PHYSICO-CHEMICAL AND ORGANOLEPTIC EVALUATIONS OF WHEAT BREAD SUBSTITUTED WITH DIFFERENT PERCENTAGE OF PUMPKIN FLOUR *(Cucurbita moschata)*

SEE EAN FANG

UNIVERSITI SAINS MALAYSIA

2008

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by

SEE EAN FANG

Thesis submitted in fulfillment of the requirements for the degree of Master of Science

March 2008

ACKNOWLEDGEMENTS

I would like to take this opportunity to thank all people who have assisted me in the completion of this project. I have immense pleasure to express my deep sense of gratitude and indebtedness to my thesis supervisor, Prof Madya Dr. Noor Aziah for her guidance, financial and moral support. Her constant support, patience, time, and effort were given, her valuable suggestions and useful criticism, constant encouragement and inspiration evinced throughout this research and in preparation of the thesis. I would also like to acknowledge the guidance and support of Dr. Ruzita Ahmad and Pn. Wan for their efforts, invaluable help and counseling.

Staff members in the department have also been extremely helpful. A special thanks goes to lab assistant Amaizan, Mr. Josheph, Ms Maizura and Najmah for their helping in every aspect of this project. I am very appreciative of the guidance, wisdom, and support provided by my fellow graduate students Joyce, Weng Kee, Mum Wai, Matthew, Ze Ching, Li Choo, Wan Teck, Yin Hui, Weng Wai, Kak Zanzila, Kak Haslina, Mardiana, Komathi and Jana. I would like to express my extreme gratitude to them for continually supported me through thick and thin, through the best and the worst of times and friendship over the years.

I am deeply grateful to Hui Chung, for friendship, shared laughters and unforgettable moments, which have made my life so enjoyable, strongly encouraged, incessant help and support, especially through the hard times.

Most importantly of all, I express my deep sense of gratitude to my most affectionate and beloved parents, See Tek Seng, Chuah Choo Hua, Kok Eng, Esther, Chin Shi Kim, Wendy, Kok Keat, Hooi Cheng, Kok Wooi, Ean Kee and Ye Horng for their love, their undying encouragement, their wisdom, their advice, and for being there in every aspect of my life has meant everything to me and will be always remembered. I honestly could not have completed this without your support.

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PENILAIAN FIZIKO-KIMIA DAN SENSORI ROTI TEPUNG GANDUM YANG DIGANTIKAN DENGAN TEPUNG LABU *(Cucurbita moschata)* PADA PERATUSAN YANG BERBEZA

ABSTRAK

Objektif kajian ini adalah untuk mengkaji sifat-sifat fiziko-kimia dan penilaian sensori roti tepung gandum yang digantikan dengan tepung labu (PF) pada peratusan yang berbeza (kawalan, 5%, 10% dan 15%). Ujian mikrobiologi hanya dilakukan semasa tempoh penstoran. Roti disediakan dengan menggunakan kaedah span dan doh. Roti dengan penggantian PF dari 5%, 10% dan 15% menunjukkan peningkatan signifikan ($p<0.05$) dalam kandungan abu dan gentian kasar. Kandungan protein dan lemak didapati menurun secara signifikan ($p<0.05$) dalam roti labu. PF menunjukkan perbezaan signifikan ($p<0.05$) hanya dalam kapasiti penyerapan air yang lebih tinggi berbanding dengan tepung gandum. Penggantian PF memberi keputusan yang lebih tinggi dalam jumlah gentian dietari (TDF), gentian dietari tak larut (IDF), gentian dietari larut (SDF) dan kanji rintang (RS) manakala kandungan jumlah kanji didapati menurun dalam lingkungan 56.5 ke 60.8% (berat kering) dalam sampel roti. Roti PF menunjukkan Glisemik Indeks (GI) yang rendah secara relatif berbanding dengan roti kawalan. Dalam kajian ini, peningkatan penggantian PF telah meningkatkan kandungan beta-karoten secara signifikan dalam roti dan ini menyebabkan peningkatan warna kuning di dalam krus dan krum roti secara signifikan ($p<0.05$). Kandungan mineral (Ca, Na, K, Fe, P dan Mg) meningkat secara signifikan ($p<0.05$) dengan peningkatan tahap penggantian PF dalam roti. Kandungan glukosa, sukrosa dan fruktosa hadir dalam ikutan susunan yang lebih tinggi ke rendah dalam roti labu. Isipadu lof dan isipadu spesifik menunjukkan perbezaan signifikan ($p<0.05$) dalam roti labu. Analisis tekstur menunjukkan roti yang mengandungi 10% dan 15% PF mempunyai nilai kekerasan, kegaman dan kekenyalan berbeza dengan signifikan ($p<0.05$) berbanding dengan roti kawalan. Mikrograf Elektron Penskanan (SEM) dalam doh kawalan dan 5% PF mempunyai struktur bersifat membran yang mempunyai gluten matriks yang tidak bersambungan manakala doh yang mengandungi 10-15% tepung labu menunjukkan struktur bersifat seperti ‘mesh-like’. SEM pada krum roti kawalan menunjukkan ruang udara yang lebih besar dan bulat berbanding dengan roti yang mengandungi 10% dan 15% PF. Penilaian sensori menunjukkan roti yang mengandungi 5% PF lebih diterima oleh panel berbanding dengan sampel roti yang lain. Keputusan ujian mikrobiologi semasa penstoran menunjukkan semua jenis roti mengandungi bilangan yis dan kulat dalam julat $<10^3$CFU/g.

PHYSICO-CHEMICAL AND ORGANOLEPTIC EVALUATIONS OF WHEAT BREAD SUBSTITUTED WITH DIFFERENT PERCENTAGES OF PUMPKIN FLOUR *(Cucurbita moschata)*

ABSTRACT

The objectives of this research were to determine the physico-chemical and organoleptic attributes of wheat bread substituted with four different percentages (control, 5%, 10% and 15%) of pumpkin flour (PF). The microbiological evaluation was conducted only on bread during storage. The bread were prepared by the sponge and dough method. Bread substitution from 5% to 15% PF showed significant increase ($p<0.05$) in ash and crude fiber content. However, protein and fat content were found to decrease significantly ($p<0.05$) in the bread. Pumpkin flour only showed significantly ($p<0.05$) higher water holding capacity than wheat flour. Substitution of wheat flour with PF resulted in significantly higher ($p<0.05$) total dietary fibre (TDF), insoluble dietary fibre (IDF), soluble dietary fibre (SDF) and resistant starch (RS) content while there was a reduction in the total starch (TS) content ranging from 56.5 to 60.8% (dry wt.) in bread. PF bread showed lower GI value in bread than the control. PF was noted to significantly increase the beta-carotene content in bread which resulted in significantly increase ($p<0.05$) in the yellowness of the crust and crumb colour of the bread. The mineral (Ca, Na, K, Fe, P and Mg) contents increased significantly ($p<0.05$) with increased percentages of PF in the bread. Glucose, sucrose and fructose were shown to be present in the orders from high to low in the pumpkin bread. Loaf volume and specific volume were significantly different ($p<0.05$) in PF bread. Texture result showed that the 10% and 15% PF bread was significantly different ($p<0.05$) in terms of hardness, gumminess and elasticity values than the control. Scanning electron microscopy (SEM) of the control and 5% dough had membrane-like structure with more discontinuous gluten matrix as compared to the 10-15% PF dough which have mesh-like structures. SEM of the control bread crumb showed larger spherical air cells as compared to the 10% and 15% PF. Sensory evaluation indicated that the 5% PF bread was the most acceptable. Microbiological evaluation during storage showed that all types of bread had mould and yeast count in the range of $<10^3$CFU/g.

CHAPTER [1]

1.0 INTRODUCTION

Pumpkin is from genus *Cucurbita* of the family Cucurbitaceae. It includes squash and cucumbers which are grown throughout the tropical and sub tropical countries. There are three common types of pumpkin world-wide, namely *Curcurbita pepo*, *Curcurbita maxima* and *C. moschata* (Lee *et al.,* 2003). Pumpkin can be found in many shapes, sizes and colours. The miniature pumpkin is *C. pepo* (Jack-o-lantern types) and the giant types of the pumpkin are 'Boston Marrow' and 'Mammoth' which tend to be *C. maxima* varieties, while the buff-coloured pie or 'Dickinson' variety and *Cucurbita moschata* are excellent for processing into pie. All pumpkins have hard skin when matured. *C. moschata* is the most commonly use in both Asia and the United States The major pumpkin producing states in Malaysia are Terengganu, Kelantan, Perak and Kedah (*http://www.bernama.com.my*) and around 10,224 tones of pumpkins are produced in Malaysia in year 2007 (MARDI, 2007).

The yellow-orange characteristic colour of pumpkin is due to the presence of carotenoid. Carotenoids are natural pigments responsible for the yellow, orange and red colour of many foods. Pumpkin provides valuable source of carotenoids, provitamin A and ascorbic acid which have major roles in nutritional aspect as well as an antioxidant. Current research indicates that a diet rich in foods containing beta-carotene may reduce the risk of developing certain types of cancer and offers protection against heart disease. The carotenoid content in Spanish pumpkin was reported to be higher than other pumpkins and even higher than carrots which contained beta- carotene (Wu and Jin, 1998). The yellow colour of the pumpkin can be used as natural colourant.

Pumpkin can be consumed in a variety of ways such as a fresh or cooked vegetable, as well as being stored frozen or canned (Figueredo *et al.*, 2000). Pumpkin can be processed into flour which has longer shelf-life. Pumpkin flour was being used because of its highly-desirable flavour, sweetness and deep yellow-orange colour. It has been used to supplement cereal flours in bakery products, soups, sauces, instant noodle, spice as well as a natural colouring agent in pasta and flour mixes.

Pumpkin is rich in carotene, vitamins, minerals, pectin and dietary fibre (Djutin, 1991). Hence, supplementation of pumpkin flour would improve the nutritional quality of bread (Ptitchkina *et al.*, 1998). Fluted pumpkin seed flour has been used as protein supplements in variety of local foods (Giami and Bekebain, 1992; Sunday and Isaac, 1999). Furthermore, El-Soukkary, 2001, reported that in-vitro protein digestibility of bread was improved with addition of pumpkin seed proteins.

Bread has always been one of the most popular and appealing food products due to its superior nutritional, sensorial and textural characteristics, ready to eat convenience as well as cost competitiveness (Giannou and Tzia, 2007). Nowadays, emphasis is on healthy bread with low glycemic index, more protein and will increase the dietary fibre intake, high resistant starch and decrease in calori and carbohydrates of baked goods. Composite breads are made from blends of wheat and non-wheat flours. Breads were prepared from composite flours such as fababean, cottonseed and sesame flour (Abdel-Aal *et al.,* 1993); corn, barley and cassava (Khalil *et al.,* 2000); breadnut flour (Oshodi *et al.,*1999); pumpkin and canola seed flour (Mansour *et al.,*1999); soybeans flour (Gahlawat and Sehgal, 1998), legume flour (Sadowska *et al*., 2003), full-fat or defatted cocoa powder (Aremu *et al*., 1995), peanuts and sunflower seed (Fagbemi *et al*., 2005); beniseed (Afolabi *et al*., 2001); sweet potato flours (Collado *et al*., 1997); coconut flour (Trinidad *et al.,* 2006). All these ingredients will impart characteristic colours, texture and nutritional value which may be favourable in bakery products, recipes and other food products. The composite flours are advantages to the developing countries because the flour could reduce wheat imports and increased the potential use of locally grown crop (Hugo *et al.,* 2003).

The objectives of this study are:

1. To determine the proximate composition of fresh pumpkin and pumpkin flour (*Cucurbita moschata*).

2. To evaluate the physico-chemical and organoleptic attributes of wheat bread substituted with different percentages of pumpkin flour.

3. To evaluate the microbial load of yeast and mould in the bread during storage.

CHAPTER [2]

2.0 LITERATURE REVIEW

2.1 Pumpkin

Pumpkin which originated from America, is a member of the family Cucurbitaceae, also includes squash, cantaloupes, cucumbers, watermelons and gourds. Pumpkin encompass a wide range of fruit types with regard to size (Tatum *et al.,* 2006) and with shapes ranging from globosely to pear-shaped, elongated or flattened; smooth, warted, ribbed or furrowed skin. Theirs colours varies from green, white and blue grey to yellow, orange or red as depending on the species (Weinstein *et al.*, 2004). The pumpkin belongs to the family of Cucubitaceae. It comprised of *Cucurbita moschata, C. Pepo, C. Maxima, C. Mixta, C. Ficifolia* and *Telfairia occidentalis Hook*. The *Cucurbita pepo L., Cucurbita maxima Duchesne* and *Cucurbita moschata Duchesne* are commercially known as ‘‘calabaza de castilla’’ or ‘‘tamalayota’’ (De Escalada Pla *et al.,* 2007) represent an economically important species cultivated worldwide with high productivity (Paris and Brown, 2005). Photo of *Cucurbita moschata* are showed in Appendix A. About 18,978,328 tones of pumpkins are produced in the world in year 2005 and around 10,224 tones of pumpkins are produced in Malaysia in year 2007 (MARDI, 2007). The five major pumpkin producing countries in the world are China, India, Ukraine, Egypt and United States (FAO, 2005). The major pumpkin producing states in Malaysia are Terengganu, Kelantan, Perak and Kedah (*http://www.bernama.com.my*). The production of pumpkin in Malaysia as reported by Malaysian Agricultural Research and Development Institute (MARDI, 2007) is shown in Table 2.1. Fresh pumpkins are very perishable and sensitive to microbial spoilage, even at refrigerated conditions; therefore they must be frozen or dried (Doymaz, 2004).

Table 2.1.The production of pumpkin in Malaysia from year 1998-2007

Year	**1998**	**2000**	**2004**	**2005**	**2006**	**2007**
Planted area (hectare)	271	382	407	582	650	720
Producing area (hectare)	271	382	407	582	650	720
Average yield (tonne/hectare)	N/A	9.0	12.2	13.8	14.0	14.2
Production (tonnes)	N/A	3425	4972	8058	9100	10224
Production value (RM)	N/A	2,158	5,596,607	9,183,133	10,371,270	11,652,290

Resources: MARDI, 2007
N/A = Not available

2.2 Uses and Nutritional Value

Pumpkins are important horticultural crops worldwide which has been used traditionally (Bezold *et al.,* 2003; Loy, 2004). Pumpkin has been used for pies, jack-O'lanterns or stock feed' (Robinson and Decker-Walters, 1999). It has numerous culinary uses either eaten fresh or vegetables (Oniang' o *et al.,* 2003) or as ingredients in pies, soups, stews, jam, sweet, marmalade (Egbekun *et al.,* 1998), beverage, baby food, ice-cream (Hassan, 2005), instant pumpkin kofta (Saxena *et al.*, 2004) and breads (Ratnayake *et al.,* 2004). It has been processed in different ways for human consumption such as fried, frozen, dried, candied or pickled (Teotia, 1992). Leaves, stems, seeds and roots have high food value and provide source of oil and raw material for variety of products. The leaves and flowers of the plant are widely cooked as vegetable soup and as well as by livestock (sheep, pigs and goats) as forage (Ladeji *et al.*, 1995; Akwaowo *et al.*, 2000). Extracts of the roots of fluted pumpkin, which are not eaten by man which have been reported to contain resins, alkaloids and saponins. Extracts of the roots are sometimes used in medical concoctions that have been shown to be lethal to rats and mice (Akwaowo *et al.*, 2000).

Preliminary investigations showed that a pumpkin-rich diet could reduce the blood glucose. The active polysaccharides from the pumpkin fruit could obviously increase the levels of serum insulin and thus reduce the blood glucose levels which improve tolerance of glucose, and therefore could be developed as new antidiabetic agent. This result the polysaccharide rich pumpkin has drawn the interest among researchers and consumers (Yang *et al.*, 2007).

Pumpkin seed has considerable nutritional value for human consumption due to its 37.8–45.4% oil and 25.2–37.0% protein. It has valuable dietetic and medicinal advantages besides being a source of edible oils, proteins and minerals of good quality (Yoshida *et al.,* 2004). The content of vitamin E in pumpkin seed is very high and the main isomers are α- and γ-tocopherols (Idouraine *et al.*, 1996; Murkovic and Pfannhauser, 2000). The seed itself can be eaten as a snack or ground as an ingredient of sauces. Pumpkin seed has also been used in traditional medicine with combination from several plants and herbs which contain fatty acids and phytosterols and are used in the treatment of benign prostatic hyperplasia (Carbin *et al.,* 1990; Zhang *et al.,* 1994; Dvorkin and Song, 2002). Pumpkin seed oil, extracted from the seed is used widespread as salad oil (Murkovic and Pfannhauser, 2000). Pumpkin seed is also rich in plant sterols which have recently become of great interest due to the serum cholesterol-lowering effect (Miettinen *et al*., 1995; Jones *et al*., 2000). The seeds may also be beneficial against colon cancer (Awad *et al.,* 1998). The composition of raw pumpkin based on United States Department of Agriculture (USDA, 2006) is shown in Table 2.2

Table 2.2: Composition of raw pumpkin (per 100g)

Nutrient	Units	Ref (A)	Ref (B)
Water	g	91.60	91.6
Energy	kcal	26	109
Protein	g	1.00	1.00
Fat	g	0.10	1.00
Ash	g	0.80	-
Carbohydrate	g	6.50	6.50
Fibre, total dietary	g	0.50	1.10
Sugars	g	1.36	-
Calcium	mg	21	-
Potassium	mg	340	-
Magnesium	mg	12	-
Phosphorus	mg	44	-
beta-carotene	mg	3.1	-
Ascorbic acid	mg	-	9.00
Thiamine	mg	-	0.05
Riboflavin	mg	-	0.11
Nicotinic acid	mg	-	0.60
Total carotene	mg	-	2.67

Resources: Ref (A) = USDA, 2006
Ref (B) = Lingle, 1993

2.3 Pumpkin Flour

Pumpkin flour prepared by drum-drying or hot air-drying techniques was reported to be stable, versatile and can be used throughout the year (Kulkarni *et al.*, 1996). Drying is one of the most widely used primary methods of food preservation (Akpinar and Bicer, 2004). Drying creates a new range of products and the flour can be stabilized by reducing the moisture content or its water activity (Boudhrioua *et al.,* 2002). Drying method was reported to provide longer shelf-life, smaller space for storage and lighter in weight during transportation (Ertekin and Yaldiz, 2004). This serves as a means of increasing the supply of pumpkin in different forms, all year round which is cost effective. The change from fresh to processed pumpkin will meet the demand for these foods throughout the year. Currently the hot air drying method resulted in a more uniform, hygienic and attractively coloured dried product which can be produced rapidly (Doymaz, 2004).

Pumpkin flour was produced from mature pumpkin *(Cucurbita moschata Duch. ex. Poir.)* contain 6.01% moisture, 3.74% protein, 1.34 % fat, 7.24% ash, 2.9% fibre, 78.77% carbohydrate and 56.04% alcohol insoluble solids. The sample has 7.29 mg/100 g beta-carotene with colour values of L* 57.81, a* 8.31, b* 34.39 and 0.24 water activity (Pongjanta *et al.*, 2006). Photo of pumpkin flour was showed in Appendix A.

Pumpkin flour can be used as a thickener in soup, gravy, fabricated snacks and as an ingredient in bakery products such as sandwich bread, sweet bread, butter cake, chiffon cake, cookies (Pongjanta *et al.*, 2006) and instant fried noodles. It has also been used as a source of beta-carotene and natural colourant (yellow colour) with most favourable in appearance, taste, texture and acceptability when supplemented in bakery products (Lee *et al.,* 2002).

Table 2.3: Composition of pumpkin flour (per 100g)

Composition	Units	Content	
		Ref (C)	Ref (D)
Moisture	%	9.1	6.01
Protein	%	9.0	3.74
Fat	%	-	1.34
Ash	%	3.8	7.24
Carbohydrate	%	-	78.77
Fibre	%	-	2.9
Water activities	-	-	0.24
beta-carotene	mg	5.60	7.29
Pectin	g	18.7	-
Cellulose	g	50.5	-
Hemicellulose	g	4.3	-
Lignin	g	4.3	-
Ascorbic acid	g	0.0683	-
Sodium	g	0.65	-
Potassium	g	1.9	-
Calcium	g	0.5	-
Phosphorus	g	0.03	-

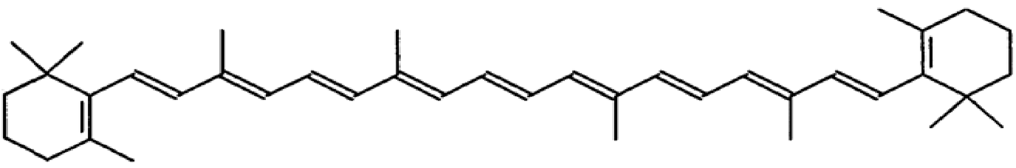
Resources: Ref (C) = Ermakov (1987)
Ref (D) = Pongjanta *et al.,* (2006)

2.4 Beta Carotene

Carotenoids have been the main group of colouring substances in nature. Carotenoids are responsible for many of the red, orange and yellow colours of fruits and vegetables (Astorg, 1997). The stability of carotenoids during processing and storage is crucial for product attractiveness and acceptabilility. Pigment degradation will affect the natural colour, nutritive value and flavour in vegetable. The common degradation pathways are isomerization, oxidation and fragmentation of the carotenoid molecules which are promoted by heat, light and acids (Bonnie and Choo, 1999; Bao and Chang, 1994; Martins and Silva, 2002; Muftugil, 1986; Sims *et al.*, 1993).

In recent year, carotenoids have increasingly become more important in man (Food and Nutrition Board, 2002; Dixon *et al.*, 2003). Beta-carotene is fat-soluble pigments of plant origin, which is essential in human nutrition. Beta-carotene is an antioxidant precursor to vitamin A in the human body is important in human eye light reception, or the beta-carotene can act directly in cancer prevention (Weinstein *et al.*, 2004). Thus, The US Cancer Institute has recommended people to increase their intake of high beta-carotene content foods (Lester and Eischen, 1996).

Pumpkin is a green-yellow vegetable rich in beta-carotene has attracted attention as a healthy food. Nineteen carotenoids were detected in *Cucurbita moschata* variety "Baianinha" and beta-carotene was the principal carotenoid. It contributes about 74% of an average total carotenoid content of 317.8 micrograms/g. In *C. maxima* variety "Jerimum Caboclo", 11 carotenoids were found with lutein and beta-carotene as the major pigments accounting for about 60% and 27%, respectively with an average total carotenoid content of 78.4 micrograms/g. The abundance of beta-carotene in the *C. moschata* variety "Baianinha" makes this squash as one of the richest sources of provitamin A. The average vitamin A value was 43,175 IU (International Units) per 100 g or 4,317 RE (retinol equivalents) per 100 g. Its vitamin A values is more than 11 times that of *C. maxima* variety "Jerimum Caboclo" and five times that of *C. moschata* cultivar "Menina Verde". The squash was previously found to be the highest in provitamin A among the Cucurbita vegetables and is the most commercialized in São Paulo (Southeastern Brazil) (Arima and Rodríguez-Amaya, 1990). The juice of pumpkin cultivars (*Cucurbita maxima*) has 49 mg/litre carotenoids content. Different concentrations and distributions of beta-carotene, α-carotene, violaxanthin, neoxanthin, all trans-lutein and isomers of 9-cis-beta-carotene and 13-cis-beta-carotene were observed (Dietrich *et al.*, 2004).



β-carotene

Figure 2.1: Structure of beta-carotene (Tee, 1988)

2.5 Dietary Fibre

The interest in foods rich in dietary fibre increased in the recent decades and this led to the development of a large market for fibre-rich products and ingredients (Drzikova *et al.,* 2005). The specific properties of DF has been reported to play an important role in the prevention and treatment of various gastrointestinal disorders (hernia, duodenal ulcer, gall stones, appendicitis, constipation, hemorrhoids, colon carcinoma), obesity, atherosclerosis, coronary heart diseases, colorectal cancer and diabetes (Bingham, 2003; De Escalada Pla *et al.*, 2007). Addition of fibre to foods is an alternative way to compensate for the existent deficiency in the diet. Apart from the nutritional application, fibre can be used for technological purposes such as bulking agent or fat substitute in foods (Guillon and Champ, 2000). The World Health Organization (WHO, 2003) currently recommends consumption of foods containing >25 grams (30-45g) of total dietary fibre/day. In fact, WHO has identified dietary fibre as the only dietary ingredient with “Convincing Evidence” showing a protective effect against weight gain and obesity.

Bread can be enriched with dietary fibre such as wheat bran (Ranhotra *et al.*, 1990; Sidhu *et al.*, 1999), gums such as guar gum and modified cellulloses (Pomeranz *et al.*, 1977) and beta-glucans (Knuckles *et al.*, 1997). Wheat flour contains 1.5–2.5% total Arabinxylans, non-starch polysaccharides of cereal which is an important source of dietary fibre where one-third to half is water-extractable and the other is water-unextractable (Su *et al.,* 2005). According to Peng *et al.,* (2002), the pumpkin polysaccharide had the function of reducing the blood sugar and showed that it had very important value in auxiliary cure for diabetes.

The dietary fibre is composed of total dietary fibre (TDF), which includes both soluble (SDF) and insoluble dietary fibre (IDF). In terms of health benefits, both kinds of fibre complement with each other. A well balanced proportion is considered when there is 70-50% insoluble and 30-50% soluble DF (Grigelmo-Miguel *et al.*, 1999).

Soluble fibre is found in fresh and dried fruit, vegetables, oats, legumes and seeds (Thebaudin *et al.*, 1997). Examples of the soluble fibre are pectins, gums, mucillages and some hemicellulose. Some soluble fibres increase the viscosity of the intestinal contents and assist in reducing cholesterol absorption. Other soluble fibres are fermented by the bacteria within the large intestine and can assist in maintaining colon health and increasing the mineral absorption. Soluble fibre fermentation results in the production of short-chain fatty acids, principally acetate, propionate and butyrate. Butyrate has been found to act as a protective agent against experimental tumor genesis of these cells. Propionate could be related to hypocholesterolemic effects (Redondo-Cuenca *et al.*, 2006).

Insoluble fibre is found in the plant cell walls of whole grain bread, whole grain cereals, fruits, vegetables, unprocessed bran and wheat germ (Thebaudin *et al.*, 1997). Examples of the insoluble fibre are cellulose, lignan and hemicellulose. Many insoluble fibres, including cellulose and psyllium, are not fermentable. Insoluble dietary fibre has a high water-holding capacity, increases the fecal bulk and reduces the gastro intestinal transit time. This effect may be related to the prevention and treatment of different intestinal disorders, such as constipation, diverticulitis, haemorrhoids and other bowel conditions (Goni and Martín-Carrón, 1998).

2.6 Resistant Starch

Resistant starch (RS) has attracted greater interest amongst both the nutritionists and the food industry, due to its contribution to the non-digestible carbohydrate component of the diet and the physiological implications, colonic fermentation, post-prandial glycemia, faecal bulking, intestinal transit time and energy value of foods (Kumari *et al.,* 2007; Gee *et al.*, 1992 and Livesey, 1990).

Human colonic bacteria ferment RS and nonstarch polysaccharides (NSP; major components of dietary fibre) to short-chain fatty acids (SCFA), mainly acetate, propionate and butyrate with potential beneficial health effects (Liljeberg *et al.*, 1996). SCFA stimulate colonic blood flow and fluid and electrolyte uptake. Butyrate is a preferred substrate for colonocytes and appears to promote a normal phenotype in these cells (Topping and Clifton, 2001).

RS1 (Resistant starch type 1) is physically inaccessible starch due to the presence of intact cell walls in grains, seeds or tubers. They are mainly found in partly milled grains, seeds and legumes. For RS1 to be digested, the seed or outer coating must be broken such that the starch granules are no longer entrapped (Tovar *et al*., 1992).

RS2 (Resistant starch type 2) is a group of the raw ungelatinized (uncooked) starches. They are classified in 3 main types: A (cereal starches, cassava starch), B (green banana and raw potato starches) and C (legume starches). RS2 is called high-amylose maize starches which are highly resistant to digestion by α-amylase until gelatinized (Sozer *et al.,* 2007).

RS3 (Resistant starch type 3) is the retrograded starch whereby when the starch has been cooked, cooled and stored for a periods of time. Examples of the RS3 are cooked and chilled potatoes or retrograded high amylose corn (*i.e.* Novelose), bread, cornflakes, canned peas or beans, food products with prolonged and repeated moist heat treatment (Englyst *et al.,* 1992). It contains mainly retrograded amylose with a melting temperature of 150 °C. This makes RS3 an appropriate candidate as a heat-stable pre-biotic food additive, which may be used in cooked or baked goods (Shamai *et al.,* 2003).

RS4 (Resistant starch type 4) refer to the chemical starch modification or thermal treatment at high temperatures. These are derived as a result of cross bonding with chemical reagents such as ethers and esters (certain modified breads, pastas and cakes). This type of resistant starches can have a wide variety of structures and are not found in nature (Tovar *et al.,* 2002).

RS may be found in both unprocessed and processed foods. Processed foods invariably undergo storage at moderate or low temperatures before consumption. Storage of foods is also a contributing factor to the changes in the available starch content of the product. The quantity of RS formed, during processing or storage depend on the severity of the processing conditions like temperature, pH, moisture, number of heating or cooking cycles adopted, condition of storage, etc. In addition, many of the processing treatments such as freezing autoclaving are also noted to have significant impact on the fermentation of RS (Goni *et al.*, 1996; Kumari *et al.,* 2007).

2.7 Bread

Bread is a dietary staple diet for the world's population (Ahlborn *et al.*, 2005; Fardet *et al.*, 2006). Bread products are well accepted worldwide because of the low cost, ease of preparation, versatility, sensory attributes and nutritional properties. Bread in human nutrition is not only a source of energy, but also a supplier of irreplaceable nutrients for the human body. It provides little fat, but high quantities of starch and dietary fibre as well as cereal protein. Apart from that, bread contains the B group vitamins and minerals which are mostly magnesium, calcium and iron (Isserliyska *et al.*, 2001).

The simplest bread can be created by basic formula dough which includes flour, water, leavening agent (yeast or chemicals) and sodium chloride (Brown, 1993; Giannou *et al.,* 2003). Other ingredients such as fat, emulsifiers, sugars and dough conditioner may be added to improve the dough and bread quality. Each ingredient has its own function in bread and if slightly changed will alter final bread quality. Therefore, a proper balance of ingredients needs to be obtained to produce high-quality bread.

2.7.1 Wheat Flour.

Flour is the most important ingredient in breadmaking because it modulates the specific characteristics of bakery products (Giannou *et al.,* 2003). It consists of protein (10–12%), starch (70–75%) and other carbohydrates, ash, non-starch polysaccharides (2–3%), in particular arabinoxylans (AX), lipids (2%), water (14%), small amounts of vitamins, minerals and enzymes (Charley and Weaver, 1998; Goesaert *et al.,* 2005). Wheat flour is the most common flour that has been used. It provides a light, palatable, well-risen loaf of bread when processed into fermented dough (Bushuk and Rasper, 1994).

Wheat flour contains starch and protein, which form the structure of the bread. There are two types of proteins in the flour, *gliadin* and *glutenin*. The prolamins of wheat (gliadin) that comprise 40–50% of the proteins are extremely sticky and inelastic is responsible for the cohesiveness of doughs. On the other hand, the glutelins was also named glutenins, provides resistance to extension (Singh and MacRitchie, 2001). The prolamins and glutelins combined during mixing to form the elastic protein gluten complex resulting in viscoelastic dough. The dough has the ability to form thin sheets that will able to retain gas and produce a light baked product (Gujral and Rosell, 2004). Wheat flour can be made from whole wheat or the germ and bran can be separated from the endosperm is then ground into flour. Without the fibrous bran and the oily germ, the resulting flour has fewer nutrients, but will keep longer and make a lighter textured, as well as higher rising bread. If the flour is allowed to age for about a month, the natural yellowish colour will fade to white due to the effects of oxygen. This aging period can allow insects to infest the flour. This can be eliminated by adding bleaching agents such as benzoyl peroxide. The strong flour has protein content in the range of 10.5% – 14.5% and the weak flour has less than 8.5% protein (Duncan, 1998). According to Cauvain (2003), hard wheat with strong gluten and high protein content in the flour has better ability to trap and retain carbon dioxide gas and resulted in higher volume of bread (Weegels *et al.*, 1996).

2.7.2 Composite Flour

Composite breads are made from blends of wheat and non-wheat flour. These flours are advantageous to developing countries because wheat imports can be reduced and elevate the use of locally grown grains (Hugo *et al.,* 2003). According to Khalil *et al.,* 2000, flours from corn, barley and cassava are the most predominant flours that have been studied in production of composite flour in breads. Abdel-Aal *et al.* (1993) studied that loaf volume and specific volume of pan breads prepared from composite flours (fababean, cottonseed and sesame flour) were 25-60% less than the control bread. Breadnut flour which contains high quality protein with total essential amino acid of 55.1% was comparable with soya flour and egg (Oshodi *et al.,* 1999). Mansour *et al.,* (1999) reported that the addition of pumpkin and canola seed flour increased the protein, lysine and mineral (Ca. P, Cu, Fe and Mg) contents of the breads by 11-38%, 90-200% and 70-135%, respectively.

The utilization of soybeans flour (Gahlawat and Sehgal, 1998), legume flour (Sadowska *et al*., 2003), full-fat or defatted cocoa powder (Aremu *et al*., 1995), peanuts, cottonseed and sunflower seed (Fagbemi *et al*., 2005), beniseed (Afolabi *et al*., 2001) as functional ingredients in breads, biscuits, chapatis, snacks and textured products has been successfully demonstrated to give extra protein and changes in texture of the product. Sweet potato flours vary widely in colour such as orange, yellow and purple colour which gives natural colourant that may be favourable for bakery products (Collado *et al*., 1997). Nochera and Caldwell (1992) reported that the organoleptic acceptability of bread and biscuits made from breadfruit flour give an acceptable in flavour, colour and texture. Coconut flour as a good source of dietary fibre can be added into the bakery products, recipes and other food products for good health (Trinidad *et al.,* 2006).

The composite flour like fermentation of sorghum flour, particularly in a sourdough bread making process, appears to have considerable potential for increasing sorghum utilization in bread. Mixing wet fermented sorghum flour directly with wheat flour (sourdough-type process) further increased the loaf volume and weight as well as reduced the crumb firmness and simplified the bread making process. It was shown that low pH of fermented sorghum flour inactivated amylases and increased the viscosity of sorghum flour which thus improved the gas-holding capacity of sorghum and wheat composite dough. In Hugo *et al.,* (2003) their studied has shown that bread made with boiled malt flour (30%) had an improved crumb structure, crumb softness, water-holding capacity and resistance to staling, as well as a fine malt flavour as compared to bread made with grain sorghum flour.

2.7.3 Salt

Salt, chemical compound, sodium chloride (NaCl), is considered as an ingredient with a functional role in the production of many bakery products (Amendola and Lundberg, 1992). One of the most important functions that salt does in bread dough is to inhibit the yeast to slow down the rate of fermentation. A small amount of salt prevents yeast from "going wild" and over-proofing the dough. Salt inhibits the action of flour proteases, which otherwise would depolimerize proteins of the gluten complex. Yeast dough without salt is sticky and difficult to manipulate.

In frozen dough products, salt slows the production of carbon dioxide by yeast by delaying the fermentation process (Charley and Weaver, 1998).

Salt also helps to toughen and strengthen gluten, which makes the dough able to stretch easier which thus improved the quality and texture of the finished bread. A small amount of salt in dough improves flavour and favours the action of amylases in helping to maintain a supply of maltose as food for the yeast (Wood, 1989). Salt is commonly added to bakery formulas at levels from 1 to 2.5% of the flour weight (Matz, 1996, Collado- Fernández, 2003).

Salt contains no calories, proteins or carbohydrates, although unrefined salt does contain traces of other minerals. The sodium and chloride of salt, along with potassium are electrolytes, which help the kidneys to regulate the body's fluid levels and the balance of acids and bases is a vital constituent of the human body (Whitney and Rolfes, 1996).

2.7.4 Sugar

Sugar belongs to the group of nutrients called carbohydrates. Sucrose is known as a disaccharide (composed of a unit of dextrose plus a unit of fructose). It is derived from sugar cane or sugar beet, which has been refined and crystallised from a concentrated solution (Cauvain and Young, 2006). Sugars are normally used by yeast during the early stages of fermentation. Later extra sugars may be added to increase gas production by the action of enzymes in the flour, to improve the crust colour and to sweeten the bread. During baking, the reducing sugars combine with amino acids from protein in a complex reaction known as Maillard reaction. This reaction gives attractive brown colours on the surface of baked goods. The higher the concentration of the reducing sugars present, the darker is the colour produced (Duncan, 1998).

According to Giannou *et al.,* (2003), sugars also act as antiplasticizers retarding the pasting of native starch or function as anti-staling ingredients inhibiting the starch recrystallization which can extend the shelf life of the bread by delaying the gelatinization and the denaturation of protein. Compounds like volatile acids and aldehyde which is responsible to enhance flavour and aroma of the bread can be developed. The addition of sugar results an increased in yield per dough (Pyler, 1973). Additions of sugar produce dough that is stable, elastic and softer and improved the crumb texture and moistness (Collado-Fernández, 2003). The addition of sugar level may reach as high as 30% of the flour weight. Increasing levels of sugar affect the gas producing ability of baker's yeast. Addition of 15% sugar based on flour weight will have more than doubles the time that it would take for a piece of dough to reach a given height in the proofer (Cauvain and Young, 2006).

2.7.5 Fat and Oil

The importance of fats and oils as baking ingredients varies with the bakery product. Fat is added at levels ranging from 2% to 8%. Different fats and oils are added into baked goods to improve the volume, soften the texture, have a uniform cell structure and tenderizing effects upon the crumb and crust as well as to facilitate dough handling and processing. Fats also increase the caloric value which enhances the flavours and aroma of the products and stabilized the cake better. The fat stays on the tongue longer which thus promote enhanced recognition at the site of the taste buds (Kamel, 1992).

However, vegetable oils or shortening are added to breads from getting stale, allowing it to be kept longer than a day before being eaten. Bhattacharya *et al.* (2002) studied that 20% waxy wheat flour could substitute for use of shortening to achieve desirable crumb softness and to retard staling upon storage.

2.7.6 Yeast

Baker's yeast like *Saccharomyces cerevisiae* strains has been selected for many years for their dough-leavening characteristics (Arlorio *et al.*, 1999). The yeast cell metabolized fermentable sugars (glucose, fructose, sucrose and maltose) in the flour, and produces carbon dioxide gas and alcohol as waste products which resulted in dough leavening and contributed the flavour and support gluten network of bread. The yeast gassing rate is critical in baking technology depends on the dough formulation, on specific fermentation parameters especially on intrinsic characteristics of baker's yeast (Randez-Gil *et al.*, 1999). Baker's yeast should be able to grow on a wide range of substrates at a high yield and have a high optimal temperature as well as excellent processing characteristics (Linko *et al.,* 1997).

According to Cauvain and Young (2006), the higher the level of yeast present in the recipe, the faster the rate at which carbon dioxide will be produced. The reaction is very temperature sensitive and increases as the temperature rises to 40-43

°C and inactivated at 55 °C. The baker's yeast should be able to grow at temperatures in the range of 25–42 °C during which dough preparation and proving under anaerobic conditions (Peres *et al.,* 2005).

Instant yeast is made from more active strains of yeast and it is dried faster and has a lower level of moisture. It can be substituted with flour and other ingredients without prior hydration. In contrast, active dry yeast has a long shelf-life at room temperature. It must be dehydrated before it is substituted with other ingredients (Charley and Weaver, 1998).

2.7.7 Milk Powder

Incorporation of dairy ingredients is long established in the baking industry. Dairy ingredients are incorporated into bread for the nutritional and functional properties. It can be readily incorporated into many food products (Crowley *et al.,* 2002). The functional benefits are including flavour, texture enhancement and storage improvement. Dairy powders used in gluten-free bread formulations resulted in improved volume, appearance and sensory aspects of the loaves (Gallagher *et al.*, 2003b).

Nutritional benefits of dairy ingredients include increased the protein enrichment and supplementation of the limiting amino acids, lysine, methionine and tryptophan in the fortified bread products. Milk and dairy foods are providing significant amounts of micronutrients, including calcium, B-group vitamins (particularly riboflavin and B_{12}, but also thiamin, niacin, B_6 and folate), vitamin A, iodine, magnesium, phosphorus, potassium and zinc. Milk and dairy foods can increase the nutrient density of the diet and play a pivotal role in ensuring that dietary intakes are nutritionally adequate. It can also help to improve bone and dental health (Wells, 2001).

2.7.8 Emulsifiers

Emulsifiers are substances possessing both hydrophobic (lipophilic) and hydrophilic properties. Emulsifiers have a fatty acid at one end that can combine with the starch, and a water-loving end that helps to keep it dispersed in the dough (Gomez *et al.,* 2004). Emulsifiers that are used in the food industry will improve texture, stability, volume, softness, aeration, homogenization and shelf life (Madsen, 2006).

Some emulsifiers are commonly used in baked goods are lecithin or phospholipids, Diacetyl tartaric acid esters of glycerides (DATA), monoglycerides (DATEM), sodium stearoyl lactylate (SSL), glycerol monostearate (GMS) and diglycerides, polyoxyethylene stearates, sugar esters of fatty acids, polyglycerol esters of fatty acids, lactic acid esters of monoglycerides, acetic acid esters of monoglycerides (Gomez *et al.,* 2004). Mono- and diglycerides are G.R.A.S. (Generally Recognized As Safe) materials which are extensively used in bakery products. SSL (sodium stearoyl-2-lactylate) is allowable in Canada at 0.38% till to 0.5% of the flour weight in the United States. In U.S.A, MYRJ 45 is not allowed in bakery products but is allowed in Canada in unstandardized bakery products at 0.4%. The maximum allowable level of ATMUL P-44 in Canada is 0.55% of the flour weight (Kamel, 1992). Kaur, 2000 reported that application of emulsifiers, GMS and