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THESIS

DEVELOPMENT OF EXTRUDED HIGH-PROTEIN, GLUTINOUS RICE-BASED SNACK

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A Thesis Submitted in Partial Fulfillment of
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The purpose of this research was to apply a linear-programming technique to design low-cost formulations containing at least 20 % protein, nutritionally adequate amount of lysine and sulphur amino acids to meet the FAO/WHO/UNU (1985). A factorial design was employed investigate the influence of feed protein content (20 and 30%), feed moisture (20%, 25%, and 30%) and barrel temperature (150 and 180 °C) on the physical, chemical and sensory qualities of extrudates. The acceptability of non-enrobe and enrobe-flavored extrudates were investigated. Result show that two mixtures formulated by linear-programming were 20 % protein formulation (71 % rice, 6 % gluten, 23 % soy) and 30 % protein formulation (54 % rice, 15 % gluten, 31 % soy). Protein content, feed moisture and barrel temperature had significant effect ($P \leq 0.01$) on the qualities of extrudate except the cystine, methionine, serine, and phenylalanine content. The feed protein content had a significant impact on all measured attributes. Increasing protein content resulted in reduced moisture content of the extrudate, more protein and NPN in the extrudate, greater bulk density and BSI, but lower expansion, and high intensity of hardness, noise, crispness but less sticky mouth coating. Increasing feed moisture content had a positive impact on final extrudate moisture, reduced the NPN, improved lysine recovery, increase bulk density and BSI, but reduced expansion, and high intensity of hardness, noise, crispness and brittle but low intensity of colour. The high barrel temperature (180°C) reduced extrudate moisture content, increased the NPN, reduced lysine content, bulk density and BSI but gave a better expansion, and reduced intensity of all sensory attributes except colour. The acceptability results indicated that changes in protein content and extruder variables affected liking scores of the final product. Increasing feed protein content significantly increased flavour, texture and overall liking. Increasing feed moisture content reduced appearance and texture liking score but increased colour and flavour liking score. The high temperature reduced appearance, colour and flavour liking of extrudate. The colour, flavour and texture of extruded snack were improved when coated with 12 % barbecue or 8% chesses seasoning resulting in high acceptability.

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Student's signature Thesis Advisor's signature

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Supat Chaiyakul

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LIST OF ABBREVIATIONS

AOAC	=	Association of Official Analytical Chemists
BSI	=	breaking strength index
BT	=	barrel temperature
°C	=	degree Celsius
EAAs	=	Essential amino acids
FAO	=	Food and Agriculture Organization
FAO/WHO/UNU	=	Food and Agriculture Organization /World Health Organization/The United Nations University
F	=	feed rate
g	=	gram
gN	=	gram Nitrogen
g/l	=	gram per liter
HCl	=	Hydrochloric acid
HTST	=	High temperature short time
% H_2O	=	percentage of water
LAL	=	lysinoalanine
LAT	=	lantionine
LP	=	linear programming
mg	=	milligram
min	=	minutes
NPN	=	non-protein nitrogen
$ NZ/kg	=	dollar New Zealand per kilogram
RPM	=	Round per minute
T	=	temperature
USFDA	=	United State Food and Drug Administration
yr	=	year

DEVELOPMENT OF EXTRUDED HIGH-PROTEIN, GLUTINOUS RICE-BASED SNACK

INTRODUCTION

Extrusion technology plays a central role in the modern cereal-based industry especially for the production of snack products from corn, wheat, rice and oats. Fewer rice-based extruded products are currently available compared to those from corn and wheat. Nevertheless, rice is the staple food for 17 countries in Asia and the Pacific, nine countries in North and South America and eight countries in Africa (FAO, 2004). Rice is non-allergenic and has a white colour and bland flavour making it useful for providing the starch matrix for expansion while allowing alternative flavours to be added. However, rice has relatively low protein content (6-8 %) and an amino acid profile that it is high in glutamic and aspartic acid, while lysine is the limiting amino acid. Thus, proteinaceous additives are needed to ensure nutritional diets. It is critical to be able to alter processing conditions and choose raw materials to keep formulation costs to a minimum, maintain high quality standards and minimise operating costs.

Linear programming (LP) can be used to identify a nutritionally adequate diet and minimal cost formulation, since price and nutrient contents are linearly related to composite ingredient weight. LP is a quantitative analysis technique for optimizing an objective function given a set of constraints (Almeida-Dominguez *et al*., 1990).

Extrusion cooking is a complex process that differs from conventional processing by using high shear rates and high temperatures (>150°C) for very short periods. A wide range of thermo-mechanical and thermo-chemical processes are involved, including shear, Maillard reactions, protein denaturation and hydrolysis (Hurrell and Carpenter, 1977). These processes result in the physical, chemical and nutritional modification of food constituents (Harper, 1981; Linko *et al*., 1981; Jowitt, 1984; Zeuthin *et al*., 1984). In a review of the effects of extrusion cooking on nutritional value, Bjorck and Asp (1984) reported reduction in total and available

lysine content from the process. Moreover, these changes influence the appearance, aroma, flavour, and texture of the extruded products. In extrusion, important process parameters for product quality include moisture content of the feed material, residence time, which is influenced by feeding rate, screw speed and configuration, die geometry, temperature and pressure. Sensory attributes of extrudates depend on a large number of machine and raw material related variables. The interactions are so complex that differentiating among the influences of individual variables on the changes in final product characteristics is impossible (Meuser *et al*., 1984). Science the most commercial extrusion process is based on empirical observations, a need for systematic research into the underlying relationships between extrusion parameters, feed composition and extrudate properties is essential.

The sensory attributes of products perceived by the human sensory systems are important determinants of consumer acceptability. Human data collected using sensory evaluation methodology provide the best models for how consumers are likely to perceive products (Meilgaard *et al*., 1999). Descriptive sensory analysis is a technique which enables the perceived sensory differences between two or more samples to be described and quantified and is useful to determine whether aspects such as changing a process variable or ingredient have significant effects on product sensory characteristics. The use of descriptive analysis to objectively measure sensory perceptions of foods has received much attention over the last two decades and the reliability of sensory descriptive analysis in providing meaningful and reproducible results is widely acknowledged by sensory professionals (Risvik *et al*., 1997). The sensory properties of these snack products were then objectively determined under controlled experimental conditions to determine the effect of protein content, extruder feed moisture and extruder barrel temperature on their sensory quality.

OBJECTIVES

1. To apply a linear-programming technique to obtain low-cost formulations containing at least 20 % protein of final food and a nutritionally adequate amount of lysine and sulphur amino acid, with glutinous rice as the major ingredient

2. To study the effect of extrusion parameters on the physical, chemical and sensory qualities of extrudates

3. To investigate the acceptability of enrobe-flavoured extrudates

LITERATURE REVIEW

1. Glutinous rice and utilizations

Rice can be separated into two types according to its category. Non-glutinous rice has a high amylose content (10-30 %) while glutinous rice has little or no amylose (< 2 %), but it is high in amylopectin (>95 %) (Juliano, 1993). Glutinous rice (*Oryza sativa var. glutinosa* or *Oryza glutinosa*) is also called sticky rice, sweet rice, waxy rice, botan rice, mochi rice, and pearl rice. It is a type of short-grained Asian rice that is especially sticky when cooked. The kernels of all glutinous rice adhere to one another, providing a product with a decidedly different texture when compared to amylose-containing rice (Bean *et al*., 1984). Glutinous rice is a type of rice grown and consumed by Lao, Northeast Thailand and Chinese. Thailand Rice is not only the staple diet of Thai people but it is also the export product of this mainly agricultural society. However, only about 10 % of the glutinous rice production is used by the food industry (Thailand Office of Agricultural Economics, 2004). If glutinous rice could be developed to another commercial product, it could generate more added value for glutinous rice and earn greater export value.

Rice is the most popular grain throughout the world. Two-thirds of the world's population relies on it for energy, fibre, antioxidant phytonutrients, vitamins and minerals essential for life. Not only is rice nutritious due to its nutrient profile, it is also highly digestible, thus rice can be enjoyed by most individuals, young and old alike, and poses no risk for those who are sensitive to or intolerant of certain proteins or other components of grains. Rice is naturally sodium and cholesterol free, and has just a trace of fat. Due to its neutral flavour, rice also compliments many other foods, including beans, soy foods, vegetables, lean beef, fish and poultry. There is increased interest using rice in novel, value-added foods (Kadan *et al*., 1997; Kadan *et al*., 2001). Abrasive or friction milling to remove the pericarp, seed-coat, testa, aleurone layer and embryo to yield milled rice results in loss of fat, protein, fibre, ash, thiamine, riboflavin, niacin and α-tocopherol (Tables 1 and 2). Available carbohydrates, mainly starch, are high in milled rice and this is responsible for the

majority of calories (at least 85%). Milled rice has about 6-7 % protein. It has reasonable quality protein, with a chemical score of 55-59. Lysine is the limiting amino acid (Table 3).

Table 1 Proximate composition of glutinous rice.

Rice fraction[1]	Crude protein (gN x 5.95)	Crude fat (g)	Crude fibre (g)	Crude ash (g)	Available carbohydrates (g)
Rough rice	5.8-7.7	1.5-2.3	7.2-10.4	2.9-5.2	64-73
Brown rice	7.1-8.3	1.6-2.8	0.6-1.0	1.0-1.5	73-87
Milled rice	6.3-7.1	0.3-0.5	0.2-0.5	0.3-0.8	77.89

[1] all rice measured at 14% moisture content

Source: Juliano (1985); Eggum *et al*. (1982); Pedersen and Eggum (1983)

Table 2 Vitamin and mineral contents of glutinous rice.

Rice fraction[a]	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)	α-Tocopherol (mg)
Rough rice	0.26-0.33	0.06-0.11	2.9-5.6	0.9-2.0
Brown rice	0.29-0.61	0.04-0.14	3.5-5.3	0.9-2.5
Milled rice	0.02-0.11	0.02-0.06	1.3-2.4	0.75-0.3

[1] all rice measured at 14% moisture content

Source: Juliano (1985); Pedersen and Eggum (1983)

Table 3 Amino acid contents of glutinous rice.

Rice[1] fraction	Rough rice	Brown rice	Milled rice
Histidine	1.5-2.8	2.3-2.5	2.2-2.6
Isoleucine	3.0-4.8	3.4-4.4	3.5-4.6
Leucine	6.9-8.8	7.9-8.5	80-8.2
Lysine+Cystine	3.2-4.7	3.7-4.1	3.2-4.0
Methionine +tyrosine	4.5-6.2	4.4-4.6	4.3-5.0
Phenyl-alanine	9.3-10.8	8.6-9.3	9.3-10.4
Threonine	3.0-4.5	3.7-3.8	3.5-3.7
Tryptoph-an	1.2-2.0	1.2-1.4	1.2-1.7
Valine	4.6-7.0	4.8-6.3	4.7-6.5
Amino acid score[2]	55-81	64-71	55-69

[1] all rice measured at 14% moisture content

[2] based on 5.8 g lysine per 16 g N as 100 % (WHO, 1985).

Source: Juliano (1985); Eggum *et al*. (1982); Pedersen and Eggum (1983).

Glutinous rice is used for a range of products. These include whole kernel products. Pinnipig (flattened parboiled waxy rice, puffed, Philippines snack), Gua-Ba (Taiwan snack) are similarly to Khao-Than (Thailand snack). Milled rice can be processed into rice flour used as a raw material for making food products such as rice noodles and snack foods. Glutinous rice flour is one of the important raw materials in the manufacture of baked or popped snacks, crackers and extruded snacks, because it expands readily and produces a more porous texture (Athapol *et al*., 1997; Jomduang and Mihamed, 1994). It has become an attractive ingredient in the extrusion industry due to its bland taste, attractive white colour, hypoallergenicity and ease of digestion (Kadan *et al*., 2003). However, rice protein levels are substantially below the levels required for child growth (Hansen *et al*., 1981). Similar to other cereals, rice seed proteins are deficient in some essential amino acids such as lysine, which is essential for human and monogastric nutrition. (Chavan and Duggal, 1978; Sotelo *et al*., 1994; Xu, 1990). Thus, the addition of proteinaceous and modification of the amino acid

profile of the rice grain to develop high nutritional rice base snack is one of the most important objectives to make nutritions foods.

2. Snack food

2.1 Snack food trend

Snacks are convenient and provide relief from short-term hunger. Rugg-Gunn *et al*. (1986) defined the term 'snack' as a food not eaten at a recognized mealtime and one which made a minor contribution to the day's dietary intake. However, there is now evidence that snacks make more than a minor contribution to the diet and this may result in eating too many high-sugar, high-fat, high-calorie, or high– sodium foods which often lack appropriate quantities of the valuable micronutrients that children need for healthy growth (Nicklas *et al*., 2001; Epstein *et al*., 2001; Astrup *et al*., 2006). A recent study of television advertising found that between 95 and 99 % of food and beverages advertised on television during children's viewing times were high in fat, sugar, or salt (Dibb and Godon, 2001). Population studies have shown an increasing energy intake related to an increase in snack consumption (Nicklas *et al*., 2001; Nielsen *et al*., 2002; Zizza *et al*., 2001). The increased consumption of energy-dense food with high levels of fat, sugar and refined carbohydrates combined with reduced physical activity is seen as the main cause of the obesity epidemic (Deckelbaum and Williams, 2001). Obesity prevalence is increasing worldwide and is a major threat to public health given its association with diabetes, hypertension, heart disease, and other serious conditions (Forslund *et al*., 2005; Bray, 2004; Healthy People 2010., 2005). The latest estimates reported that one in four children in the U.S. is overweight. The 11% of them are obese. This is an increase of almost 100% in the last twenty years (Williams *et al*., 2001). In young adults (19-24 years), the top contributors to energy from snacking were desserts, beverages, milk and salty snack foods (Drummond *et al*., 1995; Zizza *et al*., 2001). An adult survey conducted in the UK between 2000 and 2001 found that younger adults were more likely to consume chips, savoury snacks and soft drinks and less likely to eat daily portions of fruit and vegetables, wholegrain and high fibre cereals

(Henderson *et al*., 2002). This is similar to Thai school children (10-12 years) (Alavi Naini *et al*., 2006), and pre-school children (2-6 years) (Klunklin and Channoonmuang, 2006). One study of snacking patterns between obese and normal children (7-9 years) in private schools in Bangkok found that the commercial snacks such as chocolate and crisp snacks were consumed more frequently among the obese children than those of the normal children (Hakhun *et al*., 1993). In contrast, for Thai children aged 6 to 14 years old, 20% were overweight, 20% were underweight and the underweight children consumed snacks more frequently than the obese children. Crisp snacks were number one in the top 10 snacks consumed (Nutrition division, Ministry of Public Health, 2006). Thailand Food and Drug Administration (FDA) has called for manufacturers to adhere to a voluntary agreement to reduce fat, salt and sugar in snack foods. The food producer must indicate the quantity of these components on the food label (FDA Journal). Many Thai schools were concerned and had implemented some aspects of health food policies. Such involvement is important in developing healthy eating habits in young children (Korwanich *et al*., 2006). Deckelbaum and Williams (2001) suggested that trying novel approaches is more successful way to prevent and treat childhood overweight and obesity. One way may be to make snacking more healthful, not by totally replacing currently preferred snack foods, but by formulating those foods using ingredients or preparation techniques that increase the nutrient density of the snack.

Food production in the past has mainly been directed toward reduction in cost and improvement in yield, while ignoring nutritive aspects. As a previous reason, this trend has recently changed and the challenge for food scientists and nutritionists in the last few years has been produced more nutritious foods, adapted to opportunities in market segmentation, convenience aspects and increased product quality. In developed countries, sales of traditional fried salty snack products are falling because of dietary failings including high glycemic loadings and high fat levels. At the same time, sales of nutritious snack products are increasing (Burst, 2003). Japanese consumers spend more on packaged, processed, and take-out food and less on home-cooked and fresh food (Fujita and Chern, 1994; Riethmuller, 1994; Shirouzu, 1995, Gehrt and Shim, 2003). Promotions contributing to this shift in

consumption tout confectionary snack food products and salty snacks as healthy, nutritious and high-energy alternatives to other healthy types of snack food (Huthoefer, 1992; Soliman, 1994). These cases suggest that consumers today want snack foods in more healthful qualities. Thus, food manufacturers are seeking new technologies and raw materials for the production of more nutritious snacks, while trying to keep the sensory acceptance as close as possible to that of traditional foods (Loey *et al*., 1996). Some companies bake their snack foods instead of frying, to reduce fat content. Others have reduced the salt content, improved nutritional labeling. In an effort to fight the rise in childhood obesity, five of the country's largest snack food producers announced recently that they would start providing more nutritious foods to schools to replace sugary, fat-laden products in vending machines and cafeterias. Mars, best known for its M&M's, would create a new line of nutritious snacks. PepsiCo, which owns Frito-Lay, would reformulate several products to meet the guidelines. Kraft would decrease the sodium and calories in products it sells for school vending machines. Campbells would promote the benefits of soups that were lower in calories, fat and sodium and offer additional products with less sodium. Dannon would reduce the sugar content of its Danimals drinkable yogurt by 25 percent (Burros, 2006). Another interesting new development has been the launch of extruded dairy protein crisp for use by makers of healthy snack foods from Fonterra.

Improving the nutritional value of snacks not only reduces carbohydrate, sugar, fat or salt, but also increases protein, vitamins or other nutrients. Such nutritious carbohydrate-based snack foods can be produced by incorporation of a suitable protein source into the formulation to offer healthy and nutrition alternatives to snacks based solely on starchy grains such as rice, corn and potatoes. Anumber of researchers have used protein materials such as milk for supplement protein in rice-mungbean weaning food (Santos *et al*., 1993) fish to improve the nutritive value of starch-based extrudates (Suknark *et al*., 1999; Young *et al*., 2001; Choudhury and Gautam, 2003; and Pansawat *et al*., 2008), bovine lung for supplement protein in chickpea and corn flour blends (Pinto et al., 1997; Santiago *et al*. 2001), and incorporation of legumes, vegetables and fruits into the formulation to produced the nutritious snack foods (Thakur and Saxena, 2000). Many efforts have

been made to develop nutritive extruded snacks by using of cereal and legume combinations to improve the value of food proteins such as wheat, rice and fababean blends (Abdel-Aal *et al*., 1992), rice and green gram blends (Bhattacharyya, 1997), rice and soy protein blends (Ascheri *et al*., 2001), cowpea and wheat flour blends (Akubor, 2004), corn and lentil blends (Hardacre *et al*., 2005) and maize and soybean blends (Veronica *et al*., 2006; Perez *et al*., 2008). The cereal base snacks tend to be low in protein with a poor biological value because of their limited essential amino acid contents. The cereal proteins are generally low in lysine although high in sulphur amino acids (Akpapunam, 1984; Prinyawiwatkul *et al*., 1996). The products also had lower glycemic potential and higher protein content than similar products made entirely from cereal grains. However, different areas of the world often have different concerns. Nutrients missed such as dietary fibre, vitamin and mineral during mealtime can be added to a snack food. Santiago *et al*. (2001) developed the extruded chickpea, corn and bovine lung for malnutrition programs. These snacks had high quality protein content and provided 30-40 % of the iron RDA for children. Extruded snack containing 40 % banana and 60 % rice was produced with a twin screw extruder at 120 °C, 220 rpm screw speed and 12 % feed moisture (Gamlath, 2007). This is the nutritious snack which contains 6.9 g of protein, 2.3 g of fibre, 423.7 mg potassium and 139.6 g phosphorus of 100 g (dry basis) of product. Several researchers have been shown the potential use of fibre in extruded snack product. Mendonca *et al.* (2000) used corn bran as a fibre source in expanded snacks. Analysis of variance showed that the best combination of general acceptability, expansion and higher level of fibre was reached on snacks prepared with 250 g/kg corn bran, higher temperature (190 °C), lower moisture content (160 g/kg) and medium level of glycerol monostearate (4 g/kg). The radial expansion ratio, appearance and general acceptability of the product showed decreased values with increasing corn bran content. Onwulata *et al.* (2001) found that adding wheat bran fiber (125 g/kg) associated with increasing specific mechanical energy (SME) improved product quality characteristics of milk-corn extrudates. The addition of cauliflower by-product as a source of dietary fibre, antioxidants and proteins in cereal based ready-to-eat expanded snacks has been studied by Stojceska *et al*. (2008).

2.2 Direct expanded snack

Consumers today are demanding ever-broadening selections of a variety of snack foods. Extrusion has provided a means of manufacturing new and novel products and has revolutionized many conventional snack manufacturing processes. The majority of extruded snacks on the market fall into the category of direct expanded snack. The most popular raw material is corn meal. In general, direct expanded products are made on high-shear cooking extruders. The category gets its name from the fact that products expand directly out of the extruder die and require no further processing, other than drying. The puffing of direct expanded snack products is created by heating the ingredients to temperatures over 100 °C. Inside the extruder the water in the dough mass remains a liquid because the dough is under pressure. Then as the dough exits the extruder through the die openings the superheated water is exposed to atmospheric pressure. The vaporization of water into steam during this rapid pressure lost causes stretching and expansion of the starch matrix. This gives products a low density and light texture. Bulk densities typically fall in the range of 50-160 g/l. The shape and size of these products are determined by the die design, the viscoelastic properties of the extrudate and the manner in which they are cut. Moisture of the direct expanded snack is normally between 8 and 10 % moisture content on a wet basis and requires additional drying to produce the desired product crispness (Faubion and Hoseney, 1982). With drying, this moisture is brought down to 1-3 %. Directed expanded snack are often marketed as high-fiber, low-calorie, high-protein and nutritional products (Huber and Rokey, 1990; Harper and Jansen, 1985). Some examples of direct expanded snack products are corn curls, both baked and fried onion rings, potato sticks and more recently three-dimensional snacks (Frame, 1994; Raiz, 2000; Onwulata *et al*., 2001).

3. Nutrition needs for nutritious snack foods

Snack foods can offer nutritional advantages, depending on the types and quantities of nutrients they contain. Some essential nutrients are found in six classes of nutrients; carbohydrate, protein, fat, vitamins, minerals and water. These support

growth, maintenance, and repair of the body. Deficiencies, excesses and imbalances of nutrients bring on the diseases of malnutrition (Holman, 1987). The three nutrients that produce energy are carbohydrate, protein, and fat. Most of snack foods are formed from starch (rice, wheat, and other grains or starchy vegetables). They may also contain more of fat or lipid than the body needs from the diet to ensure good nutrition and health.

In general, the target consumers of snacks are children and teenagers. At these stages of life, the amount and nutritional quality of protein are particularly important because of their essential function in physical and mental development. A person who does not eat enough protein may get kwashiorkor malnutrition. The normal, healthy adult body needs at least 0.8 g of protein/kg of body weight/day, while children need at least 1.2 g of protein/kg of body weight/day (Holman, 1987; Nutrition Division, 2006). Proteins contain nitrogen. The building blocks of proteins are a combination of 22 amino acids. Some amino acid can be made in the body from other amino acids. However eight or nine amino acids, cannot be made in the human body and must be provided in foods. These are called the essential amino acids because they must be provided in the diet, in the right amount and in the right proportions. The amino acid requirements for humans at various ages are provided in the 1985 report of the Joint FAO/WHO/UNU Expert Consultation on Energy and Protein Requirement (Food and Agriculture Organization /World Health Organization/The United Nations University [FAO/WHO/UNU], 1985). These estimates, for preschool and school-age children and for adults, are summarized in Table 4.

There are two basic kinds of protein foods, animal protein and vegetable protein. Most animal proteins, such as eggs, fish, meat, milk, and dairy products, have all the essential amino acids (EAAs) in the right proportions. So these foods are already “complete proteins”. Vegetable proteins such as nuts, seeds, dry beans, dry peas, and grains such as wheat and corn and rice, all contain most of the essential amino acids, but not in the best proportions for the human body. Some are deficient in one or two amino acids; others may be low in different amino acids. A low or inadequate amino acid is called the limiting amino acid. These protein foods must be

combined (or complemented) to compensate for the limiting amino acids. For example, rice is deficient in lysine but beans have high proportion of lysine (Akpapunam, 1984).

Currently, the food nutrition labeling became mandatory for most prepackaged foods, so consumers are able to make informed food choices. At the International Conference on Nutrition-ICN meeting on December 1992, all countries including Thailand make a world declaration and plan of action for nutrition. It was deemed appropriate to have nutrition labeling for information and useful nutrition facts to consumers. In Thailand, under regulations from the Food and Drug Administration (FDA) of the Minister of Public Health, food which has nutrition claim or food which promotes high protein or low fat is required to have nutrition labeling. A product that uses the term "high", "rich in" or "excellent source" of protein, dietary fibre, vitamins or minerals (excluding sodium), the nutrient must contain $\geq$ 20% of the Thai RDI (Appendix No. 4, attached to the notification of the Ministry of Public Health No. 182 B.E. 1998).

4. Extruder technology

The use of extrusion in the snack food industry got its start in the 1930s when corn curls were first extruded. From there, the use of extruders grew in a variety of ways, such as their use for second- and third-generation snacks (Martizez-Serna *et al*, 1992). Today with the advent of twin-screw extruders, the snack food manufacturer has the capability of producing even more complex foods. Extrusion-cooking as a thermo-mechanical processing operation had a wide range of applications, assisted by (i) the number of mechanical and thermal processing steps that can take place along the screws and barrel and (ii) the high shear and pressure exerted on low moisture food mixes. Extrusion uses relatively dry materials, has a major impact on the microbial load, denatures enzymes, gelatinizes starch, polymerizes proteins and, most importantly, texturizes the end product into a desirable form (Fichtali and Van de Voort, 1989). Extrusion is a high-temperature-short-time (HTST) process then minimizes degradation of food nutrients while it can improve the digestibility of

proteins and starches (Hoyem and Kvale, 1976). Furthermore, it has no effluent (Raiz, 2000).

Table 4 Amino acid requirements for pre-school and school-aged children and adults.

Essential amino acid	Pre-school children (2-5 year)		Schoolchildren (10-12 year)		Adults (18+ year)	
	mg/kg/ day	mg/g/ protein	mg/kg/ day	mg/g/ protein	mg/kg/ day	mg/g/ protein
Histidine	-	-	-	-	8-10	16
Isoleucine	31	28	28	28	10	13
Leucine	73	66	44	44	14	19
Lysine	64	58	44	44	12	16
Methionine and Cystine	27	25	22	22	13	17
Phenylalanine and tryrosine	69	63	22	22	14	19
Threonine	37	34	28	28	7	9
Tryptophan	12.5	11	3.3	9	3.5	5
Valine	38	35	25	25	10	13
Total (except for histidine)	352	350	216	222	84	113

Source: FAO/WHO/UNU (1985)

4.1 Twin-screw extruders

Extruders are primarily divided into single- and twin-screw extruders. Twin-screw extruders can be characterized in a number of ways. In 'co-rotating' extruders the two screws rotate in the same direction. In "counter-rotating' extruders the screws move in opposite directions. Both co-rotating and counter-rotating

extruders can have fully intermeshing, partially intermeshing or non-intermeshing screws. Co-rotating twin-screw extruder with fully intermeshing screws is the extruder of choice because they are capable of exerting a high degree of mixing (Martizez-Serna *et al*, 1992). The mixing capabilities of twin-screw extruders make them well suited for use as reactors for food materials. To enhance mechanical energy dissipation as well as mixing, kneading disks are usually used (Johnston, 1978; Raiz, 2000). Twin-screw extruders are operational at very low feed moisture requiring no or minimum post-extrusion drying (Dziezak, 1991; Harper, 1981)

Extrusion cooking, although flexible, is complex because it requires close control of many variables. Any change in feed composition, influenced by the type of ingredients as well as the amount of each ingredient, and any process variable that affects physical or chemical transformations of macromolecules during extrusion can influence extrusion performance and quality of the extrudate. Therefore, new sets of optimum or desirable process conditions are required for each set of new circumstances, and these must be determined empirically because there is no fully developed theory to predict the effects of available process variables on various complex raw materials and their mixtures (Smith, 1976; Choudhury and Gautam, 2003). Control of the extrusion processing variables is vital to the success of producing any final product. Typically, extrusion experiments examine just two or three extrusion processing variables, but many factors are important as shown in Table 5. Extruder operators may select parameters for the primary factors, and these factors in turn determine a secondary set of factors (Meuser *et al.,* 1984). These factors influence the viscosity of the food within the extruder barrel, the residence time of the material in the extruder, and the shear applied to the food. Variations caused by feed composition and prior processing of the feed materials are important sources of experimental variation. The type of extruder used certainly also affects chemical reactions (Choudhury *et al*., 1997; Camire, 2000).

The screw configuration is an important parameter for determining product properties. Many twin-screw extruders have composite screws which can be built up from different types of conveying and mixing elements (reverse screw and

kneading elements). The pitch, stagger angle, length, location and spacing of screw elements are important parameters during extrusion processing. Seiler *et al*. (1980) found that there was a change in specific volume of extruded maize grits when the reverse pitch element of a Clextral BC-45 was displaced from its normal position at the end of the screw. Olkku *et al.* (1983) reported that on replacing a conveying element with a reverse pitch element in the BC-45, the result was an increased die temperature and motor current and a product of greater solubility. Published data on screw configuration effects during extrusion of rice flour have been reported by Altomare and Ghossi (1986), Erdemir *et al*. (1992) and Choudhury and Gautam (1998). Gogoi *et al*. (1996) reported the effect of location and spacing of reverse screw and kneading element combination during twin-screw extrusion of rice flour and proteinaceous blends.

Table 5 Factors influencing chemical changes during extrusion.

Primary factors	Secondary factors
barrel temperature	mass (product) temperature
die geometry	pressure
extruder model	specific mechanical energy
feed composition	
feed moisture	
feed particle size	
feed rate	
screw configuration	
screw speed	

Source: Camire (2000)

4.2 Physicochemical and nutritional changes in food during extrusion

Extruder geometry, process conditions and food mix composition

interplay to bring about various physical, chemical and nutritional modifications of the food constituents. Five general chemical or physicochemical changes can occur during extrusion cooking: binding, cleavage, loss of native conformation, re-combination of fragments, and thermal degradation (Camire, 2000; Camire *et al*., 1990). The high shear stresses and high temperatures inside the extruder screw channel lead to a variety of fast and relatively efficient chemical reactions (Gopalakrishna, 1992). Prevention or reduction of nutrient destruction, together with improvements in starch or protein digestibility, are clearly of importance in most food applications. Extrusion also permits the inactivation of several antinutritional or toxic factors, of oxidative (lipoxigenase, peroxidase) and other deterioration enzymes. The highest degree of nutritional concern is reached when extrusion is used specifically to produce foods that are nutritionally balanced or enriched, like weaning foods (Plahar *et al*., 2003), meat replacers, animal feeds and dietetic foods (Cheftel, 1986).

4.2.1 Protein

4.2.1.1 Improved protein digestibility due to protein denaturation

The nutritive value of a protein is dependent upon the ease with which it can be digested as well as its amino acid pattern. Most proteins undergo structural unfolding and/or aggregation when subjected to moist heat or shear. This often leads to insolubilization and improves the digestibility of proteins by inactivation enzyme inhibitors and denaturing the protein which may expose new sites for digestive enzyme attack. The extrusion process denatures undesirable enzymes: inactivates some antinutritional factors (trypsin inhibitors, haemagglutinins, tannins and phytates) (Fellows, 2000) and sterilises the finished product (Bhandari *et al*., 2001). In addition, this process is able to break the covalent bonds in biopolymers, and the intense structural disruption and mixing facilitate the modification of functional properties of food ingredients and/or texturizing them (Carbalho and Mitchelle, 2000). Soybeans and many legumes or oilseeds provide a good example of improved protein digestibility and bioavailability of (limiting) sulphur amino acids through (i) thermal unfolding of the major seed globulins and (ii) thermal inactivation

of trypsin inhibitors and other growth retarding factors, such as lectins in soybeans (De Muelenaere and Buzzard, 1969; Venou *et al*., 2006; Aslaksen *et al*., 2005) and sorghum (MacLean *et al*., 1983). Lectin (haemagglutinating) activity is relatively heat resistant. An aqueous heat treatment, at 60 or 70 °C for up to 90 min, did not alter the lectin activity in soybeans. However, as found with kidney bean (Grant *et al*., 1984), the lectin activity in the fully imbibed seed could be completely abolished by heating them for 5 min at 100 °C. Extrusion has been shown to be very effective in reducing or eliminating lectin activity in legume flour (Alonso *et al*., 2000a, b). Thus, extrusion cooking is more effective in reducing or eliminating lectin activity as compared with traditional aqueous heat treatment. Sing *et al*. (2000) reported that extrusion process (300 rpm screw speed, 27 kg h^{-1} feed rate, 5/32 inches die size and 93-97 °C outlet temperature) caused complete destruction of trypsin inhibitor activity in extruded blends of broken rice and wheat bran containing up to 20% wheat bran.

Similar beneficial effects are often obtained with blends of other cereals, oilseeds and legumes. Low-cost extruders are useful in developing countries for manufacturing nutritious precooked flours from local commodities (Harper and Jansen, 1985). While amino acid complementation between cereal and oilseed (or legume) usually results in high protein efficiency ratio (PER) close to that of casein, it is sometimes desirable to supplement the extruded flours with free lysine or methionine (together with vitamins and minerals). Increasing barrel temperature during extrusion has been shown to increase the digestibility of corn gluten-whey blends (Bhattacharya and Hanna, 1988), fish – wheat blends (Bhattacharya *et al*., 1988), sorghum (Fapojuwo *et al*., 1987) and bean and protein maize blends (Ruiz-Ruiz *et al*., 2008). Heating prior to extrusion may also improve digestibility. Bhattacharya and Hanna (1985) reported higher values for flash-dried corn gluten meal than for wet gluten. Screw speed and the length to diameter ratio of the screw had no effect on the digestibility of the blends. Increased screw speed may have increased the protein digestibility of extruded corn gluten because the increase in shear forces in the extruder denatured the proteins more easily, thus facilitating enzyme hydrolysis. However, varying screw speed and moisture content did not have a significant effect on the digestibility of sorghum protein (Fapojuwo *et al*., 1987).

Protein digestibility has been reported most frequently for underutilized sources of protein such as corn gluten (Buck *et al*., 1987), amaranth (Koeppe *et al*., 1987; Sanchez-Marroquin *et al*., 1986), and sorghum (Serna-Saldivar *et al*., 1988).

4.2.1.2 Protein solubility

Protein solubility in water or dilute salt solutions is decreased after extrusion. During extrusion, disulfide bonds are broken and may re-form. Electrostatic and hydrophobic interactions favour formation of insoluble aggregates. The creation of new peptide bonds during extrusion is controversial. High molecular weight proteins can dissociate into smaller subunits (Cumming *et al*., 1973). Exposure of enzyme susceptible sites improves digestibility. Although many researchers have used extrusion temperatures below 150°C, different mechanisms might occur at higher temperatures. Prudincio-Ferreira and Ares (1993) extruded soy protein at three barrel temperatures (140, 160, 180°C) and two feed moistures (30 and 40 %). Insolubility due to combined non-covalent and disulphide linkages was highest in soy extruded at 40% feed moisture. As temperature increased, protein insolubilization increased at both moisture levels.

4.2.1.3 Decreased nutritional availability of lysine

Extensive lysine loss and nutritional damage can take place when cereal flours or cereal/legume blends are extruded into biscuits, cookies, flak, crispbread, breakfast cereals or instant flours under severe conditions of temperature ($T \geq 180°C$) or screw speed (RPM > 100) at low moisture (% $H_2O \leq 15$) (Cheftel, 1986), especially in the presence of reducing sugars (Pham and Del Rosario, 1984; Bjorck *et al*., 1983), glucose (Grebaut *et al*., 1984; Solina *et al* 2007), lactose and sucrose (Racicot *et al*., 1981; Mezreb *et al*., 2006). Noguchi *et al*. (1982) found that the retention of available lysine during processing of a cereal/soy-based mixture containing 20 % sucrose ranged from 0%, to 40% at 170 °C mass temperature, 10-14 % feed moisture and 60 rpm screw speed. Sucrose, maltose and fructose were found to be much less reactive than glucose under similar extrusion conditions. There was

selective damage to lysine at low hexose contents (1-5 %). At a high-energy input to the extruder, glucose caused losses of available lysine and arginine of 61 % and 15 %, respectively. In contrast, with xylose, the loses were grater, being 70 % and 32 %, respectively (Asp and Bjorck, 1989). The chemical reaction between a reducing sugar and a free amino group on an amino acid, usually the epsilon-amino group of lysine, has important nutritional and functional consequences. This reaction, known as non-enzymatic browning, is actually a series of reactions providing a wide variety of compounds as a result (Waller and Feather, 1983; Feather, 1985). Furthermore, lysine loss has been related to extrusion process parameters such as raw material, feed moisture, screw speed, extrusion temperature, die diameter, feed rate, screw compression ratio, torque and pressure, energy input and pH (Asp and Bjorck, 1989; Camire *et al*., 1990).

An increase in RPM decreased lysine content and biological value, while an increase in feed rate had opposite effects. An increase in feed rate is known to reduce the dispersion of residence times (and the mean residence time) and also to reduce heat transfer to the food mix (Bjorck and Dahlqvist, 1984). The available lysine in the extrudates of defatted soy flour and sweet potato flour mixture ranged from 68 to 100 % (Iwe et. al., 2004). Increase in screw speed (80 to 140 rpm) and a reduction of die diameter (10 to 6 mm) enhance lysine retention. Optimum available lysine was estimated at a feed composition of 98.94 %, screw speed of 118.98 rpm, and die diameter of 2.25 mm in the extrusion of mixtures of defatted soy flour and sweet potato flour. Noguchi *et al*. (1982) noted that screw speed was not a major factor in the retention of available lysine in protein enriched biscuits. In this product, up to 40 % of the available lysine present in the mix was lost during extrusion conditions of 170 to 210 °C mass temperature and 13 % moisture biscuits. In order to keep lysine losses within the 10-15 % limit accepted in bread baking or in the drum-cooking and drying of instant flours, it is necessary: (i) to avoid extrusion above 180°C at water contents below 15 % (even if a subsequent oven-drying step is then necessary) and (ii) to avoid the presence of reducing sugars during extrusion. The nature of a protein to be extruded also influences the extent of available lysine loss. In the extrusion of wheat flour (150 °C mass temperature, 5 mm die diameter, 150 rpm

screw speed), an increase in feed rate (from 200 to 300 $gmin^{-1}$) significantly improved lysine retention (Bjorck and Asp, 1983). Pham and Del Rosario (1984) found that, at a given process temperature during extrusion cooking of cowpea and mung bean, the available lysine decreased with increasing feed moisture content at 93-167 °C barrel tempature, 30-45 % feed moisture and 100 to 200 rpm screw speed. The loss depends on extrusion conditions, increasing with temperature and decreasing with moisture content of the feed. Quantitatively, more lysine is lost from animal proteins since they generally contain more lysine than do plant protein (Yen *et al*., 1988). However, since lysine is the limiting amino acid for protein quality in cereals and some legumes, the loss of available lysine from these proteins is of greater consequence.

Proteins processed under conditions of alkaline pH and heat may develop amino acid residues that are not found in nature. Alanine is first converted to dehydroalanine, which may then react with lysine to form lysinoalanine (LAL), with cysteine to form lanthionine (LAT), or with ornithine to form ornithoalanine (Cheftel *et al*., 1985). These compounds are not well utilized, thus protein nutritive quality is reduced. Since these cross-links may develop between peptides or within a peptide, the resulting change in conformation may also affect the physical characteristics of the food containing them.

4.2.1.4 Texturization

The ability of plant proteins to be texturized during extrusion into structured bodies having a fibre-like nature has been reported (Harper, 1981; Renkema *et al*., 2000; Li *et al*., 2005). Irreversible thermal denaturation is the main process in texturization. As the temperature increases, an extensive unfolding of protein with loss of its globular three-dimensional structure occurs. The ionic, disulphide, hydrogen, and van der Waals' bonds holding the molecular structure are broken. The resulting relatively linear protein chains are free to reorient and recombine. Intramolecular amide and intermolecular disulphide and/or peptide bonds are probably rearranged (Davidek *et al*., 1990). The majority of research has focused on soy protein used as a meat analog. The water soluble protein breaks down into sub-

units during thermoplastic extrusion of soy protein and becomes redistributed and /or insoluble. Holay and Harper (1982) proposed that increasing the water content of the soy during extrusion would result in a more elastic material because protein mobility, and thus cross-linking, would increase. Higher bulk densities were also found in the corn gluten-soy protein concentrate blends when moisture was increased. Carbohydrates also play an important role in the development of texture. In the baking and starch-based snacks industry, the technological as well as the nutritive properties can be improved by adding vegetable or animal proteins. In general, the incorporation of protein into starch-based snacks led to detrimental effects on physical properties of extrudate. Matthey and Hanna (1997) extruded starch based snacks containing 30% whey protein concentrate (80 % protein) and concluded that both whey protein and amylose content should be low to obtain desirable product expansion.

4.2.2 Starch

Moist thermal treatments of cereal grains, pulses and starchy tubers induce physico-chemical modifications in starch granules and constituents that lead to (i) rheological and textural changes and (ii) increased digestibility and availability as a source of energy. Onyango *et al*. (2004) reported that in vitro starch digestibility of maize-finger millet blend in the production of *uji* (fermented cassava flour) increased from 20 mg maltose/g starch in the raw blend to about 200 mg/g after extrusion. Extrusion-cooking, depending on process conditions and food mix composition, causes swelling and rupture of starch granules, modification of crystalline spectra and DSC peaks, cold water-solubility and reduced viscosity of starch and partial, to complete, release of amylose and amylopectin. Although it is difficult to give precisely the various combinations of temperature, moisture, shear and residence time which bring equivalent degrees of starch gelatinization when applied to different food mixes, complete starch gelatinization is generally achieved at T>I20°C, $\%H_20$=20-30, or even at lower moisture levels (10-20%), provided high shear and temperatures are reached during extrusion (Linko *et al*., 1981). Chemical studies have shown that starch undergoes fragmentation during extrusion, and that the degree of fragmentation is much greater in amylopectin than in amylose (Wen *et al*., 1989). Starch with low

moisture content undergoes melting coupled with gelatinization. As the moisture content is lowered, melting becomes more pronounced; gelatinization completely disappears when the moisture level drops below 15% (Wang *et al*., 1999).

In extrusion practice, "expansion" is used to describe the events that lead to the formation of puffed, low-density cellular materials from a hot, gelatinized mass of starch which is forced under pressure through a restricted opening into the atmosphere. The most important contributors to good expansion include adequate gelatinization of the starch, development of sufficiently large pressure drops at the orifice to cause rapid boil-off of water vapour, and formation of a strong cellular structure and skin due to rapid evaporative cooling (Camire *et al*., 1990). Starch amylose and amylopectin content are known to have a large effect on the expansion of the melt. Waxy corn, which has very low levels of amylose, is reported to have superior expansion properties compared with other types of corn (Mercier and Feillet, 1975). High amylose corn, moreover, shows very poor expansion in a number of twin-screw extruder applications (Bhattacharya and Hanna, 1987).

The extrusion process has increased in vitro enzymatic digestibility and in vivo digestibility of starch. Bjorck and Dahlqvist (1984) indicated that extruded wheat flours (Creusot-Loire BC45, T= 161-171°C, %H_2O 15 or 20, RPM = 100-200, F= 200-350g per min) are as available in vitro to α-amylase as autoclaved (125 °C, 20 min) controls, and more available than boiled (20min)-and especially raw-controls. Extrusion probably increased the enzymatic availability of starch by way of gelatinization, inactivation of endogenous α-amylase inhibitor, disruption of cellular structure, size reduction and increased starch surface, partial separation from bran and protein. Rat experiments showed that both raw wheat starch and extruded wheat starch (starch from extruded wheat flours) were completely digested in vivo.

4.2.3 Lipids

Most extruded cereal foods contain less than 6-7 % lipids immediately after extrusion, because high lipid levels prevent expansion. In contrast,

small lipid levels (~5%) facilitate steady extrusion and improve the texture. Expanded extrudates can thus be considered as low calorie foods. The nutritional value of lipids could be affected during extrusion as a result of oxidation, hydrogenation, isomerization or polymerization. According to Maga (1978), the extent of hydrogenation and cis→trans isomerization of fatty acids that takes place during extrusion is too small to be nutritionally significant. The inactivation of hydrolytic enzymes is possible with extrusion processing. Higher temperatures reduce the lipase activity and moisture level, thereby decreasing the factors favouring free fatty acid development. High moisture levels and enzyme activity rapidly deteriorate the food quality of rice bran, and even mild extrusion conditions had favorable effects on the stability of rice bran stored for 6 weeks (Sayre and Nayyar, 1985). However, the expanded, porous nature of extrudates causes them to be susceptible to the development of oxidation during storage. Screw wear is a concern as metals can act as pro-oxidants. Iron content and peroxide values were higher in extruded rice and dhal compared with similar product processed by drying methods (Semwal *et al*., 1994). Oatmeal cookies with added potato peels had lower peroxide values than control samples, and higher antioxidant activity was observed for extruded peels compared with unextruded peels (Arora and Camire, 1994). Extrusion of high-fat materials is generally not adbisable, especially in the case of expanded products, as lipid levels over 5-6 % impair ectruder performance (Camine, 2000). Torque is decreased because the lipid reduces slip within the barrel, and often product expansion is poor because insufficient pressure is developed during extrusion. Lipid is released from cell owing to the high temperature and physical disruption of plant cell wall. Some lipid might be lost at the die as free oil, but this situation only occurs with high-fat materials, such as whole soy.

4.2.4 Dietary fibre

Cereals are an important source of dietary fibre. Modifications in particle size, solubility and chemical structure of the various fibre components could occur and cause changes in bacterial degradation in the intestine and in physiological properties. According to the solubility in water, total dietary fiber(TDF) can be

categorized into two groups, namely soluble (SDF) and insoluble (IDF) dietary fiber. IDF components have nutritional value as bulking agents while SDF is more chemically reactive and may bind cholesterol in the intestine (Dreher, 1987). SDF represents a good substrate for some lactic bacteria and Bifidobacteria strains, which fare beneficial for gut health (prebiotic action) (Grizard and Barthomeuf, 1999), it is able to control glycemic index (Tudorica *et al*., 2002), and it reduces plasmatic cholesterol (Brown *et al.,* 1999). The thermomechanical nature of extrusion cooking has the added potential of causing a redistribution of soluble and insoluble components of fiber in favor of the former. This would tend to improve the hypocholesterolemic properties of fiber, and therefore enhance or improve the dietary fiber profile (Ohtsubo *et al*., 2004).

Effect of extrusion cooking on TDF, SDF and IDF have been reported. Esposito *et al.* (2005) studied the dietary fibre in durum wheat bran by-products extrudates. The SDF content of the durum wheat by-product ranged between 0.9% and 4.1%; while that of IDF 21% and 64%. The water holding capacity is strictly related to the amount of IDF and to the granulometry of the by-products. Cooking-extrusion process does not affect the amount of SDF; by contrast, a significant increase of the IDF was detected. Extrusion cooking increased the TDF of barley flours. The TDF increase in waxy barley was the result of an increase in SDF (Vasantha *et al*., 2002). The change in dietary fibre profile during extrusion of barley flour may be attributed, primarily, to a shift from IDF to SDF, and the formation of resistant starch and enzyme-resistant indigestible glucans formed by transglycosidation. In contrast with results were reported by Onyango *et al*. (2004) who studied the application of a single-screw laboratory extruder to produce ready-to-eat *uji* from maize-finger millet blend. They reported that extrusion reduce TDF by 39-68 %, redistributed soluble to IDF ratios and had a negligible effect on the formation of resistant starch (less than 1 g/100g). Gajula *et al.* (2008) also observed that precooking bran-enriched wheat flour by extrusion significantly increased SDF in flours (by 22% to 73%); although in most cases it also led to a significant decrease in TDF. Larrea *et al.* (2005) modified the properties of fivre components in orange pulps using extrusion technology. They reported that the TDF of orange pulp extrudates was

decreased after extrusion. The extrusion process decreased ISF in 39.06% and SDF was increased by 80%. Ruiz-Ruiz *et al*. (2008) observed that processing conditions decreased dietary fibre content by 38% at 155 °C and 44 % at 170 °C. TDF and IDF decrease in the extrudates, but SDF increased. Increasing SDF can be caused by release of the soluble fraction from hemicelluloses as a result of heating.

Many factors influence fibre solubility. Acid and alkaline treatment, prior to extrusion, increased the SDF slightly in corn bran (Ning *et al*., 1991). Grinding doubled the soluble fibre of pea hulls to 8 % (dry basis), but all the extruded hulls contained over 10 % SDF (Ralet *et al*., 1993). Ralet *et al*. (1991) reported that extrusion reduces the molecular weight of pectin and hemicellulose molecules, resulting in increased SDF of sugar beet pulp fibre. Vasanthan *et al*. (2001) found that the content of SDF and TDF increased upon extrusion cooking of barley folurs. The increase in SDF could be due to the transformation of some IDF into SDF during extrusion and the formation of additional SDF by transglycosidation. Extrusion-cooking of white wheat flour (T = 161-171 °C, % H_2O = 15, RPM = 100-200, F= 200 g per min) caused a redistribution of insoluble to SDF (Bjorck and Dahlqvist, 1984). Thus 50-75 % of total fibre was soluble in the extruded flour, depending on process conditions, versus 40 % in the raw flour.

Rat balance experiments showed that dietary fibre in raw white wheat flour was highly available to bacterial degradation since the fecal recovery of arabinose, xylose and glucose averaged a low 22 %. After mild extrusion, the fecal recovery further decreased to 12%. The higher solubility is probably responsible for this increased fermentability. The fecal recovery of fibre constituents from raw whole grain wheat flour was higher (~42%) and remained unchanged after extrusion. Some chemical structures are therefore resistant to bacterial degradation in vivo, in spite of the intense shear treatment during extrusion.

4.2.5 Vitamins and Minerals

Several summaries of the impact of food processing on micro

nutrients have been published such as Marty and Berset (1986) reported the degradation of trans-β-carotene during heating in sealed glass tubes and extrusion cooking, Coelho (1991) vitamin stability/influencing factors during pelleting, extrusion, storage, trace minerals, Gadient and Fenster (1994) stability of ascorbic acid and other vitamins in extruded fish feeds, Suknark *et al*., (2001) studied the stability of tocopherols and retinyl palmitate in snack products, and Anderson and Sunderland (2002) studied the effect of extruder moisture and drier processing temperature on vitamin C, E and astaxanthin stability. Chaovanalikit et al. (2003) reported that the extrusion (225 rpm screw speed, 4 kg h^{-1} feed moisture) decreased anthocyanins in blueberry cereals; ascorbic acid was retained better in cereals containing blueberry concentrate than in the sweetened corn cereals.

Cereal grain products are among the most important sources of B group vitamins, and for this reason there is considerable interest in retaining the nutritional benefits of cereals during processing. Several studies have assessed the effects of extrusion cooking on the retention of B group vitamins (Cheftel, 1986; Camire *et al*., 1990; Killeit, 1994). During the extrusion of crispbread products (Cheftel, 1986) at a specific mechanical energy (SME) from 0.09 to 0.13kWh/kg, and retention times of 0.5 to 1 min at 178 °C, the levels of B group vitamins decreased. From 38% to 65% of thiamine remained, 85% of riboflavin, 80% of niacin and 71–83% of pyridoxine. Thiamine and pyridoxine were the most thermo-labile and levels decreased linearly with temperature (Beetner *et al*., 1976). Camire *et al*. (1990) obtained similar results, with thiamine losses increasing with barrel temperature, and with shear and temperature as screw speed was increased. There was some evidence that thiamine was more sensitive to heat than riboflavin and that riboflavin was more sensitive to shear. The short barrel extruders used for snack food production retain between 44% and 62% of the B group vitamins (Athar *et al*., 2006). This is considerably higher than the 20% retention for maize reported previously for long barrel extruders. The stability of the vitamins was similar, with riboflavin and niacin having the highest stability. Pyridoxine is also regarded as heat labile. Thiamine was the least stable during extrusion (Killeit, 1994). High barrel temperature (200 °C

compared with 125 °C) reduce all trans-β-carotene in wheat flour by over 50 % (Guzman-Tello and Cheftel, 1990).

Carotenoid pigments (which are also vitamin A precursors) are stable during extrusion, but are oxidised during and subsequent storage of corn starch (Creusot-Loire BC 45, T = 170-185 °C) (Berset *et al*., 1984). All trans-β-carotene was partly converted to 15-cis and 9-cis carotene as a result of extrusion, and then all three forms then decreased in concentration during storage. Ascobic acid (vitamin C) is also sensitive to heat and oxidation. Twenty to forty percent losses of vitamin C were consistently observed during extrusion, probably as a result of enhanced oxidation at high temperature. The iron content of the food mix may have a catalytic effect (Maga and Sizer, 1978). Blue berry concentrate appeared to protect 1 % added vitamin C in an extruded breakfast cereal compared with a product containing just corn, sucrose and ascorbic acid (Chaovanalikit, 1999).

The influence of extrusion on mineral absorption is complex due to many factors which affect absorption. The apparent availability of Zn, Cu and Mg was found to decrease in patients with ileostomy, after extrusion of wheat flour into crisp bread (Asp and Bjorck, 1984). Minerals added for fortification should possess high bioavailability, mix homogeneously with the food ingredients and remain compatible with processing (calcium salts reduce expansion) and post-extrusion storage. Thus, ferrous sulphate has high solubility and bioavailability but catalyses lipid oxidation. Micronized elemental iron (particle size below 15 μm) is preferable (Asp and Bjorck, 1989). Singh *et al*. (2000) and Alonso *et al*. (2001) indicated increase in mineral content during extrusion because the contamination of iron content of the flours is increased after extrusion process and it is most likely to the result of the wear of metallic pieces, mainly screws, of the extruder. The incorporation of wheat bran in broken rice flour in extrusion (300 rpm screw speed, 27 kg h^{-1} feed rate, 5/32 inches die size, 93-97 °C outlet temperature) increases of the content of calcium, phosphorus, iron and copper, which might be attributed to the addition of these minerals through water used during extrusion and also from the extruder barrel (Sing *et al*., 2000). Fortification of foods with minerals prior to extrusion poses other

problems. Iron forms complexes with phenolic compounds that are dark in colour and detract from the appearance of foods. Ferrous sulphate heptahydrate was found to be a suitable source of iron in a simulated rice product, because it did not discolour (Kapanidis and Lee, 1996). Added calcium hydroxide (0.15-0.35 %) decreased expansion and increased lightness in the colour of cornmeal extrudates (Martinez Bustos *et al*., 1998).

Extrusion cooking not only offers the ability to improve the availability of nutrients, but also the possibility of removing of reducing factors which limit the wholesomeness of food. The undesirable constituents (toxic and antinutritional) have been found to be significantly reduced by HTST extrusion. The heat produced during extrusion should be expected to destroy at least some of the microorganisms present in the raw materials (Mustakas *et al*., 1964; de Muelenaere and Buzzard, 1969; Kauffman and Hatch, 1977; Bouveresse *et al*., 1982). Grehaigne *et al*. (1983) found that aflatoxin destruction in peanut meal increased with increasing extrusion temperature and amount of ammonia added during extrusion. Furthermore, extrusion has been used to inactivate trypsin inhibitors in soybean and other legumes.

5. Optimization techniques for formulation and process

Optimization means establishing the recipe and the processing conditions for production of the best of a given food product. To find the optimum recipe and/or processing conditions for the best food products, numerical or graphical methods based on the model can be used. Mathematically, this means finding the minimum or maximum of a function of *n* variables, where *n* may be any integer greater than zero (Mackley, 1988; Nemhauser *et al*., 1989). Linear programming (LP) is the part of mathematical programming that studies optimization problems by which limited resources are allocated, selected, scheduled, or evaluated to achieve an optimal solution to a particular objective. These resources may be capital, raw material, manpower, or production facilities and the objectives may be minimum cost or maximum profit. Therefore, LP has wide application in industrial operations such as

blending, mixing, and machine tooling; and in business activities such as purchasing, planning, bidding, transportation, and distribution.

Mathematically, LP is an optimization problem of the following form: Maximize (or, sometimes, minimize) function subject to a finite set of linear constraints (Vaserstein, 2003). The simplex method has been the standard technique for solving a linear program since the 1940's. In practice, however, the method is highly efficient, typically requiring a number of steps which is just a small multiple of the number of variables. Linear programs in thousands or even millions of variables are routinely solved using the simplex method on modern computers. Efficient, highly sophisticated implementations are available in the form of computer software packages. There are numerous software packages which are dedicated to solving linear programs, of which possibly LINDO, GAMS and XPRESS-MP are the most popular. All these packages tend to be DOS based and are intended for a specialist market which requires tools dedicated to solving LPs. In recent years, however, several standard business packages, such as spreadsheets, have started to include an LP solving option and Microsoft Excel is no exception. The standard Microsoft Excel Solver uses a basic implementation of the primal Simplex method to solve LP problems.

The use of LP techniques for the minimum cost formulation of nutritionally improved products destined for human consumption has been reported previously. Ballesteros *et al*. (1984) developed a composite flour for bread manufacturing, Valencia *et al*. (1988) obtained a chickpea-based infant formula with high nutritive value, Almeida-Dominguez *et al*. (1990) obtained composite flours(corn flour, soybean meal and chickpea) for the manufacture of nutritionally improved snack foods.

Response surface methodology (RSM) is a useful statistical technique for investigation of complex process and has been widely adopted in food science research. Based on the principle of design of experiments, the methodology encompasses use of various types of experimental designs, generation of polynomial

equations and mapping of the response over the experimental domain to optimize formulation as well as processing conditions (Wang *et al*., 2008; Wu *et al*., 2008). The basic principle of RSM is to relate product characteristics to process parameters using statistical methods, yielding multivariate regression equations. The response surface is actually a surface based on the assumption that all the independent variables are continuous in the experimental area. For a better visualization, the variable effects on a definite response can be plotted in a 3D space. The model is illustrated in a 3D surface in which two factors are presented on horizontally perpendicular axes and the response on the vertical axis. If more than two independent variables are involved in the model, all other variables except the two most important variables selected must be fixed at certain, usually their middle, levels (Myers, 1995; Hu, 1999). The advantage of such methodology is in providing a rationale for simultaneous evaluation of several variables. The technique requires minimum experimentation and time, thus proving to be far more efficient and cost effective than conventional methods of product development. For implementation of RSM, factorial designs (FDs; full or fractional) are the most popular statistical designs.

RSM has been successfully applied for optimizing formulation and process conditions in several extruded food product. The examples of optimization on formulation have been shown in several extruded products such as amaranth extrudates (Iio *et al*., 1999), gum based cereal-pulse blends (Thakur and Saxena, 2000), and catfish flesh, corn flour and defatted soy flour extrudates (Rhee *et al*., 2004). Additionally, the examples of optimization on extrusion condition have also been reported in numeral extruded products such as rice flour extrudates (Hagenimana *et al*., 2006), almond sanck extrudates (Pilli *et al*., 2008), nutritionally balanced gluten-free (Ibanoglu *et al*., 2006), and oat, chickpea, carrot and hazelnut blends extrudates (Ozer e*t al*., 2006).

6. Descriptive sensory analysis

Descriptive analysis is the sensory method by which the attributes of a food or product are identified and quantified using human subjects who have been specifically

trained for this purpose. It involves the detection (discrimination) and description of both the qualitative and quantitative sensory components of a consumer product by trained panels (Meilgaard *et al*., 1991). The qualitative aspects of a product include all aroma, appearance, flavour, texture, aftertaste and sound properties of a product, which distinguish it from others. The most important attribute of extrudates is texture. The several texture attributes have been developed and studied in extruded products such as crispness, crunchiness, hardness, bittleness and sticky mouth coating. The analysis can include all parameters of the product, or it can be limited to certain aspects. This technique is appropriate for use when detailed information is required on individual characteristics of the product and material.

There are several different methods of descriptive analysis, including the Flavour Profile Method (Cairncross and Sjo¨ strom, 1950), Texture Profile Method (Brandt *et al*., 1963), Quantitative Descriptive Analysis™ (Stone *et al*., 1974), the Spectrum™ method (Meilgaard *et al*., 1991), Quantitative Flavour Profiling (Stampanoni, 1993a,b), Free-choice Profiling (Langron, 1983; Thompson and MacFie, 1983) and generic descriptive analysis.

QDA® has been applied to many diverse categories of foods including sweetener (Portmann and Lilcast, 1998; Matta *et al*., 2005; Anupama *et al*., 2003), meat flavour (Rababah *et al*., 2005; Olafsdottir *et al*., 2005), irradiated beans product (Armelim *et al*., 2006), sipaghett (Zhao *et al*., 2005), and in extruded snack products such as corn and soy blends (Faller *et al*., 1999), maize (Murray, 2001), oat-corn blends (Liu *et al*., 2000), cassava and pigeonpea blends (Rampersad *et al*., 2003).

The subjects for QDA® methodology were screened with dietary questionnaires (Sawyer *et al*., 1962). The language source is non-technical, everyday language, to avoid biasing response behaviour that may occur by providing a language. Reference standards are only used in QDA® when a problem with a particular term is identified and it is expected that subjects only need references 10% of the time (Stone and Sidel, 1993). The panel is trained over a period of perhaps 10–15 hour to understand the meaning of the attributes. The designs for descriptive

analysis are based on repeated measures and the statistical analyse is generally conducted using Analysis of Variance. Often the cobweb or spider diagram is used to graphically represent the data.

7. The texture measuring instruments for extruded snack

The texture instrumental methods are normally used to evaluate the structural properties and texture of product. The instrumental assessment, by textural equipment, has been related to sensory evaluation, such as hardness and crispness in the case of extruded snacks. However, there are several different machines and heading instruments for measure the maximum force (hardness) of extruded snacks. The Instron universal testing machine and Kramer press have been reported to evaluate the hardness of maize extrudates (Gonzalez *et al*., 2004), wheat extrudates (Case *et al*., 1992), cottonseed/maize extrudates (Camire *et al*., 1991) and adlay species and rice flour extrudates (Yang *et al*., 2008).

The using of cylinder probe with TA-XT2 texture analyser to measure crispness of extrudates has been reported by Ding *et al*. (2005; 2006) who studied the effect of extrusion conditions on the physical properties of rice and wheat expanded snacks, Mezreb *et al*. (2006) who studied the effect of sucrose on the textural properties of corn and wheat extrudates. Liu *et al*. (2000) also used this instrument to compress the sample. Texture parameters such as fracturability, cohesiveness, adhesiveness, hardness and chewiness of extruded oat-corn puff were calculated.

The method to determine the breaking strength of extrudates using a TA-XT2 texture analyzer equipped with a Werner Bratzler shear apparatus was applied for measure hardness and crispness of several extrudates such as maize extrudates (Ramırez and Wanderlei,1998), cow pea and sorghum extrudates (Falcone and Phillips, 1988), rice based extrudates (Grenus *et al*., 1993; Bhattacharya, 1996; Iio *et al*., 1999; Choudhura and Gautam, 2003; Guha and Ali, 2006), whey products in extruded corn, potato or rice snack (Onwulata *et al*., 2001) and corn-whey extrudates (Allen *et al*., 2007). Shear value was taken as the maximum forces require breaking

the extrudate. The breaking strength index of dry extrudate was determined as the ratio of the maximum force during shearing and the corresponding cross-sectional area of the extrudate.

MATERIALS AND METHODS

Materials

1. Raw materials

1.1 Polished glutinous rice was obtained from Davis Trading Co. Ltd., New Zealand (import from Thailand).

1.2 Vital wheat gluten was obtained from Davis Trading Co. Ltd., New Zealand

1.3 Toast soy grits was obtained from Oppenheimer New Zealand Ltd., New Zealand

1.4 Fish meal was obtained from ABB Co.Ltd., New Zealand

1.5 Polyethylene bag (thickness of 195 μm), size 450x750 mm, Contour Packaging and Precision Injection Moulding Co. Ltd., New Zealand

1.6 Foil pouch, thickness 99 micron (PET(12)/AL(7)/LLDPE(80)), size 200x300 mm., Contour Packaging and Precision Injection Moulding Co. Ltd., New Zealand

1.7 Barbeque and Cheese seasoning from Thai Quest Fragrances, Flavours, Food Ingredients Ltd., Thailand

2. Analytical reagents

2.1 Kjeltabs (each containing 3.5g K_2SO_4 and 0.0035g Se) was obtained from Neu-tec Group Inc., New York, USA.

2.2 Sulfuric acid (95-97 % H_2SO_4), N-free, was obtained from Biolab Limited, Auckland, New Zealand.

2.3 Boric acid (H_3BO_3), reagent grade, N-free, was obtained from Biolab Limited, Auckland, New Zealand.

2.4 Sodium hydroxide (NaOH), reagent grade, was obtained from Biolab Limited, Auckland, New Zealand.

2.5 Hydrochloric acid (HCl), reagent grade, was obtained from J.T.Baker Chemical Co., Phillipsburg, New Jersey, USA.

2.6 Bromocresol green, indicator, was obtained from Fisher Chemicals, New York, USA.

2.7 Methyl red, indicator, was obtained from Fisher Chemicals, New York, USA.

2.8 Trichloroacetic acid (TCA), reagent grade, was obtained from Biolab Limited, Auckland, New Zealand.

2.9 Formic acid, reagent grade, was obtained from Biolab Limited, Auckland, New Zealand.

2.10 Barium hydroxide octahydrate, reagent grade, was obtained from Biolab Limited, Auckland, New Zealand.

2.11 O-methylisourea (sulphate salt) reagent grade, was obtained from Biolab Limited, Auckland, New Zealand.

3. Processing equipments

3.1 Drum mills, I.M.E., Crop and Food Research Ltd., New Zealand

3.2 Thunderbird bakery mixer, Model ARM--02 supplied by Thunderbird Food Machinery Inc., Blaine, WA, USA

3.3 A co-rotating Clextral twin-screw extruder (Model BC21; Clextral, Firminy Cedex, France)

4. Analytical equipments

4.1 Kjeltec System, 1026 distilling unit and 2006 digester (Tecator AB), Sweden

4.2 Forced air oven, American

4.3 Scientific Products DK-62, USA

4.4 Flask shaker, Chiltern Inc.Super-speed refrigerated centrifuge plus Sorvall RC-5C automatic, rotor SM-24, Kendro Laboratory Product Inc.

4.5 Waters ion exchange high performance liquid chromatography system,

post column ninhydrin detection, Waters Corporation, Milford Massachusetts, USA.

4.6 Automatic speedvac AS 290 Savant Inc.

4.7 Sonicating water baths U50, Wolf Laboratories Limited , USA

4.8 Vernier caliper, B Dial Type, Electron Microscopy Sciences, USA

4.9 Texture analyzer TA-XT2 (Stable Micro Systems, Surrey, England), with a 500 N load cell and a Warner-bratzler shear cell (1-mm thick blade)

4.10 Minolta spectrophotometer CM-3500d equipped with a D65 illuminant, Minolta Co. Ltd. , USA

Methods

1. Preparation of raw material

The polished glutinous rice was milled using a drum mill and the fraction passing through a 70 mesh (210 μm diameter) sieve was taken for the experiments. Rice, wheat gluten and soy grits formulations, predetermined using linear programming were mixed, packed into polyethylene bags and allowed to equilibrate overnight at 5°C. Prior to extrusion, the moisture content of each mixture was determined in a hot air oven at 105 $^{\circ}$C for 3-5 hour according to the AOAC (1990) and these data were used to calculate the rate of added feed moisture during extrusion.

2. Extrusion process

2.1 Twin-screw extruder system

A co-rotating Clextral twin-screw extruder with intermeshing screws was used in this study (Figure 1). Screw speed, material feed rate, and temperature were monitored from a control panel (Figure 2). A 3 mm diameter circular die was used (Figure 3 (a)). The barrel which houses the twin screws had seven 100 mm long sections with induction heaters mounted on each section and bored with two 25 mm diameter holes. Length of the twin-screw was 700 mm (Figure 3 (b)). Thermal energy was provided by induction heaters mounted on each barrel section.

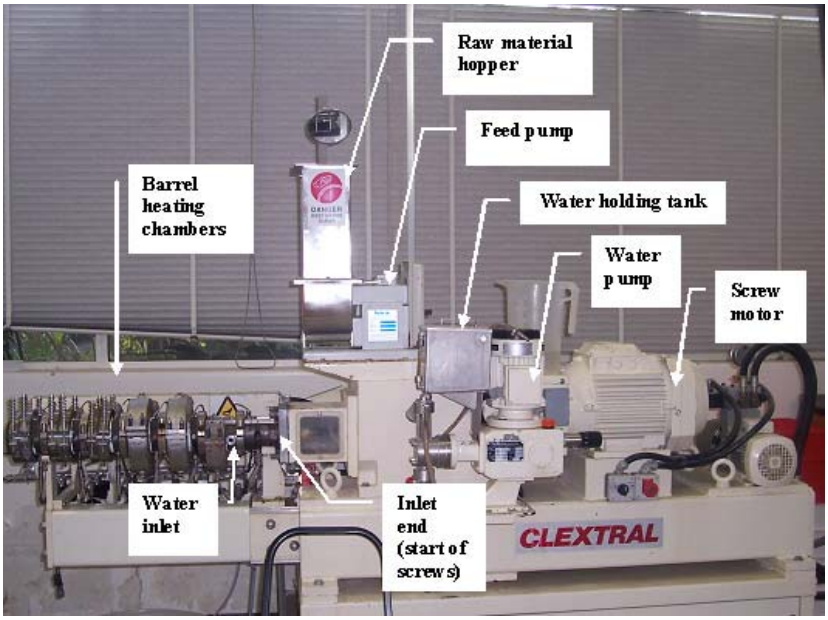


Figure 1 Overview of Clextral BC21 twin-screw extruder.

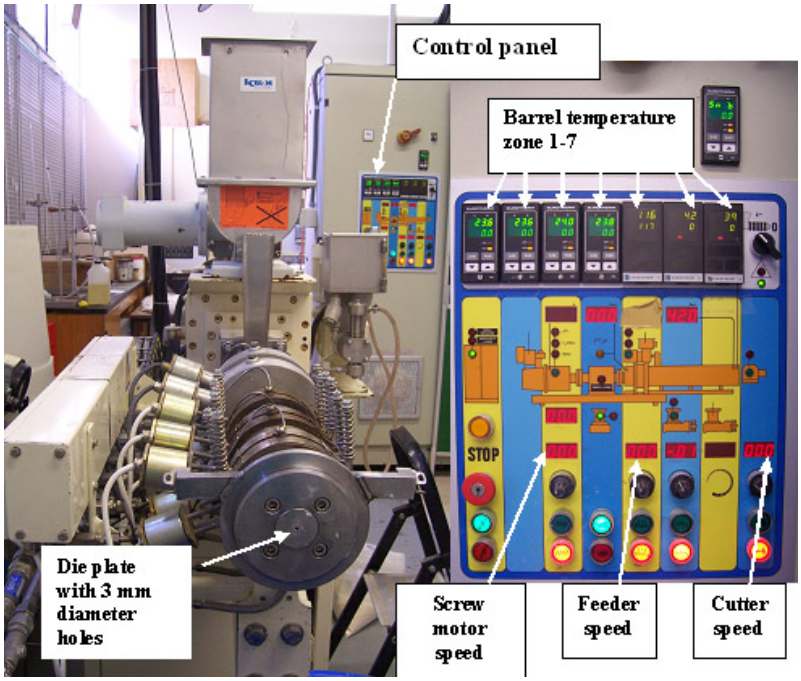
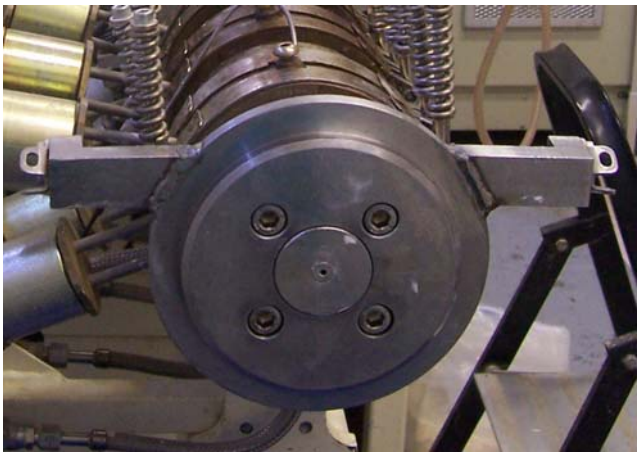
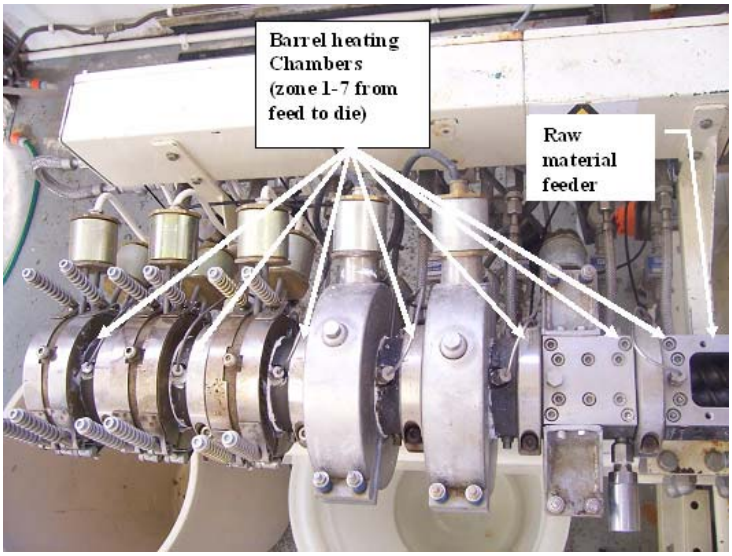


Figure 2 Control panel of the Clextral BC21 twin-screw extruder.



(a)



(b)

Figure 3 Die configuration used for Clextral BC21 extruder (a), Barrel heating elements on the Clextral BC21 twin-screw extruder (b).

The twin-screws have segmental screw elements so that mixing elements such as reverse screw element (RSE(s)) and kneading elements (KE(s)) can be placed at a desired location along the length of a splinted shaft. The RSE used was 25 mm long with a pitch of 16.6 mm. Four 6.25 mm thick KE(s) staggered alternately at 77^{O} and 103^{O} were combined to give a total length of 25 mm as shown in Figure 4 a, b.

The screw configuration was applied from previous studies of Gogoi *et al*. (1996) and Choudhury and Gautam (1998), as follows.

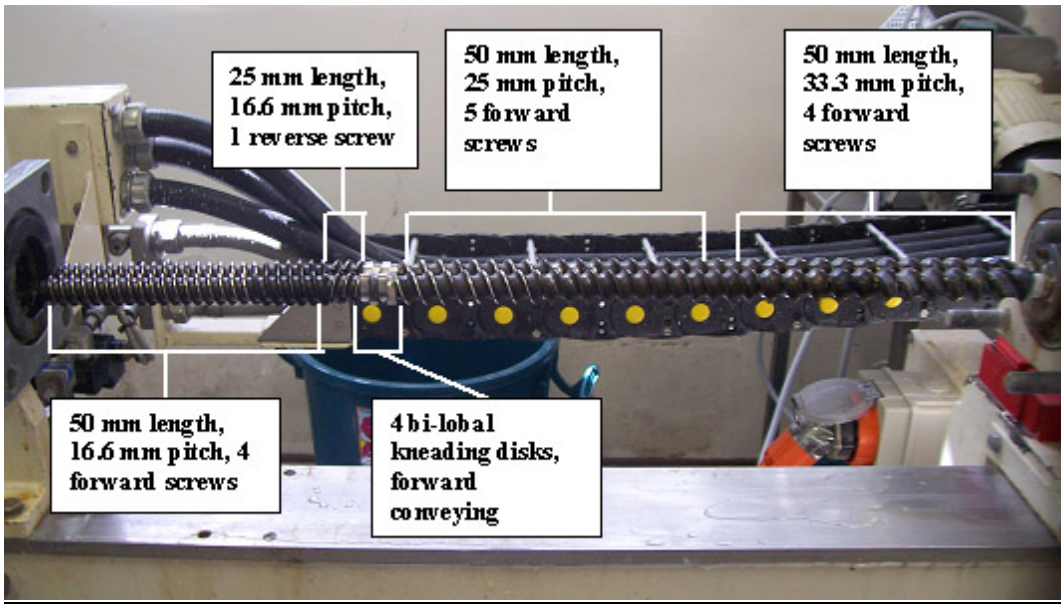
33.3/4/50[a]
25/5/50
KE/77/1/6.25[b]
KE/103/1/6.25
KE/77/1/6.25
KE/103/1/6.25
LH 16.6/1/25[c]
16.6/4/50
Total length 700 mm

[a] Pitch (mm)/ number of screw elements /length (mm).

[b] KE/stagger angle ($^{\circ}$) / number of KE/length (mm).

[c] LH, left hand pitch (RSE), pitch (mm)/ number of screw elements /length (mm).

(a)



(b)

Figure 4 Screw elements (a), Screw configuration for Clextral BC21 twin-screw extruder (b)

Raw material was fed from the raw material hopper into the extruder inlet port by a twin-screw feeder (Model T20, Clextral). The feeder was calibrated to determine the set point of the rotary feeder switch for rice flour-protein mixture to obtain a flow rate of 12 kg h^{-1} for all the experimental runs (Figure 5).

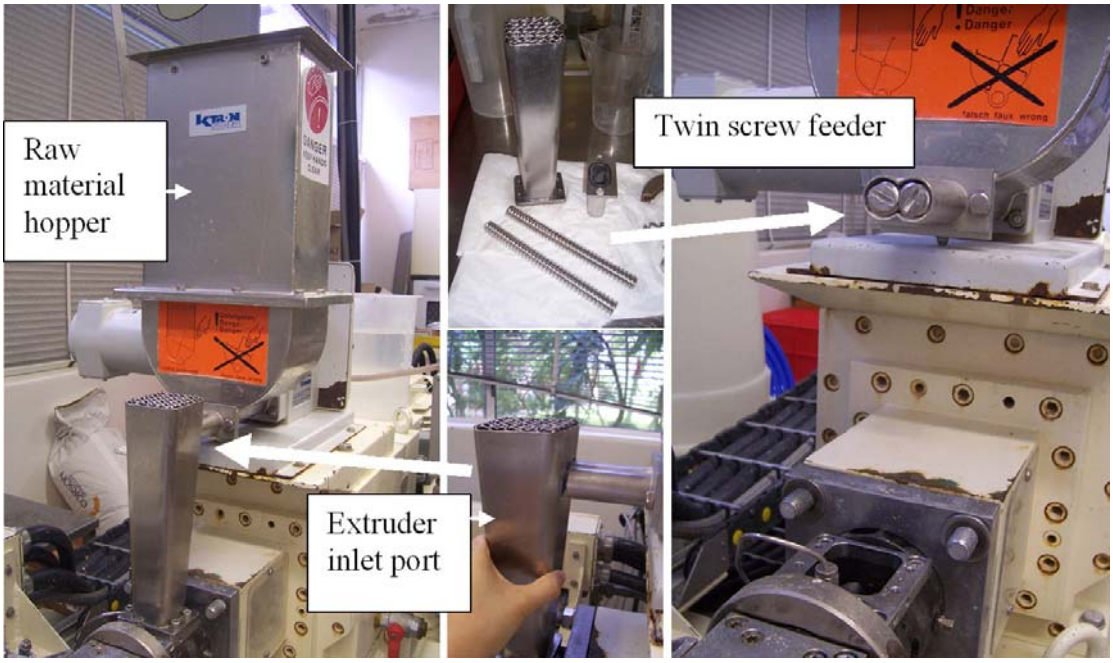


Figure 5 Feeding on the Clextral BC21 twin-screw extruder.

Water was pumped into the extruder using a variable speed water pump and was mixed with the raw material at the front set of the kneading disks (Figure 6). The moisture content of the product during extrusion prior to expansion was controlled using a control pump. The in-barrel moisture content was calculated using equation 1.

$$X_P = (FX_F + W)/ F+W \qquad (1)$$

X_P = the product moisture content during extrusion (%)

X_F = the moisture content of feed mixture (%)

W = the water flow rate (l/h) dosed to the extruder

F = the feed rate (l/h)

As the product moved through the forward kneading elements, it was mixed and kneaded by rotation of the screws. During this time, the product was heated by both the friction produced as it moved through the screws and external electrical heating in the barrels. The temperature was maintained at a constant set value by the external heating/cooling chambers thermostatically controlled via an electronic control panel. Temperatures at the extruder barrel zones 1-4 (from feed to die) were maintained constant at 30, 30, 70, and 100 ^{O}C, respectively. The next zones 5-7 were set to the desired barrel temperature.

At the discharge end of the extruder, the product was forced through the die. As it exited the extruder to the atmosphere, the product expanded because of the pressure drop across the die which allowed water to flash off as steam. Extrudates were cut to a uniform size using a cutter blade speed set at 46 RPM (Fig 7).

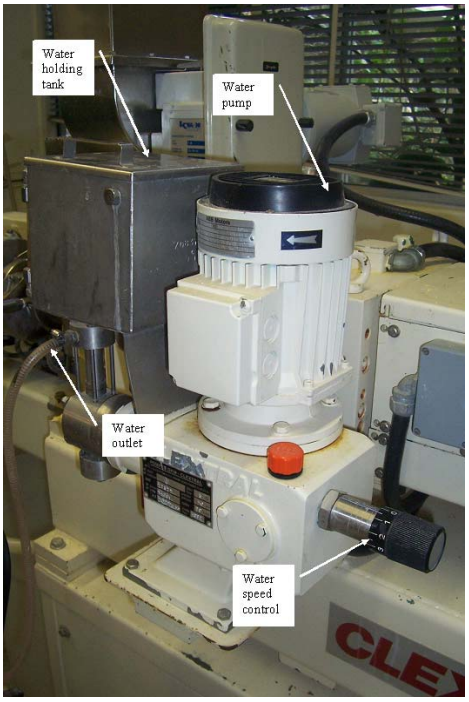


Figure 6 Water pump on the Clextral BC21 twin-screw extruder.

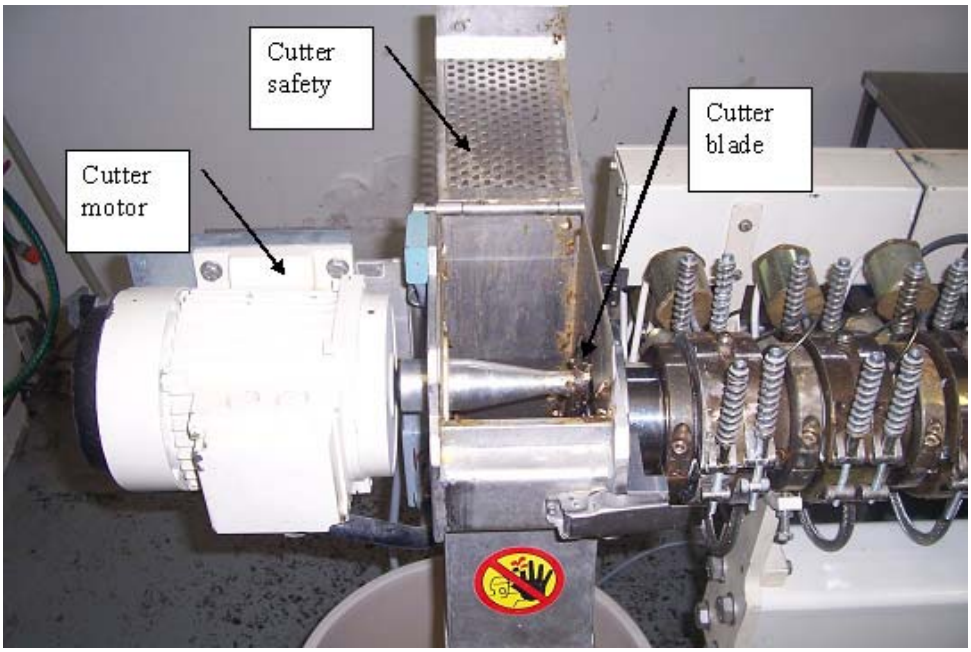


Figure 7 Cutter on the Clextral BC21 twin-screw extruder.

2.2 Twin-screw extruder start-up

The screw elements were fitted to the extruder shafts in the desired screw configuration. The extruder barrel was closed and the die plate attached. The heating chamber temperatures were set on the control panel and the chambers were heated prior to beginning the extrusion process. The raw material hopper and the water chamber were filled with mixture of materials and water, respectively. The screw speed was set to the desired speed and the water pump was set to pump at a rapid water feed rate of 1.39 $kg.h^{-1}$. Once steam exited the die, the material was feed slowly into the extruder at a rate of 0.05 $kg.h^{-1}$. When the product began to exit the die, the water feed rate was reduced and the materials feed rate was slowly adjusted to the 12 $kg.h^{-1}$ for all the experimental runs. During this time, the torque was always kept under 30 Nm so that the screws did not become blocked with product. When the materials and water feed rates were finally established, the extruder was run for a further 15 minutes to ensure that a steady torque had been reached before extrudates

were collected. The extrudates were then placed into high-density polyethylene bags (thickness of 195 μm) for physical analysis (approximately one week). Approximately 100 g of extruded samples were ground to a powder in a coffee mill and then passed through a US standard 40-mesh sieve (400 μm). The meal samples were packed in aluminium foil bags, vacuum-sealed and stored at -18 ^{O}C until required for chemical analyses. The extrudates exited the die and were stored in high density polyethylene bags (thickness 195) and vacuum-sealed for transport to Kasetsart University for sensory analysis. Upon arrival at Kasetsart University, all extrudates were stored at -18 ^{O}C until required for sensory analyses (stored about 2 mouths). All extrudate samples used in sensory section were dried at 65 $\pm$ 2 °C in a tray dryer until a final moisture content of 3-4% before sensory test.

3. Optimisation of formula and process

3.1 Preliminary study

A linear-programming (Microsoft Excel) was developed to formulate three different mixtures at minimum cost. The price, proximate composition and amino acid profile for each of the raw materials were provided by suppliers or sourced from the literature (as seen in Appendix A). The constraints were set as follows: (i) protein content was kept constant at one of three levels, namely either 20, 25 or 30 g protein per 100 g of final food; (ii) the product was to contained lysine and sulphur amino acids to meet the FAO/WHO/UNU (1985) recommended pattern (lysine 44 mg/g protein, sulphur amino acids 22 mg/g protein for schoolchildren 10-12 year); (iii) the highest single ingredient (by weight) was to be glutinous rice; (iv) each equation was also subjected to the overall material balance equation $\sum Xi = 1$ or ingredients ≥ 0. The details are presented in Table 6:

Table 6 Linear programme model for preliminary experimental formulation.

Concern	Function	Eq.
Objective: Formulation for minimum cost per kg	$Z_{\min} = \sum_{i=1}^{n} C_i X_i$	(2)
Subject to the constraints:		
Protein requirement: protein content was kept constant at one of three levels		
Formulation 1: protein content 20 g per 100 g of food	$\sum_{i=1}^{n} PiXi \geq 20$	(3)
Formulation 2: protein content 25 g per 100 g of food	$\sum_{i=1}^{n} PiXi \geq 25$	(4)
Formulation 3: protein content 30 g per 100 g of food	$\sum_{i=1}^{n} PiXi \geq 30$	(5)
Lysine requirement: lysine 4.4 g per100g of protein	$\sum_{i=1}^{n} LiXi \geq 4.4$	(6)
Sulphur amino acid requirement: sulphur amino acid 2.2 g per100g of protein	$\sum_{i=1}^{n} SiXi \geq 2.2$	(7)
Ingredient limit: the highest single ingredient (by weight) of product is glutinous rice flour	$X_1 \geq 0.5$	(8)
Overall limit: each group of equations was also subjected to the overall material balance equation	$\sum_{i=1}^{n} Xi = 1$	*(9)*

Table 6 (Continued)

Concern	Function	
Proportion ingredient limit: the proportion in which ingredient i no less than 0	$Xi \geq 0$	(10)

Where: Z = the cost per kilogram of each formulated mixture

C_i = the cost of ingredient i

Pi = the protein content of ingredient i

Li = the lysine content of ingredient i

Si = the sulphur amino acid content of ingredient i

X_i = the proportion in which ingredient i is incorporated in the formulation

X_1 = the proportion of glutinous rice flour

n = the number of ingredients included in the program

Each model was solved mathematically using Excel Solver (Microsoft Office Excel 2003, Microsoft Corporation).

A 3x3x2x2 factorial plan in CRD was employed investigate the influence of feed protein content (20, 25 and 30 %), feed moisture (20, 25, and 30 %), barrel temperature (150 and 180 °C) and screw speed (200, 400 rpm) on the chemical properties (moisture, protein content) and physical properties (bulk density, expansion and breaking strength) of extrudates.

3.2 Formulation development and optimisation

A linear-programming feasibility model was developed to formulate two different mixtures at minimum cost which the studied mixture contained 20 or 30 g protein per 100 g of final food. A 2x3x2 factorial in CRD was employed investigate the influence of feed protein content (20 and 30 g/100 g wb), feed moisture (20, 25, and 30 g/100g wb) and barrel temperature (150 and 180 °C) on the chemical

properties (moisture, protein, non protein nitrogen (NPN), lysine, cysteine and methionine content), physical properties (bulk density, expansion and breaking strength) and sensory evaluation of extrudates.

4. **Determination of quality properties of materials and extrudates**

4.1 Chemical analysis

4.1.1 Moisture content

The moisture content of materials and extruded samples were determined in a hot air oven at 105 °C for 3-5 hours according to the A.O.A.C (1990). Measurements were performed in triplicate.

4.1.2 Total protein and non-protein nitrogen content (NPN)

Raw materials and extrudate samples were assayed for total nitrogen and crude protein (Nx6.25) by the Kjeldahl method (A.O.A.C, 1990) (method as shown in Appendix B1). NPN was determined by Kjeldahl analysis following the precipitation of the protein using trichloroacetic acid (TCA) (Awolumate, 1983) as shown in Appendix B1. True Protein was calculated as the difference between total and NPN contents as recommended by Periago *et al*. (1996). Measurement was performed in triplicate.

4.1.3 Amino acid analysis

The amino acid profiles of each raw material and the extruded samples were determined by Nutrition Laboratory, Institute of Food, Nutrition and Human Health Massey University using a Waters ion exchange high performance liquid chromatography system, utilizing post column ninhydrin detection and determined from the absorbance at 570 nm (method as shown in Appendix B2). Raw materials and extrudate samples were hydrolysed in 6 M glass-distilled HCl

containing 0.1% phenol for 24 h at 110 $\pm$ 2 °C in evacuated sealed tubes. Cystine (Cysi) and Methionine (Met) were oxidized by preformic acid prior to acid hydrolysis (Spackman *et al*., 1958). Reactive lysine was determined using the guanidination method as described by Rutherfurd and Moughan (1997). The samples were incubated for 7 days in 0.6 M O-methylisourea (pH 10.6) at 21$\pm$ 2 °C in a shaking water bath, with the reagent-to-lysine ratio greater than 1000, before being dried down and analyzed. The experiment was performed in duplicate.

4.2 Physical analysis

4.2.1 Bulk density

Bulk density was measured by determining the mass and apparent volume of individual dry, cylindrical extruded rods (Ali *et al*, 1996; Alvarez-Martinez *et al*, 1988). Apparent volume was calculated as the product of length and cross-section area of the extruded rods. Six extrudate cylinders were randomly selected from each sample. Ten diameters and five lengths were measured at different points of each cylinder. The average values for 6 extrudate cylinders were used for calculation of density of each sample (Eq. (11)):

$$\text{Density}(\text{g/cm}^{-3}) = 4m / \pi D^2 L \qquad (11)$$

Where: m = mass
L = length
D = diameter

4.2.2 Expansion ratio

Sectional expansion, the ratio of diameter of extrudate and the diameter of die, was used to express the expansion of extrudate (Alvarez-Martinez *et al*, 1988; Fan *et al*., 1996). Ten measurements of diameter were taken at 90° to each

other for each of the five extrudate rods. The extrudate diameter was the average of 50 random measurements. The vernier caliper measured within + 0.01 mm.

4.2.3 Breaking strength index (BSI)

Breaking strength of extrudate was determined using a texture analyzer TA-XT2 (Stable Micro Systems, Surrey, England), with a 500 N load cell and a Warner-Bratzler shear cell (1-mm thick blade) (as shown in Appendix Figure C1). The extrudates were analyzed at a cross head speed of 0.2 mm/s. Twenty randomly collected samples of each extrudate were assayed for each treatment. Breaking strength index (BSI) was calculated using(Eq. (12)):

$$\text{BSI} = \text{Peak breaking force (N)/extrudate diameter (mm).} \qquad (12)$$

4.2.4 Colour measurement

The colour of extruded snacks was measured with a Minolta spectrophotometer CM-3500d equipped with a D65 illuminant (as show in Appendix Figure C2). The glass container of the spectrophotometer was filled with the powder and shaken for 10 seconds. The glass container was filled to the Minolta instrument and the measurement begun. After the first measurement, the container was rotated 90 degrees and the measurement performed again. The procedure was repeated at 180 and 270 degrees. The instrument automatically averaged the four measurements results. This procedure was repeated with two other samples. The average value of three measurements was reported. Color readings were displayed as $L^* a^* b^*$ values (CIE, 1976) where L^* represents lightness/darkness dimension, positive and negative a^* value indicates redness and greenness respectively and b^* indicates yellowness for positive and blueness for negative values.

4.3 Sensory evaluation

4.3.1 Descriptive analysis

4.3.1.1 Panel recruitment and screening

Candidates for the sensory panel were recruited to participate in sensitivity screening tests based on consumption of snack extrusion, interest in testing, and verbal/social skills, availability and commitment to sensory program. The panelists comprised mainly the staffs and students of the Kasetsart Univesity, who had good experience in sensory evaluation of snack products. From this group, ten were selected based on their performance in discrimination tests.

4.3.1.2 Descriptor and panel training

Descriptor generation sessions, each approximately one hour, was conducted. All assessors were present at round table discussion and the entire ranges of extruded snack product variants were available for tasting. Assessors tasted a range of the snacks and freely generated terms to describe their attributes, which were then recorded by the panel leader. Total three descriptor generation sessions of 3 h each were dedicated to lexicon development (attributes, definitions, and reference). Once decided, definitions for each attribute were established by the panel leader using existing literature (Meilgaard, 1999; Murray, 2001) and comments from each session. Assessors then amended or changed the definitions as was necessary and established the frame of comparison (reference points) by which to rate each product, in a further session.

The training involved seven sessions during which the panelists were familiarized with different attribute notes using reference samples and also trained in performing the Quantitative Descriptive Analysis (QDA) test (Stone and Sidel, 1993; Murray, 2001; Chambers IV and Wolf, 2005). The performance of panelists was checked by giving duplicate samples at the start of each testing session.

4.3.1.3 Descriptive sensory analysis

The method of intensity scaling used was QDA method (questionnaire shown in Appendix D2). The scorecard consisted of 15 cm QDA scale wherein 1.25 cm was anchored as 'low' (Recognition threshold) and 13.75 cm as 'high' (Saturation threshold). The panelists were asked to mark the perceived intensity of the attribute by drawing a vertical line on the scale and writing the code. The panelists were well trained in performing this test. All extrudate samples used in this section were dried at 65 $\pm$ 2 °C in a tray dryer until a final moisture content of 3-4%. Testing was performed in a sensory laboratory with individual booth under fluorescent lighting equal to daylight. Five pieces of extruded snack sample, at room temperature (25 $\pm$ 2 $^{\circ}$C), was served in 6x10 cm plastic bag labeled with randomly assign 3 digit numbers. Only one sample was served at a time and the presentation of the samples was randomized. A duplicate was given at each session just to check the panel performance and the data arising from this duplicate sample was not used for the final analysis. The duplication of sample was also randomized for each panelist. It was found that there was no significant difference among the duplicates, indicating good panel consistency ($P \geq 0.05$). Water was used as palate cleansing materials in between the samples. Sensory analysis of 12 samples of extruded sample was done in four sessions. All members of the panel assessed three samples and a duplicate two samples, in triplicate on different days in the sensory laboratory.

4.3.2 Acceptability test

A preliminary product selection was made in order to reduce the number of samples to be submitted to the consumer panel which was made up by untrained judges. The extruded samples, without coat seasoning, were dried at 65 $\pm$ 2 °C in a tray dryer until a final moisture content of 3-4 % . The samples were evaluated in appearance, flavour, texture, colour and overall acceptability using nine-point hedonic scale with anchor points; 1-extremely dislike to 9-extremely like (Lawless and Heymann, 1998) questionnaire shown in Appendix D3. The subjects were 50 young graduate students, between 18 and 35 years old, that declared themselves frequent consumers of extrusion snack (consuming at least 1 bag per week).

Evaluations were conducted under white fluorescent light in partitioned booths. After an oral explanation of test rules and usages of scales, the sample was located into individual booths. The volunteers received four pieces of each sample in 6x10 cm plastic bag labeled with randomly assign 3 digit numbers. All the judges tasted the twelve samples, over four sessions of three products, each one. The products were randomly presented. Water for rinsing was supplied to panels between samples.

5. Enrobed extrudates acceptability test

The selected extrudates from panalists was enrobed with barbeque or cheese seasoning (Thai Quest Fragrances, Flavours, Food Ingredients Ltd., Thailand) and rice bran oil to a final formulation of various amounts (4, 8 and 12 %) of seasoning and 15 % oil (Hanify, 2001). The consumer panelists evaluated the enrobed flavored extrudates during 2 sessions for colour, odour, saltiness, flavour, hardness and overall liking using nine-point hedonic scale with anchor points; 1-extremely dislike to 9-extremely like. A CRD was employed investigate the influence of seasoning coated on the sensory acceptance and overall lining. The panels were also asked to about right or require improvement using just-about-right (JAR) rating scale; 1-extremely decrease to 7-extremely increase (Edgar and Mona, 2005) questionnaire shown in Appendix D4. The volunteers received four pieces of each sample in 6x10 cm plastic bag labeled with randomly assign 3 digit numbers. Only one sample was served at a time and the presentation of the samples was randomized. Fifty panelists were selected on the basis of product usage of other extruded products.

6. Statistical analysis

The data were subjected to analysis of variance (ANOVA) using general linear model procedure, SPSS for Window Version 12.00 (SPSS Inc., Thailand). Means comparison was performed using Duncan's Multiple Range Test. Response surface methodology was used to determine the effects of protein content, feed moisture and

barrel temperature on chemical and physical properties by generating second-order polynomial equations (Hu, 1999) (Eq. (13)):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{22} X_2^2 \qquad (13)$$

where Y represents the experimental response, β_o, β_1, β_2, β_3, β_{12}, β_{13}, β_{23} and β_{22} are coefficients for intercept, linear, interactive and quadratic effects, respectively, and X_1, X_2 and X_3 are independent variables (X_1 = protein content, X_2 = feed moisture, X_3=barrel temperature). Response surface were plotted using the Statistica for Windows Version 5.0 (Statsoft Inc.) package.

Correlation analysis was performed on SPSS for Window Version 10.0 (SPSS Inc.) using a Bivariate Correlations procedure for chemical, physical and sensory analysis.

RESULTS AND DISCUSSION

1. Preliminary study

A linear programming model was developed to formulate three different mixtures of lowest possible cost base on glutinous rice and fulfilling the nutritious protein and technical constraints of the desired product. The composition and cost of these formulations are described in Table 7. Protein content for formulations 1, 2 and 3 was 20, 25 and 30 %, respectively. This was in agreement with the standards recommended by the FAO/WHO/UNU guidelines (1985). Formulation 1 gave the lowest cost per kilogram (35.36 baht), while formulation 3 gave the highest cost per kilogram (38.48 baht). This result means that the cost of product was increased with an increasing of protein content of extrudates, because the protein material had a high costly ingredient.

In this section, the constrain of using < 20% fish meal in the formula was added in the linear program, because the product had satisfied texture. Preliminary trails found that if more than 20 % of fish meal was used in the formula, it resulted in a sand texture in the product. The program included the maximum quantity of fish meal, gluten and soy protein necessary to increase the protein content of the final mixtures (Table 7).

The extrudates appearance is shown in Appendix Figure D1. All extruded samples had a grey colour and disagreeable taste; such as fishy smell, bitterness and acrid taste, which is the common characteristic of fish meal. The raw fish meal was made from two types of fish, whole skipjack and anchovy both caught off the coast of Peru. The skipjack is dark blue to black on the back, which becomes silvery or white below, with four to six long dark horizontal stripes on the belly. It also has bitterness and acrid taste. Therefore, we need to remove this raw material from the data study list and re-calculate new formulations.

Table 7 Minimal cost formulations calculated from linear programming.

Ingredients in mixture	Weight (%)	Protein (%wb)	Lysine (g/100g protein)	Methionine+ Cystine (g/100g protein)	Cost[1] (baht/kg)
Glutinous rice flour	75.00	4.96	2.96	2.85	
Fish meal	12.00	8.40	0.62	0.32	
Toasted soy grits	13.00	6.64	0.81	0.36	
Formulation 1		20.00	4.40	3.60	35.36
Glutinous rice flour	68.00	4.48	2.67	2.57	
Fish meal	20.00	13.64	1.01	0.52	
Vital wheat gluten	2.00	1.31	0.03	0.07	
Toasted soy grits	11.00	5.58	0.68	0.31	
Formulation 2		25.00	4.40	3.46	35.62
Glutinous rice flour	60.00	3.93	2.35	2.26	
Fish meal	20.00	13.64	1.01	0.52	
Vital wheat gluten	6.00	4.86	0.11	0.25	
Toasted soy grits	14.00	7.57	0.93	0.41	
Formulation 3		30.00	4.40	3.45	38.48

[1] Costs as of April, 2005 (1 $ = 26 baht).

The effect of protein content (20, 25, 30 %) and extrusion conditions, including feed moisture (20, 25, 30%), barrel temperature (150, 180 °C), and screw speed (200, 400 RPM) on the moisture, total protein, bulk density and expansion ratio of extrudates was investigated. Mean values and standard deviation for chemical and physical properties are shown in Table 8.The ANOVA results summarized in Table 9.

Table 8 Chemical and physical properties of glutinous rice, fish meal, soy and gluten extrudates.

Treatment	Protein (%)	Moisture (%)	Temperature (°C)	Screw speed (RPM)	Moisture (%)	Protein (%db)	Bulk density (g/cm^3)	Expansion ratio
1	20	20	150	200	7.26 ± 0.08[1]	19.41 ± 0.36[1]	0.22 ± 0.01[2]	2.85 ± 0.05[2]
2	20	20	150	400	5.26 ± 0.10	19.36 ± 0.17	0.12 ± 0.01	3.20 ± 0.08
3	20	20	180	200	6.62 ± 0.07	19.48 ± 0.05	0.17 ± 0.01	2.84 ± 0.02
4	20	20	180	400	6.29 ± 0.12	19.34 ± 0.08	0.11 ± 0.003	3.22 ± 0.05
5	20	25	150	200	9.28 ± 0.05	19.59 ± 0.40	0.30 ± 0.02	2.55 ± 0.07
6	20	25	150	400	8.73 ± 0.06	19.22 ± 0.49	0.22 ± 0.01	2.72 ± 0.04
7	20	25	180	200	7.90 ± 0.09	19.39 ± 0.13	0.23 ± 0.01	2.54 ± 0.02
8	20	25	180	400	7.44 ± 0.10	19.45 ± 0.07	0.19 ± 0.01	2.64 ± 0.02
9	20	30	150	200	10.95 ± 0.05	19.14 ± 0.03	0.43 ± 0.02	2.23 ± 0.04
10	20	30	150	400	10.20 ± 0.05	19.38 ± 0.28	0.30 ± 0.01	2.57 ± 0.06
11	20	30	180	200	9.23 ± 0.07	19.42 ± 0.15	0.26 ± 0.02	2.34 ± 0.05
12	20	30	180	400	9.03 ± 0.12	19.48 ± 0.10	0.23 ± 0.02	2.53 ± 0.05

Table 8 (Continued)

Treatment	Protein (%)	Moisture (%)	Temperature (°C)	Screw speed (RPM)	Moisture (%)	Protein (%db)	Bulk density (g/cm^3)	Expansion ratio
13	25	20	150	200	7.97 ± 0.02	24.52 ± 0.11	0.27 ± 0.03	2.30 ± 0.14
14	25	20	150	400	5.63 ± 0.04	24.18 ± 0.21	0.15 ± 0.01	2.71 ± 0.06
15	25	20	180	200	5.51 ± 0.18	24.16 ± 0.09	0.18 ± 0.01	2.38 ± 0.08
16	25	20	180	400	5.14 ± 0.11	24.24 ± 0.38	0.12 ± 0.01	3.02 ± 0.10
17	25	25	150	200	9.95 ± 0.09	24.14 ± 0.05	0.43 ± 0.03	2.04 ± 0.09
18	25	25	150	400	8.89 ± 0.12	24.34 ± 0.07	0.28 ± 0.03	2.52 ± 0.04
19	25	25	180	200	8.11 ± 0.10	24.54 ± 0.11	0.24 ± 0.03	2.47 ± 0.04
20	25	25	180	400	7.95 ± 0.07	24.35 ± 0.16	0.24 ± 0.10	2.52 ± 0.03
21	25	30	150	200	11.86 ± 0.13	24.53 ± 0.16	0.49 ± 0.02	1.97 ± 0.05
22	25	30	150	400	11.06 ± 0.10	24.41 ± 0.11	0.39 ± 0.02	2.25 ± 0.05
23	25	30	180	200	9.79 ± 0.04	24.74 ± 0.03	0.32 ± 0.01	2.25 ± 0.03
24	25	30	180	400	9.61 ± 0.05	24.33 ± 0.05	0.31 ± 0.02	2.34 ± 0.04

Table 8 (Continued)

Treatment	Protein (%)	Moisture (%)	Temperature (°C)	Screw speed (RPM)	Moisture (%)	Protein (%db)	Bulk density (g/cm^3)	Expansion ratio
25	30	20	150	200	7.45 ± 0.13	29.33 ± 0.14	0.31 ± 0.03	1.86 ± 0.07
26	30	20	150	400	5.69 ± 0.11	29.14 ± 0.32	0.17 ± 0.01	2.38 ± 0.04
27	30	20	180	200	5.39 ± 0.05	28.98 ± 0.03	0.25 ± 0.04	2.06 ± 0.08
28	30	20	180	400	5.34 ± 0.16	29.13 ± 0.23	0.15 ± 0.03	2.46 ± 0.23
29	30	25	150	200	9.69 ± 0.11	28.96 ± 0.10	0.42 ± 0.01	2.03 ± 0.03
30	30	25	150	400	8.81 ± 0.07	29.04 ± 0.12	0.31 ± 0.02	2.37 ± 0.01
31	30	25	180	200	8.30 ± 0.07	29.06 ± 0.22	0.27 ± 0.07	1.95 ± 0.32
32	30	25	180	400	7.51 ± 0.02	29.12 ± 0.16	0.21 ± 0.02	2.48 ± 0.04
33	30	30	150	200	11.33 ± 0.06	29.30 ± 0.11	0.51 ± 0.02	1.89 ± 0.06
34	30	30	150	400	10.79 ± 0.03	29.27 ± 0.29	0.39 ± 0.02	2.18 ± 0.03
35	30	30	180	200	9.39 ± 0.08	29.54 ± 0.22	0.30 ± 0.01	2.13 ± 0.07
36	30	30	180	400	8.93 ± 0.09	28.69 ± 0.03	0.26 ± 0.01	2.34 ± 0.05

[1] Mean ± standard deviation of three determinations

[2] Mean ± standard deviation of six determinations

Table 9 ANOVA summary table of chemical and physical properties of extrudates.

Variables		Moisture (%)	Total protein (%db)	Bulk density (g/cm^3)	Expansion ratio
Protein	20	8.18±1.6[a1]	19.39±0.2[c1]	0.23±0.1[b2]	2.69±0.3[a2]
(%)	25	8.46±2.1[a]	24.37±0.2[b]	0.29±0.1[a]	2.40±0.3[b]
	30	8.22±1.9[a]	29.12±0.3[a]	0.30±0.1[a]	2.17±0.2[c]
Moisture	20	6.13±0.9[c]	24.27±4.0[a]	0.19±0.1[c]	2.61±0.4[a]
(%)	25	8.55±0.8[b]	24.26±3.9[a]	0.28±0.1[b]	2.40±0.3[b]
	30	10.18±0.9[a]	24.35±4.1[a]	0.35±0.1[a]	2.25±0.2[c]
Barrel	150	8.93±1.9[a]	24.29±4.1[a]	0.32±0.1[a]	2.36±0.2[b]
Temperature ($^{\circ}C$)	180	7.64±1.6[b]	24.30±3.9[a]	0.23±0.1[b]	2.47±0.2[a]
Screw speed	200	8.66±1.8[a]	24.35±4.0[a]	0.31±0.1[a]	2.26±0.3[b]
(RPM)	400	7.90±1.9[b]	24.25±3.9[a]	0.23±0.1[b]	2.58±0.3[a]

[1] Mean ± standard deviation of three determinations

[2] Mean ± standard deviation of six determinations

[a-c] Different letters within the same column indicate significant difference ($P \leq 0.05$)

The extrusion variables (feed moisture, temperature and screw speed) had significant ($P \leq 0.05$) effect on moisture content, bulk density and expansion of extrudates. Moisture content of extrudates was decreased with increasing of extruder barrel temperature and screw speed (Table 9). The increase in protein content had significant ($P \leq 0.05$) effect on bulk density and expansion ratio of extrudates. Bulk density of extruded samples was increased with increased protein content or feed moisture (Table 9). In contrast, it was decreased with increased temperature or screw speed during extrusion. An inverse relationship between expansion ratio and density of extrudates was observed as indicated in Table 9.

2. Formulation development and optimisation

Two mixtures were formulated by the LP for fulfilling the nutritional and technical constraints of the desired product. The composition and cost of these formulations are described in Table 10. The spreadsheet table for this LP calculation contained a total thirty different ingredients such as glutinous rice, protein from milk, egg white, or cereal except fish meal (as discussed in above results); however, only three of these ingredients (glutinous rice, wheat gluten and soy) were necessary to achieve the desired optimized product formulation. The 25 % protein content was removed from the constraint, because the program selected all protein ingredients in data list for formulation, but all were not available for purchase for this study. Therefore, the constraint of protein requirement in the LP was set at one of two levels of 20 and 30 % protein content. Formulation 1 (20% protein) gave the lowest cost per kilogram (40.50 baht), and the diffence was less than five baht, between the other two experimental formulations. The cost of product was increased with an increase of protein content in mixture.

2.1 Chemical properties

2.1.1 The composition of raw materials and formulations

The protein and amino acid composition of the glutinous rice flour, vital wheat gluten and toast soy grits used in these experiments is presented in Table 11 and 12. Moisture of raw material varied from (a low of) 8.42 to 13.19 % as shown in Table 11. In the mixture preparation process, the mixtures had to equilibrate overnight at 5°C pior to analysed the moisture content and calculate the rate of added feed moisture during extrusion. Protein content of glutinous rice flour, wheat gluten and soy was 6.51, 86.55 and 54.22 %, respectively. The highest protein content was found in wheat gluten, which had higher cost (2.6 $/Kg) than soy (2.3 $/Kg) and glutinous rice (1.23 $/Kg) as shown in Appendix Table A1. These data confirms that the formulation cost was increased as a consequence of the protein addition.

Table 10 Minimal cost formulations calculated from linear programming.

Ingredients in mixture	Weight (%)	Protein (%wb)	Lysine (g/100g protein)	Methionine+ Cystine(g/100 g protein)	Cost[1] (baht/kg)
Glutinous rice flour	71.00	4.01	2.80	2.69	
Vital wheat gluten	6.00	4.77	0.11	0.24	
Toasted soy grits	23.00	11.22	1.49	0.67	
Formulation 1		20.00	4.40	3.6	40.56
Glutinous rice flour	54.00	3.06	2.14	2.06	
Vital wheat gluten	15.00	12.00	0.27	0.61	
Toasted soy grits	31.00	14.94	1.99	0.89	
Formulation 2		30.00	4.40	3.55	45.76

[1] Costs as of April, 2005 (1 $ = 26 baht).

The rice flours and protein materials exhibited wide differences in their composition of amino acid content (Table 12). Lysine was the first limiting amino acid in both cereals protein (rice and wheat), while the concentrations of this essential amino acid in wheat protein were substantially less than in rice and soy protein. Wheat protein is rich source of cystine. In order to improve the protein quality of cereal base products, blending glutinous rice flour and protein sources to their optimum concentration of total protein resulted in an increase in the amino acid content of extrudates. The lysine and sulphur amino acid (methionine and cystine) of two formulations was in agreement with the standards recommended by the FAO/WHO/UNU guidelines (1985), which minimizing cost. Essential amino acid balances also meet the minimum recommended by FAO/WHO UNU guidelines (1985) (Table 12).

Table 11 The moisture and protein content of raw materials from the specification sheet and in the formulations.

Material	Data from specification	
	Moisture content **(%)**	Total protein content (% db)
Glutinous rice flour	13.19	6.51
Vital wheat gluten	8.42	86.55
Toast soy grits	10.00	54.22
Formulation 1	12.17[1]	22.28[1]
Formulation 2	11.48[1]	33.31[1]

[1] The value by calculation from specification value of raw materials

Table 12 Amino acid composition (g/100g protein) of raw materials from specification sheet, formulations and FAO/WHO guidelines.

Typical amino acid	Data from specification			Formulation [1]		FAO/ WHO/ UNU (10-12 years)
	Gluti-nous rice flour	Vital wheat gluten	Toast soy grits	1	2	
Lysine*	3.94	1.80	6.50	4.40	4.40	4.40
Cystine	1.49	2.36	1.50	1.54	1.62	-
Methionine*	2.30	1.66	1.40	2.05	1.93	-
Total Sulphur amino acid [2]	3.79	4.02	2.90	3.60	3.55	2.20
Aspatic acid	11.67	-	11.50	-	-	-
Threonine*	4.19	3.05	4.00	4.08	3.96	2.80
Serine	6.13	-	5.00	-	-	-
Glutamic acid	23.65	-	18.10	-	-	-
Glycine	6.13	-	4.30	-	-	-
Alanine	7.48	-	4.40	-	-	-
Valine*	7.33	4.30	5.10	6.64	6.18	2.50
Isoleucine*	5.08	3.88	4.80	4.94	4.81	2.80
Leucine*	9.88	8.05	7.90	9.31	8.99	4.40
Tyrosine	3.89	-	3.50	-	-	-
Phenylalanine*	5.83	-	5.10	-	-	-
Total Aromatic amino acid [3]	9.72	-	8.60	-	-	2.20
Histidine*	2.54	2.22	2.70	2.56	2.54	-

* Essential amino acid

[1] The value by calculation from specification value of raw materials

[2] Total Sulphur amino acid: Cystine and Methionine

[3] Total Aromatic amino acid: Tyrosine and Phenylalanine

2.1.2 Effect of variables on the protein and amino acids

The effects of protein content, feed moisture content and extruder maximum barrel temperature on the chemical properties of extrudates are presented in Table 13. The ANOVA results are summarized in Table 14. The results showed the highly significant ($P \leq 0.01$) impact of protein content, extruder feed moisture and barrel temperature on moisture, protein and NPN content of extrudates.

Table 13 Result of Duncan's multiple range test of chemical properties of extrudates.

Protein content (%)	Feed moisture (%)	Barrel temperature (°C)	Moisture (%)	Total protein (%db)	Non-protein nitrogen (%db)
20	20	150	6.54±0.2^{de}	20.94±0.3^{c}	0.32±0.1^{d}
20	20	180	6.20±0.3^{e}	21.03±0.1^{c}	0.48±0.1^{b}
20	25	150	7.87±0.1^{c}	20.84±0.03^{c}	0.28±0.03^{d}
20	25	180	6.83±0.2^{d}	20.87±0.3^{c}	0.40±0.1^{c}
20	30	150	9.07±0.2^{a}	20.92±0.2^{c}	0.30±0.02^{d}
20	30	180	7.54±0.4^{c}	20.88±0.2^{c}	0.32±0.04^{d}
30	20	150	5.55± 0.1^{f}	30.91±0.1^{ab}	0.49±0.02^{b}
30	20	180	4.47±0.5^{g}	30.78±0.1^{ab}	0.59±0.01^{a}
30	25	150	6.93±0.4^{d}	30.88±0.1^{ab}	0.43±0.01^{bc}
30	25	180	5.54±0.4^{f}	30.65±0.3^{b}	0.45±0.1^{bc}
30	30	150	8.55±0.2^{b}	30.94±0.1^{ab}	0.44±0.02^{bc}
30	30	180	7.84±0.1^{c}	31.02±0.01^{a}	0.46±0.01^{bc}

[1] Mean ± standard deviation of three determinations

[a-g] Different letters within the same column indicate significant difference ($P \leq 0.05$)

Table 14 ANOVA summary table of chemical properties of extrudates.

Treatment		Moisture (%)[1]	Total protein (%db)	Non-protein nitrogen (%db)
Protein (%)	20	7.34±1.0[a]	20.91±0.2[b]	0.35±0.08[b]
	30	6.48±1.5[b]	30.86±0.2[a]	0.48±0.06[a]
Moisture (%)	20	5.69±0.9[c]	25.91±5.2[a]	0.47±0.11[a]
	25	6.79±0.9[b]	25.81±5.2[a]	0.39±0.07[b]
	30	8.24±0.7[a]	25.94±5.3[a]	0.38±0.08[b]
Barrel temperature(°C)	150	7.42±1.3[a]	25.91±5.1[a]	0.38±0.08[b]
	180	6.40±1.2[b]	25.87±5.1[a]	0.45±0.09[a]

[1] Mean ± standard deviation of three determinations

[a-c] Different letters within the same column of each treatment indicate significant difference ($P \leq 0.05$)

The seconded-order polynomial model predicted for optimisation of dependent variables (Y) was established (Eq. (13):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{22} X_2^2 \qquad (13)$$

Where β_o, β_1, β_2, β_3, β_{12}, β_{13}, β_{23} and β_{22} are coefficients for intercept, linear, interactive and quadratic effects, respectively, and X_1, X_2 and X_3 are independent variables (X_1 = protein content, X_2 = feed moisture, X_3=barrel temperature). Statistical results are summarized in Table 15. Cystine/Methionine data were not included in Table 15 because there were no significant data available. In other words, cystine and methionine content (g/100g protein) were not influenced significantly by any of the experimental treatments. There are also some second order interactions among table.

The regression models for moisture, protein, NPN and lysine content showed high R^2 of 0.93, 0.99, 0.83 and 0.89, respectively.

Table 15 Significant coefficients of regression equation for the chemical responses.

	Moisture (%)	Total protein (%db)	Non-protein nitrogen (%db)	Lysine (g/100g protein)
β_0	6.791***	25.810***	0.390***	3.859***
β_1	-0.432***	4.974***	0.0639***	-0.238***
β_2	1.279***	0.0128	-0.0467***	0.216***
β_3	-0.506***	-0.0174	0.0370***	-0.194***
β_{12}	0.312***	0.0546	0.00003	0.0608
β_{13}	-0.02	-0.0307	-0.0132	0.0165
β_{23}	-0.104	0.0104	-0.0271**	0.0863*
β_{22}	0.180	0.117	0.0349*	-0.0162
R^2 adj.	0.932	0.999	0.834	0.890
SE	0.346	0.179	0.039	0.129
F	69.199***	3966.087***	26.072***	27.614***

* Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$

*** Significant at $P \leq 0.001$

R^2 adj. = the adjusted R^2

SE = the standard error of estimation

F = the Fisher test F value

2.1.2.1 Moisture content

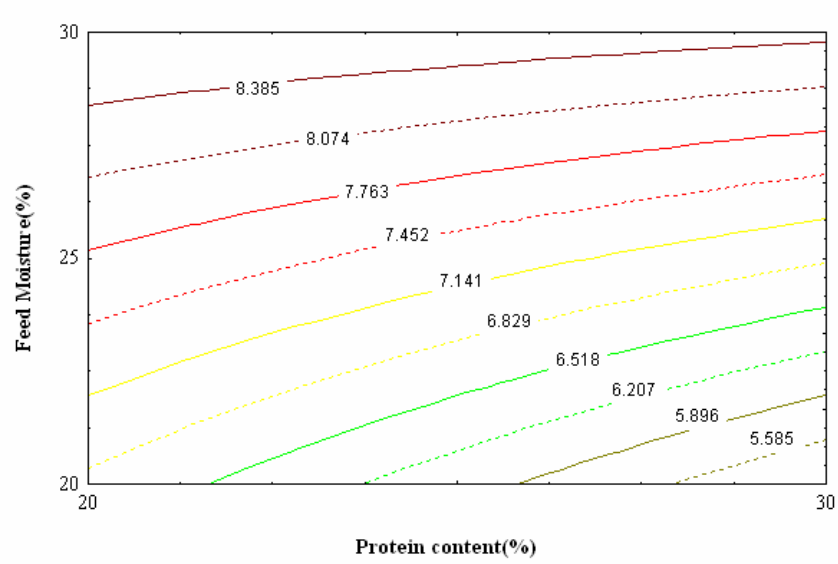
Moisture content of extrudes varied from a low of 4.47 to 8.55 %, which the highest moisture content was observed at high protein content (30%), low feed moisture (20 %) and high barrel temperature (180°C) (Table 13).

Effects of protein and extrusion conditions on moisture content are shown in Table 14. Product moisture was found to be directly related to feed moisture and inversely related to protein content and barrel temperature. Increase protein and barrel temperature or decrease feed moisture results in decreased moisture content of the extrudates.

The effect of protein content was observed that increased protein content of feed component caused a decrease in the moisture content of extrudates (Table 14). The significant interaction between protein content and feed moisture for the moisture content of extrudates was also observed (Table 15) and the representative effects of protein content and feed moisture are shown in Figure 8. Increasing feed moisture content from 20 to 30 % at a constant protein content resulted in an increase in moisture content of extrudates.

The product moisture was inversely related to barrel temperature and directly related to feed moisture (Table 14). Increasing raw material moisture content had a positive impact on final extrudate moisture. The results were contrasted with the effect of barrel temperature. The moisture content of extrudates was increased with decreased barrel temperature (Table 14). This was consistent with the observations by Falcone and Phillips (1988) who observed that increasing barrel temperature decreased the moisture content of cowpea and sorghum extrudates. Owusu-Anash et al. (1984) gave the same results as for moisture content of corn extrudates. Water inside the extruder exists in the form of superheated steam due to high temperature and pressure. When the extrudate exits the die nozzle, water vaporizes at the temperature $\geq$100 °C (Williams *et al*., 2001). The moisture remaining in the product depends on the initial (feed) moisture content and the pressure differential between the extruder and the atmosphere (Conway and Anderson, 1973).

a)



b)

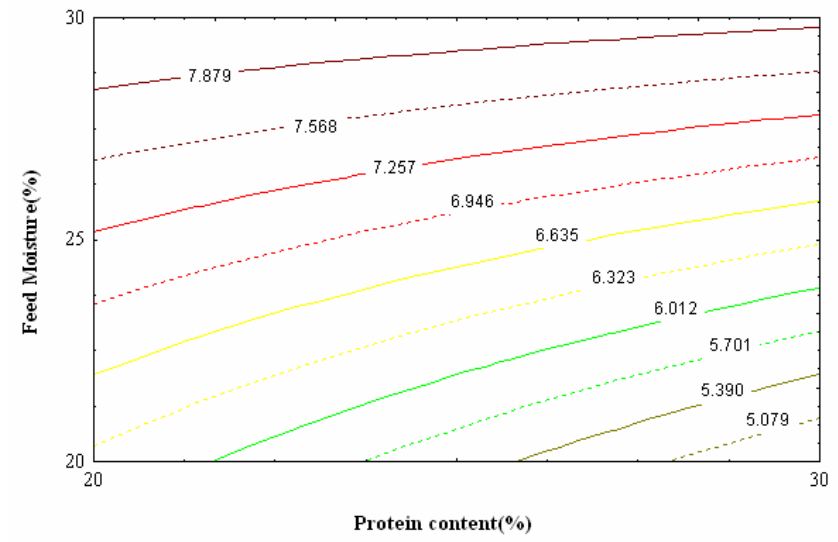


Figure 8 Moisture content of extrudates as a function of protein content and feed moisture at barrel temperature of 150 °C (a) and 180 °C (b).

2.1.2.2 Total protein and non-protein nitrogen content (NPN)

The protein content from analysis of glutinous rice flour, vital wheat gluten and toast soy grits are shown in Table 16. The protein content of the formulation 1(20% wb protein content) was 22.34 % db, and the formulation 2 (30 % wb protein content) was 33.39 % db. There were slightly differences between the specification sheet data (Table 11), used for the linear programming, and the protein composition from analysis (Table 16). Protein content of all extruded samples was significantly reduced after extrusion process. Total protein content of the 20 % protein formulation ranged from 20.84 to 21.03 % db (Table 13). The protein content of the 30% protein formulation ranged from 30.65 to 31.02 % db, which conform to the requirement.

Table 16 The moisture and protein content composition of raw materials and formulations from analysis before extrusion.

Material	Moisture (%)	Total protein (% db)
Glutinous rice flour	12.95±0.10[2]	6.48±0.06[2]
Vital wheat gluten	9.02±0.11	86.55±0.01
Toast soy grits	8.97±0.12	54.56±0.25
Formulation 1	11.16±0.12	22.34[1]
Formulation 2	11.05±0.07	33.39[1]

[1] The value by calculation from analysis value of raw materials

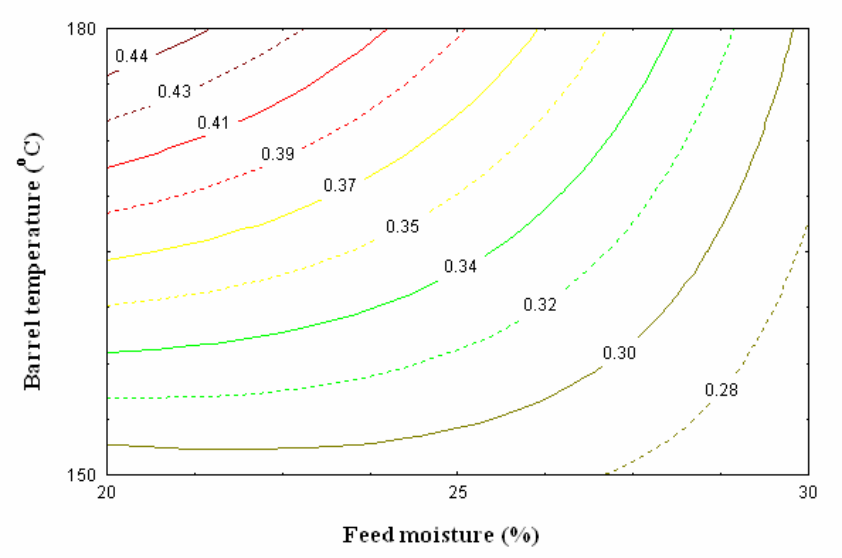
[2] Mean ± standard deviation of three determinations

For the total protein and NPN of extrudates, the results showed that the extrusion condition variable (feed moisture and barrel temperature) had no significant effect on the total protein content but it had highly significant effect on NPN (Table 14). The NPN tended to decrease with increased feed moisture or decreased extruder barrel temperature. There was significant interaction between feed

moisture content and barrel temperature for the NPN content of extrudates (Table 15). It was practically observed that an increase in the level of temperature increased NPN, especially at low feed moisture content (Fingure 9). In this study, the severe condition was observed at the lowest feed moisture (20%) and highest temperature (180 °C). The extrudates with the highest NPN content was produced at lowest moisture content and highest temperature, which related to the true protein content of extrudates. The NPN value was inversely related to protein nitrogen (true protein) value, whereas true protein was calculated as the difference between total and NPN content (Periago *et al*., 1996).

Besides protein nitrogen (PN), NPN represents an important fraction in product that determines protein quality (Bhatty, 1973; Periago *et al*., 1996). NPN involves a wide group of components, including urea, ammonium salts, amines, amides and nucleotides (Goedhart and Bindels, 1994) all of them end in ammonium as the final chemical form. This results was consistent with the observations by Conesa *et al*. (2005) who reported that industrial processing such as severe heating (> 70 °C) gave a significant increase in NPN ($P \leq 0.01$). The heating process is known to induce chemical changes in proteins, and interactions take place between the lateral groups of the amino acids, that lead to the formation of the so called ‘unnatural’ amino acids, such as lanthionine, β-aminoalanine, ornithine, ornithinoalanine, diaminopropionic acid and particularly lysinoalanine, which is known to decrease the nutritional value of foods (Hurrell and Finot, 1983; Klostermeyer and Reimerdes, 1977, Perez-Conesa *et al*., 2005).

a)



b)

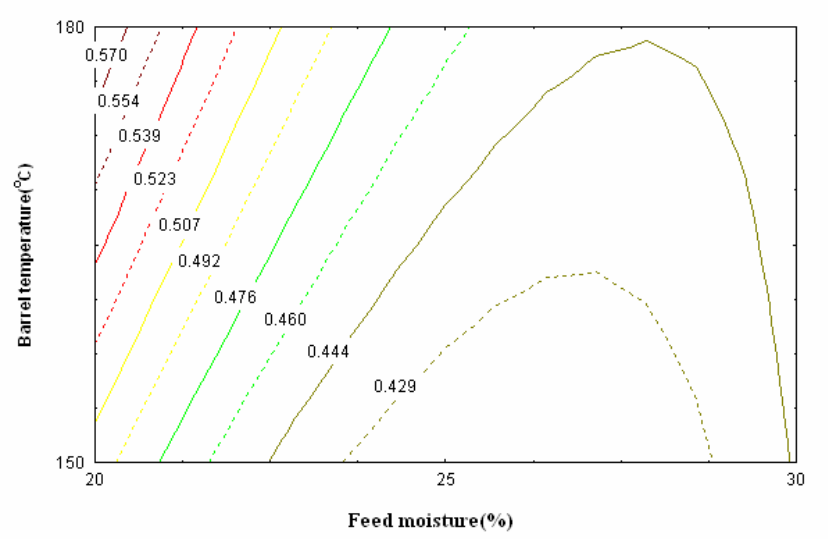


Figure 9 Non-protein nitrogen (NPN) content of extrudates as a function of feed moisture and barrel temperature at 20% protein (a) and 30% protein (b).

2.1.2.3 Amino acid analysis

The amino acid compositions of raw materials from analysis are shown in Table 17. There were significant differences between the specification sheet data (Table 7) and the data from analysis (Table 17). The variation in the chemical content may be due to the source of the samples, cultivar or variety, and method of amino acid determination which it is notoriously difficult to reproduce between laboratories even for a single sample.

A revised composition of the two formulations, based on the chemical analyses of the raw materials, is also provided in Table 17. It is shown that the lysine content from analysis of both formulations (4.15, 4.04 g/100g protein) was lower than the estimates for their composition by linear program and lower than the proposed FAO/WHO/UNU (1985) amino acid requirement pattern for the schoolchildren (4.40 g/100g protein). This is probably due to the lysine contents from analysis of wheat gluten and soy, which were lower than specification sheet data. However sulphur amino acid contents (cystine and methionine) and other essential amino acid were higher than FAO/WHO/UNU (1985) recommendation.

The amino acid compositions after extrusion at different levels of feed moisture and barrel temperature with 20% and 30 % protein extrudates are presented in Tables 18 and 19, respectively. The amino acid value of non-extrudated product (calculation from analysis value of raw materials) was also presented in Table 18 and 19. The percentage of available lysine, cysteine and methionine content in the 20% protein formulation ranged from 3.62 to 4.37, 1.37 to 1.69 and 1.67 to 1.82 g/100 g protein, respectively. And the 30% protein formulation, the percentage ranged from 3.04 to 3.99, 1.52 to 1.81and 1.41 to 1.80 g/100 g protein, respectively. The results showed that the amino acid composition of all extrudates was reduced after extrusion cooking. However, all amino acid contents except lysine was higher than the amount which is recommended by FAO/WHO/UNU (1985).

Table 17 Amino acid composition (g/100g protein) of raw materials and formulations.

Typical amino acid	Data from analysis [4]			Formulation [1]		FAO/ WHO/ UNU (10-12 years)
	Glutinous rice flour	Vital wheat gluten	Toast soy grits	1	2	
Lysine*	3.95±0.05	1.12±0.03	5.55±0.02	4.15	4.02	4.40
Cystine	1.82±0.00	2.14±0.04	1.52±0.03	1.77	1.77	-
Methionine*	2.54±0.26	1.71±0.03	1.61±0.04	2.28	2.13	-
Total sulphur amino acid [2]	4.36	3.85	3.13	4.05	3.90	2.20
Aspatic acid	13.92±1.78	3.40±0.07	13.64±0.27	13.22	12.26	-
Threonine*	4.03±0.05	2.57±0.05	4.26±0.19	4.00	3.88	2.80
Serine	5.61±0.63	4.76±0.15	5.34±0.15	5.50	5.40	-
Glutamic acid	18.82±2.32	38.89±1.12	21.02±0.50	20.53	22.51	-
Glycine	5.44±0.58	3.38±0.05	4.63±0.07	5.13	4.88	-
Alanine	7.08±0.84	2.78±0.04	5.03±0.09	6.35	5.80	-
Valine*	6.73±0.89	3.83±0.07	5.04±0.14	6.16	5.77	2.50
Isoleucine*	4.75±0.12	3.12±0.01	4.52±0.11	4.61	4.47	2.80
Leucine*	8.89±0.68	6.91±0.09	8.06±0.18	8.58	8.34	4.40
Tyrosine	5.60±0.51	3.38±0.02	3.94±0.09	5.08	4.75	-
Phenylalanine*	5.78±0.60	5.44±0.08	5.57±0.09	5.71	5.67	-
Total aromatic amino acid [3]	11.38	8.82	9.51	11.80	12.42	2.20
Histidine*	8.08±1.05	2.45±0.03	3.82±0.09	6.76	5.92	-

* Essential amino acid

[1] The value by calculation from analysis value of raw materials

[2] Total sulphur amino acid: cystine and methionine

[3] Total aromatic amino acid: tyrosine and phenylalanine

[4] Mean ± standard deviation of two determinations

A computation of the percentage values of lysine, cystein and methionine loss are shown in Table 20. The highest loss in avaliable lysine content after extrusion process was found under condition of 20 % feed moisture and 180 °C, which was lost 12.77 % and 24.38 % lysine for 20% and 30% protein formulation, respectively. Similar results were reported by Lasekan *et al*. (1996) who observed that the loss of 10 % available lysine during extrusion cooking (45 % feed moisture and 130 °C) of maize-base snack extrusion. Wiriyaumpaiworng *et al*. (2004) also observed that 20.95 % lysine in soybean was lost after treatment for twin-screw extruder at 18 % moisture and 130 °C. Another study found that the 17 % lysine content of rice protein was reduced by extrusion at 15 % feed moisture and 150 °C (Eggum *et al*., 1986). However, this study was observed that there were a slight decrease in lysine after extrusion under high feed moisture content (30%) and low barrel temperature (150°C).

Both 20 and 25 % feed moisture content at 20 % protein and 180 °C provided high loss in cystein and methionine. For 30 % protein in formulation, the highest loss of cystine and methionine was observed at low feed moisture (20%) and high barrel temperature (180 °C). Cheftel *et al*. (1985) noted that the thermal treatments at temperatures above 115 °C causes the partial destruction (irreversible chemical modification) of cysteine, cystine residues and the formation of hydrogen sulfide, dimethylsulfide, and cysteic acid. Similar observation was reported by Prudencio-Ferreira and Areas (1993) who found that free sulfhydryl groups increased and disulphide content was reduced by extrusion.

Generally, increasing protein content should result in increased amino acid content, but this is not always true. These results found that the lysine, aspatic acid, threonine, glycine, alanine, valine, isoleucine, leucine, tyrosine and histidine with 30 % protein extrudates were lower than 20% protein extrudates (Table 17, 18 and 19). This is probably due to the difference quantity of amino acid composition in protein materials. Another reason was the difference ratio of raw materials (glutinous rice flour: wheat gluten: soy grit) that were used in each formulation.

Table 18 The amino acid composition of 20 % protein extrudates (expressed on a dry basis, g/100g of protein).

Amino acid	Extruded snack product						Non-extruded formulation[1]	FAO/WHO/ UNU (10-12 years)[2]
	20% Moisture		25% Moisture		30% Moisture			
	150°C	180°C	150°C	180°C	150°C	180°C		
Lysine*	4.19±0.16$^{ab\ 3}$	3.62±0.09^{ef}	4.33±0.04^{a}	3.94±0.01^{bcd}	4.37±0.19^{a}	4.06±0.02^{abc}	4.15	4.40
Cystine^{ns}	1.65±0.11	1.49±0.04	1.65±0.03	1.37±0.06	1.69±0.01	1.53±0.01	1.77	Met+Cys 2.20
Methionine^{ns}*	1.82±0.12	1.74±0.08	1.71±0.01	1.67±0.04	1.70±0.04	1.77±0.02	2.28	
Aspatic acid	12.56±1.35^{a}	12.74±0.38^{a}	12.66±0.76^{a}	11.94±0.01^{ab}	12.89±0.25^{a}	12.55±0.65^{a}	13.22	-
Threonine*	4.01±0.00^{b}	4.28±0.07^{a}	4.02±0.01^{b}	3.97±0.01^{b}	4.00±0.05^{b}	4.06±0.05^{b}	4.00	2.80
Serine^{ns}	5.59±0.03	6.03±0.15	5.59±0.01	5.51±0.01	5.57±0.10	5.57±0.13	5.50	-
Glutamic acid	26.67±0.53^{c}	28.81±0.20^{b}	25.97±0.66^{c}	26.19±0.05^{c}	26.22±0.14^{c}	26.30±0.02^{c}	20.53	-
Glycine	4.73±0.05^{b}	5.13±0.14^{a}	4.66±0.09^{bc}	4.76±0.01^{b}	4.79±0.01^{b}	4.79±0.06^{b}	5.13	-
Alanine	5.04±0.06^{b}	5.54±0.29^{a}	5.06±0.09^{b}	5.02±0.01^{b}	5.19±0.11^{b}	5.08±0.20^{b}	6.35	-
Valine*	5.53±0.21^{bcd}	6.20±0.13^{a}	5.57±0.00^{bcd}	5.70±0.01^{b}	5.65±0.21^{bc}	5.71±0.30^{b}	6.16	2.50
Isoleucine*	4.69±0.20^{bc}	5.15±0.01^{a}	4.53±0.12^{bcd}	4.66±0.00^{bc}	4.78±0.06^{b}	4.63±0.13^{bc}	4.61	2.80
Leucine*	8.49±0.03^{b}	9.26±0.02^{a}	8.30±0.15^{bcd}	8.55±0.01^{b}	8.53±0.08^{b}	8.41±0.14^{bc}	8.58	4.40

Table 18 (Continued)

Amino acid	Extruded snack product						Non-extruded formulation[1]	FAO/WHO /UNU (10-12 years)[2]
	20% Moisture		25% Moisture		30% Moisture			
	150°C	180°C	150°C	180°C	150°C	180°C		
Tyrosine	4.39±0.11^{cd}	4.84±0.17^{a}	4.24±0.04^{cde}	4.73±0.01^{ab}	4.52±0.04^{bc}	4.39±0.09^{cd}	5.08	Try+Phe 2.20
Phenylalanine^{ns}*	5.96±0.06	6.51±0.27	5.83±0.16	6.10±0.01	6.02±0.08	5.95±0.08	5.71	
Histidine*	4.96±0.88^{a}	4.54±0.30^{abc}	4.88±0.02^{a}	4.34±0.01^{abcd}	4.66±0.02^{ab}	4.33±0.51^{abcd}	6.76	-

* Essential amino acid

[1] The values were calculated from analysis value of raw materials

[2] Food and Agricultural Organization (FAO) standards for protein requirements (FAO/WHO/UNU, 1985)

[3] Mean ± standard deviation of two determinations

$^{a-g}$ Different letters within the same row of each amino acid indicate significant difference ($P \leq 0.05$); ^{ns} not significant ($P > 0.05$)

Table 19 The amino acid composition of 30 % protein extrudates (expressed on a dry basis, g/100g of protein).

Amino acid	Extruded snack product						Non-extruded formulation[1]	FAO/WHO/ UNU (10-12 years)[2]
	20% Moisture		25% Moisture		30% Moisture			
	150°C	180°C	150°C	180°C	150°C	180°C		
Lysine*	3.66±0.25$^{ef\ 3}$	3.04±0.01^{g}	3.71±0.03^{def}	3.45±0.28^{f}	3.99±0.05^{bcd}	3.81±0.09^{cde}	4.02	4.40
Cystine^{ns}	1.75±0.32	1.56±0.05	1.81±0.01	1.68±0.01	1.52±0.46	1.81±0.10	1.77	Met+Cys 2.20
Methionine* ^{ns}	1.63±0.05	1.57±0.04	1.68±0.03	1.69±0.01	1.41±0.45	1.80±0.01	2.13	
Aspatic acid	11.22±0.23^{bc}	10.55±0.00^{c}	10.62±0.21^{c}	10.16±0.23^{c}	10.77±0.10^{bc}	10.74±0.15^{bc}	12.26	-
Threonine*	3.79±0.09^{c}	3.66±0.01^{d}	3.75±0.10^{cd}	3.66±0.08^{d}	3.82±0.01^{c}	3.80±0.07^{c}	3.88	2.80
Serine^{ns}	5.53±0.17	5.37±0.02	5.51±0.05	5.44±0.06	5.59±0.11	5.55±0.03	5.40	-
Glutamic acid	29.73±0.50^{ab}	28.91±0.02^{b}	30.02±0.24^{a}	28.78±0.83^{b}	30.22±0.20^{a}	30.39±0.54^{a}	22.51	-
Glycine	4.56±0.05^{cd}	4.27±0.05^{g}	4.42±0.07^{efg}	4.37±0.12^{fg}	4.53±0.05^{cde}	4.53±0.06^{cde}	4.88	-
Alanine	4.62±0.06^{c}	4.26±0.02^{d}	4.43±0.10^{cd}	4.34±0.06^{cd}	4.54±0.05^{c}	4.55±0.03^{c}	5.80	-
Valine*	5.24±0.09^{de}	5.06±0.02^{e}	5.07±0.14^{e}	5.01±0.31^{e}	5.30±0.01^{cde}	5.25±0.05^{de}	5.77	2.50
Isoleucine*	4.56±0.08^{bcd}	4.46±0.12^{cd}	4.32±0.02^{d}	4.34±0.24^{d}	4.49±0.02^{cd}	4.43±0.01^{cd}	4.47	2.80
Leucine*	8.34±0.16^{bc}	8.06±0.23^{de}	8.03±0.01^{e}	7.98±0.16^{e}	8.30±0.14^{bcd}	8.21±0.14^{cde}	8.34	4.40

Table 19 (Continued)

Amino acid	Extruded snack product						Non-extruded formulation[1]	FAO/WHO/UNU (10-12 years)[2]
	20% Moisture		25% Moisture		30% Moisture			
	150°C	180°C	150°C	180°C	150°C	180°C		
Tyrosine	4.11±0.32^{de}	4.20±0.06^{de}	4.12±0.05^{de}	3.95±0.15^{e}	4.20±0.01^{de}	4.12±0.09^{de}	4.75	Try+Phe 2.20
Phenylalanine* ^{ns}	5.99±0.26	5.99±0.02	5.86±0.09	5.84±0.20	6.15±0.07	5.91±0.04	5.67	
Histidine*	3.95±0.03^{bcd}	3.72±0.13^{d}	3.81±0.08^{cd}	3.65±0.09^{d}	3.81±0.08^{cd}	3.99±0.06^{bcd}	5.92	-

* Essential amino acid

[1] The values were calculated from analysis value of raw materials

[2] Food and Agricultural Organization (FAO) standards for protein requirements (FAO/WHO/UNU, 1985)

[3] Mean ± standard deviation of two determinations

[a-g] Different letters within the same row of each amino acid indicate significant difference ($P \leq 0.05$); [ns] not significant ($P > 0.05$)

Table 20 The loss of lysine, cysteine and methionine content under different extrusion conditions.

Protein content (%)	Feed moisture (%)	Barrel temperature (°C)	Lysine (%)	Cystine (%)	Methionine (%)
20	20	150	1.20	6.78	20.18
20	20	180	12.77	15.82	23.68
20	25	150	0.72	6.78	25.00
20	25	180	5.06	15.25	23.24
20	30	150	0.24	4.52	25.44
20	30	180	2.17	13.56	22.37
30	20	150	8.96	1.13	23.47
30	20	180	24.38	11.86	26.29
30	25	150	7.71	1.12	21.13
30	25	180	14.18	5.08	20.66
30	30	150	0.75	0.56	20.18
30	30	180	5.22	3.95	15.49

Table 21 ANOVA summary table of amino acid of extruded snacks.

Treatment		Lysine*	Aspatic acid	Threonine*	Glutamic acid
Protein	20	4.09±0.3[a 1]	12.56±0.6[a]	4.06±0.1[a]	26.69±1.1[b]
(%)	30	3.61±0.4[b]	10.68±0.4[b]	3.75±0.1[b]	29.67±0.7[a]
Moisture	20	3.63±0.5[c]	11.77±1.1[a]	3.94±0.3[a]	28.53±1.2[a]
(%)	25	3.86±0.4[b]	11.34±1.1[a]	3.85±0.2[a]	27.74±1.9[b]
	30	4.06±0.2[a]	11.74±1.1[a]	3.92±0.1[a]	28.28±2.2[a]
Barrel	150	4.04±0.3[a]	11.79±1.1[a]	3.90±0.1[a]	28.14±1.9[a]
temperature	180	3.65±0.4[b]	11.45±1.1[a]	3.90±1.1[a]	28.23±1.6[a]
(°C)					

* Essential amino acid

[1] Mean ± standard deviation of two determinations

[a-c] Different letters within the same column of each treatment indicate significant difference ($P \leq 0.05$)

Table 21 (Continued)

Treatment		Glycine	Alanine	Valine*	Isoleucine*
Protein	20	4.81±0.2[a]	5.15±0.2[a]	5.73±0.3[a]	4.74±0.2[a]
(%)	30	4.39±0.1[b]	4.46±0.1[b]	5.15±0.2[b]	4.44±0.1[b]
Moisture	20	4.68±0.3[a]	4.87±0.5[a]	5.51±0.5[a]	4.46±0.2[c]
(%)	25	4.55±0.2[b]	4.71±0.4[a]	5.34±0.4[a]	4.58±0.2[b]
	30	4.66±0.1[a]	4.84±0.3[a]	5.48±0.3[a]	4.71±0.3[a]
Barrel	150	4.62±0.1[a]	4.82±0.3[a]	5.39±0.2[a]	4.56±0.2[a]
temperature	180	4.64±0.3[a]	4.80±0.5[a]	5.49±0.5[a]	4.61±0.3[a]
(°C)					

* Essential amino acid

[1] Mean ± standard deviation of two determinations

[a-c] Different letters within the same column of each treatment indicate significant difference ($P \leq 0.05$)

Table 21 (Continued)

Treatment		Leucine*	Tyrosine	Histidine*
Protein	20	8.59±0.3[a]	4.52±0.2[a]	4.62±0.4[a]
(%)	30	8.15±0.2[b]	4.12±0.1[b]	3.82±0.1[b]
Moisture	20	8.55±0.5[a]	4.38±0.3[a]	4.29±0.6[a]
(%)	25	8.22±0.3[c]	4.26±0.3[a]	4.17±0.5[a]
	30	8.36±0.2[b]	4.31±0.2[a]	4.20±0.4[a]
Barrel	150	8.33±0.2[a]	4.26±0.2[a]	4.34±0.6[a]
temperature	180	8.41±0.5[a]	4.37±0.4[a]	4.09±0.4[b]
(°C)				

* Essential amino acid

[1] Mean ± standard deviation of two determinations

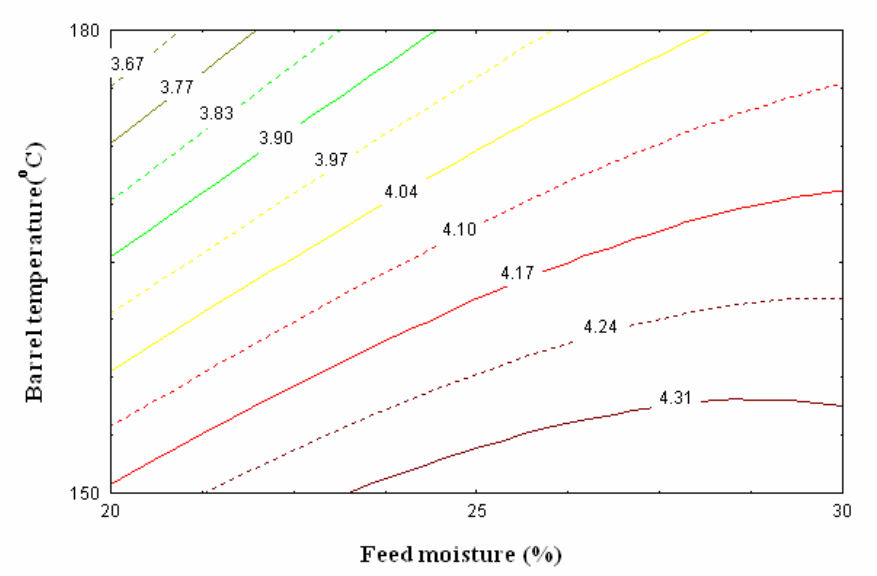
[a-c] Different letters within the same column of each treatment indicate significant difference ($P \leq 0.05$)

Results observed that the lysine content was significantly reduced by decreasing feed moisture content or increasing the barrel temperature during extrusion (Table 21). The significant interactions ($P \leq 0.01$) were also found between feed moisture content and barrel temperature on lysine content of extrudates (Table 15) and the representative effect of protein content and feed moisture are shown in Figure 10. The surface plot was generated from the model for lysine at 20% protein (Figure 10 (a)), and 30 % protein (Figure 10 (b)). The figure revealed that lysine decreased with decreasing feed moisture content or increasing the barrel temperature. High feed moisture content provided less lysine loss than low feed moisture content. Thus, feed moisture content was a major factor in the retention of available lysine in protein enriched extrudates. However, lower barrel temperature (< 150 °C) also enhanced lysine retention, especially at an increase level of feed moisture content during extrusion (Figure 10). In other words, available lysine was decreased with increase barrel temperature level. The essence lysine is lost because of a heat induced reaction between the reducing groups of sugars and amino groups of protein polypeptide chains (Acquistucci, 2000). Typically both terminal α-NH_2 groups and the ε-NH_2 groups of lysine side chains are involved (Iwe *et al*., 2004; Lei *et al*., 2007). Beside that lysine may also form cross-linkages with other amino acid residues forming lysinoalanine and lanthionine (Sternberg *et al*., 1975; Camire *et al*., 1990). These compounds reduced digestibility and, together with the loss important amino acids, decreased protein nutritional values (Camire et al., 1990).

Effect of extrusion condition on others amino acid are also shown in Table 21. The result showed that feed moisture content also affected the amount of glutamic acid, glycine, isoleucine and leucine. Glutamic acid, glycine and isoleucine were decreased with the decrease of feed moisture content, while leucine was decreased with the increase of feed moisture content. The barrel temperature had no significant effect on amino acid content except lysine. Similar results had been reported by Prudencio-Ferreira and Areas (1993) who observed that the glutamic acid and aspatic of soya protein extrudates was decreased in extrusion at 140 °C. Previous results have shown that foodstuffs with low moisture required higher energy inputs for extrusion and the high friction produced in the extruder resulted in a high degree

of molecular fragmentation and irreversible association (Bjork and Asp, 1983; Campos and Areas, 1993; Cheftel, 1986). These chemical transformations could promote loss in nutritional quality of protein.

a)



b)

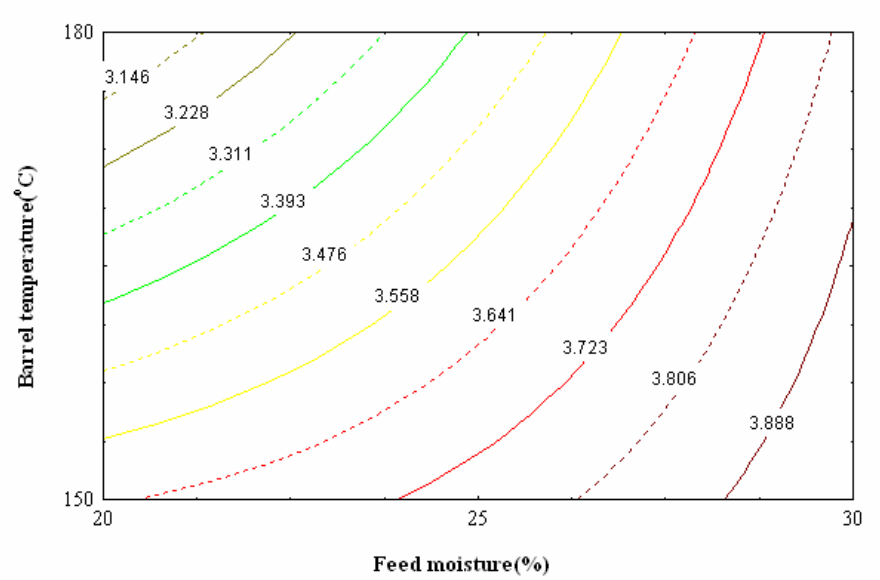


Figure 10 Lysine content of extrudates as a function of feed moisture and barrel temperature at 20% protein (a) and 30% protein (b).

2.2 Physical properties

Effect of addition of protein, extruder feed moisture and barrel temperature on bulk density, expansion and BSI are given in Table 22. Significantly, the highest bulk density, BSI and the lowest degee of expansion were observed in extrudates prepared from high protein content (30 %) and low temperature (150°C). The ANOVA results are shown in Table 23. Data with respect to coefficients of regression models for density, expansion and BSI as a function of protein content, feed moisture and barrel temperature are presented in Table 24. The regression models for product bulk density, expansion ratio and BSI showed high R^2 of 0.94, 0.95 and 0.98, respectively.

Table 22 Result of Duncan's multiple range test of physical properties of extrudates.

Protein content (%)	Feed moisture (%)	Barrel temperature (°C)	Bulk density (g/cm^3)	Expansion ratio	BSI (N/mm)
20	20	150	0.10 ± 0.01^{f}	3.72 ± 0.05^{b}	1.04 ± 0.01^{h}
20	20	180	0.07 ± 0.01^{h}	3.87 ± 0.12^{a}	0.64 ± 0.02^{j}
20	25	150	0.13 ± 0.01^{e}	3.51 ± 0.06^{c}	1.28 ± 0.02^{g}
20	25	180	0.08 ± 0.01^{gh}	3.82 ± 0.13^{ab}	0.73 ± 0.03^{i}
20	30	150	0.19 ± 0.02^{b}	3.01 ± 0.10^{d}	1.53 ± 0.05^{f}
20	30	180	0.09 ± 0.01^{fg}	3.43 ± 0.06^{c}	0.80 ± 0.01^{i}
30	20	150	0.16 ± 0.03^{c}	2.96 ± 0.22^{d}	2.32 ± 0.16^{c}
30	20	180	0.14 ± 0.02^{d}	3.01 ± 0.10^{d}	1.66 ± 0.05^{e}
30	25	150	0.21 ± 0.02^{b}	2.71 ± 0.09^{e}	2.69 ± 0.08^{b}
30	25	180	0.17 ± 0.01^{c}	2.89 ± 0.06^{d}	1.82 ± 0.04^{d}
30	30	150	0.29 ± 0.01^{a}	2.41 ± 0.06^{f}	3.52 ± 0.09^{a}
30	30	180	0.19 ± 0.01^{b}	2.70 ± 0.07^{e}	1.82 ± 0.05^{d}

[1] Mean ± standard deviation of six determinations

[2] BSI = breaking strength index, Mean ± standard deviation of twenty determinations

[a-j] Different letters within the same column indicate significant difference ($P\leq0.05$)

Table 23 ANOVA summary table of physical properties of extrudates

Treatment		Bulk density (g/cm^3)[1]	Expansion ratio[1]	BSI $(N/mm)^2$
Protein (%)	20	0.11 ± 0.04^b	3.56 ± 0.31^a	1.00 ± 0.32^b
	30	0.19 ± 0.04^a	2.78 ± 0.23^b	2.31 ± 0.66^a
Moisture(%)	20	0.12 ± 0.04^c	3.39 ± 0.44^a	1.41 ± 0.66^c
	25	0.14 ± 0.05^b	3.23 ± 0.47^b	1.63 ± 0.74^b
	30	0.19 ± 0.07^a	2.89 ± 0.39^c	1.92 ± 1.02^a
Barrel temperature(°C)	150	0.18 ± 0.06^a	3.05 ± 0.46^b	2.06 ± 0.88^a
	180	0.12 ± 0.04^b	3.28 ± 0.47^a	1.24 ± 0.54^b

[1] Mean ± standard deviation of six determinations

[2] BSI = breaking strength index, Mean ± standard deviation of twenty determinations

[a-c] Different letters within the same column of each treatment indicate significant difference ($P\leq0.05$)

Table 24 Significant coefficients of regression equation for the physical responses.

	Bulk density (g/cm^3)	Expansion ratio	BSI (N/mm)
β_0	0.145***	3.230***	1.633***
β_1	0.041***	-0.390***	0.652***
β_2	0.036***	-0.251***	0.252***
β_3	-0.028***	0.115***	-0.410***
β_{12}	0.007**	0.039*	0.089***
β_{13}	0.002	-0.029*	-0.128***
β_{23}	-0.018***	0.064***	-0.171***
β_{22}	0.011**	-0.092**	0.0332
R^2 adj.	0.949	0.951	0.981
SE	0.014	0.106	0.1158
F	189.020***	195.884***	519.286***

* Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$

*** Significant at $P \leq 0.001$

R^2 adj. = the adjusted R^2

SE = the standard error of estimation

F = the Fisher test F value

Where β_o, β_1, β_2, β_3, β_{12}, β_{13}, β_{23} and β_{22} are coefficients for intercept, linear, interactive and quadratic effects, respectively, and X_1, X_2 and X_3 are independent variables in coded values (X_1 = protein content, X_2 = feed moisture, X_3=barrel temperature)

2.2.1 Bulk density

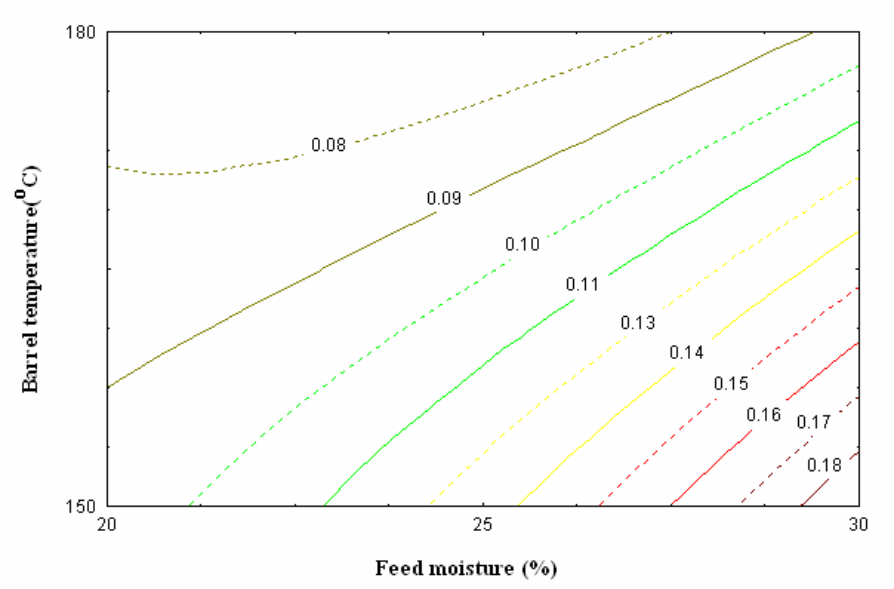
Bulk density of extrudates range from 0.07 to 0.29 g/cm^3 (Table 22). The protein content had major effects on bulk density. High protein content in mixtures gave a higher bulk density than low protein content (Table 23). As protein content of the feed material increases, the final structure becomes more rigid, with protein also participating in the network (Guy and Horne, 1988; Areas, 1992; Battacharya and Prakash, 1990; Areas, 1992). Similar observations were reported by Veronica *et al*. (2006) who studied qualities of extruded puffed snack from maize/soybean mixture and Allen *et al*. (2007) who studies influence of protein level on an extrusion-expanded whey product. They found that increasing protein cocentration significantly reduced air cell size, water solubility index and increased extrudate density and breaking force. In addition, our preliminary trials revealed that a high level of protein content interfered with extrusion, causing die head blockage. The sufficient screw speed (more than 350 rpm) in the cooking section generates sufficient pressure for the feed to flow through the extruder resulting in no die head blockage.

The results found that increasing feed moisture content also increased bulk density of extrudates (Table 23). The significant ($P \leq 0.01$) interaction between protein content and feed moisture was observed for bulk density (Table 24). At low moisture contents, more frictional heat would be generated within the barrel, which would cause to increase in product temperature, which more highly superheated product exited the die and was exposed to atmopheric pressure. The extrudates would expand to a greater extent, thus reducing its bulk density (Wang *et al*., 1999). However, the insufficient amount of water available for vaporization in protein-starch base extrudates appeared to be the main cause of low expansion and high bulk density values (Park, 1993). Our results supported the hypothesis that the amount of water and temperature during extrusion cooking was an important factor affecting density and expansion of extrudates. Similar results were observed by Bhattacharya and Hanna (1986) who reported that increasing product moisture content lead to a less puffed and hence a denser product. This agrees with an

observation by Ding *et al*. (2006) that increasing feed moisture content results in extrudates with a higher density, lower expansion, and higher hardness.

Conversely, the bulk density decreased with an increase in barrel temperature (Table 23). The inverse relationship between temperature and bulk density was confirmed in previous study (Cumming *et al*., 1972; Wang *et al*., 1999). Interaction effect between feed moisture and barrel temperature on bulk density was also significant (Table 24). The response surfaces plot showed that increasing barrel temperature leads to decrease in bulk density, especially at low feed moisture (Figure 11). So the extrudate with lowest density was produced at low feed moisture (20%) and high barrel temperature (180 °C). Similar results were observed for corn starch (Chinnasway and Hanna, 1988) and soy flour (Holay and Harper, 1982) and rice base expanded snack (Ding *et al*., 2006). For starchy systems, Gomez and Aguilera (1984) found that puffing was directly related to temperature and inversely related to moisture. Sacchetti et al. (2004) reported that the increase in barrel temperature increased starch gelatinization. According to Mercier and Feillet (1975) and Case *et al*., (1992), as gelatinization increases, the volume of extruded products increases and bulk density decreases.

a)



b)

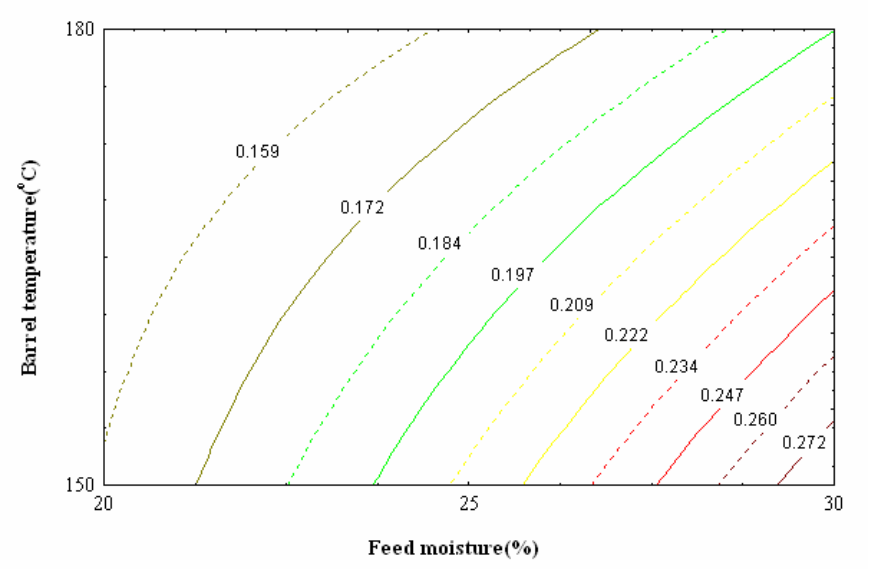


Figure 11 Bulk density of extrudates as a function of feed moisture and barrel temperature at 20% protein (a) and 30% protein (b).

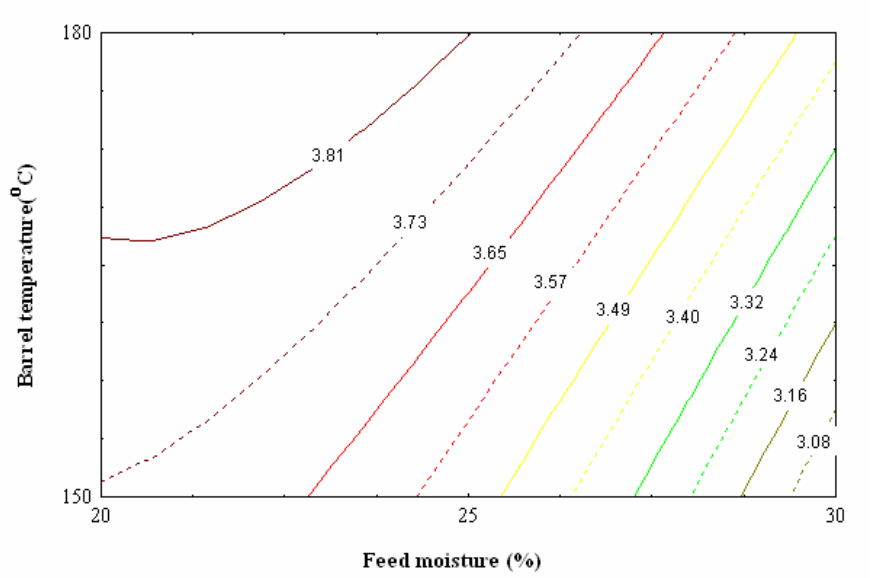
2.2.2 Expansion ratio

The addition of protein in formulation at up to 30 % decreased expansion ratio of extrudates (Table 22). The expansion ratio of 20 % formulation range from 3.01 to 3.87, while 30 % formulation range from 2.41 to 3.01. The highest expansion was obsorved at high protein (30%), high feed moisture content (30%) and low barrel temperature (150 °C). High protein content in mixture provides more expansion due to its high bulk density and small cell size. An inverse relationship between expansion ratio and density of extrudates has been earlier reported (Singh *et al*., 1996) as also observed in this work. Increasing protein increased bulk density but reduced expansion of extrudates. During extrusion cooking, starch or protein undergoes physicochemical changes (such as starch gelatinization, protein denaturation and hydrogen bond rupture) resulting in mechanical and textural properties of extrudates (Ravindra *et al*., 2004). Gelatinization of starch may be influenced by the presence of protein. Proteins have an effect on expansion through their ability to affect water distribution in the matrix and through their macromolecular structure and they also contribute to extensive networking through covalent and non-bonding interactions that take place in extrusion (Moraru and Kokini, 2003). Champenois *et al*.(1998) noted that the presence of a three-dimensional protein network, entrapping starch granules inside the dough, both decreases swelling because it hinders water diffusion and provides a mechanical resistance. Therefore, starch gelatinization should be less favoured, and a slower reaction velocity and higher transition temperature are then expected. In addition, the protein materials also reduced the extensibility of the starch polymer foam during its expansion at the die exit, resulting in the reduction of extrudate expansion (Derby *et al*., 1975).

The effect of extrusion condition on expansion is show in Table 23. Increasing feed moisture content or decreasing barrel temperature significantly decreased expansion of extrudates. The regression coefficients for the expansion model are presented in Table 24. The significant ($P<0.01$) interaction of feed moisture and barrel temperature was observed. The regression model of expansion ratio (Table

24) was used to generate surface plot (Figure 12). Graphic representation of the expansion ratio showed that increasing barrel temperature at costant feed moisture content caused increase expansion ratio of extrudate, espectially at low feed moisture content. The most expansion products were obtained at 180 °C and 20% moisture of the feed (Figure 12). This study found that feed moisture and barrel temperature was the main factor affecting extrudate density and expansion. This is consistent with the observations by Miller (1985) who studied the effect of cooking moisture on product characteristics of low moisture extrusion. They found that the expansion occurring in a food material depended on the pressure differential between the die and atmosphere. Foods with lower moisture tend to be more viscous than those with higher moisture and; therefore, the pressure differential would be smaller for higher moisture foods, leading to a less expanded product. Pansawat *et al*. (2007) also observed that the increasing feed moisture decreased radial expansion of fish, rice-based snacks extrudates. Singh *et al*. (2007) studied the effect of feed moisture (18–24%), extrusion temperature (130–170 °C) and level of pea grits (0–30%) on the density and expansion of rice grits. They also found that the important factors affected these properties were feed moisture and barrel temperature. The reports of Chinnaswamy and Hanna (1988) suggested that extruded at low moisture condition had higher average die pressures, whereas the higher pressure could cause more moisture evaporation leading to higher expansion degree. Low moisture content in feed may restrict flow of the material and increase shearing rate and residence time, and also the degree of superheating of water in the extruder would increase at higher temperatures, which might increase the degree of gelatinization and expansion (Chinnaswamy and Hanna, 1990). Moreover, the high dependence of bulk density and expansion on feed moisture would reflect its influence on elasticity characteristics of the starch-based material. Decreasing feed moisture content during extrusion may increase the elasticity of the dough through plasticization of the melt, resulting in increased SME and therefore increased gelatinization, increasing the expansion and decreasing the density of extrudate (Ding *et al*., 2006).

a)



b)

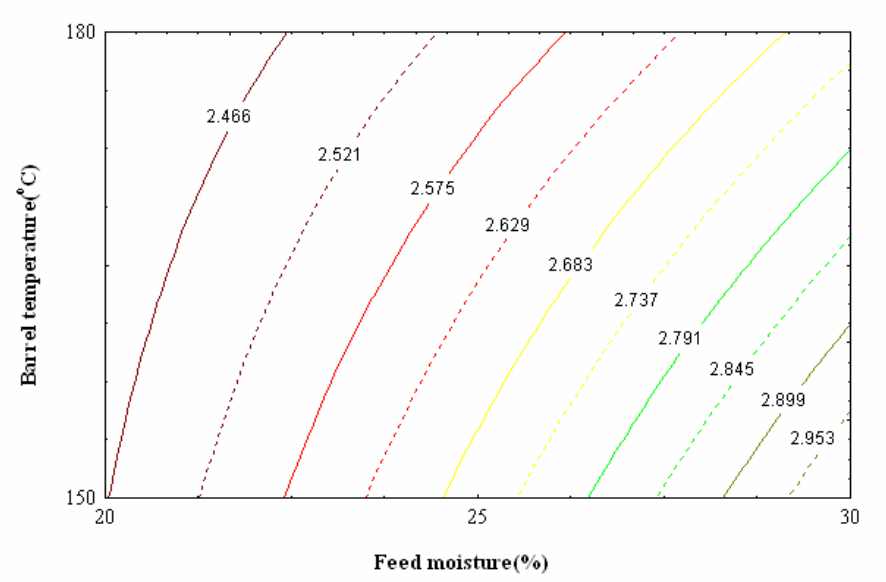


Figure 12 Expansion of extrudates as a function of feed moisture and barrel temperature at 20% protein (a) and 30% protein (b).

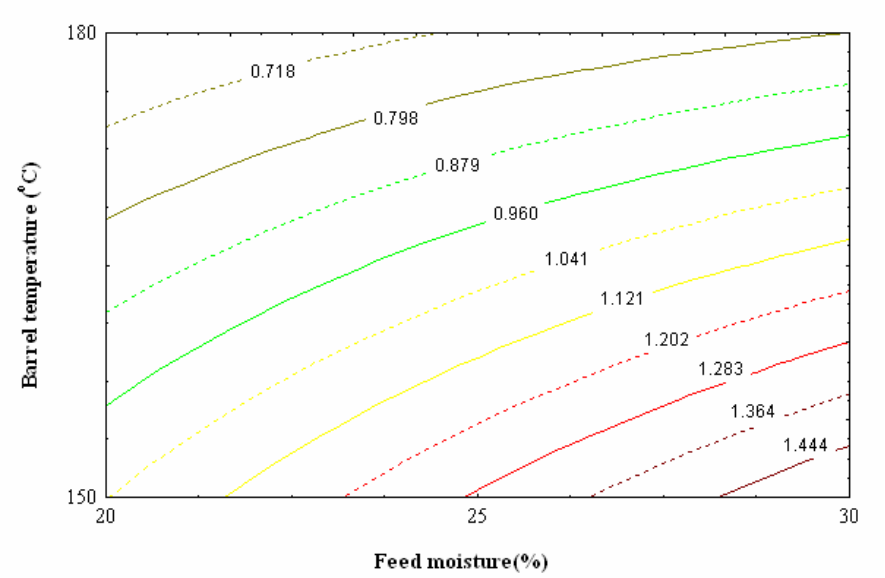
2.2.3 Breaking strength index (BSI)

The BSI was found to be directly related to bulk density and inversely related to expansion ratio of extrudates. The high expansion, low bulk density and low shear strength of both formulations were obtained under the condition of 20 % feed moisture and 180 °C (Table 23). The BSI of 20% and 30 % protein formulation ranged from 0.64 to 1.53 N/mm, and 1.66 to 3.52 N/mm, respectively (Table 23). These results observed that high protein content in extrudates gave a higher BSI than low protein content. Therefore, the extrudate hardness was found to be strongly influenced by the protein content. Increased protein content in feed material produced a less expanded product and more rigid network, resulting in the higher resistance to shear. Barrett *et al*. (1994) reported that the cellularity and bulk density in extrudates influenced strength and fracture properties. Similar results was reported by Veronica *et al*. (2006) about extruded maize and soybean and Allen *et al*. (2007) who studied the influence of protein level and starch type on an extrusion-expanded whey product. They found that increasing protein concentration and normal cornstarch significantly reduced extrudate expansion ratio, air cell size, and increased extrudate density and breaking force.

The effect of protein content and extrusion variable are shown in Table 24. The BSI increased with the increasing of protein content, feed moisture or decreased barrel temperature. The regression model showed positive relationship between protein content and feed moisture, and a negative relationship between feed moisture and barrel temperature (Table 24). The surface plot for BSI (Figure 13) shows that increasing feed moisture content increased BSI, while increasing barrel temperature reduced it. Similar observations were reported by Taylor *et al*., (2006) who studied the influence of barrel temperature on the properties of expanded extrudates containing whey protein concentrate. They reported that breaking strength of extrudates was influenced by a level of protein content and barrel temperature in cooking zone. Increased temperature may have increased both flashing off moisture from the extrudate upon exiting the die and the content of gelatinized starch, both of which are correlated to increased extrudate expansion and decreased breaking strength

of extrudates. These results are consistent with the observations of Jauregui et al, (2000) who found that increasing amaranth protein, increasing feed moisture or decreasing barrel temperature resulted in an increase in shear strength, shearing stress and bulk density but decrease in expansion of amaranth extrudates.

a)



b)

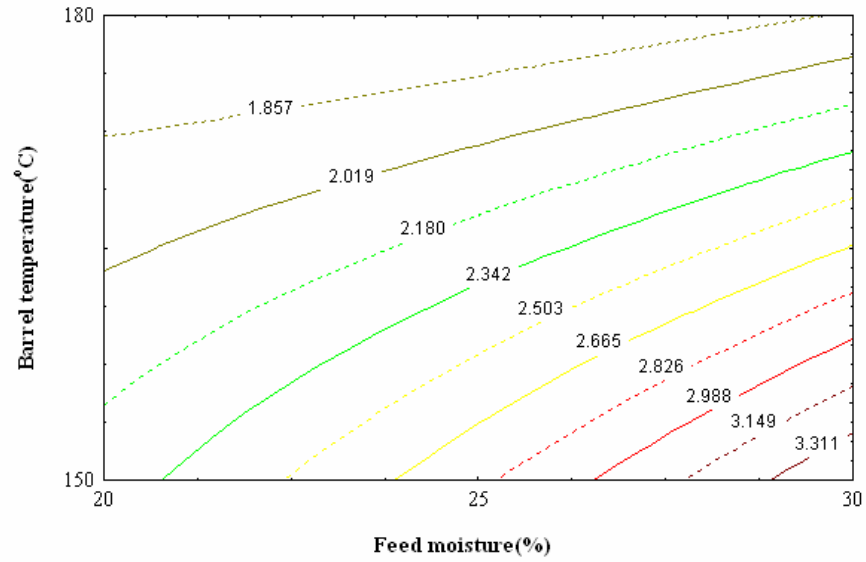


Figure 13 Breaking strength index (BSI) of extrudates as a function of feed moisture and barrel temperature at 20% protein (a) and 30% protein (b)

2.2.4 Colour measurement

The effect of extrusion cooking on the colour parameters of extrudates are shown in Table 25 and ANOVA results are presented in Table 26. The coefficients of regression model for lightness (*L**), redness/greenness (*a**) and yellowness/blueness (*b**) parameters as a function of protein content, feed moisture and barrel temperature are reported in Table 27.

Table 25 Result of Duncan's multiple range test of instrument colour measurements of extrudates.

Protein content (%)	Feed moisture (%)	Barrel temperature (°C)	*L**	*a**	*b**
20	20	150	81.36 ± 0.01^{b}	2.92 ± 0.02^{fg}	23.85 ± 0.06^{g}
20	20	180	75.51 ± 0.07^{j}	5.98 ± 0.06^{a}	26.00 ± 0.19^{c}
20	25	150	81.44 ± 0.05^{a}	2.52 ± 0.06^{i}	24.47 ± 0.36^{f}
20	25	180	77.66 ± 0.03^{h}	4.62 ± 0.07^{c}	25.43 ± 0.02^{d}
20	30	150	81.05 ± 0.05^{c}	2.52 ± 0.12^{i}	24.12 ± 0.23^{g}
20	30	180	79.94 ± 0.02^{e}	3.74 ± 0.03^{e}	24.98 ± 0.03^{e}
30	20	150	80.08 ± 0.01^{d}	2.99 ± 0.01^{f}	$25.43+0.02^{d}$
30	20	180	75.58 ± 0.02^{i}	5.89 ± 0.03^{b}	27.62 ± 0.04^{a}
30	25	150	80.03 ± 0.02^{d}	2.65 ± 0.04^{h}	23.88 ± 0.13^{g}
30	25	180	77.71 ± 0.01^{h}	4.65 ± 0.02^{c}	26.44 ± 0.09^{b}
30	30	150	79.43 ± 0.02^{f}	2.84 ± 0.06^{g}	24.83 ± 0.18^{e}
30	30	180	78.34 ± 0.03^{g}	4.06 ± 0.02^{d}	26.02 ± 0.17^{c}

[1] Mean ± standard deviation of three determinations

[a-j] Different letters within the same column indicate significant difference ($P\leq0.05$)

*L**, index of lightness/ brightness ; *a**, index of redness/greenness; *b**, index of yellowness/blueness.

Table 26 ANOVA summary table of instrument colour measurements of extrudates.

Treatment		*L**	*a**	*b**
Protein (%)	20	79.49±2.27[a 1]	3.72±1.29[b]	24.81±0.79[b]
	30	78.53±1.62[b]	3.85±1.19[a]	25.70±1.23[a]
Moisture (%)	20	78.13±2.75[c]	4.45±1.56[a]	25.72±1.41[a]
	25	79.21±1.68[b]	3.62±1.07[b]	25.06±1.03[b]
	30	79.69±1.02[a]	3.29±0.66[c]	24.98±0.73[b]
Barrel temperature(°C)	150	80.56±0.78[a]	2.74±0.19[b]	24.43±0.60[b]
	180	77.45±1.59[b]	4.83±0.87[a]	26.08±0.86[a]

[1] Mean ± standard deviation of three determinations

[a-j] Different letters within the same column indicate significant difference ($P \leq 0.05$)

*L**, index of lightness/ brightness ; *a**, index of redness/greenness; *b**, index of yellowness/blueness.

Table 27 Significant coefficients of regression equation for the colour responses.

	*L**	*a**	*b**
β_0	79.211***	3.615***	25.057***
β_1	-0.484***	0.064***	0.448***
β_2	0.779***	-0.578***	-0.370***
β_3	-1.557***	1.041***	0.826***
β_{12}	-0.251***	0.085***	-0.181***
β_{13}	0.236***	-0.022*	0.164*
β_{23}	1.017***	-0.442***	-0.287***
β_{22}	-0.300***	0.254***	0.298*
R^2 adj.	0.988	0.998	0.887
SE	0.2211	0.0588	0.3741
F	407.127***	2177.29***	40.298***

* Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$

*** Significant at $P \leq 0.001$

R^2 adj. = the adjusted R^2

SE = the standard error of estimation

F = the Fisher test F value

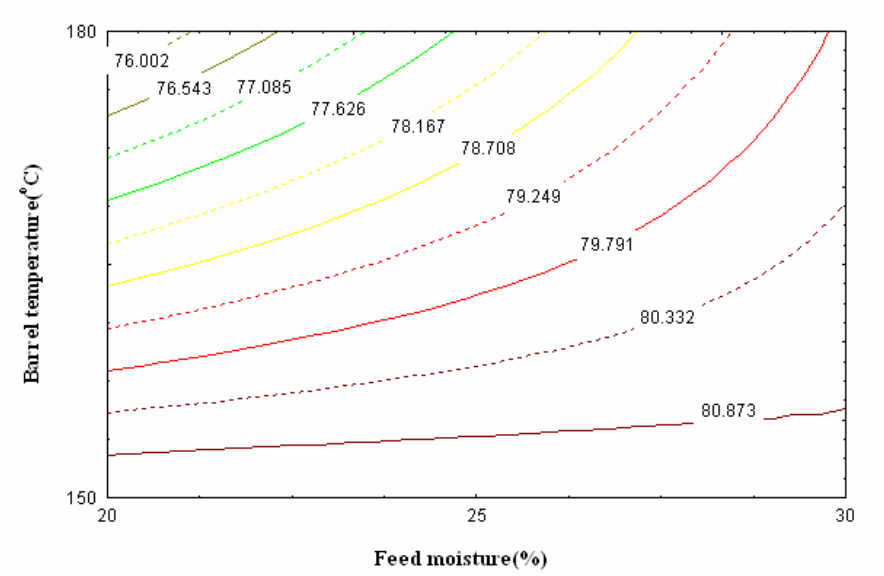
Where β_o, β_1, β_2, β_3, β_{12}, β_{13}, β_{23} and β_{22} are coefficients for intercept, linear, interactive and quadratic effects, respectively, and X_1, X_2 and X_3 are independent variables in coded values (X_1 = protein content, X_2 = feed moisture, X_3=barrel temperature)

The colour parameters (*L**, *a**and *b** value) of extrudates produce from different level of protein content, feed moisture content and barrel temperature presented in Table 25. The result showed that protein content and extrusion condition had significant effect ($P \leq 0.01$) on *L**, *a** and *b** values of extrudates. Increasing protein content in the initial blend resulted in decreased snack's lightness (*L**) but increased redneess (*a**) and yellowness (*b**) of the extrudates (Table 26). In other

word, the high protein extrudate (30% protein) had higher colour intensity than low protein extrudate (20% protein). This is probably due to the non-enzymatic browning reaction (Maillard reaction) which is brown colour of wheat gluten and soy grits.

Increasing feed moisture content increased *L** but decreased a* and b* values. In contrast, increasing barrel temperature decreased *L** but increased *a** and *b** values (Table 26). The interactive effects of the three independent variables are a determining factor in testing the statistical significance of the models (R^2 contribution are 0.988, 0.988 and 0.887, respectively, for *L*, a** and *b** indices). The highly significant interactions ($P \leq 0.01$) were also found between feed moisture and barrel temperature on *L*, a** and *b** value of extrudates (Table 27). The response surfaces of *L*, a** and *b** parameters are represented in Figure 14, 15 and 16, respectively. It was found that increasing feed moisture content resulted in increased *L** (Figure 14) but decreased a* (Figure 15) and *b** (Figure 16) values, particularly at high temperature. These studies indicated that the decreasing feed moisture resulted in the dark yellow colour of extrudates due to browning reaction. Lawton *et al*. (1985) suggested that water in the extruder works as a heat sink/trap, lubricant and reduced shear strength. On the other hand, a low feed moisture lead to increase power requirements and surging (Conway and Anderson, 1973) and can also result in excessive browning and stoppage of extrudate at die nozzle opening. The impact of feed moisture and barrel temperature on colour of extrudates was related to the degree of browning in the products, which was also observed in studies of MacDougall and Granov (1998) and Sacchetti *et al*. (2004). It was expected due to the known influence of temperature and moisture on non-enzymatic browning reaction (Maillard reaction) in products, which contain substantial levels of protein and substantial of carbohydrates (Berset, 1989; Hurrell and Carpenter, 1977).

a)



b)

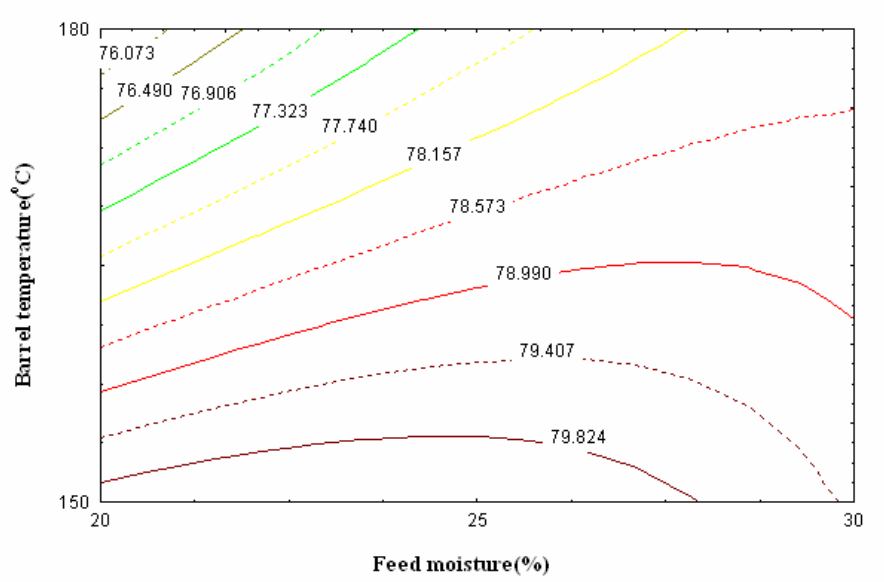
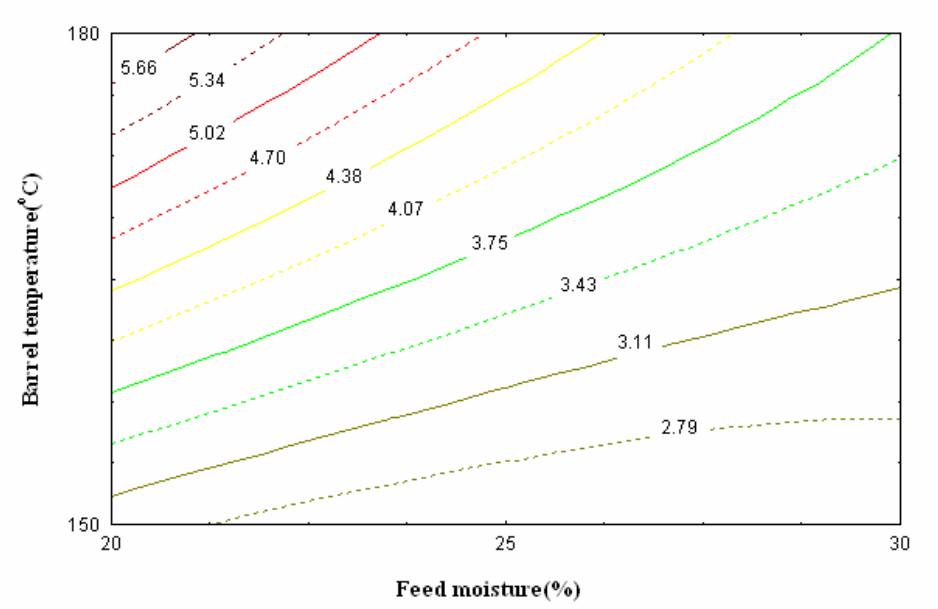


Figure 14 Lightness (L^*) of extrudates as a function of feed moisture and barrel temperature at 20% protein (a) and 30% protein (b).

a)



b)

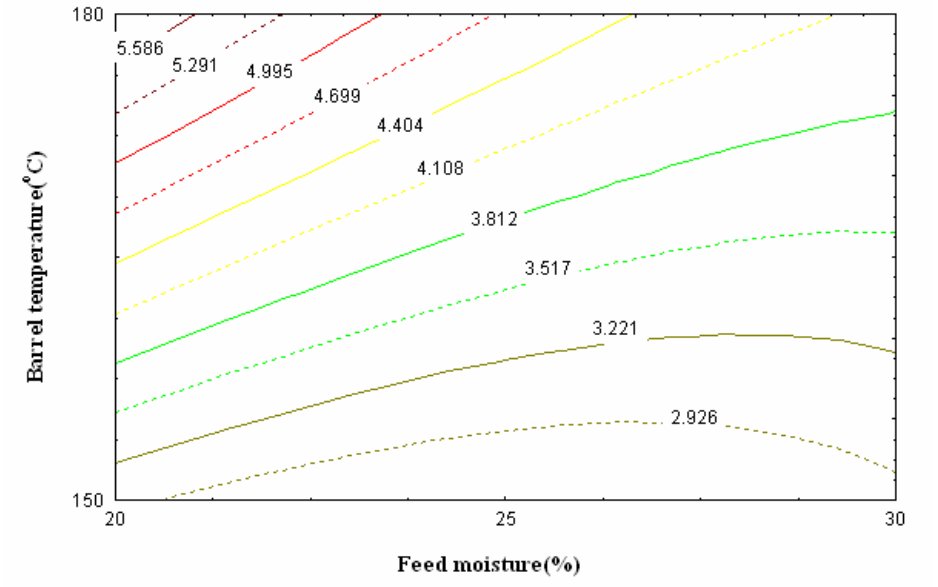
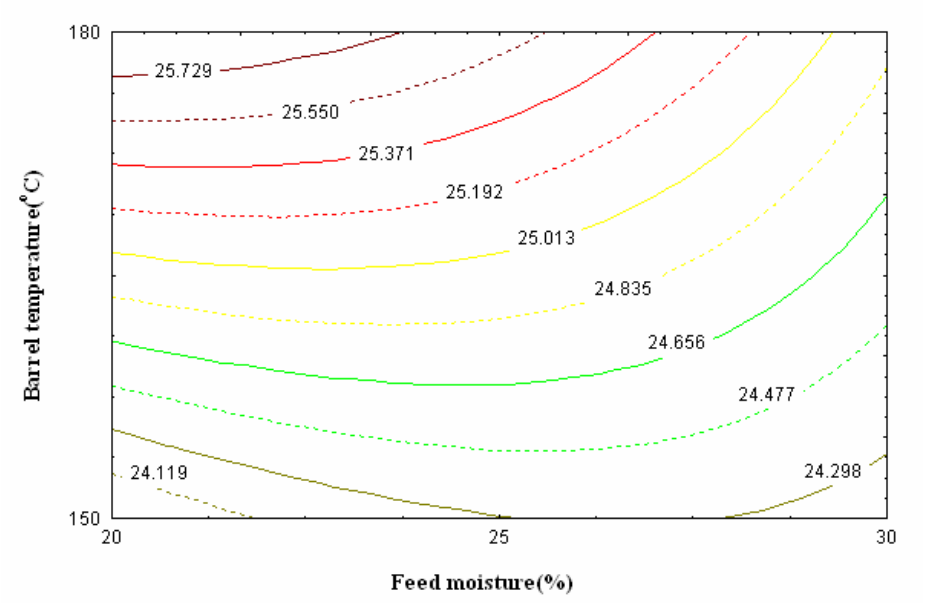


Figure 15 Redness/greenness (*a**) of extrudates as a function of feed moisture and barrel temperature at 20% protein (a) and 30% protein (b).

a)



b)

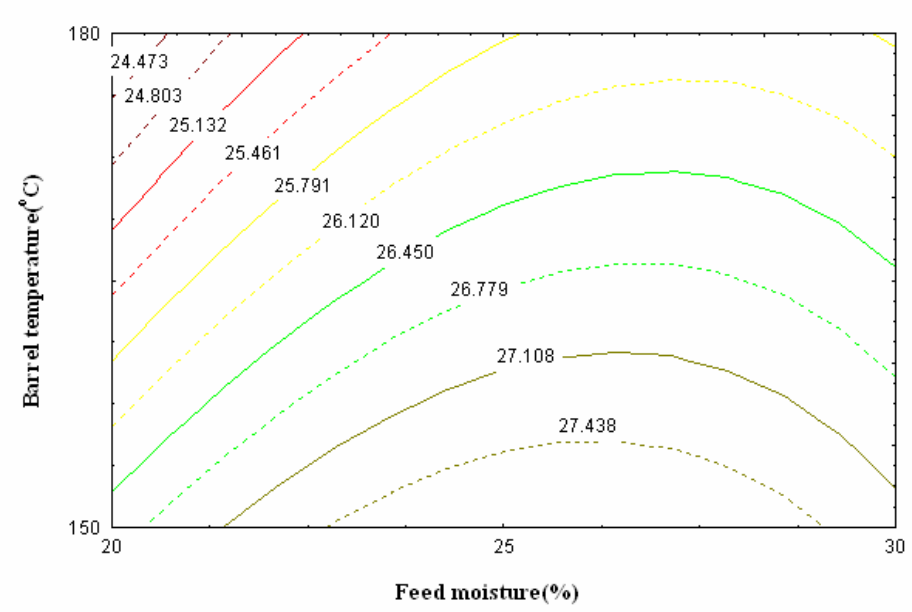


Figure 16 Yellowness/blueness (*b**) of extrudates as a function of feed moisture and barrel temperature at 20% protein (a) and 30% protein (b).

2.3 Sensory properties

2.3.1 Descriptive analysis

Extruded snack samples (12 treatments), produced under different conditions of protein content, feed moisture and temperature were characterised by ten trained panels using descriptive sensory analysis. The generated attributes, difinition established and references used are presented in Appendix Table D1. Each treatment was evaluated for colour, noise, hardness, crispness, brittleness and sticky mouth coating. Mean scores for the descriptive sensory analysis are shown in Table 28. The highest hardness, noise, crispness and brittleness were observed at high protein content (30%), high feed moisture (30%) and low barrel temperature (150°C) because the extudates had high bulk density, high breaking strength and low expansion. However, the lowest sticky mouth coating was observed at 30 % protein content but extruded under condition of 180°C. The statistical analysis of protein effects in Table 30 confirmed that sticky mouth coating of extrudates decreased with an increase of protein content in the mixtures. This is probably due to the water absorption index (WAI) and water solubility index (WSI) of starch and protein after extrusion process. A high WAI level is correlated to poor consumer acceptability, especially in crisp snack product, because unbroken starch rapidly absorbs water and reduces product crispiness and sticky (or starchy) in the mouth and covers the teeth after chew (Sohkey *et al*., 1996). As with WAI, increased WSI is correlated to poor consumer acceptability, WSI is a measure of the extent the extrudate is solubilized during hydration (Jin *et al*., 1995; Sohkey *et al*., 1996).

The extrusion of starchy foods results in gelatinization, partial or complete destruction of the crystalline structure and molecular fragmentation of starch polymers, as well as protein denaturation, and formation of complexes between starch and protein (Colonna and Mercier,1989; Ho and Izzo, 1992; Mercier and Feillet, 1975; Mercier *et al*., 1980). The WAI of extruded products is related to the level of gelatinization of starch and to the length of the starch chains (Kandan *et al*. 2003). WSI often used as an indicator of degradation of molecular components (Kirby et al.,

1988). The WAI increases with an increase in gelatinization and decreases with either a decrease in gelatinization or starch dextrinization (Nijoki and Faller 2001; Li *et al*., 2003). Sing *et al*. (2007) reported that WAI and WSI of rice grits increased by extrusion cooking. Similar results were reported by Mercier and Feillet (1975) who found that soluble starch increased after extrusion.

The WAI and WSI decreased with addition of protein materials (soy grits and wheat gluten) in mixture may be due to the dilution of starch in blends. Jones *et al*. (2000) reported a decrease in WAI with decrease in starch content. It has been observed that incorporation of pea grits in feed material decreased WAI and WSI of rice based extrudates (Sing *et al*., 2007). Generally, extrusion cooking leads to disorder in the structure of the protein and also a loss in solubility (Kilara and Sharkasi, 1986). Similar observations were reported by Hager (1984) who studied the effect of extrusion upon soy concentrate solubility. Ding *et al.* (2006) observed that the WAI and WSI of wheat based extrudates decreased by extrusion cooking. This is due to during wheat dough cooking a physical competition between protein coagulation into a continuous network and starch gelatinization will take place. The formation of a gluten network would have a restrictive role on starch gelatinization and result in greater dextrinization. Similar results were reported by Hagenimana et al. (2006) who observed that using soy protein concentrate (SPC) in combination with rice flour (RF), not only to provide a useful alternative in highly nutritious food products, but also to improve the physicochemical, functional and sensory characteristics of the products. This result was consistent with the report of Veronica *et al*. (2005) who studied the qualities of extruded puffed snacks from maize/soybean mixture. The level of insoluble protein and soluble carbohydrate are likely the result of protein-starch interactions. Ravindra *et al*., (2004) reported that the interaction of starch and protein plays an important role in macroscopic properties of food products in terms of flow, stability, texture and mouth feel.

An increase of protein content from 20% to 30%, samples became significantly ($P \leq 0.05$) increased hardness of the extrudates (Table 29). This is probably due to the presence of gluten protein. Harper (1981) suggested that the

gluten protein is important factor related to hardness of extrudate. Similar results were reported by Ding *et al*. (2006) who obserbed that the wheat extrudates gave a harder product compared to rice extrudate. However, addition of soy protein isolate in cornmeal snack also increased hadness of extrudate (Camire and Kinge, 1991). Bhattacharya *et al* (1986) studied the textural properties of extruded corn gluten meal and soy protein blends. They found that increased protein content of feed component caused an increase of hardness of extrudates.

The feed moisture content had significant ($P \leq 0.01$) effect on all descriptive attributes (Table 29). Increasing feed moisture content resulted in increased hardness, noise, crispy and brittleness but decreased sticky mouth coating of extrudates. These results were contrast with the effect of barrel temperature, which high barrel temperature (180 °C) caused to reduce hardness, crispness and brittleness and less biting noise and also less stick mouth coating (Table 29). The results in this study found that the feed moisture and barrel temperature were the main factor affecting the density and expansion of the extrudate. It would be expected that high density and low expansion would produce a harder extrudated. These results are consistent with the observations by Badrie and Mellowes (1991) who studied the sensory and physical properties of cassaba flour extrudates. Faller and Heymann (1996) who studied the physical properties of extruded potato puffs, Liu *et al*. (2000) who studied the physical properties of extruded oat-corn puff and Sacchetti *et al*. (2004) who studied the physical properties of chestnut and rice flour-based snack-like products, also agree with these results.

For the colour attribute, both of two fromulation was extruded under low feed moisture (20%) and high barrel temperature (180°C) had the highest darkening colour (Table 28). The colour intensity of extrudate increased with the decrease in feed moisture and an increase in barrel temperature (Table 29). This result was most likely caused by the formation of Maillard reaction products, which is promoted by condition of low moisture and high temperatures (Bredie *et al*., 1998).

The significant ($P \leq 0.05$) interactions effects are presented in Appendix Figures D3, D4 and D5. There were distinct significant ($P \leq 0.05$) interaction between protein content and barrel temperature on sticky mouth coating (Figure D3 (a)). Increasing barrel temperature at high protein content (30%) resulted in sharp decreasing sticky mouth coating than that of low protein content (20%).

Table 28 Result of Duncan's multiple range test of descriptive analysis of extruded snacks.

Protein content (%)	Feed moisture (%)	Barrel temperature (°C)	Hardness	Noise	Crispness
20	20	150	4.8±0.6^{f1}	7.2±0.3^{d}	7.3±0.4^{fg}
20	20	180	3.2±0.5^{h}	6.6±0.2^{e}	6.9±0.3^{g}
20	25	150	5.3±0.3^{f}	7.5±0.4^{cd}	7.7±0.2^{de}
20	25	180	3.4±0.5^{h}	7.0±0.4^{de}	6.3±0.4^{h}
20	30	150	7.9±0.9^{c}	8.5±0.7^{b}	8.1±0.7^{bc}
20	30	180	4.2±0.6^{g}	7.1±0.4^{d}	6.1±0.3^{h}
30	20	150	7.7±0.5^{c}	7.9±0.5^{c}	8.3±0.5^{b}
30	20	180	5.9±0.4^{e}	7.4±0.4^{cd}	7.5±0.5^{ef}
30	25	150	8.4±0.7^{b}	8.7±0.5^{ab}	9.1±0.7^{a}
30	25	180	6.5±0.6^{d}	7.7±0.5^{c}	7.9±0.4^{de}
30	30	150	10.2±0.6^{a}	8.9±0.7^{a}	9.3±0.6^{a}
30	30	180	6.8±0.5^{d}	7.8±0.8^{c}	8.0±0.3^{bc}

Table 28 (Continued)

Protein content (%)	Feed moisture (%)	Barrel temperature (°C)	Brittleness	Sticky mouth coating	Colour
20	20	150	9.2±0.5^{bc}	9.3±0.8^{a}	3.7±0.3^{e}
20	20	180	9.1±0.7^{bc}	8.8±0.9^{ab}	6.6±0.7^{a}
20	25	150	9.4±0.7^{bc}	8.7±1.2^{ab}	3.2±0.8^{fg}
20	25	180	9.0±0.6^{bc}	8.5±0.8^{bc}	5.1±0.5^{c}
20	30	150	9.6±0.5^{ab}	8.1±1.1^{bc}	3.1±0.3^{g}
20	30	180	9.2±0.7^{bc}	8.1±0.6^{bc}	4.4±0.5^{d}
30	20	150	9.1±0.6^{bc}	8.8±1.1^{ab}	3.7±0.3^{e}
30	20	180	8.7±0.7^{c}	7.5±0.4^{cd}	5.6±0.5^{b}
30	25	150	9.6±0.7^{ab}	8.3±0.9^{bc}	3.6±0.3^{ef}
30	25	180	9.2±0.7^{bc}	7.2±0.8^{d}	5.1±0.4^{c}
30	30	150	10.1±0.9^{a}	8.2±0.5^{bc}	3.4±0.3^{ef}
30	30	180	9.4±0.5^{b}	6.9±0.5^{d}	4.1±0.4^{d}

[1] Mean ± standard deviation of ten panelists evaluated three replicates of each sample (30)

[a-h] Different letters within the same column indicate significant difference ($P \leq 0.05$)

All data input on 15 cm line scales wherein 1.25 cm anchored as "low" and 13.75 cm as "high"

Table 29 ANOVA summary table of descriptive analysis of extruded snacks.

Treatment		Hard-ness	Noise	Crisp-ness	Brittle-ness	Sticky mouth coating	Colour
Protein (%)	20	4.8±1.7[b1]	7.3±0.7[b]	7.1±0.8[b]	9.2±0.6[a]	8.9±0.9[a]	4.3±1.3[a]
	30	7.6±1.5[a]	8.1±0.8[a]	8.3±0.8[a]	9.4±0.8[a]	7.8±0.9[b]	4.2±0.9[a]
Mois-ture (%)	20	5.4±1.8[c]	7.2±0.6[c]	7.5±0.7[b]	9.0±0.6[b]	8.6±1.1[a]	4.9±1.3[a]
	25	5.9±1.9[b]	7.7±0.7[b]	7.7±1.1[a]	9.3±0.7[ab]	8.1±1.1[b]	4.2±0.9[b]
	30	7.3±2.3[a]	8.1±0.9[a]	7.9±1.2[a]	9.8±0.7[a]	7.8±0.9[b]	3.7±0.7[b]
Barrel temp-erature (ºC)	150	7.4±1.9[a]	8.1±0.8[a]	8.3±0.9[a]	9.5±0.7[a]	8.6±1.0[a]	3.5±0.5[b]
	180	4.9±1.6[b]	7.3±0.6[b]	7.1±0.8[b]	9.1±0.7[b]	7.9±0.9[b]	5.1±0.9[a]

[1] Mean ± standard deviation of ten panelists evaluated 12 extrudates differing in protein content, feed moisture and barrel temperature in three replicates of each sample

[a-c] Different letters within the same column of each treatment indicate significant difference ($P \leq 0.05$)

2.3.2 Acceptability test

2.3.2.1 Non-enrobed extrudates acceptability test

The extruded samples, without seasoning coating, evaluated for appearance, flavour, texture, colour and overall acceptability using a 9-point hedonic scale by 50 untrained panelists are shown in Table 30. Both formulations (20 % and 30 % protein), extruded at 30% feed moisture and 180 °C received the highest ratings for all attributes and overall liking compared to all other snack samples.

The ANOVA results are summarized in Table 31. Increasing protein content resulted in a decrease in hedonic rating scores for appearance and colour attributes. This was probably because the shapes of extrudates product from 30% protein were not uniform, and the surfaces were rough, uneven and pockmarked, which influenced the appearance acceptance (as shown in Appendix Figure D2). The results also observed that increasing protein content from 20% to 30 % in the formulation had no significant effect on rating for flavour acceptance (Table 31). However, the 30% protein extrudates received a significantly higher rating for texture than 20% protein extrudates. This was due to the unacceptable snacks which had more sticky mouth coating. In addition, the high protein extrudates (30%) had more bulk (cellular structure) for chewing. This sample also received a high rating for overall liking. Similar results were reported by Singh *et al*. (1991) who observed that the addition of small amounts of proteins (<5% of milk protein) in a corn base extruded snack enhances the textural properties of extruded products. Faller *et al*. (1999) suggest that an added 15-19 % of soy increased hedonic rating scores for overall liking of extruded corn snacks.

The influence of extrusion conditions on hedonic ratings for sensory attributes and overall liking of extrudates are also shown in Table 31. The results found that the rating for colour and flavour of extrudates increased with an increase in feed moisture content or decrease in barrel temperature. The interaction between feed moisture and barrel temperature for affecting colour and flavour was

also observed (Figure 19 (b) and (c)). The rating for colour was increased, when feed moisture increased from 20 to 30 %. Meanwhile, it had an inverse effect for low barrel temperature (150 °C) (Figure 19 (b)). This was because the product had low expansion and pale yellow colour. Similar observations were reported by Liu et al. (2000) who observed that higher feed moisture content (ranged from 18 to 21%) reduced expansion, and increased the lightness of extruded oat-corn puff. Ding et al. (2005) also reported that increasing feed moisture content results in rice-based extrudates with higher density, lower expansion, higher hardness and lower crispness. Similar observations were reported by Sing et al. (2007) who studied the effect of moisture and temperature on rice-based extrudates.

The hedonic rating for texture of extrudates decreased with an increase in feed moisture content. The interaction plot between protein content and barrel temperature for texture and overall liking scores are shown in Figure 18. The figures indicates that there was a downward trend in ratings for texture (Figure 18 (a)) and overall liking (Figure 18 (b)) of 30 % protein extrudates as the barrel temperature was increased to 180°C because the product became rigid and less expanded.

According to above results, the appearance, colour and flavour were not considered as important attributes for non-enrobe extrudates. Most panels liked extrudates with less sticky mouth coating and more bulk (cellular structure) for chewing. These characteristics are consistent with the extrudates product from 30% protein content. The formulation contained 30 % protein, extruded under the conditions of 30 % moisture at 180 °C was selected for further study because this formulation had high protein content and high hedonic scores rating for all attributes.

Table 30 Result of Duncan's multiple range test of hedonic ratings for sensory attributes and overall acceptance of non-enrobed extrudates.

Protein content (%)	Feed mois-ture (%)	Barrel temp-erature (°C)	Appea-rance	Colour	Flavour	Texture	Overall liking
20	20	150	5.9±1.2[a1]	6.0±1.1[a]	4.5±1.2[a]	5.6±1.2[ab]	5.8±1.2[ab]
20	20	180	4.3±0.8[c]	5.0±1.1[c]	3.7±1.2[b]	4.0±1.2[c]	4.6±1.1[c]
20	25	150	5.8±1.2[a]	6.1±1.0[a]	4.4±1.2[a]	5.5±1.2[ab]	5.6±1.1[ab]
20	25	180	5.0±1.1[b]	5.3±1.3[bc]	4.4±1.2[a]	4.2±1.0[c]	4.9±1.0[c]
20	30	150	4.0±1.0[c]	5.7±1.2[b]	4.6±1.1[a]	3.9±0.9[c]	4.8±1.1[c]
20	30	180	5.9±1.2[a]	6.0+1.1[a]	4.5±1.2[a]	5.9±1.1[ab]	5.9+1.2[a]
30	20	150	5.6±1.1[a]	5.7±0.9[ab]	4.4±1.1[a]	5.7+1.2[ab]	5.8±1.2[ab]
30	20	180	4.2±1.0[c]	4.9±1.0[c]	3.6±1.2[b]	5.4±1.1[b]	5.4±1.2[b]
30	25	150	5.5±1.1[a]	5.6±1.2[ab]	4.7±1.2[a]	5.6±1.2[ab]	5.5±1.2[ab]
30	25	180	4.8+1.1[b]	5.0+0.9[c]	4.6±1.2[a]	5.9±1.1[ab]	5.8±1.2[ab]
30	30	150	4.1±0.8[c]	5.4+1.1[bc]	4.5±1.2[a]	4.0±1.2[c]	4.7±1.2[c]
30	30	180	5.6+1.2[a]	5.7+1.2[ab]	4.7±1.1[a]	6.0±1.2[a]	6.0±1.2[a]

[1] Mean ± standard deviation of fifty panelists evaluated of each sample

[a-c] Different letters within the same column indicate significant difference ($P \leq 0.05$)

Sensory data collected using nine-point hedonic scale with anchor points; 1-extremely dislike to 9-extremely like.

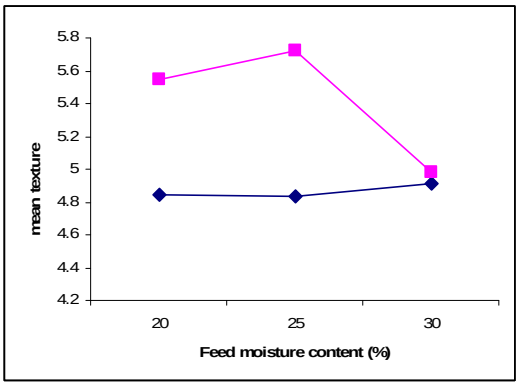
Table 31 ANOVA summary table of hedonic ratings for sensory attributes and overall acceptance of non-enrobed extrudates.

Treatment		Appearance	Colour	Flavour	Texture	Overall liking
Protein (%)	20	5.2±1.3[a 1]	5.7±1.2[a]	4.3±1.2[a]	4.9±1.4[b]	5.3±1.2[b]
	30	4.9±1.2[b]	5.4±1.1[b]	4.4±1.2[a]	5.4±1.3[a]	5.5±1.3[a]
Moisture (%)	20	5.0±1.3[b]	5.4±1.1[b]	4.1±1.2[b]	5.2±1.3[a]	5.4±1.3[a]
	25	5.3±1.2[a]	5.5±1.2[ab]	4.5±1.1[a]	5.3±1.3[a]	5.5±1.2[a]
	30	4. 9±1.4[b]	5.7±1.2[a]	4.6±1.1[a]	5.0±1.2[b]	5.4±1.3[a]
Barrel temp-erature (°C)	150	5.2±1.4[a]	5.8±1.1[a]	4.5±1.2[a]	5.1±1.4[a]	5.3±1.2[a]
	180	5.0±1.2[b]	5.4±1.2[b]	4.2±1.2[b]	5.2±1.4[a]	5.4±1.3[a]

[1] Mean ± standard deviation of fifty volunteer young graduate students (18-35 years old) participated at Kasetsart University evaluating 12 samples, four sessions of three products.

[a-b] Different letters within the same column of each attribute indicate significant difference ($P \leq 0.05$)

Sensory data collected using nine-point hedonic scale with anchor points; 1-extremely dislike to 9-extremely like.

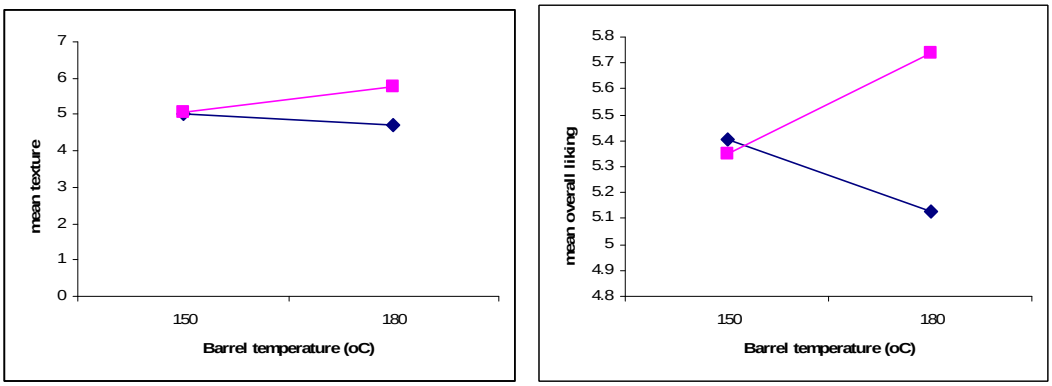


- Sensory data collected from 50 panelists
- Extrusion moisture contents 20, 25, 30 % and protein contents 20, 30 %
- For plots (a) through (c) the symbols correspond to the following protein contents:

-♦- 20 % protein

-■- 30 % protein

Figure 17 Interaction plots of protein content by feed moisture for texture scores.



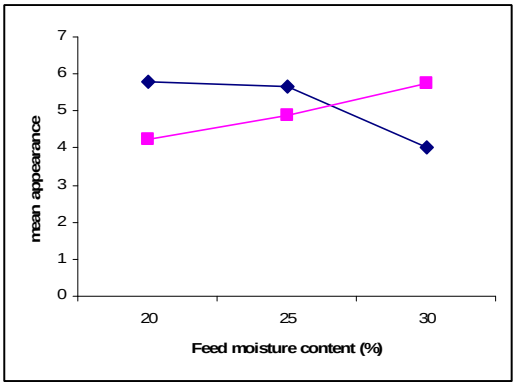
a) Texture b) Overall liking

- Sensory data collected from 50 panelists
- Extrusion barrel temperature 150, 180°C and protein contents 20, 30 %
- The symbols correspond to the following protein contents:

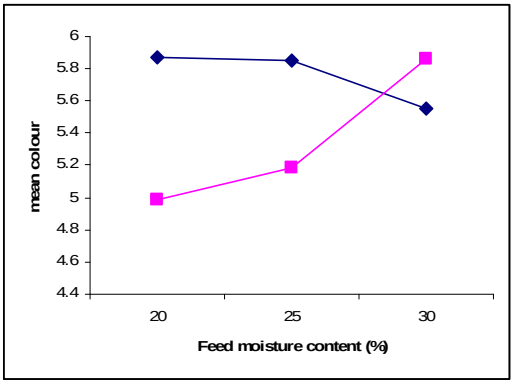
-♦- 20 % protein

-■- 30 % protein

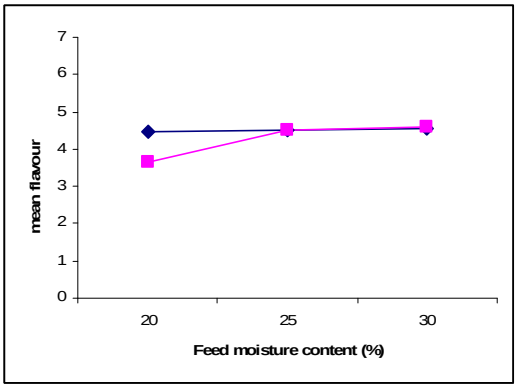
Figure 18 Interaction plots of protein content by barrel temperature for the texture and overall liking scores.



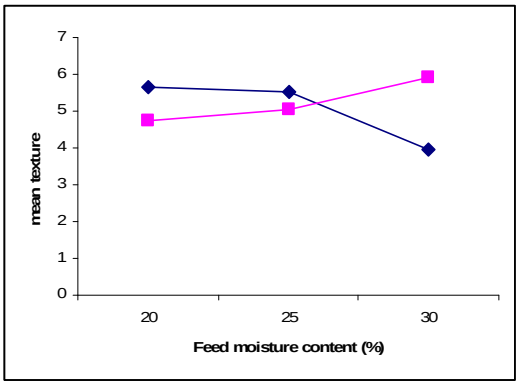
a) Appearance



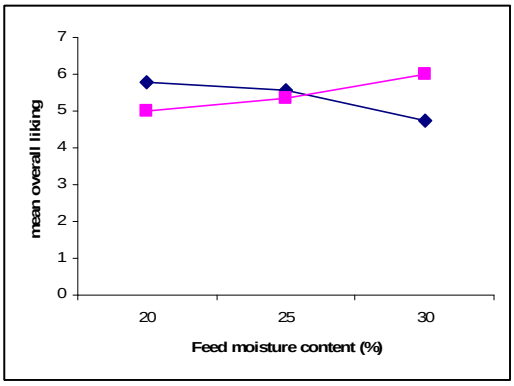
b) Colour



c) Flovour



d) Texture



e) Overall liking

- Sensory data collected from 50 panelists
- Extrusion moisture content 20, 25, 30 % and barrel temperature 150, 180°C
- The symbols correspond to the following protein contents: -♦- 150 °C -■- 180 °C

Figure 19 Interaction plots of feed moisture by barrel temperature for the hedonic rating scores

2.3.2.2. Enrobed extrudates acceptability test

Among extrudates containing 30 % protein content, extruded under condition of 30 % feed moisture, at 180 °C was selected from the non-enrobed extrudates acceptability test. This product was enrobed with two seasoning ingredients (barbecue or cheese seasoning). The amount of seasoning in a final formulation was contained 4, 8 or 12 %, respectively . The appearance of enrobed extrudates is shown in Appendix Figure D1. Means of hedonic rating scores for odour, salty flavour, hardness and overall liking are presented in Table 32.

The colour, flavour and texture of extruded snacks were improved when coated with seasoning, compared with scores of non-enrobed extrudate (Table 32 and 30). The results showed that consumers rated for overall liking of both enrobed extrudates ranged from 5.5 to 7.5 (like slightly to like moderately) (Table 32), whereas non-enrobed extrudates raged from 4.6 to 6.0 (dislike slightly to like slightly) (Table 30). Similar observations was reported by Clark (1996) who also observed that the texture and sensory response of exturdates was modified by coatings with seasoning material. The 12 % barbecue extrudates had the highest ($P \leq 0.05$) rating score for all sensory attributes and for overall liking. However, the extrudates coated with 8% and 12 % cheese extrudates were not significantly different but a had high rating for all sensory attributes, when compared with scores of extrudates coated with 4 % cheese.

The results from just-about-right (JAR) rating scale for barbecue seasoning are shown in Table 33 and for cheese seasoning are shown in Table 34. Overall, the majority of the panelists gave scores of 4 (just about right) for 12 % barbecue for all the sensory attributes (Table 33). Conversely, the “about right” of cheese seasoning level was 8 % cheese (Table 34). Extrudates with 30% protein content extruded at 30% feed moisture, 180 °C, coated with 12 % barbecue had higher preference levels for colour, odour, salty, flavour, hardness and overall liking.

Table 32 Consumer acceptability of 30% protein extruded snacks enrobed with three levels in each seasoning types.

Seasoning level (%)	Colour[2,3]	Odour	Salty	Flavour	Hardness	Overall liking
Barbecue						
4	6.5±1.0^{c} [1]	5.6±1.3^{c}	4.6±1.2^{c}	4.6±1.3^{c}	6.20±1.16^{c}	5.53±1.30^{c}
8	6.7±0.9^{bc}	6.7±1.1^{b}	6.5±1.5^{b}	6.2±1.4^{b}	6.27±1.17^{c}	6.43±1.10^{b}
12	7.7±0.8^{a}	7.4±0.9^{a}	7.6±0.9^{a}	7.3±1.2^{a}	7.56±1.13^{a}	7.46±1.07^{a}
Cheese						
4	5.9±1.2^{d}	5.7±1.0^{c}	5.0±1.3^{c}	5.1±1.1^{c}	6.70±1.12^{bc}	5.50±1.19^{c}
8	7.1±0.8^{b}	6.8±1.1^{b}	6.9±1.1^{b}	6.8±0.8^{ab}	6.83±1.15^{b}	6.90±0.99^{ab}
12	6.8±1.2^{bc}	6.4±1.5^{b}	6.5±1.2^{b}	6.5±1.2^{b}	7.07±1.01^{ab}	6.70±1.08^{b}

[1] Mean ± standard deviation of fifty volunteer young graduate students (18-35 years old) participated at Kasetsart University evaluating 6 samples, two sessions of three products

[a-c] Different letters within the same column of each treatment indicate significant difference ($P \leq 0.05$)

Sensory data collected using nine-point hedonic scale with anchor points; 1-extremely dislike to 9-extremely like.

Table 33 Percentage of the respondents for barbecue seasoning level assessment of coated extrudates.

Level of Season-ing	Scale	Panelists[1] (%)				
		Colour	Odour	Salty	Flavour	Hard-ness
4 % Barbe-cue	Very much stronger (1)	-	-	-	-	-
	Much stronger (2)	-	-	3	-	-
	Very slightly stronger (3)	3	7	7	3	17
	Just about right (4)	50	30	13	13	60.00
	Very slightly weaker (5)	40	33	27	33	10
	Much weaker (6)	4	23	27	37	10
	Very much weaker (7)	3	7	23	14	3
8 % Barbe-cue	Very much stronger (1)	-	-	-	-	-
	Much stronger (2)	3	-	-	-	-
	Very slightly stronger (3)	4	7	7	7	7
	Just about right (4)	70	50	73	37	70
	Very slightly weaker (5)	20	33	7	43	13
	Much weaker (6)	3	10	3	13	7
	Very much weaker (7)	-	-	10	-	3
12 % Barbe-cue	Very much stronger (1)	-	-	-	-	-
	Much stronger (2)	-	-	-	-	-
	Very slightly stronger (3)	10	3	10	7	7
	Just about right (4)	80	67	67	60.	77
	Very slightly weaker (5)	7	20.	13	26	7
	Much weaker (6)	3	7	3	7	6
	Very much weaker (7)	-	3	7	-	3

[1] Fifty volunteer young graduate students (18-35 years old) participated at Kasetsart University evaluating 6 samples, two sessions of three products

Table 34 Percentage of the respondents for cheese seasoning level assessment of coated extrudates.

Level of Season-ing	Scale	Panelists[1] (%)				
		Colour	Odour	Salty	Flavour	Hard-ness
4 % Cheese	Very much stronger (1)	-	-	-	-	-
	Much stronger (2)	-	-	-	3	-
	Very slightly stronger (3)	-	3	-	-	10
	Just about right (4)	20	7	10	3	73
	Very slightly weaker (5)	53	67	50	40	14
	Much weaker (6)	27	20	20	37	3
	Very much weaker (7)	-	-	20	17	-
8 % Cheese	Very much stronger (1)	-	-	-	-	-
	Much stronger (2)	3	-	-	-	-
	Very slightly stronger (3)	7	27	10	13	-
	Just about right (4)	73	53	57	50	87
	Very slightly weaker (5)	14	10	23	30	10
	Much weaker (6)	3	7	7	7	3
	Very much weaker (7)	-	-	3	-	-
12 % Cheese	Very much stronger (1)	-	-	-	-	-
	Much stronger (2)	-	3	7	3	3
	Very slightly stronger (3)	37	33	40	23	10
	Just about right (4)	53	50	43	57	77
	Very slightly weaker (5)	3	14	10	17	7
	Much weaker (6)	7	-	-	-	3
	Very much weaker (7)	-	-	-	-	-

[1] Fifty volunteer young graduate students (18-35 years old) participated at Kasetsart University evaluating 6 samples, two sessions of three products

2.4. Correlations among extrudate properties

The correlation matrix of the mean chemical, physical and sensory properties are shown in Table 35. The results showed that the moisture content was negatively correlated to NPN ($r = -0.63$, $P \leq 0.01$), but positively correlated to lysine content ($r = 0.80$, $P \leq 0.01$). This result indicated that the high moisture content product had high lysine content. This is probably due to the water retarded the reaction rate of non-enzymatic browning (Millard reaction). Eichner and Karel (1972) suggested that the browning rate decreased with increasing water content in product. This result supported the colour values.The high moisture content in extrudates, the lightness (L^*) was also high but redness (a^*) and yellowness (b^*) were low value, which was low intensity of colour. The colour intensity (sensory) was correlated best with the L^* ($r = -0.89$, $P \leq 0.01$), a^* ($r = 0.93$, $P \leq 0.01$), *and* b^* ($r = 0.74$, $P \leq 0.01$). In addition, the moisture content was positively correlated to density ($r = 0.42$, $P \leq 0.05$), noise ($r = 0.41$, $P \leq 0.05$), and brittleness ($r = 0.47$, $P \leq 0.01$). However, the moisture content had no significant correlated with hardness of extrudates ($r = 0.32$). This result shown that the feed moisture content was an important factor affecting lysine retention, physical and sensory properties of extrudates.

Protein content was negatively correlated to lysine content ($r = -0.63$, $P \leq 0.01$). Generally, the protein content increase should result in increased lysine content but conversely observed in this study. This is probably due to the difference quantity of amino acid composition in protein material and the difference ratio of raw materials used in each formulation. The amount of protein content was correlated moderate with density ($r = 0.62$, $P \leq 0.01$), expansion ($r = -0.79$, $P \leq 0.01$) and BSI ($r = 0.79$, $P \leq 0.01$). This may be due to the fact that increasing protein content in feed resulted in an increase in protein content of extrudates and also increase shear strength and bulk density, but decrease expansion of extrudates. Similar results were observed by Veronica et al. (2006) and Allen et al. (2007) who studied influence of protein level on the physical properties of extrudates.

The amount of protein content was also correlated moderate with hardness ($r = 0.64$, $P \leq 0.01$), crispness ($r = 0.72$, $P \leq 0.01$) and sticky mouth coating ($r = -0.51$, $P \leq$

0.01). Additional, the hardness and crispness were mostly explained by instrumental density, expansion ratio or BSI measurements, which is consistently with the finding from correlation analysis. Similar result was reported by Bouvier *et al*. (1997) who observed that the hardness and crispness of expanded extrudate is a perception of the human being and is associated with the expansion and cell structure of the product.

The amount of lysine was not significant correlation with instrumental texture measurements and sensory (texture) but it was correlated with *L**(r = 0.77, $P \leq 0.01$), *a** (r = -0.70, $P \leq 0.01$), *b** (r = -0.79, $P \leq 0.01$) and colour intensity (r = -0.69, $P \leq 0.01$). In severe condition extrusion (20% feed moisture, 180 °C), the extrudates had high lysine loss, high redness (*a**) and yellowness (*b**) values and high colour intensity (sensory). The serious problem for lysine is thermal destruction in processing because of the well known Maillard or browning reaction involving ϵ-amino group (Aylward and Morton, 1970; Carpenter, 1973). It is possible to use the colour parameter from instrumental measurements or colour intensity from sensory as the indicator to estimate the lysine loss during extrusion process.

Table 35 Coefficients for correlation between measured expanded extrudate characteristics.

		Chemical properties			
		Moisture	**Protein**	**NPN**	**Lys**
Chemical properties	Moisture[1]	1.00			
	Protein	-0.32	1.00		
	NPN	-0.63**	0.68**	1.00	
	Lys	0.80**	-0.63**	-0.88**	1.00
Physical properties	Density	0.42*	0.62**	0.11	0.01
	Expansion	-0.21	-0.79**	-0.31	0.23
	BSI	0.16	0.79**	0.29	-0.15
	*L**	0.58**	-0.24	-0.74**	0.77**
	*a**	-0.62**	0.05	0.62**	-0.70**
	*b**	-0.65**	0.40*	0.73**	-0.79**
Sensory properties (QDA)	Hardness	0.32	0.64**	0.13	0.003
	Noise	0.41*	0.30	-0.17	0.32
	Crispness	0.76	0.72**	0.28	-0.13
	Brittle	0.47**	-0.06	-0.30	0.41*
	Sticky mouth coating	0.05	-0.51**	-0.30	0.44*
	Colour	-0.63**	0.05	0.56**	-0.69**

[1]Protein= total protein (%db); NPN = non-protein nitrogen (%db); Lysine (g/100 g protein); Density= bulk density (g/cm^3); BSI = breaking strength index (N/mm); *L**, index of lightness/ brightness; *a**, index of redness/greenness; *b**, index of yellowness/blueness;

** Correlation is significant difference ($P \leq 0.01$)

* Correlation is significant difference ($P \leq 0.05$)

Table 35 (Continued)

		Physical properties					
		Density	**Expansion**	**BSI**	***L****	***a****	***b****
Chemical properties	Moisture[1]						
	Protein						
	NPN						
	Lys						
Physical properties	Density	1.00					
	Expansion	-0.93**	1.00				
	BSI	0.92**	-0.89**	1.00			
	*L**	0.24	-0.06	0.18	1.00		
	*a**	-0.45**	0.27	-0.42*	-0.96**	1.00	
	*b**	-0.16	-0.09	-0.10	-0.86**	0.85**	1.00
Sensory properties (QDA)	Hardness	0.93**	-0.87**	0.92**	0.33*	-0.54**	-0.23
	Noise	0.73**	-0.64**	0.72**	0.49**	-0.65**	-0.47**
	Crispness	0.80**	-0.75**	0.86**	0.19	-0.40*	-0.12
	Brittle	0.43**	-0.33**	0.39**	0.28	-0.36*	-0.43**
	Sticky mouth coating	-0.32**	0.47**	-0.26*	0.27	-0.22	-0.47**
	Colour	-0.51**	0.39**	-0.45**	-0.89**	0.93**	0.74**

[1]Protein= total protein (%db); NPN = non-protein nitrogen (%db); Lysine (g/100 g protein); Density= bulk density (g/cm^3); BSI = breaking strength index (N/mm); *L**, index of lightness/ brightness; *a**, index of redness/greenness; *b**, index of yellowness/blueness;

** Correlation is significant difference ($P \leq 0.01$)

* Correlation is significant difference ($P \leq 0.05$)

Table 35 (Continued)

		Sensory properties					
		Hardness	**Noise**	**Crispness**	**Brittleness**	**Sticky mouth coating**	**Colour**
Chemical properties	Moisture[1]						
	Protein						
	NPN						
	Lys						
Physical properties	Density						
	Expansion						
	BSI						
	*L**						
	*a**						
	*b**						
Sensory properties (QDA)	Hardness	1.00					
	Noise	0.80**	1.00				
	Crispness	0.82**	0.76**	1.00			
	Brittleness	0.31**	0.38**	0.39**	1.00		
	Sticky mouth coating	-0.17	-0.11	-0.08		0.05	
	Colour	-0.57**	-0.52**	-0.43**	-0.28**	-0.11	1.00

[1]Protein= total protein (%db); NPN = non-protein nitrogen (%db); Lysine (g/100 g protein); Density= bulk density (g/cm^3); BSI = breaking strength index (N/mm); *L**, index of lightness/ brightness; *a**, index of redness/greenness; *b**, index of yellowness/blueness;

** Correlation is significant difference ($P \leq 0.01$)

* Correlation is significant difference ($P \leq 0.05$)

CONCLUSION AND RECOMMENDATION

Conclusion

From the experimental results and discussion of this study, the conclusion can be drawn as following:

Two extruded high-protein glutinous rice-based snacks were designed using a linear-programming technique to minimize total cost while meeting the FAO/WHO/UNU (1985) requirements for lysine and sulphur amino acids (cystine and methionine). The final product containing blends of glutinous rice, vital wheat gluten and toast soy grits was produced with a twin screw extruder.

The extrusion condition variables (20, 25 and 30 % feed moisture and 150 and 180 °C barrel temperature) had no effect on the total protein but it was highly significant influenced on NPN. Increasing feed moisture and decreasing barrel temperature resulted in a decrease in NPN content. The amino acid compositions of all extrudates were reduced after extrusion cooking. Lysine was significantly reduced by decreasing feed moisture content or increasing the barrel temperature during extrusion. Cysteine and methionine content were not significantly influenced by any of the experimental treatments. This information provided a quantity and nutritional change of proteins and lysine during extrusion processing, and should help design new approaches for controlling and predicting qualities and characteristics of extruded rice snack.

For the physical properties, extrusion with increasing amount of protein, increasing feed moisture or decreasing in barrel temperature resulted in decreased expansion and increased bulk density and hardness of extrudates. The colour intensity was confirmed by the colour measurement, which the formulation contained 30 % protein extruded under the conditions of 20 % moisture, at 180 °C had the lowest lightness (L^*) and the highest redness (a^*) and yellowness (b^*).

For the sensory properties, the protein content, feed moisture content and barrel temperature significantly affected sensory properties of extrudates. The high protein snack extrudate had high hardness, noise, crispness and brittleness. Increasing the amount of protein in component resulted in reduce the sticky mouth coating of extrudates. The extrudate produced under low feed moisture content and high barrel temperature, had low hardness, noise, crispness and brittleness but high colour intensity.

The acceptability results indicated that changes in protein content and extruder variables affected hedonic rating scores of the final product. Increasing feed protein content significantly increased hedonic rating scores for flavour, texture and overall liking. Increasing feed moisture content reduced hedonic rating scores for appearance and texture but increased hedonic rating score for colour and flavour. The high temperature reduced hedonic rating score for appearance, colour and flavour of extrudate.

The sensory attributes of extruded snack were improved when coated with seasoning (barbecue or chesses) resulting in high acceptability. In this study, highly acceptable snack products were obtained by 30 % protein content extruded under the condition of 30% feed moisture content, at 180 °C, enrobed with 12 % barbecue seasoning. These snacks had high protein content and provided essential amino acid value in agreement with 10 -12 years children food specifications outlined by FAO/WHO/UNU (1985). It is possible to produce a highly acceptable snack of high quality protein glutinous rice-based snack that can be useful in nutritional programs against malnutrition.

Recommendation

One problem yet to be solved is that the finished product must be carefully controlled, because the nature of the formulation method can cause variations in the content of ingredients as prices change. The constraints for lysine content in linear programming model should be added the amount of lysine loss during extrusion

processing, which differ in each extrusion condition. Nevertheless, certain ranges of ingredients can be established to allow greater flexibility in the formulation.

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APPENDICES

APPENDIX A

Data specification sheet of raw materials

Appendix Table A1 The company name and data information of raw material for linear programming to formulation

Material	Company name	Cost[a] ($ /Kg)	Proximate composition (%)					Typical amino acids (g/100 g protein)		Sulfur amino acid	Lysine
			Mois-ture	Prot-ein	Fat	Ash	Cabo-hydrate	Cysteine	Methi-onine		
Glutinous rice flour	Davis Trading Co. Ltd.	1.23	13.19	6.51	0	-	81.8	1.49	2.3	3.79	3.94
Ion exchange whey protein	Nutra life Co. Ltd.	47.33	-	99.79	0.5	-	0.5	2.03	2.03	4.06	9.26
Whey protein isolate	NZ low CARB	60	-	96.41	-	-	-	3.04	2.44	5.48	12.24
Wheat gluten (SWP 500)	Cerestar	3.4	5	88.42	11	2	9	1.69	1.37	3.06	1.24
Calcium caseinate 380	Fonterra	17	3.9	96.36	1	3.9	0.1	0.5	2.6	3.1	7.7
Sodium caseinate 180	Fonterra	13	4.3	96.87	0.7	3.6	0.2	0.5	2.6	3.1	7.7
Whey protein concentrate 312	Fonterra	12	4.6	83.86	4.6	3.4	7.4	2.9	2.1	5	10
Wey protein isolate 894	Fonterra	13	5.2	95.46	0.2	3.1	1	3.3	2.8	6.1	10.4
Lactalbumin Alatal 825	Fonterra	14	4.1	93.74	3.3	-	0.4	2.9	2.4	5.3	11.5
Total milk protein 1180	Fonterra	15	4.2	96.35	0.7	3.7	0.1	0.7	2.8	3.5	8.1
Vital wheat gluten Flourg25	Davis trading	2.6	8.42	86.55	6	1.5	6	2.36	1.66	4.02	1.8
Calcium caseinate	DMV international	22	5	95.26	0.8	3.5	-	0.4	3.0	3.4	8.3
Magnesium caseinate	DMV international	22	4	95.31	0.8	3.5	-	0.4	3	3.4	8.3
Potassium caseinate	DMV international	22	5	94.21	0.8	4.5	-	0.4	3	3.4	8.3
Sodium caseinate	DMV international	22	5	94.74	0.8	4	-	0.4	3	3.4	8.3
Collagen hydrolysate	GELITA Sol D	15	9	106.59	-	1	-	1.2	0.6	1.8	4
Collagen hydrolysate	GELITA Sol D	15	9.0	106.59	-	1.0	-	1.2	0.6	1.8	4
Isolated soy protein PRO-FAM646	AMD	8.7	6.0	95.74	4.0	5.0	-	1.2	1.4	2.6	6.4
Isolated soy protein PRO-FAM780	AMD	9	6.0	95.74	4.0	6.0	-	1.2	1.3	2.5	6.4

Appendix Table A1 (Continued)

Material	Company name	Cost[a] ($ /Kg)	Proximate composition (%)					Typical amino acids (g/100 g protein)		Sulfur amino acid	Lysine
			Moisture	Protein	Fat	Ash	Cabohy-drate	Cysteine	Methio-nine		
Isolated soy protein PRO-FAM781	AMD	9	6.0	95.74	4.0	6.0	-	1.2	1.3	2.5	6.4
Isolated soy protein PRO-FAM825	AMD	9	6.0	95.74	4.0	5.0	-	1.2	1.3	2.5	6.4
Isolated soy protein PRO-FAM873	AMD	9	6.0	95.74	4.0	5.0	-	1.2	1.4	2.6	6.4
Soy protein concentrate Arcon F	AMD	5	9.0	75.82	3.0	-	-	1.3	1.3	2.6	6.1
Soy protein concentrate Arcon S	AMD	5.75	6.0	76.60	4.0	5.0	-	1.4	1.3	2.7	6.9
Soy protein concentrate Arcon SM	AMD	6.35	6.0	74.47	3.0	5.0	-	1.4	1.5	2.9	6.9
Toasted soy grits, defatted	AMD	2.3	10.0	54.22	3.0	-	-	1.5	1.4	2.9	6.5
Egg albumen powder	Zeagold	19.6	8.0	86.96	0.0	-	-	2.102	2.79	4.892	5.515
Dried autolysed yeast powder	Swift	15	6.0	59.79	4.0	5.0	17	0.9	1.4	2.3	8
Isolated soy protein SUPRO 670IP	Tari	9.96	5.5	95.24	5.5	5.0	0.5	1.3	1.3	2.6	6.3
Soy protein concentrate DANPRO HTS	Tari	4.54	5.0	73.68	0.5	6.5	17.5	1.5	1.5	3	6.3

[a] Cost as of April 2005

Appendix B

Chemical analysis methods

Appendix B1 Method for determine protein nitrogen and non-protein nitrogen (Awolumate, 1983 and Periago *et al*., 1996)

Apparatus

- Kjeltec System 1026 distilling unit and 2006 Digester (Tecator AB), Sweden
- Forced air oven, American Scientific Products DK-62, USA
- Flask shaker, Chiltern Inc.
- Super-speed Refrigerated Centrifuge plus Sorvall RC-5C Automatic, Rotor SM-24, Kendro laboratory product Inc.

Reagents

- Trichloroacetic acid (TCA
- Kjeltabs (each containing 3.5g K_2SO_4 and 0.0035g Se)
- Sulfuric acid (95-97 % H_2SO_4)
- Boric acid (H_3BO_3)
- Sodium Hydroxide (NaOH)
- Hydrochloric acid (HCl)
- Bromocresol green
- Methyl red

Procedure

1. Total protein nitrogen content

Place weighed sample (0.7 - 2.2 g) in digestion flask. Add two Kjeltabs and then 15 ml concentrated H_2SO_4. Place flask in inclined position and heat gently until frothing ceases, boil briskly until solution clears and then $\geq$ 30 min longer. Cool, add 200 ml H_2O, cool < 25°C, then connect to digestion tube to the distilled in position. Add 25 ml of the 4% boric acid solution and 5-7 drops indicator. Place the receiver

conical flask on the platform. The automatic addition of NaOH (For each 10 ml H_2SO_4 used, add 15 g solid NaOH or enough solution to make contents strongly alkaline), then heat until all NH3 has distd ($\geq$ 150 ml distillate). Remove receiver, wash tip of condenser, and titrate sample with 0.1M or 0.01M HCl to a grey-mauve end point. Correct for blank digestion on reagents. Calculation of % Nitrogen in samples:

%N = [(ml HCl x normality HCl) – (ml std NaOH x normality NaOH)] x 1.4007/g sample

2. Non-protein nitrogen content

100 mg of sample was solubilized in 5 ml of a 0.2% NaOH (w/v) solution, shaken for 20 min, and centrifuged at 6,000g for 5 min at room temperature. The pellet was washed with 4 ml of 0.2% NaOH (w/v) solution, and added to the supernatant after a second centrifugation under the same conditions. TCA (6ml) was added to the collected supernatants and left for 2 h at 4 ^{O}C occasional shaking prior to centrifugation at 12,000g for 20 min at room temperature. The precipitate was washed with 5 ml of ethyl ether, dried for 1 h at 105 ^{O}C and weighed. Nitrogen content of the precipitate was then determined by the micro-Kjeldahl method. Non protein nitrogen (NPN) was calculated as the difference between TN and PN.

Appendix B2 Method for amino acid analysis

(Spackman *et al*, 1958; Rutherfurd and Moughan, 1997)

1. Acid hydrolysis

Apparatus

- Hydrolysis tubes
- Oxygen/methane torch.
- Tweezers
- Matches
- Marker pen
- Small spatula
- Balance (5 decimal places)
- Savant Speedvac
- Vacuum pump with cold trap
- 110^{o}C Oven
- 0.45μm filters and cartridges
- 1ml syringes
- 10ml syringe
- Sonicating
- Vortex mixer
- Waters 4ml vials, liners and caps

Reagents

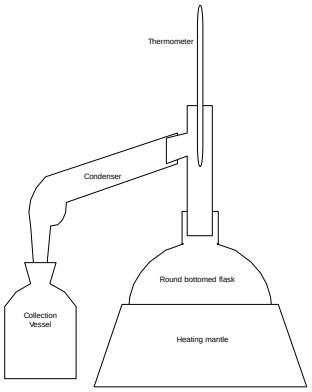
- 6 M HCl containing 0.1% phenol
- Norleucine solutions
- Loading Buffer

Procedure

1. Preparation of Redistilled 6M HCl containing 0.1% phenol

1.1 Distillation of the HCl

- conc. HCL is added to an equal volume of Nanopure water in a round bottomed flask (Appendix Figure 9).
- The flask is connected to a distillation condenser with thermometer inlet.
- The mixture is heated until it reaches approximately 109 ^{O}C at which time the distillate is collected.
- When the temperature rises to above 110 ^{O}C the concentration of the distillate is checked using titration against 6M NaOH using phenylphathalein as an indicator.
- If the concentration of the HCl is below 5.8M then collection of the distillate continues until the concentration of the HCl distillate reaches 5.8M.
- A final concentration of 5.8-6.0M HCl is acceptable.
- Label container according to protocol “labelling reagents and solutions”.
- The solution will be stored in the dark at room temperature for no more than 2 years.
-



Appendix Figure B1 Apparatus for distillation of HCl.

1.2 Preparation of Redistilled 6M HCl containing 0.1% phenol

- 1g of phenol is dissolved in 1 litre of redistilled HCl .
- 2. Store the solution in the dispenser bottle labelled 6M HCl containing 0.1% phenol.
- Label container according to protocol “labelling reagents and solutions”.
- Store the solution in the dark at room temperature for no more than 3 months.

2. Norleucine solutions

2.1 High concentration Norleucine standard

- Weigh out exactly, approximately 524.8mg of norleucine into a 100 ml volumetric flask.
- Make up to 100ml with Nanopure water and allow the norleucine to dissolve.
- The concentration will be approximately 40mM but it is necessary to calculate the concentration exactly.
- Label container according to protocol “labelling reagents and solutions”.
- Store the solution in 10 ml aliquots in the freezer for no more than 2 years and store the working aliquot in the refrigerator no more than 6 months.

2.2 Low concentration Norleucine standard

- Weigh an aliquot of the high concentration Norleucine standard into a container. Add 9 times that weight with Nanopure water.
- Calculate the actual concentration of the solution.
- Label container according to protocol “labelling reagents and solutions”.

- Store the solution in 20 ml aliquots in the freezer for no more than 2 years and store the working aliquot in the refrigerator no more than 6 months.

2.3 Loading Buffer

- Weigh out 98g of sodium citrate dihydrate and 5g of phenol into a 5 litre beaker.
- Add 4.5 litres of Nanopure water and dissolve.
- Adjust the pH to 2.2 with the careful addition of conc HNO_3.
- Filter the solution through a 0.45μm filter using the water aspirator filtration unit.
- Label container according to protocol "labelling reagents and solutions".
- Store the solution in the refrigerator no more than 12 months. The working solution is stored in a calibrated dispenser bottle at room temperature in the dark for no more than 2 month.

3. Sample preparation and weighing

Sample hydrolysis tubes should be pyrolysed at 500 ^{O}C for at least 2 hours prior to use. After pyrolysis the tubes can be stored in a closed plastic bag indefinitely. Samples that arrive wet but are not water soluble must be freeze dried and ground to less than 1mm mesh. If samples are kept in a refrigerator or freezer allow the samples to warm to room temperature before weighing.

3.1 Sample weighing

- If the samples are dry then weigh between 0.5 - 4mg of "protein" (the amount of sample weighed should be between 2 and 15 mg) into the hydrolysis tube on a balance set to measure to 5 decimal places. If the protein is in the form of a solution then using a autopipette aliquot a

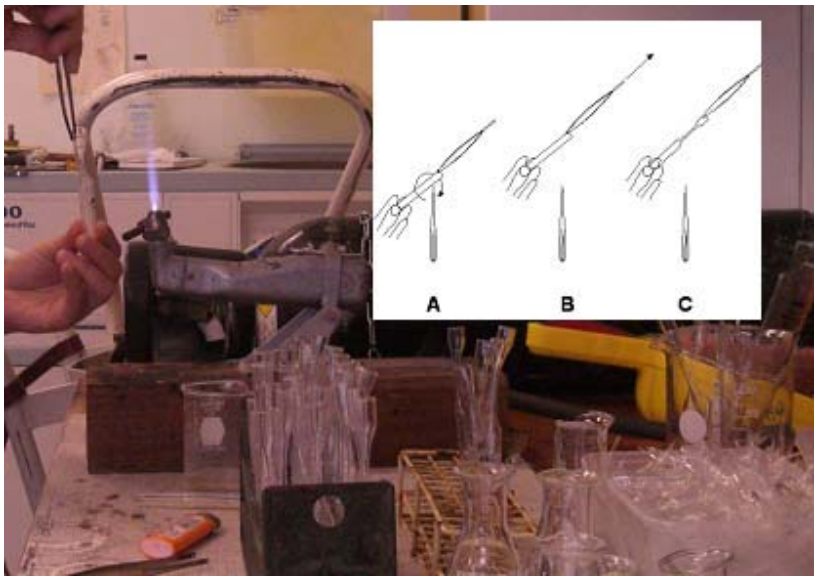
similar amount of protein into the hydrolysis tube and dry the sample in the Savant Speedvac.

- Samples should be hydrolysed at least in triplicate.
- Avoid, as much as possible, getting sample on the side of the tube when adding the sample to the tube.
- Dry sample on the side of the tube can often be dislodged by tapping the tube gently on a bench.
- A duplicate sample of lysozyme must also be weighed out an hydrolysed as an external control.

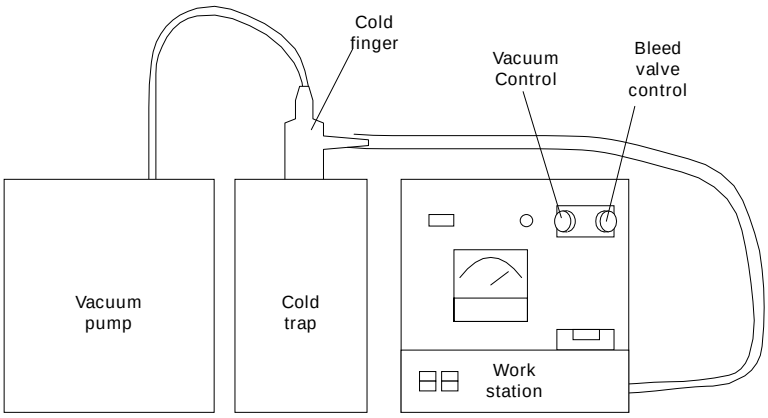
3.2 Adding the acid and sealing the tube

- Using the calibrated dispenser provided add 1 ml of redistilled 6M HCl containing 0.1% phenol to the tube.
- Light the oxygen/methane torch according to the instructions above the torch.
- Hold the bottom of the tube with your fingers and the top with a pair of tweezers (Appendix Figure 10A).
- Heat the tube with the torch approximately 1-1.5 cm below the top of the tube until it becomes soft, be sure to rotate the tube continuously to ensure even softening around the tube (Appendix Figure 10A).
- Pull the top of the tube with a pair of tweezers until the soft glass is stretched to approximately 5 6 cm in length (Appendix Figure 10B, C).
- Allow the tube to cool.
- Prepare the hydrolysis workstation by filling the cold trap with liquid air (Appendix Figure 11). The liquid air is transported in a portable dewar flask and you must be accompanied by someone else when getting liquid air. Caution liquid air is explosive and extremely cold and must be handled with the utmost care, use gloves and full face mask when pouring the liquid air into the dewar flask. When carrying the liquid air back to the lab wear safety glasses and gloves. Using gloves and a full face mask, carefully pour the liquid air in to cold trap flask.

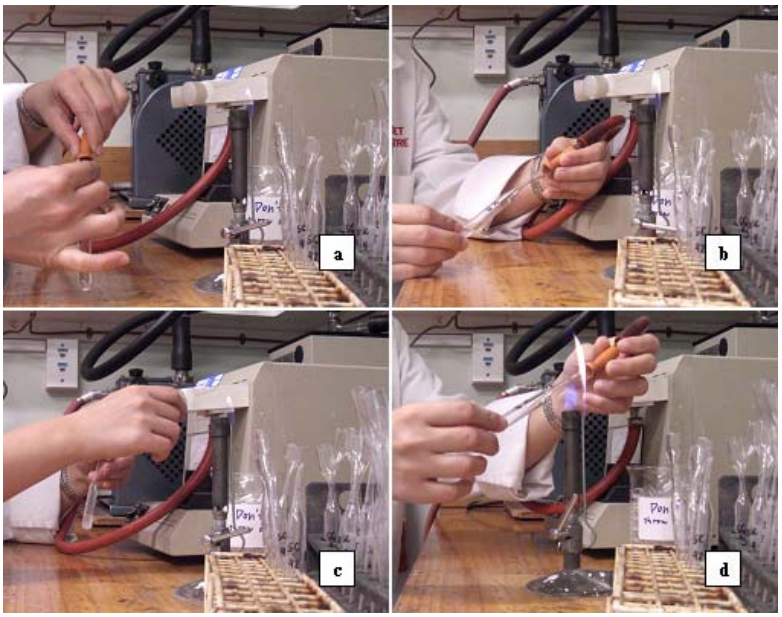
- Place the protective covers over the flask and slowly lower the cold finger into the flask.
- Wait about a minute for the cold finger to cool and turn on the vacuum pump at the wall.
- Turn off the vacuum control and bleed valve control and attach the rubber hose to the tube.
- Turn on the bleed valve control until the valve is open and turn the vacuum control to full open.
- Turn the bleed valve control very slowly off until the sample starts to bubble, flicking the bottom of the tube to remove bubbles (Appendix Figure 12).
- Allow bubbling to subside then turn off the bleed valve until bubbling starts again.
- Continue the last two steps until the acid stops bubbling and the bleed valve is completely turned off.
- Using a bunsen burner, melt the stretched part of the tube and when the glass is soft pull the bottom of the tube away from the top of the tube. Stop pulling when the molten part of the tube has formed a thin neck about 5-8 cm in length.
- Allow the tube to cool.
- Place the sealed evacuated tube in the 110oC oven for 24 hours.
- After 24 hours remove the tube from the oven, allow it to cool.
- Centrifuge the tube in the bench centrifuge located in the Nutrition lab at 3000 rpm for 3 minutes.



Appendix Figure B2 Stretching the neck of the hydrolysis tube



Appendix Figure B3 Acid Hydrolysis Workstation.



Appendix Figure B4 Degassing the Tube.

3.3 Cracking the tube open

- Using a glass pen scratch a line approximately 2-5mm long into the tube about 1-1.5 cm below the shoulder of the tube.
- Place a small drop of water onto the scratch.
- Using the oxygen/methane torch, heat a glass rod until it is white hot.
- Then place the molten part of the rod firmly onto the glass tube just next to the water droplet and the tube should crack around its circumference.
- If the tube does not crack then repeat the previous three steps until it does crack. If the tube doesn't crack after three attempts, rescratch the tube and try again.
- Add Norleucine to the amino acid hydrolysate (50µl of low concentration Norleucine standard to tubes that contain less than 2 mg of protein and add 50µl of high concentration Norleucine standard to tubes containing over 2 mg of protein).

- Dry down the sample using the Savant Speedvac or other suitable device.
- Add 2ml of loading buffer to the tube using the dispenser provided.
- The suspension is sonicated using the sonicating bath and mixed using the vortex mixer to dissolve the amino acids.
- Filter the sample through at least a 0.45µm syringe filter into a Waters 4 ml vial using a 1ml syringe.
- Flush through the sample left in the filter with an air filled syringe.
- The hydrolysis tubes can be discarded into the "glass only" rubbish bin. Note: the vial must contain at least 1.6ml of solution.
- A liner is placed into the cap (teflon side down) which is then screwed onto the vial.
- The sample can be stored in the refrigerator if it is to be analysed within 72 hours otherwise store in the freezer.

2. Performic acid oxidation of cysteine and methionine

Apparatus

- Hydrolysis tubes
- Savant Speedvac

Reagents

- 88% formic acid
- 30% hydrogen peroxide

Procedure

1. Performic acid preparation

1.1 88% formic acid

- Add 880 ml of formic acid to 120 ml of deionised water.
- Label container according to protocol “labelling reagents and solutions”.
- Store the solution at room temperature in an amber bottle for no more than 6 months.

1.2 Performic acid

- This solution should be made up fresh
- One volume of 30% hydrogen peroxide is added to Nine volumes of 88% formic acid at room temperature.
- The solution is mixed and allowed to sit at room temperature for 30 minutes.
- The solution is then cooled in an ice-bath for 30 min.

2. Sample preparation and weighing

Sample hydrolysis tubes should be pyrolysed at 500oC for at least 2 hours prior to use. After pyrolysis the tubes can be stored in a closed plastic bag indefinitely. Samples that are not water soluble must be freeze dried and ground to less than 1mm mesh. If samples are kept in a refrigerator or freezer allow the samples to warm to room temperature before weighing.

- Weigh between 0.5 - 1mg of “protein” into the hydrolysis tube on a balance set to measure to 5 decimal places.
- The amount of sample weighed should be between 2 and 6 mg.
- Samples should be hydrolysed at least in triplicate.
- Avoid, as much as possible, getting sample on the side of the tube when weighing.

- Sample that is on the side of the tube can often be dislodged by tapping the tube gently on a bench. A duplicate sample of lysozyme must also be weighed out an hydrolysed as an external control.

3. Oxidation step

- Cool the tube containing the sample to ice temperature.
- Add 1 ml of freshly made ice-cold performic acid to the tube.
- Cover the tubes with parafilm.
- Incubate in an ice bath in the refrigerator for 16 hrs.
- Slowly add 150□l of ice-cold HBr to the tube sitting in ice cold water and allow the mixture to sit on ice for 10-30 min or until bubbling subsides.
- Sonicate the tube for 5 minutes if there is excessive bubbling.
- Dry down the sample in the Savant speedvac or other suitable device.
- Hydrolyse the sample as described in the section "ACID HYDROLYSIS".

3. Reactive Lysine

Apparatus

- Hydrolysis tubes
- Savant Speedvac

Reagents

- 0.6M OMIU

Procedure

1. 0.6M OMIU preparation

This procedure makes 20ml of 0.6M OMIU. The procedure can be scaled up or down accordingly. This solution must be made up fresh just prior to use.

- Boil 30ml of nanopure water in a beaker until it volume reaches approximately 18-20ml.
- Weigh 4g of barium hydroxide octahydrate into an another beaker.
- Weigh 2 g of O-methylisourea (sulphate salt) in a 40 ml centrifuge tube.
- When the volume of the water reaches 18-20ml quickly add the barium hydroxide into the water.
- Swirl and heat the solution until is just starts to boil again. Be very careful, the solution will boil over very quickly after it has started boiling.
- Quickly pour the solution into the centrifuge tube containing the OMIU.
- Put the lid tightly onto the centrifuge tube and invert the tube a couple of times. Carefully loosen the cap to release any pressure buildup and repeat a couple more times.
- Let the solution cool for 30 min.
- Centrifuging at 6400g for 10 min in the high speed centrifuge in the nutrition laboratory.
- Retain the supernatant and check the pH. If the pH of the solution was lower than 12 then it was assumed that conversion of the sulphate salt to the free base is incomplete and the solution must be remade. However, if the pH was above 12 then the pH was adjusted to the appropriate pH for guanidination (pH 10.6 for ingredients and diets, 11.2 for digesta.
- Filter the solution through a 0.45□m syringe filter.
- Label container according to protocol “labelling reagents and solutions”.
- Use the solution immediately.

2. Sample preparation and weighing

Sample hydrolysis tubes should be pyrolysed at 500oC for at least 2 hours prior to use. After pyrolysis the tubes can be stored in a closed plastic bag indefinitely. Samples that are not water soluble must be freeze dried and ground to less than 1mm mesh. If samples are kept in a refrigerator or freezer allow the samples to warm to room temperature before weighing.

- Weigh between 0.5 - 1mg of "protein" into the hydrolysis tube on a balance set to measure to 5 decimal places.
- The amount of sample weighed should be between 2 and 6 mg.
- Samples should be hydrolysed at least in triplicate.
- Avoid, as much as possible, getting sample on the side of the tube when weighing.
- Sample that is on the side of the tube can often be dislodged by tapping the tube gently on a bench.

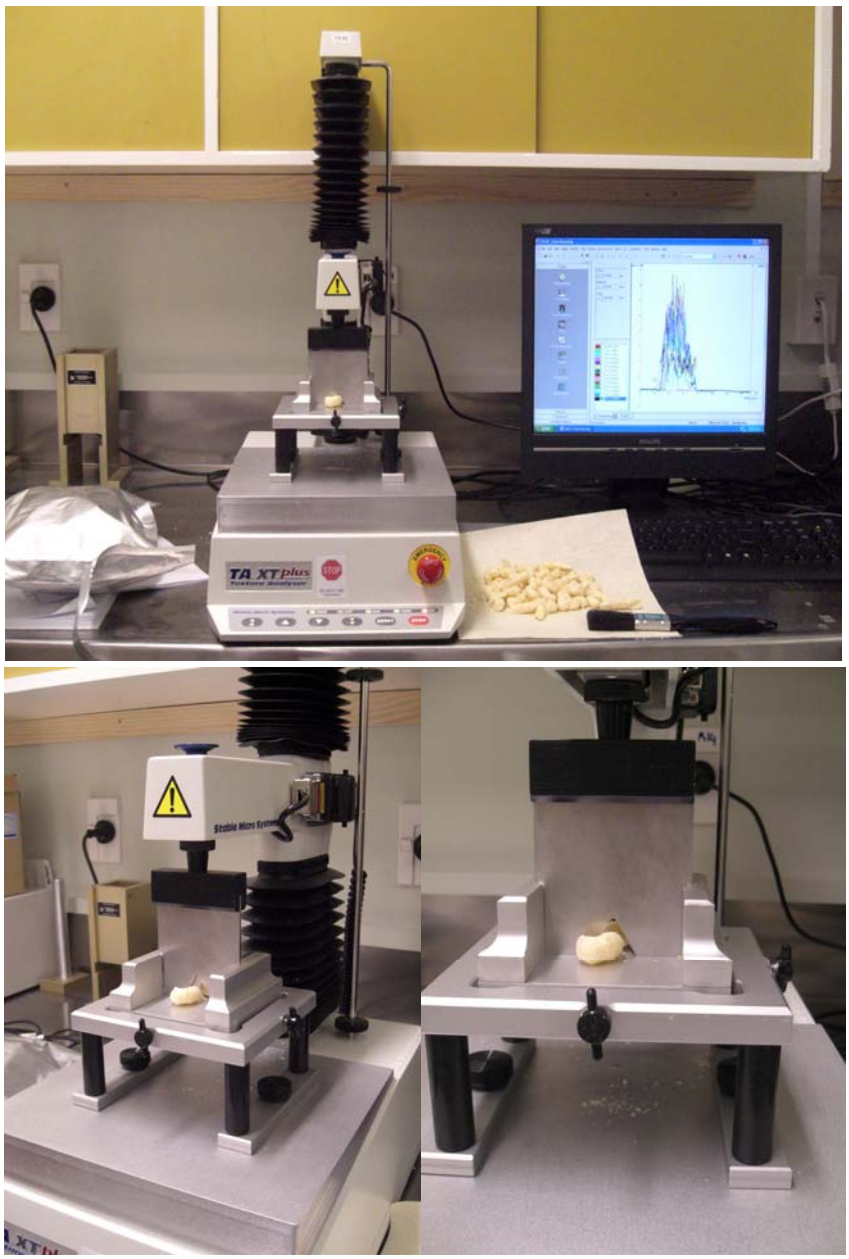
A duplicate sample of lysozyme must also be weighed out an hydrolysed as an external control.

3. Guanidination step

- Add 1 ml of freshly made 0.6M OMIU to the tube.
- Cover the tubes with parafilm.
- Incubate in an shaking water bath (Chromatography Laboratory) for 7 days.
- Dry down the sample in the Savant speedvac or other suitable device.
- Hydrolyse the sample as described in the section "ACID HYDROLYSIS".

Appendix C

Physical analysis



Appendix Figure C1 Texture analyzer (TA-XT2) with Warner-Bratzler shear cell ready for measurement

Appendix Figure C2 Minolta spectrophotometer CM-3500d) ready for measurement

Appendix D

Sensory analysis

Appendix Table D1 Descriptive vocabulary and Standard reference intensity ratings used in descriptive tests for extruded snack samples.

Attribute	Definition	Reference samples	
Colour	Yellow colour from Munsell book of colour varying from light to dark. The low end of the scale represents a light yellow colour and the high end represents the dark yellow colour.	2.0 6.0 10.0 15.0	5 Y 9/2 2.5 Y 8.5/6 2.5 Y 7/8 2.5 Y 5/6
Noise	Measure the level of noise by biting through the sample with incisor teeth	0.0 Sandwich bread	Farmhouse 1/2 in. cube
		6.0 Wafer	1/3 piece, Bissin coffee wafers, Thai president foods Co.
		10.0 Potato chips	1 piece, Testo, Berli jucker Co.
Hardness	A texture sensation associated with solid and dense products which represents the force required to bite initially through a product. The lower end of the scale represents very soft samples and the upper end of the scale represents very hard samples. This attribute is probably perceived in the initial stages of biting and chewing.	2.5 Egg white	Hard cooked, 1/2 in. cube
		7.0 Frankfurter	Large, cooked 5 min, 1/2in.cube, CP Co.
		9.5 Peanuts	1 nut, Tong garden Co.
		14.5 Hard Candy	1 pieces, one colour, Clorets hard candy, Cranbury Adams (Thailand) Ltd.

Appendix Table D1 (Continued)

Attribute	Definition	Reference samples	
Crispness	A texture sensation associated with highly aerated products with many air bubbles. Crispy products tend to be associated with high pitched sounds and are likely to melt in the mouth. The low end of scale represents samples low in crispiness, the upper end of the scale represents very crispy samples. This is probably perceived during the initial stages of biting.	2.0 Granola bar	Chewy chunk, 1/3 bar, Nature valley, USA
		5.0 Cracker	1 pieces, Rosy cracker, imperial general food industry Co.Ltd.
		14.0 Corn flakes	1 oz. Kellogg's corn flakes cereal
Brittleness	A texture sensation which describes how easily a snack product breaks, fractures or splinters in the mouth. When broken or fractured brittle products may be abrasive on the tongue. The lower end of the scale represents samples which are not brittle and the upper end of the scale represents very brittle samples. This attribute is probably perceived during the first bite.	1.0 Muffin	1/2 in cube, Big C bakery
		4.0 Crackers	1/2 in cube, Nabisco
		10.0 Wafer	1/2 in square,Bissin coffee wafer, Thai president Ltd.
		14.5 Hard candy	1 piece, Clorets hard candy, Cranbury Adams (Thailand) Ltd.

Appendix Table D1 (Continued)

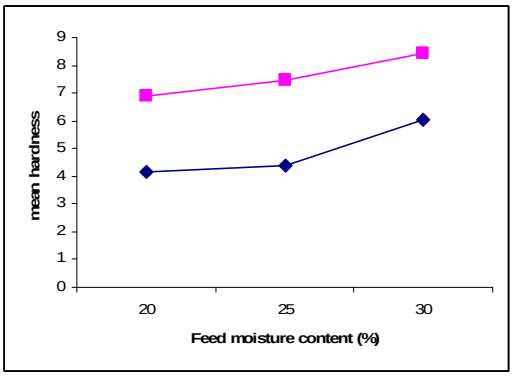
Attribute	Definition	Reference samples	
Sticky mouth coating	A sensation which relates to extent to which the sample become sticky (or starchy) in the mouth and covers the teeth. The lower end of the scale represents samples which are not sticky mouth coating, the upper end represents samples which are very sticky mouth coating. Perceived during chewing.	1.0 Carrot	Uncooked, fresh, unpeeled 1/2 in slice.
		7.0 Cracker	1 piece, Rosy crackers, Imperial general food industry Co., Ltd.
		15.0 Mango leather	1 piece, Ruckthai food product Co.

Appendix Figure D1 Non-enrobed of glutinous rice, fish meal, soy and gluten extrudates

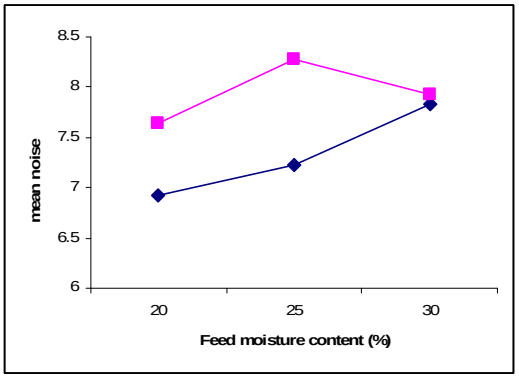
Appendix Figure D1 (Continued)

Appendix Figure D1 (Continued)

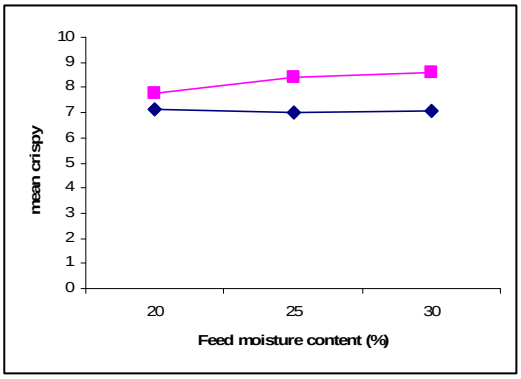
Appendix Figure D2 Non-enrobed of glutinous rice, soy and gluten extrudates



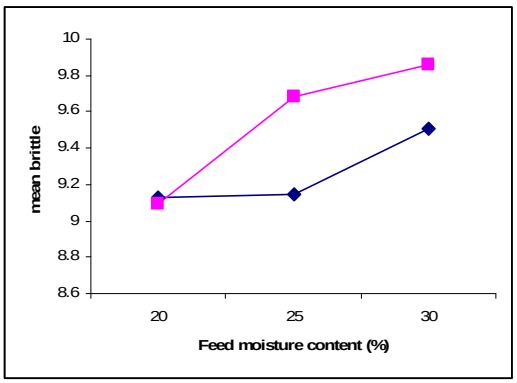
a) Hardness



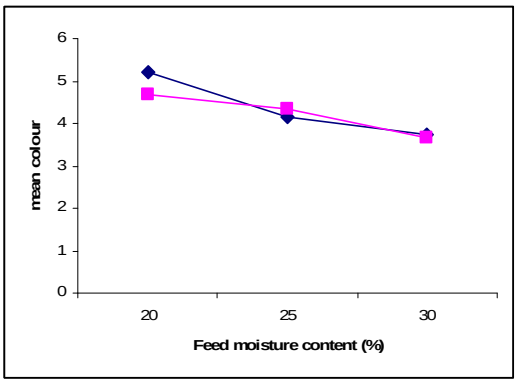
b) Noise



c) Crispness



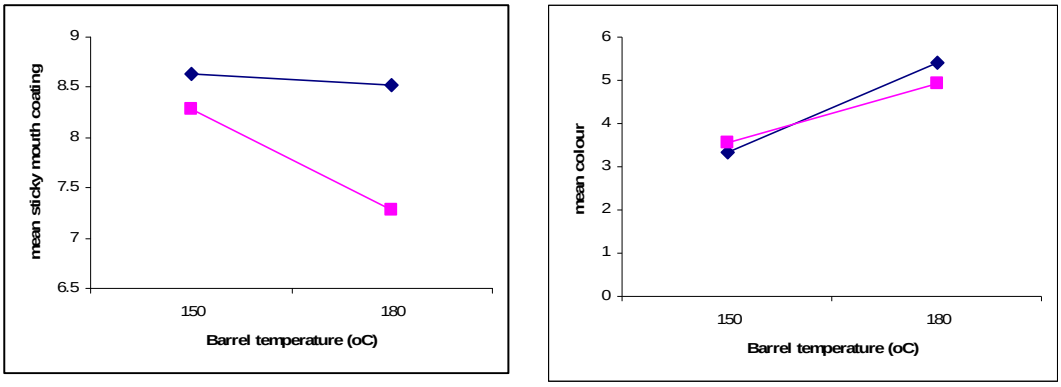
d) Brittle



e) Colour

- Sensory data collected from 10 panelists evaluate each sample in 3 replicates.
- Extrusion moisture contents 20, 25, 30 % and protein contents 20, 30 %
- For plots (a) through (c) the symbols correspond to the following protein contents:
  - -♦- 20 % protein
  - -■- 30 % protein

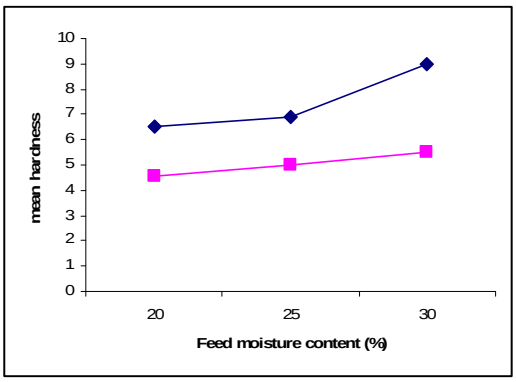
Appendix Figure D3 Interaction plots of protein content by feed moisture for QDA sensory.



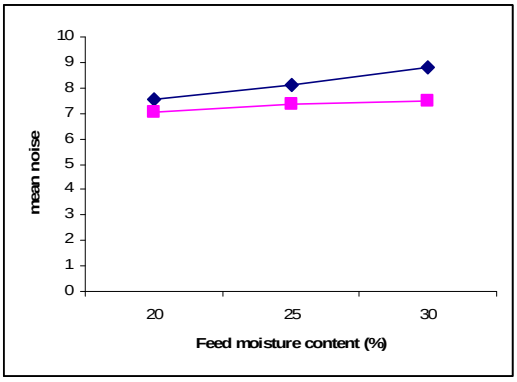
a) sticky mouth coating b) colour

- Sensory data collected from 10 panelists evaluate each sample in 3 replicates.
- Extrusion barrel temperature 150, 180°C and protein contents 20, 30 %
- The symbols correspond to the following protein contents:
  - -♦- 20 % protein
  - -■- 30 % protein

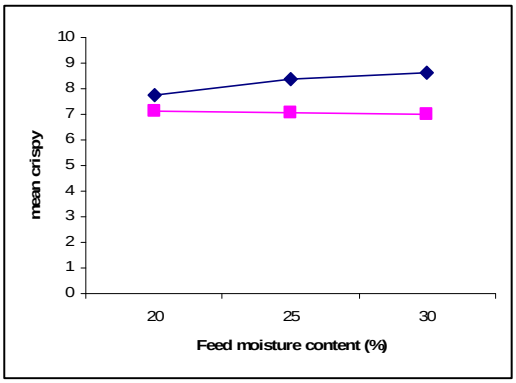
Appendix Figure D4 Interaction plots of protein content by barrel temperature for the QDA sensory.



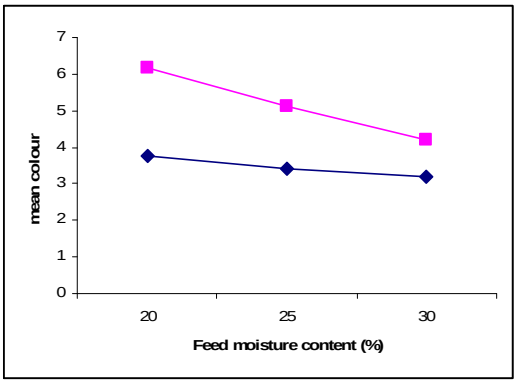
a) Hardness



b) Noise



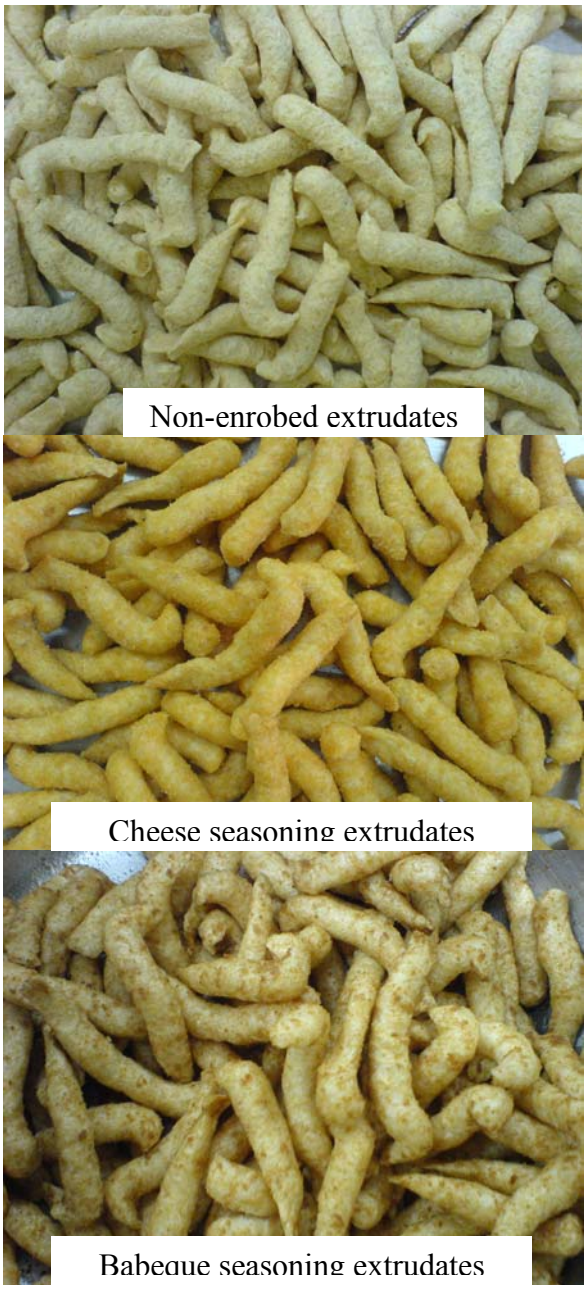
c) Crispness



d) Colour

- Sensory data collected from 10 panelists evaluate each sample in 3 replicates.
- Extrusion moisture content 20, 25, 30 % and barrel temperature 150, 180°C
- The symbols correspond to the following protein contents: -♦- 150 °C -■- 180 °C

Appendix Figure D5 Interaction plots of feed moisture by barrel temperature for the QDA sensory.



Appendix Figure D6 Non-enrobed and enrobed extrudates

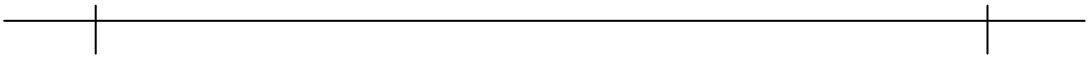
Appendix D1 Questionnaire for sensory evaluation by Quantitative descriptive analysis (QDA)

Product sample: high protein extruded snack form glutinous rice, soy protein and wheat gluten

Panelist's Name_____Date_____ Code: _____

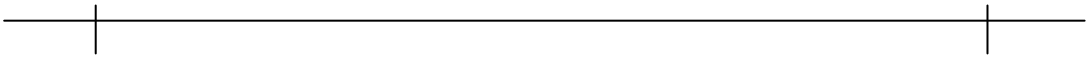
Please evaluate the samples in the following order:

Yellow Colour:



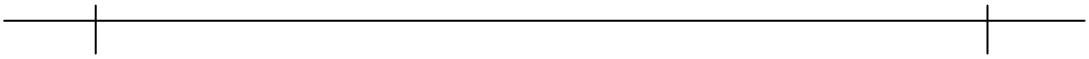
slight intense

Hardness:



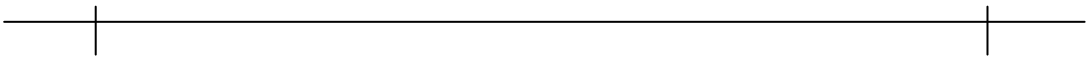
slight intense

Noise:



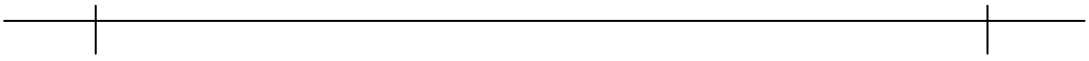
slight intense

Crispy:



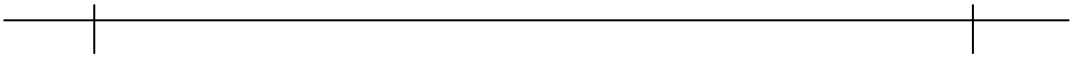
slight intense

Brittle:



slight intense

Sticky mouth coating:



Comments:_____

_____

Thank you very much

Appendix D2 Questionnaire for screening extrudates using 9-point hedonic scale

Product sample: high protein extruded snack form glutinous rice, soy protein and wheat gluten without enrobe seasoning

Panelist's Name_____Date_____

Please determine your preferences toward each sensory characteristic and evaluate overall acceptance to the product, using 9 hedonic scale.

Like Extremely	Like very much	Like Moderately	Like Slightly	Neither like nor dislike	Dislike Slightly	Dislike Moderately	Dislike very much	Dislike Extremely
9	8	7	6	5	4	3	2	1

Sample code	**Preference score 1-9**				
	Appearance	**Colour**	**Flavour**	**Texture**	**Overall liking**

Comments:_____

_____

_____

_____

Thank you very much

Appendix D3 Questionnaire for enrobed extrudates acceptability test using 9-point hedonic scale and just-about-right (JAR) rating scale.

Product sample: high protein extruded snack form glutinous rice, soy protein and wheat gluten, enrobed with ……..

Name_____Date_____

Please evaluate the snack samples in the following order:

Indicate how much you like or dislike each sample using 9 hedonic scale;

Like Extremely 9 | Like Very much 8 | Like Moderately 7 | Like Slightly 6 | Neither like nor dislike 5 | Dislike Slightly 4 | Dislike Moderately 3 | Dislike Very much 2 | Dislike Extremely 1

And please indicated the requirement to improve product using JAR rating scale;

Very much weaker 7 | Much weaker 6 | Very slightly weaker 5 | Just about Right 4 | Very slightly stronger 3 | Much stronger 2 | Very much stronger 1

Attribute						
	liking	**about right**	**liking**	**about right**	**liking**	**about right**
colour						
odour						
salty						
flavour						
hardness						
overall liking		-		-		-

Comments:_____

_____

_____

Thank you very much

APPENDIX E

Statistic results

Appendix Table E1 ANOVA of chemical properties of extrudates.

Source[1]	df.	Moisture (%)		Protein (% db)	
		MSS	*F*	MSS	*F*
F.Prot	2	0.80	0.22	853.91	15219.34**
Error	105	3.66		0.06	
Total	108				
Mc	2	149.56	3982.84**	0.83	7.06
BT	1	45.38	1208.39**	0.00	0.02
SP	1	15.54	413.87**	0.26	2.16
F.Prot x Mc	4	0.90	23.89**	0.08	0.71
F.Prot x BT	2	1.28	34.09**	0.06	0.53
Mc x BT	2	1.74	46.28**	0.10	0.82
F.Prot x Mc x BT	4	0.65	17.31**	0.05	0.41
F.Prot x SP	2	0.02	0.65	0.03	0.23
Mc x SP	2	1.04	27.70**	0.06	0.47
F.Prot x Mc x SP	4	0.11	2.88	0.15	1.29
BT x SP	1	4.93	131.32**	0.03	0.26
F.Prot x BT x SP	2	0.18	4.69	0.03	0.24
Mc x BT x SP	2	1.46	38.96**	0.25	2.08
F.Prot x Mc x BT x SP	4	0.04		0.12	
Sampling error	72	0.01		0.04	
Total	108				

* Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$

[1] Source of variance : F.Prot= protein content, Mc = feed moisture content, BT = barrel temperature, SP = screw speed

Appendix Table E2 ANOVA of physical properties of extrudates.

Source[1]	df.	Bulk density (g/cm^3)		Expansion ratio	
		MSS	*F*	MSS	*F*
F.Prot	2	9.59×10^5	9.36**	4.68	60.64**
Error	213	1.02×10^5		0.08	
Total	216				
Mc	2	4.94×10^4	217.24**	2.30	27.29**
BT	1	4.43×10^4	195.03**	0.59	6.95*
SP	1	3.52×10^4	154.79**	5.57	65.96**
F.Prot x Mc	4	5.23×10^6	2.30	0.52	6.18
F.Prot x BT	2	7.46×10^6	3.28	0.19	2.27
Mc x BT	2	4.03×10^5	17.76*	0.03	0.38
F.Prot x Mc x BT	4	2.16×10^6	0.95	0.02	0.21
F.Prot x SP	2	3.74×10^6	1.65	0.08	0.93
Mc x SP	2	2.76×10^6	1.21	0.23	2.77
F.Prot x Mc x SP	4	1.26×10^6	0.55	0.06	0.66
BT x SP	1	6.90×10^5	30.36**	0.06	0.66
F.Prot x BT x SP	2	4.81×10^6	2.12	0.02	0.24
Mc x BT x SP	2	2.95×10^6	1.30	0.04	0.52
F.Prot x Mc x BT x SP	4	2.27×10^6		0.08	
Sampling error	180	8.937×10^7		0.01	
Total	216				

* Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$

[1] Source of variance : F.Prot= protein content, Mc = feed moisture content, BT = barrel temperature, SP = screw speed

Appendix Table E3 ANOVA of descriptive analysis of extrudates.

Source[1]	df.	Hardness		Noise		Crispness		Brittleness		Sticky mouth coating		Colour	
		MSS	*F*	MSS	*F*	MSS	*F*	MSS	*F*	MSS	*F*	MSS	*F*
Panelist[2]	9	1.54	2.81**	2.32	5.56**	1.79	5.04**	11.77	19.98**	8.09	6.29**	1.01	2.686**
Replication	2	1.24	2.27	0.39	0.94	0.07	0.21	0.07	0.12	0.09	0.08	0.066	0.174
F.Prot	1	677.06	1238.64**	58.89	141.33**	127.81	358.29**	7.43	12.59**	56.76	44.09**	1.72	4.579*
Mc	2	96.78	177.06**	22.62	54.29**	4.29	12.04**	9.85	16.71**	14.50	11.26**	47.12	125.280**
BT	1	542.43	992.35**	66.22	158.94**	131.41	368.39**	20.78	35.27**	27.49	21.36**	260.95	693.869**
F.Prot x Mc	2	3.13	5.73**	1.43	3.44*	6.79	19.06**	2.49	4.23*	0.56	0.44	3.80	10.110**
F.Prot x BT	1	0.36	0.65	0.00	0.00	0.58	1.64	0.51	0.86	17.44	13.55*	10.44	27.755**
Mc x BT	2	25.85	47.29**	4.65	11.16**	7.96	22.32**	1.39	2.36	0.97	0.76	14.63	38.902**
F.Prot x Mc x BT	2	0.25	0.46	1.74	4.19*	2.49	7.01**	0.02	0.30	0.32	0.25	1.39	3.701*
Error	337	0.55		0.42		0.38		0.59		1.29		0.38	
Total	360												

* Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$

[1] Source of variance: F. Prot= protein content (20, 30 %), Mc = feed moisture content (20, 25, 30 %), BT = barrel temperature (150, 180 °C)

[2] Ten panelists evaluated 12 extrudates differing in protein content, feed moisture and barrel temperature in three replicates of each sample

Appendix Table E4 ANOVA of hedonic ratings for sensory attributes and overall acceptance of non-enrobed extrudates.

Source[1]	df.	Appearance		Colour		Flavour		Texture		Overall linking	
		MSS	*F*	MSS	*F*	MSS	*F*	MSS	*F*	MSS	*F*
Panelist[2]	49	1.65	1.48*	2.08	1.83**	2.87	2.42**	1.94	1.59**	1.76	1.36
F.Prot	1	7.94	7.09**	12.62	11.09**	1.04	0.88	46.48	38.03**	11.48	8.88**
Mc	2	8.95	7.99**	3.93	3.46*	15.22	12.85**	5.93	4.85**	0.38	0.29
BT	1	6.62	5.91*	25.23	22.18**	10.40	8.78**	4.68	3.83	0.48	0.37
F.Prot x Mc	2	0.31	0.272	0.34	0.29	1.90	1.61	9.29	7.59**	3.53	2.73
F.Prot x BT	1	0.02	0.01	0.38	0.33	0.82	0.69	39.02	31.92**	17.00	13.15**
Mc x BT	2	144.22	128.79**	20.05	17.63**	11.25	9.49**	120.35	98.47**	55.55	42.95**
F.Prot x Mc x BT	2	1.60	1.43	0.46	0.40	0.35	0.29	8.18	6.69**	1.85	0.033
Error	539	1.12		1.14		1.18		1.22		1.29	
Total	600										

* Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$

[1] Source of variance : F. Prot= protein content (20, 30 %), Mc = feed moisture (20, 25, 30 %), BT = barrel temperature (150, 180 °C)

[2] Fifty volunteer young graduate students (18-35 years old) participated at Kasetsart University evaluating 12 samples, four sessions of three products.

Appendix Table E5 ANOVA of enrobed extrudates acceptability test

Source	df.	Colour		Odour		Salty		Flavour		Hardness		Overall linking	
		MSS	*F*	MSS	*F*	MSS	*F*	MSS	*F*	MSS	*F*	MSS	*F*
Samples[1]	5	10.66	10.11**	14.33	10.74**	40	28.43**	32.37	23.30**	7.86	7.2**	18.22	14.11**
Panelist[2]	49	0.95	0.89	1.68	1.26	1.75	1.25	1.10	0.79	2.14	1.97**	1.23	0.95
Error	245	1.05		1.34		1.41		1.39		1.09		1.29	
Total	300												

* Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$

[1] Two types of seasoning (BBQ and Cheese) varied 3 levels, total 6 samples

[2] Fifty volunteer young graduate students (18-35 years old) participated at Kasetsart University evaluating 6 samples, two sessions of three products.

CIRRICULUM VITAE

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