EFFECTS OF PARTIAL SUBSTITUTION OF WHEAT FLOUR WITH CHEMPEDAK (*Artocarpus integer*) SEED FLOUR ON THE PHYSICOCHEMICAL, ORGANOLEPTIC AND MICROBIOLOGICAL ATTRIBUTES OF THE BREAD

by

MARDIANA BINTI AHAMAD ZABIDI

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KESAN PENGGANTIAN SEPARA TEPUNG GANDUM DENGAN TEPUNG BIJI CEMPEDAK (*Artocarpus integer*) DI DALAM ROTI TERHADAP SIFAT-SIFAT FIZIKOKIMIA, ORGNOLEPTIK DAN MIKROBIOLOGI

ABSTRAK

Objektif kajian ini adalah untuk menentukan kesan penggantian separa tepung gandum dengan tepung biji cempedak (*Artocarpus integer*) di dalam roti terhadap sifat-sifat fizikokimia, organoleptik dan mikrobiologi. Tepung gandum telah digantikan dengan tepung biji cempedak (CSF), iaitu sumber gentian tempatan di dalam formulasi roti pada tahap yang berbeza (0, 10, 20 dan 30% w/w). Penilaian terhadap sifat-sifat fizikokimia dan organoleptik roti pada tahap penggantian tepung biji cempedak yang berbeza telah dijalankan. Penilaian tersebut melibatkan penentuan kandungan proksimat iaitu kandungan lembapan, protein, gentian dietari kasar, abu dan lemak; gentian dietari total; kandungan mineral; kandungan kanji total dan kanji rintang; kandungan antinutrien; kandungan oligosakarida; kandungan amilosa dan amilopektin; dan penentuan nilai anggaran indeks glisemik. Sifat-sifat fizikal roti yang ditentukan ialah isipadu roti, berat roti, densiti roti, isipadu spesifik, 'oven spring', penilaian sensori dan analisis tekstur fizikal roti. Kandungan lembapan, gentian kasar, abu, kanji rintang, gentian dietari total dan beberapa jenis mineral penting telah meningkat secara signifikan ($p<0.05$) apabila tahap penggantian tepung biji cempedak di dalam roti ditingkatkan. Kandungan lemak kasar, karbohidrat dan nilai kalori didapati menurun secara signifikan ($p<0.05$) apabila tahap penggantian tepung biji cempedak meningkat. Peningkatan penggantian tepung biji cempedak di dalam roti telah menurunkan nilai anggaran indeks glisemik (EGI) secara signifikan ($p<0.05$). Kandungan amilosa didapati menurun secara signifikan ($p<0.05$) manakala kandungan amilopektin meningkat secara signifikan ($p<0.05$) dengan peningkatan penggantian tepung biji cempedak di dalam roti. Penggantian tepung biji cempedak ke dalam roti menunjukkan kandungan antinutrisi (asid fitik, perencat tripsin, fenolik total dan tanin) dan oligosakarida (rafinosa dan stakiosa) adalah rendah untuk memberi kesan negatif kepada kesihatan. Keputusan

SEM menunjukkan struktur sel krum roti adalah lebih terbuka apabila tahap penggantian tepung biji cempedak ditingkatkan. Peningkatan penggantian tepung biji cempedak di dalam roti mempengaruhi parameter fizikal apabila isipadu lof, isipadu spesifik dan 'oven spring' berkurangan secara signifikan ($p<0.05$). Analisis warna menunjukkan warna krum roti menjadi semakin gelap manakala kekerasan krum meningkat secara signifikan ($p<0.05$) apabila tahap penggantian tepung biji cempedak ditingkatkan. Penerimaan keseluruhan terhadap roti yang digantikan dengan tepung biji cempedak pada tahap yang berbeza menunjukkan roti 10% CSF adalah tidak berbeza secara signifikan ($p>0.05$) dibandingkan dengan roti kawalan. Penambahan maltodekstrin dan α-amilase tidak memanjangkan tempoh penstoran roti yang digantikan dengan tepung biji cempedak pada tahap yang berbeza. Tempoh penstoran roti yang digantikan dengan tepung biji cempedak pada tahap yang berbeza adalah selama 4 hari. Hitungan plat total (TPC) didapati melebihi had selamat pada hari ke-5.

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ABSTRACT

The main objective of this study was to determine the effects of partial substitution of wheat flour with chempedak (*Artocarpus integer*) seed flour on the physicochemical, organoleptic and microbiological attributes of the bread. Chempedak seed flour (CSF) was substituted at different levels (0, 10, 20 and 30% w/w) in bread formulation. The effects on physicochemical and organoleptic attributes of bread with different substitution levels of CSF were investigated. The effects on chemical composition of bread includes proximate, total dietary fibre, mineral, total starch and resistant starch, antinutrient, oligosaccharides, amylose and amylopectin contents, and the estimated glycemic index (EGI) value. The physical attributes of the bread were determined by loaf volume, loaf weight, density, loaf specific volume, oven spring, sensory evaluation and physical texture analysis. At higher CSF substitution level, bread samples resulted in significantly increased ($p<0.05$) moisture, crude fibre, ash, resistant starch, total dietary fibre and certain essential mineral contents. Crude fat, carbohydrate content and calorie values were decreased significantly ($p<0.05$) with higher CSF substitution level in bread. Subsequently, the estimated glycemic index (EGI) value of bread at higher substitution levels of CSF was found to decrease significantly ($p<0.05$). Amylose content was found to decrease significantly ($p<0.05$) while amylopectin content increased significantly ($p<0.05$) with elevated substitution level of CSF in bread. The value of antinutritional factors and oligosaccharides in bread with different substitution levels of CSF were low to exert deleterious effect on health. Scanning electron microscopy (SEM) of bread crumb indicated distinguishable open pore structures with increased CSF substitution level. Loaf qualities (loaf volume, specific volume, oven spring) were significantly decreased ($p<0.05$) at higher substitution level of CSF. Meanwhile, colour analysis showed that bread with higher CSF substitution level produced darker crumb colour ($p<0.05$) and

the crumb firmness was significantly increased ($p<0.05$). Overall acceptability of bread with different substitution CSF levels showed that the 10% CSF bread resulted in no significant difference ($p>0.05$) as compared to the control. Addition of maltodextrin and α-amylase did not delay the staling rate of bread with different substitution levels of CSF. The shelf life of bread with different substitution levels of CSF was found to lasts for only 4-days as the total plate count (TPC) was exceeding the safety limit on Day 5.

CHAPTER 1 INTRODUCTION

Health consciousness is increasing around the world population due to the growing incidence of diabetes, coronary heart disease, obesity and certain types of cancer (Rosell, 2003). The awareness is well publicized and significantly affects changes in food habit in term of consumption, and resulted in emerging various health products to meet the nutritional demand and preferences of consumers.

Recently, the focus of interest and significant efforts have been emphasized on production and development of food related to by-products or wastes and underutilized agricultural products. Apparently, such utilization and development embark production of various new food products by maximizing the available resources to contribute the recommended dietary fibre intake and fulfill the consumer's expectations.

Malaysian bakery and confectionery products were reported to reach RM2 billion in 2004, growing by 3% over the previous year at similar current value period (Danish Trade Council, 2005). However, to date, the food market in Malaysia such as cereal and cereal products is highly dependent on importation from foreign countries i.e. Australia, USA, Thailand and Argentina to satisfy the needs of the population.

Development of new generation bread products derived from diverse sources of non-wheat flour provides an alternative towards healthier bread products. The objective of supplementation alternative ingredients in bread formulation was to fortify the deficiency of nutritional value in wheat flour particularly essential amino acids, minerals, vitamins and dietary fibre (Hallén *et al.*, 2004).

The fundamental need of the human body is energy, which is derived from various substances to maintain optimum functionality. The metabolism from carbohydrate, protein and fat are the sources of energy, while other micro- and macro nutrients such as vitamins, minerals, dietary fibre and fluid are vital for normal regulation of body systems.

Extensive studies have been conducted by using different types of non-wheat flour to enhance the nutritional value without sacrificing the quality and palatability of the bread. The substitution level of non-wheat flours were reported to be as high as 15% w/w of wheat flour without significant deleterious effect on the physical textural (McWatters *et al.*, 2004). The organoleptic qualities of the bread concomitantly with other additive ingredients aid the product properties (Hallén *et al.*, 2004; Rosell, 2003). Various sources of non-wheat flour were used in bread making include chickpea, cowpea, lupin, soy and soy hulls, legumes, seeds, rice straw and rice bran, barley, cassava and other sources of fibres (Shittu *et al.*, 2007; Dalgetty and Baik, 2006; Kutoš *et al.*, 2004; McWatters *et al.* 2004; Sangnark and Noomhorm, 2004a; Doxastakis *et al.*, 2002; Gill *et al.*, 2002; Dhingra and Jood, 2001; Kadan *et al.*, 2001; Abdul-Hamid and Luan, 2000; El-Adawy, 1997; Defloor *et al.*, 1993).

However, apart of its health claim, substituting non-wheat flour into the bread formulation exerted adverse effects such as increase antinutritional factors, impaired physical and textural qualities, and reduced bread shelf life. Hence, significant approaches have been developed to counteract the negative impact upon substituting non-wheat flour into the bread formulation to improve the nutritional and quality of the bread. Enzymes (Caballero *et al.*, 2007; Blaszczak *et al.*, 2004, Martínez-Anaya, 1996) and additives such as hydrocolloids, guar gum and celluloses (Rosell *et al.*, 2001) are added in the bread ingredients to yield better loaf, nutritional value and organoleptic properties (Rosell, 2003).

This study is to determine the potential of underutilized by-product of local fruit source i.e. chempedak seed as a functional ingredient in processed food products, particularly for bread products.

Hence, the main objectives of this study are:

i. to characterize the chemical composition of chempedak seed and chempedak seed flour

ii. to study the effect of partial substitution of chempedak seed flour in the bread in term of chemical composition, physical textural and organoleptic attributes

iii. to study the effect bread staling by using maltodextrin and α-amylase in bread with different substitution level of chempedak seed flour to improve the physical and organoleptic of the bread.

CHAPTER 2 LITERATURE REVIEW

2.1 CHEMPEDAK

2.1.1 Background of chempedak

Chempedak (*Artocarpus integer* (Thunb.) Merr., *Artocarpus integrifolia* L. f., *Artocarpus polyphema* Persoon, *Artocarpus champeden* (Lour.) Stokes) belongs to the Moraceae family, the same family as jackfruit (*Artocapus heterophyllus* Lam.) and breadfruit (*Artocarpus altilis*). Chempedak (English), cempedak (Malay), bankong (wild), sonekadat (Myanmar) or champada (Thailand) is native to South East Asia and is widely distributed and cultivated in Burma, Indonesia, Peninsular Thailand and Peninsular Malaysia particularly in Perak and Kedah (Nakasone and Paull, 1998; Jansen, 1991). It is strictly tropical in growing requirements and is always restricted to regions without a distinct dry season (Jansen, 1991).

Chempedak is an evergreen monoecious tree and commonly found in abundant in primary lowland rain forest in its area of natural occurrence (Jansen, 1991). However, chempedak is usually grown in home gardens and sometimes in mixed orchard. The tree can grow up to 20 metres tall and is seldom buttressed. Chempedak is more seasonal than jackfruit as some flowers may be found at any time of the year. In Peninsular Malaysia, the flowers tend to bloom concentratively around the months of February to April and/or August to October while the fruits are harvested in between June and August. Meanwhile, in Sarawak, chempedak fruits ripen towards the end of the year in most years (Nakasone and Paull, 1998; Jansen, 1991).

Since the crop is restricted to wet regions, chempedak is not widespread as jackfruit. The demands for chempedak fruit are rather small and often regarded as a locally orientated fruit. However, chempedak is a promising new tropical fruit outside of its current area of distribution. In Malaysia, a number of chempedak selections have been cloned such as CH29 cultivar, which produces attractive orange flesh while other cultivars, CH26, CH27 and CH28 are high-yielding cultivars. Jackfruit and chempedak occasionally hybridized and a clone has been selected in

Malaysia called 'Nangka-Chempedak CH/NA' (Jansen, 1991). Chempedak plantation area, production and production value in states of Malaysia on 2003 is presented in Table 2.1.

Table 2.1: Plantation area, production and production value of chempedak in states of Malaysia, 2003

State	Chempedak			
	Planted area (Ha)	Producing area (Ha)	Production (Mt)	Value of production (RM '000)
Johor	1,093.7	758.4	4,186.3	8,373
Kedah	1,472.9	1,007.5	6,920.6	13,841
Kelantan	489.4	92.2	340.5	681
Malacca	372.7	240.7	1,925.2	3,850
Negeri Sembilan	269.9	151.6	756.9	1,514
Pahang	939.0	287.3	1,244.8	2,490
Perak	1,113.4	340.7	1,629.6	3,259
Perlis	25.7	19.1	86.6	173
Penang	296.0	207.2	1,243.2	2,486
Selangor	677.4	623.6	3,693.1	7,386
Terengganu	707.0	105.2	287.3	575
Peninsular Malaysia	7,457.1	3,833.4	22,314.1	44,628
Sabah	1,229.3	645.5	3,810.8	7,622
Sarawak	2831.0	1,539.9	9,239.4	18,479
W. P. Labuan	138.0	100.0	323.0	676
Malaysia	11,655.4	6,118.8	35,687.3	71,375

Source: Jabatan Pertanian, Malaysia (2006).

The production value of chempedak in Malaysia is relatively low as compared to Thailand. However, under the Ninth Malaysia Plan, the government is reinforcing the agriculture sector to reduce the food trade deficit and to increase annual growth of the agro-food production sector.

2.1.2 Composition of chempedak

Chempedak fruit weigh from 600 g to 3500 g and is generally smaller than jackfruit. The total edible portion (perianths + seeds) amounted for 25-50% of fresh fruit weight with total weight of all perianths in fresh fruit varies from 100-1200g. Chempedak composition is very similar to jackfruit. According to Nakasone and Paull (1998), the fruit is a good source of carbohydrate and vitamin A and a fair source of protein. The chemical composition of the edible portion of chempedak is shown in Table 2.2.

Table 2.2: Chemical composition value of edible portion of chempedak per 100 g

Chemical composition	Value of edible portion (per 100 g)
Energy	117 kcal
Moisture	66.7 g
Protein	2.5 g
Crude fat	0.4 g
Crude fibre	3.4 g
Ash	1.2 g
Carbohydrate	25.8 g

Source: Hassan (1999).

The total weight of chempedak seeds per fruit range from 65 g to 880 g, with weight of each seed ranging from 1 g to 12 g. The chemical composition of the seed based on dry weight is approximately: protein 10.0-13.0%, fat 0.5-1.5%, fibre 4.0-6.0%, ash 3.0-4.0% and the moisture content is 46.0-78.0% (Jansen, 1991). The comparison of chemical composition in chempedak seed and jackfruit seed are shown in Table 2.3.

Table 2.3: Chemical composition of chempedak seed and jackfruit seed

Chemical composition (% dry weight)	Chempedak seed	Jackfruit seed
Moisture	57.2	63.0
Protein	6.6	4.7
Crude fat	0.6	0.5
Crude fibre	1.4	1.6
Ash	1.4	1.3
Carbohydrate	32.8	28.9

Source: Siong (1985).

Most of the chemical compositions for chempedak and jackfruit seeds are comparable. Thus, these fruits may be exploited in the development of value-added food products.

2.1.3 Utilization of chempedak

The consumption of chempedak flesh and its seed are considered as under-utilized to the consumers. Both chempedak's flesh and its seed are considered as edible as the flesh is usually eaten either raw or cooked such as chempedak fritters as a delicacy, or the flesh is creamed to be used in making jams and cakes. Young chempedak fruits are cooked in coconut milk and eaten as curried vegetable or soup (Thulaja, 2003). Meanwhile, the chempedak seeds are

normally discarded or eaten either roasted or boiled in salty water. The utilization of chempedak seed with its nutritional properties is a new source in food products such as in bakery products, particularly in bread to exert health benefits.

2.2 BREAD MAKING PROCESSES

Bread represents a substantial part of the daily food around the world. Continuous improvement in baking technology and introduction of new materials and ingredients to the bread composition resulted in better quality product which enhance its' nutritional value (Mondal and Datta, 2007).

2.2.1 Introduction

The value-added products in the health food sector are significantly expanding and gaining popularity in Malaysia due to the increase consciousness in health. Various types of high-fibre food products are found in the market. High dietary fibre content of bread and baked products are well accepted by the consumers for its health claim.

However, bread and baked products with high dietary fibre content required new technology to satisfy the quality and palatability of the products. In recent years, baking technology has advanced drastically to meet the preference of consumers needs.

In the modern baking industries, bread making technology evolved significantly to suit the large scale production and increased demand of consumers on high qualities, yet maintaining the cost efficiency for the industry itself (Mondal and Datta, 2007; Giannou *et al.*, 2003).

2.2.2 Major bread making process methodologies

Generally, the process of bread making can be divided into three basic operations i.e. mixing, fermentation (resting and proving) and baking (Sahlström and Bråthen, 1997). Mixing entrains gas cells into the dough; proving inflates these gas cells with CO_2 generated by yeast during fermentation; and baking transforms the foam structure containing discrete bubbles into a

sponge of interconnected gas cells, and sets the structure (Campbell, 2003). However, different processing methods vary in the aforementioned operations and responded differently to diverse ingredient qualities and formulations (Cauvain, 1998b).

The simplest bread making procedure is the straight-dough method whereby all the ingredients in bread formulation are mixed to form developed homogenous dough in one-step (Sahlström and Bråthen, 1997). Dough formation for straight-dough method require low amount of energy during mixing process to produce a suitable bread quality (Cauvain, 1998b). Subsequently, the resting periods of the dough in this method varied depending on the flour quality, yeast level, dough temperature and the specificity in types of bread produced (Mondal and Datta, 2007). A typical white wheat flour protein content used in this bread making procedure is 12% or higher to obtained an optimum dough development. However, addition of non-wheat flour resulted in lower bread quality due to lower flour quality and strength (Cauvain, 1998b).

Sponge and dough method is another type of bread making processes which includes two-stages of mixing process. Leavening agent consists of yeast and certain amount of water and flour are mixed to form homogenous soft dough i.e. the sponge (Mondal and Datta, 2007). The leavening agent is left to develop, depending on flavour requirements and later mixed with the remainder of the ingredients to form homogenous dough (Cauvain, 1998b).

In typical sponge-dough methods, combinations of high-protein and low-protein flours were used to obtain a satisfactory loaf. Stronger gluten bread flours are commonly used in the sponge state, as the sponge is subjected to double mixing and extended fermentation. Meanwhile, in the dough stage, the remainder weaker gluten flour is added to preferment and mixed to obtain optimum dough development (Hareland and Puhr, 1998).

Hareland and Puhr (1998) hypothesized that the adjustment of weaker gluten flour (non-bread flour) used in the dough stage will be made by stronger gluten bread flour used in the sponge stage. However, the differences of crumb firmness were observed attributed by the water-binding capacity of different flour blends.

The invention of mechanical dough development or Chorleywood Bread Process (CBP) from 'no-time dough' method was to achieve optimum dough qualities in an ultrahigh mixer for a few minutes (Mondal and Datta, 2007). The energy expenditures are capable in breaking the disulphide bonds, which modified the protein structure in the dough and thus improved its ability to stretch and retained gas from yeast fermentation in the prover (Cauvain, 2003; Cauvain, 1998b). In the CBP, mixing process carried out under partial vacuum condition gives fewer bubbles in the loaf, resulting in a finer gas cell structure (Campbell, 2003). However, in the CBP method, bakery fat or shortening is an obligatory ingredient in the formulation for production of acceptable final product (Campbell, 2003; Gan *et al.*, 1995).

The CBP method was adopted in modern baking industries to produce similar dough consistency and bread qualities even with lower protein content flour due to mechanical mixing actions (Cauvain, 2003).

2.2.3 Mixing

Mechanical and enzymatic degradation involved during bread making are necessary to eliminate the starchy residual taste of flour (Martínez-Anaya, 1996). Mixing is considered as the critical control point in bread making, which in turn determined the quality of the final product (Campbell, 2003). Mixing is the homogenization of ingredients for uniform dispersion, development of the gluten structure in the dough and incorporation of air bubbles within the dough (Cauvain, 2003; Autio and Laurikainen, 1997). Mixing is a comprehensive series of compressing and stretching (kneading) process of the ingredients (Cauvain, 2003) to impart the necessary work for formation of extensibility and cohesive strength of the dough for subsequent processing (Gan *et al.*, 1995).

During dough mixing, wheat flour is hydrated and starch from flour absorbs almost 46% of total water (Goesaert *et al.*, 2005). As a consequence of the mechanical energy input, distinct masses of gluten proteins were disrupted and transformed into a continuous cohesive

viscoelastic gluten protein network (Keetles *et al.*, 1996). Other ingredient interactions such as lipid, salt, non-starch polysaccharides and starch itself contributes significantly to the formation of gluten matrix for optimum dough development (Giannou *et al.*, 2003).

During mixing, the dough resistance began to increase gradually until optimum level is reached and further mixing decreased the dough resistance, a condition of 'over-mixing' (Goesaert *et al.*, 2005). Over-mixing affects the gluten protein network, which certain disulphide bonds disrupted to form thiol radicals and gluten proteins are partially depolimerized (Giannou *et al.*, 2003), thus increased solubility of proteins and decreased extractability of lipids, which resulted in a sticky dough (Autio and Laurikainen, 1997).

Mixing conditions is highly dependant on the rapid processing, homogeneity and temperature (Giannou *et al.*, 2003), as well as atmospheric conditions (Cauvain, 2003) to form dough with good rheological properties and bread characteristics (Autio and Laurikainen, 1997). Types of mixers are crucial in determining the structure of the final bread product. High-speed mixers with blades shear the dough effectively and produce small bubbles, which results in fine-structured bread, while low-speed mixers, such as spiral-type mixer occlude more air but result in uneven pore size distribution (Autio and Laurikainen, 1997).

2.2.4 Proofing

Proofing is stipulation for dough resting period allows time under favourable conditions to activate the yeast and enzymes in the flour. The purpose of proofing is to produce dough that are sufficiently soft, extensible and relaxed for optimum rheological properties (Giannou *et al.*, 2003). Proofing link the bubbles size distribution created in the mixer to the bubble distribution apparent in the baked loaf, through the dynamics of CO_2 generation by yeast and its mass transfer into gas cells and further coalescence (Campbell, 2003).

Flander *et al.*, (2007) reported that the proofing time is more pronounced in determination of specific volume and firmness of bread than the proofing temperature. Relaxation

time of the dough is one of the important rheological properties which is related to disappearance of free liquid water at certain temperature (Mondal and Datta, 2007).

Proofing mainly attributed to the yeast action regarded as dough maturing or ripening (Giannou *et al.*, 2003). During proofing, starch from the flour progressively converted into dextrins and sugars by enzyme actions (Cauvain, 2003). Proofing process further changes the gluten protein network by becoming less extractable. Gluten protein network of fermenting dough is essential in retaining the CO_2 production during fermentation period as CO_2 production contributes to dough expansion and the initial stages of baking (Goesaert *et al.*, 2005).

The gas phase of a proofing dough exists as a dispersion of discrete gas cells comprising of starch, gluten and other minor constituents (Gan *et al.*, 1995). The proportion of gas retention depends on the development of a suitable gluten matrix within the dough which the expanding gas can be held (Cauvain, 1998b). Hence, gas stabilization and gas retention stimulates the crumb structure and volume of bread (Giannou *et al.*, 2003).

During proofing, the dough expands by a factor of three or four to its almost final volume. However, the dough expansion is restricted by the walls of the tin, which determine the shape and orientation of the cells in the final product (Wiggins, 1998). The growth of gas cells during proofing depends partly on the size of the cells. Greater pressure is needed to expand a small gas cell than the larger cells, while the smallest gas cell presumably will not undergo gas expansion at all (Autio and Laurikainen, 1997).

2.2.5 Baking

During all processing steps of bread making, various complex chemical, biochemical and physical transformation occurs, which affect and are affected by the diverse flour constituents (Goesaert *et al.*, 2005). Baking is the last but the most important stage in bread making procedure. Time and temperature of the baking process determine the quality and shelf life of the bread products. Temperature affects various physicochemical changes, which increase in baking

temperature promotes the formation of protein cross-links to set the loaf during baking (Mondal and Datta, 2007).

Meanwhile, Campbell (2003) stated that baking contributed to additional leavening action and bread dough, which experienced a structural transformation from foam into an open sponge structure, containing a porous interconnected network of fine gas cells separated by thin walls through rupture of starch-protein matrix and gas diffusion (Keetles *et al.*, 1996; Gan *et al.*, 1995). In addition, protein denaturation and starch gelatinization both affect the water diffusion by releasing and absorbing water, hence contributing to transformation from dough to crumb (Mondal and Datta, 2007). Breadcrumb has a porous structure, mainly consisting of open polyhedral cells with very small cells enclosed together thus forming solid elastic sponge material (Keetles *et al.*, 1996).

Simultaneously, several conversion activities take place during baking such as evaporation of water, formation of porous structure, starch gelatinization, protein denaturation, melting of fat crystals, volume expansion, crust formation and browning reaction. In addition, incorporation into the surface of air cells, rupture of gas cells and sometimes fragmentation of cell walls occur during baking process (Mondal and Datta, 2007; Giannou *et al.*, 2003; Autio and Laurikainen, 1997). Baking process alters the physical properties of wheat flour through a series of changing procedure, known as gelatinization (Mondal and Datta, 2007; Primo-Martín *et al.*, 2006) and the flour properties are continuously modified until the structure of final product is achieved (Giannou *et al.*, 2003). Thermal reactions during baking, including caramelization and non-enzymatic browning promote crust flavour and colour (Martínez-Anaya, 1996).

The role of baking is purposely to alter sensory properties of food products, to improve palatability and to extend the range of tastes, aromas and textures in food products from its raw material (Giannou *et al.*, 2003). According to Campbell (2003), baking resulted in structure having a solid outer crust and a soft, delicate crumb comprising of cell walls, which surround the gas cells and determine the mechanical properties of the loaf. The internal and external

appearance, compressibility and fracture mechanics of the loaf are the main indicators in determining its aesthetic appeal, apparent freshness and performance.

2.2.6 Bread ingredients

Advances in bread making technology facilitated new ingredients to enhance the physicochemical attributes of breads. Bread quality is determined by the complex interactions of the raw materials, their qualities and quantities used in the bread formulation and the processing method employed (Cauvain, 2003).

2.2.6.1 Flour

Wheat flour is the most important ingredient in bread formulation, as it is responsible for formation of viscoelastic dough when hydrated with water, is capable of supporting gas cells and retaining gas (Maforimbo *et al.*, 2006; He and Hoseney, 1991). Strong (hard-wheat) flour in which the high protein content ranged from 9% to 15% of dry weight is the basic ingredient for most baked products (Wilde, 2003).

Wheat flour consists of starch, gluten, non-starch poysaccharides, lipids and trace amounts of minerals. Starch, a major component of wheat flour, making up to 80% of wheat flour dry weight, significantly affects the dough rheological properties, particularly the starch gelatinization upon heating in the presence of water. Available water content has been suggested to modify the structural properties of the dough (Angioloni and Rosa, 2004).

Martínez-Anaya (1996) stated that wheat flour contains considerably low amounts of sugar, about 1.55-1.84% (0.19-0.26% sucrose, 0.07-0.10% maltose, 0.01-0.09% glucose, 0.02-0.08% fructose and 1.26-1.31% oligosaccharides (fructosans and maltooligosaccharides)).

Typically, wheat flour contains two types of amylases i.e. α-amylase and β-amylase. Both amylases degrade the wheat starch producing dextrins and maltose sugars. Almost 85% of starch is converted to sugars, ready for transformation by yeast into carbon dioxide (CO_2) and alcohol during dough fermentation (Belderok, 2000).

Most of the lipids contained in wheat flour are surface active, which will compete with proteins to stabilize the gas cells in the dough (Gan *et al.*, 1995). Lipids in the wheat flour are classified as starch lipids and are found as free non-starch lipids (NSL) and bound non-starch lipids (NSL). According to Goesaert *et al.*, (2005), the constituents of the starch lipids are of importance as these lipids exert positive correlation with amylose content, forming amylose-lipid complexes during starch gelatinization. However, during bread making, NSL prominently affected the dough rheological properties through gas cell stabilization and crumb colour (Goesaert *et al.*, 2005).

The unique bread making properties attributed mainly to the water-insoluble gluten proteins in wheat flour to form a cohesive viscoelastic mass when hydrated with water (Dervas *et al.*, 1999). The physical properties of hydrated wheat proteins resulted from covalent (disulfide bonds) and non-covalent interactions (hydrogen, ionic and hydrophobic bonds) of wheat gluten proteins (Robertson *et al.*, 2006). Proteins constitute 8-18% of wheat flour (Oates, 2001) and its content exerted significant effect on the loaf volume (Lai *et al.*, 1989a). Goesaert *et al.* (2005) asserted that the quantity and quality (compositions) of proteins in the wheat flour are the important parameters for bread making performance.

Functional properties of proteins is highly dependant on their solubility to form gels and to stabilize emulsions and foams (Gan *et al.*, 1995). Protein present in wheat flour are classified into four groups i.e. albumins, globulins, gliadins (prolamines) and glutenin (Patient and Ainsworth, 1994). Monomeric gliadins and polymeric glutenins are the main functionally distinct groups of gluten proteins, the determinants for optimal development of dough (Goesaert *et al.*, 2005).

Gluten protein constitute about 85% of wheat flour proteins (Oates, 2001), and their structures and interactions are responsible for the development of the extensibility and elasticity in doughs (Wilde, 2003). Furthermore, the gliadin/glutenin ratio and the quality of glutenin

fractions of the gluten proteins are the main factors in determining the gluten protein quality in bread making (Goesaert *et al.*, 2005). Glutenin polymers form a continuous network that provides strength (resistance to deformation) and elasticity to the dough, while monomeric gliadins act as plasticizers of the glutenin polymeric system, contributing to the viscosity and extensibility of dough (Goesaert *et al.*, 2005; Wieser, 2003). Gluten proteins are water insoluble complex, and the molecular insolubility originated from glutenins formed network structures crosslinked by disulphide bonds involving cysteine residues, which plays an important role in the development and stabilization of gluten (Patient and Ainsworth, 1994; Parker and Ring, 2001).

The functional properties of dough depend greatly on the proteins forming the gluten network. Gluten network holds the carbon dioxide (CO_2) produced by yeast fermentation (Belderok, 2000). Gluten significantly contributes to the gas retention by slowing the gas diffusion through dough phase (Gan *et al.*, 1995). The type of protein being cross-linked appeared to be more important than the cross-links agent or type of cross-linked formed and it is highly correlated to the character of qualitative changes in the final product (Caballero *et al.*, 2007).

Hence, the reduction of bread making potential upon substitution of non-wheat flour into the bread formulation was due to the deterioration of viscoelastic properties. Dilution of gluten structure is the primary rationale of the adverse effect exhibited by the bread products substituted with non-wheat flour due to the weakening effect of foreign proteins on wheat flour dough (Dervas *et al.*, 1999).

According to Oates (2001), the discontinuous gluten network for weak flours is formed by gluten proteins which tend to interact strongly with starch granules through cross-linking and apparently decreased the flow properties of poor quality dough. Thus, the dough structures appeared to be ruptured gluten membranes with many visible open pores (Oates, 2001).

2.2.6.2 Yeast

Yeast's roles in bread making are crucial by acting as a leavening agent, strengthen and developing gluten in dough and contributing to the flavour generation in the bread. *Saccharomyces cerevisiae* is the most common yeast species used in bread making. The suggested amount of yeast for optimum dough rheology and crumb texture is 2% w/w of flour (Mondal and Datta, 2007; Giannou *et al.*, 2003).

The yeast species have a saturated kinetics for hexoses and maltose, and possess α-glucosidase and β-fructosidase (Martínez-Anaya, 1996). Yeast growth process is encouraged by reproduction, provided with optimum conditions i.e. warm water ($30^{o}C$) and nutrients (sugar) (Williams and Pullen, 1998).

Yeast cells metabolize the fermentable sugars (glucose, fructose, sucrose and maltose) under anaerobic conditions producing carbon dioxide, which acts as a leavening agent and enhances dough volume (Giannou *et al.*, 2003). Sugar and warm water were added to the yeast for initiation of fermentation (Mondal and Datta, 2007). The actions of yeast may be simplified as follows:

$$\underset{\text{(Simple sugar)}}{C_6H_{12}O_6} \longrightarrow \underset{\text{(Ethyl alcohol)}}{2C_2H_5OH} + \underset{\text{(Carbon dioxide)}}{2CO_2}$$

According to Martínez-Anaya (1996), the complex alcoholic fermentation processes which predominates in white bread made from commercial yeast. Glucose and fructose are fermented by yeast at similar rate. However, when both sugars are present at similar level, glucose is more preferable and fermented at a faster rate than fructose. Meanwhile, sucrose is hydrolyzed 200 times faster than the other fermented hexoses, and is not detected after mixing process. Fermentation of maltose by yeast occurred at the lowest rate when the levels of monosaccharides are low. However, in bread making, limited oxygenation fermented the glucose and produced carbon dioxide and ethanol molecules at lower energy efficiency (Martínez-Anaya, 1996).

Apart from alcoholic fermentative processes, yeast acta as an insulating agent by preventing surplus rise of breadcrumb and excessive moisture evaporation upon high temperature during baking process (Mondal and Datta, 2007).

Bakers' yeast is available in different forms including compressed, granular, cream, dried pellet, instant, encapsulated and frozen. Commercial active cells of yeast are commonly available as compressed yeast and dried yeast (Belderok, 2000). Compressed yeast comprises 70% of moisture and is highly perishable unless it is refrigerated. Active dried pellet yeast is produced to contain lower moisture levels by extruding compressed cake yeast. Meanwhile, instant yeast contains even lower moisture content from active dried pellet yeast, faster drying process and is produced from more active yeast strains (Giannou *et al.*, 2003).

Active dry yeast has a longer shelf life and easily stored at room temperature. However, upon usage in the bakery, dried yeast need to be hydrated preceding incorporation of other ingredients. Conversely, instant yeast can be incorporated with the flour and other ingredients without prior hydration (Giannou *et al.*, 2003; Williams and Pullen, 1998).

2.2.6.3 Salt

The presence of salt (sodium chloride) primarily contributes to the improvement of bread flavour. According to Angioloni and Rosa (2004), addition of salt at optimum level helps in conditioning the dough by improving its tolerance to mixing process, subsequently producing a more stable and stiff dough by affecting the dough rheological properties.

Salt has an inhibiting effects on the formation of gluten during mixing (Cauvain, 2003) and further restrict the gas expansion by yeast conversion in the dough system (Mondal and Datta, 2007). Hence, salt in bread formulation consequently strengthened the dough through protein interactions, presumably shielding charges on the dough gluten protein network by retaining the CO_2 from the leavening agent (Lombard *et al.*, 2000).

The normal level of salt added in the bread formulation is about 2% of flour weight (Williams and Pullen, 1998). According to Swanson and Penfield (1988), the increase of salt addition level at higher non-wheat flour substitution level marked an increment of loaf volume. However, a higher level of salt addition in the bread formulation affect the yeast activities through osmotic pressure, thus requiring longer proofing time to achieve optimum dough development (Williams and Pullen, 1998) and shifting the flavour profile of bread to saltiness.

Furthermore, salt has been reported to be directly involved with water content in the dough system in lowering the water activity and increasing the energy necessary for chemical and physical reactions. Heat-induced reactions subsequently delay the starch gelatinization and protein coagulation in the dough (Angioloni and Rosa, 2004).

2.2.6.4 Sugar (sucrose)

Sugar, particularly sucrose provides the characteristics of sweetness of the bread. The common practice of sugar level added in the bread is up to 4% of total flour. Sugar normally is used as the fermentable carbohydrate for the yeast during initiation of fermentation (Belderok, 2000).

Later, additional sugar is released for further gas production by the action of enzymes in the flour (Giannou *et al.*, 2003). However, higher levels of sugar may inhibit the yeast activity although it is fermentable (Cauvain, 2003).

Sugar also acts as anti-plasticizers by retarding pasting of native starch or functions as anti-staling ingredients through inhibition of starch recrystallization (Giannou *et al.*, 2003). Addition of sucrose liberates competition for water between starch and sucrose, which consequently alters the swelling of the native starch in the presence of sucrose (Le Meste *et al.*, 2001).

In certain cases, the sugar level was being increased to produce more gas production and to improve the crust colour through the caramelization and Maillard reactions during baking

process (Giannou *et al.*, 2003). Fermentation of sugars by yeast generated a large number of volatile compounds that is responsible for the distinctive characteristics associated with bread flavour (Martínez-Anaya, 1996).

2.2.6.5 Shortening

Shortening is often added to the dough to obtain a softer crumb, improvement in loaf volume and to act as anti-staling effect, which may extend the shelf life of loaf. Shortening is a term used in the baking industries to describe fats, oils and their derivatives to improve the bread quality (Stampfli and Nersten, 1995).

Addition of shortening allows the weaker flour to be used in the formulation by aiding the increment of the dough strength and stability, and gas retention (Stampfli and Nersten, 1995). Hence, by adding shortening in high-fibre breads increased the loaf volume (Autio and Laurikainen, 1997). Conversely, Lai *et al.*, (1989a) reported the elevated amount of shortening stimulated little effect on augmentation of loaf volume with the addition of bran in the bread formulation.

Fat crystals were suggested to induce gas retention of bread doughs. Liquid oil originated from melted solid fat, flows over the inner surface of the gas cells forming hybrid interface comprising the oil layer in addition to the protein and/or polar lipid layer due to the increased in temperature during baking. The oil layer helps in maintaining the continuity of the gas/liquid interface in the dough expansion and hence aids gas retention during oven spring (Gan *et al.*, 1995).

Partial substitution of non-wheat flour into the bread formulation required higher level of shortening due to the disruption of gas cell network in the dough (Williams and Pullen, 1998; Cauvain, 2003). Solid fat facilitates a better stabilization of gas cells in the dough system through its numerous small fat crystals with higher melting point, which increase the baking performance (Autio and Laurikainen, 1997; Gan *et al.*, 1995).

According to Schiraldi and Fessas (2001), the mechanism of anti-staling effect by monoglycerides is due to interaction with gluten. During dough mixing, monoglycerides were trapped by the gluten phase and subsequently released towards the starch gel during baking. Monoglycerides form complexes with leached amylose and/or amylopectin. The complexes result in inhibition of linkages between starch granules and amylopectin recrystallization, hence ultimately retarding starch retrogradation (Autio and Laurikainen, 1997).

Bhattacharya *et al.*, (2002) conferred the significant effect on reducing crumb firmness upon storage by addition of shortening in the waxy wheat flour blends. Stampfli and Nersten (1995) stated that adjuncts of monoglycerides and shortening interact with starch molecules, consequently decreased the starch swelling during baking process. Monoglycerides, shortening and water profoundly decrease the crumb firmness through gluten plasticization, thus reducing the swelling of starch granules (Stampfli and Nersten, 1995) resulting in less crystallization (Goesaert *et al.*, 2005).

2.2.6.6 Improvers

The use of improvers in bread making has been practiced to improve dough handling properties, increase the quality of fresh breads and extend the shelf life of stored bread (Rosell *et al.*, 2001).

Improvers are added to improve dough strength which results in higher loaf volume and better crumb texture. Improvers in the bread formulation may contain one or combination with other ingredients, depending on the functionality of the additive in the bread making (Cauvain, 2003).

Oxidative flour improvers such as ascorbic acid and potassium bromate are widely used in the bakery industry. Oxidative agent shows synergistic effect on cross-linking gluten protein and improves gas retention abilities of the dough (Cauvain, 2003). Hence, the breads resulted in considerable increase in loaf volume and improved crumb softness.

However, the usage of potassium bromate was reported to impair human health. Consequently, its use is almost completely replaced by ascorbic acid (Belderok, 2000). Ascorbic acid enhance the strength, handling and baking properties of dough (Goesaert *et al.*, 2005). The effect of ascorbic acid (a reducing agent) as bread improver in wheat breads is achieved by the enzymic oxidation, which ascorbic acid is converting to dehydro-ascorbic acid (DAA) and becomes an oxidizing agent (Maforimbo *et al.*, 2006).

Reducing agents such as L-cysteine and sodium metabisulfite may be added to weaken the dough structure by reducing the dough resistance to deformation, thus assisting in moulding and shaping without structural changes (Goesaert *et al.*, 2005).

2.2.6.7 Water

Water is essential for the formation of dough and responsible for its fluidity. Water is vital throughout the bread making processes for dissolution of salt and sugar, assisting the dispersion of yeast cell, starch and sucrose hydrolysis, activation of enzymes to form new bonds between the macromolecules in the flour and consequently alters the rheological properties of the dough (Giannou *et al.*, 2003).

The optimum water level is crucial in determining the dough properties and subsequently the final quality of bread. The aqueous phase is necessary for dissolving the soluble flour components and providing the medium for various reactions to take place throughout the bread making process (Gan *et al.*, 1995). Adjustments of water levels during dough mixing were in accordance to the water absorption capacity of the flour to compensate for the changes of the dough consistency (Cauvain, 1998b). Insufficient water addition to the flour resulted in inadequate cohesiveness of the dough, which is unable to sustain mechanical stresses. Conversely, excessive amount of water added to the flour resulted in weak and sticky dough (Masi *et al.*, 2001).

Water acts as a lubricant by occupying the space between the various flour components (Masi *et al.*, 2001). Furthermore, water is an efficient plasticizer in the amorphous regions of starch deliberating the slow changes of water-sorption capacity of the baked gluten. Hence, the effects on crumb plasticity and staling perception prominently extend the bread shelf life (Cesàro and Sussich, 2001).

Thorvalsson and Skjöldebrand (1998) elucidated the good correlation between the increase in loaf volume and the reduction in water content. Water content plays a significant role in browning of the crust and development of bread aromas through the Maillard reaction (Thorvalsson and Skjöldebrand, 1998).

However, studies reported that a higher substitution level of non-wheat flour (composite flour) relatively increased the water uptake of the dough (Lai *et al.*, 1989a). The proportion of damaged starch granules and wheat proteins were altered, resulting in an increase in water absorption by five times than 100% wheat flour (Cauvain and Young, 2003). Higher water content in bread consequently increased the CO_2 bubbles, hence resulted in coarser crumb (Mondal and Datta, 2007). Gan *et al.*, (1995) emphasized that the optimal moisture content should ranged from 35% to 44% of the total dough mass in order to provide effective gas retention, subsequently the crumb structure.

2.2.7 Implementation on partial composite flour substitution in bread

Composite flours have been developed from locally grown non-wheat agricultural products such as rice, cassava, sorghum, barley, soybean, banana and potato flour to stimulate the local economic growth. Composite flours are promoted to reduce the demand and dependability on imported wheat flour (McWatters *et al.*, 2004; Dhingra and Jood, 2001; Nochera and Caldwell, 1992).

Developments in baking technology stimulate various products from wheat flour, such as bread, pastries and other baked products. Even though bread from wheat flour provides a good source of calories and other nutrients, however wheat flour is considered as nutritionally poor

(Dhingra and Jood, 2001). Addition of composite flour into food products is vital in complementing the deficiency of essential nutrients in wheat flour, in particular of essential amino acid, minerals and B-complex vitamins (Muhammad Anjum *et al.*, 2006; Dhingra and Jood, 2001).

Bread is considered as the appropriate vehicle for incorporation of composite flour into the diet. Higher substitution levels of composite flours into the bread formulation imparted improvement in nutritional value of breads with respect to dietary fibre, essential amino acid, essential minerals and subsequent calorie reduction. McWatters *et al.*, (2004) reported the breads substituted partially with cowpea flour exerted higher protein and ash content as compared to 100% of wheat flour bread. Meanwhile, Škrbić and Filipčev (2008) found that wholegrain breads with the addition of sunflower seed contained substantially higher crude fibre and ash contents and significantly lower starch content than white bread with the addition of sunflower seed.

2.2.7.1 Physical characteristics of bread

Nutrient compositions of flour incorporated into the bread formulation are important as indicators of quality and quantity of bread consumed (Musaiger *et al.*, 1988). Partial substitution of composite flour into bread formulation in amounts that are of health benefits can be expected to exert detrimental effects on the physical characteristics in term of loaf yield, loaf volume, texture and flavour (Katina, 2003).

Gluten proteins in the flours are responsible for differences in bread making quality (Hayta and Schofield, 2004). According to Oates (2001), gluten in wheat flour constitutes approximately 80-90% of total wheat protein fractions, which are water insoluble while proteins in non-wheat flour are water-soluble (Goesaert *et al.*, 2005). Exposure of the gluten proteins to the heating conditions, shear and tensile stresses; affect the protein structure such as conformation and molecular size.