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Influence of Rice Bran Oil and Rice Flours on Physicochemical Properties of a Mozzarella Cheese Analog

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ABSTRACT

The mozzarella cheese market has been growing considerably in recent years, due to the increasing demand for pizzas. Owing to the high cost of traditional mozzarella cheese and consumer preference towards healthier products, cheese analogs containing low levels of saturated fatty acid and cholesterol can be used to make additional varieties of mozzarella cheese. The current study attempted to use rice bran oil (RBO) and rice flours in the production of milk fat-free mozzarella cheese analogs made with sodium caseinate (SCN). The SCN was physicochemically modified by lactic acid hydrolysis with limited water content (30-50%). It was found that mozzarella cheese analogs retained similar stretchability and meltability characteristics to those of commercial cheese and could be prepared by emulsifying 20% RBO in an aqueous suspension containing 16% SCN, 7.75% MSCN (4.12 mg TCA soluble peptide/g SCN), 1.25% waxy rice flour and 51% aqueous phase. Confocal laser scanning microscopy showed dispersed strands of gelatinised waxy rice starch separated from the protein phase after cheese melting. The multi-phase structure thus helped control the meltability and stretchability of the mozzarella cheese analogs containing RBO. Consequently, RBO could be used to replace milk fat in cheese products.
Keywords: cheese, flour, hydrolysis, rice bran oil, sodium caseinate

INTRODUCTION

Mozzarella cheese production has grown considerably in recent years. The impetus for the dynamic growth of mozzarella usage has been the growing demand for pizzas. 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 when baked (Bachmann, 2001).

A cheese analog provides an ideal model system for examining the interactions between major food components, such as protein, fat, starch and water. Basically, they are oil–in–water (o/w) emulsified protein curds (Mounsey and O'Riordan, 2008a). Although the basic curd structure in all cheese varieties can be described as emulsified protein gel, mozzarella cheeses possess unique characteristics obtained from the stretching process in hot water or brine during their manufacturing. The stretching process converts the three-dimensional network of protein into parallel-aligned protein bundles or fibers, embedded with a partially aligned serum phase and fat globules. Kiely *et al*. (1993) and Kindstedt and Guo (1997) indicated that the meltability of traditional mozzarella cheese was associated with the

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proteolytic hydrolysis of caseins during aging. The increased casein hydrolysis improved cheese meltability, increased free oil and decreased the apparent viscosity of mozzarella cheese.

Upon heating, the hydrolysed caseins in cheese (Kiely *et al*., 1993; Kindstedt and Guo, 1997), together with melted milk fat (Joshi *et al*., 2004), regulate the viscosity and the unique stretchability and meltability characteristics of mozzarella cheese. Therefore, the alteration of the protein and fat ingredients may influence these attributes during cheese melting.

Modification of the microstructure by the addition of pre-gelatinised starch, as well as the distribution of fat and protein matrix, could increase firmness and brittleness, while decreasing the flowability of melted cheese (Mounsey and O'Riordan, 2008b; Ye *et al*., 2009). In traditional cheese, hardness could be increased in the presence of wheat, potato and maize starches. However, the hardness was lowered by waxy maize and rice starches. It is apparent that the physical characteristics of traditional cheese are governed by ingredient interactions.

Recently, the influence of granular rice flour and waxy rice flour and their protein matrices in the composite network of protein-starch mixtures has been investigated, as the storage rice protein network could form another separated matrix (Isarakarn and Hongsprabhas, 2008; Likitwattanasade and Hongsprabhas, 2010) and a gelatinized rice starch phase could enhance the hardness of the protein-starch matrix (Hongsprabhas and Dit-udom-po, 2006). In this study, it was hypothesized that cheese analogs could be prepared using a matrix of phosphorylated caseins (in the form of sodium-caseinate) and vegetable oil. The melting characteristics of mozzarella cheese analogs could be manipulated by controlling the phase separation of sodium-caseinate, vegetable oil, starch and water in the composite structure governed by thermodynamic incompatibility of the biopolymers.

The objective of the current study was to investigate the roles of protein and lipid characteristics on the meltability and stretchability of mozzarella cheese analogs and the influence of flour substitution on the continuous protein phase in controlling cheese behavior during melting. The understanding gained of the interplay among protein-starch-oil constituents in the emulsified composite structure may help in the effective design of a food matrix with desirable characteristics.

MATERIALS AND METHODS

Materials

Sodium-caseinate (SCN) (ECCO 2300, International, Inc. Erie, IL, USA) and food grade lactic acid (88% w/v) were obtained from Vicchi Consolidated Thailand Co., Ltd. The SCN contained 2.13±0.02% fat, 93.47±1.23% protein, 4.47±0.02% moisture (AOAC, 2000) and had a pH of 7.1±0.00 (53.33% suspension in distilled water). Rice bran oil (RBO, King, Thai Edible Oil Co., Ltd, Thailand), rice flour and waxy rice flour (Fish brand, Thai Flour Industry Co., Ltd, Thailand), palm oil (PO, Oleen, Oleen Co., Ltd, Thailand) were purchased from a local supermarket. The rice flour (RF) contained 10.5% protein and 24.50% amylose while the waxy rice flour (WRF) contained 10.3% protein and 4.30% amylose. The amylose contents were determined using the method of colorimetric quantification of amylose, as described by Chrastil (1986), using potato amylose as a standard. Sodium hydrogen phosphate, sodium tripolyphosphate, sodium citrate and guar gum of food grade were obtained from Aditdaya Birla Chemical Phosphate, Thailand. Food-grade sodium chloride was purchased from Thai Refined Salt Co., Ltd, Thailand.

Effect of protein phase characteristics on the properties of Mozzarella cheese analogs

Preparation of modified sodium caseinate (MSCN)

Sodium-caseinate (SCN) was modified by the method described by Tudthong *et al.* (2007). Briefly, the moisture content of the SCN was adjusted to 30, 40 or 50% w/w, then lactic acid (88% w/v) was added, to obtain 1% lactic acid in the SCN by weight. This was mixed thoroughly and incubated at room temperature (25°C) for 30 min and dried at 50°C in a tray-dryer, until the moisture content reached 6% w/w (Reliance Tech – Service Co., Ltd, Bangkok, Thailand). The resulting modified SCN (MSCN) was ground in a mortar, screened through a 100-mesh sieve, and kept at 10°C before use.

Small molecular weight (MW) proteins in the SCN and MSCN were analysed by the method described by Henn and Netto (1998). The SCN or MSCN (2% w/v) was dispersed in distilled water and stirred until no sedimentation was observed. Trichloroacetic acid (TCA) was added to obtain 10% w/w and the suspension was stirred for 10 min and centrifuged at 14,000 rpm for 10 min (Spectrafuge, 16M, LABNET International, NJ, USA). Proteins in the supernatant were analysed by the Kjedahl method (AOAC, 2000) and designated as TCA-soluble proteins.

Preparation of mozzarella cheese analogs containing MSCN

A mozzarella cheese analog was made by heating 20 mL of RBO at 85°C, and then 25 g of SCN or MSCN at a specified ratio and 0.2 g of guar gum were added. The dispersion was heated at 85°C for 2 min and subsequently 51 mL water and emulsifying salts were added to obtain the final concentration of salt in the cheese analogs as 0.75% sodium chloride, 0.2% sodium citrate, 0.2% sodium hydrogen phosphate and 0.2% sodium tripolyphosphate. The emulsion was heated at 85°C for 2 min, 0.35 mL lactic acid (88% w/v) was added and blended in a high-speed blender to obtain a smooth mozzarella o/w emulsion, which was then poured into a mould (100 × 100 × 100 mm) to form mozzarella cheese analogs that were then kept at 10°C for 24 h before analysis.

Determination of stretchability

Stretchability of the mozzarella cheese analogs was analyzed by the method described by Guinee and O'Callaghan (1996). The sample was shredded into blocks 2 × 5 × 2 mm in size, which were loaded onto a pre-cut rectangular (45 × 50 mm) bread base, using a load of 25 g shredded cheese/mm^2. The bread base was placed in a thermostatically controlled electric oven at 250°C for 4 min to simulate the way cheese was heated in practice. The stretchability test was performed at room temperature (25°C) within 1 min after the cheese had been removed from the oven. The heated sample was gripped with a pair of self-tightening grips (TG20, from Lloyd TA Plus, Lloyd Instruments Ltd., West Sussex, UK). The stretching speed was set at 0.075 m s^{-1} and the sample was stretched until the extended string of the melted cheese mass was broken completely. The distance of complete strand breakage was recorded as stretchability (mm).

Determination of meltability

Meltability was analysed using the Schreiber test (Mounsey and O'Riordan, 2008a). Each sample was cut into disks 8 mm thick with a diameter of 41 mm, placed on a Petri dish and baked in an oven at 250°C for 5 min. After cooling for 5 min at room temperature, the dimension of the melted cheese was measured at six positions and the average of the measurements recorded. Meltability was defined as the per cent increase in the diameter of the melted cheese compared with the original diameter.

Effect of vegetable oil phase on the characteristics of mozzarella cheese analogs

Preparation of mozzarella cheese analogs containing MSCN and rice bran oil-

palm oil blends

A mozzarella cheese analog was prepared from the SCN and MSCN with a ratio of 16.7:8.3, using three different ratios of protein to oil of 20:25, 22.5:22.5 and 25:20. The oil fraction was prepared using different ratios between the RBO and PO of 0:100, 25:75, 50:50, 75:25 and 100:0. Other ingredients were formulated as described previously. Mozzarella cheese analogs were evaluated for stretchability and meltability using the methods described above.

Thermal characteristics of rice bran oil and palm oil blends

The melting range and solid fat content (SFC) of the RBO, PO, their blends and anhydrous milk fat were analyzed using a differential scanning calorimeter (DSC, Pyris1, Perkin-Elmer, Norwalk, CT, USA), using the method described by Ortiz-Gonzalez *et al*. (2007). The RBO and PO were blended at different ratios of RBO to PO of 0:100, 25:75, 50:50, 75:25 and 100:0. Each sample was subjected to the following temperature programs: holding at 60°C for 3 min, cooling from 60°C to -60°C at the rate of 10°C /min, and holding at -60°C for 3 min. The temperature change in the samples was at a rate of 10°C/min during the heating and cooling cycles between 60°C and -60°C. The SFC was calculated by dividing the partial area under the thermograms by the total area from -30 to 40°C and multiplying it by 100. The SFC from 0 to 40°C was calculated at 5°C intervals.

Effect of rice flour on the characteristics of mozzarella cheese analogs

Preparation of mozzarella cheese analogs containing emulsified rice bran oil and rice flours

The protein constituent in the cheese analog was substituted with rice flour (RF) or waxy rice flour (WRF) at 0, 2.5, 5.0, 7.5 and 10% (w/w) to inhibit the flow of liquid oil in the mozzarella cheese analog during melting. The cheese analog was prepared as previously described using the RBO as the lipid source. The stretchability and meltability of the mozzarella cheese analogs were evaluated using the methods described above. The microstructure of the melted cheese analogs was evaluated by confocal laser scanning microscopy (CLSM).

Microstructure of mozzarella cheese analogs

A thin slice (approximately 1 mm thick) was cut from the mozzarella cheese analogs or the heated samples (at 250°C) for microstructural analysis using CLSM (Axio Imager MI, Carl Zeiss Pte Ltd, Germany). Each sample was mixed with 0.05% Nile blue A in 95% ethanol, which fluoresced green in the lipid phase, and 0.01% rhodamine B in distilled water, which fluoresced red in the protein phase. A HeNe laser with an excitation wavelength of 488 and 543 nm was used. CLSM digital images were acquired using the LSM 5 PASCAL program.

Statistical analysis

The experiments were carried out in three separate trials. Each trial was run in triplicate. The data were analyzed by analysis of variance (ANOVA) with the significance level set at $p<0.05$. All statistical analyses were performed using the SPSS Software, Version 12.

RESULTS AND DISCUSSION

The use of the RBO within the range of concentrations investigated in this study of mozzarella cheese analogs did not affect the meltability of cheese ($p \geq 0.05$; Figure 1). However, the cheese analog had a much lower stretchability within the range 125-175 mm. The commercial cheese could be stretched up to 224 mm. Nevertheless, increasing the protein-to-oil ratio resulted in greater stretchability. This indicated that more protein helped form stretchable fibers during cheese melting, which could withstand the tension better than those samples with a low protein-to-oil ratio.

Due to the fact that the stretchability of the mozzarella cheese analog was quite low, the MSCN was substituted with SCN at different levels in the formulae. Figure 2 illustrates that substitution of the SCN with 33% (wt/wt) MSCN effectively improved stretchability. Although the stretchability was improved, the meltability was impaired with higher levels of MSCN substitution. It is likely that the MSCN, of which some phosphorylated caseins were hydrolyzed (observed as the increased TCA soluble fraction in Table 1), were less able to withstand the tension, due to the smaller MW proteins (Sheehan and Guinee, 2004).

The degree of SCN hydrolysis was controlled by the water content during

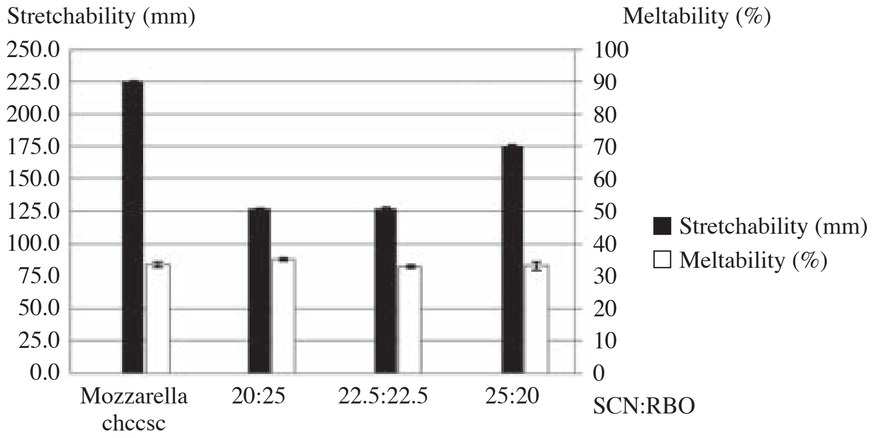

Figure 1 Effect of the ratio of sodium caseinate (SCN) to rice bran oil (RBO) on the characteristics of mozzarella cheese analogs. The mozzarella cheese analog contained 20% SCN, 20-25% RBO and 51% water. Bars represent standard deviation.

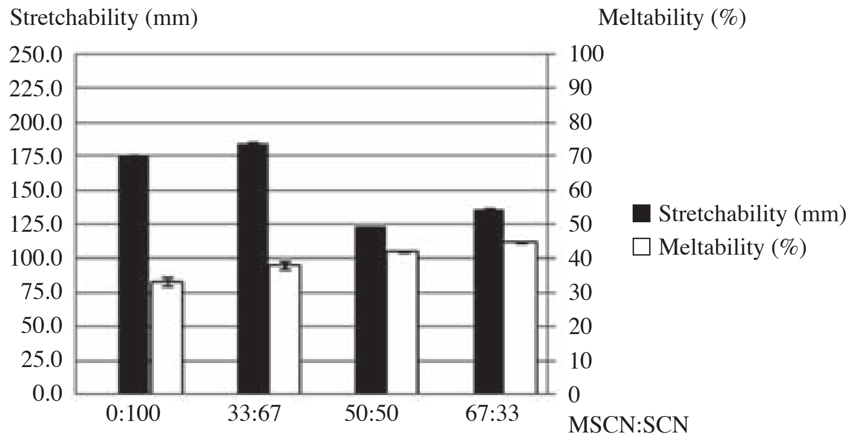

Figure 2 Effect of the ratio of modified sodium caseinate (MSCN) to sodium–caseinate (SCN) on the characteristics of mozzarella cheese analogs. The MSCN was modified at 30% moisture content. The mozzarella cheese analog contained 8.3-16.7% SCN, 8.3-16.7% MSCN, 20% RBO and 51% water. Bars represent standard deviation.

modification. Increasing the moisture content during the modification process increased the TCA-soluble protein content in the MSCN (Table 1), which subsequently lowered the stretchability and enhanced the meltability (Table 2). The latter caused detrimental effects on the characteristics of the mozzarella cheese. Small MW peptides were likely involved in the stretchability of mozzarella cheese analogs investigated in this study. Nevertheless, using liquid oil RBO impaired the melting characteristics of the cheese analog, since the casein network could not hold lipid oil

Table 1 Effect of lactic hydrolysis at limited water content on TCA-soluble protein content of modified sodium caseinate (MSCN).

Water content during acid hydrolytic modification of SCN	TCA - soluble protein content (mg/100g SCN or MSCN)
Unhydrolysed SCN (control)	8.7[d]
MSCN modified at 30% water content	21.5[c]
MSCN modified at 40% water content	35.0[b]
MSCN modified at 50% water content	51.8[a]

Means followed by different superscript letters are significantly different ($p<0.05$).

Table 2 Effect of water content during modification of sodium caseinate (SCN) on the characteristics of mozzarella cheese analogs. The mozzarella cheese analog contained 16.7% SCN, 8.3% MSCN, 20% RBO and 51% water.

Water content during the modification by by acid hydrolysis	Stretchability (mm)	Meltability (%)
Mozzarella cheese	224.93 ± 0.57[a]	33.89 ± 0.79[b]
MSCN modified at 30% water content	183.33 ± 1.00[b]	38.33 ± 1.36[b]
MSCN modified at 40% water content	229.72 ± 1.77[a]	51.94 ± 1.04[a]
MSCN modified at 50% water content	168.40 ± 0.71[c]	53.89 ± 0.40[a]

Means in the same column followed by different superscript letters are significantly different ($p<0.05$).

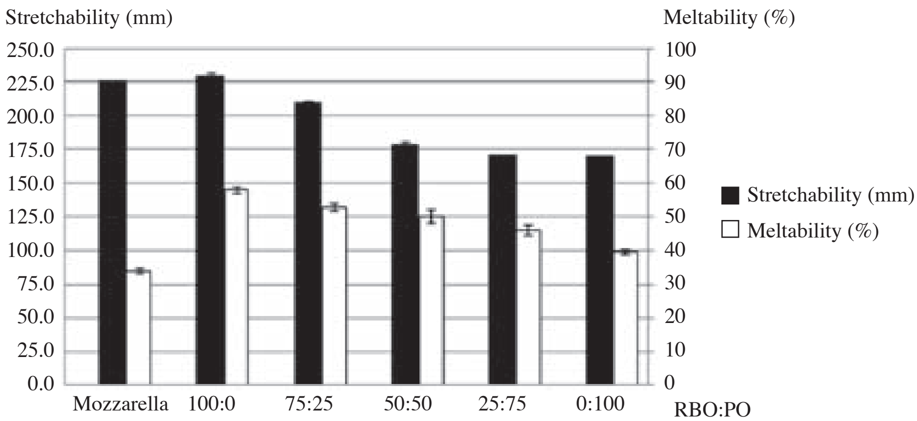

Figure 3 Effect of the ratio of rice bran oil (RBO) to palm oil (PO) on the characteristics of mozzarella cheese analogs. The mozzarella cheese analog contained 16.7% SCN, 8.3% MSCN, 20% vegetable oil and 51% water. Bars represent standard deviation.

effectively in the presence of the small MW proteins in the MSCN.

The influence of lipid types on the stretchability and meltability was confirmed when the RBO was substituted with PO (Figure 3). Increasing the ratio of PO, which contained more monounsaturated fatty acid than did the RBO (Kallio *et al*., 2001; Mayamol *et al*., 2007), lowered the stretchability and meltability of the cheese analogs, because the PO had a higher melting temperature range (Table 3) and solid fat content than did the RBO (Figure 4). Nevertheless, both vegetable oils were inferior to milk fat in terms of promoting the desirable high SFC at high temperature for melted mozzarella cheese analogs.

To enhance the desirable melting characteristics of the mozzarella cheese analog containing the RBO, substitution of the protein fraction with flour was investigated. Substitution of the SCN by RF could decrease the meltability of the mozzarella cheese analog ($p< 0.05$; Figure 5). After melting, the gelatinised RF expanded in size and altered the continuous protein phase. The alterations in the continuous protein phase subsequently lowered the stretchability when the substitution level was increased to 5% (Figure 5).

However, the influence of WRF on the characteristics of the mozzarella cheese analog were different from those of RF. Although the meltability of the cheese analog could be decreased in the presence of the WRF (Figure 6), the influence of the WRF concentration on

Table 3 Melting temperature range of anhydrous milk fat, rice bran oil (RBO) and palm oil (PO) blends determined by differential scanning calorimetry.

Oil blends	Melting temperature range (°C)
Anhydrous milk fat	21.29 - 40.08
RBO:PO = 100:0	-22.57 - 4.7
RBO:PO = 75:25	-6.16 - 6.18
RBO:PO = 50:50	-0.48 - 9.80
RBO:PO = 25:75	-2.54 - 11.67
RBO:PO = 0:100	-2.47 - 14.16

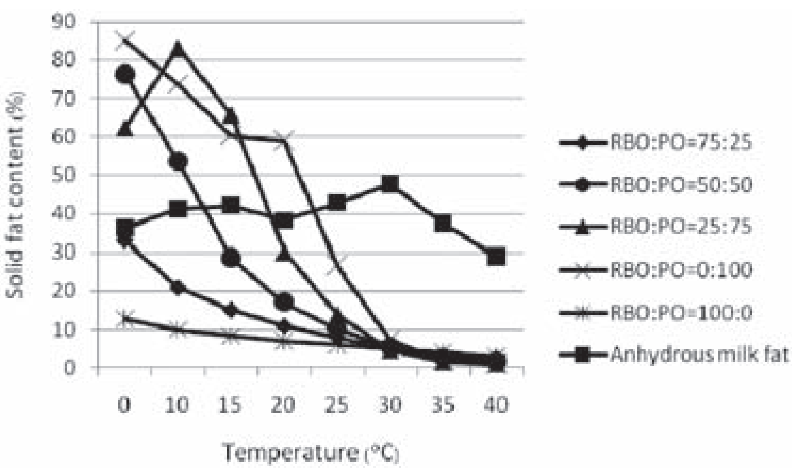

Figure 4 Solid fat content of oil blends prepared from rice bran oil (RBO) and palm oil (PO) compared to that of anhydrous milk fat.

stretchability seemed to be inconsistent. Nevertheless, using WRF substitution to the SCN at 1.25% resulted in a cheese analog possessing melting characteristics (239 mm stretchability and 50.8 % meltability) close to those of commercial cheeses.

To understand the roles of gelatinised WRF, the distribution of oil droplets and flour in the protein matrix of the mozzarella cheese analogs was observed under CLSM (Figure 7). The micrographs showed that oil droplets and flour were in dispersed phases, and protein was in a continuous phase. In the mozzarella cheese analogs, the oil was dispersed in the protein matrix and had a particle size of around 1 μm (Figure 7A). After the mozzarella cheese analog had been heated, the oil droplets melted and coalesced to a larger size of around 3 μm. The protein matrix could still hold the melted oil droplets and retained its continuity (Figure 7B). However, the addition of WRF resulted in another dispersed phase in the protein matrix. The oil droplet size of the cheese analog containing WRF was slightly larger (5 μm) than in the absence of WRF, (Figure 7C). After the cheese analog had melted, the WRF gelatinized and the starch dispersed phase appeared as a large

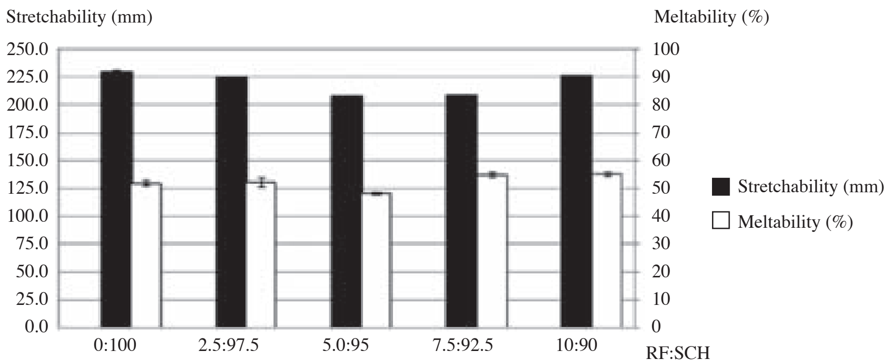

Figure 5 Effect of rice flour (RF) substitution for sodium-caseinate (SCN) on the characteristics of mozzarella cheese analogs. The mozzarella cheese analog contained 15.07-16.7% SCN, 7.43-8.3% MSCN, 20% RBO, 0-2.5% RF and 51% water. Bars represent standard deviation.

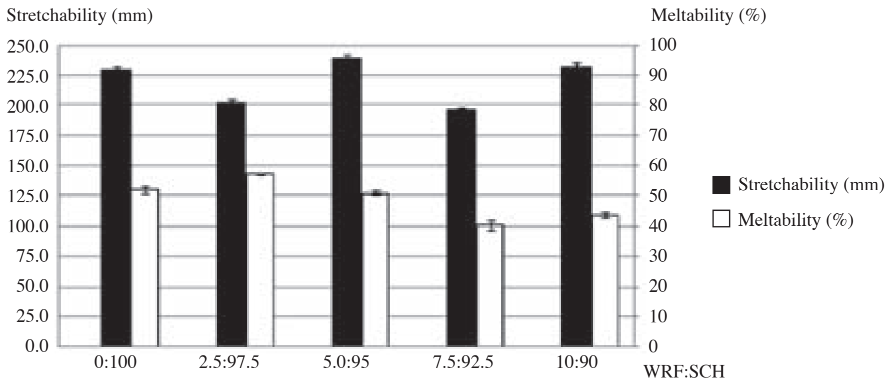

Figure 6 Effect of waxy rice flour (WRF) substitution for sodium-caseinate (SCN) on the characteristics of mozzarella cheese analogs. The mozzarella cheese analog contained 15.07-16.7% SCN, 7.43-8.3% MSCN, 20% RBO, 0-2.5% WRF and 51% water. Bars represent standard deviation.

separated dark gray phase (Figure 7D). The gelatinised WRF phase may have been responsible for lowering the meltability of the cheese analog.

This study demonstrated the significance of the interplay among protein-starch-liquid oil constituents in the composite structure of mozzarella cheese. Despite the low solid fat content in the RBO, it could still be used as a replacement for anhydrous milk fat to enhance the meltability of cheese analogs. However, the continuous protein phase composed of the SCN and MSCN needed to be disrupted by a gelatinised starch phase to reduce excessive flow in the protein phase during heating.

This study showed the potential use of vegetable oil as a replacement for solid fat in milk products that require high stability of o/w emulsified gel and stretchability at high baking temperatures. RBO is known to contain high level of phytosterols, γ-oryzanol and tocols, which have the capacity to lower serum cholesterol and tocols and are known as powerful antioxidants associated

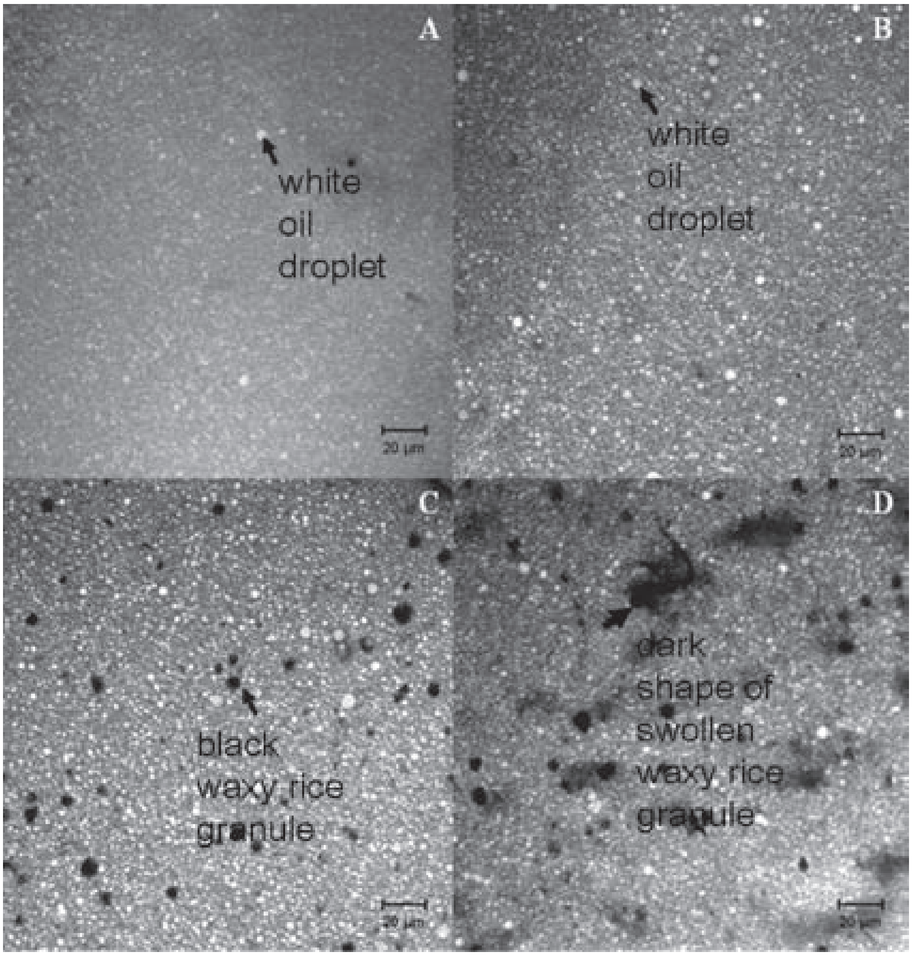

Figure 7 Confocal laser scanning micrographs of mozzarella cheese analogs: A) mozzarella cheese analog, B) heated mozzarella cheese analog, C) mozzarella cheese analog containing waxy rice flour, D) heated mozzarella cheese analog containing waxy rice flour. Bar scale = 20 µm. White area = fluoresced oil droplets; gray background = fluoresced protein network; and black area = waxy rice starch granules, which are not fluoresced.

with the prevention of cardiovascular diseases and some cancers (Piironen *et al.*, 2000). RBO also has an appropriate ratio of saturated: monounsaturated:polyunsaturated fatty acid, as recommended by the American Heart Association (Piironen *et al.*, 2000; Van Hoed *et al.*, 2006). The replacement with RBO for the milk fat in the cheese analog did not only reduce the amount of saturated fatty acid and cholesterol, but also fortified the cheese analog with a high level of phytochemicals from rice bran oil.

CONCLUSIONS

A multi-phase matrix of oil and starch dispersed in the protein matrix could be used in the preparation of mozzarella cheese analogs. Despite the use of liquid oil to replace butter fat, the mixed SCN and MSCN could hold high amounts of oil within the protein network during and after heating. Heating at a temperature of 250° C in the Shreiber test, with observation under CLSM, indicated that the dispersed gelatinised rice flour phase could decrease the meltability of cheese analogs by disrupting the liquid oil from flowing and coalescencing.

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