PHYSICAL DEVELOPMENT AND PHYSICOCHEMICAL CHARACTERIZATION OF A SOY-BASED CREAM CHEESE

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by

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LIST OF ABBREVIATIONS

Abbreviations	Caption
ACE	Angiotensin I-converting enzyme
ANOVA	Analysis of variance
CHD	Coronary heart disease
DCC	Commercial dairy cream cheese
DE	Dextrose equivalent
FAME	Fatty acid methyl ester
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GC-FID	Gas chromatography-flame ionization detector
GDL	Glucono-δ-lactone
HDL	High-density lipoprotein
HDLC	High-density lipoprotein cholesterol
IEP	Isoelectric point
LDL	Low-density lipoprotein
LDLC	Low-density lipoprotein cholesterol
LVE	Linear viscoelastic
MD	Maltodextrin
MTG	Microbial transglutaminase

MUFA	Monounsaturated fatty acids
OFS	Oscillatory frequency sweep
OSS	Oscillatory stress sweep
OTS	Oscillatory time sweep
PUFA	Polyunsaturated fatty acids
RSM	Response surface methodology
SAOS	Small amplitude oscillatory shear
SCC	Soy-based cream cheese
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEM	Scanning electron microscope
SFA	Saturated fatty acids
SPI	Soy protein isolate
S1	SCC with firmness similar to that of the DCC
S2	SCC with minimum concentration of MTG
S3	SCC with minimum concentration of MD
S4	SCC with minimum concentration of SPI
S5	SCC with minimum concentrations possible of MTG, MD and SPI as predicted by the model.
TFA	*Trans* fatty acids
TG	Transglutaminase
TPA	Texture profile analysis
UFA	Unsaturated fatty acids

WHC	Water holding capacity
WHO	World Health Organization

PERKEMBANGAN FIZIKAL DAN PENCIRIAN FISIKOKIMIA KEJU KRIM SOYA

ABSTRAK

Keju krim soya (SCC) dihasilkan dengan tekstur yang menyerupai keju krim tenusu (DCC) dengan penambahan enzim mikrob transglutaminase (MTG), soya protein terasing (SPI) dan maltodekstrin (MD). Kaedah permukaan gerak balas (RSM) digunakan untuk menentukan kesan-kesan MTG, MD dan SPI terhadap kepadatan (*firmness*) SCC. Model tertib kedua menunjukkan keputusan yang signifikan dengan variasi serendah 9.76% dan model ini berjaya meramalkan dan menghasilkan model SCC dengan kepadatan yang menyerupai DCC. MTG dan SPI didapati meningkatkan kepadatan SCC manakala MD mengurangkan kepadatan SCC. Analisis Profil Tekstur (TPA) menunjukkan ciri-ciri tekstur SCC dipengaruhi oleh kepekatan MTG dan SPI. Pengukuran reologi menunjukkan SCC mempunyai kecenderungan kepada ciri-ciri pepejal pada suhu bilik, tetapi kurang kekenyalan pada suhu penyejukkan dibandingkan dengan DCC. Mikrograf yang diperolehi daripada penilaian mikroskop elektron penskanan (SEM) mengesahkan perubahan tekstur SCC adalah disebabkan oleh enzim hubung silang. Profil elektroforesis poliakrilamida gel natrium dodecyl sulfat (SDS-PAGE) SCC mengilustrasikan kesan-kesan daripada hubung silang dalam protein soya oleh MTG. Analisis fizikokimia menunjukkan SCC mengandungi nilai pemakanan yang tinggi dibandingkan dengan DCC disebabkan oleh komposisi lemak yang rendah (kurang daripada 2% w/w), pengurangan asid lemak tepu, pengurangan nisbah asid lemak tepu kepada asid lemak tidak tepu (SFA/UFA) dan kandungan lemak trans yang rendah (kurang daripada 0.01% w/w)

PHYSICAL DEVELOPMENT AND PHYSICOCHEMICAL CHARACTERIZATION OF A SOY-BASED CREAM CHEESE

ABSTRACT

Soy-based cream cheese (SCC) was developed with textural properties similar to those of commercial dairy cream cheese (DCC) via the addition of microbial transglutaminase (MTG), soy protein isolate (SPI) and maltodextrin (MD). Response surface methodology (RSM) was employed to determine the effects of MTG, MD and SPI on firmness of SCC. The second-order model was significant with only a 9.76% variation not explained by the model and this model successfully predicted and developed a SCC model with similar firmness as that of DCC. MTG and SPI were found to increase the firmness of SCC, whereas MD decreased the firmness of SCC. Texture profile analysis (TPA) showed that the textural properties of SCC were affected by the concentrations of MTG and SPI. Rheological measurements revealed that SCC was more solid-like at room temperature, but less elastic at refrigerated temperature compared to DCC. The micrographs obtained from the scanning electron microscope (SEM) affirmed the textural changes of SCC attributed to the cross-linking. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profile of SCC illustrated the effect of the cross-linking of soy proteins by MTG. Physicochemical analyses also revealed that SCC possessed higher nutritional values due to its lower fat composition (less than 2% w/w), reduced saturated fatty acid, lower ratios of saturated fatty acids to unsaturated fatty acids (SFA/UFA) and low level of *trans* fat content (less than 0.01% w/w) compared to DCC.

1.0 INTRODUCTION

1.1 Background

Commercial dairy cream cheese (DCC) is a soft unripened acid coagulated cheese that contains at least 33% of fat and less than 55% of moisture content. Conventional production of cream cheese mainly uses butterfats from dairy milk to obtain a creamy and smooth texture, thus exhibits better mouthfeel (Musfirah *et al*., 2008). However, lactose intolerance is the main setback of the consumption of dairy products. Lactose intolerance refers to gastrointestinal symptoms associated with the incomplete digestion or limited digestion of lactose. It has been estimated that up to 75% of the world's adult population have a genetically controlled limited ability to digest lactose in milk and other dairy foods. In lactase deficient individuals, the ingestion of one or two glasses of milk leads to a variety of symptoms including abdominal bloating, rumbling, cramping, flatulence and often diarrhea (Norton & Rosensweig, 1969; Savaiano & Levitt, 2000).

With the changeable food market, natural cheeses such as Mozzarella, Cheddar or cream cheese are disadvantaged due to their high production and storage costs, compositional and nutritional inflexibility (Mounsey & O'Riordan, 2008). Thus, analogue cheese and imitation cheese have emerged as an alternative to natural cheese. Additionally, the influence of health-related issues has also prompted the development of processed cheese and low-fat cheese, where the milk fat and milk protein are partially or fully replaced by non-milk based components (Cunha *et al*., 2010).

Recently, the production of soy-based foods as alternatives to dairy products has attracted much attention (Farnworth *et al*., 2007). Soy-based products provide a range of health benefits to consumers compared to dairy milk, including reduced level of cholesterol and saturated fat as well as the absence of lactose (Donkor *et al*., 2007). Soy-based product has also been categorized as a functional food attributed to soy isoflavones that have been associated with the decreased risk of breast cancer, prostate cancer and colon cancer, cardiovascular diseases, bone health problems and postmenopausal symptoms (Setchell, 2000).

Over the past decade, there has been a tendency to consume low fat products to reduce the prevalence of several chronic diseases such as obesity, cardiovascular diseases and cancers. Fats and oils of animal origin are composed primarily saturated fatty acids (SFA). High consumption of SFA is associated with hypercholesterolemia, which in turn is responsible for cardiovascular diseases (Kromhout *et al*., 1995), whereas unsaturated fatty acids (UFA) is hypocholesterolemic and lowers plasma total cholesterol when substituted for SFA (Jonnalagadda *et al*., 1995).

In order to reduce the SFA content, vegetable fat sources are used frequently. Vegetable oils are often used as the substitution of dairy fat in soy-based products because they contain high concentration of UFA which is heart healthy. However, incorporation of vegetable oils cause some problems related to firmness and overall acceptability due to its low melting point. Thus, partial hydrogenation is usually applied to vegetable oil to obtain the desired functionality. However, *trans* fatty acids are produced upon partial hydrogenation and they have been reported to raise total serum cholesterol and low-density lipoproteins (LDL) cholesterol levels (Judd *et al*., 1994).

Soy-based cream cheese (SCC) was developed in this study using response surface methodology (RSM). RSM is a robust mathematical tool and effective in analyzing responses that are affected by multiple factors and their interactions. RSM is advantageous compared to the conventional one-factor-at-a-time experiments, due to reduced number of time and experiment trials needed to evaluate single, multiple and interaction effects (Myers and Montgomery, 1995).

In order to mimic the texture of DCC, cross-linking enzyme was incorporated into SCC. Textural characteristics and viscoelastic properties of SCC were further evaluated via texture analyzer and oscillatory rheometer. To our knowledge, no information is available on the utilization of cross-linking enzyme to develop cream cheese using soy as a base.

1.2 Aim and Objective of Research

The aim of this study was to develop a non-dairy cream cheese from soy, that is texturally comparable to commercial dairy cream cheese and was *trans* fat free. Thus, the specific objectives of this study were:

1. To develop a soy-based, *trans* fat free cream cheese.
2. To determine the physical properties of developed soy-based cream cheese.
3. To characterize the physicochemical properties of the developed soy-based cream cheese.

2.0 LITERATURE REVIEW

Parts of section 2.0 have been published. *Yeo, SK, Ooi, LG, Lim, TJ & Liong, MT. (2009). Antihypertensive properties of plant-based prebiotics. Int J Mol Sci, 10, 3517-3530.*

2.1 Commercial dairy cream cheese (DCC)

2.1.1 General description and cream cheese varieties

Cream cheese is defined as the soft, mild, rich, unripened cheese with a creamy white colour and a mild acid flavour. It is usually prepared from cream or a mixture of cream and milk, skim milk, concentrated milk, concentrated skim milk and non fat dry milk solids and coagulated by acidification with starter culture (Gigante *et al*., 2006). Traditionally, cream cheese mix is pasteurized, homogenized, inoculated with lactic culture with or without rennet and held at 23 °C until it attains a pH of approximately 4.6 (Sainani *et al*., 2004). The curd is heated to kill the bacteria and subsequently stabilizers, emulsifiers and salt are added. Gum karaya, gum tragacanth, carob bean gum, locust bean gum, gelatin and alginate can be used as stabilizers and thickeners.

Cream cheese is often categorized based on the different fat contents in the initial mix and the final composition (Phadungath, 2005). In the United States, the Food and Drug Administration (FDA) regulations states that cream cheese has to have at least 33% of fat and not more than 55% of moisture content. In France, the cream cheese has to have at least 75% of fat in dry matter while the Canadian standard for cream cheese requires at least 30% of fat content (Sanchez *et al*., 1996). According to the Malaysian Food Act 1983 and Regulation 1985, cream cheese shall contain not more than 55% of water and 0.5% of stabilizer or emulsifier as permitted food conditioner and shall contain not less than 65% of milk fat on a water free basis. Cream cheese is one of the most famous soft cheese products in North America. It is used as a spread due to its smooth texture and spreadability at refrigerated temperature. Cream cheese is also used as a salad dressing and as an ingredient in baked goods, desserts and other food products (Sainani *et al*., 2004; Phadungath, 2005).

2.1.2 Issue of lactose intolerance and fat content

Lactose is the principle carbohydrate in human and animal milk and it cannot be absorbed as such but a unique intestinal enzyme, lactase, a beta-galactosidase is needed to hydrolyze lactose. Lactase breaks the bonding between glucose and galactose thus freeing them for absorption (Saavedra & Perman, 1989). Among dietary sugars, lactose is least hydrolyzed due to a lack of lactase enzyme, leading to lactose maldigestion. The majority of the undigested lactose would reach the colon and metabolized by indigenous microorganisms to short chain organic acids, hydrogen, methane, and carbon dioxide gases. Some of the organic acids are absorbed into bloodstream, while some may be excreted in the feces (Savaiano & Levitt, 2000).

Lactose intolerance refers to gastrointestinal symptoms associated with the incomplete digestion or limited digestion of lactose. In lactase deficient individuals, the ingestion of dairy milk leads to a variety of symptoms including abdominal bloating, rumbling, cramping, flatulence and often diarrhea. Several strategies are available for lactose maldigesters to improve lactose tolerance while consuming dairy foods. Yogurt with active microbial cultures, lactose hydrolyzed milk or commercial oral enzyme replacement therapy are often applied to improve the digestion of lactose (Jiang *et al*., 1996; Mustapha *et al*., 1997). The concern of lactose intolerance has introduced other alternatives such as soy products. Varieties of soy products such as soy-based infant formulae, calcium fortified soymilk, soy-based cheese alternatives and non-dairy frozen desserts have been developed for lactase deficient individuals (Golbitz & Jordan, 2006).

The apparent relationship between dietary fat and development of cardiovascular diseases, hypertension and obesity has prompted to demand for reduced fat products. Fats and oils of animal origin, such as butter and lard comprise primarily SFA. The high consumption of SFA, together with that of cholesterol has been found responsible for hypercholesterolemia and increased risk of metabolic diseases (Woodside & Kromhout, 2005). Thus, animal fat has been progressively replaced with vegetable fat sources, which are heart healthy and lead to a reduction of the risk of coronary heart diseases (CHD) (Muguerza *et al*., 2003).

2.2 Non-dairy alternatives for cheeses

2.2.1 Dairy substitutes: Low-fat cheese and processed cheese

The eating habits of consumers have changed since the 1980s, largely influenced by health-related concerns. There are thus much demand to reduce the amount of fat, sugar, cholesterol, salt and certain additives in diets (Muir *et al*., 1999). In recent years, consumer demand for dairy substitutes for cheeses has encouraged research on cheese products in which milk fat, milk protein or both are partially replaced by non milk-based components.

Consumer interest in decreasing fat intake has led manufacturers to develop low-fat and reduced-fat cheeses. However, fat is an important component for desirable flavour and textural characteristics. Low-fat and reduced-fat cheeses are usually identified as bland, rubbery and defective in colour (Drake & Swanson, 1995). In low-fat cheeses, fat globules within protein matrix are usually smaller than in full-fat cheese. Additionally, the protein dominated microstructure in low-fat cheeses produce harder and rubbery characteristics (Mistry & Anderson, 1993). Thus, low-fat cheeses and reduced-fat cheeses are usually developed via the replacement of milk fat with fat replacers.

Many commercial fat replacers are now available to produce superior low-fat cheeses. Fat replacers that are suggested for use in cheese can be categorized as protein-based and carbohydrate-based microparticulates (McMahon *et al*., 1996). Carbohydrate-based fat replacers, such as cellulose, dextrins, fruit-based fibre, grain-based fibre, hydrocolloid gums, maltodextrin, pectin and polydextrose have been typically used in dairy-type products. Similarly, protein-based fat mimetics, such as microparticulate protein and modified whey protein concentrate are incorporated in dairy products including reduced-fat butter, low-fat cheese and fat-free ice creams (Jonnalagadda & Jones, 2005).

Past studies have showed that the application of fat replacers in low-fat and reduced-fat cheeses provided the desirable qualities of traditional full-fat cheese. Sipahioglu *et al*. (1999) previously reported that the fat reduction in Feta cheese via the combination of tapioca starch and lecithin as fat replacers improved overall acceptability of reduced-fat and low-fat Feta cheeses. In another study, Lobato-Calleros *et al*. (2001) reported no significant difference in sensory characteristics between the full-fat and low-fat Manchego cheese containing whey protein concentrate as a fat replacer. McMahon *et al*. (1996) also studied the development of low-fat Mozzarella cheese using protein-based and carbohydrate-based fat replacers. The addition of fat replacers into low-fat Mozzarella cheese did not significantly affect the apparent viscosity. Additionally, a greater overall meltability was achieved compared to those low-fat Mozzarella cheese without the incorporation of fat replacers.

Processed cheese is a dairy product which differs from natural cheese in the fact that processed cheese is not made directly from milk. Although the main ingredient of processed cheese is natural cheese, other protein and fat sources are replacing a portion of the natural cheeses (Kapoor & Metzger, 2008; Lee *et al*., 2004). Processed cheese is produced by blending one or more natural cheeses in the presence of emulsifying salts and other non-dairy ingredients into a smooth homogeneous product (Černíková *et al*., 2008) followed by heating and continuous mixing processes. Thus, potential pathogenic and spoilage microorganisms will be killed, and thereby extends the shelf-life of the product (Guinee, 2007). However, the availability and cost of natural cheese (type and age) are the main constraints that manufacturers have to deal with. Thus, various non-fat whey-based ingredients such as whey powder, dried permeated and whey protein concentrate have been used as a dairy substitute ingredients in processed cheese (Lee *et al*., 2006).

2.2.2 Cheese-like products: Analogue cheese and imitation cheese

Analogue cheese is defined as products made by blending individual constituents, including non-dairy fats and proteins to develop a cheese-like product to meet specific regulatory requirements (Bachmann, 2001). The milk fat and milk protein are partially or wholly replaced by non-milk based components in an analogue cheese. Calcium caseinates, sodium caseinates and vegetable oils are being widely used in the manufacturing of analogue cheese as protein sources instead of milk protein (Cavalier-Salou & Cheftel, 1991; Kiely *et al*., 1991). Additionally, the utilization of vegetable fat in analogue cheese products has gained increased popularity due to the increased awareness of the detrimental effects of cholesterol found in animal fats.

Analogue cheese is being used increasingly due to their cost-effectiveness, simplicity in production compared to natural cheese and the replacement of selected milk ingredients by cheaper and healthier vegetable products (Cunha *et al*., 2010). Such cheese-like products have gained importance in different aspects including nutritional, textural and economical. Additionally, developing cheese-like products by substituting the higher priced milk-derived ingredients with lower priced ingredients from vegetable sources can lower the overall production cost (Bachmann, 2001). The short supply of milk production in certain areas has also led to increased interest in the utilization of substitute ingredients from vegetable sources in producing analogue cheese. Analogue cheese contains less total fat, saturated fat, cholesterol and calories which are useful in reducing the risk of heart and artery diseases (Ahmed *et al*., 1995).

Similarly, imitation cheese is defined as a dairy product based on skim milk and vegetable or non-milk fat. Imitation cheese is widely used as an ingredient in prepared foods such as pizza, lasagna, cordon bleu products. Several studies have showed that replacing the protein rennet casein in imitation cheese with different native starch types resulted in desirable thermoplastic properties (Mounsey & O'Riordan, 2001; 2008). In another study, Yusoff *et al*. (1995) reported that vegetable palm oil can be used in the production of imitation cheese with organoleptic properties comparable to that of natural cheese.

In addition to the currently available analogue and imitation cheeses, there is also an increasing demand for more cheese alternatives. Thus, the utilization of soy as a base for the development of cheese-like products could be another alternative that is well suited for consumers with a preference for soy products and desire for soy health benefits.

2.3 Soy protein

2.3.1 General description

Approximately 10% of the world's soybeans are used for the production of food products such as tofu, soymilk, natto and miso. In addition, soybeans are also further processed into soy protein ingredients which are used in infant formula, dairy and meat alternatives, nutritional supplements and energy bars (Golbitz & Jordan, 2006). Soybeans are an inexpensive, high quality protein source and have gained much attention in the efforts to reduce consumption of animal products. Soybeans are the most important representative of legume proteins due to their high protein levels and high amount of the essential amino acids needed for human health. In general, soy protein is considered to be equivalent to animal protein. Soybeans contain higher amount of fat compare to other beans, grains and cereals but the fat found naturally in soybeans is categorized as healthy and approximately 50% of the fat in soybeans is linoleic acid, an essential polyunsaturated fat that lowers blood cholesterol levels (Golbitz & Jordan, 2006).

2.3.2 Beneficial effects of soy

Soy has gained much attention due to their health promoting effects. Soybeans contain numerous biologically active compounds such as isoflavones, phytate, phytosterols and saponins. These compounds account for the hypothesized health benefits of soy foods but much evidence have suggested that isoflavones are primarily responsible for many of these benefits (Messina & Barnes, 1991). Isoflavones are subclass of flavonoids and classified as phytoestrogens. Messina (2006) showed that soybeans contain approximately 1.2 to 3.3 mg of isoflavones per gram dry weight and every gram of protein in soy foods is associated with approximately 3.5 mg of isoflavones. Isoflavones are naturally present in soybean primarily in their beta glycoside form, genistein, daidzein and glycitein. An acetyl and malonyl group can be attached to the glucose molecule, thus resulting in a total of 12 different soybean isoflavones isoforms.

Isoflavones in soy have been credited with lowering the incidence of several types of cancer. Epidemiologic studies have reported that consumption of soy protein during adolescence could reduce adult risk of breast cancer by approximately 50% (Shu *et al*., 2001). Wu *et al*. (2002) also showed that high consumption of soy throughout life was associated with a one third reduction in the risk of breast cancer. Additionally, the International Prostate Health Council concluded that isoflavones could prevent latent prostate cancer from progressing to a more advanced level (Griffiths, 2000). Recent studies have illustrated that soy foods could reduce osteoporosis in perimenopausal or postmenopausal women and bone loss (Messina *et al*., 2004). Nagata *et al*. (2001) examined the effects of soy foods on menopausal symptoms and suggested that isoflavones help to alleviate pre-menopausal hot flashes. The United States FDA also approved a health claim for the cholesterol lowering effects of soy protein. FDA established that a 25 g of soy protein per day is necessary for cholesterol reduction. A study by Anderson *et al*. (1995) found that soy protein could lower total cholesterol level and a 12.9% decrease in low-density lipoprotein cholesterol (LDLC) levels. The authors also found that soy protein could modestly raise high-density lipoprotein cholesterol (HDLC).

2.3.3 Current soy foods and soy-based products

The consumption of soy worldwide varies. The Asian populations primarily utilize soybeans as traditional foods namely tofu, soymilk and fermented products. On the other hand, the Western nations consume soy in the form of refined soy protein ingredients used in food processing.

Soybeans have been transformed into various forms, among which tofu being the most widely accepted soy food in the world today. Tofu is a gel-like soy food with a soft texture, white, almost cheese-like and is favoured for its versatility, mild flavour and high nutritional value (Prabhakaran *et al*., 2006). Several types of tofu are commonly consumed, namely soft, regular, firm and extra firm tofu. The soft tofu contains lower protein and fat levels and the higher water content compared to firm tofu. According to Golbitz & Jordan (2006), tofu contains between 10% to 15% of protein and 5% to 9% of fat. It is relatively low in carbohydrates and in fibre due to the removal of okara (soy pulp). Soy milk, the liquid extract of soybeans has been consumed daily as a nutritious beverage and the demand for processed and packaged soy milk has grown increasingly worldwide. Soy milk generally contains most of the active phytochemicals including high amounts of isoflavones. Soybeans fermented products such as natto and miso are consumed daily in soup broth, salad dressing and food toppings in Japan, while in Indonesia, tempeh is the most popular fermented soy food.

Soy-based products have been incorporated into various cuisines due to their health benefits. The increasing of demand for alternatives to dairy milk due to problems with allergy and the desire for vegetarian alternatives have also gained much attention. Several new commercial soy-based products are available in the market such as soy yogurt, made from pasteurized soy milk and fermented by *Lactobacillus* and *Bifidobacterium* (Farnworth *et al*., 2007). Soy-based infant formulae have also been established as a solution for those with lactose intolerance or allergic to cow's milk protein (Mendez *et al*., 2002). van Vliet *et al*. (2002) also showed that soy protein has a great potential in the substitution of dairy proteins and meat such as ground meat, chicken and fish. The other popular soy-based products are soy ice cream, energy bars, soy pasta and soy frozen desserts.

2.4 Soy-based cream cheese (SCC): Definition and ingredients

2.4.1 Definition

Soy-based cream cheese (SCC) is established by mixing hard tofu with refined cooking palm oil, hydrocolloids and salt. In order to produce a SCC with texture similar to that of the DCC, some enzymes and fat replacers are added. Interaction of ingredients and compatibility between them is crucial to produce desired textural properties and product stability.

2.4.2 Microbial transglutaminase (MTG)

Transglutaminase (TG: EC 2.3.2.13, protein glutamine:amine γ-glutamyl-transferase) are a group of thiol enzymes that catalyses an acyl-transfer reaction between the γ-carboxyamide group of peptide-bound glutamine residue as acyl donors and primary amines as receptors, including the ε-amino group of lysine residues in certain proteins to form ε(γ-glutamyl)lysine bonds (G-L bonds). If amine substrates are not available, TG catalyses the deamination of glutamine residues where water molecules act as acyl receptors (Motoki & Seguro, 1998). TG can modify proteins by means of amine incorporation, cross-linking and deamination. New intra- and inter-molecular cross-links and high molecular weight of polymers are produced as a result of these reactions. Such reactions can modify the structure and functionality of proteins (Gauche *et al*., 2008). Microbial transglutaminase (MTG) does not require calcium for reactivity, shows a broad substrate specificity and can be produced at relatively low cost. These properties are advantages for industrial applications and MTG is currently produced by fermentation using a *Streptomyces* strain.

MTG has been widely used in different food industries as a cross-linking agent, in products such as meat, fish, dairy, bakery, noodles and soy derivatives. Soy proteins such as 11S and 7S globulins, have been found to be good substrates for MTG reaction (Tang *et al*., 2006). MTG treatment has been found to increase water holding properties of tofu and resulted in a smoother and firmer texture (Nonaka *et al*., 1994). Studies of Kwan & Easa (2003) also demonstrated that MTG improved the quality and smoothness of retort tofu. Hence, MTG offers the potential of texture modulation of foods containing soy proteins.

2.4.3 Maltodextrin (MD)

Maltodextrin (MD) is an oligosaccharide consisting of α-1,4-linked glucose units and contains a mixture of maltose, maltotriose, maltotetraose, maltopentaose and higher oligosaccharides. It is produced upon incomplete hydrolysis of corn starch and comprises 13% to 27% of reducing sugars, calculated as anhydrous dextrose and expressed on a total solid basis. FDA defined MD as non sweet, nutritive saccharide polymers consisting of D-glucose units linked primarily by α-1,4 bonds with dextrose equivalent (DE) of less than 20 (Roller, 1996).

The chemical property of MD is based on the DE value. The DE value is an empirical measurement of the amount of reducing sugar present and is a reflection of the extent of starch hydrolysis. In general, the presence of free glucose and oligosaccharides with degree of polymerization below 8 will increase as the DE increases, whereas the viscosity, cohesiveness and the ability to prevent the formation of large crystal will increase as the DE decreases (Roller, 1996).

MD is commonly used as a carbohydrate-based fat mimetic, typically used in a variety of foods, including dairy type products, frozen desserts, salad dressing and spread. MD absorbs water and form gels, thus providing texture and mouth feel similar to fat and could mimic some of the functional characteristics of fat (Jonnalagadda & Jones, 2005). MD, a nutritive polysaccharide not only acts as a bulking agent replacing fat and sugar volume but has also been reported to act as fat mimetics in flour-based dry mixes, baking systems, fillings and icings. It has been substituted for 25% to 35% of fat in food products without comprising sensory characteristics, thus commonly used in low-fat formulations.

The low-DE MD (DE range of 1 to 10) has been found particularly useful in fat replacement. Additionally, one part of a low-DE MD and three parts of water can often replace four parts of fat and oil. Several commercially available low-DE MD developed specifically for fat replacement have been launched, including Paselli SA2, Instant N-Oil and Maltrin® M100 derived from potato starch, tapioca starch and corn starch, respectively (Harkema, 1996).

MD as an oligosaccharide plays the role as prebiotics and as an ingredient in functional foods. Prebiotics are non-digestible food ingredients that can escape digestion under the harsh conditions of the upper gastrointestinal tract and reach the lower gut as substrates for the fermentation by selective indigenous gut microflora. Dietary treatment has gained much attention lately as the alternative antihypertensive approach, thus prebiotics such as MD is seen as better alternatives than drug therapy to treat hypertension considering that prebiotics has a long history of safe use and has been a natural component present in our foods (Yeo *et al*., 2009).

2.4.4 Soy protein isolate (SPI)

Soy protein isolate (SPI) comprises 85% to 90% of pure soybean protein. SPI is obtained from defatted soy flake that is deprived of fat, soluble sugars, insoluble sugars and dietary fibre. Isolated soy protein is very low in flavour, highly digestible and easy to use in food processing (Golbitz & Jordan, 2006). The functional properties of SPI reflect the composition and structure of their globulins. The two major globulins in soy protein are β-conglycinin, or 7S, and glycinin, or 11S, which represent 27% and 34% respectively, of the proteins occurring in the isolate (Iwabuchi & Yamauchi, 1987). The glycinin subunits consist of two polypeptide components linked via disulfide bonds, while β-conglycinin is a trimeric glycoprotein consists of at least six combinations of three subunits, namely α, α' and β (Braga *et al*., 2006). It has been found that soy protein globulin reduced plasma cholesterol concentrations by 35% and has a marked inhibitory effect on atherosclerosis (Clair & Anthony, 2005).

SPI has been widely used in food industries as a functional ingredient due to its highly functional qualities. SPI disperse easily in water and work well as emulsifiers, helping to bind water and fat to improve textural properties. SPI has been applied in a wide range of food products due to their ability to form gels with good water holding capacity (WHC) upon heating (Braga *et al*., 2006). Walsh *et al*. (2003) also showed that SPI has good gelation, emulsifying, foaming and water absorption properties. SPI has also been widely applied in non-dairy creamers and infant formulas.

The formation of protein networks in SPI can be induced by acidification, enzymatic hydrolyzing and cross-linking processes. By manipulating SPI under various conditions, the network formation among the biopolymers can alter the physical properties of the final products and meet the specific functional properties of foods. The coagulation and gelation induced by SPI-MTG has been found to produce aggregates which were mainly composed of basic subunits glycinin and new formed high molecular weight of biopolymers. This cross-linking resulted in the polymerization of protein molecules which led to desired textural changes (Tang *et al*., 2005).

2.5 Fatty acids

Fatty acids are hydrocarbon chains with terminal methyl and carboxyl groups. There are numerous fatty acids that occur naturally with different chain length, number of double bonds and type of double bonds (Woodside & Kromhout, 2005). Among those fatty acids, probably less than twenty are quantitatively important in the human diet. Most of the natural food sources contain a wide variety of saturated fatty acids (SFA) and unsaturated fatty acids (UFA) (Baylin *et al*., 2007). Dietary fatty acids are required for membrane synthesis, development of various structural elements in cells and tissues and as an energy source (German & Dillard, 2004).

2.5.1 Saturated fatty acids (SFA)

Saturated fatty acids (SFA) have no double bonds between carbon atoms of the fatty acid chain and are fully saturated with hydrogen atoms. Fats and oils of animal origin such as butter and lard are composed primarily of SFA (Priego-Capote *et al*., 2007). High-fat diets usually refer to the increased intakes of SFA. Dietary fat is commonly perceived as unhealthy and is based on the fact that high consumptions of SFA could lead to an elevated blood cholesterol level. This would subsequently lead to an increased risk of atherosclerosis, cardiovascular disease and CHD (German & Dillard, 2004).

Epidemiological studies have suggested that SFA increased the concentration of total cholesterol and LDL-cholesterol levels in the bloodstream and subsequently elevated cholesterol concentrations heighten the risk of heart disease (Hu & Willett, 2001). Hegsted *et al*. (1993) previously found that SFA are hypercholesterolemic and that elevated plasma and LDL-cholesterol levels increased the risk of CHD. In another study, Lauer *et al*. (2000) evaluated the lowering dietary intake of SFA in children and found that dietary changes were effective in achieving modest lowering of LDL-cholesterol.

However, it is impossible to achieve a nutritionally adequate diet that has no SFA. Since there is no upper limit for the intake level of SFA at which there is no adverse effect is set, the recommendation is to keep the intake of SFA as low as possible. The most recent recommendation for all adults is to limit the intake of SFA to less than 10% of calories (German & Dillard, 2004).

2.5.2 Unsaturated fatty acids (UFA)

Unsaturated fatty acids (UFA) contain at least one double bond within the fatty acid chain. UFA is classified as monounsaturated fatty acids (MUFA) if it contains one double bond, whereas polyunsaturated fatty acids (PUFA) possess more than one double bond. The biological effects of MUFA depend on their configuration of double bond. *Cis*-MUFA is inversely associated with the risk of CHD, but *trans*-MUFA has been shown to increase LDL-cholesterol and decrease high density lipoprotein (HDL) cholesterol (Mensink *et al*., 2003; Woodside & Kromhout, 2005). Over the past years, long chain PUFA has attracted much attention attributed to their heart healthy properties. Several studies suggest that PUFA may improve serum lipid profile and possess beneficial effects on cardiovascular disease (Préstamo & Fontecha, 2007).

Muller *et al*. (2003) previously evaluated the effects of fatty acids in the diet by comparing high SFA diet and diet with high content of MUFA and PUFA consumed by women. This study showed that total and LDL-cholesterol was lower in the women with high UFA diet compared to those with high SFA diet. In another study, DeDeckerre *et al*. (1998) reported that diet replaced with PUFA and particularly linolenic acid reduced blood cholesterol and decreased the risk of heart disease.

Although it is commonly believed that UFA is a healthy fat, excessive PUFA intake has been implicated as contributing to cancer and heart disease (Felton *et al*., 1994). Diniz *et al*. (2004) previously also showed that diet rich in PUFA increased oxidative stress in the heart compared to a low-fat diet. Thus, daily intake of PUFA as more than 10% of total energy is not recommended (Eritsland, 2000).

2.5.3 *Trans* fatty acids (TFA)

Trans fatty acids or *trans* fat (TFA) are unsaturated fatty acids containing at least one ethylenic double bond in the *trans* configuration (Destaillats *et al*., 2007). *Trans* isomers of octadecenoic acid are the major contributor of TFA intake and these compounds are found in both vegetable and animal fats. TFA is produced by anaerobic fermentation of PUFA in the rumen of lactating cows (Bauman & Griinari, 2003). Ruminant fats from dairy products (80% - 90%) and secondarily from meat fat (10% - 20%) constitute the main dietary source of *trans*-18:1 acids. Vaccenic acid (*trans*-11 18: isomer) is the main constituent of *trans*-18:1 acids in ruminant fats (Precht *et al*., 2001). Hence, the occurrence of vaccenic acid is a very unique feature of milk fat. Partially hydrogenated vegetable oil is another dietary source of TFA. Alonso *et al*. (2000)

reported that approximately one-third of all edible fats and oils in the world are hydrogenated in order to obtain higher melting point and greater stability. Part of the *cis* double bonds are isomerized into *trans* form upon partial hydrogenation. (van de Vijver *et al*., 2000).

Epidemiological study has found a strong positive association between TFA intake and risk of CHD (Hu *et al*., 1997). Clinical studies have also shown that a high TFA diet causes adverse changes in the plasma lipoprotein profile (Priego-Capote *et al*., 2007). Mensink & Katan (1990) reported that TFA increased the concentrations of total and atherogenic LDL-cholesterol and decreased the concentrations of beneficial HDL-cholesterol, thus resulting in increased risk of CHD and atherosclerosis. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) recommend that fats for human consumption should contain less than 4% of *trans* fat of the total fat (Priego-Capote *et al.,* 2007). FDA has also decreed that manufacturers must label *trans* fat on the nutrition facts beginning in January 2006 (Food and Drug Administration, 2003). The Malaysian Food Act 1983 & Food Regulation 1985 has also made provisions for nutrient content claims on low and free *trans* fat in foods. Regulation 18c, Fifth A schedule (Table 1) stated "*trans* fat free" product must contain less than 0.1 g of *trans* fat per 100 g (solids) or 0.1 g *trans* fat per 100 mL (liquids).

2.6 Response surface methodology (RSM) in product development

Response surface methodology (RSM) is a group of important statistical and mathematical techniques for developing, improving and optimizing various processes. It usually contains three stages: (1) design of experiment, (2) response surface modeling through regression, and (3) optimization (Myers & Montgomery, 1995). Study of a process often focuses on the relationship between the response and the input factors, thus response surface framework has become the standard approach for much of the experimentation carried out in industrial research, development, manufacturing and technology commercialization (Myers *et al*., 2004). RSM designs are aimed at obtaining improved process settings, troubleshoot process problems and weak points, and at making a product or process more robust against external and non-controllable influences. The main advantage of RSM is the reduced number of experiment trials needed to evaluate multiple parameters and their interactions. RSM has been successfully utilized in optimization of manufacturing conditions of food products, such as dairy tofu (Chen *et al*., 2005), extraction of pectin (Pinheiro *et al*., 2008) and production of low-fat frankfurters (Pappa *et al*., 2000). It has also been effectively utilized to optimize composition of the fermentation medium (Liew *et al*., 2005) and growth of probiotics (Fung *et al*., 2008).

Response surface often influenced by various variables, thus factorial designs are used for screening in order to select significant variables that affect the changes of responses (Myers & Montgomery, 1995). Factorial designs are more efficient than one-factor-at-a-time experiments. A factorial design is necessary when interactions may be present to avoid misleading conclusions. Additionally, factorial designs allow effects of a factor to be estimated at several levels of the other factors, resulting conclusions that are valid over a range of experimental conditions (Montgomery, 2001).

The 2^k factorial design is one of the general factorial designs widely used in research work. This design involves k factors, each at only two levels where these levels may be quantitative or qualitative. On the other hand, 3^k factorial design is a factorial arrangement with *k* factors each at three levels. Three levels of the factors are referred as low, intermediate and high. In the 3^k system of designs, the low, intermediate and high levels of factors are denoted by -1, 0 and +1, respectively. The ±1 notation is preferred because it facilitates the geometric view of the design and directly applicable to regression modeling, blocking and the construction of fractional factorials (Montgomery, 2001).

2.7 Rheological study

Rheological measurements can be categorized as small deformations and large deformations. Small deformation testing generally investigates viscoelastic parameters and frequently uses specialized dynamic mechanical analytical equipment. On the other hand, large deformations generally involves compression test and tension test, thus texture analyzer is usually employed (Lelievre *et al*., 1992). Rheological test methods for the assessment of gel characteristics is generally categorized into three groups, namely fundamental, empirical and imitative (Smewing, 1999).

2.7.1 Fundamental method

Rheology aims at measuring properties of materials that control their deformation and flow behaviour upon subjection of external forces. Thus, rheological study is mainly concerned with the relationship between strain, stress and time. The subjection of external forces will lead a deformation of solids particles (truly elastic materials) and flow in liquids (truly viscous materials) (Gunasekaran & Ak., 2002).