsible for forming a porous structure and capillaries within the endosperm and the presence of more damaged starch granules could be the reason for the increasing WAI of pasta (Sharma *et al.*, (2011); Zhu *et al.*, (2010). According to

Kruger *et al.* (1996), dough is transferred in the extrusion screw, pressure builds up and the dough temperature rises locally. The structural transformations were, therefore, a consequence of both the mechanical (shearing stress) and thermal forces involved during pasta making. Addition of 10% chickpea to wheat and barley based pasta have showed noticeable effect in WAI of samples.

Table 6.3 Firmness of wheat and barley based pasta (g)

Sample	Firmness
Wheat Control	19.62 ± 4.86 ^{cd}
Wheat+10% Chickpea	16.1 ± 1.89 ^d
Wheat+20% Chickpea	51.53 ± 2.08 ^a
Wheat+10% Green pea	24.38 ± 1.35 ^c
Wheat+20% Green pea	37.25 ± 3.43 ^b
Barley Control	211.0 ± 11.47 ^c
Barley+10% Chickpea	246.1 ± 19.03 ^{bc}
Barley+20% Chickpea	300.6 ± 5.28 ^a
Barley+10% Green pea	238.4 ± 20.43 ^c
Barley+20% Green pea	280.4 ± 8.73 ^{ab}

* Values are expressed as mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other (p > 0.05).

6.3.5 Firmness:

The firmness (Table 6.3) of the control wheat pasta was significantly lower compared to the control barley pasta. It seems possible that the cooking process weakened the gluten network of wheat pasta and made the pasta soft (Foschia *et al.*, 2015a), as legumes contain low or no gluten, their additions resulted in the increasing firmness. Similarly, in the barley control pasta the reduction in the amounts of gluten or different gluten composition may be responsible for the high firmness of the pasta. Sissons *et al.*, (2005) showed that gluten content increased spaghetti firmness with no consistent

trends relating to glutenin/gliadin composition. The legume additions also significantly increased the firmness of both wheat and barley based pasta compared to the control. The observations suggests that the fortified spaghetti firmness might have increased as the protein and amylose contents increased due to legume additions. As legumes contain low, or no gluten, the legume flour additions diluted the wheat gluten network and increased the firmness of wheat pasta. Studies by Sudha and Leelavathi (2012) have demonstrated that adding fibre such as wheat bran or oat bran reduced the firmness of pasta. Moreover, it has been reported that the high moisture content of non-starch polysaccharides, such as β -glucan, can result in a softening of pasta (Cleary and Brennan, 2006). In this study it was observed that addition of legumes increased the firmness of wheat and barley pasta. It was suggested that the addition of legumes strengthened the inner structure of pasta and resulted in higher firmness. It is generally considered that texture is the main criteria to assess the overall acceptability of pasta and assessing the firmness of pasta was one of the factor.

6.3.6 Starch digestibility:

Figs 6.1 and 6.2 illustrate the reducing sugar release from wheat and barley based pasta, respectively.

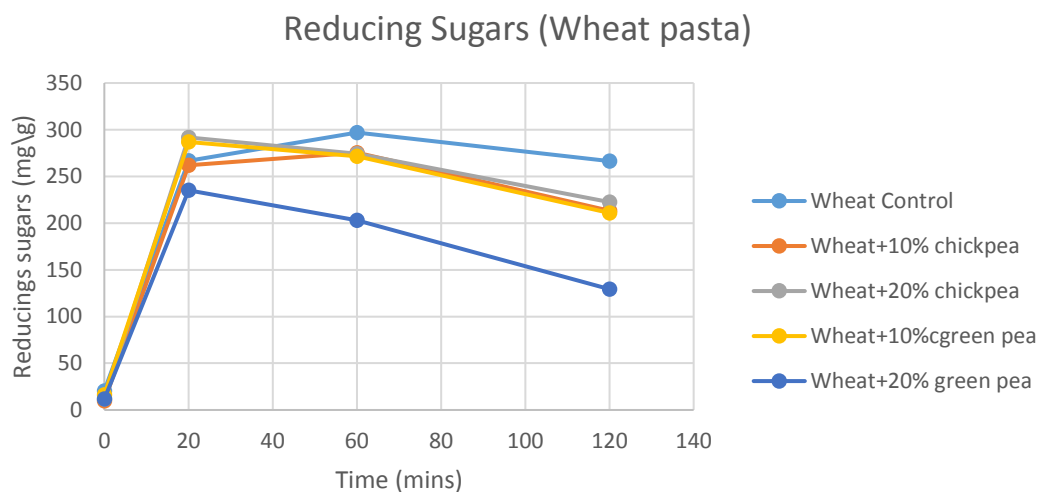


Fig 6.1 The amount of reducing sugars released (mg/g of starch) during *in vitro* digestion; control and 10% and 20% wheat replaced by green pea flour and chickpea flour

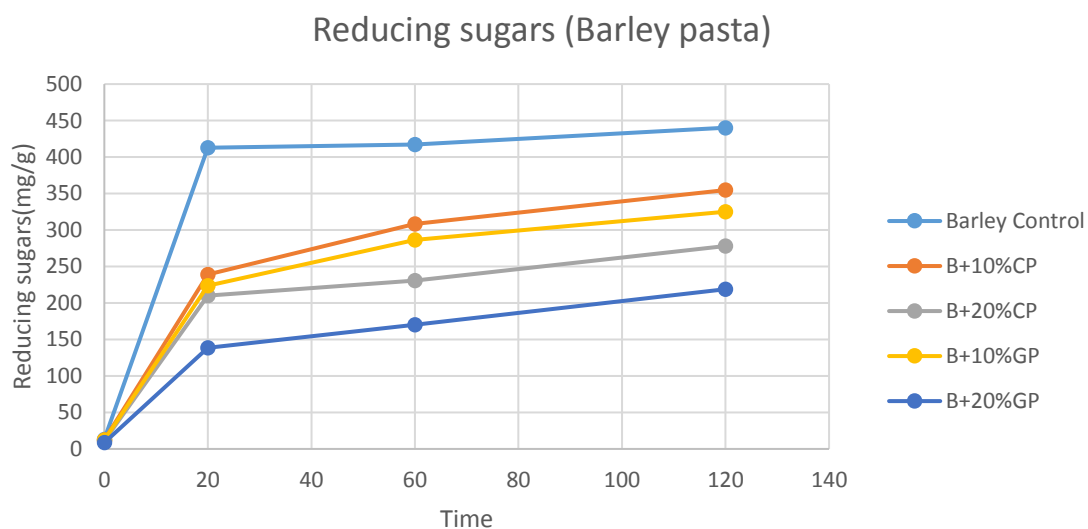


Fig 6.2 Amount of reducing sugars released (mg/g of starch) during *in vitro* digestion; control and 10 % and 20% barley replaced by green peas and chickpeas

Additions of chick pea and green pea lowered the reducing sugar release from the wheat and barley based pasta (Figs. 6.1 and 6.2). The rates of starch digestibility were significantly higher in control samples than the legume fortified pasta samples. It can be seen that legume addition altered the rate of starch digestion. The lowest value was recorded in barley + 20% green pea and wheat + 20% green pea. This suggested that green pea starches can manipulate reducing sugar release during starch digestion due to their high fibre and resistant starch contents at 20% incorporation level. Recently published results by Brennan *et al.* (2012a); Foschia *et al.* (2014); Gallegos-Infante *et al.* (2010); Lu *et al.* (2016); Rathod and Annapure, (2016) supported our findings that high fibre and higher amounts of resistant starch can negatively affect sugar release during starch digestion. Moreover, the uneven distribution of water among the starches during cooking delayed, or prevented the encapsulation of the protein-starch matrix and reduced starch digestion. The anti-nutritional factors and slowly digestible starch (SDS) and residual starch (RS) present in legumes may also have affected enzyme activity during starch digestion of extruded lentil splits (Rathod and Annapure, 2016). Previously, researchers have suggested that due to the compact nature of pasta, starch granules become “trapped” within the protein matrix, slowing the enzyme activity for starch digestion (Foschia *et al.*, 2014). Dietary fibre may also encapsulate starch granules during pasta making and this restricted the swelling and triggered the retrogradation of starch granules during cooking. This mechanism might also have affected starch digestibility (Brennan and Tudorica, 2008). However, Brennan and Samyue, (2004) observed slight increases in sugar release in dietary fibre (inulin) enriched biscuits. So, the type of dietary fibre, processing method and level of dietary fibre addition can have a great impact on sugar release from the samples.

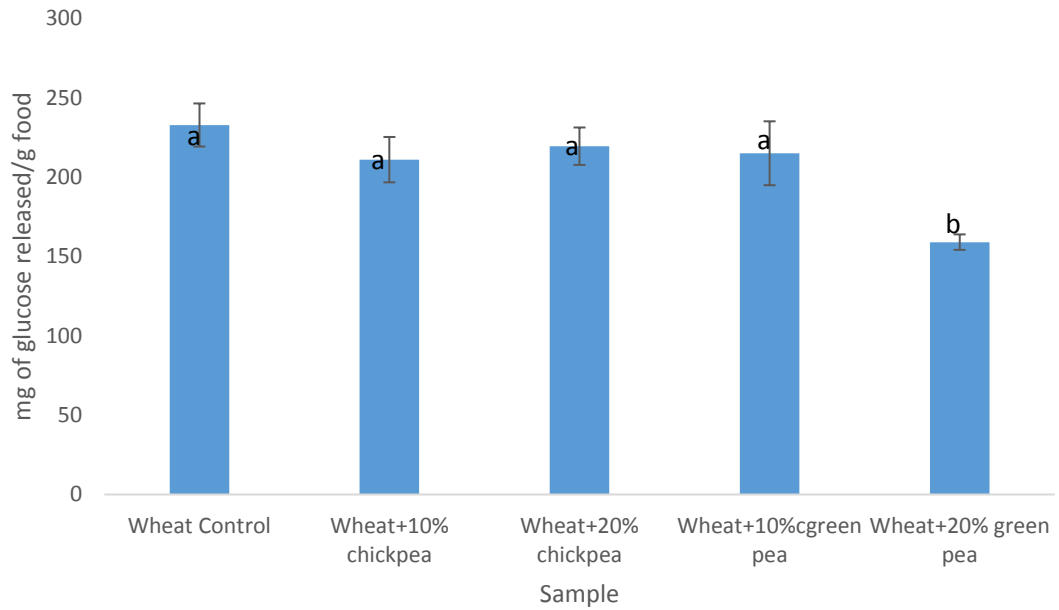


Fig 6.3. Values for area under the curve (AUC) comparing the wheat control and legume-added pasta made with different levels of chickpea and green pea flour

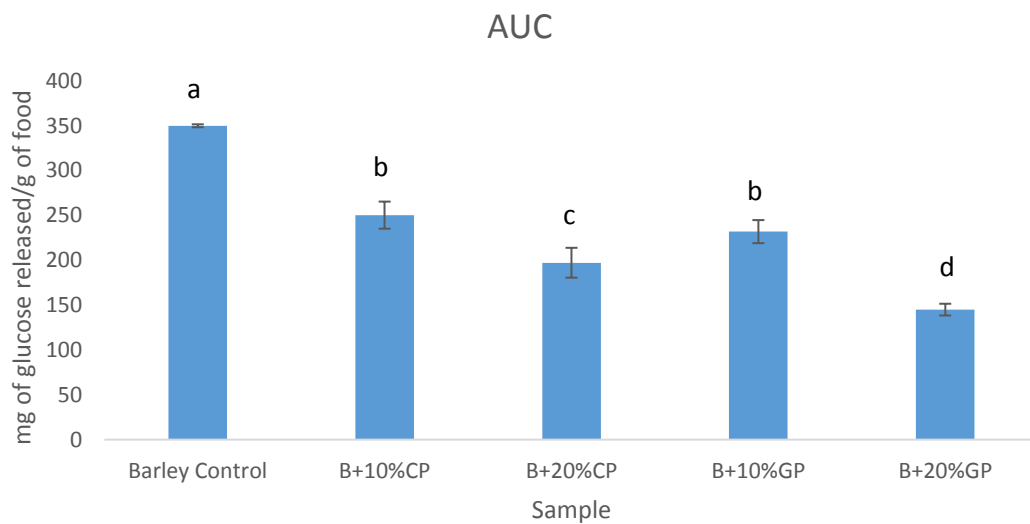


Fig 6.4. Values for area under the curve (AUC) comparing the barley control and legume-added pasta made with different levels of chickpea and green pea flour

Similarly, the predicted glycaemic response (Figs 6.3 and 6.4) was also reduced after adding legumes.

The AUC values of barley based pasta samples decreased with the addition of 10% and 20% legumes. The lowest AUC values were observed in barley + 20% green pea. The change in AUC values can be linked with the dietary fibre and resistant starch contents of legumes (Rathod and Annapure, 2016). Moreover, the composition (dietary fibre and β -glucan) of barley (Barley + 20% green pea) could be the reason for the low glycaemic response (Brennan *et al.*, 2016). β -glucan might increase the viscosity of the samples during digestion and reduce enzyme activity (Cleary and Brennan, 2006). Moreover, barley fibre and β -glucan may control starch gelatinisation due to better water binding capacity (Brennan *et al.*, 2016; Cleary and Brennan, 2006).

6.4 Conclusions:

The results of this study indicated that green pea and chickpea additions have significant effects on pasta cooking and nutritional quality. The physico-chemical, textural and nutritional properties suggested that the application of chick peas and green peas in this study had the potential to increase resistant starch content of both wheat and barley based pasta. Legume incorporation significantly increased the WAI, firmness and decreased the SI. It was suggested that the change in physical properties and nutritional properties differed according to the chemical composition and nature of the raw material. The addition of 20% green peas had significantly ($p > 0.05$) reduced the glycaemic response of both the wheat and barley pasta. It can be seen that a 20% green pea addition can play a vital role in manipulating the glycaemic response of wheat- and barley-based pasta. The study showed that starch and protein had subsequent

structural changes during the manufacture of pasta, and the encapsulation of starch granules with protein was the main factor affecting the glucose response. Legume fortification might also increase the fat content of pasta samples. Various levels of what and green pea needs to be investigated further in terms of textural and nutritional properties.

Chapter 7

Conclusion

Main features from introduction chapter 1

- A background on human diet and current trends of human diet due to changing lifestyles was described in this chapter.
- The importance of plant based nutrition in human diet were discussed in terms product development.
- Current trends in the consumption of RTE, as well as nutritional profile of current available RTE products and future scope of nutritional RTE product development, were discussed.

Main features from literature review chapter 2:

- This chapter presented an overall results and investigations done by previous researches related to our study. For instance, the disadvantages of current food trends related consumption of high calories foods, significance of legumes and cereals in daily consumption.
- Potential use of New Zealand grown seed in utilisation of food development was discussed.
- Tables containing the composition of legumes and cereals which are utilised in this study was shown.

- The mechanisms behind protein and starch hydrolysis were discussed in detail which gives a thorough understanding of human physiology of digesting starch and protein.
- Importance of the amino acids content and the glucose response in protein and starch digestibility respectively. Also, the impact of presence of fibres and fatty acids during digestion.
- Tables illustrating the relation of starch and protein digestion to human body were shown illustrating various *in vitro* protein and starch digestibility methods to show use of various enzymes in *in vitro* digestion over the past few years.
- The significance of extrusion cooking and combining cereal and legume blends in development of extruded snacks was discussed which illustrated the effect of extrusion on protein digestion as well as glycaemic response of extruded snack.

Importance of snacks in human diet, current snacking pattern among population, negative outcomes of consumption of high calorie snacks and importance of inclusion of cereals and legumes in development of healthy snack product was discussed. Detailed explanation of extrusion technology in terms of development of novel food product was given including history and various type of extrusion. Furthermore, the various methods of starch and protein digestibility were reviewed. In particular, this study focused on various aspects of development of extruded products and popularity of processed foods due to cultural changes and low cost society are causing adverse impacts on human health in urban population. During writing the literature review it

was found that U.S. and U.K. are the most prevalent countries in regular snack consumption. Most of consumed (chips, chocolates, popcorns) snacks are rich in calories which resulting health problems such as obesity. It was also found that regular consumption of calorie rich foods increases diseased population (CVD, blood pressure, cancer). It was also reported that snack consumption is expected to grow in near future. Most of the current available products were found to be low in vital nutrients and have low satiety, such factors created a need to develop a novel product which rich in vital nutrients such as proteins, dietary fibres as well as they can be available in affordable prices.

Developing healthy snack alternatives can be useful in overcome health problems. Taking this in to account in this study the effect of hot and cold extrusion were undertaken to produce snack products by using locally grown legumes and cereals which are easily available in affordable prices around Canterbury region. Meat and dairy provides considerable amount of protein to human body. However, increase intake of animal oriented protein sources can increase the risk of heart diseases and these sources contains significant amount of cholesterol. Whereas legumes such as peas and lentils are inexpensive, they contain easily digestible proteins and are also low in fats and cholesterol. This chapter also highlighted that wheat protein diet helps in decrease uric acid, LDL cholesterol and try glycerol in human body.

Cereals have significant amount of dietary fibres that helps in limiting glycaemic response of the product and legumes contains complex carbohydrates which may help in reduction in blood glucose levels. Legume consumption ranks second in after cereals and contains higher amount amino acids than the cereals. Thus combining

cereals and legumes to develop a novel product can be an advancing option for meeting the nutritional requirement of the society. Moreover, in terms of dietary pattern cereals and legumes are resides in significant position and are considered as the best combination in delivering nutritional food, particularly in developing countries where plant based sources are available abundantly in very low cost (Ndidi *et al.*, 2014).

This part of the study also reviewed the importance and diet requirement of protein and carbohydrates to the human body. The content of amino acids and digestibility of proteins are the responsible factors that decides quality of protein. For example, protein present in red meat is less digestible than whey protein. Proteases denatures protein during digestion and they converted into amino acids in stomach, thus the digestibility is act as the tool for protein turnover. Although, around 25 - 30% protein consumption (energy basis) is vital from nutritional viewpoint, the level of amino acids can be differ according to protein sources.

Starch is the most common carbohydrate required by human body and it is a major factor responsible for glycaemic response. This study also focused on the quantity and digestibility of legume and cereal starch. It has been suggested that adding various legumes to traditional cereal based snack. Based on the finding of previous studies, it has been identified that adding considerable amount of legumes to traditional cereals based snack reduces glycaemic response of snack as well as it increase protein content of the product. Moreover, the processing factors such as mixing facilitates the protein granules to surround the starch granules to form protein starch matrix and the matrix weakens ability of starch digestive enzymes and leads to reduction in starch

hydrolysis. This delay in starch digestion responsible for slow sugar release that lowers glucose levels in blood stream.

Although, cereals (wheat and barley) are rich source of dietary fibres, adding legume to traditional cereals snack can have a significant impact on dietary fibre levels and structure of snacks. It has been identified that protein and dietary fibres in legumes combine surrounds starch granules and reduce the starch hydrolysis and glucose release during digestion. Combining cereals and legume in extrusion blends could break down the polymers bond to form starch-protein matrix. This matrix develop protein may create a wall around the starch granules to reduce activity of carbohydratase, the low moisture content avoids complete gelatinization of product and formation of resistant starch due to retrogradation during extrusion process. However, extrusion works differently with protein molecules. Presence of tannin, phytates in legumes can greatly affects protein digestibility and it can be concluded from the review that extrusion temperature plays vital role in restriction of antinutritional factors in protein digestion. Extrusion parameters breaks down protein complexes and further lead to denaturation of protein where it increase digestibility and bioavailability of protein after digestion.

Presently, health and nutrition is the most emphasized subject by food researchers and most of the nutritional literature is focused on dietary requirements and potential sources for development of novel food products. The main aim of investigating the literature was to assess work of some of the well-known food researchers and to summarize their work to extend our knowledge before undertaking this study. The quoted references in the literature confirms that combining cereals and legumes

blends is promising accomplishable in formation of innovative food product. This review has also discussed different aspects of our study such as potential of extrusion technique in generation quality snacks, change in structure occurred in the product due to extrusion parameters and it helped us to enhance our understanding about aims our study.

Main findings from chapter 4

The effect of fortification of legumes and extrusion on protein digestibility of wheat based snacks

- The study indicated that the presence of legumes in the extruded formulations had no effect on product moisture and fat of the extruded pellets, which shows that it is possible to include legumes into cereal extrudates without affecting the nutritional fat content in the foods.
- The obtained results indicate that the use of different amounts of legumes to wheat based extrudates have significantly increased the protein content.
- The values are indicative of the effect that extrusion processing has impact on the protein digestibility of the samples with significant increases in protein digestibility being observed after extrusion processing.

The aim of this part of this study was to prepare wheat-based extrudates using four different legume flours: lentil, chickpea, green pea, and yellow pea flour. Whole grains of green pea flour and chickpea flour were used at 0%, 5%, 10% and 15% (on weight basis) with whole wheat (Zentofan) flour, organic barley flour were used in the production of extrudates. The standard moisture was retained the highest value observed was in wheat + 10% yellow pea (9.36%). The fat content in all samples was

less than 1% except in wheat + 10% green pea (1.03%). The lowest fat content was shown by wheat + 15% lentil composition. There was no significant differences were noted between the samples in the concentration of fat. Protein values (Table 3) testify that the inclusion of legume grains to the extruded samples enhances the protein content of all the products. Among all our testing composition samples and control samples, wheat + 15% yellow pea and wheat + 15% green pea samples showed statistically significant ($p > 0.05$) protein content.

The protein content of legumes was considerably higher than that of wheat. It ranged from 20.27% to 25.33% for legumes, whereas that for wheat it was 14.47%. The IVPD of the control samples was relatively low as compared to other combinations in both raw flour mixes and the extrudates. In samples with only the raw flour mixes, the maximum level of digestibility was obtained in wheat + 5% green pea. However, in the extruded samples, the wheat + 15% green pea product also showed the highest level of protein digestibility. No significant differences were noted in tested samples that had increased legume content, e.g. extruded wheat samples with 5% lentil addition also non - significantly differ in their protein digestibility level than those samples with 15% lentil addition. It is known that the extrusion process itself generally increases the digestibility of protein within the samples. It therefore can be suggested that in this set of experiments the extrusion processing has led to a denaturation of protein structures leading to increased ease of digestion by inactivation the enzyme inhibitors and exposing protein molecules to the enzyme attack, this supports the observation of Dahlin and Lorenz, (1993). The mechanical and chemical processing factors from extrusion technology play a major role in protein digestibility. There is also a possibility that some other factors like grain structure and cell wall components of the seed

contribute to the solubility and digestibility of protein in seed. Besides the protein reactions with non-protein components of seed during processing may influence the digestibility rates (Duodu *et al.*, 2003).

The effects of adding legumes to wheat-based snacks at different levels (0%, 5%, 10%, and 15%) during extrusion were investigated in terms of protein digestibility. It was observed that fortification of snacks with legumes caused a slight increase in the protein content by 1%–1.5% w/w, and the extrusion technique increased the protein digestibility by 37%–62% w/v. The product developed by extrusion was found to be low in fat and moisture content.

Main findings from chapter 5

Investigation of the combination of legumes and cereals in the development of extrudate snacks and its effect on physico-chemical properties and *in vitro* starch digestion

- A possible reason for the low *WAI* could be that the feed moisture rate during extrusion might restrict starch gelatinization during extrusion process and this mechanism led to low *WAI* of samples containing barley. The higher moisture content in samples containing rice might increase gelatinization and it further led to higher *WAI*.
- High levels of soluble dietary fibre may be responsible for the variation in *BD*, *PV* and *FV* of legume enhanced samples.
- The dietary fibre may coat the starch granules of a cereal-based product, inhibiting enzyme penetration during starch digestion. Besides that the viscous

nature of fibre may affect enzyme functionality in starch degradation and hence reduce *AUC*.

As detailed in Table 5.1 statistically significant differences ($p > 0.05$) were noted in moisture levels of maize - based extrudates after addition of legumes. The peak value was observed in the sample containing 5% green pea addition ($8.78 \text{ g}\cdot\text{kg}^{-1}$). Moisture content of samples containing wheat or rice indicated significant decrease in moisture levels on addition of yellow pea. Green pea (5% addition) reduced the moisture content ($7.57 \text{ g}\cdot\text{kg}^{-1}$) of rice-based extrudates, while the value in wheat-based samples was matching with the corresponding value in samples with 10% yellow pea ($5.57 \text{ g}\cdot\text{kg}^{-1}$). It is acceptable to consumers, since earlier reports indicated that higher extrusion temperature being directly proportional to moisture loss from the feed, and increase in feed moisture helps to decrease moisture loss during extrusion (Brennan *et al.*, 2016, Rashid *et al.*, 2015) and moisture retention during extrusion can have negative effect on consumer acceptance of snack foods by altering product's physical properties such as hardness and bulk density.

The effect on water solubility and water absorption indices (*WAI*) were presented in Table 5.2 and Table 5.3. Substitution of cereals with legumes were without significant ($p > 0.05$) effect on *WAI* of samples containing maize and wheat. Both the samples containing barley and rice showed significant variations ($p > 0.05$) in *WAI* on inclusion of legumes. The rice-containing samples with 15% lentil estimated, a value of $4.40 \text{ ml}\cdot\text{kg}^{-1}$, whereas the barley-containing samples with 15 % yellow pea the value was $5.04 \text{ ml}\cdot\text{kg}^{-1}$. *WSI* of samples containing rice showed insignificant change, after legume addition. However, there was a significant ($p > 0.05$) variation in maize-, barley and wheat-based extrudates as compared to the values of control products. Inclusion

of 10% and 15% yellow pea shows significant ($p > 0.05$) increase in *WSI* of samples containing wheat as compared to the values of control sample. Substitution by legumes negatively affected *WSI* of samples containing barley, with significant ($p > 0.05$) lower *WSI* values noted in legume-added samples. A possible reason for this observation could be that the feed moisture rate during extrusion might restrict starch gelatinization during extrusion process and this mechanism led to low *WAI* of samples containing barley (Sharma *et al.*, 2011, Sharma and Gujral, 2012). The higher moisture content in samples containing rice might increase gelatinization and it further led to higher *WAI*.

The pasting properties of cereal-based extrudate samples are shown in Table 5.4, 5.5 and 5.6. There were no significant variations noted in peak viscosity (PV) (Table 5.4) and breakdown viscosity (BV) (Table 5.5) of samples containing maize, barley or rice after legume addition. Substitution by legumes altered final (Table 5.6) of the extrudates. Final viscosity (FV) of samples containing maize with 5% green pea addition showed a significant decrease as compared to the corresponding values in maize control sample, whereas samples containing maize with 15% green pea addition resulted in higher FV as compared to the corresponding values of control maize sample. Substitution by legumes shows significant ($p > 0.05$) increase in FV of barley-based samples. The highest increase in FV is noted in samples containing barley with 10% yellow pea.

Significant ($p > 0.05$) decreases were obtained in the values of FV of rice-based extrudates on adding legumes. Variations are observed in FV of wheat-based samples. Wheat samples with 10% green pea addition showed an increase in FV, whereas a reduction in FV was noted in wheat samples with yellow pea addition. The variation

in FV of wheat-based extrudates can be explained on the causes noted in work of Balasubramanian *et al.*, (2012), who reported that legume - fortified cereal-based extrudate products developed a complex formation of starches that led to low *PV* and *BD*. However, higher FV values were observed in wheat-based samples than in control samples, which reinforce our present observation. Our findings also showed that an increase in legume addition to barley-based extrudates increased FV of the final product. Furthermore, a high content of non-starch polysaccharides, such as β -glucan, may affect the pasting properties of barley-based extrudates. Similarly, Sharma *et al.*, (2011) have shown a positive correlation between final viscosity and total β -glucan content of barley samples. The comparison of these studies with our observations on maize- and rice-based extrudates points out a slight variation in viscosity after the addition of a legume. This was likely to be associated with starch gelatinization, disruption to shear and temperature (Parada *et al.*, 2011). The addition of legume (fibre) to cereal-based products is reported to tend to reduce the viscosity of extrudates (Brennan and Samyue, 2004, Brennan *et al.*, 2004, Brennan *et al.*, 2012a, Brennan *et al.*, 2008, Balasubramanian *et al.*, 2012). However, this effect on viscosity is not observed to be followed for rice and maize based extrudates.

The starch digestibility and predictive glycaemic response results in the present work was studied using *in vitro* enzymatic starch digestion (mimicking the human digestive system). The values for reducing sugar during *in vitro* digestion varied according to cereal and the addition of different legume material to it (Table 5.7). A reduction in *AUC* indicated lower release of reducing sugars of maize- and wheat-based extrudates (5%, 10% and 15% amount of green pea, yellow pea or chick pea addition, while in the case of barley-based extrudates, legumes such as yellow pea (5 - 15%) and chick

pea (5%) show *AUC* values high over the control. Table 5.7 presented the effect of addition of various legumes to cereal-based extrudates on standardized *AUC* values. The strongest decrease was noted for samples containing wheat and maize with 5% green pea addition. Nevertheless the addition of lentil grains non-significantly alters the *AUC* values of wheat- and maize-based extrudates, although lower *AUC* is obtained for rice-based extrudates with 10% and 15% lentil fortification. It was well studied that legumes contain considerable amounts of resistant and slowly-digestible starch, resulting in lower digestibility of legume starches compared to cereal starches (Hoover and Zhou, 2003, Sandhu and Lim, 2008). The presence of slowly-digestible and resistant starch in legumes may increase the dietary fibre content of foods containing legumes (Tosh and Yada, 2010). Extrusion process tends to increase *in vitro* starch content and depolymerize the starch in the product at high extrusion temperatures. The process also contributes to rapid starch digestion and increases in the glycaemic response (Brennan *et al.*, 2013). The investigations by Alonso *et al.*, (2000) has shown that an increase in *in vitro* starch digestion due to a reduction in α -amylase inhibitors such as tannins, polyphenols and phytic acid, hence findings Brennan *et al.*, (2004) confirms that manipulating product composition can be useful for increasing levels of slowly-digestible starch in a product. It seems that dietary fibre may coat the starch granules of a cereal-based product, inhibiting enzyme penetration during starch digestion. Besides it is known that the viscous nature of fibre may affect enzyme functionality in starch degradation and hence reduce *AUC* (Brennan, 2005, Brennan *et al.*, 2011, Brennan *et al.*, 2012a). In conclusion addition of various quantities of legumes to cereal-based extrudates altered significantly the physical and nutritional properties of the products. Production of combined legume-and cereal

extrudates with low moisture (less than 9%) and low glucose response could be achieved. It is observed that combined effect of legume addition, non-starch polysaccharides, fibre content, extrusion temperature and shear stress could affect the pasting and physico-chemical properties of the product. Reduction in *AUC* sugar response could also be achieved by legume addition. It is concluded that legume fortification in cereal products for making extruded snacks has the potential to increase the nutritional quality, simultaneously decreasing its glycaemic nature.

Main findings from chapter 6

The effect of adding chickpea (*Cicer arietinum* L.) or green pea (*Pisum sativum* L.) on physicochemical properties and predictive glycaemic response of wheat and barley based pasta

- The addition of chickpea and green pea has increased the protein content in both barley and wheat based pasta.
- It was observed that disruption of protein-starch matrix, uneven disruption of water during cooking of pasta also had an impact on cooking properties of pasta. Also, increase in resistant starch due to legumes addition variation in cooking loss of wheat and barley pasta was observed.
- In our study was observed that addition of legumes had increased firmness of wheat and barley pasta. It is possible that legumes addition had strengthened inner structure of pasta and resulted in higher firmness.
- The amount of dietary fibres, nature of starches and degree of starch gelatinisation could found to be responsible for decrease in swelling index.

- In this study it was observed that the compact nature of pasta, allowed starch granules to trap within protein matrix with slow enzyme activity for starch digestion. Also, dietary fibre encapsulate starch granules during pasta making and restricts the swelling and triggered retro gradation of starch granules during cooking. These mechanisms together possibly reduced the starch digestibility.

Table 6.1 presents proximate composition of pasta made with different grains and legumes. From the data, a variation in amount of water can be observed. In wheat based pasta addition of 10% green pea significantly reduces moisture content; interestingly no significant variation is recorded in other treatments. On the other hand in barley based pasta (except barley + 10% chickpea) moisture content in all the samples is significantly increased after adding legumes as compared to the moisture content of control.

The protein content of wheat and barley pasta as observed was 15.0% and 10.42% respectively. It can be observed from the data that addition of chickpea and green pea increased the protein content in both barley and wheat based pasta. A similar trend was observed by Zhao, (2005) and Wood, (2009).

The fat contents of pasta samples are less than 2%. Wheat based pasta showed no significant variation in fat content after adding legumes as compared to corresponding values in control. In barley based pasta samples legumes addition had a significant increase fat content as compared to fat contents in control samples. Results of Bouasla (2016), Wojtowicz and Moscicki (2014) agree with current findings. These lower values of fat content in processed pasta may be the result of amylose-lipids

complex formation during the extrusion-cooking leading to the reduction of lipids extractability or this variation or legumes addition such as chickpea, lentils and peas that potentially contain slight amount of fat which may be changing the fat content of product as suggested by Bouasla *et al.*, (2016). The composition of raw material responsible for variation in fat and moisture. The major change in fat and moisture content can affect the consumer's acceptance and shelf life as well as bulk density and hardness characteristics of product (Brennan *et al.*, 2012b), and hence present results are in the bracket of the products that may get positive response of consumers.

In the present study there was no significant change in cooking loss (Table 6.2) of barley pasta recorded on adding chickpea and green pea. Addition of 20% chickpea showed a significant reduction in cooking loss of wheat pasta. Legume starches in general were higher in resistant starch content than cereal starches (Wood, 2009) and increase in solid loss with increasing legumes addition has been reported by Zhao and colleagues (2005). While some researchers (Foschia *et al.*, 2014) have shown legumes increase coupled with high amylose content of the products with subsequent increase in the cooking loss; There are also reports (Sissons *et al.* 2005) with no relation between amylose content and cooking loss of pasta in the study of role of gluten in cooking quality of spaghetti.

In our study reduction in cooking loss with addition chickpea are in agreement with the results of Wood, (2009), who on addition of chickpea has noted reduction in cooking loss of spaghetti. Woods findings suggests that amylose content is not a major component in influencing cooking loss. The results can also be explained on protein-polysaccharide matrix bound amylose during cooking which might reduce cooking loss (Wood, 2009). In the case of pasta, disruption of protein-starch matrix and uneven

disruption of water during cooking of pasta also has impact on cooking properties of pasta (Foschia *et al.*, 2014).

In wheat based pasta addition 10%, 20% of chickpea and 20% green pea reduced the swelling index of wheat pasta (Table 6.2). The 20% seems to be threshold of green pea fortification. While in barley based pasta 10% chickpea addition had significant increase swelling index. This is in agreement with results obtained with other preparations (Foschia *et al.* 2014, 2015a). The high fibre and protein content of legumes tend to absorb and retain water within starch protein network during cooking as per Lu *et al.* (2016). Possibly in wheat based pasta fibre and starch both seem to be responsible for water absorption during pasta formation, with loss in reduction in starch swelling. The nature of starches and degree of starch gelatinisation could be responsible for decrease in swelling index as suggested by other workers (Brennan and Samyue, 2004, Brennan *et al.*, 2012a). The encapsulation of starch granules with protein molecules might further truncate the swelling of starch during cooking which results in net reduction in swelling index of wheat pasta (Petitot *et al.*, 2009).

The water absorption index (WAI) (Table 6.2) of pasta results agree with results of some recent studies (Foschia *et al.*, 2015a, Foschia *et al.*, 2014, Lu *et al.*, 2016,). WAI of pasta shows increase in all legume added samples except Wheat + 10% green pea. The structural change in protein network and increase in fibre content of pasta seems to be responsible for the increase in WAI. Adding 10% green pea was probably below the threshold level to affect the WAI of wheat based pasta. Foschia *et al.* (2014) illustrated that 20% pea flour or more was the optimum level which can affect the

WAI of pasta. The change in WAI values can be the impact of adding legume fibre fractions, pasta enriched with fibre tend to absorb and retain water within a very well developed starch-protein-polysaccharide network in comparison with the control samples as per Lu *et al.* (2016). In addition the screw speed and presence of heat during pasta manufacture might be responsible for the formation porous structure and capillaries within endosperm and presence of more number of damaged starch granules could be the reason for increasing WAI of pasta (Sharma *et al.*, 2011, Zhu *et al.*, 2010).

The firmness (Table 6.3) of control wheat pasta was very low compared to the control barley pasta. It seems possible that to the cooking process might have weakened the gluten network of wheat pasta and it made the pasta soft (Foschia *et al.*, 2015a), as legumes contains no gluten this it might resulted in decreasing firmness. In barley control pasta high content of dietary fibre may have been responsible for the high firmness of pasta. Sissons *et al.*(2005) showed that as gluten content increased, spaghetti firmness also increased. The legume addition also significantly increased the firmness of both wheat and barley based pasta compared to control. This suggests that the fortified spaghetti firmness should have increased as protein content and amylose content increased due to legume addition. Adding fibre such as wheat bran or oat bran reduces the firmness of pasta (Sudha and Leelavathi, 2012).

Non starch polysaccharides such as β -glucan, high moisture content makes pasta soft (Cleary and Brennan, 2006). In our study it has been observed that addition of legumes had increased firmness of wheat and barley pasta. It can be suggested that legumes addition had strengthened inner structure of pasta and resulted in higher firmness.

Fig. 6.1 and Fig. 6.2 illustrates reducing sugar release of wheat and barley based pasta respectively. Addition of chick pea and green pea have lowered the reducing sugar release of the wheat and barley based pasta. The rates of starch digestibility were significantly higher in control samples than the legume fortified pasta samples. The lowest value was recorded in barley + 20% green pea and wheat + 20% green pea. This suggests that the green pea starches can manipulate the reducing sugar release during starch digestion may due to high fibre and resistant starch content. Recently published results in other products (Brennan *et al.*, 2012b, Foschia *et al.*, 2014, Gallegos-Infante *et al.*, 2010, Lu *et al.*, 2016, Rathod and Annapure, 2016) have similar findings.

The compact nature of pasta, allows starch granules to trap within protein matrix with slow enzyme activity for starch digestion. Dietary fibre encapsulate starch granules during pasta making and restricts the swelling and triggers retro gradation of starch granules during cooking. All these mechanisms together might affect the starch digestibility (Brennan and Tudorica, 2008).

Similarly, the predicted glycaemic response (Fig 6.3 and 6.4) was reduced after adding legumes. A decreasing trend on AUC values of pasta is seen after addition of 10% and 20% legumes. Lowest AUC values are in barley + 20% green pea. The change in AUC values can be linked with dietary fibre content and resistant starch content of legumes (Rathod and Annapure, 2016). The composition (dietary fibre and β -glucan) of barley could be the reason for low glycaemic response. In this case it can be suggested that β -glucan, dietary fibre and effect of extrusion have net effect in manipulation of barley pasta (Brennan *et al.*, 2016). β -glucan may be reason to increase the viscosity of sample during digestion and reduction in the enzyme activity (Cleary and Brennan,

2006). Barley fibre and β -glucan also control starch gelatinisation due to better water binding capacity (Brennan *et al.*, 2016, Cleary and Brennan, 2006). All these reasons explain the present values.

In conclusion the result of this study indicates that green pea and chickpea addition has a significant effect on pasta cooking and nutritional quality. The physicochemical, textural and nutritional properties suggest the application of chickpea and green pea in this study has the potential to increase resistant starch content of both wheat and barley based pasta. Legume incorporation significantly increased the WAI, firmness and decreased the SI. It can be suggested that the change in physical properties and nutritional properties differ according to chemical composition and nature of raw material. Addition of 20% green pea has significantly reduced the glycaemic response of both wheat and barley pasta. It can be concluded from present observation that 20% green pea can play vital role in manipulating the glycaemic response of wheat and barley based pasta. The study showed that starch and protein undergo subsequent structural change during manufacturing of pasta and encapsulation of starch granules with protein is the main factor affecting glucose response. However, legume fortification might increase fat content marginally of pasta samples.

Overall Conclusion

The findings of this investigation compliment those of earlier studies and have several practical implications. The contribution of this study has been to confirm that retaining nutritional elements of both cereals and legumes (since in both the cases raw flour was used) can be accomplished. Retaining these properties in marketable product for rational cost is possible. This can be provided to school children as

acceptable breakfast snacks. Wheat + 20% green pea was proven to be best combination in pasta making in terms of physiological and nutritional properties. Also, in terms of hot collets addition on 15 % legumes have altered the properties. A key strength of this study was the use of local grains asserts that such products can bring wide market from other regions. The rice + 15 chickpea and barley + 15% chickpea combinations could not be tested in our study. As it couldn't be developed by using present processing conditions. These combinations need to be studied with different processing conditions.

In further efforts these protein rich products in gluten free or other as per market demand form can be modified and tested.

Chapter 8

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