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THESIS

FACTORS AFFECTING ON STRETCHABILITY AND MELTABILITY OF IMITATION MOZZARELLA CHEESE

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Mozzarella production has grown considerably in recent years. The impetus for the dynamic growth of Mozzarella consumption has been the growing popularity of pizza. However, Mozzarella cost is high. Imitation Mozzarella cheese may offer an excellent opportunity to substitute a traditional product with lower cost by using caseinate. Imitation Mozzarella is manufactured by blending rice bran oil and sodium – caseinate into a smooth emulsion. Imitation Mozzarella cheese with high protein to oil ratio has high stretchability, but it is not as long as commercial Mozzarella. For meltability, imitation Mozzarella cheese with different protein to oil ratios did not show any difference in meltability. Substitution lactic acid modified sodium – caseinate at limited moisture content to sodium – caseinate could improve stretchability. Using high moisture to modify sodium – caseinate resulted in high stretchability. During modification, lactic acid and moisture cut the protein molecules into small fractions. Increasing moisture content used to modify sodium – caseinate caused more small molecular weight proteins which could slip through each other. Small molecular weight proteins also promoted imitation cheese to melt and flow leading to high meltability. Palm oil has higher melting range than rice bran oil. It could decrease meltability of imitation cheese. Moreover, palm oil could reduce stretchability of imitation cheese, due to its higher solid fat content, which might disrupt stretching fibrous of protein. Substitution rice flour and waxy rice flour to protein was done in order to improve stretchability and meltability of imitation cheese. Gelatinised starch in waxy rice flour could decrease meltability of imitation cheese. Imitation Mozzarella cheese with similar stretchability and meltability to those of commercial one could be prepared by emulsifying 20% rice bran oil in an aqueous suspension containing 16% sodium – caseinate, 7.75% modified sodium – caseinate, 1.25% waxy rice flour and 51% water.

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Student's signature   Thesis Advisor's signature

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FACTORS AFFECTING ON STRETCHABILITY AND MELTABILITY OF IMITATION MOZZARELLA CHEESE

INTRODUCTION

Mozzarella production has grown considerably in recent years. The impetus for the dynamic growth of Mozzarella consumption has been the growing popularity of pizza. The functional attributes of Mozzarella cheese include the ability to shred cleanly, melt rapidly, and exhibit the desired degree of flow, stretchability, chewiness, oiling – off and browning on baking (Bachmann, 2001). Due to it's expensive cost, imitation Mozzarella cheese may offer an excellent opportunity to substitute a traditional product and offers the same or better nutritional and textural characteristics with lower cost by using caseinate as casein micelle and using vegetable fat in place of butter fat (Lobato-Calleros *et al.,* 1997). As imitation cheese is used mainly as an ingredient in food preparation, their properties, as related to stretchability and meltability during heat treatment, are very important.

The public's increase awareness of many dangers of cholesterol found in animal fats, imitation cheese contains vegetable fat replacing butterfat has gained an increase popularity. Rice bran oil is considered to be very healthy promoting material, because of it's high level of active components such as phytosterols, γ-oryzanol and vitamin E complex (Nicolosi *et al*., 1994).

Sheehan and Guinee (2004), studied stretchability of Mozzarella cheese kept at different storage times. They found that, increasing storage time, increased stretchability and meltability of melted cheese because all storage cheese had higher level of protein breakdown than fresh cheese.

In this study we hypothesizes that small molecular weight protein occurred during acid hydrolysis of sodium caseinate might promote the stretchability of imitation mozzarella cheese and the gelatinised starch might stop the fat phase from flowing during melting process resulting in lower meltability. Therefore, our objective was to produce imitation Mozzarella cheese without milk fat by using sodium caseinate and rice bran oil which has same or better characteristics than commercial Mozzarella cheese.

OBJECTIVES

1. To study the effects of sodium – caseinate and rice bran oil ratio on the characteristics of imitation Mozzarella cheese.

2. To modify sodium – caseinate with lactic acid at limited moisture content and determine its effect on the characteristics of imitation Mozzarella cheese.

3. To investigate the effects of oil blends on the characteristics of imitation Mozzarella cheese.

4. To determine the effects of rice flour and waxy rice flour on the characteristics of imitation Mozzarella cheese.

LITERATURE REVIEW

Mozzarella cheese

Mozzarella cheese was traditionally made from water buffalo high fat milk. It is usually round or pear – shaped, soft and white with a lively surface sheen. When cut open, the cheese has a slightly juicy, milky appearance. The flavor is bland and slightly acid. Mozzarella cheese sales are continually growing due to the widespread popularity of pizzas. On cooking, cheese may be required to melt, flow, stretch and oiling off to varying degrees depending on its applications.

Functional properties

Cheese that used to be an ingredient in food should have some useful characteristics such as low moisture. Mozzarella cheese has a good melt when heated, this characteristic is suitable for making pizza or any food that require melting characteristic.

1. Functional properties before heating

1.1 Shreddability

Mozzarella cheese is usually produced in block type and must therefore be comminuted (shredded or diced) before it can be used. The term 'shreddability' is used in reference to several important functional characteristics, including the ease with which the cheese block is processed through a shredding machine; the geometry and integrity of the shreds and the ability of shreds to resist matting and remain free flowing. Normally, shredded machine is used to cut the cheese, so that shredded cheese coming out from the machine does not form gummy balls. Problems related to shreddability may occur when the body of the cheese is soft and pasty or wet, causing clog on the shredding machine and resulting shreds with ragged edges and deformed geometry. In other hand, excessively firm and dry Mozzarella cheese may take longer time to process through the shredding machine (Kindstedt *et al.*, 2004).

There are several empirical and imitative tests reported for measuring shreddability. Shreddability of cheese can be measured by using quantitative image analysis data to calculate a shreddability index as a function of size, shape and number of shredded fragments obtained upon shredding. The image analysis was correlated with sensory panel visual assessment made on the basis of length of the shreds, amount of fragments present and degree of stickiness between the shreds. The proposal of the empirical method for shreddability measurement was based on the ability of cheese particles to penetrate down through a stack of vibration sieves of decreasing mesh size. Sticky cheese that mats excessively is retained by the larger sieves, whereas cheese that remains free – flowing may penetrate to the bottom of the stack. The aggregation index calculated as a by weighted average of sieve size x mass of cheese retained by each sieve. Guinee and O'Callaghan (1996) studied the shreddability of Mozzarella cheese and Cheddar cheese by penetrating cheese through the sieve, and they found that Mozzarella cheese (3.95) had aggregation index lower than Cheddar cheese (4.04).

1.2 Texture

The texture characteristics of Mozzarella cheese could be obtained by the texture analyzer. Many researchers have used texture profile analysis (TPA) to characterize the hardness or firmness of Mozzarella cheese. Zisu and Shah (2006) studied the hardness of Mozzarella cheese made from different processes and aging times, and found that Mozzarella cheese tended to be softer with increasing aging time (Figure 1).

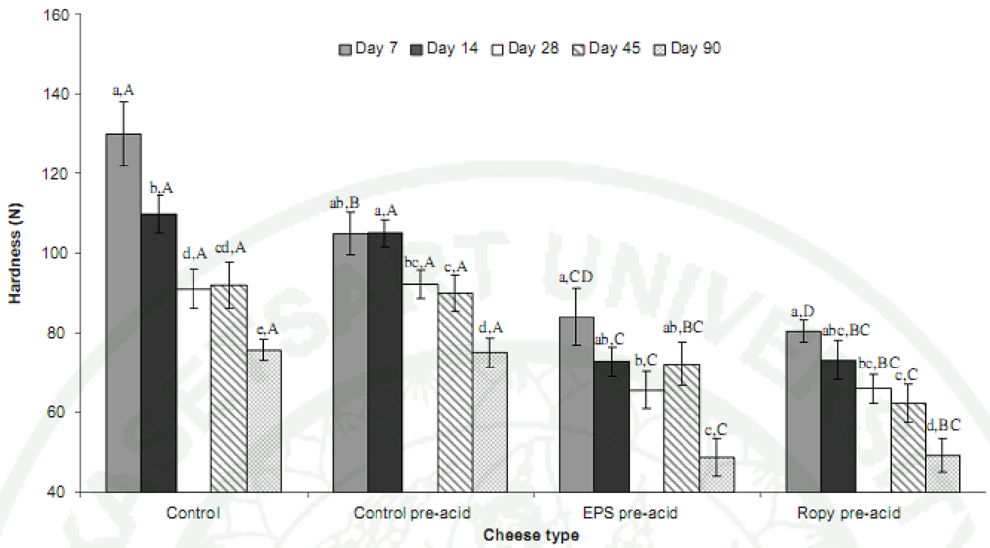

Figure 1 Hardness of Mozzarella cheese made from different processes and aging times

Source: Zisu and Shah (2006)

2. Heat – induced functional properties

Heat – induced functionalities are essential determinants of the quality and acceptability of Mozzarella cheese that is used as an ingredient in cooking applications such as for pizza. This characteristic makes it different from other cheeses that also melt such as Cheddar and Provalone (Guinee and O'Callaghan, 1996). Important heat – induced characteristics include meltability, stretchability, elasticity, oily off and baking color. In general, Mozzarella cheese should melt, flow readily to form a continuous melt with complete loss of shred identity, giving a stretching fibrous and chewy consistency.

2.1 Meltability

Meltability or flowability is the basic characteristic of Mozzarella cheese. The term 'meltability' refers to the way melted cheese flows and spreads upon heating. The meltability of cheese has been evaluated extensively by empirical tests such as Schreiber test and Arnott test. The Schreiber test measures the increasing dimension of melted cheese under standard condition. The disadvantages of this method are the noncircular cheese spread and unpredicted shape of melted cheese (Muthukumara *et al.*, 1999). Another method is the method of Olson and Price that measures the increasing distance of melted cheese in horizontal pyrex glass tube (Kosikowski, 1982).

Sameen *et al.* (2008) measured meltability of Mozzarella cheese made from buffalo milk, cow milk and combination by using Schreiber test and found that Mozzarella cheese made from the combination of buffalo and cow milk had higher meltability than that made from only buffalo or cow milk (Figure 2).

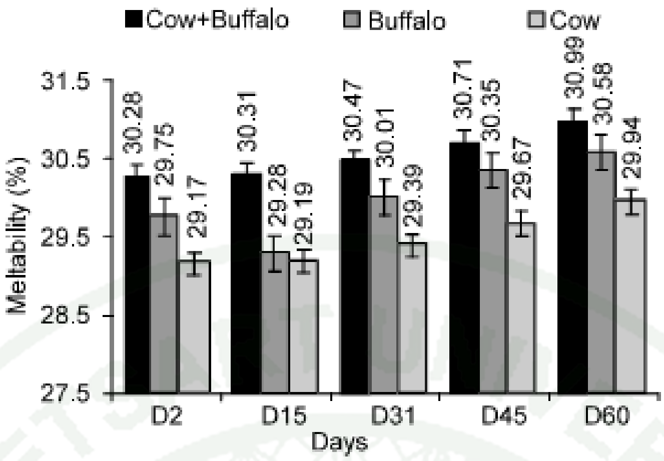

Figure 2 Meltability of Mozzarella cheese made from buffalo milk, cow milk and combination

Source: Sameen *et al.* (2008)

1.3 Stretchability

One of the most important characteristics of Mozzarella cheese is stretchability or elongational, which makes it different from other cheeses. The term 'stretchability' refers to the ability of the molten cheese to stretch and form strings when extended. There are many empirical and fundamental test methods have been proposed to measure the stretchability properties of Mozzarella cheese. Guinee and O'Callaghan (1996) developed the method to measure the distance to which the melted cheese could be stretched in horizontally before it was broken down. The method most commonly used by cheese manufacturers and pizza companies is the fork test, in which cheese is baked on pizza and then tested for how far it would stretch. In this test, shredded cheese is placed on pizza. After baking, a fork is inserted into the melted cheese and raised vertically until the cheese strands break. Fife *et al.* (2002) developed the tool for measuring stretchability of Mozzarella cheese, called tri – probe (Figure 3). The tri – probe or a three – prong spindle was lowered into the melted cheese and pulled vertically.

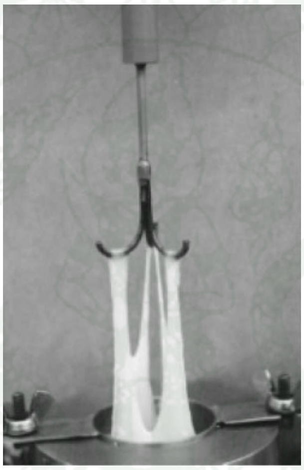

Figure 3 Strands of cheese being pulled upward during the Tri –probe stretch test

Source: Fife *et al.* (2002)

Guinee *et al.* (2001) studied the stretchability of low moisture Mozzarella cheese (LMMC) and found that the stretchability of cheese increased with increased aging times (Figure 4).

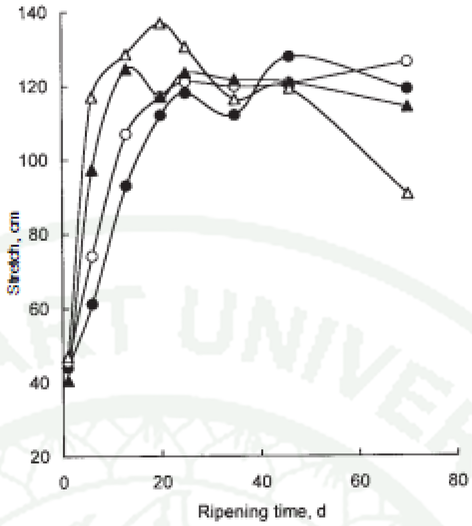

Figure 4 The stretchability of low moisture Mozzarella cheese with difference aging time

Source: Guinee *et al.* (2001)

From Figure 4, when the aging time increased, Mozzarella cheese could be stretched longer because *para* casein's water holding capacity increased during aging period.

1.4 Oiling off

Oiling off is caused by the release of free oil from the body of melted cheese. Excessive oiling off results in pools of liquid fat at the surface and throughout the body of the melted cheese, giving the cheese glossy appearance and mouthfeel. A moderate release of free oil contributes to desirable melting characteristics by creating a hydrophobic film on the cheese surface during baking, slowing down evaporative loss of moisture. Excessive dehydration during melting, as occurs when insufficient free oil is released, results in the formation of tough skin on cheese surface that inhibits flow and scorches readily.

Free oil could be measured empirically by two different approaches: (1) melting a disk of cheese on filter paper and then measuring the area of the oil ring that diffuse into the filter paper or (2) melting the centrifuging cheese to recover the free oil. In general, oiling off of Mozzarella cheese has been shown to increase with increasing fat content, decreasing salt content and increasing time of storage and level of proteolysis (Kindstedt and Rippe, 1990).

Homogenization milk before making cheese reduced oiling off because the shear of homogenizer produced a finer dispersion of fat within the cheese structure. Moreover, free oil was reduced by the addition of buttermilk solids to the cheese milk, presumably due to phospholipid – mediated enhancement of emulsification (Poduval and Mistry, 1999).

1.5 Baking color

Browning is a property of cheese that results in patches of darkened color on the cheese surface during baking before consumption. It is widely believed that the browning of cheese during baking is mainly caused by Maillard reaction which involves an interaction between reducing sugars and amino compounds. As a heat induced process, the browning of cheese starts to occur during processing and slow cooking after processing. Mozzarella cheese that contains both reducing sugars and proteolysis products is susceptible to Maillard browning reactions at high temperatures, such as pizza baking (Wang and Sun, 2003).

The browning of cheese has been evaluated objectively by reflectance colorimetry after heating of cheese. After heating and cooling, the cheese may be analyzed for three color indices, L* (light to dark), a* (red to green) and b* (yellow to blue), from which an evaluation of the intensity of brownness can be made (Kindstedt *et al.,* 2004).

Wang and Sun (2003) have been studied the effect of baking time (0 – 20 minute) on color of Mozzarella and Cheddar cheese by using reflectance colorimetry. They found that Mozzarella cheese (baked 2 – 4 minute) had more intense browning color than Cheddar cheese (baked 8 – 12 minute).

Imitation cheese

During the last decade, consumers have become increasingly aware of the importance of maintaining adequate nutrition, and on limiting their consumption of fat – rich foodstuffs to avoid obesity and heart diseases (Lobato-Calleros *et al.,* 1998). Imitation cheese offers an excellent opportunity for substituting a traditional product with a new one which offers the same or better nutritional and texture characteristics, by using caseinate as protein source and the use of vegetable fat or oil. The imitation cheese product is defined as one in which both the protein and lipid systems are derived from non – dairy sources such as soy protein, peanut protein, soy bean oil, coconut oil or palm oil, and may use hydrocolloid or gum to improve the texture characteristic (Bachmann, 2001).

Caseinate

Caseinate is a derivative of casein, which is the main protein in milk. Caseinate plays an important role in structure and texture in imitation cheese. Commercial caseinate is made from skimmilk by one or two general methods – precipitation by acid or coagulation by rennet. As much of the fat, whey proteins, lactose and minerals as possible must be removed by multistage washing in water, as they reduce the quality of the caseinate as well as its keeping quality. Dried, property produced caseinate has a relatively good keeping quality and it is used mainly in foods (Figure 5). Caseinate is usually divided into the following types;

- Rennet casein, obtained by enzymatic precipitation.
- Acid casein, obtained by acidifying skimmilk to the isoelectric point (pH 4.6 – 4.7).
- Sodium – caseinate, obtained from acid casein dissolved in sodium hydroxide.

- Calcium – caseinate, obtained from acid casein dissolved in calcium hydroxide.

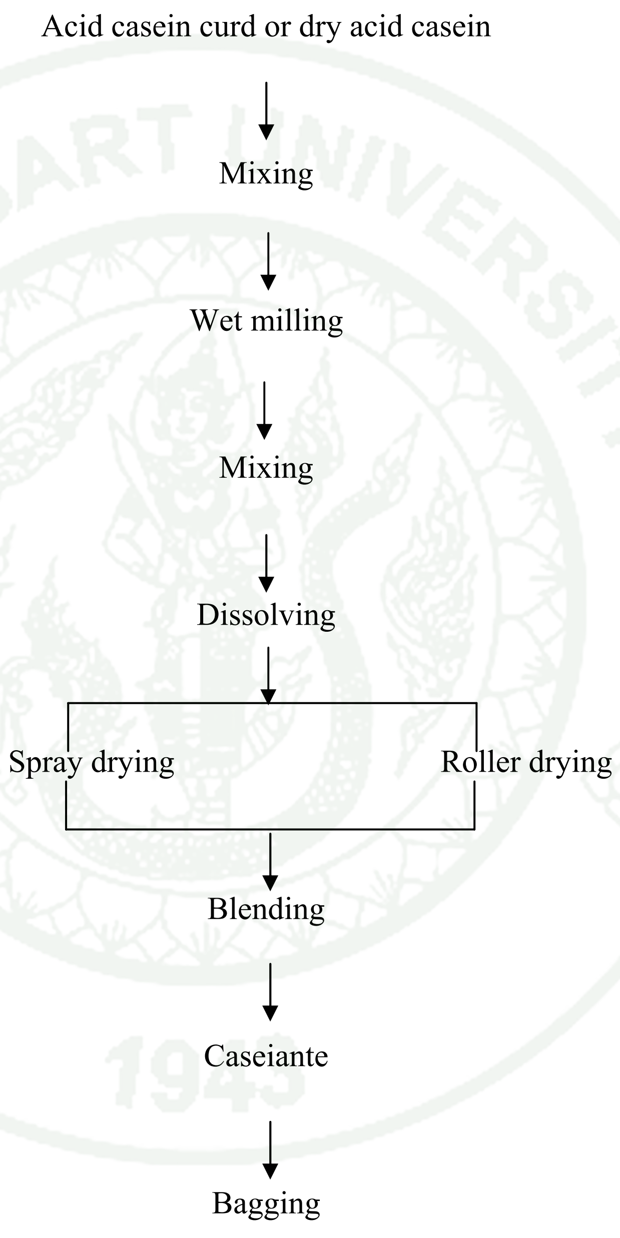

Figure 5 Caseinate productions from acid caseine

Source: Modified from Southward (1986)

Sodium – caseinate and calcium – caseinate are being widely used in the manufacture of imitation cheese. Sodium – caseinate is easily dissolved in a diluted alkali. It is often used as an emulsifier in cured meats and is found in a number of new products, such as milk and cream substitutes. For certain applications, calcium – caseinate may be chosen instead of sodium – caseinate, one reason being the wish to reduce the sodium content of the product to a minimum and viscosity of calcium – caseinate is lower than that of sodium – caseinate at the same concentration (TetraPak Processing System, 2000).

The composition of sodium – caseinate and calcium – caseinate is shown in Table 1. They have similar protein content, 91.4% and 91.2%, respectively. Sodium – caseinate contains 1.2 – 1.4 % sodium, and 0.1% calcium. In contrast, calcium – caseinate contains 1.3 – 1.6% calcium. Sodium – caseinate has lower pH than calcium – caseinate, so it can better dissolve in water (100% and 90 – 98% respectively) and it hydrates 2 times more. The difference in composition of two types of caseinates play the important role in quality of products, sodium – caseinate; highly hydrates and dissolves in water producing better emulsion and softer imitation cheese. Calcium – caseinate gives a firm imitation cheese due to bonds between calcium ion and protein molecule (Southward, 1986).

Table 1 Sodium – caseinate and calcium – caseinate composition

	Sodium caseinate (%)	Calcium caseinate (%)
Moisture	3.8	3.8
Protein (X 6.38)	91.4	91.2
Ash	3.6	3.8
Lactose	0.1	0.1
Fat	1.1	1.1
Sodium	1.2 - 1.4	<0.1
Calcium	0.1	1.3 - 1.6
Iron (mg/kg)	3.0 - 20.0	10.4
Copper (mg/kg)	1.0 - 2.0	1.0 - 2.0
Lead (mg/kg)	<1	<1
pH	6.5 - 6.9	6.8 - 7.0
Solubility	100	90 - 98
Water adsorption	271	143

Source: Southward (1986)

Curd formation

Hoke *et al*. (1982) studied curd formation model of calcium – caseinate by dissolving calcium – caseinate in ethyl alcohol. In this condition, calcium – caseinate particles are in droplet form same as when dispersing calcium – caseinate in oil; non – polar solvent. If water was added to this system, calcium – caseinate will coalesce into curd. Electron microscopic image showed the birefringent of the calcium – caseinate curd indicating good molecule arrangement in curd. Disruption the curd such as applied shear force or agitation causes loss of birefringent.

There are 2 stages of curd formation; first stage is hydration and flocculation, second stage is syneresis. In a non – polar solvent, caseinate will show the hydrophobic groups contacted to solvent and calcium ion bonded to protein molecule resulting in coalesce of curd into a sticky ball and syneresis.

Factors affect quality of imitation cheese

1. Protein sources and its substitute

Caseinate may be defined as a chemical compound of casein and light metals, *e.g.* monovalent sodium (Na+) or divalent calcium (Ca++). Caseinates can be produced from freshly precipitated ("wet") acid casein curd or from dry acid casein by reaction with any of several diluted solutions of alkali. Sodium – caseinate and calcium – caseinate are highly used in imitation cheese production. From Table 1, sodium – caseinate can hydrate and solubilized in water more than calcium – caseinate. Solution of sodium – caseinate is higher viscous than that of calcium – caseinate. It also has higher emulsifying capacity value than calcium – caseinate (Southward, 1986). When prepared imitation cheese from sodium – caseinate instead of calcium – caseinate, the following characteristics were noted: higher pH, lower firmness, higher degree of fat emulsification and higher degree of casein dissociation (Bachmann, 2001).

Many researches used starch in partial or total replacement of caseinate. Starch plays an important role in macroscopic properties of imitation cheese such as flow, stability, texture and mouthfeel. Mounsey and O'Riordan (2001) compared native starches from different botanical origins – potato, rice, wheat, maize and waxy – maize – as casein substitute on imitation cheese rheology. The starches with higher amylose contents (maize, potato and wheat) increased imitation cheese hardness but reduced meltability.

Mounsey and O'Riordan (2008) showed the effect of starch – protein interactions in imitation cheese containing 3% native (amylose levels of 2.5%, 15% or 28%), pregelatinised, cross – linked or cross – linked and stabilized rice starch on the rheology and microstructure of imitation cheese. Scanning electron microscopy images showed smaller (more emulsified) fat globules in products containing 15% amylose starch, while those containing 2.5% amylose starch appeared less emulsified. Products containing waxy or cross – linked acetylated waxy starch had large starch particles within the protein matrix, indicating high degree of starch hydration and disruption the protein network. They suggested that the influence of starch on the physical properties of imitation cheese was affected by the amylose content, their ability to swell, shape and size of starch granules.

2. Moisture

Functional properties of imitation cheese are controlled by chemical composition, including moisture content. Moisture affects interaction of protein such as hydration, network formation and solubilization. Water also acts as a plasticizer in the cheese network and so has impact on texture properties. Typical imitation cheeses have moisture contents in the region of 48 – 52% (Duggan *et al.,* 2008). It has been reported that high – moisture processed cheese has a weak body and grainy texture, with a tendency for fat separation, whereas at low moisture level the cheese is firm, dry and brittle (Hennelly *et al*. 2004).

Hennelly *et al*. (2004) studied the effect of moisture content on texture of imitation cheese and found that imitation cheese with 46% moisture content (no. 1) has highest hardness (Table 2).

Table 2 Hardness of imitation cheese with different moisture contents

Variable	Imitation cheese			
	1	2	3	4
Hardness (N)	447.3	300	226.4	152.7
Moisture content (g/100g)	46.42	50.29	52.54	54.15

Source : Modified from Hennelly *et al*. (2004)

3. Fat type and content

Fats impart a wide range of texture characteristics to dairy products, including firmness, springiness, creaminess and mouthfeel. Cheese microstructure consists of a continuous protein matrix with loose and open structure with spaces occupied by fat globules dispersed through the protein network. The structure arrangement of the network determines the texture characteristics of cheese and is affected by factors such as composition, cheese making process, proteolysis during ripening and fat droplet size and distribution. The fat content influences the texture characteristics of cheese and imitation cheese. The reduction in fat content of cheese has been recommended to obtain foodstuffs low in cholesterol and saturated fatty acids. Hennelly *et al.* (2004) found that reducing fat content caused the imitation cheese to become harder, with the lowest fat cheese (12%) having a hardness value considered undesirable from a sensory perspective.

The possibility of substituting animal fats by vegetable fats and oils, which are cholesterol free and contain low saturated fatty acids level, has been explored extensively and imitation cheese products wherein the butterfat is replaced with a vegetable fat have increased popularity. The use of vegetable fats can give the cheese a consistency that makes it more suitable for certain applications. Soybean fat conferred hardness and adhesiveness to the imitation cheese, but decreased their cohesiveness and springiness, while the opposite effect was due to soybean oil and butterfat (Lobato-Calleros *et al.,* 1998).

Rice bran oil

In addition to being a source of high quality vegetable oil, rice bran oil is an excellent, and in some cases unique, source of many valuable derivatives and concentrate. The predominant commercial process for producing rice bran oil is solvent extraction with hexane followed by refining steps including bleaching, dewaxing, winterization, and deodorization. Rough rice contains 0.6 – 3.5 % oil. The oil is concentrated primarily in the germ but present in the bran as well (Nicolosi *et al*., 1994).

Composition of rice bran oil

Rice bran oil comprises about 20% saturated fatty acids and an even balance of monounsaturated and polyunsaturated fatty acid (40:40). Rice bran oil contains relatively large amounts of unsaponifiable components (4 – 5%). The minor constituents of the oil consist of phospholipids, glycolipids, waxes, sterols, ferulic esters of sterols (oryzanol), tocopherols, tocotrienols, color pigments, hydrocarbons and squalene (Kochhar, 2002). A typical fatty acid composition of rice bran oil is oleic acid, linoleic acid and palmitic acid (Table 3). The major triacylglycerol molecular species of rice bran oil comprises PLO, PLL and OOO. Most of the unsaponifiable material consists of sterol, sterol ester and acylsterol glycosides (Kochhar, 2002).

Table 3 TAG composition of rice bran oil and palm oil

	Fatty acid content (wt %)						
	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3
RBO[a]	-	0.7	18.2	1.6	43	34.7	1.11
PO[b]	0.30	1.0	34.9	3.8	47.0	12.0	0.3

Source : a Mayamol *et al.,* 2007

b Kallio *et al.,* 2001

Health benefits of rice bran oil

Rice bran oil is in steady demand as a so – called 'healthy oil'. A number of studies in humans and animals have shown that rice bran oil is an effective as other vegetable oils in lowering plasma cholesterol levels. In some cases, rice bran oil lowered plasma cholesterol more effectively than other commonly used vegetable oils rich in linoleic acid. This effect can be attributed to the occurrence of specific components in rice bran oil, γ – oryzanol (and its constituents, triterpene alcohol) and perhaps tocotrienols. γ – oryzanol which is a group of ferulic acid ester of triterpene alcohols and plant sterols, has been demonstrated to have certain beneficial effects. For example, γ – oryzanol has a hypocholesterolemic effect in primary type II hyperlipoproteinemia, reduce plasma cholesterol and enhance cholesterol excretion when fed to rats and inhibitory effects on platelet aggregation (Sugano and Tsuji, 1997). Phytosterols carry out functions in plants in equivalent to those of cholesterol in animals being thus required as necessary components of cell membranes and as precursors of important biomolecules, including sex hormones and vitamins. There are about 44 phytosterols known to exist in plants, the most abundant being β – sitosterol, campesterol and stigmasterol. The food sources with the highest total phytosterol contents, as the sum of these compounds (in mg / 100 g oil), are the oil of rice bran (Lerma –Garcia *et al.,* 2009). Rice bran oil have cycloartenol and 24 – methylene cycloartenol; major component terpene alcohols. Rice bran oil also contains campesterol and β – sitosterol at a relatively high level (Table 5) (Sugano and Tsuji, 1997).

Rice bran oil is also a rich source of tocotrienols, which range from 72 to 612 ppm. The possible regulatory role of tocotrienols in cholesterol dynamics including hypocholesterolemic action which has been reported (Nicolosi *et al.*, 1994).

Hyperlipoproteinanemias are heritable disorders associated with increased plasma concentrations of cholesterol, higher LDL – cholesterol than normal, implying a high risk of cardiovascular disease. Rice bran oil and its main components have demonstrated an ability to improve the plasma lipid pattern of humans, reducing total plasma cholesterol and triglyceride concentrations and increasing the HDL cholesterol level (Lerma –Garcia *et al.,* 2009).

Table 4 Sterol and triterpene contents in different edible oils

Oils	Campesterol	Stigmasterol	β-Sitosterol	Cycloartanol	Cycloartenol	24-Methylene -cycloartanol
	mg/100 g oil					
Rice bran	506	271	885	106	482	494
Saffflower	45	31	181	1	34	7
Corn	410	110	1180	4	8	11
Sunflower	31	31	235	-	29	16
Cottonseed	17	4	400	-	10	17
Sesame	117	62	382	4	62	107
Soybean	72	72	191	-	156	8
Peanut	36	21	153	1	11	16

Source : Sugano and Tsuji (1997)

More recently, Lerma –Garcia *et al*. (2009) had investigated the anti – inflammatory effects of γ – oryzanol, cycloartenyl *trans* – ferulate and *tran* – ferulic acid (as possible metabolite of γ – oryzanol *in vivo*) severe colitis was induced in mice, and a series of indices were monitored. Both γ – oryzanol and cycloartenyl *trans* – ferulate markedly inhibited the inflammatory reactions. Thus, phytosteryl ferulates could be effective as therapeutic and preventive agents for gastrointestinal inflammatory disease.

Rice

Rice (*Oryza sativa* L.) is one of the most important cereal grains in the world. It is the staple food in most Asian countries where more than 90% of the World's rice crop is produced and consumed. Milled rice provides 90% starch and 8% protein, so rice provides as much as 80% of the daily caloric intake for human body. Not only does rice supply more energy than other cereals, it also contains better protein quality (Luh, 1991; Juliano, 1998). Due to increasing demand of rice exporting, industries are facing problem with numerous broken rice, which are low-value from milling process. Currently broken rice are often used as raw material for rice flour manufacturing, and the utilization of rice flour provides a diversity of rice products, depending upon the different rice varieties with specific flour properties (Luh, 1991).

Classification of rice

Rice can be classified into many categories based on their prominent characteristics, i.e. kernel length, shape, variety, amylose content, cooking properties and industrial use. Based on amylose content, rice is classified as waxy (1-2%), very low amylose (2-12%), low amylose (12-20%), intermediate amylose (20-25%) and high amylose (25-33%) (Juliano, 1979). Another characteristic widely used to classify rice is cooked rice properties. According to these properties, rice can be categorized into two types as follow:

1. Glutinous rice or waxy rice : Waxy rice is essentially composed of amylopectin, resulting in the white and opaque kernel. When cooked, this rice provides very chewy texture and the cooked grains stick to one another.

2. Non – glutinous rice or non-waxy rice : Non-waxy rice contains 70-80% amylopectin and 10-30% amylose and provides firm, dry texture and fluffy when cooked.

Rice flour

Rice flour in Thailand is made from broken rice, from both glutinous and non – glutinous types. There are three kinds of rice flour process; wet-milled rice flour, dry milled rice flour and mixed milled rice flour. Wet-milled rice flour is widely used at present. Wet-milled rice flour is very popular for consumers and it was reported to have better properties than dry-milled rice flour. Dry-milled rice flour is produced using a hammer mill. This type of milling process is uncomplicated with a lower cost and requires less water than does the wet milling process. Mixed-milled rice flour is produced from broken glutinous rice which has been soaked and steamed until fully cooked. This type of flour is finer than normally ground flour.

Flour has composed a range of particle size. The varying in particle size differs in chemical and physical properties. The particle size of rice flour plays a key role in flour properties which influence the application of rice flour. Particle size is an important parameter in flour milling, affecting the texture quality and taste of products. It also influences functional characteristic of final product quality (Chen *et al*., 1999).

Rice starch granules is one of the smallest granules among the cereal grains, ranging in size from 3 to 5 μm in the mature grain. These granules are polyhedral and constitute approximately 90% of milled rice (dry weight). Most starch granules grow as a single entity inside an amyloplast or chloroplast of cell. Rice, however, contains only compound granules in which many granules have developed within a single amyloplast. The amyloplast contains 20 – 60 of the small granules forming a spherical to ellipsoidal cluster, varying from 7 to 39 μm in diameter (Champagne, 1996).

Amylose is in recent years known as a linear and branched glucose polymer. Rice amyloses have a β-amylolysis limit of 73-81%, indicating them to be slightly branched molecules with three to four chains on average. This β-amylolysis limit is considerably higher than that of amylopectin (55-60%). Rice starch amyloses have degree of polymerization (DP) values of 1,000-1,100 and average chain-lengths of 250-320. The amylose content of rice is specified as waxy (0-2%), very low (5-12%), low (12-20%), intermediate (20-25%) and high (25-33%).

Amylopectin is a highly branched polymer having on the average 96% of α-1, 4 bonds and 4% α-1, 6 bonds. The molecules have 104 – 105 individual chains, and the ratio of unbranched to branched chain is about 1.0. Approximately 22-25 chains form each cluster, comprising the crystalline regions of starch granules. In waxy rice, 80-90% of the amylopectin chains probably constitute a single cluster, while the remaining 10 – 20% forms intercluster connections, which are mainly between adjacent clusters (Champagne, 1996).

MATERIALS AND METHODS

Materials

1. Raw materials

1.1 Palm olein oil (Oleen, Oleen Co., Ltd., Thailand)

1.2 Rice bran oil (King, Thai Edible Oil Co., Ltd., Thailand)

1.3 Rice flour (Fish brand, Thai Flour Industry Co., Ltd., Thailand)

1.4 Sodium – caseinate (Erie Foods, USA)

1.5 Waxy rice flour (Fish brand, Thai Flour Industry Co., Ltd., Thailand)

2. Chemicals

2.1 Chemicals for sample preparation

2.1.1 88% Lactic acid (Vicchi Consolidated Thailand Co., Ltd., Thailand)

2.1.2 Guar gum (Aditdaya Birla Chemical Phosphate, Thailand)

2.1.3 Sodium chloride (NaCl, Prung Thip, Thai Refined Salt Co., Ltd., Thailand)

2.1.4 Sodium citrate (Rama Chemical, Thailand)

2.1.5 Sodium hydrogen phosphate (Na_2HPO_4, Food Grade, Aditdaya Birla Chemical Phosphate, Thailand)

2.1.6 Sodium tripolyphosphate ($Na_5P_3O_{12}$, Food Grade, Aditdaya Birla Chemical Phosphate, Thailand)

2.2 Reagent for modifying of sodium – caseinate

2.2.1 88% Lactic acid (Vicchi Consolidated Thailand Co., Ltd., Thailand)

2.3 Reagents for amylose content determination

2.3.1 Amylose (Amylose Type III: From potato, SIGMA, Germany)

2.3.2 Iodine (I_2, analytical grade, Asia Pacific Specially Chemicals Limited, Australia)

2.3.3 Methanol (CH_3OH, analytical grade, MERCK, Merck KGaA, Germany)

2.3.4 Potassium iodide (KI, analytical grade, UNIVAR, Ajax Finechem, Australia)

2.3.5 Sodium hydroxide (NaOH, analytical grade, MERCK, Merck KGaA, Germany)

2.3.6 Trichloroacetic acid (CCl_3COOH, analytical grade, MERCK, Merck KGaA, Germany)

2.4 Reagents for protein content determination

2.4.1 Boric acid (H_3BO_3, analytical grade, MERCK, Merck KGaA, Germany)

2.4.2 Bromocresol green ($C_{21}H_{14}Br_4O_5S$, analytical grade, LABCHEM, Ajax Finechem, New Zealand)

2.4.3 Copper sulfate ($CuSO_4.5H_2O$, analytical grade, Fisher Chemicals, Fisher Scientific UK Limited, UK)

2.4.4 Methyl red ($C_{15}H_{15}N_3O_2$, analytical grade, Panreac, Panreac Quimica S.A., Spain)

2.4.5 Potassium sulfate (K_2SO_4, analytical grade, MERCK, Merck KGaA, Germany)

2.4.6 Sodium hydroxide (NaOH, commercial grade, THASCO, THASCO Chemical Co., Ltd., Thailand)

2.4.7 Sulfuric acid (H_2SO_4, analytical grade, Mallinckrodt Chemicals, Mallinckrodt Baker, Inc., USA)

2.5 Reagent for fat content determination

2.5.1 Petroleum ether (C_6H_6, analytical grade, Mallinckrodt Chemicals, Mallinckrodt Baker, Inc., USA)

2.6 Reagent for TCA – soluble protein

2.6.1 Trichloroacetic acid (CCl_3COOH, analytical grade, MERCK, Merck KGaA, Germany)

3. Instruments and apparatus

3.1 Instruments and apparatus for sample preparation

3.1.1 Test sieve (Aperture size 150 Mic., Endorotis Limited, England)

3.1.2 Tray dryer (Reliance Tech – Survice Co., Ltd., Thailand)

3.1.3 Weight balance (OHAUS, model GT 4100, OHAUS Coorparation, USA)

3.2 Instruments and apparatus for sample analysis

3.1.1 Differential Scanning Calorimeter (DSC, Pyris1, Perkin-Elmer, USA)

3.1.2 Electric – oven (Kitchen Master, USA)

3.1.3 Kjeldahl apparatus (BUCHI, B-324, LabortechnikAG, Switzerland)

3.1.4 Muffle furnace (model Tactical 308, Gallenkamp, UK.)

3.1.5 pH meter (OMEGA, model PHB-62, Omega Engineering Inc., USA)

3.1.6 Soxtec system (model HT 1043, Tecator, Sweden)

3.1.7 Texture Analyser (TA Plus, Lloyd Instruments Ltd., England)

3.1.8 Weight balance (OHAUS, model GT 4100, OHAUS Coorparation, USA)

Methods

1. Effect of sodium – caseinate : rice bran oil ratio

1.1 Preparation of imitation Mozzarella cheese

Imitation Mozzarella cheese was produced from sodium – caseinate (SCN) and rice bran oil (RBO) at 3 different ratios of protein to oil; 20:25, 22.5:22.5 and 25:20 with other ingredients as shown in Table 5. Formulation which yielded highest stretchability and lowest meltability was selected for further experimental studies.

Table 5 Formulations of different ratios of sodium – caseinate to rice bran oil

Ingredients (%)	SCN:RBO		
	20:25	22.5:22.5	25:20
RBO	25	22.5	20
SCN	20	22.5	25
Water	51	51	51
Guar gum	0.2	0.2	0.2
Emulsifying salts	3.45	3.45	3.45
Lactic acid	0.35	0.35	0.35

Imitation Mozzarella cheese was made by heating RBO at 85^{o}c, and mixture of SCN and guar gum was added, and pasteurized at 85^{o}C for 2 minutes.

Water and emulsifying salts (0.75% sodium chloride, 0.2% sodium citrate, 0.2% sodium hydrogen phosphate and 0.2% sodium tripolyphosphate) were mixed and pasteurized at 85^{o}C for 2 minutes, then these 2 mixtures were mixed by blending for 2 minutes and lactic acid was added during mixing. Finally, a smooth emulsion was formed and kept at 4^{o}C, 24 hr before analyzing (modified from Mounsey and O'Riordan, 2008).

1.2 Measurements of imitation Mozzarella cheese properties

Stretchability of imitation Mozzarella cheese was analyzed by modified method from Guinee and O'Callaghan (1996). Imitation Mozzarella cheese size 0.25 g/cm^2 was loaded onto a rectangular (4.5 x5 cm) bread base, which was cut into half along the short axis. Imitation Mozzarella cheese, stored at 10^{o}C, was grated to 1 – 2 mm shred length and distributed over the bread base that was placed in a thermostatically controlled electric fan oven at 250 ^{o}C for 4 minutes. So as to simulate the way cheese is eaten in practice, the stretch test was performed at room temperature in 1 minute after removing cheese from the oven. The cooked bread base was placed on the platform unit of the stretch apparatus. Stretching speed was set at 0.075 m/s and stretched until the extended string of the melted cheese mass completely broken. Stretchability was defined as the distance of complete strand breakage.

Meltability was analyzed by Schreiber test (Mounsey and O'Riordan, 2008) by using a modified procedure. Imitation Mozzarella cheese was cut into 8 mm thick disk of 41 mm diameter, placed on a petri dish and baked in 250°C oven for 5 minutes. After 30 minutes cooling at room temperature, the dimension of the melted cheese was measured at 6 positions and the average of the dimension taken. Meltability was defined as the increasing dimension of the melted cheese.

1.3 Determination of rice bran oil properties

Melting range and solid fat content (SFC) of rice bran oil (RBO) was analyzed by DSC by the method of Ortiz-Gonzalez *et al.,* 2007. Sample was subjected to the following temperature programs: holding at 60°C for 3 min, cooling from 60 °C to -60 °C at rate 10 °C /min, and holding at -60 °C for 3 minutes. The sample was heated from -60 °C to 60 °C at rate 10 °C/min. heating and cooling thermograms were recorded. SFC was calculated by dividing the partial area under thermogram curve by the total area from -30 to 40°C and multiplied by 100. SFC from 0 to 40 °C was calculated at 5 °C intervals.

1.4 Determination of sodium – caseinate properties

Proximate analysis including fat and protein contents was determined using the Standard AOAC (2000) Method. The nitrogen factor used for protein calculation was 6.38. Moisture content of sodium - caseinate was determined by oven drying at 130°C (AACC, 1995).

2. Effect of modified sodium – caseinate

2.1 Preparation of modified sodium – caseinate

Sodium – caseinate was modified by method from Tudthong *et al.* (2007). Firstly, it's moisture content was adjusted to 30% wt/wt, then added 1% lactic acid by volume, placed at room temperature for 30 minutes, and tray dried at 55^{o}C until moisture content reached 4.5% wt/wt. Modified sodium – caseinate (MSCN) was ground and screened by 100 mesh sieve.

2.2 Measurements of modified sodium - caseinate properties

Proximate analysis including moisture, fat, protein and pH was determine as described above.

Small molecular weight proteins in MSCN were analyzed by the modified method from Henn and Netto (1998). MSCN was soluble in water and stirred until clear solution obtained, 10% wt/wt trichloroacetic acid (TCA) was added with stirring for 10 minutes. After that centrifuged at 14,000 rpm for 10 minutes. Protein in supernatant was analyzed by Kjeldahl method. Small molecular weight proteins were defined as TCA – soluble protein.

2.3 Preparation of imitation Mozzarella cheese using modified sodium – caseinate substitution to sodium – caseinate

Imitation Mozzarella cheese was made from SCN and MSCN substitution at 33, 50 and 67% where moisture content was fixed at 30% (Table 6). When effect of moisture contents (at 30, 40 and 50%) in imitation Mozzarella cheese was studied, quantity of modified sodium – caseinate was fixed at 33% (Table 7).

Table 6 Formulations with varying modified sodium – caseinate contents

Ingredients (%)	0MSCN	33MSCN	50MSCN	67MSCN
RBO	20	20	20	20
SCN	25	16.7	12.5	8.3
MSCN	0	8.3	12.5	16.7
Water	51	51	51	51
Gour gum	0.2	0.2	0.2	0.2
Emulsifying salts	3.45	3.45	3.45	3.45
Lactic acid	0.35	0.35	0.35	0.35

Table 7 Formulations with varying moisture contents used to modify sodium – caseinate

Ingredients (%)	MSCN-MC30	MSCN-MC40	MSCN-MC50
RBO	20	20	20
SCN	16.7	16.7	16.7
MSCN	8.3	8.3	8.3
Water	51	51	51
Gour gum	0.2	0.2	0.2
Emulsifying salts	3.45	3.45	3.45
Lactic acid	0.35	0.35	0.35

2.4 Measurements of imitation Mozzarella cheese using modified sodium – caseinate substitution to sodium – caseinate properties

Stretchability and meltability were determined as described in 1.2.

3. Effect of oil blends

3.1 Preparation of imitation Mozzarella cheese containing oil blends

Imitation Mozzarella cheese was produced using different ratios of rice bran oil (RBO) and palm oil (POO) at 0:100, 25:75, 50:50, 75:25 and 100:0.

3.2 Measurements of imitation Mozzarella cheese containing oil blends properties

Stretchability and meltability were determined as described in 1.2.

3.3 Measurements of palm oil properties

Melting range and SFC were determined as described in 1.3.

4. **Effect of rice flour and waxy rice flour**

4.1 Preparation of imitation Mozzarella cheese containing rice flour and waxy rice flour

Protein in imitation Mozzarella cheese was substituted by rice flour (RF) and waxy rice flour (WRF) at 0, 2.5, 5.0, 7.5 and 10% and prepared as in 1.1.

4.2 Measurements of imitation Mozzarella cheese containing rice flour and waxy rice flour properties

Stretchability and meltability were determined as described in 1.2.

4.3 Measurements of rice flour and waxy rice flour properties

4.3.1 Proximate analysis

Commercially available RF and WF purchased from a local supermarket were proximately analyzed for protein contents (Appendix A).

4.3.2 Amylose content

RF and WF were analyzed for amylose content by colorimetric quantitation. Flours (10 – 20 mg) were mixed with 5 ml of 85 % methanol, heated at 60°C for 15 min, discarded supernatant and collected the solid. This process was repeated twice to remove lipids in sample. Lipid-free samples were solubilized with 2 ml of 0.4 N NaOH and 4 ml of distilled water, and heated at 95°C for 30 min. 0.1 Ml solubilized solutions were added into 5 ml of 0.5% TCA and mixed immediately with 0.05 ml of 0.01 NI_2-KI solution. The absorbance of sample was read at 620 nm (Appendix A).

4.4 Sensory evaluation test

Imitation Mozzarella cheese containing 3.75, 5.0 and 6.35% WRF was made. The difference in sensory stretchability by hand and mouthfeel of imitation Mozzarella cheese was determined using Difference from Control test (Mucci *et al.,*2004) by 50 assessors. Assessors received four samples. One was coded with word “control” (commercial Mozzarella cheese) and the other three were coded with three – digit codes (imitation Mozzarella cheese containing 3.75, 5.0 and 6.35% WRF). Assessors had to score each coded sample on a 0 – 5 scale as equal to control (0) or very different from control (5). Presentation orders of the coded samples were balanced over assessors. They were instructed to taste the sample on their left and memorize it as the control sample. Then they tasted the next from the left sample and compared with the control. As the cheese sample was prepared by loading cheese on bread base, assessor had to score only the cheese sample.

4.5 Confocal scanning microscopy

A thin slice of approximately 1 mm thickness was cut from imitation Mozzarella cheese for microstructural analysis using Confocal Laser Scanning Microscopy or CLSM (Axio Imager MI, Carl Zeiss PTe Ltd, Germany). The samples were stained using 0.05% Nile Blue A (in 95% ethanol), which stains the fat phase, and 0.01% Rhodamine B (in distilled water), which stains the protein phase. A HeNe laser with an excitation wavelength of 488 and 543 nm was used. CLSM digital image were acquired using the LSM 5 PASCAL program.

5. Statistical Analysis

The experiments were carried out in three separated trials. Each trial was run in triplicates. The data were analyzed by Analysis of Variance (ANOVA) with significance at $p<0.05$. All statistical analyses were performed using the SPSS Software Version 12.

PLACE AND DURATION

Place

At the Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University (Bangkhen campus), Thailand

Duration

From June 2007 to December 2009

RESULTS AND DISCUSSION

1. Effect of sodium – caseinate : Rice bran oil ratio

Imitation Mozzarella cheese made from sodium – caseinate (SCN) and rice bran oil (RBO) was firm and flexible, similar to commercial Mozzarella cheese. On cooking, Mozzarella cheese requires melting, flowing and stretching, which are the properties of sodium – caseinate (Bachmann, 2001). Owing to its good solubility, surface activity, heat resistance and water – holding properties, sodium – caseinate is widely used as an emulsion stabilizing agent in foods. The stearic stabilizing casein layer protects fine droplets against intermediate recoalescence, and it confers long – term stability during processing and storage (Dickinson, 1999).

During the last decade consumers have become increasingly aware of the importance of maintaining adequate nutrition. Substituting animal fats by vegetable fats and oils help to lower health risks. If unsaturated fats are used then the analogues may offer certain health or dietary advantages commonly associated with unsaturated and polyunsaturated fatty acids. Rice bran oil gives a number of health benefits, for instance, it could lower level of serum cholesterol, and prevent cardiovascular disease and a source of vitamin E complex (Piironen *et al.*, 2000), therefore, using rice bran oil to make imitation cheese can create lot of advantages for health.

Imitation Mozzarella cheese was manufactured as described previously. Sodium caseinate (SCN) was mixed in 85^{o}C RBO. Hydrated caseinate swelled and clumped, forming circular like particles and associated with the surface of oil droplet. Water and emulsifying salts were mixed at 85^{o}C and blend into the oil. Salt ion in emulsifying salts bonded to protein molecule resulting in coalesces of curd (Hoke *et al.,* 1982). SCN was unfolded and acted as an emulsifier during blending. SCN adsorbed strongly on fluid interfaces, water and RBO. Emulsion was formed with continuous blending. During emulsification, the matrix also appeared more cohesive, although the surface still appeared slightly oily. Further blending, the liquid was found to become milky, indicated that the hydrated proteins emulsified the free fat in the liquid phase. After blending for 2 minutes, the visually free water and oil were imbibed by the protein matrix, and an increase in viscosity was observed. Finally, a smooth emulsion was formed. This imitation cheese is an oil – in – water emulsion in which, fat droplets are incorporated in a protein matrix which functions as an emulsifier.

The composition requirements of Mozzarella cheese including moisture, protein, fat, salt and pH are 30 – 45%, 20 – 25%, 20 – 25%, 1.2 – 2.0 and 5.0 – 5.4, respectively (USDA Commodity Requirements for Mozzarella Cheese, 2005). Table 8, shows the composition of imitation Mozzarella cheese (calculation from composition of sodium – caseinate) which largely determines its texture. Three imitation Mozzarella cheeses had similar composition to the requirements, except moisture content, pH and salts (emulsifying salts). pH of Mozzarella cheese dropped during storage from 6.4 to 5.4, due to proteolysis in cheese, the higher proteolysis reaction, the lower pH resulted (Guinee *et al.,* 2001). Moisture content is a function of water – binding capacity of the proteins. The water in the protein network acted as a plasticizer making cheese more elastic and less fracture (Bachmann, 2001). When moisture increased, Mozzarella cheese became softer and less shreddable. Salts influences texture through ionic strength, so increasing salt content could cause a significant decrease in cohesiveness and springiness but increase firmness. The way fat and protein distributed through cheese also impacted on the texture of imitation cheese.

Table 8 Composition of imitation Mozzarella cheese with different SCN: RBO ratios

SCN: RBO ratio	pH	Moisture (%)	Protein (%)	Fat (%)	Salts (%)
20:25	6.45	55.47	18.69	27.13	3.45
22.5 :22.5	6.45	55.47	21.03	25.63	3.45
25:20:00	6.45	55.47	23.37	22.13	3.45

When SCN and RBO were used to make imitation Mozzarella cheese at different ratios, it affected the stretchability and meltability of imitation Mozzarella cheese when compared with commercial Mozzarella cheese (Figure 6).

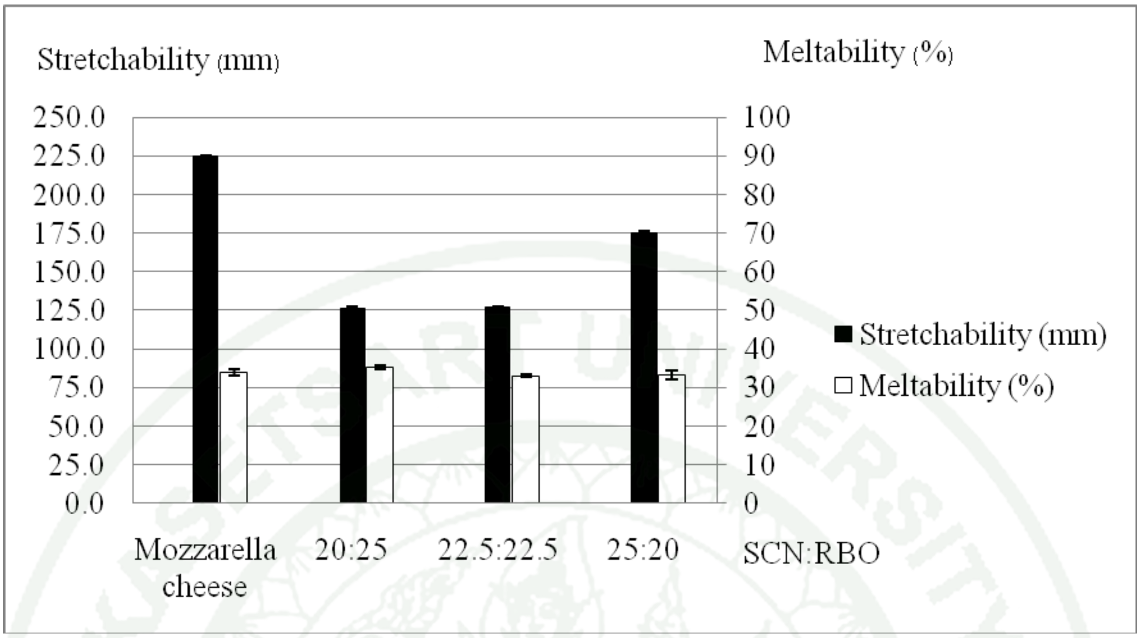

Figure 6 Effect of sodium – caseinate to rice bran oil ratios on imitation Mozzarella cheese quality

From Figure 6, commercial Mozzarella cheese could stretch 224 mm. long, alter melting its dimension increased 33%. Stretchability was defined as the length that cheese could stand under tension. Imitation Mozzarella cheese with higher protein to oil ratio gave higher stretchability, but shorter than commercial Mozzarella cheese. Because high protein to oil ratio in imitation Mozzarella cheese had higher protein to form a stretching fibrous which could stand under tension. Moreover, in high fat product, fat droplets might disrupt the protein fibrous resulting in low stretchability, so product with higher protein and low fat content gave higher stretchability.

Meltability depended on the fat phase because cheese structure consisted of continuous protein matrix with a space occupied by fat globules dispersed through the protein network. The melt test measures the ability of cheese particles to flow passing one another when heating. Fat acts as a lubricant and increases the ability of cheese particles to flow (Fife *et al.,* 2002). From Figure 6, there was no statistically difference in melt among the imitation cheese and commercial Mozzarella cheese, although there were some differences at different fat contents. High level of RBO might increase meltability of imitation Mozzarella cheese. Although, in high protein product, SCN which acted as an emulsifier could emulsify more fat, the fat which trapped by SCN was hard to melt and flow, the meltability of high protein imitation cheese was low. So, imitation Mozzarella cheese with high fat content and low protein content gave high meltability.

Imitation Mozzarella cheese with SCN: RBO ratio at 25:20 gave high stretchability and low meltability, this formula was used as the base formulation for next experiment.

2. Effect of modified sodium – caseinate

Sheehan and Guinee (2004) studied the molecular pattern of Mozzarella cheese with different storage times, and found that Mozzarella cheese with longer storage time had high level of α_{s1} – casein and β – casein breakdown. And this storage cheese had high stretchability value. Breaking down of caseins was the main factor affecting the age – related changes which occurred in Mozzarella as reflected by increases in meltability and stretchability and decreased apparent viscosity (Feeney *et al.,* 2001). Imitation Mozzarella cheese made from SCN yielded unsatisfied stretchability. Small molecular weigh proteins occurred might be the factor promoting better quality in imitation Mozzarella cheese.

Hydrolysis of SCN by lactic acid and moisture occurred during drying the SCN in tray dryer at 55^{o}c. Lactic acid concentration, moisture content, time during acid hydrolysis and temperature modified protein molecules in SCN by breaking into small fractions. The higher moisture content, the higher lactic acid concentration used (1% lactic acid wt/v) and the more time during hydrolysis used.

Increasing moisture content, acid concentration, and time during hydrolysis resulted in more small molecular weight proteins. When protein was precipitated by TCA, protein fractions with molecular weight more than 14kDa precipitated out and soluble protein with molecular weight less than 14 kDa left in the supernatant. TCA – soluble proteins of modified sodium – caseinate (MSCN) at different moisture contents is shown in Table 10. MSCN modified at 50% moisture content had higher TCA – soluble protein than MSCN modified at 40% and 30% moisture contents, respectively. The higher moisture content used to modify SCN, the more TCA – soluble protein occurred (Table 9).

Table 9 Effect of lactic hydrolysis at limited water content on TCA-soluble protein content of modified sodium caseinate (MSCN)

Moisture content at initial acid hydrolysis	TCA - soluble protein content (mg/100g MSCN)
Unhydrolysed SCN	8.7 ± 0.0d
30	21.5 ± 0.0c
40	35.0 ± 0.1b
50	51.8 ± 0.0a

MSCN was used to substitute SCN to improve stretchability. Changes in the functional characteristics of the different levels of MSCN substitution to SCN are shown in Figure 7. Substitution of MSCN up to 33% could improve stretchability of imitation Mozzarella cheese. Since MSCN had small molecular weight proteins which act like lubricant and could slip through each other in promoting imitation Mozzarella cheese to form stretchable fibrous and withstand the tension pressure. More than 33% substitution retarded this quality attribute, because of high level of small molecular weight protein resulted in condensed protein fibrous which could not stand under tension. Moreover, these small molecular weight proteins could also melt and flow, promoting the meltability of imitation cheese. The results from Figure 7 showed that imitation Mozzarella cheese with more amount of MSCN yielded higher meltability.

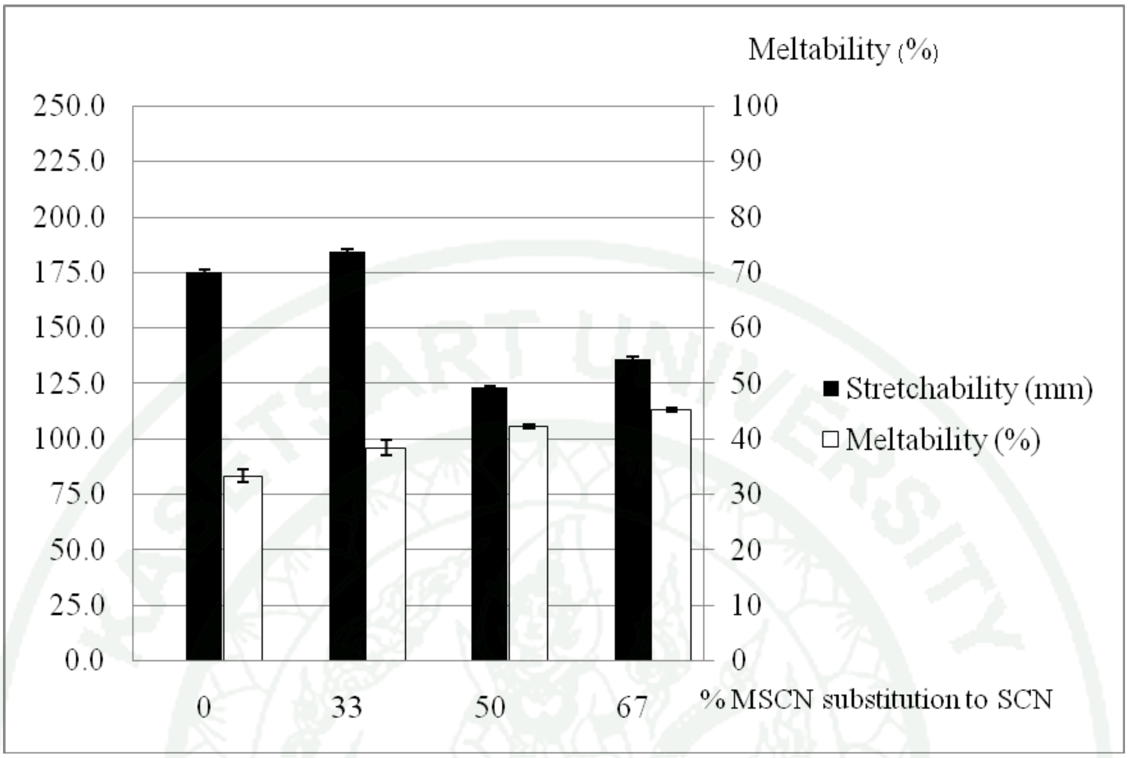

Figure 7 Effect of MSCN on imitation Mozzarella cheese quality. The moisture at initial modification was 30%.

Moisture used to modify MSCN affected qualities of the product (Table 10), especially in meltability. MSCN modified with high moisture, gave high level of small molecular weight proteins, due to high content of water, lactic acid concentration and time during hydrolysis breaking protein molecule into smaller fractions (Table 9). Small molecular weight proteins promote cheese to better flow and causing high meltability. Higher moisture content and acid concentration in MSCN resulted in higher percent increased dimension in melted cheese that resulted in higher meltability.

Table 10 Effect of moisture used to modified sodium – caseinate on imitation Mozzarella cheese quality

Moisture content at initial preparation of MSCN by acid hydrolysis	Stretchability (mm.)	Meltability (%)
Mozzarella cheese	224.9 ± 0.6a	33.9 ± 0.8b
30	183.3 ± 1.0b	38.3 ± 1.4b
40	229.7 ± 1.8a	51.9 ± 1.0a
50	168.4 ± 0.7c	53.9 ± 0.4a

3. Effect of oil blends

Blending rice bran oil (RBO) and palm oil (PO) changed their normal physical properties, i.e. melting range (Table 11) and solid fat content (Figure 8) at different temperatures. Solid fat content is equivalent to the percentage of solid fat remaining at the selected temperature. The ratio of solid to liquid fat is the amount of fat that is crystallized. The amount of fat crystals was determined by calculating the ratio of solid to liquid fat under DSC curve as described previously. The calculation of the solid fat content involved the implicit assumption that the heat of melting of triacylglycerol (TAG) was constant. In fact, the heat of melting had a positive correlation with melting range and the proportion of solid was overestimated at high solid fat content. From Table 11, it can be seen that RBO had the widest and the lowest melting range (from - 22.57 to 4.7^{o}C) when comparing to anhydrous milk fat

(AMF) and PO. The difference in melting range was due to the triacylglycerol in oil. Increase in C14:0 and C16:0 were directly correlated with increased melting range, whereas increased C18:2 content was associated with decreased melting range (Table 3). PO consisted of higher content of saturated fatty acid, its melting range was narrower than RBO. RBO also contained lowest solid fat content when measured at room temperature (25^{o}C). High solid fat content can be mainly constituted of high melting point TAG, that is, TAG with saturated long chain fatty acid such as palmitic acid (C16:0). Solid fat content of oil blends increased when the proportion of PO increased due to high solid fat content in PO (Figure 8).

Table 11 Melting range of oil blends

Oil blends	Melting range
Anhydrous milk fat	21.29 - 40.08 ^{o}C
RBO	-22.57 – 4.7 ^{o}C
PO25	-6.16 – 6.18 ^{o}C
PO50	-0.48 – 9.80 ^{o}C
PO75	2.54 – 11.67 ^{o}C
PO100	-2.47 – 14.16 ^{o}C

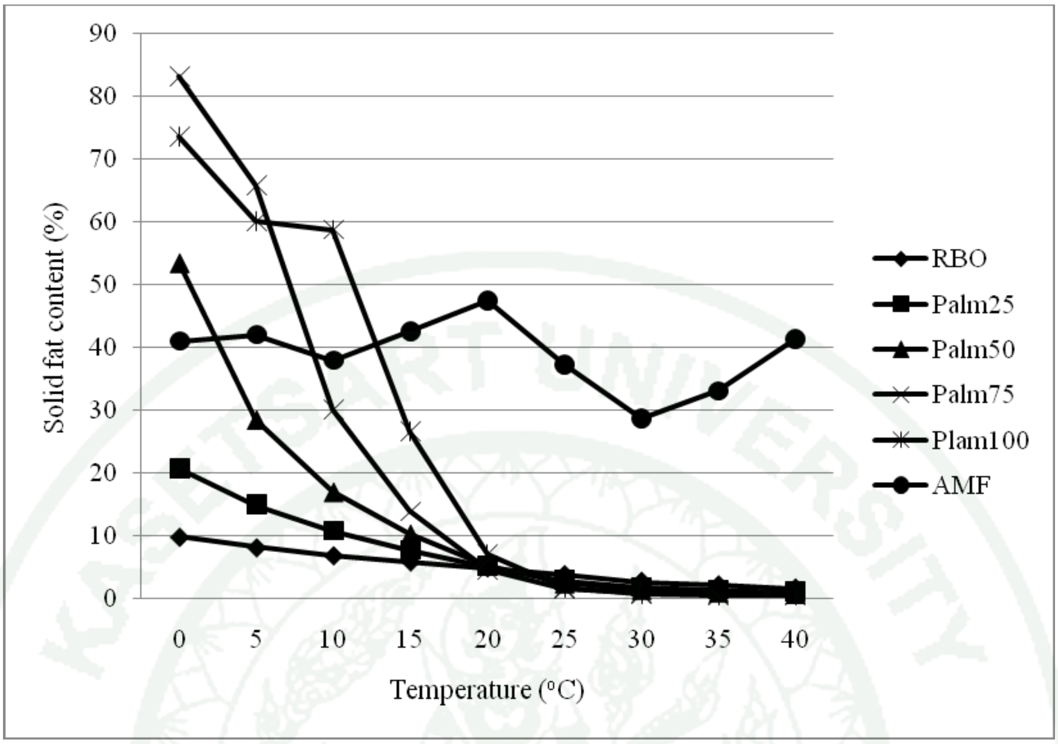

Figure 8 Solid fat content of oil blends prepared from rice bran oil and palm oil

From previous results, MSCN could improve stretchability of imitation Mozzarella cheese, but it retarded meltability. PO had higher melting range and solid fat content than RBO and oil blends of RBO and PO. Imitation Mozzarella cheese made from oil blends of RBO and PO could enhance meltability. Effect of oil blends on imitation Mozzarella cheese was studied and the result shown in Figure 9.

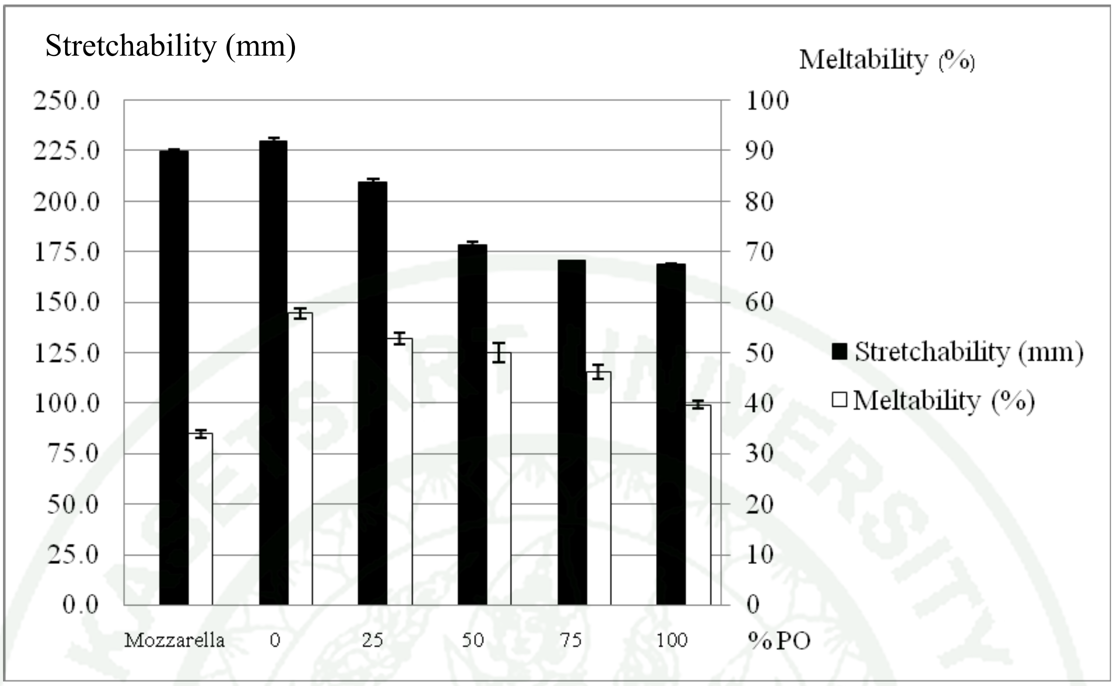

Figure 9 Effect of oil blends on imitation Mozzarella cheese qualities

When using blends of the different oil types, the behavior exhibited by a given textural characteristic was an average of that provided by each oil on its own. Oil blends with different in fatty acid profile, triacylglycerol (TAG) composition and polymorphism have difference physical characteristic *e.g.* melting range, solid fat content and polymorphism (Mayamol *et al.,* 2007; Ortiz-Gonzalez *et al.,* 2007). Oil blends from RBO and PO which different in fatty acid profi % PO glycerol (TAG) composition and degree of saturation (Table 3) have different characteristic due to the proportion of oil in the blends. Fatty acid composition mainly in PO is palmitic acid which is saturated fatty acid, but RBO contain higher degree of unsaturated oil such as oleic acid and linoleic acid (Table 3). Therefore, increasing PO in oil blends caused higher melting range (Table 11). Moreover, as shown in Figure 7, PO100 had higher solid fat content than PO75, PO50, PO25 and RBO, respectively

Substitution RBO by PO could lead to lower stretchability and meltability. For meltability, PO had higher melting range than RBO. Therefore, increasing PO in oil blends caused lower meltability. Solid fat in oil blend with high proportion of PO may stop the oil phase from flowing when heated cheese, so high quantity of PO substitution lowered dimension of melted imitation cheese. Moreover, solid fat in PO disrupted the protein fibrous causes low stretching property of imitation cheese manufactured from oil blend with high quantity of PO.

From the result, high proportions of PO reduced stretchability and meltability of imitation Mozzarella cheese.

4. Effect of rice flour and waxy rice flour

Rice flour (RF) contained 10.5% protein and 24.50% amylose, while waxy rice flour (WRF) contained 10.3% protein and 4.30% amylose. Substitution rice flour (RF) and waxy rice flour (WRF) to protein in order to enhance the meltability of imitation Mozzarella cheese was studied. Results showed that rice flour could decrease stretchability and meltability (Figure 10). Waxy rice flour also decreased meltability of imitation cheese. Moreover, waxy rice flour could increase stretchability of the product (Figure 11).

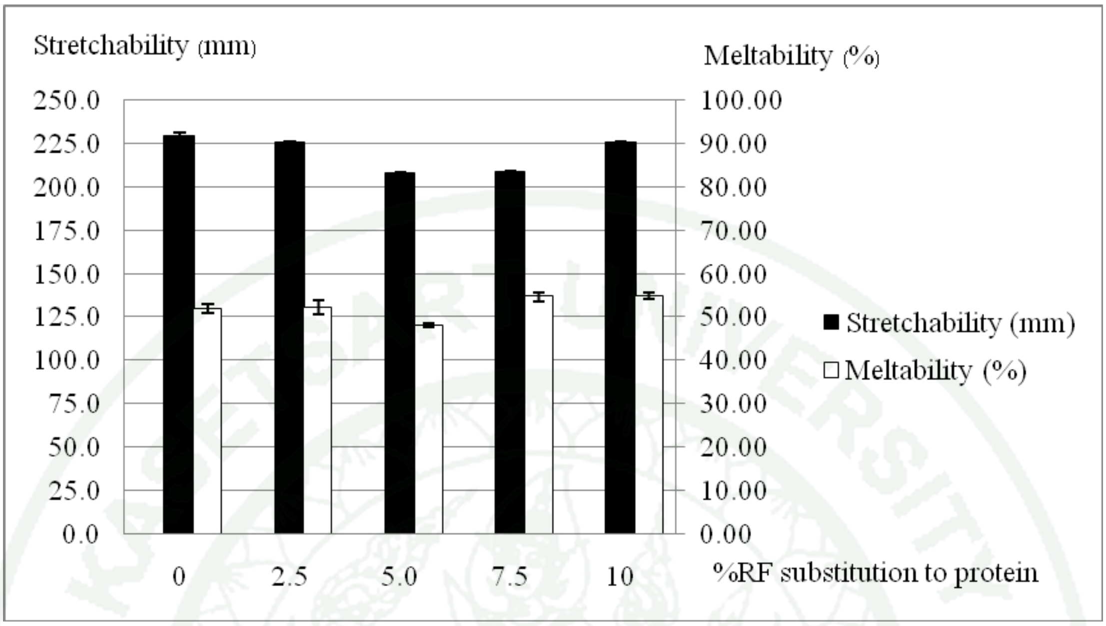

Figure 10 Effect of rice flour on imitation Mozzarella cheese quality

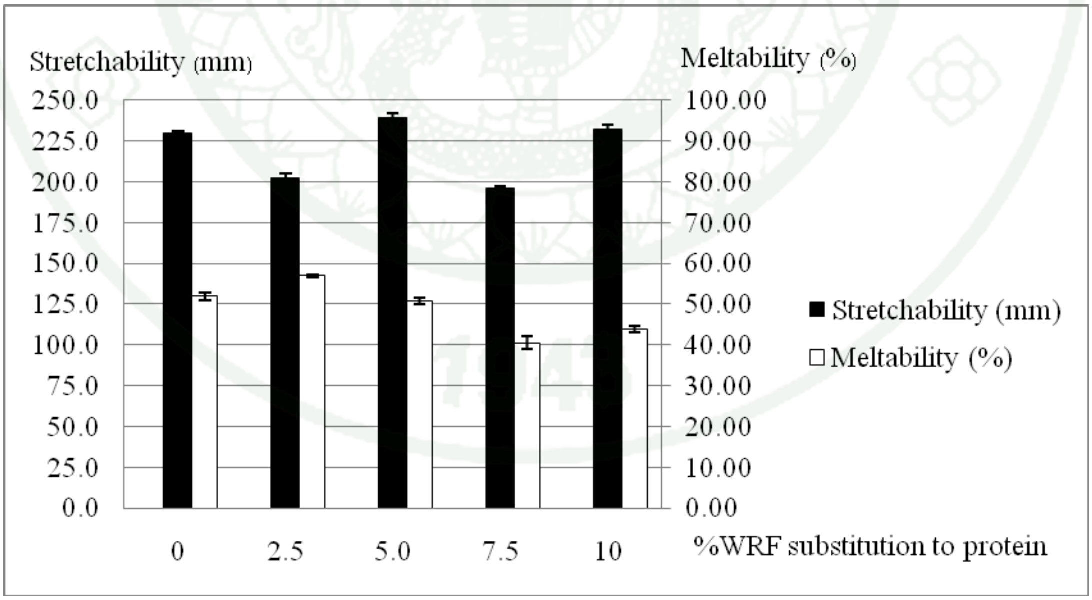

Figure 11 Effect of waxy rice flour on imitation Mozzarella cheese quality

Addition of RF and WRF during cheese making process imparted certain functional characteristics such as meltability and stretchability of the end product, and partially substitute casein, could thereby reduce formulation costs.

When making imitation Mozzarella cheese, flour was hydrated and swelled. This swollen flour expanded in size and disrupted the continuously protein matrix causing low stretchability. Furthermore, flour had higher water – binding capacity than SCN and MSCN. Caseinate could not hydrate and unfold to act like emulsifier around oil surface resulting in large fat globules and unsatisfied emulsified product. Imitation Mozzarella cheese with less emulsifying capacity protein would be high in meltability. In contrast, flour might stop fat phase from flowing, when it melted by its swelling. Substitution flour to protein probably decreased meltability of imitation Mozzarella cheese.

Starch is one of the chemical constituents of flour, this substance is important in obtaining the desired characteristics of final product. Starch is a polysaccharide which is one of three major constituents of rice kernel (the others are proteins and lipids). Starch consists of a branched fraction, amylopectin, and a linear fraction, amylose. Starch granules of RF was more strength than WRF (Noisuwan *et al.,* 2008). When making imitation Mozzarella cheese at 85^{o}C starch granules of WRF was broken but granules of RF still remained. Starch granules in RF might disrupt the protein fibrous in imitation cheese resulting in low stretchability and it also stopped the fat phase from flowing resulting in low meltability.

Difference from control test was set to obtain the difference between commercial Mozzarella cheese and imitation Mozzarella cheese, WRF 3.75, WRF 5.0 and WRF 6.25. Assessors could discriminate the difference between commercial Mozzarella cheese and imitation cheese both in stretching by hand and mouthfeel (cheese) attributes (Table 12). In both stretching by hand and mouthfeel attributes, when increased WRF contents in samples, the difference score increased.

Table 12 Score of Difference from Control test of cheese

Samples	Size of the difference	
	Stretch by hand	Mouthfeel (cheese)
WRF 3.75	1.88 b	1.68 b
WRF 5.0	2.24 b	1.62 c
WRF 6.25	2.90 a	1.76 a

Substitution WRF to protein in imitation Mozzarella cheese in order to lower its price should not be more than 3.75%. Assessors could discriminate the difference between commercial Mozzarella cheese and imitation Mozzarella cheese containing WRF more than 3.75%.

The distributions of oil droplets and flour in the protein matrix of Mozzarella cheese analogues are shown in the CLSM micrographs in Figure 12. From the micrographs, oil and flour were in a disperse phase and protein was in a continuous phase. In imitation Mozzarella cheese, the oil was dispersed in the protein matrix as small particles (1 µm) (Figure 12A). When heated imitation Mozzarella cheese, the fat phase melted and oil droplets coalesced (3 µm) but not present in large cluster indicating that protein matrix was able to hold the melted oil droplets and form continuously fibrous without disruption of larger oil droplets (Figure 12B).

Oil was dispersed as size smaller than 10 µm in imitation Mozzarella cheese without added flour. In present of flour, more large oil droplets size distribution was observed (5 µm). Due to the greater swelling power and water-binding capacity of waxy rice flour impaired the hydration and functionality of sodium-caseinate, fat coalescence would occur (Figure 12C).

At 5% added waxy rice flour, the flour was dispersed through the protein matrix as particles of irregular shape, the size was larger than oil droplet (Figure 12C). When heated Mozzarella cheese with 5% waxy rice flour, the flour dispersed in particles and was presented as strands and large cluster (Figure 12D). This flour tended to link together but protein was still a continuous phase.

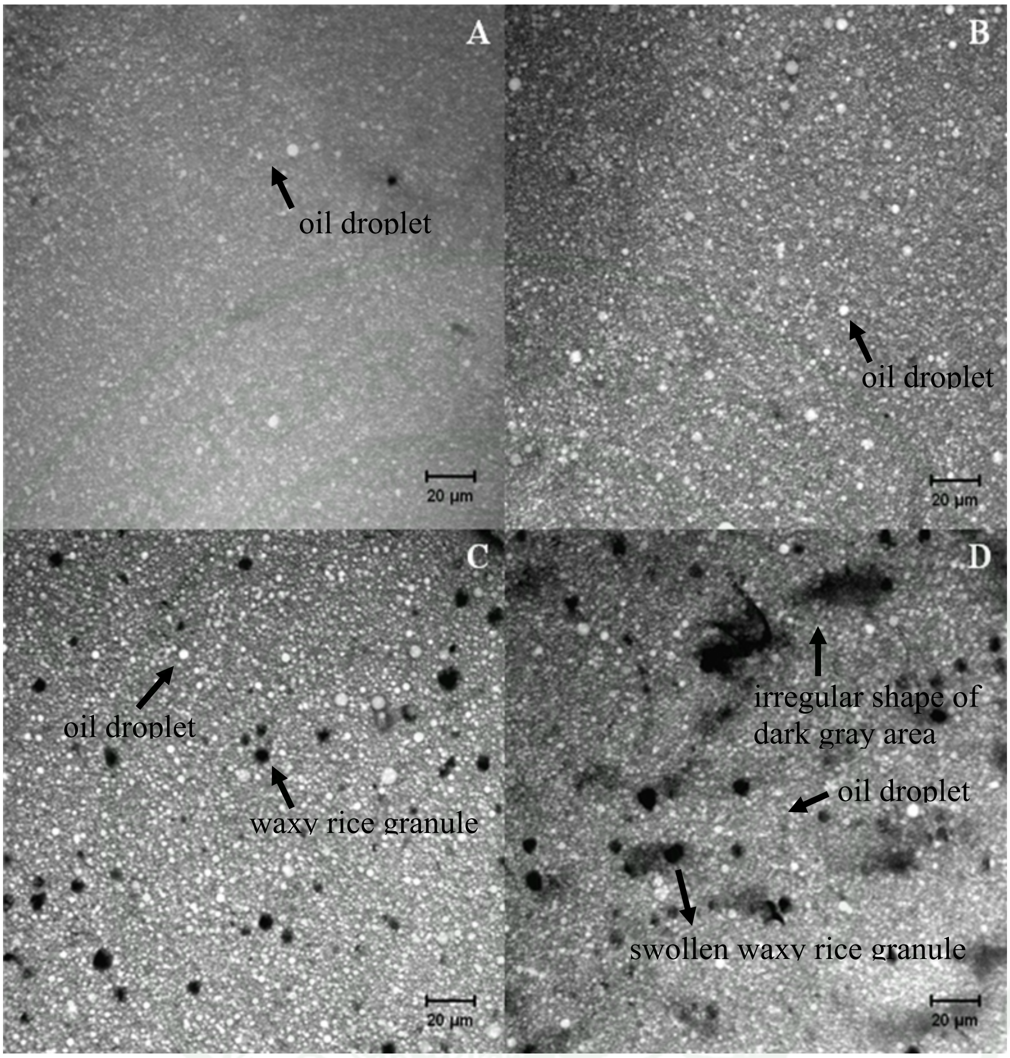

Figure12: Confocal laser scanning micrographs of imitation Mozzarella cheese: a) imitation Mozzarella cheese, b) heated imitation Mozzarella cheese, c) imitation Mozzarella cheese with 5% waxy rice flour, d) heated imitation Mozzarella cheese with 5% waxy rice flour. White area represents fluoresced oil droplets while the gray background indicates fluoresced protein network and black area represents waxy starch granules, which are not fluoresced.

CONCLUSION AND RECOMMENDATION

Imitation Mozzarella cheese was made using sodium – caseinate and rice bran oil. High protein to oil ratio gave a desirable quality of imitation Mozzarella cheese. Modified sodium – caseinate substituted sodium caseinate could improve its stretchability, but impaired meltability. High moisture content and lactic acid concentration during modification of sodium – caseinate gave more small molecular weight protein to MSCN. Replacement of rice bran oil with palm oil could decrease meltability of imitation cheese due to high melting range of palm oil. However, more work is needed to clarify the roles of palm oil in lowering stretchability of the product. Substituting rice flour to protein diminished imitation Mozzarella cheese quality, but gelatinised waxy rice starch enhanced the meltability of the product. Confocal scanning micrograph revealed that imitation Mozzarella cheese is an o/w emulsion in which proteins are the continuous phase, oil and flour are the disperse phase. It was found that imitation Mozzarella cheese with similar stretchability and meltability to those of commercial one could be prepared by emulsifying 20% RBO in an aqueous suspension containing 16% SCN, 7.75% MSCN, 1.25% WRF and 51% water. This study showed the potential use of vegetable oil i.e. RBO to replace solid fat in milk products that require high stability of o/w emulsified gel and stretchability at high baking temperature. The replacement of RBO to milk fat in the imitation cheese did not only reduce the amount of saturated fatty acid and cholesterol, but also fortified the imitation cheese with high level of phytochemicals from rice.

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APPENDICES

Appendix A

Analytical Method

Analytical methods

1. Moisture content determination (AACC, 1995)

1.1 Instruments and apparatus

1.1.1 Moisture can and cover

1.1.2 Hot air oven

1.1.3 Dessicator

1.1.4 Weight balance

1.2 Procedure

1.2.1 Dry the moisture can by heating in hot air oven at 130°C for 1 h

1.2.2 Cool in dessicator and weight

1.2.3 Weigh the sample (2-3 g) and place into the moisture can

1.2.4 Heat uncover moisture can for 1 h

1.2.5 Remove the can from oven, cool in dessicator and weigh

1.2.6 Repeat step 1.2.4 – 1.2.5 to constant weight

1.3 Calculation

$$\% \text{ Moisture} = \frac{A \times 100}{B}$$

Where, A = g of moisture loss

B = g of original sample

2. Determination of protein content (AOAC, 2000)

2.1 Instruments and apparatus

2.1.1 Weight balance

2.1.2 Kjeldahl digestion flask

2.1.3 Digestion unit

2.1.4 Scrubber

2.1.5 Distillation unit

2.1.6 Flasks

2.2 Chemicals

2.2.1 Concentrated Sulfuric acid

2.2.2 2% Boric acid

2.2.3 Copper (II) sulfate

2.2.4 Potassium sulfate

2.2.5 40% Sodium hydroxide

2.2.6 Mix indicator (0.1 g bromocresol green and 0.1 g of methyl red in 100 ml of ethanol)

2.3 Procedure

2.3.1 Place weighted sample (0.5-1 g) in a Kjeldahl digestion flask

2.3.2 Add 5 g of catalyst consisted of copper (II) sulfate and potassium sulfate (1:9 w/w) in the flask with a few glass beads

2.3.3 Add 20 ml H_2SO_4 in the flask

2.3.4 Connect the flask with digestion unit and heat (At first heating was provided gently until all the water was removed and charring is completed. The heat is then gradually increased so that the solution is brought to constant boiling with slight bubbling) until solution clears and then for at least 15-20 min longer.

2.3.5 Cool and remove the flask to distillation unit

2.3.6 Add 20 ml distilled water and 60 ml of 40% NaOH

2.3.7 Place the receiving flask containing 60 ml of 2% boric and acid 2-3 drops of mixed indicator in the distillation unit

2.3.8 Distill for 3 min

2.3.9 Titrate the solution in receiving flask with standard 0.1 N H_2SO_4 until the color changes from green to colorless

2.3.10 Correct for blank determinations on reagents

2.4 Calculation

$$\% \text{ Nitrogen} = \frac{(S - B) \times N \times 1.401 \times 100}{W}$$

Where, S = ml sulfuric acid titration of sample titer

B = ml sulfuric acid titration of blank titer (ml of standard)

N = Normality of sulfuric acid

W = g of sample weight

% protein = % Nitrogen X 6.38

Where, 6.38 is conversion factor for milk protein

3. Determination of fat content (AOAC, 2000)

3.1 Instruments and apparatus

3.1.1 Soxtec System HT2; Extraction unit

3.1.2 Cooling generator

3.1.3 Extraction cup

3.1.4 Extraction thimble

3.1.5 Filter paper

3.1.6 Dessicator

3.2 Chemicals

3.2.1 Petroleum ether (boiling point 40 – 60°C)

3.3 Procedure

3.3.1 Weigh 2 g of sample, place on a filter paper and fold the filter paper to cover the sample

3.3.2 Transfer sample to extraction unit

3.3.3 Add 60 ml petroleum ether into an extraction cup and connected it to extraction unit

3.3.4 Dip the extraction thimble containing the sample into petroleum ether that was constantly heated to boiling for 20 min

3.3.5 Lift the extraction thimble up and rinse by petroleum ether for 45 min

3.3.6 Evaporate petroleum ether for 10 min

3.3.7 Remove extraction cup and place in hot air oven at 100°C for 1 h

3.3.8 Cool in dessicator and weigh

3.4 Calculation

$$\% \text{ Fat} = \frac{(W_3 - W_2) \text{ X } 100}{W_1}$$

Where, W1 = g of sample

W2 = g of extraction cup

W3 = g of extraction cup and fat

4. Determination of amylose content (Chrastil, 1987)

4.1 Instruments and apparatus

4.1.1 Water bath

4.1.2 Vortex

4.1.3 Refrigerated centrifuge

4.1.4 Spectrophotometer

4.2 Chemicals

4.2.1 85% Methanol

4.2.2 0.4 N NaOH

4.2.3 0.13 N NaOH

4.2.4 0.5% Trichloroacetic acid

4.2.5 Iodine solution (0.127% I_2 and 0.3% KI in distilled water)

4.2.6 Potato amylose

4.3 Procedure

4.3.1 Preparation of test sample

1) Weigh 10-20 mg of sample and transfer to test tube
2) Add 5 ml 85% methanol in the test tube, cap the tube and vortex thoroughly
3) Heat for 15 min in a water bath at 60 ^{o}C
4) Centrifuge at 3000xg for 15 min and discard the supernatant
5) Repeat step 1) – 4)
6) Add 2 ml 0.4 N NaOH and then vortex
7) Add 4 ml distilled water and vortex
8) Cap the tube and heat for 30 min in a water bath at 95°C
9) Cool and use it as a test sample (Solution A)

4.3.2 Solution for standard curve

1) Weigh 50 mg of potato amylose and transfer to test tube

2) Add 8.3 ml 0.4 N NaOH in the test tube and vortex thoroughly

3) Adjust volume to 25 ml by adding distilled water and cap the tube

4) Heat for 30 min in a water bath at 60°C

5) Cool and use it as a stock (100% concentration)

6) Use the stock to prepare working solutions at 20, 40, 60 80 and 100% (dilute stock solution to 5 ml by 0.13 NaOH) (Solution B)

7) Iodine color measurement for test sample (Solution A) and standard curve (Solution B)

8) Pipette 5 ml of 0.5% Trichloroacetic acid in test tube (for blank, prepare test tube using 5 ml 0.13 N NaOH)

9) Add 0.1 ml Solution A or Solution B

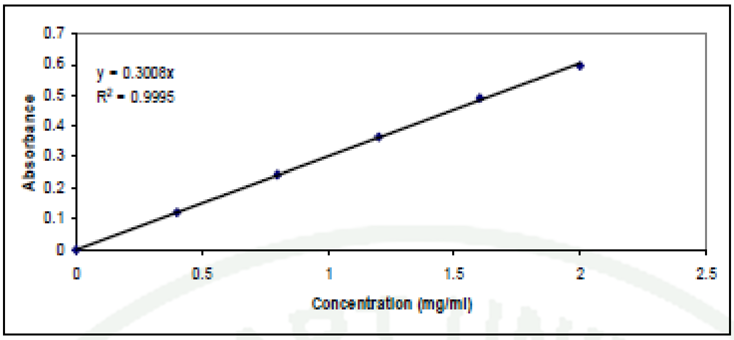
10) Add 0.05 ml iodine solution

11) Read color absorbance at 620 nm, using the blank to zero the spectrometer

4.4 Calculation

4.4.1 Plot absorbance at 620 nm against amylose concentration of working solutions for a standard curve

4.4.2 Use absorbance values obtained from the test sample to calculate the apparent amylose content of the sample from the equation obtained from the standard curve (Appendix Figure A1)



Appendix Figure A1 Standard curve for amylose content determination.

Appendix B

Characterization of imitation Mozzarella cheese

Characterization of imitation Mozzarella cheese

1. Stretchability (modified from Guinee and O'Callaghan, 1996)

1.1 Instruments and apparatus

1.1.1 Texture analyzer

1.1.2 Electric oven

1.2 Procedure

1.2.1 Imitation Mozzarella cheese, stored at 10°C, was grated to 1 – 2 mm shred length.

1.2.2 Imitation Mozzarella cheese size 0.25 g/cm^2 was loaded on to a rectangular (4.5 x5 cm) bread base, which was cut into half along the short axis.

1.2.3 Placed in a thermostatically controlled electric fan oven at 250 °C for 4 minutes, and performed at room temperature in 1 minute after removing from the oven.

1.2.4 The cooked bread base was placed on the platform unit of the stretch apparatus. Stretching speed was set at 0.075 m/s and stretched until the extended string of the melted cheese mass completely broken. Stretchability was defined as the distance of complete strand breakage.

2. Meltability (Mounsey and O'Riordan, 2008)

2.1 Instruments and apparatus

2.1.1 Petri dish

2.1.2 Electric oven

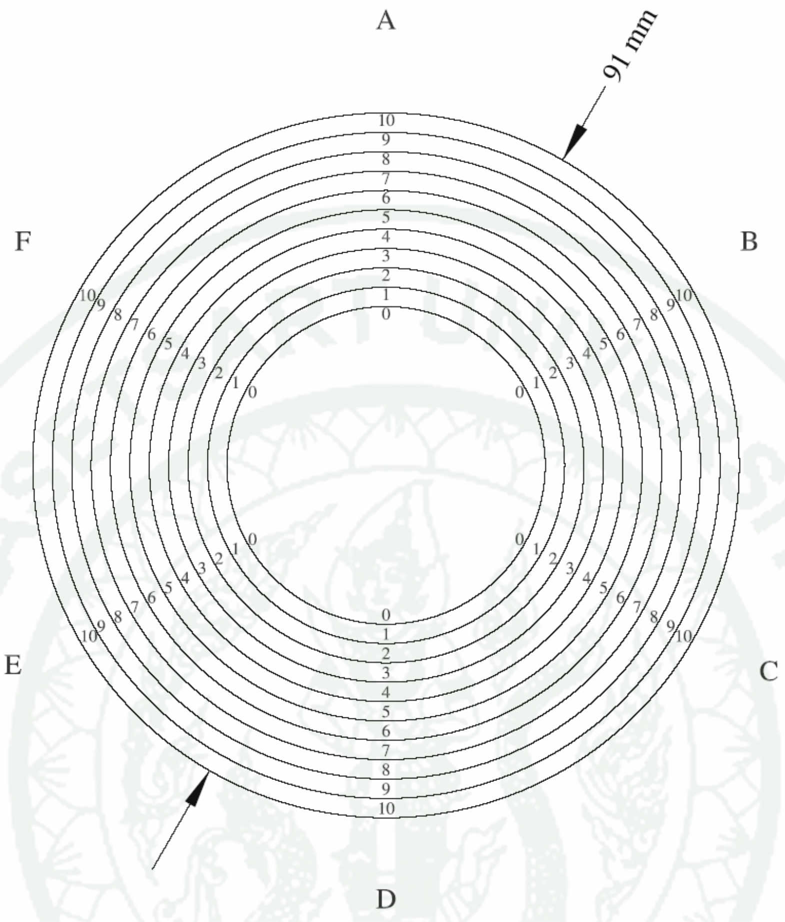
2.1.3 Schreiber test paper

2.2 Procedure

2.2.1 Imitation Mozzarella cheese was cut into 8 mm thick disk of 41 mm diameter, placed on a petri dish.

2.2.2 Baked in 250^{o}C oven for 5 minutes, and maintain at room temperature for 5 minutes.

2.2.3 The dimension of the melted cheese was measured at 6 positions using Schreiber test paper and the average of the dimension taken. Meltability was defined as the increasing dimension of the melted cheese.



Appendix Figure B1 Schreiber test paper

Appendix C

Sensory analysis

Questionnaire for sensory analysis

Different from Control Test: Difference analysis of imitation Mozzarella cheese and commercial Mozzarella cheese

Name..Date.................................Product: Mozzarella cheese

Instruction

1. Taste the sample marked "control" first.
2. Taste the sample marked with three digit code 591, 874, 426.
3. Assess the sensory difference between the three samples using the scale below.
4. Mark the scale to indicate the size of difference.

0 = no difference

1 = very slight difference

2 = slight difference

3 = moderate difference

4 = large difference

5 = very large difference

Stretch by hand (stretch in horizontal)

	0	1	2	3	4	5
591						
874						
426						

Mouthfeel (cheese)

	0	1	2	3	4	5
591						
874						
426						

Comments

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CIRRICULUM VITAE

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