LOW HEAT FULL-FAT SOY FLOUR: NUTRITIONAL VALUES, FUNCTIONAL PROPERTIES AND POTENTIAL FOOD APPLICATION

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Pimpamas Panyajiva

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ABSTRACT

The Institute of Food Research and Product Development, Kasetsart University has developed low heat full-fat soy flour using low temperature of roasting. This study aims to evaluate the nutritional values, the antinutritional factors and the functional properties of this low heat full-fat soy flour. In addition, the potential applications of the soy flour in food products were investigated.

The results showed that low heat full-fat soy flour contained 45.6% protein, 19.4% fat, 5.8% moisture, 5.8% ash, 23.5% carbohydrate, 11.2% total dietary fiber and 451 kcal energy content. Trypsin inhibitor activity of low heat full-fat soy flour (34 mg / g) was significantly lower than that of raw soy flour, while phytate content (1,092 mg / 100 g) was not different from raw soy flour. Low heat full-fat soy flour was better in some functional properties than the commercial full-fat soy flour. These include water and oil binding capacity, foaming capacity and stability, emulsifying activity and stability, gelation and nitrogen solubility index. The color of low heat full-fat soy flour was also lighter than the commercial full-fat soy flour, leading to good appearance and less interference when added to foods. Incorporation of low heat full-fat soy flour into food products showed that 25% replacement of wheat flour with low heat full-fat soy flour resulted in a cake doughnut which was acceptable by sensory evaluation. At this level, soy flour helped to increase protein, provide moistness and freshness, and reduce oil uptake (20.5%) in the product. At 30% of low heat full-fat soy flour replacement of wheat flour in butter cake, the product was acceptable by sensory evaluation. At this level, soy flour helped to increase protein without causing any differences in quality of the product. At 10% replacement of pork by low heat full-fat soy flour, the formulated pork patties were acceptable by sensory evaluation. At this level, soy flour also increases protein and moisture content, and markedly decreases fat content in the product. In addition, low heat full-fat soy flour could also be beneficial in increasing yield as well as reducing shrinkage of the patties.

KEY WORDS: LOW HEAT FULL-FAT SOY FLOUR / PROTEIN / FUNCTIONAL PROPERTY / ANTINUTRITIONAL FACTOR / CAKE DOUGHNUT / BUTTER CAKE / PORK PATTIES / SOY PROTEIN

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คุณค่าทางโภชนาการ คุณสมบัติการทำงาน และการนำไปใช้ประโยชน์ในผลิตภัณฑ์อาหารของแป้งถั่วเหลืองไขมันเต็มอุณหภูมิต่ำ (LOW HEAT FULL-FAT SOY FLOUR: NUTRITIONAL VALUES, FUNCTIONAL PROPERTIES AND POTENTIAL FOOD APPLICATION)

พิมพมาส ปัณยาชีวะ 4436580 RANU / M

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บทคัดย่อ

สถาบันค้นคว้าและพัฒนาผลิตภัณฑ์อาหาร มหาวิทยาลัย เกษตรศาสตร์ ได้มีการพัฒนาแป้งถั่วเหลืองไขมันเต็มอุณหภูมิต่ำโดยใช้อุณหภูมิในการอบต่ำ จุดประสงค์ของการศึกษานี้คือ เพื่อศึกษาคุณค่าทางโภชนาการ สารต้านคุณค่าทางโภชนาการ และคุณสมบัติการทำงาน ของแป้งถั่วเหลืองไขมันเต็มนี้ และความสามารถในการนำไปใช้ในผลิตภัณฑ์อาหาร

ผลการทดลองแสดงให้เห็นว่า แป้งถั่วเหลืองไขมันเต็มอุณหภูมิต่ำมีองค์ประกอบของโปรตีน 45.6 เปอร์เซ็นต์ ไขมัน 19.4 เปอร์เซ็นต์ ความชื้น 5.8 เปอร์เซ็นต์ เถ้า 5.8 เปอร์เซ็นต์ คาร์โบไฮเดรต 23.5 เปอร์เซ็นต์ ใยอาหาร 11.2 เปอร์เซ็นต์ และ พลังงาน 451 กิโลแคลอรี นอกจากนี้ ค่าทริปซิน อินฮิบิทเตอร์ของแป้งถั่วเหลืองไขมันเต็มอุณหภูมิต่ำมีค่า 34 มก. / ก. มีปริมาณต่ำกว่าแป้งถั่วเหลืองดิบอย่างมีนัยสำคัญทางสถิติ ส่วนปริมาณไฟเตท (1,092 มก. / 100 ก.) ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับถั่วเหลืองดิบ แป้งถั่วเหลืองไขมันเต็มอุณหภูมิต่ำสามารถแสดงคุณสมบัติการทำงานบางประการได้ดีกว่าแป้งถั่วเหลืองไขมันเต็มในระดับทางการค้า ซึ่งประกอบไปด้วย ความสามารถในการอุ้มน้ำ ความสามารถในการดูดซับไขมัน ความสามารถในการเกิดโฟม ความคงตัวของโฟม ความสามารถในการเกิดอิมัลชัน ความคงตัวของอิมัลชัน ความสามารถในการเกิดเจล และ ดัชนีการละลายของไนโตรเจน สีของแป้งถั่วเหลืองไขมันเต็มอุณหภูมิต่ำมีความอ่อนกว่าแป้งถั่วเหลืองไขมันเต็มในระดับทางการค้า ซึ่งนำไปสู่ลักษณะทางภายนอกที่ดีของผลิตภัณฑ์อาหารและลดการรบกวนของสีเมื่อเติมลงไปในผลิตภัณฑ์อาหาร ในการใช้แป้งถั่วเหลืองไขมันเต็มอุณหภูมิต่ำในผลิตภัณฑ์อาหาร ในโดนัทเค้ก สามารถแทนที่แป้งสาลีด้วยแป้งถั่วเหลืองไขมันเต็มอุณหภูมิต่ำได้ที่ 25 เปอร์เซ็นต์ ซึ่งได้รับการยอมรับในการทดสอบทางประสาทสัมผัส แป้งถั่วเหลืองยังช่วยเพิ่มโปรตีน ให้ความชุ่มชื้น และลดการอมน้ำมันระหว่างการทอดได้ 20.5 เปอร์เซ็นต์ ในเค้กเนยสด สามารถแทนที่แป้งสาลีด้วยแป้งถั่วเหลืองไขมันเต็มอุณหภูมิต่ำได้ที่ 30 เปอร์เซ็นต์ ซึ่งได้รับการยอมรับในการทดสอบทางประสาทสัมผัส แป้งถั่วเหลืองยังช่วยเพิ่มโปรตีน โดยไม่ทำให้เกิดความแตกต่างของคุณภาพ ในแพททตี้ส์ หมู สามารถแทนที่เนื้อหมูด้วยแป้งถั่วเหลืองไขมันเต็มอุณหภูมิต่ำได้ที่ 10 เปอร์เซ็นต์ ซึ่งได้รับการยอมรับในการทดสอบทางประสาทสัมผัส แป้งถั่วเหลืองยังช่วยเพิ่มโปรตีน ความชื้น ลดปริมาณไขมัน เพิ่มผลผลิต อีกทั้งลดการหดตัวระหว่างการทอดด้วย

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CHAPTER I

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill, family Leguminosae, subfamily Papilionoidae] originated in Eastern Asia, probably in north and central China. It is believed that cultivated varieties were introduced into Korea and later into Japan some 2000 years ago. Soybeans have been grown as a food crop in countries of East and South East Asia and constitute to this day an important component of the traditional popular diet in these regions. Although the U.S.A. and Brazil account today for most of the soybean production of the world, the introduction of this crop to Western agriculture is quite recent. Soybean is truly a useful plant. It provides us with cooking oil and valuable protein. It grows in temperate as well as in tropical and sub-tropical locations. Because it is a legume, it requires much less fertilizer than other crops (1).

Soybean is also a significant economic crop of Thailand, because it can be used as a raw material in many kinds of industry. Soybean can be produced at various locations all over the country. From the prediction of the Office of Agricultural of Economics in year 2002/3, the north area can produce soybean 69.66 % of the production area (1,649 m^2), northeast area is 17.31%, central area is 11.73%, and the other area is 1.3 %. Soybean can be cultivated in 3 seasons, the beginning of rainy season, the end of rainy season, and summer. The production capacity of the country is 330,952 tons (224 kg/m^2). Every part of the plant can be used such as leaf, stem, hull (2-4) and seed for application in both of agriculture and industry (5).

Proximate analysis of the whole soybean from Coppock (6) shows that protein (40%) and oil (21%) make up about 60%, the remaining third consisting of nonstarchy carbohydrate, including polysaccharides, starchyose (3.8%), raffinose (1.1%), and sucrose. According to The Concise Thai Food Composition Tables (composition per 100 g edible potion), soybean contains protein 34.2 g, fat 16.7 g, and total carbohydrate 33.9 g (7). Soybean and some soybean products contain a variety of nutrients, but the particular attraction of the soybean is its protein. Soybean proteins are made of an essential amino pattern, maybe one of the best among vegetable protein sources (1). In addition, for many years, researchers have documented the health benefits of soybean protein. The benefits relate to the reduction of cholesterol levels, menopause symptoms, and the reduction of the risk for several chronics diseases, i.e., cancer, heart disease, and osteoporosis (8). However, the nutritional quality of soy products is determined not only by the quantity and bioavailability of amino acids, which make up the protein of such products, but is also markedly affected by the processing conditions which are employed in their manufacture. The most important factor in this regard is the application of some form of heat treatment that serves to inactivate a number of naturally occurring constituents of soybean. These substances which can nevertheless elicit adverse physiological response in animals, and unless destroyed, can detract the full nutritional potential of soy protein. The known and certainly the most studied of these factors are the trypsin inhibitors, which play the key role in the digestion of protein in the animals. Also present in soybean are several other components including phytic acid (phytate) (9).

Increasing in consumer awareness has prompted food manufacturers to develop varieties of soybean protein products. Technological advances have made it possible to have soybean protein accessible in various forms: as whole seeds, soy flours and grits, textured soy protein products, soy concentrate and soy isolate (10). Furthermore, the soybean industry is able to produce a wide variety of products with specific functionality properties to meet the targeted needs required by the food industry. It is important to recognize that certain of these soybean proteins have and perform specific functions in the food products, such as texture forming, gelation, fat and water binding, and emulsification. However, the limits of application of soy protein technology are based on maintaining the traditional specification of the food products (11).

The production of full-fat soy flour occurs at many levels of production capacity in Thailand, such as school level and commercial level (12). At commercial level, the roasting temperature of the process is rather high, which might affect the quality of soy flour resulting in poor functionality properties. Recently, the Institute of Food

Research and Product Development, Kasetsart University has developed low heat full-fat soy flour using low temperature of roasting. Nevertheless, there is no research on the nutritional value, the functional properties and applications of this soy flour in food products.

As a result, this study aims to evaluate the functional properties, nutritional values and antinutritional factors of this low heat full-fat soy flour. In addition, the potential applications of the soy flour in food products to improve the food qualities are also investigated as a guideline for using this soy flour in other foods.

CHAPTER II

OBJECTIVES

2.1 General objective

To evaluate nutritional values, functional properties and antinutritional factors of low heat full-fat soy flour, which is a soy protein product, and to study the possibility of using low heat full-fat soy flour in food products.

2.2 Specific objective:

This study aims to:

2.2.1 Evaluate nutritive values (main nutrients) and antinutritional factors (trypsin inhibitor and phytate) of low heat full-fat soy flour.

2.2.2 Evaluate some functional properties including water/oil binding capacity, foaming capacity/stability, emulsifying capacity/stability, gelation, nitrogen solubility index of low heat full-fat soy flour.

2.2.3 Study the possibility to incorporate low heat full-fat soy flour into two categories of food products (bakery and meat products).

CHAPTER III

LITERATURE REVIEWS

3.1 Physicochemical properties of amino acid and protein (13)

Proteins are complicated macromolecules that form in combination to several hundred elemental units, definition as amino acids, connected together by substituted amide bonds named peptide bonds. Each one of amino acid contains an amino group ($-NH_3$) and a carboxylic group (-COOH) attach to a central carbon called the alpha carbon. A hydrogen atom and an R-group (or side chain) are also attached to the alpha carbon (**Figure 1**). Twenty amino acids have been acknowledged as components of nearly all proteins. These amino acids vary from each other in the nature of the R-group attached to the alpha carbon.

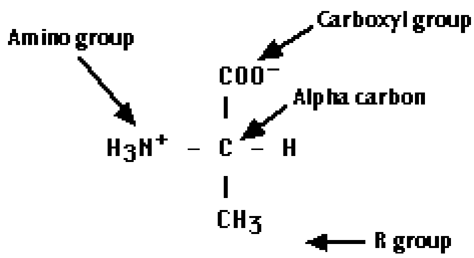


Figure 1 Amino acid (13.)

The primary structure is a simpler linear sequence of amino acid linked by peptide bonds (-CO-NH-), created by the condensable of the amino group of one amino acid and the carboxyl group of another (**Figure 2**(a)). The covalent linkages are also capable created by disulfide bonds (**Figure 2**(b)). The structural of the amino acid chain in a three-dimension configuration is called as the secondary structure. Some models of this structure are the α–helix, β- pleated sheet and the collagen triple helix (**Figure 2**(c)). The structure is become stable by H bonds between the hydrogen of the NH-group and oxygen of the CO-groups (**Figure** 2(d)). In the tertiary structure the helices are twisted in short segments, which are grouped in a specific way to provide globular of fibrous proteins for example lyzozyme. The structure of this protein is seized by hydrogen bonds, salt linkages, hydrophobic interactions (**Figure 3**) and covalent disulfide linkages.

The three structures expressed consist of only one polypeptide chain. When numerous chains act jointly to form a native protein molecule, a quaternary structure is formed, e.g., haemoglobulin (**Figure 4**). The stabilizing bonds are related to those of the tertiary structures. The essential character of these bonds is resulting from the structure and explicit the properties of the amino acid. Proteins for example amino acids, are amphoteric, in the other words they have both a positive and negative charge which be influenced to pH and ionic strength (**Figure 5**). This net charge can provide the interaction of the proteins with other substances.

The R group or side chains also effort as a vital affect on protein behavior. The properties of the side chains can be separated into four groups. Firstly, polar uncharged (hydrophilic) R group that hydrogen able to bond with water as well as are soluble in aqueous solution for example hydroxyl group of serine which are threonine and tyrosine, the sulfidryl group of cysteine and the amide group of asparagine and glutamine. Secondly, non-polar (hydrophobic) R group which are fewer soluble in aqueous solutions such as the amino acids like tryptophan leucine and proline. Thirdly, net positively charged R group, for example the amino group of lysine and the guanidine group of arginine at pH 6-7. Fourthly, the negatively charged R group, for example the dicarboxylic amino acids, aspartic and glutamic acids at neutral pH.

The kinds of bonds, reactive groups, and charge of the proteins can find out their behavior, and the functional properties in a product.

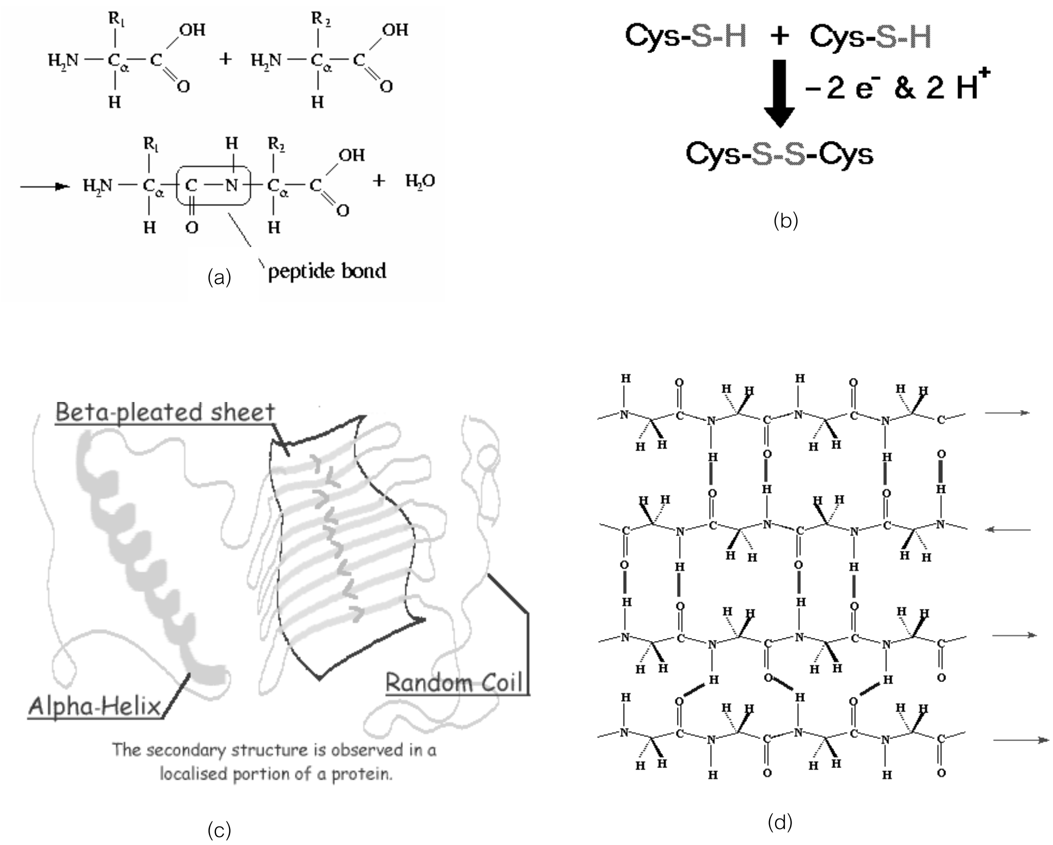


Figure 2 (a) Peptide bond, (b) Disulphide bond, (c) the Secondary structure, (d) H bonds between the hydrogen of the NH-group and oxygen of the CO-groups (13).

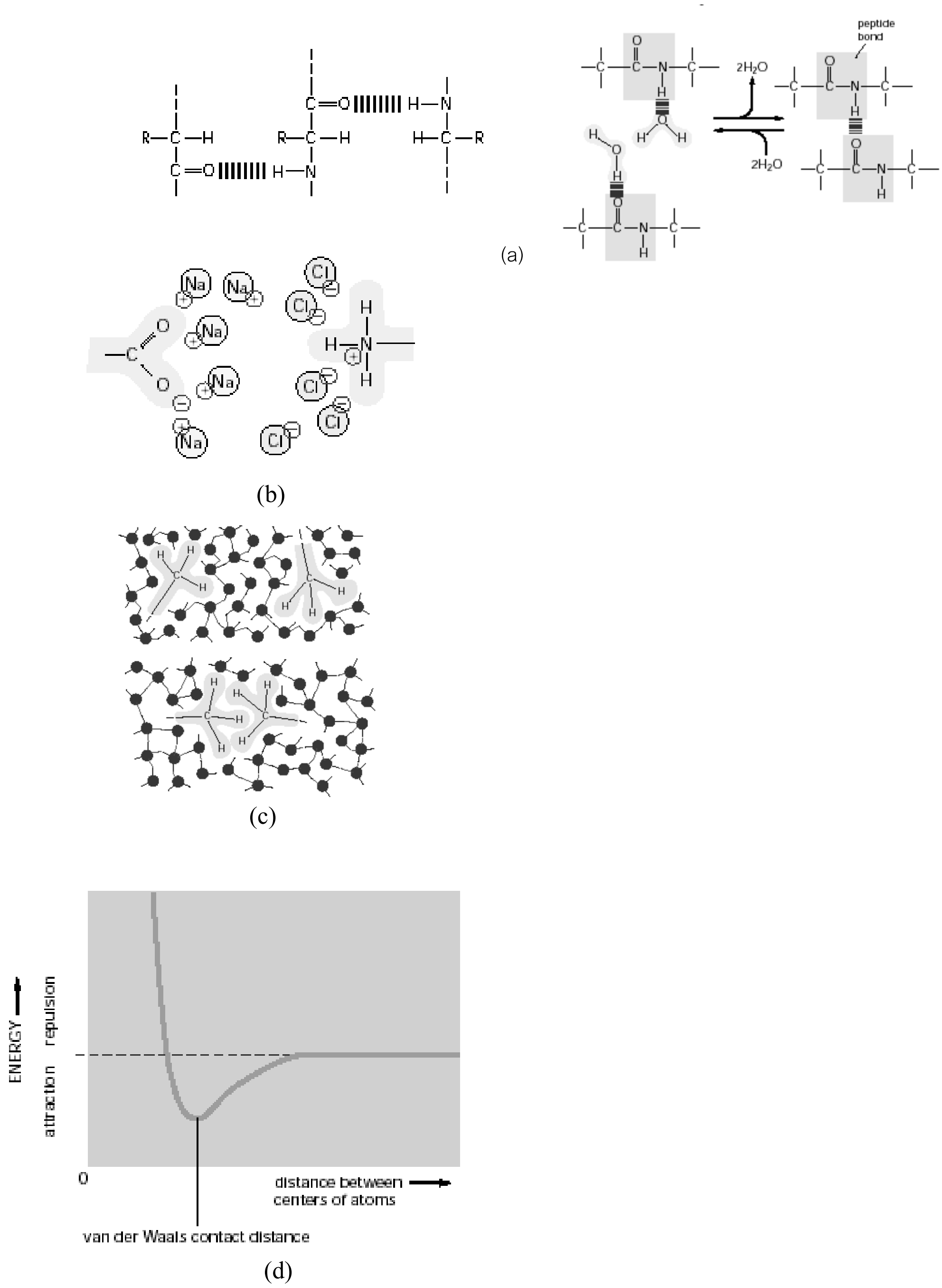


Figure 3 Types of noncovalent bonds which stabilize protein structure: (a) Hydrogen bonds; (b) Salt linkages; (c) Hydrophobic interactions; (d) Van Der Waals interactions (13)

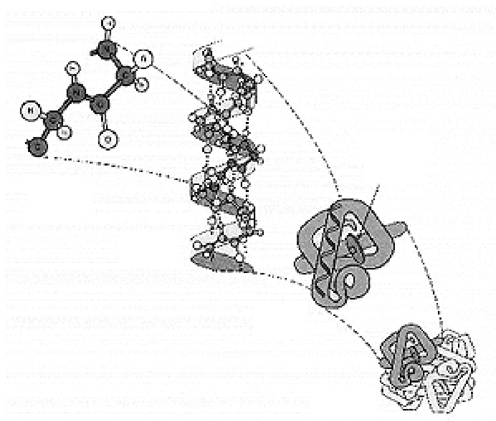


Figure 4 The quaternary structure of protein (Haemoglobulin) (13)

$$\begin{array}{c} {}^{\oplus}NH_3 \\ | \\ R \text{ ---- } C \text{ ---- } COO^{\theta} \\ | \\ H \end{array}$$

Figure 5 Amino acid zwitterion (13)

3.2 Functional properties of proteins (14)

Proteins, as macromolecules, perform important roles in functionality in food and pharmaceuticals, as well as in biological system. Therefore, the growing demand for proteins as important ingredients in formulated food or in pharmaceutical and industrial mixtures has created a necessary for protein with specific and consistent functional properties. Proteins exhibit many functional properties governed by their physiochemical activities in bulk liquid phase (14). The functional properties are also related to the amino acid composition and sequence (primary structure) as well as the spatial configuration of protein molecule and the inter-molecule forces (secondary and tertiary structures)(1).

Basic concept of functionality of proteins in food system has defined functionality as any properties of a food or food ingredient, except its nutritional ones, that influences its utilization, which include the performance of proteins in food systems. The functionality of proteins most prized by the food manufacturer for processing, storage, preparation and formulation of products, old and new. Some significant functional properties of food protein are discussed below (14).

3.2.1 Solubility

Among the functional properties of proteins, solubility is one of primary importance due to its significant influence on the other functional properties of proteins. In general, proteins used for functionality are required to have high solubility, in order to provide good emulsion, foam, gelation and whipping properties.

Protein solubility is, thermodynamically, the protein concentration in the solvent in a single- or two-phase system (protein solution in liquid-liquid phases or in liquid-solid phases) at the equilibrium state. It is a function of many factors (i.e. pH, temperature, pressure, nature and concentration of salts, and concentration of protein). In general, proteins go into solution until a maximum concentration is reached, after which the soluble concentration remain constant and a solid phase appears, as further protein is added the two phases remain at equilibrium under a given experimental condition. Degree of solubility of a protein is scientifically the amount of protein present in liquid phase in relation to amount of protein in liquid and solid phase at equilibrium. Technically, however, protein solubility is an operational parameter determined by retention of protein in the supernatant after centrifugation of the solution for a given time and centrifugal force.

Different terms are used to express protein solubility, such as protein dispersibility index (PDI), water soluble nitrogen (WSN), water soluble protein (WSP), protein solubility index (PSI) and Nitrogen solubility index (NSI). For Nitrogen solubility index, this method determines the dispersible nitrogen under the conditions of test. The original method was developed for soy protein. Percent water soluble nitrogen (%WSN) is calculated and further expressed as nitrogen solubility index (NSI):

$$\%WSN = \frac{\text{Weight of soluble nitrogen}}{\text{Weight of sample}} \times 100$$

$$NSI = \frac{\text{\% Water soluble nitrogen}}{\text{\% Total nitrogen}} \times 100$$

3.2.2 Water and fat binding capacity

Determining the water and fat binding (or holding) capacity of protein-based food is of utmost importance to the food industry, both in marketing exiting products and in developing new products. Water and fat determine not only the acceptability of a given food product (texture, juiciness, mouth feel) but also the profit margin. Since most conventional food products contain more than 50% water and sometimes up to 95% (gelatin desserts), good holding capacity is essential. Consumers tend to avoid products that show ‘free’ water in the package such as in fresh meat trays, yogurt containers, etc. Furthermore, formulations that result in poor water and fat holding capacity (WHC/FHC) directly translate to liquid losses during processing (cooking, freezing, etc.).

WHC is one of the functionalities that has also been used to describe different properties, some of which have been used synonymously in the literature. Term such as water binding, hydration capacity, water retention, water absorption, water imbedding, suction potential and swelling have been used to describe the phenomenon of WHC.

Protein composition and conformation have significant effects on WHC and FHC, and have been reviewed by various researchers. In general terms, water held within a protein structure (gel, powder) can be divided into two categories. The first water bound to the protein molecule, which is no longer available as a solvent; this is referred to by some as the absorbed water. The second is the water trapped within the protein matrix; this can be regarded as the retained water. While binding of the absorbed water is largely dependent on the physicochemical properties (type of amino acid, pH), the retained water is more affected by the structure of the matrix (pore size).

FHC is a protein-based food is governed by the protein's matrix structure (strand size, pore size) as well as the type of fat and its distribution (fat droplet size, inclusion /absence of an emulsifying agent) interactions between the proteins and fat are also vary important since they can affect the gel structure, as well as the distribution and/or emulsification of the fat. In food systems, the fat is usually dispersed within an aqueous protein phase (at least prior to cooking) and its flocculation/creaming is undesirable. Therefore, fat distribution patterns, fat globule size and the presence of an interfacial protein film (coating the fat) or cellular structure (fat cells in meat) are one of major importance in FHC.

3.2.3 Emulsifying properties

One of the primary functional requirements of several food systems is the ability to form emulsions. An emulsion is defined as a dispersion or suspension of two immiscible liquids but the structure of most food emulsions is much more complex. Food emulsions can be products such as margarines and butter, which are water-in-oil (W/O) emulsions, or milk where the water is continuous phase, i.e. an oil-in-water emulsion (W/O).

A variety of tests have been applied to indicate the value of protein in an emulsion, for example, measurement of droplet size, Emulsifying activity (EA), Emulsion capacity (EC), Emulsion stability (ES), and Oil phase volume (OPV). Only EA and ES will be discussed here.

Emulsifying activity (EA) is defined as the difference between the conductivity of the protein solution and the minimum conductivity of the emulsion during homogenization for one minute. The idea of emulsifying activity as an indication of emulsion capacity of protein, the method is the same as for emulsion stability except that no heat is involved.

Emulsion stability (ES) is commonly measured in term of the oil and/or cream separating from an emulsion during a certain period of time at a stated temperature and gravitational field, with is commonly expressed as follows.

$$ES = \frac{\text{Volume final emulsion}}{\text{Volume initial emulsion}} \times 100$$

3.2.4 Gelation

Gelation is one of the most important of food proteins as many foods are made of protein gels, e.g. yogurt, cheese, pudding, sausage, felly, etc. Some traditional orient foods are also based on protein gels, for instance *tofu*, *kamaboko* (fish meat).

In the mass production process in modern food industries strict control of the processing conditions is necessary to maintain the constant high quality of such traditional food. To establish the processing conditions properly, fundamental and practical studies must be made on gelling properties of the food proteins which constitute these traditional foods. Furthermore, there is now an increased demand for new protein gel foods in order to cope with the diverse nutritional needs and tastes of people.

Gel formation can be defined as a protein aggregation phenomenon in which attractive and repulsive forces are so balanced that a well-ordered tertiary network or matrix, capable of holding much water, is formed. If attractive forces predominate, a coagulum is formed and eater is expelled from the gel matrix. If repulsive forces predominate, no network will be formed.

Gelation consists of two steps: (i) conformational change or partial denaturation of protein molecules; and (ii) the following gradual association or aggregation of the individual denatured proteins. The second step should be slow relative to the first one, so that a well-organized gel is formed. The association of denatured protein molecules and the formation of the gel network is based on a good balance of attractive and repulsive chemical forces. The important chemical forces involved in the protein gelation phenomena are hydrophobic interactions, hydrogen bonds, electrostatic inter actions, disulphide bonds, and rheological aspects.

3.2.5 Foaming properties

Foams are being found in a huge variety of diverse applications including the food industry, large-scale construction (e.g. insulation) firefighting. Applications involving protein stabilized of foams are more limited, probably due to their shelf life. Their largest field of application is food industry, where they are widely used in meringues, mousses, a variety of whipped products and beer (14).

3.3 Soy protein (15)

Since the 1960s, soy protein products have been used as nutritional and functional food ingredients in every food category available to the consumer. Soy protein products are an ideal source of some of the essential amino acids used to complement food proteins. At present, soy proteins are more versatile than many other food proteins in various worldwide nutrition programs.

There is strong incentive for using low-cost vegetable sources of protein in the world economy. This has prompted segments of the U.S. food industry to focus on vegetable proteins in food formulations. Soy protein products offer more than just the obvious economic advantages that vegetable proteins have over animal proteins.
Advances in soy ingredient technology have resulted in products that can perform many functions in foods such as emulsification, binding, and texture. Soy protein product acceptance has grown because of such functional properties, abundance, and low cost. The excellent nutritional value of soy protein products has recently been recognized by both the Food and Drug Administration (FDA) and the United States Department of Agriculture's (USDA) School Lunch Program (15).

3.4 Soy protein and health (16)

Soy foods are a source of high-quality protein. In addition, consumption of soy protein provides health benefits that may help prevent or treat certain chronic diseases. Currently, a great deal of research is being conducted to investigate possible health benefits of soy, especially for those who take soy protein daily (16).

3.4.1 Coronary heart disease

Coronary Heart Disease (CHD) is the most common, most frequently reported and the most serious form of CVD. The study of CHD is complicated because there is no single cause. There are a number of nonmodifiable risk factors (inheritance, sex, and age), and modifiable risk factors, including elevated blood lipid levels, hypertension, cigarette smoking, and lack of physical exercise, obesity and diabetes. There may also be unidentified factors, which contribute to CHD. However, reduction of elevated blood LDL- cholesterol levels is given the highest priority in the prevention and treatment of arteriosclerosis and CHD (16).

Studied conducted over the past several years have shown that soy protein is hypocholesterolemic. Many studies have found that adding soy protein to the diet or replacing animal protein in the diet with soy can lower blood cholesterol. The cholesterol-lowering effect of soy has been attributed to isoflavones, a class of phytochemicals found in soybeans. Soy protein drinks that contain naturally occurring high levels of isoflavones reduce total cholesterol and LDL cholesterol in patients who had high cholesterol levels despite consuming a low-fat, heart-healthy diet. Some studies reported vary large decreases in cholesterol in response to soy protein, whereas others reported only minor effects. Several Studies promote the cholesterol-lowering effects of soy protein as a weapon in the fight against coronary heart disease. The incidence of coronary heart disease is lower in nations consuming soy products as a major component of the diet. These findings are particularly good news for consumers with elevated cholesterol level and/or a history of heart disease. Every 1 % reduction in cholesterol values is associated with an approximate 2-3 % reduction in the risk of coronary heart disease (17).

3.4.2 Calorie control

Obesity is a genuine health concern in the minds of an informed populace. Soy protein products can make a significant contribution to weight reduction, mainly by providing essential high quality protein in a concentrated form for specially designed, low-calorie/high nutrient density meals.

3.4.3 Cancer

Soy foods fit the dietary guidelines for reducing cancer risk, and they contain anticarcinogens that may prove to be protective. Epidemiological studies show that populations which consume a typical Asian diet have lower incidences of breast, prostate, and colon cancers than those consuming a Western diet. The Asian diet includes mostly plant foods, including legumes, fruits, and vegetables, and is low in fat. The Japanese have the highest consumption of soy foods. On the other hand, the typical Western diet includes large amounts of animal foods, is lower in fiber and complex carbohydrates, and is high in fat. Soy foods are dietary staples in the Orient, but are not commonly included in the Western diet. Japan has a very low incidence of hormone-dependent cancers. The mortality rate from breast and prostate cancers in Japan is about one-fourth that of the United States. There is evidence that suggests the difference in cancer rates is not due to genetics but rather to diet. Migration studies have shown that when Asians move to the United States and adopt a Western diet, they ultimately have the same cancer incidence as Americans (15).

There is research has suggested that protease inhibitors and acid-two of the nonnutritive compounds in soybeans—contributed to the observed anticarcinogenic effect of consuming soy. Even more promising are recent observations involving phytochemicals, naturally occurring compounds in fruits, vegetables, and legumes, including soybeans. Initial research on phytochemicals indicates that they may play a variety of roles in preventing the development of cancer. Some researchers contend that the large amounts of phytochemicals, such as isoflavones, found in vegetables contribute to reduced incidence of cancer in Asian countries and in vegetarians in Western countries. There are some review the *in vivo* and *in vitro* studies of soy consumption and cancer and concluded that the relatively low rates of colon, breast, and prostate cancer in China and Japan may be attributed to the higher consumption of soy foods in these countries. Since then, much of the research on isoflavones has concentrated on studying the potential health-promoting isoflavones found only in soybeans and soy protein—genistein and daidzein (16).

3.4.4 Menopause symptoms and osteoporosis

The hormonal changes that occur during menopause can cause a variety of symptoms and increase risk for heart disease and osteoporosis. Soy foods, which contain phytoestrogens, are being studied for possible efficacy in decreasing the negative effects of menopause. During peri-menopause, women experience fluctuations in estrogen levels. This can cause uncomfortable symptoms such as hot flashes, night sweats, insomnia, vaginal dryness, or headaches. Changes in estrogen levels have surprisingly wide ranging effects throughout the body. Hormone replacement therapy (HRT) is commonly prescribed to help prevent the negative health effects of menopause. However, many women do not want to take HRT because of the possible increased risk for breast cancer. The evidence is accumulating for several health benefits of soy. Soy contains phytoestrogens in the form of the isoflavones (genistein and daidzein), these are known to have weak estrogenic effects when consumed by animals and humans. Researchers are studying the physiological effects of the isoflavones to find out whether they can serve some of the same functions as physiological estrogens and thereby decrease the health risks associated with menopause. A cross-cultural study of menopause found that women in Japan rarely reported the symptoms of peri-menopause that are common in the West. Post-menopausal Japanese women also have lower rates of osteoporosis and heart disease, and a longer life expectancy. These facts have fueled an interest in research designed to clarify the relationship between soy consumption and health. (15)

Soy protein may play a role in the prevention of osteoporosis, a chronic disease characterized by a loss of normal bone density. Osteoporosis is typically found in women and is related to aging and hormone deficiency. It has been suggested that the high protein content of the Western diet is one of the causative factors. However, studies have indicated that soy protein does not result in an increased loss of calcium in the urine. Additionally, the isoflavones in soy may inhibit the resorption of bone. (16).

3.4.5 Food allergies

Though uncommon, food allergies can have serious consequences. The incidence of true food allergy is about 1% to 2% in adults, and 5% to 8% in young children.

Soy foods often take the place of the more allergenic foods, such as cow's milk and eggs. However, some people are also allergic to soy. Those who are allergic to soy may be able to tolerate some soy foods but not others. It is important for these people to read food labels and familiarize themselves with the ingredients.

Children often outgrow their allergies within a few years. The most common food allergies are to cow's milk, eggs, peanuts, and fish, although any food can be potentially allergenic. The best treatment for food allergy is complete avoidance of the allergenic food

This means that alternative foods must be found to provide the missing nutrients. Identifying alternative foods is especially crucial in the case of young children because they are in a phase of rapid growth and development. Soy-based infant formulas have been used since 1929 to feed infants with cow's milk protein allergies.

Today's soy formulas are equivalent to cow's milk formulas in digestibility, nutrition profile, and acceptability. In healthy infants, soy formulas promote normal growth, nutritional status, and bone mineralization (15).

3.4.6 Diabetes and kidney disease

There is some research evidence that soy foods may help with blood sugar control in diabetics. It is interesting that the use of soy foods for diabetes control was one of the first health benefits noted for soy.

Soy may also help lower risk for some of the complications of diabetes, such as kidney disease. Legumes, especially soybeans, have a very low glycemic index and are valuable foods to include in a diabetic diet.

Regardless of source, the total amount of carbohydrate in the diet needs to be within the patient's recommended limit. Blood sugar control may also be improved by choosing carbohydrates that are high in soluble fiber. Some researchers believe that fiber has no measurable benefit unless it is added to the diet in very large amounts.

Soy fiber is extremely fermentable in humans and therefore may have more physiological benefits than some other types.

Supplemental soy fiber may also help by slowing absorption of sugars. In kidney disease, a soy-based diet may be preferable to the traditional low-protein diet for decreasing renal damage. Soy provides high-quality protein without stimulating hyperfiltration and proteinuria. It may also help prevent kidney damage by lowering serum LDL cholesterol levels.

Cardiovascular disease is two to four times as common in diabetics as in the general population. Therefore it is important for diabetics to follow the standard recommendations for heart health. More research is needed to clarify the possible benefits of soy foods in a diabetic diet (15).

3.5 Antinutritional factors in soy food (18)

In soybean and other legumes, antinutritional factors such as inhibitors of digestives enzymes, hemagglutinin as well as poor digestibility, and secondary metabolite such as phytate, have all been reported to lower nutritional value.

3.5.1 Trypsin inhibitor

Protease inhibiting proteins are widespread in nature, but the trypsin inhibitors of soybeans are the best known and most thoroughly studied. Inhibition of trypsin by raw soybeans has been reported more than 50 years ago and the first soybean trypsin inhibitor was isolated and crystallized in the early forties.

Soybeans contain two types of trypsin inhibitors. Both bear the names of scientists who first isolated and characterized them. They are respectively known as the Kunitz inhibitor with a molecular weight in the range of 20000, and the Bowman-Birk inhibitor which is a much smaller polypeptide in the 8000 dalton range. Both types consist of a number of differentiable proteins. The amino acid sequence and spatial structure of these proteins have been elucidated.

It has been known for a long time that raw soybeans or unheated soybean meal will impair growth when fed to young rats or chicks. This effect is completely eliminated when the soybean component is properly heated. Since trypsin inhibitors are also heat labile, it was concluded that their presence in the diet is responsible for the suppression of growth. In fact, growth is retarded if the inhibitors are added to diets containing heat-treated soybean meal.

A logical explanation for the harmful effect of the inhibitors could be that the inhibition of trypsin in the digestive track of the animal impairs protein digestibility and utilization. There was the study observed that trypsin inhibitor preparations did impair growth when fed with diets containing completely pre-digested proteins. Inhibition of trypsin is not the only physiological effect of the trypsin inhibitors. It has been observed that their ingestion can result in increased pancreatic secretion and hypertrophy of the pancreas. Increased secretion of enzymes into the digestive tube represents an internal loss of protein. Since the proteins excreted by the pancreas are particularly rich in sulphur containing amino acids, this internal loss could be especially important if the diet is marginal in methionine / cystine.

Although the bulk of the available information on soybean trypsin inhibitors biochemical, physiological and nutritional properties stems from experimentation with animals or from *in vitro* investigations, there is no direct evidence as to the physiological effect of the inhibitors on humans. Nevertheless, it has become customary to take the necessary precautions for the removal or inactivation of trypsin inhibitors from soybean products intended for human consumption (1).

3.5.2 Hemagglutinin

Liener discovered a class of bioactive glycoproteins in soybeans, which agglutinate red blood cell, the so-call hemagglutinin or lectins (17). Hemagglutinin are proteins (or glycoproteins) of non-immunoglobin nature capable of specific recognition of and reversible binding to carbohydrate moieties of complex glycoconjugates without altering the covalent structure of any of the recognized glycosyl ligands. Soybean hemagglutinin binds specifically to *N*-acetyl-Dgalactosamine (including its glycosides and oligosaccharides containing a terminal *N*-acetyl-D-galactosamine residue) with greatest affinity and also binds to galactose and its derivatives with lesser affinity. The hemagglutinin is localized in the protein bodies of cotyledon cells in soybean seeds. It is a tetrameric glycoprotein with a molecular weight (MW) of 120 KDa and consists of four identical subunits. Each subunit carries an oligosaccharide chain $Man9(GlcNAc)_2$ and has a MW of 30 kDa.

Hemagglutinin may be detrimental to food animals and poultry since it is known to induce antinutritional effects in laboratory rodents. Most ingested dietary hemagglutinin are able to resist gut proteolysis to varying degrees and bind to their target glycoproteins on the intestinal surface. In particular, soybean hemagglutinin is known to survive enzyme degradation in the gut to a considerable extent. Hemagglutinin that survive intestinal enzyme degradation bind to their target glycoprotein receptors in the small intestine, thereby causing disruption of the brush border membrane. This membrane disruption usually results in antinutritional effects such as loss of brush border enzymes, reduced nutrient digestibility, reduced growth, and occasionally death. Studies investigating the antinutritional effects of soybean lectin have been done mostly with laboratory rats. A number of researchers have observed a significant reduction in feed consumption and growth of rats and mice fed diets containing purified soybean hemagglutinin or raw soybeans.

Among farm animals, the poultry industry is the largest consumer of soybean products as animal feed. Some experiments investigating the antinutritional effect of hemagglutinin have also been done with chickens. These investigators observed that feeding raw conventional soybean meal (SBM) or raw trypsin inhibitor-free SBM to chickens reduced weight gain. In addition, they found that soybean hemagglutinin contributed to the growth-depressing effects observed. In fact, some researchers reported that soybean hemagglutinin accounted for approximately 15% of the growth depression observed for raw soybeans in chicks. Hemagglutinin and trypsin inhibitor are the major antinutrients present in soybeans due their high levels and to the severity of the antinutritional effects they cause (18).

3.5.3 Phytate

Phytate or phytic acid, *myo*-inositol hexakisphosphate, is widely distributed in nature because it is the major storage form of phosphorus in cereals, legumes, and oil seeds. It is typically found in the outer (aleuron) layers of cereal grains and in the endosperm of legumes and oil seeds (19).

As soy proteins replace traditional protein sources in our diet, and as fiber and whole grain products gain popularity, researchers must consider how these changing dietary patterns affect nutrient bioavailability. Of particular interest is the impact of soy consumption on total nutrition, since trace minerals from vegetable proteins are less readily bioavailable for use than those from animal products. At the same time, many current investigators agree that certain factors (e.g. phytate and fiber) interact in such a complex manner that it is difficult to predict the bioavailability of a mineral in a food. For example, the availability of iron from soy flour and soy isolates is higher than that from some other plant foods with lower phytate contents, indicating that phytate may not be a major factor in determining iron bioavailability.

Some investigators have focused on the specific effects of increased soy consumption in human nutrition. They have concluded that, while phytate content appears to inhibit zinc availability, the situation is more complex and may involve other components. The combination of dietary phytate and a high calcium intake may have a greater impact on availability of trace minerals, such as iron and zinc, than phytate in combination with lower dietary calcium levels. Hence, the total diet must be considered in assessing the nutritional significance of phytate content of food and its relationship to mineral availability. In human studies, ingestion of soy concentrate at a level equivalent to about 23 grams of protein a day did not result in any unfavorable trends in calcium, magnesium, zinc, or iron assimilation (16).

3.6 Soy flours and grits

Soy flours and grits are produced by grinding and screening soybean flakes either before or after the oil is removed. Protein contents of these products are between 40 – 54 %. Soy flours and grits are the least refined forms of soy protein ingredients used for human consumption and may vary in particle size distribution, fat content, and degree of protein denaturation. Depending on the type and level of oil present, soy flours and grits are commonly divided into full-fat flours, high fat flour, low-fat flours, defatted flour and lecithinated soy flour (20).

Protein solubility is a measure of the percentage total protein soluble in water under controlled conditions and is a measure of the degree of heat treatment to which the soy flour has been subjected (21). Protein solubility index (PSI) refers to the percentage of total protein that will dissolve in water under standardized conditions. The PSI of raw, unprocessed soy flour is in the range of 80 to100%, and that of toasted soy flour products, 0 to 20%. Between these two extreme a continuous spectrum of PSI value can be made. This is shown in **Figure 6**. Although the PSI value is empirical, nonetheless it can be related to certain functional properties (22).

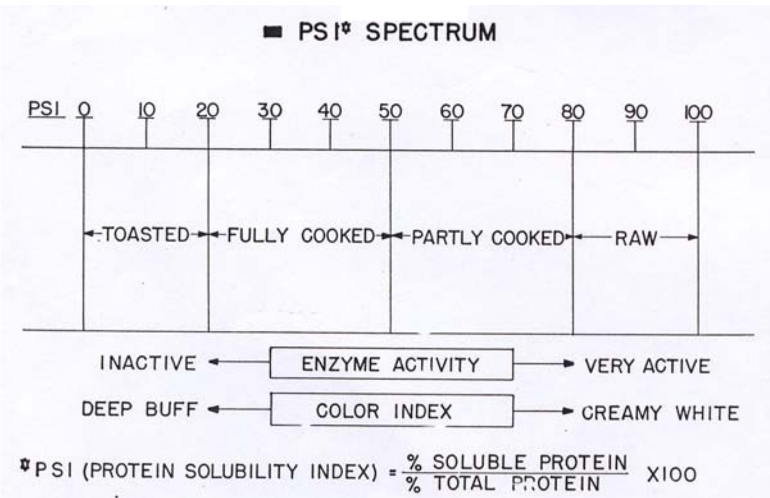


Figure 6 PSI spectrum (22)

Commercial soy flours and grits are further classified according to their Nitrogen Solubility Index (NSI), or their Nitrogen Dispersibility Index (NDI). It will be recalled that these parameters indicate the extent of protein denaturation and hence the intensity of heat treatment which has been applied to the starting material. Flours made from "white flakes" have NSI values of about 80%, while those made from toasted flakes show NSI levels of 10 to 20 %. Other grades are available over of NSI reflects, in fact, a compromise between the need to maintain the functional properties of the soy proteins or some enzyme activity, and the desire to inactivate anti-nutritional factors and eliminate the beany taste, all in function of the end use. In this literature, only full-fat soy flours will be described (1).

3.6.1 Full-fat soy flours

Full-fat soy flours are becoming increasingly important in food. With steadily improving production technology and intensive training in applications, full-fat soy flours are finding wide application as a food or food ingredient. Full-fat soy flours are the least refined soy protein ingredients, containing about 40% protein. They are prepared by grinding dehulled cotyledons to specific size. They are produced primarily in Europe and Asia for baking industry and production of soymilks. There are two types of flours are used; enzyme-active and enzyme-inactive (20).

3.6.1.1 Enzyme-active full-fat soy flour

Enzyme-active full-fat soy flour is prepared without heat treatment and has a high NSI value around 80%. It is used in bakery products (white bread and rolls) mainly for its lipoxidase activity. Lipoxidase catalyses oxidative bleaching of the carotenoid pigments in wheat flour. Enzyme-active soy flour is a valuable "natural flour" bleaching agent, especially where the use of chemical bleaching agents has been prohibited. Lipoxidase activity is also beneficial to the mechanical properties of the dough. Since soybean product is added in relatively small quantities (up to 0.5% on flour basis in bread and buns in the U.S.A) the beany flavor of unheated soybeans is not a limiting factor. Usually, enzyme-active full fat soy flour is not sold as such, but rather in mixture containing other ingredients such as corn flour (1).

3.6.1.2 Enzyme-inactive full-fat soy flour

Enzyme-inactivated (heated) full-fat soy flour, alone or with re-fatted and lecithinated soy flours, is mainly used in the heavier type of cake batters, such as sponge cake. It contributes to the richness of the cake while increasing the proportion of water that can be added to the mix. Due to their oil and phospholipids content, these flours exert egg and shortening sparing effects and act as emulsifiers. In these formulae, soybean flours are used at the level of 3-5%, based on flour weight. Full-fat or lecithinated soy flour with high nitrogen solubility (NSI of 80%) has been found to improve eating quality and reduce fat absorption in doughnuts (1).

3.6.2 Full-fat soy flour produced by the Institute of Food Research and Product Development, Kasetsart University

In this study, low heat full-fat soy flour (60 ºC) from the Institute of Food Research and Product Development, Kasetsart University, was evaluated for its functional properties, the antinutritional factors and the possibility to incorporate into to some food products. The process of soy flour production, the proximate analysis, and the essential amino acid composition of full-fat soy flour are shown below comparing with a commercial product (**Figure 7**, **Table 1**, and **Table 2**, respectively) (23).

Kasetsart University low heat soy flour

Soybean seeds
↓
Removing of defective seeds and other contaminants
↓
Washing with water until clean
↓
Drying in cabinet dryer at 60 °C for 8 hours.
↓
Dried soybean
↓
Removing hulls by a blower
↓
Grinding by Pin Mill
↓
Low heat treated full-fat soy flour
~ 80 – 100 mesh
~ 80 – 90 % yield

A commercial soy flour

Soybean seeds
↓
Removing contaminants by separator
↓
Selected and cleaned soybean
↓
Drying in cabinet dryer at 130 °C for 30 minutes.
↓
Dried soybean
↓
Removing hulls by a blower
↓
Grinding by Pin Mill
↓
Heat treated full-fat soy flour
~ 80 – 100 mesh
~ 80 – 85 % yield

Figure 7 The process of full fat soy flour production (23)

Table 1 Proximate composition of two types of full-fat soy flour (23)

Chemical composition	Full-fat soy flour	
	Low – heat[1]	High – heat[2]
Moisture (%)	6.14	4.86
Fat (%)	25.73	20.39
Protein (%)	45.55	46.96
Ash (%)	5.68	4.33
CHO which include DF (%)	16.9	23.46
Energy (kcal / 100 g)	418	465

[1] Low heat full-fat soy flour from the Institute of Food Research and Product Development, Kasetsart University
[2] A commercial full-fat soy flour

Table 2 Essential amino acid composition of two types of full-fat soy flour (23) and FAO Standard (1)

Essential amino acid	Amino acid (mg / g of protein)		
	Full fat soy flour		FAO
	Low - heat[1]	High - heat[2]	
Isoleucine	36	28	48
Leucine	69	37	65
Lysine	62	39	57
Methionine + Cystine	23 (66)*	13 (37)*	29
Phenylabanine + Tyrosine	83	42	81
Thronine	38	28	42
Tryptophan	15	8	18
Valine	37	30	46

* Limiting amino acid with chemical score in parenthesis

[1] Low heat full-fat soy flour from the Institute of Food Research and Product Development, Kasetsart University

[2] A commercial full-fat soy flour

3.7 Functional properties of soybean protein and food application (20)

Soybeans have been an integral part of the diet of people in the Far East for more than 5000 years. However, the history of soy protein products; flours, concentrate, isolates, and their derivatives, is relatively short. In early years, soy protein products were mainly used to meet nutrition needs, but more recently they have been used primarily for their unique functional characteristics. Today, many thousands of tons of concentrated forms of functional soy protein ingredients and products are used by the food industry, feed manufacturers, and other nonfood, nonfeed industries in a variety of applications.

Soy protein ingredients have become an important protein source, not only for human food, but also for animal feed and industrial applications. Although soy protein is used in animal feed, human feed, human food use and industrial applications have increased steadily over the past several years. Soy protein ingredients are important in food and industrial applications principally because of their functional characteristics.

Functional characteristics are generally dependent on intrinsic physicochemical properties, which are affected greatly by the methods used for their manufacture, storage, handling, and use. It is, therefore, very important to clearly understand the detailed conditions of manufacturing of soy protein ingredients as they affect the composition, structure, and functional properties of the component proteins.

Various manufacturing procedures for the basic soy protein ingredients, i.e., soy flours/grits, soy protein concentrate, soy protein isolates, and textured soy proteins. The functional properties of soy protein products are summarized in **Table 3** (16). Only soy flours and grits are briefly reviewed.

Table 3 Functional properties of soy protein products in food [1] (16)

Functional property	Mode of action	Food system	Preparation used
Solubility	Protein solvation, pH dependent	Beverages	F, C, I, H
Water absorption and binding	Hydrogen-bonding of H_2O, entrapment of H_2O, no drip	Meat, sausages, breads, cakes	F, C
Viscosity	Thickening, H_2O binding	Soups, gravies	F, C, I
Gelation	Protein matrix formation and setting	Meats, curds, cheese	C, I
Cohesion-adhesion	Protein acts as adhesive material	Meats, sausages, baked goods, pasta products	F, C, I
Elasticity	Disulfide links in gels deformable	Meats, bakery	I
Emulsification	Formation and stabilization of fat emulsions	Sausages, bologna, soup, cakes	F, C, I
Fat adsorption	Binding of free fat	Meats, sausages, donuts	F, C, I
Flavor-binding	Adsorption, entrapment, release	Simulated meats, bakery	C, I, H
Foaming	Forms stable films to entrap gas	Whipped toppings, chiffon desserts, angel cakes	I, W, H
Color control	Bleaching of lipoxygenase	Breads	F

[1] Abbreviations: F, C, I, H, W denote soy flour, concentrate, isolate, hydrolyzate and soy whey, respectively.

3.7.1 Application of soy protein ingredients in food products

Using soy protein ingredients successfully in traditional foods depends on formulating products in such a manner that the traditional characteristics of that product are maintained and the antinutritional factors are inactivated. When substitute protein ingredient replaces the original proteins, it is critical that traditional food characteristics and quality not be changed. In new foods, soy ingredients must also contribute to the overall appeal of the product. Protein affects the sensory properties of foods, i.e. the appearance, color, flavor, taste, and texture, which are key attributes determining consumer acceptance. The flavor of soy protein ingredients, and their interaction with both desirable and undesirable flavors, is extremely critical. This determines the application of soy protein ingredients and suggests choices between products and usage level. Table 4 outlines the major food uses of soy protein ingredients in food products (16).

Table 4 Uses of soy protein ingredients in food products(16)

Product	**Soy protein isolate**	**Soy protein concentrate**	**Soy flour (grits)**	**Textured soy protein**
Bakery products				
Milk products	X	X	X	
Bread, rolls			X	
Breads (specialty)	X	X	X	
Cakes, cake mixtures	X	X	X	
Cookies, biscuits, crackers, pancakes, sweet pastry, snacks, etc.	X	X	X	
Doughnuts		X	X	
Pasta products	X	X	X	
Breakfast cereals				
Dairy-type products				
Beverage powders	X	X	X	
Cheeses		X		
Coffee whiteners		X		
Frozen desserts		X		
Whipped toppings	X	X		
Infant formulas	X	X	X	
Milk replacers for young animals		X	X	
Meat food products				
Emulsified meat products				
Bologna, frankfurters	X	X		
Miscellaneous sausage	X	X		
Luncheon loaves	X	X		
Luncheon loaves (canned)	X	X		
Seafoods	X	X		
Coarsely ground meat products				
Chili con carne, sloppy joes	X	X	X	X
Meat balls	X	X	X	X
Patties	X	X	X	X
Pizza toppings	X	X	X	X
School lunch/military	X	X		X
Seafood				
Whole muscle meat				
Analogs	X	X		X
Ham		X		
Meat bits (dried)				X
Poultry breast		X		
Seafood (surimi)	X	X		X
Stews	X	X		X
Miscellaneous applications				
Candies, confection, desserts	X	X	X	
Dietary items	X	X	X	
Asian foods		X		
Pet foods			X	X
Soup mixes, gravies	X	X		X

3.7.1.1 Bakery products

Soy protein ingredients are being used for a variety of functional and nutritional reasons (20).

a. Doughnuts

Soy flour (or soy protein) is added to the mix at level of 4-10% (24, 25). The purpose of adding the soy flour is to prevent fat absorption during the frying operation. It has been shown that as little as a 4% level of addition decreased the fat absorption by 25%. The freshness of the product is increased by the retention of higher moisture in the finished product. The type of soy flour used may be a defatted flour, full-fat flour, or a defatted flour with addition of 5-15% soy lecithin (26).

b. Cakes and cake mixes

By using information of functional property, several researchers have studied the possible of using soy flour in cake type products

Cotton (25) reported that, about seven million pounds soy flour with varying fat level is used in doughnut mixed and cakes. Levinson and Lemancik (26) demonstrated that, in bakery-type products such as pancakes, waffles, and cake, soy flour will absorb about 2-3 times its weight of water and will retain the moisture under baking and other food processing conditions. Not only is a greater product yield obtained, but shelf life and freshness also increased. Pringle (27) explained that the heat processed flour is used widely in cake mixing, thus sparing more expensive ingredients, like egg, fat, and milk. In addition, Dubios and Hoover (21) clarified that, high fat, full fat and lecithinated soy flours are often used in heavier cakes, such as sponge cake and pound cake, because of the increase richness and emulsification function they provide.

3.7.1.2 Meat products

The use of soy protein ingredient in processed meat system is increasing due to changing attitudes of consumers, processor, and regulatory agencies. Soy protein ingredients are used as partial replacements of meat, binders, flavor enhancers, emulsifiers, brine ingredients, and meat analogs and contribute to nutrition, flavor, and critical functional properties. Most of the current meat applications of soy protein ingredients are in the area of processed (comminuted and coarsely ground) meat products. (20).

a. Pork patties

Soy flour, the finely ground soy protein product, has been used in cooked sausage and non-specific loaves (hamburgers, patties, and chili-type products) for several years. Its primary purpose has been to extend meat, and it was used because it was an inexpensive product high in nutritious protein. It was recognized early that soy flour has the advantage of holding both the meat juices and the fat. Its main disadvantage has been its taste and mouthfeel. These factors tended to limit its use.

Of the various types of soy flours available, the toasted products are preferred in meat applications. White soy flours have high lipoxygenase, urease, and trypsin inhibitor activities that can cause problems in emulsion systems. This is specially so in sausages cooked in smokehouse where temperatures are insufficient to destroy these activities (28).

CHAPTER IV

MATERIALS AND METHODS

4.1 Low heat full-fat soy flour

Low heat full-fat soy flour was prepared by drying washed soybean at low temperature (60 ºC) for 8 hours according to the process shown in **Figure 8** (23). The raw material used was soybean seeds supplied by the Institute of Food Research and Product Development, Kasetsart University. The soy flour was prepared in 3 lots. Each lot was collected and evaluated to obtain an average value and standard deviation for all tests.

A commercial full-fat soy flour was purchased from the supermarket in Bangkok, and was tested to compare with the low heat full-fat soy flour.

Soybean seeds

↓

Removing of defective seeds and other contaminants

↓

Washing with water until clean

↓

Drying in cabinet dryer at 60^{o} C for 8 hours

↓

Dried soybean

↓

Removing hulls by a blower

↓

Grinding by Pin Mill

↓

Low heat treated full-fat soy flour
~ 80 – 100 mesh
~ 80 – 90 % yield

Figure 8 The process of low heat full-fat soy flour production (23)

4.2 Chemical analysis of low heat full-fat soy flour

Low heat full-fat soy flour was subjected to chemical analysis to evaluate its main nutrients and antinutritional factors contents.

4.2.1 Main nutrients

a. Crude protein (total N × 6.25) was determined using the Macro-kjeldahl technique (29). (Appendix A)

b. Crude fat was determined by acid hydrolysis and solvent extraction in a soxhlet apparatus (continuous extraction using Soxtec System HT) (29). (Appendix B)

c. Moisture was determined by drying to constant weight in a hot air oven (29). (Appendix C)

d. Ash was determined by incinerating in a muffle furnace (550 °C) until constant weight (29). (Appendix D)

e. Total dietary fiber (TDF) was determined by the enzymatic-gravimetric method (AOAC, 2000) (29). (Appendix E)

f. Carbohydrate content (including dietary fiber) was calculated by subtracting the percentage of crude protein, crude fat, moisture and ash from 100.

g. Energy content was calculated using factors 4, 4, and 9 for the energy provided by each gram of protein, carbohydrate, and fat, respectively.

4.2.1 Antinutritional factor

Low heat full-fat soy flour was focused to evaluate its antinutritional factor which include trypsin inhibitor activity as an indicator of heat treatment and phytate.

a. Trypsin inhibitor activity (TIA) was evaluated in terms of the extent to which trypsin inhibitor in a portion of an aqueous extract of the soy flour inhibited the action of trypsin on benzoyl - DL – arginine - ρ - nitroanalide (BAPA) (30). (APPENDIX F)

b. Phytate was extracted from duplicate test portions of soy flour using dilute HCl. Extract is mixed with EDTA-NaOH solution and placed on ion-exchange column. Phytate is eluted with 0.7 M NaCl solution and wet digested with mixture of concentrated HNO_3-H_2SO_4 to release P, which is measured by calorimetrically. Amount of phytate in original test portion is calculated as hexaphosphate equivalent (29). (APPENDIX G)

4.3 Functional and physical properties evaluation of low heat full-fat soy flour

Low heat full-fat soy flour was subjected to evaluate its functional and physical properties which include water/oil binding capacity, foaming capacity/stability, emulsifying capacity/stability, gelation, nitrogen solubility index, and color.

4.3.1 Water binding capacity (WBC) and oil binding capacity (OBC) were evaluated by the method of Beachet (31). (APPENDIX H)

4.3.2 Foaming capacity and foaming stability were evaluated by the method of Coffman (32). (APPENDIX I)

4.3.3 Emulsifying activity (EA) and emulsifying stability (ES) were evaluated by the method of Yatsumatsu (33). (APPENDIX J)

4.3.4 Gelation was evaluated by the method of Coffman (32). (APPENDIX K)

4.3.5 Nitrogen solubility index (NSI) was evaluated by the method of AOCS (30). (APPENDIX L)

4.3.6 Color was measured by using Spectro Colorimeter (JUKI model JS555, Color Techno System Cooperation, Japan).

4.4 Incorporation of low heat full-fat soy flour into food products

The formula of three kinds of food product, which were cake doughnut, butter cake and pork patties, were prepared from basic cookbook recipes. Low heat full-fat soy flour was used as food ingredient by substituting part of wheat flour in the bakery formulas and substituting pork in case of patties formula. These developed formulas were evaluated for their quality and compared to the control formula (no added soy flour).

4.4.1 Cake doughnut

4.4.1.1 Preparation of cake doughnut

Low heat full-fat soy flour was used to replace wheat flour at a level of 25 % by weight. The ingredients of cake doughnut is shown in **Table 5**

Wheat flour, soy flour, baking powder and baking soda were sifted together (as shown in **Figure 9**). Butter, granulated sugar, salt and an emulsifier were beaten at high speed in a Hobart Kitchen Aid mixture (Model KSM5) (speed 8-9 approximately 2 minutes). Eggs were added one at a time, and the mixture was mixed together at medium speed (speed 5-7 approximately 5 minutes). Wheat flour then milk (milk and vanilla flavor) was added a little at a time until completed, mixed at low speed (speed 1-2 approximately 5 minutes).

The batter was poured into doughnut dropper apparatus. The holder of the apparatus was pressed for 5 seconds each time to drop the dough in a brass kettle containing about 4 inches of palm oil that was preheated to 180 °C. After frying for 1 minute each side, the doughnut was removed from the frying oil and allowed to cool on a paper towel. Three different lots were prepared for each formula then pooled together for the analysis.

Table 5 Cake doughnut formula

Ingredients	Control formula (g)	Soy flour formula (g)
Soy flour (Low heat full-fat soy flour size about 80-100 mesh)	-	125
Wheat flour (Red Lotus cake flour, United Flour Mill Co., Ltd.)	500	375
Granulated sugar (Mitr Phol, Mitr Phol Sugar Co. Ltd.)	185	185
Milk (unsweetened condensed milk) (Carnation, Nestle (Thailand) Ltd.)	165	165
Baking powder (Imperial Baker's Choice, Kim Chua Trading Ltd.)	5	5
Salt (Prung Thip, Nakhornrajsema)	3.3	3.3
Baking soda (McGarrett, Ben&Co.)	3	3
Butter (no salt) (Orchid, The Thai Dairy Industry Co., Ltd.)	100	100
Vanilla flavor (Winner's GreatHill Co., Ltd.)	5	5
Patco-3 (Patco-3, United Flour Mill Co., Ltd.)	1	1
Whole eggs	275	275

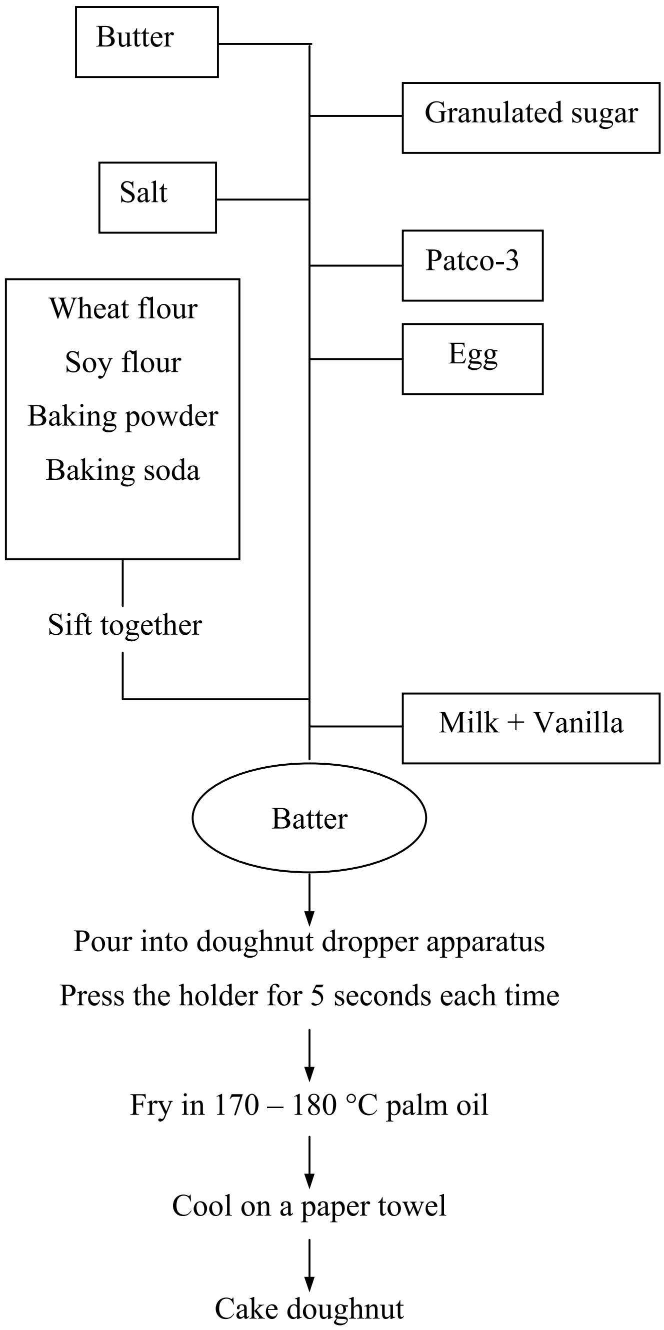


Figure 9 Diagram of doughnut preparation

4.4.1.2 Quality evaluation of cake doughnut

For chemical analysis, the three lots of sample were pooled into one composite sample while each lot was evaluated individually for physical quality.

a. Crude protein (total × 6.25) was determined according to Appendix A.

b. Crude fat was determined according to Appendix B.

c. Moisture was determined according to Appendix C.

d. Oil uptake was determined by method of Pinthus (34) (Appendix M).

e. Texture The firmness of doughnut was measured as the maximum force of resistance to compression by texturometer (Chatillon model DGFIS50, Chatillon Force Measurement, USA) using the compression head. Twelve measurements were carried out for each formula and the mean and SD was calculated.

f. Color was measured by using Spectro Colorimeter (JUKI model JS555, Color Techno System Cooperation, Japan). Measurements were performed for each formula and the mean and SD was calculated.

4.4.2 Butter cake

4.4.2.1 Preparation of butter cake

Low heat full-fat soy flour was used to replace wheat flour at a level of 30 % by weight. The ingredients of butter cake is shown in **Table 6**.

Wheat flour, soy flour, baking powder, and salt were sifted together (the mixture A) (**Figure 10**). Butter, shortening, and sugar (1) were beaten at medium speed in a Hobart Kitchen Aid mixture (Model KSM5) (speed 6 - 7 approximately 2 minuets). Egg yolk were added and the mixtures beaten at high speed (speed 8-9 approximately 3 minutes). Mixture A, and then milk was added a little at a time until completed and mixed at low speed (speed 2-3 approximately 3 minutes). Egg white and cream of tartar were added into dry mixing bowl and beaten for 5 minutes at high speed (until egg white was set). Sugar (2) was added in at high speed (speed 8-9 approximately 2 minutes).

Egg white mixture was poured into flour mixture, whisking softly until well mixed. The mixture was then poured into a baking tray and baked, about 160 – 170 °C until cooked (35 – 45 minuets). Three different lots were prepared for each formula.

Table 6 Butter cake formula

Ingredients	Control formula (g)	Soy flour formula (g)
Soy flour (Low heat full-fat soy flour size about 80-100mesh)	-	45
Wheat flour (Royal Fan cake flour, United Flour Mill Co., Ltd.)	150	105
Baking powder (Imperial Baker's Choice, Kim Chua Trading Ltd.)	1.3	1.3
Butter (no salt) (Orchid, The Thai Dairy Industry Co., Ltd.)	66.7	66.7
Shortening (Olympic Kream Brand, Ketvanit Industral Ltd.)	33.4	33.4
Granulated sugar (1), (2) (Mitr Phol, Mitr Phol Sugar Co. Ltd.)	75	75
Salt (Prung Thip, Prung Thip Co. Ltd.)	1.5	1.5
Egg yolk	42.5	42.5
Egg white	87.5	87.5
Milk (unsweetened condensed milk) (Carnation, Nestle (Thailand) Ltd.)	45	45
Water	45	45
Cream of tartar (Mcgarrett, Spice Product CO. Ltd.)	0.75	0.75
Vanilla flavor (Winner's GreatHill Co., Ltd.)	2	2

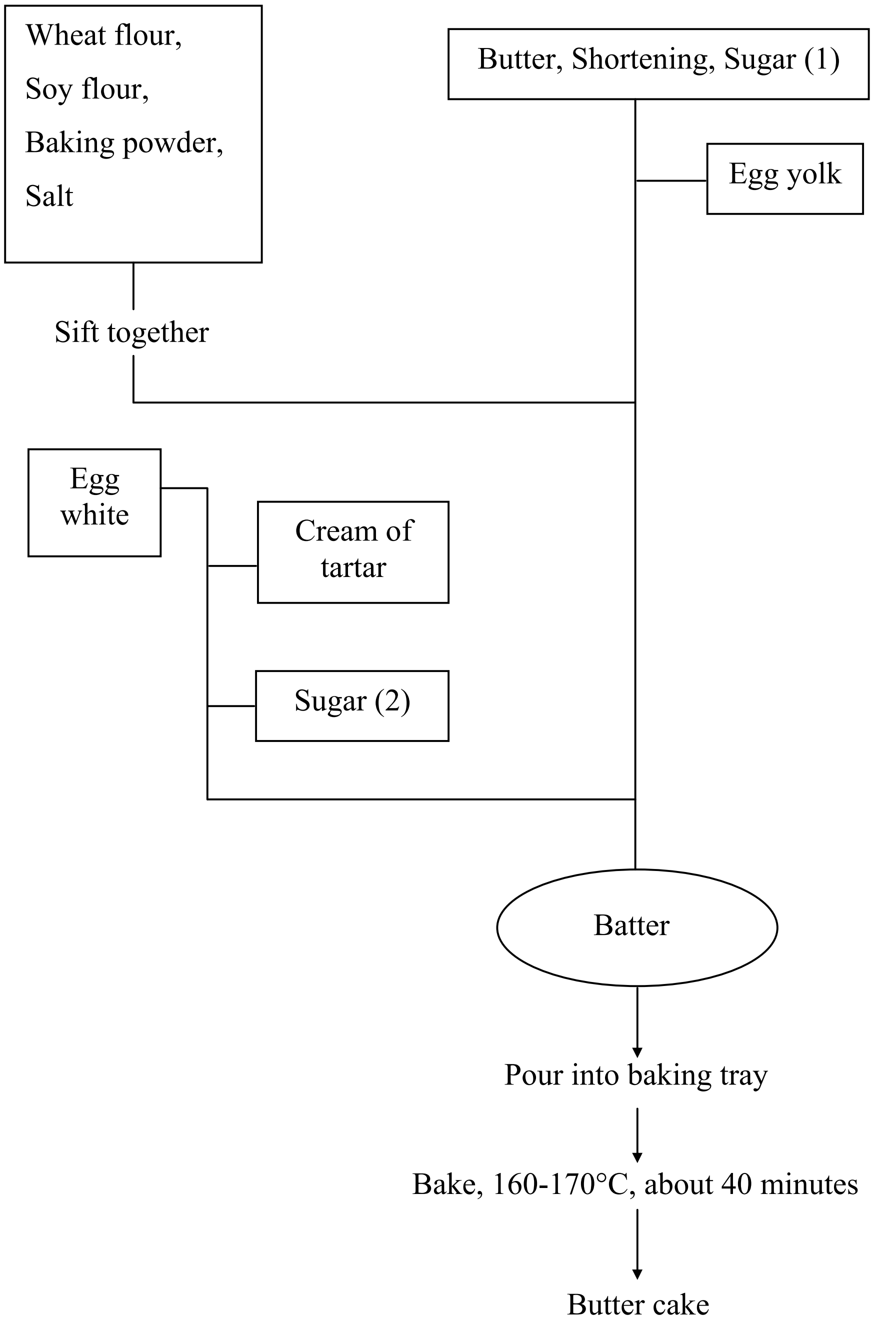


Figure 10 Diagram of butter cake preparation

4.6.3.2 Quality evaluation of butter cake

For chemical analysis, the three lots of sample were pooled into one composite sample while each lot was evaluated individually for physical quality.

a. Crude protein (total × 6.25) was determined according to Appendix A.

b. Crude fat was determined according to Appendix B.

c. Moisture was determined according to Appendix C.

d. Volume was measured by displacement method (35). It was expressed as difference of the volume of low density seeds (sesame seeds) in a container with and without the cake. Three samples of each formula were measured.

e. Texture The firmness of doughnut was measured as the maximum force of resistance to compression by texturometer (Chatillon model DGFIS50, Chatillon Force Measurement, USA) using the compression head. Twelve measurements were carried out for each formula and the mean and SD of firmness was calculated.

f. Color was measured by using Spectro Colorimeter (JUKI model JS555, Color Techno System Cooperation, Japan). Measurements were performed for each formula and the mean and SD was calculated.

4.4.3 Pork patties

4.4.3.1 Preparation of pork patties

Low heat full-fat soy flour was used to replace the sirloin pork at the level of 10 % by weight. The ingredients of pork patties are shown in **Table 7**

Pork patties were prepared by adding 10 % (w/w) of soy flour to meat using a Hobart Kitchen Aid mixture (Model KSM5) equipped with a cake paddle. The meat was mixed with the bread for 3 minutes, soy flour was added and mixing continued for 2 minutes. Seasoning sauce, soy sauce and sugar were added, and the meat was kneaded until mixed well. Celery, coriander root, garlic, pepper were added, and then kneaded until well mixed.

Pork patties (about 10 g) were formed by making into a ball and compressed the meat into a round shape, 1 cm thick and having a diameter of about 4 cm. The patties were cooked on a greased Teflon-coated pan until inside temperature reached 60 °C, which took about 5 minutes each. The diagram of pork patties preparation is shown in **Figure 11**. Three different lots were prepared for each formula.

Table 7 Pork patties formula

Ingredients	Control formula (g)	Soy flour formula (g)
Soy flour (Low heat full-fat soy flour size about 80-100mesh)	-	30
Minced sirloin pork	300	270
Sugar (Mitr Phol, Mitr Phol Sugar Co. Ltd.)	3	3
Soy sauce (Yan Wal Yun, Yan Wal Yun Co. Ltd.)	7.5	7.5
Seasoning sauce (Golden Mountain, Thai Theppa Rot Co. Ltd.)	13.5	13.5
Small pieces slice bread, soak in water, and squeeze until almost dry (Farmhouse, Thai President Bakery Co. Ltd.)	30	30
Pepper	3	3
Finely chopped celery	6	6
Finely chopped coriander roots	10	10
Finely chopped garlic	10	10

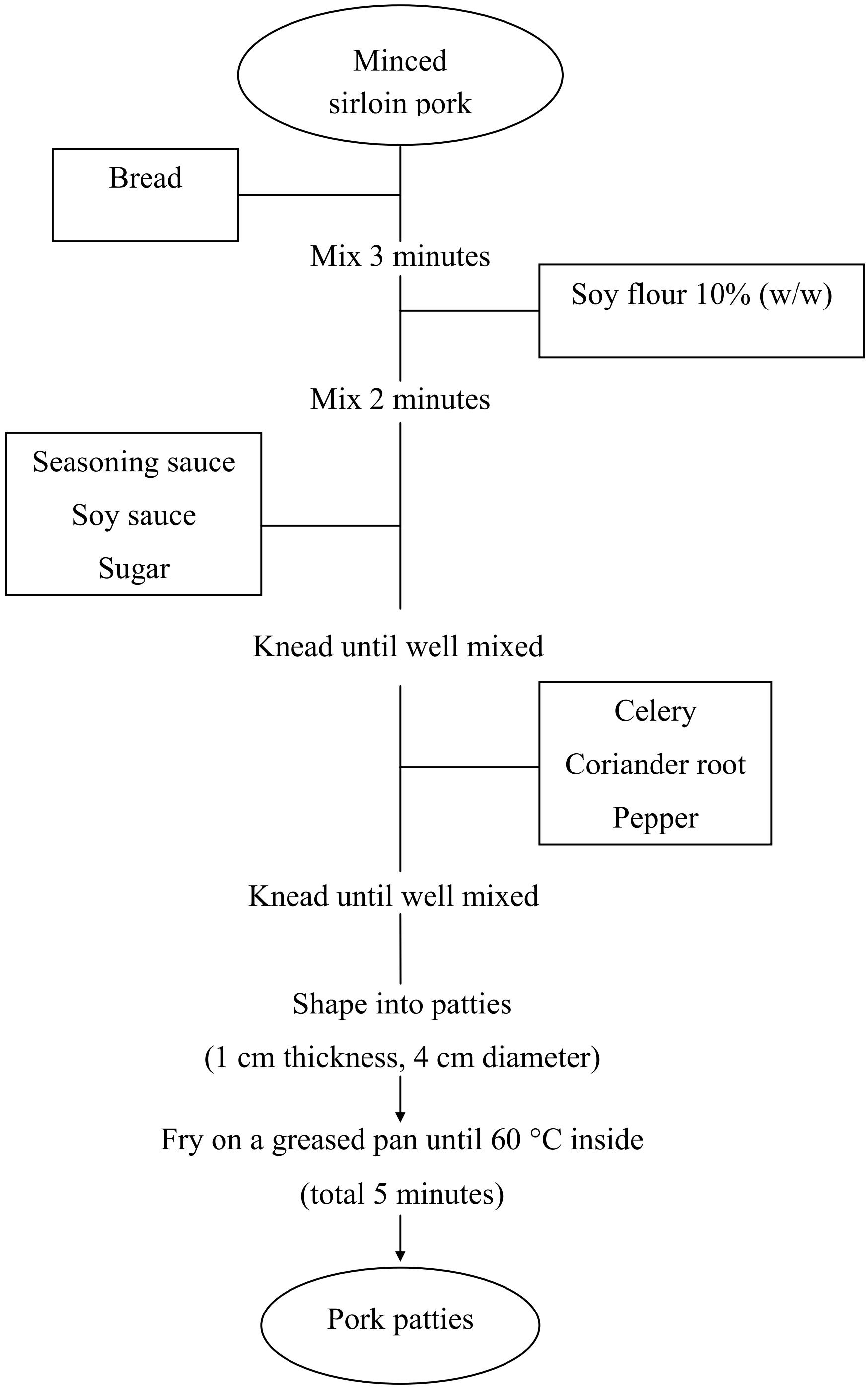


Figure 11 Diagram of pork patties preparation

4.6.4.2 Quality evaluation of pork patties

For chemical analysis, the three lots of sample were pooled into one composite sample while each lot was evaluated individually for physical quality.

a. Crude protein (total × 6.25) was determined according to Appendix A.

b. Crude fat was determined according to Appendix B.

c. Moisture was determined according to Appendix C.

d. Texture The firmness of doughnut was measured as the maximum force of resistance to compression by texturometer (Chatillon model DGFIS50, Chatillon Force Measurement, USA) using the compression head. Twelve measurements were carried out for each formula and the mean and SD was calculated.

e. Technological yield, yield of cooked pork patties was measured as percentage of weight retained after cooking. Yield was given as:

$$Y = \frac{\text{Weight after cooking (g)}}{\text{Weight before cooking (g)}} \times 100$$

f. Shrinkage was measured as difference of diameter and thickness before and after cooking in centimeters.

4.5 Sensory evaluation of low heat full-fat soy flour enriched products

4.5.1 Part I: Preliminary trial

The purpose of this trial was to identity an optimum amount of soy flour to be used in food products. The sensory test was preformed in testing boot at the Sensory Science Laboratory, Food Science and Technology Section, the Institute of Nutrition, Mahidol University, under daylight fluorescent bulb, air-conditioned and free from cooking odors and noise. The panelists were not allowed to communicate with each other. Twenty-five subjects who were recruited from staff and graduate students of the Institute of Nutrition, Mahidol University were in the panelist.

Acceptability of different characteristics including appearance, shape, color, flavor, texture, and overall acceptability of cake doughnut and butter cake were determined using nine-point hedonic scale (9 = like extremely, 5 = neither like nor dislike, and 1 = dislike extremely) (36). The questionnaire used in the sensory evaluation is shown in APPENDIX N (cake doughnut) and APPENDIX O (butter cake).

Acceptability of different characteristics including appearance and overall acceptability of pork patties was determined using nine-point hedonic scale (9 = like extremely, 5 = neither like nor dislike, and 1 = dislike extremely) (36). Color, flavor and texture were determined using five-point just-about-right scale (5 = much too strong, 3 = just about right, and 1 = much too weak) (37). The questionnaire used in the sensory evaluation is shown in APPENDIX P.

The samples were prepared on the day before the evaluation, sliced and sealed in the plastic bag, stored at room temperature for cake doughnut and butter cake, for pork patties, the samples were stored in the refrigerator (5 – 7 °C). All samples were served on white melamine plates, labeled with three-digit number code selected from a random number table. Five samples were served to each panelist during the test, however only one sample was served at a time. The order of sample presentation for a panelist was randomized. Panelist was asked to rinse his/her mouth with water for cake doughnut and butter cake and drinking soda for pork patties between samples.

The results from this part of the study were applied as guidelines in developing the final soy flour-enriched food product formulas.

Control formulas obtained from the conventional recipes were tested for sensory acceptability. The formulas which were accepted by the consumers would be selected as control formulas. Mean values of the sensory acceptability scores of each product were evaluated for significant difference at $p \leq 0.05$. A sample with an overall acceptability score above 5 would be selected for further study.

Acceptability of food products developed by modifying some ingredients in the control formula was evaluated. The samples of food products with varying amount of soy flour were tested. The experimental design and all testing techniques were similar to the acceptability test of the control formula. A sample with an overall acceptability score above 5 would be selected for further study in **4.5.2**.

The results of the sensory test would provide the formula containing maximum amount of soy flour which can be accepted by panelists.

4.5.2 Part II: In-house consumer test

In this part, sensory evaluation was conducted on food products made from the selected recipes of control formula and soy flour-enriched food product formulas in **4.5.1**. The evaluation was performed by fifty panelists who were recruited from staff and graduate students of the Institute of Nutrition, Mahidol University. The experimental design and all testing techniques were similar to the acceptability test which was performed in **4.5.1**.

4.6 Statistics

All experiments were repeated at least 3 times with duplicate measurements. Statistical program, SPSS^{TM} software for Windows version 12.0 was used to analyze data. Treatment effects were tested by analysis of variance. Significant differences between means were identified by the least significant difference procedures. In case that data were not normally distributed, Kruskal Wallis Test was employed to test treatment effect and Mann-Whitney U Test was used to test the difference between two treatment effects. Significant level was set at $p \leq 0.05$.

CHAPTER V

RESULTS

5.1 Chemical analysis of low heat full-fat soy flour

Low heat full-fat soy flour was evaluated for main nutrients and antinutritional factors.

5.1.1 Main nutrients

Low heat full-fat soy flour (LHFF) was analyzed for main nutrients and compared with a commercial full-fat soy flour. The results of the analysis are shown in **Table 8**. Low heat full-fat soy flour contains more protein than the commercial full-fat soy flour and was lower in fat and energy content.

Table 8 Proximate composition of low heat full-fat soy flour (per 100 g wet basis) compared with a commercial soy flour

Type of flour	Energy (kcal)	Moisture (g)	Crude protein (g)	Crude fat (g)	CHO (including DF) (g)	Total dietary fiber (g)	Ash (g)
LHFF soy flour	451	5.8	45.6	19.4	23.5	11.2	5.8
Commercial soy flour	474	2.8	43.8	22.5	24.0	14.6	5.9

5.1.2 Antinutritional factor

Trypsin inhibitor activity and phytate content of low heat full-fat soy flour are reported in **Table 9**. These data showed that low heat full-fat soy flour contained less trypsin inhibitor activity (34 TIU/ mg) than raw soy flour, but there was no difference in the phytate content.

Table 9 Antinutritional factor content of low heat full-fat soy flour [1, 2]

Type of flour	Trypsin inhibitor activity (TIU / mg) [3]	Phytate (mg /100 g sample)
LHFF soy flour	34 ± 2.78 [a]	1,092 ± 51[a]
Raw soy flour	65 ± 2.63 [b]	1,161 ± 118[a]

[1] Mean ± standard deviation of at least three replicate determinations of three independent samples
[2] Means within the same column having the same superscripts are not significantly different ($p > 0.05$)
[3] TIU/ mg = Trypsin inhibitor unit / mg sample

5.2 Functional and physical properties evaluation of low heat full-fat soy flour

Low heat full-fat soy flour was evaluated for its functional and physical properties which include water/oil binding capacity, foaming capacity/stability, emulsifying capacity/stability, gelation, nitrogen solubility index, and color. The properties were compared with those of a commercial soy flour.

5.2.1 Water binding capacity (WBC) and oil binding capacity (OBC)

The results of WBC and OBC are shown in **Table 10** as g of water or oil retained. Low heat full-fat soy flour hold less amount of water than the commercial full-fat soy flour and no difference was found in oil binding capacity. Both flours tended to hold less oil than water.

Table 10 Water and oil binding capacity of low heat full-fat soy flour compared with a commercial full-fat soy flour [1,2]

Type of flour	Water binding capacity (g water / g soy flour)	Oil binding capacity (g oil / g soy flour)
LHFF soy flour	3.24 ± 0.28^{a}	2.94 ± 0.04^{a}
Commercial soy flour	4.12 ± 0.06^{b}	2.94 ± 0.02^{a}

[1] Mean ± standard deviation of at least three replicate determinations of three independent samples

[2] Means within the same column having the same superscripts are not significantly different ($p > 0.05$)

5.2.2 Foaming capacity and foaming stability

Foaming capacity was expressed as the percentage increase in foam volume measured immediately after blending. Foam stability was determined according to residual foam volume at specific time after blending. **Figure 12** shows that foaming capacity of low heat full-fat soy flour was much higher than the commercial full-fat soy flour.

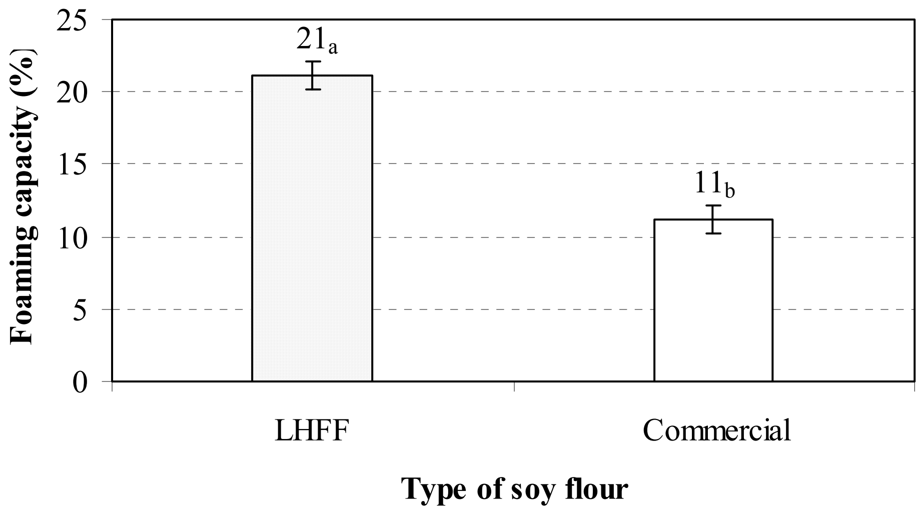


Figure 12 Foaming capacity of low heat full-fat soy flour compared with a commercial full-fat soy flour [1, 2]

[1] Results are means of triplicate determinations of three independent samples

[2] Different latter shows significant difference at $p \leq 0.05$

Foam destabilization for two kinds of full-fat soy flour was followed by measuring the decreasing in the volume of proteins as shown in **Figure 13**. Low heat full-fat soy flour exhibited better of foam stabilization than the commercial full-fat soy flour.

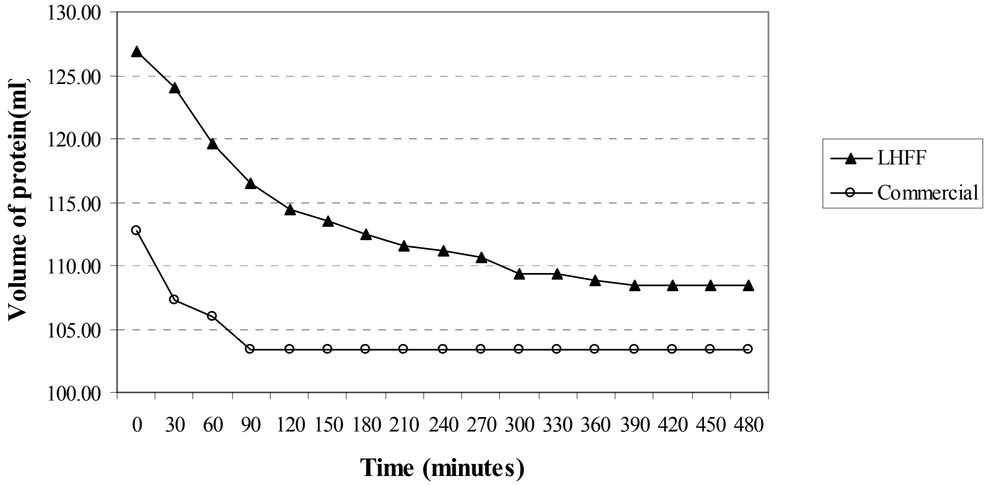


Figure 13 Foam destabilization low heat full-fat soy flour and commercial full-fat soy flour [1]

[1] Results are means of triplicate determinations of three independent samples

For foaming stability at 480 minutes (8 hours), the results exhibited the same tendency as foaming capacity. Low heat full-fat soy flour gave better foaming stability property than the commercial full-fat soy flour as shows in **Figure 14**.

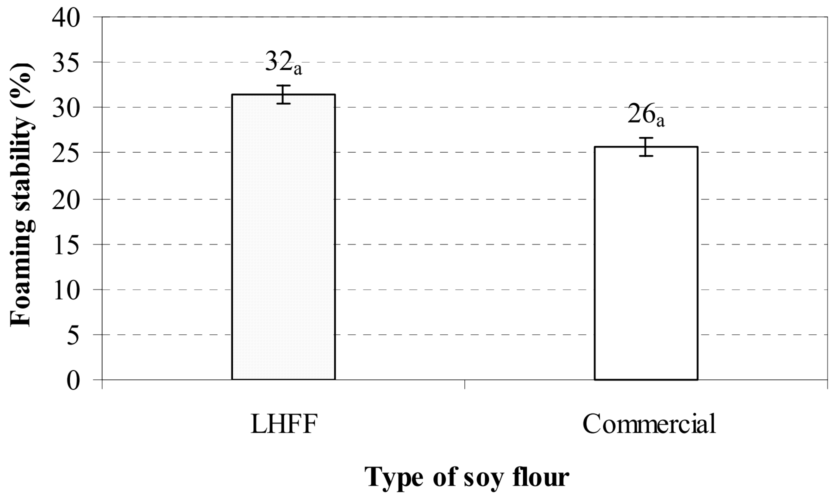


Figure 14 Foaming stability after 480 minutes of low heat and commercial full-fat soy flour [1, 2]

[1] Results are means of triplicate determinations of three independent samples
[2] Different subscript shows significant difference at $p \leq 0.05$

5.2.3 Emulsifying activity (EA) and emulsifying stability (ES)

Emulsifying activity was expressed as percentage of the emulsified layer volume of the entire layer in the centrifuge tube. For emulsion stability, this functional property was expressed as percentage of the remaining emulsified layer of the original emulsion volume. Low heat full-fat soy flour presented higher emulsifying activity than the commercial full-fat soy flour (**Table 11**). There was also significantly higher emulsifying stability with low heat full-fat soy flour whereas the commercial soy flour showed lower emulsifying stability.

Table 11 Emulsifying activity and stability of low heat full-fat soy flour compared with the commercial soy flour [1, 2]

Type of flour	Emulsifying activity (%)	Emulsifying stability (%)
LHFF soy flour	51.12 ± 0.49 [a]	56.35 ± 2.68 [a]
Commercial soy flour	44.64 ± 3.57 [b]	19.64 ± 6.84 [b]

[1] Mean ± standard deviation of at least three replicate determinations of three independent samples

[2] Means within the same column having the same superscripts are not significantly different ($p > 0.05$)

5.2.4 Gelation

The least protein concentration (w/v) which could form gel for low heat full-fat soy flour was 8 % which was slightly lower than the commercial full-fat soy flour (10 %). A complete gelation of low heat full-fat soy flour started at 16 %, and 18 % for the commercial full-fat soy flour (**Table 12**).

Table 12 Gelation of low heat and commercial full-fat soy flour [1, 2]

Protein concentration (%)	Low heat full-fat soy flour	A commercial soy flour
2	−	−
4	−	−
6	−	−
8	+ −	−
10	+ −	+ −
12	+ −	+ −
14	+ + −	+ −
16	+ + +	+ + −
18	+ + +	+ + +
20	+ + +	+ + +

[1] Results from triplicate analyses of three independent samples

[2]

Gelling		Gel structure
−	No gelling	Liquid
+ −	Some floccules	Pourable
+ + −	Almost homogenous gel	Gel remains fixed on turning the tube upside down
+ + +	Complete gelation	Gel remains fixed on shaking the tube upside down

5.2.5 Nitrogen solubility index (NSI)

The difference in nitrogen solubility index of low heat and commercial is shown in **Table 13**. Low heat full-fat soy flour exhibited much higher nitrogen solubility index (11.55 %) than the commercial full-fat soy flour (2.31 %) at the neutral pH (6.5-6.6).

Table 13 Nitrogen solubility index of low heat and commercial full-fat soy flour [1, 2]

Type of flour	Nitrogen solubility index (pH 6.5-6.6) (%)
LHFF soy flour	11.55 ± 1.28 [a]
Commercial soy flour	2.31 ± 0.00 [b]

[1] Mean ± standard deviation of at least three replicate determinations of three independent samples

[2] Means within the same column having the same superscripts are not significantly different ($p > 0.05$)

5.2.6 Color

The color of low heat full-fat soy flour is presented in **Table 14**. The data shows that low heat full-fat soy flour was slightly lighter in color than the commercial full-fat soy flour. The image of low heat full-fat soy flour is shown in **Figure 15**.

Table 14 Color of low heat full-fat soy flour[1]

Type of flour	L[2]	a[2]	b[2]
Commercial soy flour	86.95±0.26	0.31±0.09	24.8±0.13
LHFF soy flour	89.27±0.81	-1.32±0.64	23.52±3.40

[1] Mean ± standard deviation of at least three replicate determinations of three independent samples

[2] L: ↑ 100 bright
↓ 0 dark
a: ↑ + red
↓ - green
b: ↑ + yellow
↓ - blue

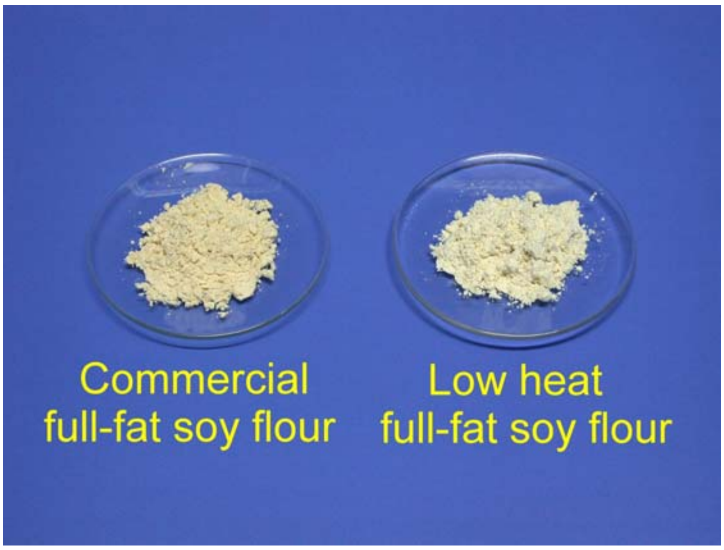


Figure 15 A commercial full-fat soy flour and low heat full-fat soy flour

5.3 Incorporation of low heat full-fat soy flour into food products

Low heat full-fat soy flour was used as food ingredient by substituting part of wheat flour in the bakery formulas whereas substituting pork in case of patties formula. The study for each product was divided into two steps; 1) selection of suitable level of low heat full-fat soy flour to be incorporate in food products, 2) evaluation for their sensory, chemical and physical properties and compared to the control formula (no soy flour added).

5.3.1 Sensory evaluation

5.3.1.1 Cake doughnut

In the preliminary study, 10%, 20% and 30% were selected as the level of low heat full-fat soy flour to incorporate into cake doughnut compared with the control formula. The results are shown in **Table 15**. The acceptability scores from before tasting part (general appearance, color and shape) were not significantly different in all tests. The overall acceptability score of the control formula was 6.60 which was between “like some little” and “like moderately”.

Nevertheless the range of adding low heat full-fat soy flour was rather wide (20% - 30%), so an additional trial was conducted to define a suitable level of replacement. Therefore, 20%, 22.5%, 25% and 27.5% were the level of low heat full-fat soy flour selected to incorporate into cake doughnut. The results are shown in **Table 16**. The overall acceptability and flavor at 27.5% replacement showed a significant difference from control. As a result, the next lower level (25% replacement) was selected for further trial.

For In-house consumer test, 25% was the level of low heat full-fat soy flour selected to incorporate into cake doughnut compared with the control formula. The results are shown in **Table 17**. There was no significant difference between control formula and 25% replacement in overall acceptability and flavor. **Figure 16** shows the picture from both formulas.

Table 15 Sensory acceptability score[1, 2] from preliminary trial of low heat full-fat soy flour enriched-cake doughnuts as compared with control formula

Formula	Before tasting			After tasting		
	General[6] appearance	Color[6]	Shape[6]	Overall[6] acceptability	Flavor[6]	Texture[6]
Control	6.20 ± 1.63^{a}	6.88 ± 1.49^{a}	6.36 ± 1.58^{a}	6.60 ± 1.53^{a}	6.64 ± 1.50^{a}	6.56 ± 1.50^{a}
10% replacement[3]	6.12 ± 1.39^{a}	6.20 ± 1.39^{a}	6.12 ± 1.54^{a}	5.84 ± 1.40^{b}	6.52 ± 1.33^{a}	6.00 ± 1.76^{ab}
20% replacement[4]	5.88 ± 1.39^{a}	6.16 ± 1.34^{a}	5.92 ± 1.47^{a}	5.68 ± 1.62^{b}	6.52 ± 1.41^{a}	5.84 ± 1.60^{ab}
30% replacement[5]	5.68 ± 1.44^{a}	5.92 ± 1.63^{a}	5.84 ± 1.31^{a}	5.64 ± 1.47^{b}	5.52 ± 1.66^{b}	5.60 ± 1.63^{b}

[1] Mean ± standard deviation from 25 subjects

[2] Mean with the same column having the same subscripts are not significantly different ($p > 0.05$)

[3] 10% replacement = cake doughnut which contains 10% of wheat flour as low heat full-fat soy flour

[4] 20% replacement = cake doughnut which contains 20% of wheat flour as low heat full-fat soy flour

[5] 30% replacement = cake doughnut which contains 30% of wheat flour as low heat full-fat soy flour

[6] Use a nine – point hedonic scale (1 = dislike extremely, 5 = neither like or dislike, 9 = like extremely)

Table 16 Sensory acceptability score[1, 2] from preliminary trial of low heat full-fat soy flour enriched-cake doughnuts

Formula	Before tasting			After tasting		
	General[7] appearance	Color[7]	Shape[7]	Overall[7] acceptability	Flavor[7]	Texture[7]
20.0% replacement[3]	6.80 ± 0.91 ᵃ	6.88 ± 1.01 ᵃ	6.76 ± 1.13 ᵃ	6.52 ± 1.53 ᵃ	6.64 ± 1.52 ᵃ	6.48 ± 1.73 ᵃ
22.5% replacement[4]	6.72 ± 1.14 ᵃ	6.72 ± 1.02 ᵃ	6.60 ± 1.15 ᵃ	6.48 ± 1.39 ᵃ	6.52 ± 1.58 ᵃ	6.48 ± 1.66 ᵃ
25.0% replacement[5]	6.40 ± 1.29 ᵃ	6.24 ± 1.39 ᵃ	6.20 ± 1.44 ᵃ	6.36 ± 1.44 ᵃ	5.88 ± 1.54 ᵃ	5.80 ± 1.66 ᵃ
27.5% replacement[6]	6.24 ± 1.36 ᵃ	6.60 ± 1.12 ᵃ	6.60 ± 1.47 ᵃ	5.06 ± 1.41 ᵇ	5.56 ± 1.73 ᵇ	5.92 ± 1.85 ᵃ

[1] Mean ± standard deviation from 25 subjects

[2] Mean with the same column having the same subscripts are not significantly different ($p > 0.05$)

[3] 20.0% replacement = cake doughnut which contains 20.0% of wheat flour as low heat full-fat soy flour

[4] 22.5% replacement = cake doughnut which contains 22.5% of wheat flour as low heat full-fat soy flour

[5] 25.0% replacement = cake doughnut which contains 25.0% of wheat flour as low heat full-fat soy flour

[6] 27.5% replacement = cake doughnut which contains 27.5% of wheat flour as low heat full-fat soy flour

[7] Use a nine – point hedonic scale (1 = dislike extremely, 5 = neither like or dislike, 9 = like extremely)

Table 17 Sensory acceptability score[1, 2] from In-house consumer test of low heat full-fat soy flour enriched-cake doughnuts as compared with control formula

Formula	Before testing			After testing		
	General[4] appearance	Color[4]	Shape[4]	Overall[4] acceptability	Flavor[4]	Texture[4]
Control	7.34 ± 1.02 [a]	7.76 ± 0.85 [a]	7.30 ± 1.02 [a]	7.40 ± 1.16 [a]	7.26 ± 1.14 [a]	7.76 ± 0.96 [a]
25% replacement[3]	7.22 ± 0.95 [a]	7.38 ± 0.92 [a]	7.22 ± 0.97 [a]	7.30 ± 1.13 [a]	7.18 ± 1.12 [a]	7.36 ± 0.98 [b]

[1] Mean ± standard deviation from 50 subjects

[2] Mean with the same column having the same subscripts are not significantly different ($p > 0.05$)

[3] 25% replacement = cake doughnut which contains 25% of wheat flour as low heat full-fat soy flour

[4] Use a nine – point hedonic scale (1 = dislike extremely, 5 = neither like or dislike, 9 = like extremely)

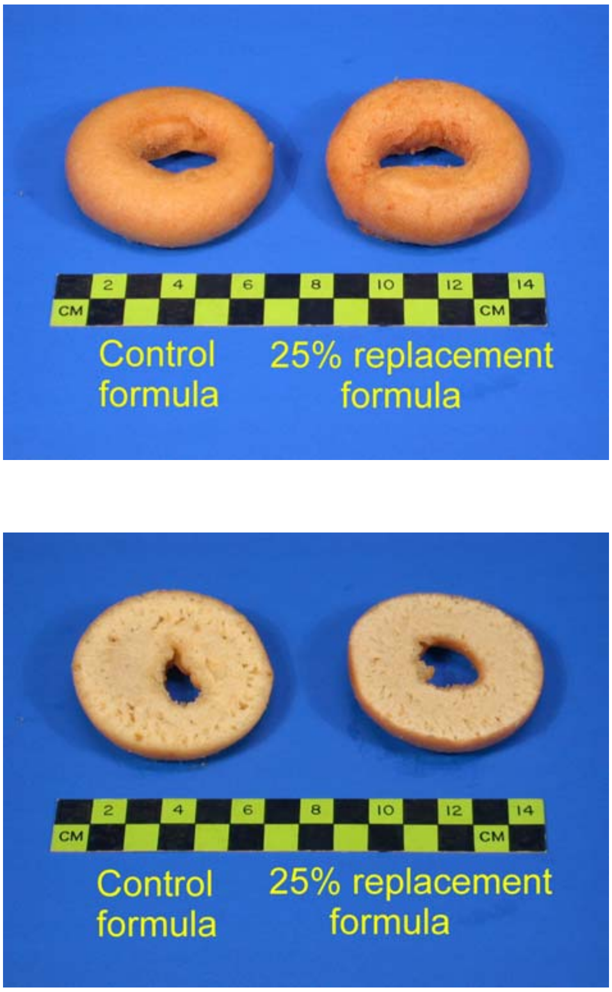


Figure 16 The control formula cake doughnut and 25% replacement formula cake doughnut

5.3.1.2 Butter cake

In the preliminary study, 10%, 20%, 30% and 40% were the selected level of low heat full-fat soy flour to incorporate into butter cake compared with the control formula. The results are shown in **Table 18**. At 40% replacement, a significant difference was found in the texture score compared to the rest of the formulas.

However, the range of adding low heat full-fat soy flour was rather wide (30% - 40%). The next trial was then set up to define a suitable level of replacement. Therefore, 30%, 32.5%, 35% and 37.5% were the level of low heat full-fat soy flour selected to incorporate into butter cake. The results are shown in **Table 19**. The overall acceptability at 30% replacement showed a significant difference from other formulas. As a result, this level (30% replacement) was selected for further trial.

For In-house consumer test, 30% was the level of low heat full-fat soy flour selected to incorporate into butter cake compared with the control formula. The results are shown in **Table 20**. There was no significant difference between control formula and 30% replacement formula in the overall acceptability, shape, flavor and texture scores. **Figure 17** shows the picture of butter cake from both formulas.

Table 18 Sensory acceptability score[1, 2] from preliminary trial of low heat full-fat soy flour enriched-butter cakes as compared with control formula

Formula	Before tasting			After tasting		
	General[7] appearance	Color[7]	Shape[7]	Overall[7] acceptability	Flavor[7]	Texture[7]
Control	7.12 ± 1.17^{a}	6.92 ± 1.29^{a}	6.80 ± 1.44^{a}	6.80 ± 1.53^{a}	6.72 ± 1.72^{a}	6.88 ± 1.90^{a}
10% replacement[3]	6.60 ± 1.44^{ab}	6.80 ± 1.19^{ab}	6.20 ± 1.41^{ab}	6.12 ± 1.69^{ab}	6.16 ± 1.57^{ab}	6.64 ± 1.58^{a}
20% replacement[4]	6.20 ± 1.78^{ab}	6.12 ± 1.83^{ab}	6.20 ± 1.71^{ab}	5.68 ± 1.99^{b}	6.04 ± 1.65^{ab}	6.52 ± 1.85^{a}
30% replacement[5]	6.20 ± 1.66^{b}	6.04 ± 1.57^{b}	6.20 ± 1.66^{ab}	5.64 ± 1.73^{b}	5.84 ± 1.62^{b}	6.44 ± 1.87^{a}
40% replacement[6]	6.12 ± 1.51^{b}	6.00 ± 1.96^{b}	5.76 ± 1.51^{b}	5.60 ± 1.76^{b}	5.72 ± 1.65^{b}	5.44 ± 1.83^{b}

[1] Mean ± standard deviation from 25 subjects

[2] Mean with the same column having the same subscripts are not significantly different ($p > 0.05$)

[3] 10% replacement = butter cake which contains 10% of wheat flour as low heat full-fat soy flour

[4] 20% replacement = butter cake which contains 20% of wheat flour as low heat full-fat soy flour

[5] 30% replacement = butter cake which contains 30% of wheat flour as low heat full-fat soy flour

[6] 40% replacement = butter cake which contains 40% of wheat flour as low heat full-fat soy flour

[7] Use a nine – point hedonic scale (1 = dislike extremely, 5 = neither like or dislike, 9 = like extremely)

Table 19 Sensory acceptability score[1, 2] from preliminary trial of low heat full-fat soy flour enriched-butter cakes

Formula	Before tasting			After tasting		
	General[7] appearance	Color[7]	Shape[7]	Overall[7] acceptability	Flavor[7]	Texture[7]
30.0% replacement[3]	6.53 ± 1.50^{a}	6.77 ± 1.30^{a}	6.63 ± 1.47^{a}	6.73 ± 1.46^{a}	6.47 ± 1.85^{a}	6.60 ± 1.63^{a}
32.5% replacement[4]	6.00 ± 1.58^{a}	6.30 ± 1.39^{a}	6.40 ± 1.45^{a}	5.83 ± 1.72^{b}	6.03 ± 1.52^{a}	6.33 ± 1.69^{a}
35.0% replacement[5]	5.93 ± 1.44^{a}	6.17 ± 1.29^{a}	6.27 ± 1.68^{a}	5.77 ± 1.72^{b}	5.63 ± 1.85^{ab}	6.10 ± 1.35^{a}
37.5% replacement[6]	5.80 ± 1.32^{a}	6.03 ± 1.35^{a}	5.93 ± 1.46^{a}	5.47 ± 1.87^{b}	5.27 ± 1.76^{b}	5.87 ± 1.38^{a}

[1] Mean ± standard deviation from 25 subjects

[2] Mean with the same column having the same subscripts are not significantly different ($p > 0.05$)

[3] 30.0% replacement = butter cake which contains 30.0% of wheat flour as low heat full-fat soy flour

[4] 32.5% replacement = butter cake which contains 32.5% of wheat flour as low heat full-fat soy flour

[5] 35.0% replacement = butter cake which contains 35.0% of wheat flour as low heat full-fat soy flour

[6] 37.5% replacement = butter cake which contains 37.5% of wheat flour as low heat full-fat soy flour

[7] Use a nine – point hedonic scale (1 = dislike extremely, 5 = neither like or dislike, 9 = like extremely)

Table 20 Sensory acceptability score[1,2] from In-house consumer test of low heat full-fat soy flour enriched-butter cakes as compared with control formula

Formula	Before tasting			After tasting		
	General[4] appearance	Color[4]	Shape[4]	Overall[4] acceptability	Flavor[4]	Texture[5]
Control	7.56 ± 1.09^{a}	7.78 ± 0.91^{a}	7.56 ± 0.99^{a}	7.70 ± 0.99^{a}	7.70 ± 0.95^{a}	7.74 ± 0.88^{a}
30% replacement[3]	7.16 ± 1.04^{b}	7.42 ± 0.93^{b}	7.50 ± 0.95^{a}	7.46 ± 0.93^{a}	7.64 ± 0.94^{a}	7.70 ± 0.84^{a}

[1] Mean ± standard deviation from 50 subjects

[2] Mean with the same column having the same subscripts are not significantly different ($p > 0.05$)

[3] 30% replacement = butter cake which contains 30% of wheat flour as low heat full-fat soy flour

[4] Use a nine – point hedonic scale (1 = dislike extremely, 5 = neither like or dislike, 9 = like extremely)

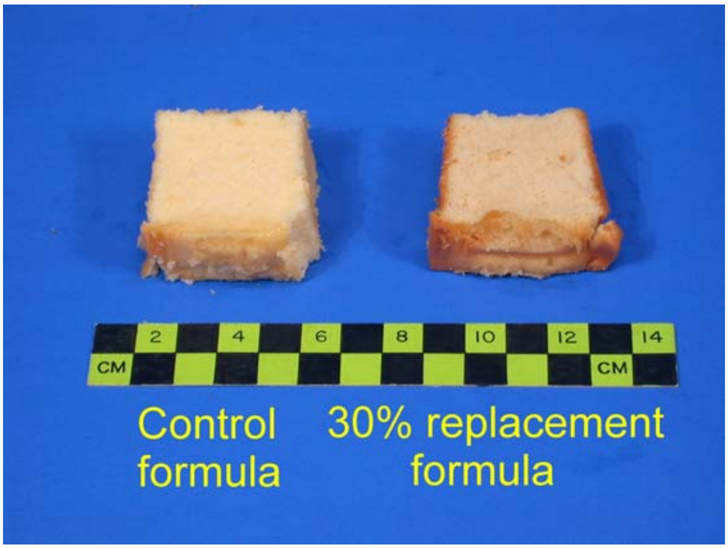


Figure 17 The control formula butter cake and 30% replacement formula butter cake

5.3.1.3 Pork patties

In the preliminary study, 10%, 20% and 30% were the selected level of low heat full-fat soy flour to incorporate into pork patties compared with the control formula. The results are shown in **Table 21**. At 20% replacement, the overall acceptability score started become lower than that of the control. Therefore, the possible range was 10% - 20% replacement.

However, the range of adding low heat full-fat soy flour was still wide (10% - 20%). The next trial was then set up to define the level of replacement. Therefore, 10%, 12.5%, 55% and 17.5% were the level of low heat full-fat soy flour selected to incorporate into pork patties. The results are shown in **Table 22**. The overall acceptability scores at all higher levels of replacement was significantly lower than that of the 10% replacement formula. As a result, 10% replacement level was selected for further trial.

For In-house consumer test, 10% was the level of low heat full-fat soy flour selected to incorporate into pork patties compared with the control formula. The results are shown in **Table 23**. There was no significant difference between control formula and 10% replacement in the overall acceptability, flavor and texture score. **Figure 18** shows the picture of pork patties from both formulas.

Table 21 Sensory acceptability score[1, 2] from preliminary trial of low heat full-fat soy flour enriched-pork patties as compared with control formula

Formula	Before tasting		After tasting		
	General[6] appearance	Color[7]	Overall[6] acceptability	Flavor[7]	Texture[7]
Control	6.11 ± 1.62^{a}	3.04 ± 1.04^{a}	6.36 ± 1.81^{a}	2.93 ± 0.94^{a}	3.07 ± 0.72^{a}
10% replacement[3]	5.82 ± 1.87^{a}	2.79 ± 1.17^{a}	6.07 ± 1.25^{a}	2.75 ± 0.80^{a}	2.96 ± 0.84^{a}
20% replacement[4]	5.57 ± 1.97^{a}	2.61 ± 0.88^{a}	5.11 ± 1.85^{b}	2.54 ± 1.04^{a}	2.89 ± 0.96^{a}
30% replacement[5]	5.54 ± 1.71^{a}	2.50 ± 0.88^{a}	5.00 ± 1.94^{b}	2.46 ± 0.96^{a}	2.82 ± 1.09^{a}

[1] Mean ± standard deviation from 25 subjects

[2] Mean with the same column having the same subscripts are not significantly different ($p > 0.05$)

[3] 10% replacement = pork patties which contains 10% of sirloin pork as low heat full-fat soy flour

[4] 20% replacement = pork patties which contains 20% of sirloin pork as low heat full-fat soy flour

[5] 30% replacement = pork patties which contains 30% of sirloin pork as low heat full-fat soy flour

[6] Use a nine – point hedonic scale (1 = dislike extremely, 5 = neither like or dislike, 9 = like extremely)

[7] Use a five – point just-about-right scale (1 = much too weak, 3 = just-about-right, 5 = much too strong)

Table 22 Sensory acceptability score[1, 2] from preliminary trial of low heat full-fat soy flour enriched-pork patties

Formula	Before tasting		After tasting		
	General[7] appearance	Color[8]	Overall[7] acceptability	Flavor[8]	Texture[8]
10.0% replacement[3]	6.73 ± 1.55 ^{a}	3.03 ± 0.93 ^{a}	6.87 ± 1.43 ^{a}	3.00 ± 0.91 ^{a}	3.03 ± 1.07 ^{a}
12.5% replacement[4]	6.27 ± 1.62 ^{a}	2.97 ± 0.93 ^{a}	6.07 ± 1.57 ^{b}	3.00 ± 0.69 ^{a}	2.97 ± 0.67 ^{a}
15.0% replacement[5]	6.10 ± 1.67 ^{a}	2.90 ± 1.12 ^{a}	5.90 ± 1.83 ^{b}	2.93 ± 0.78 ^{a}	2.90 ± 0.80 ^{a}
17.5% replacement[6]	5.90 ± 1.67 ^{a}	2.83 ± 1.05 ^{a}	5.73 ± 1.66 ^{b}	2.90 ± 0.84 ^{a}	2.87 ± 1.01 ^{a}

[1] Mean ± standard deviation from 25 subjects

[2] Mean with the same column having the same subscripts are not significantly different ($p > 0.05$)

[3] 10.0% replacement = pork patties which contains 10.0% of sirloin pork as low heat full-fat soy flour

[4] 12.5% replacement = pork patties which contains 12.5% of sirloin pork as low heat full-fat soy flour

[5] 15.0% replacement = pork patties which contains 15.0% of sirloin pork as low heat full-fat soy flour

[6] 17.5% replacement = pork patties which contains 17.5% of sirloin pork as low heat full-fat soy flour

[7] Use a nine – point hedonic scale (1 = dislike extremely, 5 = neither like or dislike, 9 = like extremely)

[8] Use a five – point just-about-right scale (1 = much too weak, 3 = just-about-right, 5 = much too strong)

Table 23 Sensory acceptability score[1,2] from In-house consumer test of low heat full-fat soy flour enriched-pork patties as compared with control formula

Formula	Before tasting		After tasting		
	General[4] appearance	Color[5]	Overall[4] acceptability	Flavor[5]	Texture[5]
Control	7.06 ± 1.15^{a}	3.06 ± 0.79^{a}	7.14 ± 1.16^{a}	3.10 ± 0.99^{a}	3.04 ± 0.49^{a}
10% replacement[3]	6.46 ± 1.46^{b}	2.94 ± 0.84^{a}	6.98 ± 1.19^{a}	3.04 ± 1.03^{a}	2.98 ± 0.55^{a}

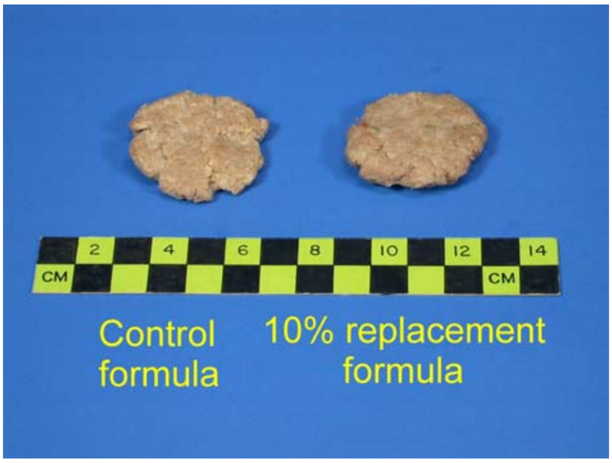
[1] Mean ± standard deviation from 50 subjects

[2] Mean with the same column having the same subscripts are not significantly different ($p > 0.05$)

[3] 10% replacement = pork patties which contains 10% of sirloin pork as low heat full-fat soy flour

[4] Use a nine – point hedonic scale (1 = dislike extremely, 5 = neither like or dislike, 9 = like extremely)

[5] Use a five – point just-about-right scale (1 = much too weak, 3 = just-about-right, 5 = much too strong)



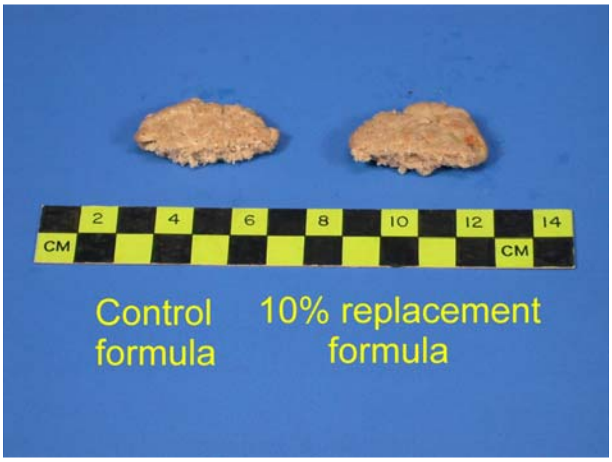


Figure 18 The control formula pork patties and 10% replacement formula pork patties

5.3.2 Quality evaluation

5.3.2.1 Cake doughnut

Protein content of low heat full-fat soy flour enriched-cake doughnut at 25% replacement of wheat flour was higher than that of the control formula as presented in **Table 24**.

This part of the study also aimed to examine the effect of soy flour addition on oil absorption in the final product by using oil uptake ratio as the criterion. Wheat flour was replaced with low heat full-fat soy flour at the level of 25% by weight. The results of initial moisture and fat content, and final moisture and fat content (%) of each formula are shown in **Table 25**. Initial fat content and final fat content were lower than control formula, while final moisture content was higher than control formula.

From the results, the final doughnut moisture content, water removed, oil uptake, and the derived oil uptake ratio;U_R, which were calculated at the end of frying and recorded, are shown in **Table 26**. Water removed and oil uptake of low heat full-fat soy flour enriched-cake doughnut were lower than control formula leading to a higher U_R ratio than control formula.

Table 24 Protein content of low heat full-fat soy flour enriched-cake doughnut as compared with control formula[1]

Doughnut formulation	Protein content (%)
Control	7.10
25% replacement [2]	11.04

[1] Results are means of duplicate analysis from three lots of cake doughnut pooled into one composite sample

[2] 25% replacement = cake doughnut which contains 25% of wheat flour as low heat full-fat soy flour

Table 25 Initial moisture and fat content, and final moisture and fat content (% wet basis) of low heat full-fat soy flour enriched-cake doughnut as compared with control formula[1]

Doughnut formulation	Initial[3]		Final[4]	
	Moisture content (%)	Fat content (%)	Moisture content (%)	Fat content (%)
Control	33.52	11.77	16.58	30.67
25% replacement [2]	31.76	9.59	21.91	24.39

[1] Results are means of duplicate analysis from three lots of cake doughnut pooled into one composite sample

[2] 25% replacement = cake doughnut which contains 25% of wheat flour as low heat full-fat soy flour

[3] Initial moisture and fat content = moisture and fat content of the batter before frying (%)

[4] Final moisture and fat content = moisture and fat content of the final doughnut after frying (%)

Table 26 Final moisture content, water removed, and oil uptake of low heat full-fat soy flour enriched-cake doughnut as compared with control formula[1]

Doughnut formulation	Water removed [3] (%)	Final moisture content (%)	Oil uptake [4] (%)	U_R [5]
Control	16.97	16.58	18.90	1.12
25% replacement [2]	9.85	21.91	14.80	0.85

[1] Results are means of duplicate analysis from three lots of cake doughnut pooled into one composite sample

[2] 25% replacement = cake doughnut which contains 25% of wheat flour as low heat full-fat soy flour

[3] Water removed = Initial moisture content (g) – Final moisture content (g)

[4] Oil uptake = Final fat content (g) – Initial fat content (g)

[5] U_R ratio = Oil uptake ratio between the weight of oil uptake and the weight of water removed

The fat content of the final product in control and low heat full-fat soy flour enriched-cake doughnuts was used to calculate % reduction in fat absorption as shown in **Table 27**. Compared to the control formula, 25% replacement of wheat flour could reduce fat absorption by 20.5%.

Table 27 Moisture and fat content of control formula and low heat full-fat soy flour enriched-cake doughnut formula and % reduction of fat absorption of low heat full-fat soy flour enriched-cake doughnut formula compared with control formula[1]

Doughnut formulation	Moisture content (% wet basis)	Fat content (% wet basis)	% Reduction of fat absorption
Control	16.58	30.67	-
25% replacement [2]	21.91	24.39	20.48

[1] Results are means of duplicate analysis from three lots of cake doughnut pooled into one composite sample

[2] 25% replacement = cake doughnut which contains 25% of wheat flour as low heat full-fat soy flour

The physical characteristics i.e texture and color of low heat full-fat soy flour enriched-cake doughnut were as well being tested and compared with control formula. The results are shown in **Table 28** and **Table 29**, respectively. The result showed no significant difference in texture, but the color of the control formula was slightly lighter than soy flour enriched formula.

Table 28 Texture[1] of low heat full-fat soy flour enriched-cake doughnut as compared with control formula[2, 3]

Doughnut formulation	Size (cm^3)	Texture (N)
Control	2.0 × 2.0 × 2.0	6.40 ± 0.20 ^{a}
25% replacement [4]	2.0 × 2.0 × 2.0	6.47 ± 0.31 ^{a}

[1] Compression

[2] Mean ± standard deviation of at least three replicate determinations of three independent samples

[3] Mean with the same column having the same subscripts are not significantly different ($p > 0.05$)

[4] 25% replacement = cake doughnut which contains 25% of wheat flour as low heat full-fat soy flour

Table 29 Color of low heat full-fat soy flour enriched-cake doughnut as compared with control formula[1]

Doughnut formulation		L[3]	a[3]	b[3]
Control	outside	50.51±0.58	5.60±0.34	32.00±0.41
25% replacement [2]	outside	53.22±0.46	6.65±0.22	31.48±0.19
Control	inside	71.45±0.37	-0.11±0.21	29.62±0.17
25% replacement [2]	inside	67.30±0.30	0.69±0.25	28.33±0.14

[1] Mean ± standard deviation of at least three replicate determinations of three independent samples

[2] 25% replacement = cake doughnut which contains 25% of wheat flour as low heat full-fat soy flour

[3] L: ↑ 100 bright
↓ 0 dark
a: ↑ + red
↓ - green
b: ↑ + yellow
↓ - blue

5.3.2.2 Butter cake

Protein content of low heat full-fat soy flour enriched-butter cake at 30% replacement of wheat flour was higher than that of the control formula as presented in **Table 30**.

Table 30 Protein, fat and moisture content (%wet basis) of low heat full-fat soy flour enriched-butter cake compared with control formula[1]

Cake formulation	Protein content (%)	Fat content (%)	Moisture content (%)
Control	6.35	21.52	19.82
30% replacement [2]	9.04	21.98	19.01

[1] Results are means of duplicate analysis from three lots of butter cake pooled into one composite sample

[2] 30% replacement = butter cake which contains 30% of wheat flour as low heat full-fat soy flour

The physical characteristics i.e volume, texture, and color of low heat full-fat soy flour enriched-butter cake were tested and compared with control formula. The results are shown in **Table 31**, **Table 32** and **Table 33**, respectively.

Volume and texture were not significantly different from control formula, whereas color was lighter both of inside and outside of butter cake.

Table 31 Volume of low heat full-fat soy flour enriched-butter cake compared with control formula[1, 2]

Cake formulation	Volume (ml)
Control	326.67 ± 15.28^{a}
30% replacement [3]	273.33 ± 15.28^{a}

[1] Mean ± standard deviation of at least three replicate determinations of three independent samples

[2] Mean with the same column having the same subscripts are not significantly different ($p > 0.05$)

[3] 30% replacement = butter cake which contains 25% of wheat flour as low heat full-fat soy flour

Table 32 Texture[1] of low heat full-fat soy flour enriched-butter cake as compared with control formula[2, 3]

Cake formulation	Size (cm^3)	Texture (N)
Control	4.0 × 4.5 × 1.8	11.60 ± 0.40 [a]
30% replacement [4]	4.0 × 4.5 × 1.8	11.53 ± 0.23 [a]

[1] Compression

[2] Mean ± standard deviation of at least three replicate determinations of three independent samples

[3] Mean with the same column having the same subscripts are not significantly different ($p > 0.05$)

[4] 30% replacement = butter cake which contains 30% of wheat flour as low heat full-fat soy flour

Table 33 Color of low heat full-fat soy flour enriched-butter cake as compared with control formula[1]

Cake formulation		L[3]	a[3]	b[3]
Control	outside	64.43±0.65	4.34±0.47	36.32±0.30
30% replacement [2]	outside	52.59±0.72	9.79±0.58	36.21±0.38
Control	inside	73.65±0.55	-0.25±0.33	28.98±0.37
30% replacement [2]	inside	66.61±0.64	2.03±0.41	27.51±0.48

[1] Mean ± standard deviation of at least three replicate determinations of three independent samples

[2] 30% replacement = butter cake which contains 30% of wheat flour as low heat full-fat soy flour

[3] L: ↑ 100 bright
↓ 0 dark
a: ↑ + red
↓ - green
b: ↑ + yellow
↓ - blue

5.3.2.3 Pork patties

Protein, fat and moisture content of low heat full-fat soy flour enriched-pork patties at 10% replacement of pork was higher than that of the control formula, whereas fat content was lower, as presented in **Table 34**.

Table 34 Protein, fat and moisture content of low heat full-fat soy flour enriched-pork patties compared with control formula[1]

Patties formulation	Protein content (%)	Fat content (%)	Moisture content (%)
Control	20.11	19.15	48.70
10% replacement [2]	22.85	11.07	56.43

[1] Results are means of duplicate analysis from three lots of pork patties pooled into one composite sample

[2] 10% replacement = pork patties which contains 10% of sirloin pork as low heat full-fat soy flour

The physical characteristics i.e texture, technological yield, and shrinkage of low heat full-fat soy flour enriched-pork patties was tested and compared with control formula. The results are shown in **Table 35**, **Table 36**, and **Table 37**, respectively. Data showed that 10% replacement of pork in pork patties formula resulted in higher values for texture meaning that the texture became firmer with soy flour addition. Nevertheless, adding soy flour increased technological yield of patties. For shrinkage, there was no significant difference in % change of thickness, but adding soy flour helped to reduce shrinkage in term of diameter.

Table 35 Texture[1] of low heat full-fat soy flour enriched-pork patties compared with control formula[2, 3]

Patties formulation	Size (cm^3) [5]	Texture (N)
Control	3.63 × 0.7	13.00 ± 0.20 [a]
10% replacement [4]	3.87 × 0.7	16.67 ± 0.31 [b]

[1] Compression

[2] Mean ± standard deviation of at least three replicate determinations of three independent samples

[3] Mean with the same column having the same subscripts are not significantly different ($p > 0.05$)

[4] 10% replacement = pork patties which contains 10% of sirloin pork as low heat full-fat soy flour

[5] Diameter (cm.) × thickness (cm.)

Table 36 Technological yield of low heat full-fat soy flour enriched-pork patties compared with control formula[1, 2]

Patties formulation	Technological yield (%)
Control	82.38 ± 1.41 [a]
10% replacement [3]	86.33 ± 0.47 [b]

[1] Mean ± standard deviation of at least three replicate determinations of three independent samples

[2] Mean with the same column having the same subscripts are not significantly different ($p > 0.05$)

[3] 10% replacement = pork patties which contains 10% of sirloin pork as low heat full-fat soy flour

Table 37 Shrinkage of pork patties and changes after cooking of low heat full-fat soy flour enriched-pork patties as compared with control formula[1, 2]

Patties formulation	Thickness (cm)			Diameter (cm)		
	Before cooking	After cooking	Change (%)	Before cooking	After cooking	Change (%)
Control	0.77±0.06	0.67±0.06	-13.10 [a] ±1.03	4.00±0.00	3.63±0.12	-9.17 [a] ±2.89
10% replacement[3]	0.80±0.00	0.70±0.00	-12.50 [a] ±0.00	4.00±0.00	3.87±0.06	-2.50 [b] ±0.00

[1] Mean ± standard deviation of at least three replicate determinations of three independent samples

[2] Mean with the same column having the same subscripts are not significantly different ($p > 0.05$)

[3] 10% replacement = pork patties which contains 10% of sirloin pork as low heat full-fat soy flour

CHAPTER VI

DISCUSSION

6.1 Chemical analysis

6.1.1 Proximate analysis

Low heat full-fat soy flour could be considerably varied in composition and nutritive value, depending not only on the composition of soybean but also on the method or process of production. The composition of low heat full-fat soy flour was compared with a commercial full-fat soy flour as given in **Table 8**. Since the moisture content of the products may vary during storage, the percentage figures for protein, fat, total dietary fiber and ash should be examined on a moisture free basis (1, 38, 39, 40, 41, 42 and 43). Low heat full-fat soy flour in this study showed basic composition in close proximity to the values reported by other researches. The low heat full-fat soy flour was shown to be a good source of protein, fat, and dietary fiber, as expected.

6.1.2 Antinutritional factor

Several components associated with soybean proteins have been found to exert specific physiological effects. These are for example, trypsin inhibitors and phytate. It has been known for a long time that raw soybeans or unheated soybean protein product will impair growth when fed to young rats or chicks. This effect is completely eliminated when the soybean component is properly heated (1).

The bulk of the available information on their biochemical, physiological and nutritional properties stems from experimentation with animals or from *in vitro* investigations. There is no direct evidence as to the physiological effect of the inhibitors on humans. Nevertheless, it has become customary to take the necessary precautions for the removal or inactivation of trypsin inhibitors from soybean products intended for human consumption (1).

In this study, trypsin inhibitor activity of low heat full-fat soy flour is 34 TIU/mg or 75 TIU/mg protein (**Table 9**) which was significantly lower than that of the raw soy flour (65 TIU/mg or 141 TIU/mg protein). This is similar to the study of Anderson and Wolf (44) while reported the values 16-27 TIU/mg or 34-122 TIU/mg protein for whole soybean flour and the study of Kakade *et al* (45) which gave the value 100-178 TIU/mg protein of petroleum ether extracted-whole soybean flour. Moreover, the study of Turner and Liener (46) indicated the value 98 TIU/mg protein of raw soy flour. The variation of trypsin inhibitor activity among soy flours could arise from the different of the temperature application used for roasting or drying soy flour.

Trypsin inhibitor activity remained in low heat full-fat soy flour in this study could be considered relatively moderate to high when compared with other soy flours. Nevertheless, application of this flour in food products which involve heat treatment such as deepfat frying (cake doughnut), baking (butter cake) and pan frying (pork patties) could help to further reduce the effect from trypsin inhibitor activity.

For phytate, the result showed that low heat full-fat soy flour contained 1,092 mg /g sample, which was not different from the raw soy flour (1,160 mg / 100 g sample). Anderson and Wolf (44) reported the phytate content of 1.0-2.30 g/ 100 g raw soybean flour (or 1,000-2,300 mg / 100 g) which was in the same range of the phytate level in this study. In contrast, Mason *et al* (47) obtained relatively low phytate content 0.11-1.12 g/100 g (or 110 -1,120 mg / 100 g) in soy flour. The level of phytate may vary among different soy flour products due to the difference in the raw soybean seeds.

6.2 Functional and physical properties evaluation

6.2.1 Water binding capacity (WBC) and oil binding capacity (OBC)

Both low heat full-fat soy flour and the commercial full-fat soy flour could bind slightly more water than oil. WBC of low heat full-fat soy flour was significantly lower than that of the commercial full-fat soy flour. Basically, the differences in WBC among these soy flours might be attributed to the different protein conformation and the variations in the number and nature of the water binding-sites on protein molecules (48). The enhanced WBC of the commercial full-fat soy flour which was derived from higher heat-processed seeds, may be due to the denaturation of proteins that facilitates additional binding sites available for water binding (49, 50).

Oil binding capacity is the binding of oil or fat by nonpolar sited chains of proteins (51). Low heat full-fat soy flour gave oil binding capacity similar to the commercial full-fat soy flour. The ability of low heat full-fat soy flour to bind fat is likely due to nonpolar side chains that bind hydrocarbon chains, thereby contributing to oil absorption (52).

Since many tests of WBC and OBC can be performed under different conditions and the results will depend on these conditions. This is a very important issue in considering the result of the testing. Such large variations in test condition made it impractical to compare of results from different studies (14).

6.2.2 Foaming capacity and foaming stability

Foaming capacity and foaming stability of low heat full-fat soy flour were 21.1 ± 0.5 % and 31.5 ± 1.9 %, respectively. The commercial full-fat soy flour had foaming capacity and foaming stability 11.2 ± 1.8 % and 25.7 ± 4.9 %, respectively. Foaming capacity of low heat full-fat soy flour was significantly higher than the commercial full-fat soy flour. No significant differences in foam stability were found between low heat full-fat soy flour and the commercial full-fat soy flour.

The ability of protein to form and stabilize foams depends on several parameters such as type of protein and degree of denaturation, other compositions, the presence or absence of calcium ions, pH, temperature, and whipping methods (53). The greater ability of low heat full-fat soy flour to form foams around neutral pH (6.51) can be attributed to its higher solubility than the commercial full-fat soy flour (**Table 13**). Protein solubility requires high net charges, which influence the adsorption of proteins at the air-water interface (50). Higher temperature of heat treatment of the commercial full-fat soy flour considerably reduces foaming capacity because heat processing diminishes the protein solubility by denaturation (49). Moreover, foaming stability of both full-fat soy flours diminished through time (30, 60, …, 480 minutes) (**Figure 13**).

6.2.3 Emulsifying activity (EA) and emulsifying stability (ES)

Low heat full-fat soy flour exhibited better emulsifying activity and emulsion stability than the commercial full-fat soy flour (51.1 ± 0.5 and 44.6 ± 3.6, respectively). Surface hydrophobicity is an important factor in determining the emulsifying properties. When compared with that of the commercial full-fat soy flour, the higher EA of low heat full-fat soy flour might be due to its higher hydrophobicity value. This would facilitate the interaction between proteins and oils, resulting in an increase in emulsifying properties (54).

Soluble proteins are surface active and known to promote oil-in-water emulsions as well (55). The result was in agreement with the general correlation between EA and nitrogen solubility found in the study of Hung and Zayas (56).

ES was significantly higher for the low heat full-fat soy flour than the commercial full-fat soy flour (56.4 ± 2.7, and 19.6 ± 6.5, respectively). In this case, the high ES, which involved heating of protein at 80ºC for 30 minutes, might be attributed to the dissociation of some proteins, and the resulting subunits formed had more hydrophobic groups which interacted more strongly with the lipid phase (57). In contrast the high heat full-fat soy flour had undergone a more severe heat treatment which might have caused damage to the protein structure. This result indicates that low heat full-fat soy flour could be an effective emulsifier, making it useful in applications such as sausage, mayonnaise, and seasonings manufacture, especially in products that require heating, because the protein-lipid interaction is favored by the temperature increase (greater than 60ºC) (58).

6.2.4 Gelation

Low heat full-fat soy flour exhibited a gel-starting point at a lower protein concentration than the commercial full-fat soy flour (8% and 10%, respectively). The protein concentration to form gel completely for low heat full-fat soy flour protein was 16%, while the commercial full-fat soy flour could form completed gelation at 18%.

Gel strength can be improved with an increasing protein concentration, but a minimum protein concentration is necessary for gelation (59). The gelling time is, however, dependent on temperature. These results show that the differences in protein concentration have an influence on gelation (55).

Solutions of most globular proteins in foods (e.g., egg white, soybean, and whey protein) form aggregates and heat-set gels as temperature increases. Gel viscosity increases with increased temperature used for heating the protein solutions investigated (50). Kato *et al* (60) reported that the strengthening of gel formed from egg white is mainly attributed to hydrogen bonding and hydrophobic interactions and partially to the formation of intermolecular disulfide bonds.

6.2.5 Nitrogen solubility index (NSI)

Among the functional properties of proteins, solubility is probably the most critical because it affects other properties such as emulsification, foaming and gelation (61, 62). Higher protein solubility enhances such properties. Under neutral condition, low heat full-fat soy flour exhibited higher nitrogen solubility index (11.55%) than did the commercial full-fat soy flour (2.31%). Hence, it also exhibited better emulsifying, foaming and gelling property as shown earlier.

A lower in protein solubility in the commercial full-fat soy flour was the result of heat processing. Proteins are sensitive to heat and undergo denaturation that result in low solubility, as in the case of heat processed sunflower seeds (63), rapeseeds (64) and soybean seeds (65).

6.3 Incorporation of low heat full-fat soy flour into food products

Low heat full-fat soy flour was used as food ingredient by substituting part of wheat flour in the bakery formulas whereas substituting pork in case of patties formula. The study for each product was divided into two steps; 1) selection of suitable level of low heat full-fat soy flour to be incorporate in food products (n = 25), 2) evaluation for their sensory (n = 50), chemical and physical properties and compared to the control formula (no soy flour added).

6.3.1 Cake doughnut

In preliminary trial, the sensory acceptability scores both before tasting and after tasting were well-accepted. Moreover, the results showed no significant differences (p>0.05) for acceptability mean scores of general appearance, overall acceptability, color, shape, flavor, and texture among the samples between control formula and low heat full-fat soy flour enriched-cake doughnut formula (**Table 15** and **Table 16**). The overall acceptability scores of most low heat full-fat soy flour enriched-cake doughnut formulas were, however, lower than control formula. These results might be caused by the off-flavor (beany) and the inferior texture i.e. hard texture that made the low heat full-fat soy flour enriched-cake doughnut less palatable when compared with control formula. Previous studies also reported negative effect of soybean supplementation on the sensory quality of doughnut (2, 3, 23, 24, 40 and 66).

For In-house consumer test, the result of sensory acceptability test of control and low heat full-fat soy flour enriched-formula showed that the mean value of overall acceptability, general appearance, color, shape, and flavor were not significantly different (p>0.05). For texture, however, low heat full-fat soy flour enriched-formula had lower score than the control formula. This result might be caused by the undesirable effect of soybean on texture as discussed earlier. Addition of low heat full-fat soy flour particles produced a coarser texture in the finished product.

In term of nutrition, low heat full-fat soy flour enriched-cake doughnut formula provided 35.7 % more protein than control (**Table 24**). For the effect on oil absorption in the final product by using oil uptake ratio (U_R) as the criterion, low heat full-fat soy flour at 25% replacement in the product contributed effectively in reducing oil uptake 20.5% (**Table 26**), and consequently lowered U_R of the product from 1.12 down to 0.85. Many studies previously reported about this occurrence as soy flour could prevent fat absorption during the frying operation, or had capacity to reduce fat absorption compared with the control formula because low heat full-fat soy flour possessed hydrophilic property and therefore could absorb and retain water. These results agreed well with the higher WBC compared to OBC value (**Table 10**) (24, 25 and 42).

The high WBC of low heat full-fat soy flour also resulted in enriched-cake doughnut formula had higher moisture content than the control formula. Low heat full-fat soy flour can bind water molecules making the strong hydrogen binding between water molecules and low heat full-fat soy flour molecules. This could provide moisture and freshness to cake doughnut. The result agreed well with the result of oil uptake since oil uptake depended on the exchange of oil for water removed from the product.

For physical quality evaluation, low heat full-fat soy flour enriched-cake doughnut formula did not generate the difference in texture (measured as compression) as compared to the control formula (**Table 28**). The colorimeter could detect the color difference between low heat full-fat soy flour enriched-cake doughnut formula and the control formula, but there was no significant difference in sensory evaluation.

6.3.2 Butter cake

Thirty percent replacement of low heat full-fat soy flour in enriched-butter cake formula was the selected level among other levels of replacement. The product was rated as like slightly to like moderately in all sensory characteristics. Previous studies had reported the effect of soybean supplementation on the sensory quality of cake. Cotton (24), Levinson (25) and Dendy (70) reported that formulation with soy flour in cake was accepted when soy flour was used about 2-3 % replacement wheat flour. This was similar to the study of Dubois (27) which reported that adding 3-5 % soy flour to replace wheat flour in cake did not produce undesirable effects. Gilbertson and Porter (66) suggested that 25% replacement of egg white protein with soy flour in chocolate cake was possible. Hence, the level of low heat full-fat soy flour used in this study was relatively high and could be beneficial in term of providing higher protein content.

For In-house consumer test, the result of sensory acceptability test of control and low heat full-fat soy flour enriched-formula showed that the mean value of overall acceptability, shape, flavor and texture were not significantly different ($p>0.05$). General appearance and color scores, however, were lower for low heat full-fat soy flour enriched-formula. This result might be caused by the addition of low heat full-fat soy flour led to an undesirable change from the original light brown color to darker color. Although the full-fat soy flour enriched-butter cake received lower score than the control butter cake, the product was accepted by panelists with the overall acceptability score being 7.46 (like moderately to like very much).

Nutritionally, low heat full-fat soy flour enriched-butter cake contained more protein (**Table 30**), but did not affect moisture content of the product. Hence, low heat full-fat soy flour could not help to retain moisture of the cake. The fat content in the low heat full-fat soy flour enriched-butter cake was also similar to that of the control formula. Although soy flour contained more fat than wheat flour which it was used to replace, the resulting products were similar in term of fat content. The reason may because fat was partially expressed out of the low heat full-fat soy flour enriched-butter cake during baking. It was observed by the researcher that the soy flour enriched cake was readily loosen from the pan while the control formula was more different to remove from the pan.

For physical evaluation, low heat full-fat soy flour enriched-butter cake did not generate the difference in volume and texture as compared to the control formula (**Table 31** and **Table 32**, respectively), while Kiattheerachai (3) found significant difference in both volume and texture of 4% soybean hull enriched-butter cake. The colorimeter as well as sensory evaluation could detect the color difference between low heat full-fat soy flour enriched-butter cake formula and the control formula. The color and general appearance of the soy flour enriched formula was interior to the control because of the addition of large amount of low heat full-fat soy flour (30%) leading to more brown color in the finished product.

6.3.3 Pork patties

In the preliminary trial, since up to 20% replacement of pork with low heat full-fat soy flour in pork patties formula caused significant differences compared to the control formula in overall acceptability, the range between 10%-17.5% replacement was further investigated.

Ten percent replacement of pork with low heat full-fat soy flour in enriched-pork patties formula was the best level compared to other level of replacement because it was good in all sensory characteristics and showed significantly ($p \leq 0.05$) higher score in overall acceptability. Previous studies reported the effect of soybean supplementation on the sensory quality of pork patties. Rakosky (28) reported the possibility of using 8% soy flour in meatball. Other studies also reported the utilization of soy flour at a level of 1-4% in meat based product (11, 67, 68 and 69).

For In-house consumer test, the mean value of overall acceptability, shape, color, flavor and texture were not significantly different ($p > 0.05$) between low heat full-fat soy flour enriched formula and control formula. General appearance score, however, was lower for low heat full-fat soy flour enriched-formula. This result might be caused by the addition of low heat full-fat soy flour led to rougher surface of the pork patties than the control formula. Although the full-fat soy flour enriched-pork patties received lower score than the control pork patties, the product was accepted by panelists with the overall acceptability score being 6.98 (like moderately).

In quality evaluation, low heat full-fat soy flour enriched-pork patties formula exhibited an increase in protein (13.6%) and moisture content (15.9%) (**Table 34**), and a marked decrease in fat content (42.2%). Low heat full-fat soy flour could improve moisture retention of the patties resulting in good texture acceptability score. Moreover, replacing pork with low heat full-fat soy flour also contributed to better nutritional quality of patties i.e increasing protein while decreasing fat content. In particular, saturated fat content would also decrease and unsaturated fat content increase in the food product.

For physical quality evaluation, low heat full-fat soy flour enriched-pork patties formula provided a higher yield as compared to the control formula (**Table 35** and **Table 36**, respectively). There was a significant reduction in diameter shrinkage but no difference in thickness shrinkage as shown in **Table 37**. Prakongpan (71) reported that the small size fiber from pineapple core added in beef burger improved yield and texture. Cooked beef burger had a smaller diameter, but increasing in their thickness after cooking. Hence, addition of soy flour to pork patties could be beneficial in increasing yield as well as reducing shrinkage of the patties.

CHAPTER VII

CONCLUSION

7.1 Proximate analysis

Low heat full-fat soy flour contained 45.6 g protein, 19.4 g fat, 5.8 g moisture, 5.8 g ash, 23.5 g carbohydrate, 11.2 g total dietary fiber and 451 kcal per 100 g. The proximate composition of low heat full-fat soy flour was comparable to the commercial full-fat soy flour and was also in close proximity to the values reported by previous researches studied.

7.2 Antinutritional factor

In this study, trypsin inhibitor activity of low heat full-fat soy flour was significantly lower than that of raw soy flour. The level found was similar to other study. Generally, trypsin inhibitor activity can vary among different soy flour samples due to variation in temperature application used for drying or toasting soy flour.

When soy flour was added into food products that require high temperature processing, trypsin inhibitor activity can be further reduced.

For phytate, the result for low heat full-fat soy flour was not different from raw soy flour and was in the same range reported by other researchers.

7.3 Functional and physical properties evaluation

Low heat full-fat soy flour exhibited better functional properties than the commercial full-fat soy flour. These include several important properties namely; water binding capacity, oil binding capacity, foaming capacity, foaming stability, emulsifying activity and emulsifying stability, gelation and nitrogen solubility index. Therefore, the low heat full-fat soy flour could perform better when applied in food products according to the functional properties required. The color of low heat full-fat soy flour was also lighter than the commercial full-fat soy flour, leading to good appearance and less interference when added to foods.

7.4 Food application of low heat full-fat soy flour

7.4.1 Cake doughnut

The expectation of adding the soy flour in cake doughnut is to increase protein content, prevent fat absorption during the frying operation and increase the freshness of the product by retention of moisture in the finished product.

Twenty five percent replacement of wheat flour by low heat full-fat soy flour resulted in cake doughnut which was acceptable to the panelists in sensory evaluation. At this level, soy flour markedly increased protein content, as well as provided moisture and freshness and reduced oil uptake in the product. Therefore, addition of low heat full-fat soy flour to cake doughnut in this study not only provide nutritional benefits but also improve the quality of the product.

7.4.2 Butter cake

The expectation of adding the soy flour in butter cake is to increase protein content, retain moisture content of the product and provide better product yield.

Thirty percent replacement of wheat flour by low heat full-fat soy flour resulted in butter cake which was acceptable to the panelists in sensory evaluation. At this level, soy flour markedly increased protein content in the product although it did not increase product yield. Hence, in this study low heat full-fat soy flour could improve nutritional value of butter cake while maintaining the quality of the control formula.

7.4.3 Pork patties

The expectation of adding the soy flour in pork patties is to extend meat and provide better moisture retention.

Ten percent replacement of pork by low heat full-fat soy flour resulted in pork patties which was acceptable to the panelists in sensory evaluation. At this level, soy flour increased protein and moisture content, and markedly decreased fat content in the product. It could also increase yield as well as reduce shrinkage of the patties.

Therefore, adding low heat full-fat soy flour to patties helped to improve nutritional quality of pork patties as well as could increase their yield.

7.5 Recommendation on further study

Low heat full-fat soy flour shows a good potential to be a functional component in bakery and meat products. Further studies should be carried out to formulate other types of food product such as beverage, soup, etc., in order to expand the possibility of using this soy flour. Moreover, studies should be undertaken to look at other beneficial compounds in low heat full-fat soy flour such as isoflavones.

REFERENCES

1. The Soybean: Berk Z, editor. Technology of production of edible flours and protein products from soybeans. 1992. FAO Agricultural service bulletin No.97. Food and Agriculture Organization of the United Nations, Rome. Available from: http://www.fao.org/docrep/t0532e/t0532e00.htm#con [Accessed 2003 Dec 9].
2. Arpanantikul M. Formulation of high fiber bakery products and Thai traditional [M.S. Thesis in Nutrition]. Bangkok: Faculty of Graduate Studies, Mahidol University; 1993.
3. Kiattherachai P. Study on the extraction of dietary fiber from soybean from soybean hulls and its application [M.S. Thesis in Food and Nutrition for Development]. Bangkok: Faculty of Graduate Studies, Mahidol University; 2000.
4. Sukkra S. Formulation of fiber-enriched doughnuts using ground soybean hulls and the effect on absorption of frying oil [M.S. Thesis in Food and Nutrition for Development]. Bangkok: Faculty of Graduate Studies, Mahidol University; 1999.
5. พืชเศรษฐกิจที่สำคัญ-สถานการณ์พืชเศรษฐกิจ (พืชไร่-ถั่วเหลือง). 2574. กรมส่งเสริมการเกษตร. กระทรวงเกษตรและสหกรณ์. แหล่งข้อมูล: http://www.doae.go.th/plant/soybn.htm [วัน เดือน ปี ที่เข้าถึงข้อมูล 19 มี.ค 2004].
6. Coppock J. Soy Proteins in Foods-Retrospect and Prospect. JAOCS. 1974;51: 59A-62A.

7. Institute of Nutrition, Mahidol University. Thai Food Composition Tables. 1 st ed. Bangkok: Paluk Tai; 1999.

8. Riaz MN. Soybeans as functional foods. Cereal Food World. 1999;44(2):88-92.

9. Liener IE. Factors Affecting the Nutritional Quality of Soya Products. JAOCS. 1981;58:406-15.

10. Bressani R. The Role of Soybeans in Food System. JAOCS. 1981;58:392-400.

11. Waggle DH, Decker CD, Kolar CW. Soy products in meat, poultry and seafood. JAOCS. 1981;58:341-3.

12. การพัฒนาผลิตภัณฑ์จากถั่วเหลือง. 2547. สถาบันค้นคว้าและพัฒนาผลิตภัณฑ์อาหาร. มหาวิทยาลัยเกษตรศาสตร์. แหล่งข้อมูล: http://www.ku.ac.th/ED/book/001/ foodint.html [วัน เดือน ปี ที่เข้าถึงข้อมูล 19 มี.ค 2004].

13. Tutorial on peptide and protein structure, Introduction. 1996. Department of Biochemistry & Molecular Biology, The University of Georgia. Available from: http://bmbiris.bmb.uga.edu/wampler/tutorial/prot1.html [Accessed 2004 Jan 19].

14. Hall GM, editor. Methods of testing protein Functionality. London: Blackie academic & Professional; 1996.

15. The Soy Protein Partners. Soyfood Guide. 2003. United Soybean Board and the Soy Protein Partners, USA. Available from: http://www.soybean.org/sfg03.pdf [Accessed 2003 Dec 15].

16. Endres JG, editor. Soy Protein Products: Characteristics, Nutritional, Aspects, and Utilization (Revised and Expanded Edition). 2001. Soy protein Council, USA. Available from: http://www.spcouncil.org/SoyProtein.pdf [Accessed 2003 Dec 9].

17. Friedman M, Brandon DL. Nutritional and health benefits of soy proteins. J Agric Food Chem 2001;49(3):1069-86.

18. Fasina YO, Swaisgood HE, Garlich JD, Classen HL. A semi-pilot-scale procedure for isolating and purifying soybean *(Glycine max)* lectin. J Agric Food Chem 2003;51:4532-8.

19. Bohn T, Davidsson L, Walczyk T, Hurrell RF. Phytic acid added to white-wheat bread inhibits fractional apparent magnesium absorption in humans. Am J Clin Nutr 2004;79:418–23.

20. Rhee KC. Functionality of soy proteins. In: Hettiarachchy NS, Ziegler GR, editors. Protein functionality in food systems. New York: Marcel Dekker, Inc.; 1994. p.311-24.

21. Dubios DK, Hoover WJ. Soya Protein Products in Cereal Grain Foods. JAOCS. 1981;58:343-6.

22. Hafner FH. Adapting soy protein products to food use. Cereal Science Today. 1964;9(5):163-6.

23. Varanyanond W, Praphawat S, Jurunuch C, Runggaeng S, Temtrakool K, Paosungthong U. Progress report on low heat full-fat soy flour production: The Institute of Food Research and Product Development, Kasetsart University; 2004.

24. Martin ML, Davis AB. Effect of soy flour on fat absorption by cake donuts. Cereal Chemistry. 1986;63(3):252-255.

25. Cotton RH. Soy products in bakery goods. JAOCS. 1974;51:116A-119A.

26. Levinson AA, Lemancik JF. Soy Protein Products in Other Foods. JAOCS. 1974;51:135A-7A.

27. Pringle W. Full-Fat Soy Flour. JAOCS 1974;51:74A-6A.

28. Rakosky J. Soy grits, flour, concentrate, and isolate in meat products. JAOCS. 1974;51:123A-7A.

29. Association of Official Analytical Chemists (AOAC). 16th ed. Washington, DC: Association of Official Analytical Chemists; 2000.

30. American Oil Chemists Society (AOCS). 5th ed. St. Paul, Minnesota: American Oil Chemists Society; 1998.

31. Beuchat LR. Functional and electrophoretic characteristic of succinylated peanut flour properties. J. Agric. Food Chem. 1977;15:258-61.

32. Coffman CW, Garcia VV. Functional properties and amino acid content of protein isolate from mung bean flour. J Food technol (U.K.). 1977;12:473-85.

33. Yasumatsu K, Sawada K, Moritaka S, Misaki M, Toda J, Wada T, *ea al*. Whipping and emulsifying properties of soybean products. Agric Biol Chem. 1972;36:719-27.

34. Pinthus EJ, Weinbergs N, Saguy S. Criterion for oil uptake during deep fat frying. J Food Sci. 1993;58(1):204-5.

35. Penfield MP, Campbell AM. Experimental food science. 3 rd ed. San Diago (CA): Academic Press, Inc.;1990.

36. Peryan DR, Pilgrim FS. Hedonic scale method of measuring food preference. Food Tech .1957;9:14-9.

37. Stone H, Sindel JL. Sensory evaluation practices. Food Science and Technology. 1985;20: 338-41.

38. Garcia C, Marina L, Laborda F and Torre M. Chemical characterization of commercial soybean products. Food Chemistry. 1998;62(3):325-31.

39. Pringle W. Full-fat soy flour. JAOCS. 1974;51:74A-6A.

40. Lusas EW and Niaz MN. Soy protein products: Processing and use. Journal of Nutrition. 1995;125:573S-802S.

41. Bailey LH., Capen RG and Leclere JA. The composition and characteristics of soybeans, soybean flour, and soybean bread. Cereal Chemistry. 1935;12(5):441-71.

42. Wolf WJ. Soybean proteins: Their functional, chemical, and physical properties. J Agric Food Chem. 1970;18(6):969-76.

43. Albert WJ, Mustakas GC, Maghee JE and Griffin EL. A simple method for making full-fat soy flour. Cereal Since Today. 1967;12(3):81-3.

44. Andersin RL and Wolf WJ. Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. Journal of Nutrition. 1995;125:581S-86.

45. Kakade ML, Simons NR, Liener IE and Lamber JW. Biochemical and nutrition assessment of different varieties of soybeans. J Agric Food Chem. 1970;20(1):87-90

46. Turner R and Liener IE. The effect of the selective removal of haemagglutinins on the nutritive values of soybeans. J Agric Food Chem. 1975;23(1):484-87.

47. Mason AC, Weaver CM, Kimmel S and Brown RK. Effect of soybean phytate content on calcium bioavailability in mature and immature rats. J Agric Food Chem. 1993;41(1):246-49.

48. Chau CF, Cheung CK and Wong YS. Functional Properties of protein concentrates from three Chinese indigenous legume seeds. J Agric Food Chem. 1997;45(7):2500-3.

49. Amita M, Bhardwa S and Dua S. Traditional Processing treatments as a promising approach to enhance the functional properties of Rapseed (*Brassica campestris* Var. *toria*) and sesame seed (*Sesamum indicum*) meals. J Agric Food Chem. 1999;47(8):3093-8.

50. Ahmedna M, Prinyawiwatkul W and Rao RM. Solubilized wheat protein isolate: functional properties and potential food applications. J Agric Food Chem. 1999;47(8):1340-5.

51. Santi SK, Desphande SS and Salunkhe DK. Functional properties of winged bean proteins. J Food Sci. 1982;47:503-9.

52. Lin CS and Zayas JF. Functionality of defatted corn germ proteins in a model system: fat binding capacity and water retention. J Food Sci. 1987;52(5):1308-11.

53. Townsend A and Nakai S. Relationship between hydrophobicity and foaming characteristics of food proteins. J. Food. Sci. 1983;48:588-94.

54. Wang M, Hettiarachchy NS, Qi M, Burks W and Siebenmorgen T. Preparation and functional properties of rice bran protein isolate. J Agric Food Chem. 1999;47(2):411-6.

55. Padilla FC, Alvarez MT and Alfaro MJ. Functional properties of barinas nut flour (*Caryodendron orinocense* Karst., Euphorbiaceae) compared to those of soybean. Food Chemistry. 1996;52(2):191-6.

56. Hung SC and Zayas JF. Emulsifying capacity and emulsion stability of milk protein and corn germ protein flour. J Food Sci. 1991;56:1216-18, 1223.

57. Mahajan A and Dua S. Functional properties of rapeseed protein isolates. J Food Sci Technol. 1995;32:162-5.

58. Pretruccelli S and Anon M. Relationship between the method of obtention and the structural and functional properties isolate. 2. Surface properties. J Agric Food Chem. 1994;42(10):584-91.

59. Mulvihill DH and Kinsella JE. Gelation characteristics of whey proteins and β-lactoglobuline. Food Technol. 1987;41:102-11.

60. Kato A, Ibrahim HR, Watanbe H, Honma K and Kobayashi K. Structural and gelling properties of dry-heating egg white proteins. J Agric Food Chem. 1990;38:32-7

61. Hettiarachchy NS, Griffin VK and Gnanasambandam R. Preparation and functional properties of protein isolate from defatted wheat germ. Cereal Chem. 1996;73(3):364-7.

62. Kinsella JE. Functional properties of proteins in foods: a survey. CRC Crit Rev Food Sci Nutr. 1976;7:219-80.

63. Lin MJY, Humbert ES and Sosulski FW. Certain functional properties of sunflower meal products. J Food Sci. 1971;39:368-70.

64. Sosulski FW, Humbert ES, Bui K and Jones JD. Functional properties of rapeseed flours, concentes and isolates. J Food Sci. 1976;4:1349-51.

65. Mcwatters KH and Cherry JP. Functional properties of peanut paste as affected by heat treatment of full fat peanut. J Food Sci. 1975;3:1205-9.

66. Gilberston DB and Porter MA. Replacing eggs in bakery goods with soy flour. Cereal Foods World. 2001;46(9):431-5.

67. Lockmiller NR and Stanley AE. Increased utilization of protein in foods. Cereal Science Today. 1973;18(3):77-81.

68. Robert LH. Utilization of high levels of soy protein in comminuted processed meat products. JAOCS. 1974;51:195A.

69. Lacourt A. Soy proteins in French products with meat base. JAOCS. 1974;51:190A-2A.

70. Dendy D. Use of soy flour in bakery products. JAOCS. 1974;51:185A-6A.

71. Prakongpan T. The extraction and application of dietary fiber and cellulose from pineapple cores [M.S. Thesis in Food and Nutrition for Development]. Bangkok: Faculty of Graduate Studies, Mahidol University; 1999.

72. Deshpande SS, Sathe SK, Cornforth D and Salunkhe OK. Effects of dehulling on functional properties of dry bean (*Phaseolus vulgaris* L.) flours. Cereal Chem. 1982;59:396-401.

APPENDIX

APPENDIX A

Crude protein analysis

This Kjeldahl method determines total nitrogen in nitrate - containing materials. The sample is digested in sulfuric acid; ammonia is distilled; and excess acid is titrated by using boric acid as the indicator solution. Steps in the measurement of crude protein is described below

Weight accurately 0.5 - 3 g of sample and transfer to digestion tube. Add about 9 g of crystal mix and 1 glass brad. Add 20 ml of concentrate sulfuric acid and place the digestion tube in the digester. Digest of additional 30 minutes after the solution clear. Cool to the room temperature.

Place 500 ml Erlenmeyer flask contains 100 ml of 2 % boric acid and few drops of indicator in the distillation unit with a tip of the condenser extending below the surface of the acid solution. Connect the digestion tube to the distillation unit. Add 100 ml of 30 % NaOH to the digested tube by pressing a start button. Distilled for 4 minutes, then lower the receiver flask so that the tip of the condenser is above the solution, wash down the deliver tube with the water and allow the washing to drain the flask.

Titrate the ammonia in the flask back to the original purplish color with standardized 0.1 N HCl. Carry out a blank follow exactly the same method as the sample. Crude protein content of sample was calculated as shown in the equation below.

$$\text{N (g \%)} = \frac{\text{titer (sample} - \text{blank)} \times \text{N of HCl} \times 14.007 \times 100}{\text{weight of sample} \times 100}$$

$$\text{Protein (g \%)} = \text{\%N} \times \text{appropriate converting factor}$$

$$= \text{\%N} \times 6.25$$

APPENDIX B

Crude fat analysis

The modified method of crude fat analysis (29) was used to determine fat content in material. The sample previously digested with hydrochloric acid and was utilized to measure fat content by a Soxhlet extraction method.

The prepared sample was weighed accurately 3-5 g into 500-ml Erlenmeyer flask. Fifty-ml of 4 N hydrochloric acid was added to the sample. The flask was then equipped with the air condenser and refluxed with gentle boiling for 1 hour. The digested sample was cooled to warm temperature and filtered though a filter paper (Schleicher Schuell no. 595). The remaining sample on the filter paper was washed with hot water until the filtrate had no acid (testing with pH paper). The washed sample remaining on the filter paper was dried at 100 °C in a hot-air oven for 30 minutes.

The dried sample was transferred to the thimble. The extraction container containing 50 ml of petroleum ether and the thimble were equipped to the Soxtec System HT (Tecator, model 1043 extraction unit and model 1046 service unit). The extraction was preformed with 30 minutes immersing and 1 hour rinsing. After finishing, the thimble was removed and the container was dried at 60 °C in a hot-air oven for 30 minutes, then cooled in the desiccator to room temperature and weighed. Crude fat content of sample was calculated as shown in the equation below

$$\%\text{ crude fat} = \frac{\text{weight of fat (g)}}{\text{weight of sample (g)}} \times 100$$

APPENDIX C

Moisture content

In wet samples with crude fat content less than 10%, the moisture content was determined by hot air oven method (29). The representative sample was heated under carefully specified conditions and the loss of weight was taken as a measure of the moisture content of the sample. Acid washed sand was mixed with the sample prior to drying in order to increase the surface area for rapid and complete evaporation of water from sample. The duration of evaporation depends on the two successive weightings not differing by over 5 mg when the residual solids amount in 2-5 g. Steps in the measurement of moisture content are summarized in **Figure 19**.

Weigh acid washed sand 20 g into a porcelain dish

↓

Dry at 105 °C, 30 minutes

↓

Cool in a desiccator ⟶ weight = a g

↓

Add 2 - 2.5 g of homogenized sample ⟶ reweigh = b g

↓

Evaporate off the water on a water bath

↓

Dry the sample at 105°C, 2 hours

↓

Cool in a desiccator ⟶ weight = c g

↓

Calculation of moisture (w/w) = $\frac{b-c}{b-a} \times 100$

Figure 19 Flow diagram of moisture content determination (wet sample)

In dry sample, the finished sample with crude fat content higher than 10%, the moisture content were determined by hot air oven at 105 °C (29). About 1 g of sample was dried under specified condition for 2 hours, then cooled in the desiccator and weight. The sample was returned to the oven and dried until a constant weight was obtained (reweighed every 30 minutes). The weight difference should not be more than 5 mg and the percent of moisture was expressed as:

$$\% \text{ moisture} = \frac{\text{weight of water lost on drying (g)}}{\text{weight of wet sample (g)}} \times 100$$

APPENDIX D

Ash

This method determines ash (total mineral) content in the sample after incineration as described below.

Weigh 2-3 ± 0.0001 g of sample into porcelain ashing dish that has been ignited, cool in desiccator, and weighed soon after attaining room temperature.

Heated the dried sample over a low frame of Bunsen burner until clear for burning away some of the organic matter.

Place in muffle furnace at 550 °C, incinerate until light gray ash is obtained or to constant weight. Ash must not be allowed to fuse. Cool in desiccator and weight soon after room temperature is attained.

If the sample is not completely ashed, 2 ml of 50% nitric acid is carefully added and mixed. Evaporate off the acid and dry the sample over the flame and re-incinerate until complete ashing is obtained.

Ash content of sample was calculated as shown in the equation below.

$$\text{Ash (\%)} = \frac{\text{Weight of residue}}{\text{Sample weight}} \times 100$$

APPENDIX E

Total dietary fiber analysis

The sample of dried foods, fat extracted if containing more than 10% fat, are gelatinized with heat stable α-amylase and then enzymatically digested with protease and amyloglucosidase to remove the protein and starch present. Then 95% ethanol is added to precipitate the soluble dietary fiber. The total residue is filtered, wash with 78% ethanol, 95% ethanol, and acetone. After drying, the residue is weighed. Content of residue is analyzed for protein and the ash determined. Total dietary fiber is weight of the residue less the weight of the protein and ash present. Steps in the measurement of TDF are summarized in **Figure 20**.

Calculation

$$\text{TDF, \%} = [(\text{wt residue sample} - P - A - B)/\ \text{wt sample}] \times 100$$

B = average of residue weight (mg) for duplicate blank

P = weight (mg) of protein

A = weight (mg) of ash weight (mg) of protein

Weight dry sample 0.5 g in centrifuge tube

Add phosphate buffer, pH 6.0 (25 ml)

↓ Alpha-amylase incubation

Add 50 µl Alpha-amylase enzyme, 15 minutes At 100 °C

↓

Cool solution at room temperature

↓

Adjust to pH 7.5 with 0.275 N NaOH

↓ Protease incubation

Add 50 µl Protease enzyme, 30 minutes at 60 °C

↓

Cool solution at room temperature

↓

Adjust to pH 4.5 with 0.325 N HCl

↓ Amyloglucosidase incubation

Add 100 µl Amyloglucosidase enzyme, 30 minutes at 60 °C

↓

Precipitate with 280 ml 95 % alcohol

↓ Overnight

Filter and wash with 78%, 95% alcohol and acetone

↓

Dry at 105 °C, overnight

↓

Record residue weight

Kjeldahl protein determination — Ash determination

↓

Calculation of total dietary fiber

Figure 20 Total dietary fiber determination

APPENDIX F

Trypsin inhibitor activity

Objective

This method determines the total and residual trypsin inhibitors in raw and toasted soybean meals and flours, soy protein concentrates and isolates, and soy products.

Principle

Trypsin inhibitor activity is determined by incubating the raw and heat-treated sample with a know substrate (BAPA) and trypsin. Trypsin activity is indicated by an increase in absorbance at 410 nm. Inhibition of trypsin, by the inhibitor present in the sample, decreases the absorbance increase. The method can be modified slightly to determine trypsin inhibitor content in other food and feed products and plant materials.

Apparatus

1. Water bath maintained at 37 ± 0.5 °C.
2. Spectrophotometer with good accuracy at 410 nm.
3. Test tubes.
4. Vortex stirrer.
5. Magnetic stirrer.

Reagents

1. Tris buffer (0.05M, pH 8.2) containing $CaCl_2$. Dissolve 6.05 g tris (hydroxymethylamino) methane and 2.94 g $CaCl_2{\cdot}H_2O$ in 900 ml water. Adjust pH to 8.2, and dilute volume to liter with water.

2. Substrate solution containing 40 mg benzoyl-DL arginine-*p*-nitroanalide (BAPA) hydrochloride in 100 ml reagent 1. Dissolve 40 mg BAPA in 1 ml dimethyl sulfoxide and dilute to 100 ml with reagent 1, prewarmed to 37°C.

3. Trypsin solution containing 4 mg trypsin (salt-free) in 200 ml 0.001M HCl.

4. Acetic acid solution containing 30 ml glacial acetic acid in 70 ml water.

Procedure

1. Preparation of sample

1.1 Grind sample to at least 100 mesh, preferably 200 mesh, without generating heat.

1.2 Whole soybeans and full-fat soybean meals and flours should be defatted by extraction with pentane-hexane at room temperature.

1.3 Extract sample (not to exceed 1 g) with 50 ml 0.01N NaOH/g sample for 3 hours, with magnetic stirrer at low setting.

2. Determination

2.1 Pipette portions (0, 0.6, 1.0, 1.4, and 1.8 ml) of diluted suspension into duplicate of test tubes and adjust to 2.0 ml with water. See Note 2.

2.2 Add 2 ml trypsin solution (reagent 3) to each test tube and place in water bath at 37 °C. Mix.

2.3 Add 5 ml substrate solution (reagent 2) previously warmed to 37 °C, and exactly 10 minutes later, stop reaction by adding 1 ml acetic acid solution (reagent 4). Mix

2.4 Blank preparation: Add 5 ml reagent 2 to 2 ml sample extract, incubate mixture at 37 °C for 10 minutes, and add 1 ml reagent 4 followed by addition of 2 ml reagent 3.

Expression of activity

One trypsin unit is arbitrarily defined as increased of 0.01 absorbance unit at 410 nm per 10 ml of reaction mixture under conditions use herein. Trypsin inhibitor activity is expressed in terms of trypsin inhibitor unit (TIU).

Notes

1. pH of suspension should be between 8.4 and 10.0.
2. Solutions should be diluted to the point where 1 ml produces trypsin inhibition of 40-60%. This reduces relative standard deviation.
3. Prepare BAPA daily and keep it at 37°C while in use.
4. TIU is different between 0 ml extract reading and reading for each volume of extract (ml) taken minus blank reading. TIU/ml is TIU/ml of extract taken.

APPENDIX G

Phytate

Principle

Phytate is extracted from duplicate test portions of dried foods using dilute HCl. Extract is mixed with EDTA-NaOH solution and placed on an ion-exchange column. Phytate is eluted with 0.7 M NaCl solution and wet-digested with mixture of concentrated HNO_3-H2SO_4 to release P, which is measured calorimetrically. Amount of phytate in original test portion is calculated as hexaphosphate equivalent.

Apparatus

1. Glass barrel columns: 0.7 x 15 cm, equipped with value (Econo-columns, Bio-Red Laboratories, or equivalent).
2. Anion exchange resin: AG1-X4, 100-200 mesh, chloride form (bio-Red Laboratories). Check resin (according to method below) by measuring recovery of purified sodium phytate.
3. Micro-Kjeldahl flasks: 100 ml, or 25 x 200 mm digestion tubes.
4. Micro-Kjeldahl digestion rack
5. Spectrophotometer: to read at 640 nm

Reagents

1. HCl - 2.4 %, Add 54 ml to 1 L volumetric flask and test portion to volume with H_2O.
2. NaCl solutions – 0.1 and 0.7 M

3. Phosphate standard solution – 80 μm/ml. Weigh 0.350 g dried, desiccated potassium acid phosphate (primary standard) into 1 L volumetric flask, add 500 ml H_2O and 10 ml 5 M H_2SO_4 and dilute to volume with H_2O.

4. Molybdate solution – 2.5 % ammonium molybdate in 0.5 M H_2SO_4. Dissolve 12.5 g ammonium molybdate in 200 ml H_2O. Transfer to 500 ml volumetric flask, add 50 ml H_2SO_4 and dilute to volume with H_2O. Solution is stable.

5. Sulfonic acid reagent (1- amino -2- naphthol - 4 – sulfonic acid) – Dissolve 0.16 g 1- amino -2- naphthol - 4 – sulfonic acid, 1.92 g Na_2SO_3, and 9.60 g $NaHSO_3$ in 90 ml H_2O. Quantitatively transfer to 100 ml volumetric flask. Heat to dissolve if necessary. Dilute to volume with H_2O. (Store in brown bottle in refrigerator, prepare fresh weekly.)

6. Na_2EDTA-NaOH reagent – In 250 ml flask, stir 10.23 g Na_2EDTA (0.11 M) and 7.5 NaOH (0.75 M). Dilute to volume with H_2O. Solution is stable.

Preparation of phosphate standard curve

1. Adjust spectrophotometer to 640 nm, and equilibrate ≥ 15 minutes.

2. Pipet 1.0, 3.0, and 5.0 ml P standard solution into 50 ml volumetric flasks. Add about 20 ml H_2O, mix thoroughly. Add 2 ml molybdate solution, mix well, dilute to volume with H_2O, and mix well. Wait 15 minutes, and read in spectrophotometer at 640 nm. See **Table 38** for calculations for typical standard curve.

Procedure

1. Accurately weigh about 2.0000 g test portion and place in 125 ml erlenmeyer. Add 40 ml 2.4 % HCl (20 ml of 2.4 % HCl/g test portion). Cover flask and shake vigorously 3 hours at room temperature.

2. Meanwhile, prepare columns. Add 3 ml H_2O to empty mounted column and then pour H_2O slurry of 0.5 g resin into column. After resin bed has formed, wash column with 15ml of 0.7 M NaCl. Wash column with 15 ml H_2O.

3. Remove test solution from shaker and filter with vacuum through Whatman NO.1 paper. Extract is stable at least 1 week if refrigerated.

4. Prepare blank by mixing 1 ml 2.4 % HCl with 1 ml Na_2EDTA-NaOH reagent, dilute to 25 ml with H_2O, and pour mixture onto column.

5. Pipet 1.0 ml filtrate into 25 ml glass - stoppered graduate. Add1.0 ml Na_2EDTA-NaOH reagent, dilute to 25 ml with H_2O. Mix and quantitatively transfer to column; discard eluate. Elute with 15 ml H_2O; discard eluate. Elute with 15 ml 0.1 M NaCl; discard eluate. Elute with 15 ml 0.7 M NaCl; collect this 0.7 M fraction in digestion vessel. Add 0.5 ml H_2SO_4 and 3.0 ml HNO_3 to flask. Add 15 ml H_2O through column. After 1 week or 3 test solutions, discard old resin and replace with fresh resin.

6. Digest under hood on micro - Kjeldahl rack over medium heat until active boiling cases and cloud of thick yellow vapor fills neck of flask. Heat contents 5 minutes more on medium heat, 5 minutes on low heat, then off burner.

7. When flask is cool, add about 10 ml H_2O, swirl, or heat flask on low temperature setting if necessary to dissolve salt. Continue heating flask on low temperature 10 minutes. Let solution cool. Quantitatively transfer solution to 50 ml volumetric flask. Add 2.0 ml molybdate solution; mix well. Add 1.0 ml sulfonic acid reagent; mix well. Dilute to volume, mix well, let stand 15 minutes, and read absorbance at 640 nm. Calculate phytate concentration:

$$\text{Phytate (mg / 100 g sample)} = \frac{\text{“mean } K\text{”} \times A \times 20 \times 100}{0.282 \times 1000 \times \text{sample wt.}}$$

where

A = absorbance

mean K = standard P (µg)/A/n(standards)

Phytate = 28.2 % P

Table 38 Calculations for typical standard curve

ml, standard	μg P	A	Concentration / *A (K)*
1.0	80	0.1805	443.21
3.0	240	0.5160	465.12
5.0	400	0.852	469.4
"Mean *K*"			459.27

APPENDIX H

Water/oil binding capacity

0.5 g of sample was weighed in 50 ml centrifuged tube then sharked into 10 ml of distilled water at 350 rpm for 1 hour at room temperature.

This protein suspension was then centrifuged at 6000 rpm for 20 minutes, after that decanting the supernatant at 45° slanting for 15 seconds, and the weight of sample was measured.

Water holding capacity was expressed as gram of water per gram of protein sample.

Oil holding capacity was expressed as gram of soybean oil held per gram of protein sample.

$$\frac{\text{Weight of sample after shaking (g)}}{\text{Weight of sample at the initiation (g)}}$$

APPENDIX I

Foaming capacity and stability

Foaming capacity and foaming stability were determined in triplicate, 100 milliliters of the solution (2 % w/v in deionized water) of soy flour was blended for 5 minutes using a high speed blender, poured into a 250 ml graduated cylinder, and immediately recorded for the volume of foam and liquid. Protein foams were left undistributed at 25 °C for each 30 minutes, standing for 8 hours, the volume of liquid generated beneath the foam was measured for calculation foaming stability, using the following equations:

Foaming capacity (%)

$$= \frac{(\text{volume after whipping} - \text{volume before whipping})}{\text{volume before whipping}} \times 100$$

Foaming stability (%)

$$= \frac{\text{foam volume after time } t}{\text{Initial foam volume}} \times 100$$

APPENDIX J

Emulsion activity and emulsion stability

Emulsion activity

Sample of 100 ml of 7 % (w/v) suspension in 600 ml graduated beaker was stirred by magnetic stirrer until well mixed. Then 100 ml of soybean oil was added, the mixture was homogenized using a homogenizer (IKA ULTRA TURAX-T25 with dispersing tool, S25-25F) at maximum speed for 1 minute. The emulsion was centrifuged in four 50 ml graduated centrifuged tubes (25 ml) at 1300 rpm for 5 minutes, and the volume of the emulsion left was measured. Emulsion activity (EA) was calculated as follow:

$$EA = \frac{\text{volume of emulsified layer}}{\text{volume of whole layer in centrifuge tube}} \times 100$$

Emulsion stability

To determine the emulsion stability (ES), emulsion prepared by the above procedures were heated at 80 °C (using water bath) for 30 minutes, cooled to room temperature, and centrifuged in four 50 ml graduated centrifuged tubes (25 ml) at 1300 rpm for 5 minutes. ES was calculated as follow:

$$ES = \frac{\text{volume of remaining emulsified layer}}{\text{volume of whole layer in centrifuge tube}} \times 100$$

APPENDIX K

Gelation

To determine gelation or gel formation, water dispersions of the sample at concentrations in the range 2-20 % (w/v) with increments of 2 % were prepared. Aliquots of 5 ml were transferred to each of three test tubes for each concentration, and heated for 1 hour in a boiling water bath, followed by rapid cooling under running tap water and then stored at 4 °C for 2 hours. The least gelation concentration when the sample did not fall or slip from the inverted test tube.

The time needed to form a gel was measured by Deshpande (72). Suspensions of the samples were prepared in the previously determined minimum flour concentration, placed in a water bath at 80 ºC, and observations made at 2 minutes interval. The following indices are used to describe the observed situation.

Gelling		Gel structure
−	No gelling	Liquid
+ −	Some floccules	Pourable
+ + −	Almost homogenous gel	Gel remains fixed on turning the tube upside down
+ + +	Complete gelation	Gel remains fixed on shaking the tube upside down

APPENDIX L

Nitrogen solubility index

To determine nitrogen solubility index (NSI), weight 5 g of the sample into a 500 ml beaker. Measure 200 ml of distilled water at 30 °C. Add a small portion of the water at a time and disperse it thoroughly with a stirring rod. Stir in the remainder of the water, using the last of it if to wash off the stirring rod.

Transfer the mixture to a 250 ml volumetric flask by carefully washing out the contents of into the flask. Shake the mixture at 120 rpm with the mechanical shaker for 120 minutes at 30 °C with the volumetric flask immersed in the 30 °C water bath.

Allow standing for a few minutes and decant off about 40 ml in to a 50 ml centrifuge tube. Centrifuge 10 minutes at 1500 rpm and decant supernatant through a funnel containing a plug of glass fiber (be careful not to transfer any of the centrifuged solids to the filter). Collect the clear filtrate in a 100 ml beaker.

Pipet 25 ml of the clear liquid into a Kjeldahl flask; then proceed according to standard practice for determining protein nitrogen and total nitrogen in the sample.

$$\text{Water – soluble nitrogen, \%} = \frac{(B - S) \times N \times 0.14}{\text{Mass of sample}} \times 100$$

B = volume, ml of alkali back titration of blank

S = volume, ml of alkali back titration of sample

N = normality of alkali used

Nitrogen solubility index (NSI)

$$\text{NSI} = \frac{\text{\% water – soluble nitrogen}}{\text{\% total nitrogen}}$$

APPENDIX M

Oil uptake

The oil uptake criterion, U_R, was utilized to evaluate the effectiveness of each dietary fiber to reduce oil absorption during frying (34). The value of U_R was derived from the equation below. The difference between moisture content before and after frying was used to calculate water removed and oil uptake as a difference between the initial and final fat concentration of the products.

$$U_R = \frac{\text{oil uptake (g)}}{\text{water removed (g)}}$$

APPENDIX N

แบบสอบถามการประเมินผลทางประสาทสัมผัส
ผลิตภัณฑ์ขนมโดนัทเค้กเสริมแป้งถั่วเหลือง

รหัสตัวอย่าง........ วันที่............................ เวลา................ อายุ................ปี เพศ...................

ตอนที่1 เมื่อท่านได้รับผลิตภัณฑ์ ****อย่าเพิ่งชิม**** กรุณาให้คะแนนความชอบของท่าน***โดยการมอง*** และขีดเครื่องหมาย √ ลงในช่องที่ตรงกับความเห็นของท่านมากที่สุด

<u>ตอนที่ 2</u> หลังจากที่ท่าน **_*ได้ชิมผลิตภัณฑ์แล้ว*_** * กรุณาขีดเครื่องหมาย √ ลงในช่องที่ตรงกับความรู้สึก และความเห็นของท่านมากที่สุด

1. ความชอบต่อลักษณะโดยรวมเมื่อได้ชิมผลิตภัณฑ์	2. ความชอบเฉพาะรสชาติ (กลิ่นและรส)	3. ความชอบในลักษณะเนื้อสัมผัส (กรุณาอ่านหมายเหตุ **)
.... ชอบมากที่สุด	.... ชอบมากที่สุด	.... ชอบมากที่สุด
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เฉยๆ* หมายถึง ความรู้สึกระหว่างชอบและไม่ชอบ

หมายเหตุ ** ความชอบในลักษณะเนื้อสัมผัส ขึ้นอยู่กับชนิดของผลิตภัณฑ์นั้นๆ เช่น

ขนมโดนัท ให้ท่านสังเกตความละเอียดของเนื้อ ฟองอากาศและความนุ่ม

APPENDIX O

แบบสอบถามการประเมินผลทางประสาทสัมผัส
ผลิตภัณฑ์ขนมเค้กเสริมแป้งถั่วเหลือง

รหัสตัวอย่าง....... วันที่......................... เวลา................. อายุ...............ปี เพศ...................

ตอนที่1 เมื่อท่านได้รับผลิตภัณฑ์ ***อย่าเพิ่งชิม*** กรุณาให้คะแนนความชอบของท่าน***โดยการมอง*** และขีดเครื่องหมาย √ ลงในช่องที่ตรงกับความเห็นของท่านมากที่สุด

1. ความชอบต่อลักษณะโดยรวมเมื่อได้เห็นผลิตภัณฑ์	2. สีของผลิตภัณฑ์	3.ลักษณะของผลิตภัณฑ์ เช่น ความนูน ความฟู ความละเอียด/หยาบของเนื้อ
.... ชอบมากที่สุด	.... ชอบมากที่สุด	.... ชอบมากที่สุด
.... ชอบมาก	.... ชอบมาก	.... ชอบมาก
.... ชอบปานกลาง	.... ชอบปานกลาง	.... ชอบปานกลาง
.... ชอบเล็กน้อย	.... ชอบเล็กน้อย	.... ชอบเล็กน้อย
.... เฉยๆ*	.... เฉยๆ*	.... เฉยๆ*
.... ไม่ชอบเล็กน้อย	.... ไม่ชอบเล็กน้อย	.... ไม่ชอบเล็กน้อย
.... ไม่ชอบปานกลาง	.... ไม่ชอบปานกลาง	.... ไม่ชอบปานกลาง
.... ไม่ชอบมาก	.... ไม่ชอบมาก	.... ไม่ชอบมาก
.... ไม่ชอบมากที่สุด	.... ไม่ชอบมากที่สุด	.... ไม่ชอบมากที่สุด
ข้อเสนอแนะ..............................	ข้อเสนอแนะ..............................	ข้อเสนอแนะ..............................
....	....	....
....	....	....

เฉยๆ* หมายถึง ความรู้สึกระหว่างชอบและไม่ชอบ

****ต่อหน้าหลัง****

<u>ตอนที่ 2</u> หลังจากที่ท่าน ****ได้ชิมผลิตภัณฑ์แล้ว**** * กรุณาขีดเครื่องหมาย √ ลงในช่องที่ตรงกับความรู้สึก และความเห็นของท่านมากที่สุด

1. ความชอบต่อลักษณะโดยรวมเมื่อได้ชิมผลิตภัณฑ์	2. ความชอบเฉพาะรสชาติ (กลิ่นและรส)	3. ความชอบในลักษณะเนื้อสัมผัส (กรุณาอ่านหมายเหตุ **)
.... ชอบมากที่สุด	.... ชอบมากที่สุด	.... ชอบมากที่สุด
.... ชอบมาก	.... ชอบมาก	.... ชอบมาก
.... ชอบปานกลาง	.... ชอบปานกลาง	.... ชอบปานกลาง
.... ชอบเล็กน้อย	.... ชอบเล็กน้อย	.... ชอบเล็กน้อย
.... เฉยๆ*	.... เฉยๆ*	.... เฉยๆ*
.... ไม่ชอบเล็กน้อย	.... ไม่ชอบเล็กน้อย	.... ไม่ชอบเล็กน้อย
.... ไม่ชอบปานกลาง	.... ไม่ชอบปานกลาง	.... ไม่ชอบปานกลาง
.... ไม่ชอบมาก	.... ไม่ชอบมาก	.... ไม่ชอบมาก
.... ไม่ชอบมากที่สุด	.... ไม่ชอบมากที่สุด	.... ไม่ชอบมากที่สุด
ข้อเสนอแนะ..............................	ข้อเสนอแนะ..............................	ข้อเสนอแนะ..............................
....	....	....
....	....	....

เฉยๆ* หมายถึง ความรู้สึกระหว่างชอบและไม่ชอบ

หมายเหตุ ** ความชอบในลักษณะเนื้อสัมผัส ขึ้นอยู่กับชนิดของผลิตภัณฑ์นั้นๆ เช่น
ขนมโดนัท ให้ท่านสังเกตความละเอียดของเนื้อ ฟองอากาศและความนุ่ม

APPENDIX P

แบบสอบถามการประเมินผลทางประสาทสัมผัส
ผลิตภัณฑ์เบอร์เกอร์หมูเสริมแป้งถั่วเหลือง

รหัสตัวอย่าง...............วันที่............................เวลา........................อายุ....................เพศ........................

<u>ตอนที่1</u> เมื่อท่านได้รับผลิตภัณฑ์ ***อย่าเพิ่งชิม*** กรุณาให้คะแนนความชอบของท่าน ***โดยการมอง*** และขีดเครื่องหมาย √ ลงในช่องที่ตรงกับความเห็นของท่านมากที่สุด

1. ความชอบต่อลักษณะโดยทั่วไป หมายถึง ความชอบของท่านเมื่อได้เห็นผลิตภัณฑ์

....................ชอบมากที่สุด
....................ชอบมาก
....................ชอบปานกลาง
....................ชอบเล็กน้อย
....................เฉยๆ*
....................ไม่ชอบเล็กน้อย
....................ไม่ชอบปานกลาง
....................ไม่ชอบมาก
....................ไม่ชอบมากที่สุด

ข้อเสนอแนะ...
....
....

2. สีของผลิตภัณฑ์

....................เข้มมาก
....................เข้มเล็กน้อย
....................กำลังดี
....................อ่อนเล็กน้อย
....................อ่อนมาก

ข้อเสนอแนะ..
....
....

เฉยๆ* หมายถึง ความรู้สึกระหว่างชอบและไม่ชอบ

<u>ต่อหน้าหลัง**</u>**

ตอนที่2 หลังจากที่ท่าน ***ได้ชิมผลิตภัณฑ์แล้ว*** กรุณาขีดเครื่องหมาย √ ลงในช่องที่ตรงกับความรู้สึกและ ความเห็นของท่านมากที่สุด

1. ความชอบโดยรวม

...............ชอบมากที่สุด
...............ชอบมาก
...............ชอบปานกลาง
...............ชอบเล็กน้อย
...............เฉยๆ*
...............ไม่ชอบเล็กน้อย
...............ไม่ชอบปานกลาง
...............ไม่ชอบมาก
...............ไม่ชอบมากที่สุด

ข้อเสนอแนะ...............................
....
....

2. รสชาติของผลิตภัณฑ์

...............เข้มไป มาก
...............เข้มเล็กน้อย
...............กำลังดี
...............อ่อนเล็กน้อย
...............อ่อนไป มาก

ข้อเสนอแนะ...........................
....
....

3.ลักษณะเนื้อสัมผัสของผลิตภัณฑ์

..........แข็ง/แน่น/แห้งไป มาก
..........แข็ง/แน่น/แห้งไป
..........กำลังดี
..........นิ่ม/เละ/แฉะไป
..........นิ่ม/เละ/แฉะไป มาก

ข้อเสนอแนะ.............................
....
....

เฉยๆ* หมายถึง ความรู้สึกระหว่างชอบและไม่ชอบ

BIOGRAPHY

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