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The Effect of Casein Modification and Inulin on Physicochemical Properties of Mozzarella Cheese Analogue Microwavable

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Abstract : The effect of casein modification on physicochemical characteristics of Mozzarella cheese analogue microwavable was studied. The research was divided into three stages. The first stage was to find out a better physicochemical properties of casein with cross linking modification, while the second stage was to find out a better physicochemical properties of Mozzarella cheese analogue with the addition of casein modified and inulin at several ratios. The third stage was to study the influence of microwave power level on physicochemical properties of Mozzarella cheese analogue. Experimental results showed that the effect of pH value and level of $CaCl_2$ of modified casein did not give a significantly differenceeffect ($p>0.05$) on protein solubility, however it gave a significantly difference effect on calcium solubility at a confidence level of 95%. Furthermore, the pH values and level of $CaCl_2$ on the rheological parameters showed that storage modulus (G') and loss modulus (G") decreased with the increasing of coagulation temperature, approximately 5 – 40^0C, and theresults obtained using SDS-PAGE indicated that the interaction between casein micelles at pH 4.2 and 4.6 was more intensive than at pH 5.0. Additionally, ratio of casein modified and inulin did not give a significantly difference effect ($p>0.05$) on meltability and gave a significantly difference effect ($p<0.05$) on fracture point and stretchability of Mozzarella cheese analogue. The porosity structure of Mozzarella cheese analogue was attained when ratio of casein modified:inulin 80:20, while for the ratio 70:30; 60:40 and 50:50, the size and porosity structure was less and more swollen. Furthermore, statistical analysis revealed that microwave power level gave a significantly differenceeffect ($P<0,05$) on water activity, colour(L*a*b* values), fracture point, and expansion of Mozzarella cheese.
Keywords: modified casein, Mozzarella cheese, crosslink, inulin, microwave, physicochemical properties.

Introduction

Cheese and fermented milk products are among the great food of the world. Mozzarella cheese analogues are defined as products made by blending food ingredients, including milk fat, non-dairy fats or proteins to produce a cheese like product to meet specific requirement. Mozzarella cheese analogues have gained increase acceptance with food processors and consumers because is also used extensively in cooking applications, e.g. its primary use on the pizza toping, cheese burgers and used as an ingredient of ready to eat foods. They are being used increasingly due to their cost effectiveness and attributable to the simplicity of their manufacture. Analogue cheese products are made using similar technique to those used for processed cheese manufacture with the aid of heat, agitation and emulsifying salts.[1]

The physical characteristics of Mozzarella cheese like body, texture, meltability, stretchability, and colour are altered by the factors like milk composition, starter culture, and ripening conditions prevalent during the cheese preparation.[2]The functional properties of importance for cheese analogue include the hardness and exhibiting the desired degrees of meltability.

The main protein source in dairy – based cheese analogue is casein. Rennet casein or sodium and/or calcium play an important role in manufacturing as well as functional properties and desirable flavour of Mozzarella cheese. The water soluble phosphate groups of the caseinate are located at one end of the protein, while the other end carries non polar fat soluble groups, which called emulsifier salts. Emulsifier salts as a calcium chelating agents by increasing its hydrosolubility.[3]

The addition of calcium increases casein–casein interaction and reduces casein hydration. Previous studied that the addition of calcium, at a level of 0 – 1.4%, w/w to Mozzarella cheese by high pressure injection of a 40% (w/w) $CaCl_2$ solution resulted in marked weeping and water loss, a reduction in pH value and a more aggregated para-casein matrix interspersed with large voids containing free water.[4]

Proteins are well known to be versatile with the pH value. In the manufacture of cheese, the conditions of low pH and high temperature are conducive to limited aggregation of para casein and the formation of para-casein fibers of relatively hightensile strength.[5] Casein micelles in milk are remarkably stable systems that can withstand the rigorous conditions applied during commercial processing.[2] However, under certain conditions of temperature and pH value, the colloidal integrity of the casein micelles can be disrupted, resulting in decreased stability, as manifested through visible flocculation, gelation, or protein separation.[6]

Casein can often be modified by chemical or biochemical cross linkers to achieve protein network formation, to form micro size aggregates and decrease the solubility of the protein in Mozzarella cheese manufacture.[7,8]Inulin is a food ingredient that belongs to a class of carbohydrates known as fructans. Inulin is used in industrially processed dairy and non- dairy products because it is a bulking agent for use in fat replacement, textural modification, to form the gel structure and organoleptic improvement.[9,10]

Inulin has many technological benefits and for prevention of syneresis, inulin especially long chain has an excellent water binding capacity which prevents syneresis in spreads and fresh cheese. Therefore, the aim of this study was to investigate physicochemical properties of casein with cross linking modification, the influence of casein modified and inulin on the physicochemical properties of functional Mozzarella cheese analogue after microwave cooking.

Materials and Methods

Isolation of Casein.

Milk was subjected to centrifugation at 3000 rpm for 10 minutes to remove particulate matter. Beaker glass containing 500 ml of supernatant was then heated on a water bath to about 40^0C and the temperature of the milk solution was monitored with a thermometer. When the mixture has reached 40^0C, then add the 10% acetic acid glacial drop wise to the warm milk. Using the spatula, push the precipitated casein into the side of the beaker glass so that most of the liquid drains from the solid. Collect the casein with suction filtration and place the casein in a beaker, then add with 25 ml ethanol to the casein precipitate and stir the solution for 5 minutes. Collect the casein by suction filtration and place the casein between several layers of paper towels to dry.[11]

Casein modification.

The method of modifying acid casein was prepared using lactic acid solution at pH value of 4.2; 4.6; and 5.0, and the solution were incubated at 4^0C. Lactic acid solution was withdrawn at specified pH value and then adding $CaCl_2$ at concentration 0.025, 0.05, and 0.075%, respectively. Subsequently, the casein modification was obtained by soaking casein in $CaCl_2$ with the appropriate pH value, and then was incubated at 4^0C for 30 minutes. Casein was kept at room temperature for 10 minutes before stored at 4^0C.[12]

Manufacture of Mozzarella cheese analogue.

Mozzarella cheese analogue was manufactured by heating the raw milk at 35^{0}C and 0.05% citric acid and 0.025% protease enzyme were added, and held at this temperature for 15 minutes. The milk was then allowed to coagulate and this takes approximately 30 minutes. The coagulum was then cut into small cube-shaped particles and stirred while the temperature of the vat was increased to the desire cooking temperature. Cheese analogue was manufactured by blending the 1% salt, 1% sodium citrate, 0.1% citric acid, 0.25% sodium tripolyphosphate, 0.25% CMC, and casein modification at concentration 50, 60, 70, and 80% respectively. The curd was cooked and stretched to obtain the elastic and stringy character of Mozzarella cheese. When the temperature had reached 50^{0}C, then inulin was added at concentrations of 50, 60, 70, and 80% respectively and stretched to the desired temperature 75 – 80^{0}C. The cheese was then cooled and wrapped in a plastic and stored at temperatures between -2 and 5^{0}C.[13]

Characterization of Mozzarella cheese analogue microwavable.

Mozzarella cheese was then cut into small cube-shaped particles, wrapped with aluminium foil and allowed heated in a microwave for 2 minutes at a wavelength of heat low, medium low, medium, medium high and high. The Mozzarella cheese analogue microwavable was then determined its water activity[14], colour[15], fracture point[16]and expansion.[14]

Statistical analysis.

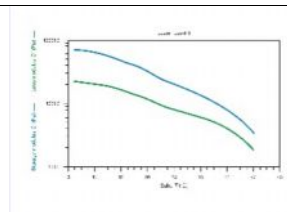
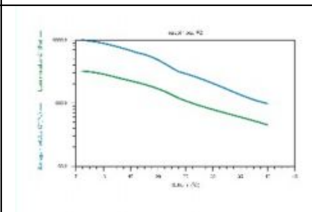
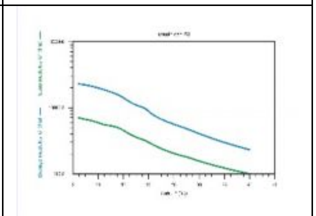
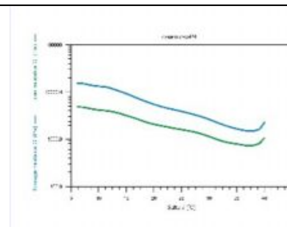
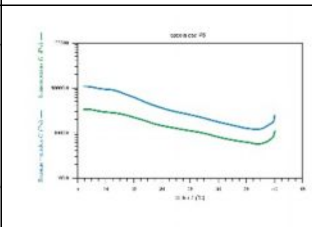
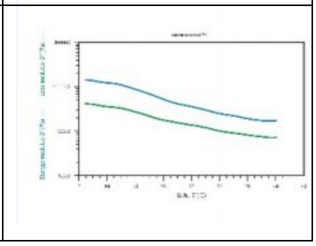
The experiment was replicated 3 times in a completely randomized design. The ANOVA was carried out to determine the differences between data means at 5% significant level.

Results and discussion

Effect of pH value and levels of $CaCl_2$ on rheology of modified casein.

The effect of pH value and levels of $CaCl_2$ on the rheological parameters was seen in Figure 1.A, 1.B, and 1.C. Figure 1.A, 1.B, and 1.C showed that storage modulus (G') and loss modulus (G") decreased with the increasing of coagulation temperature from approximately 5 – 40 ^{0}C. It was probably caused by the influence of the plasticity of casein, thus leading to increased hydration of casein to water. Decreasing of storage modulus and loss modulus due to influence of plasticity and increase hydration of matrix protein thus lead to increasing of water content.[10]While increasing storage modulus and loss modulus showed cohesiveness and stability of casein micelles at high temperatures.

Hydrophobic interactions, which influence the aggregation of casein particles, are favored at higher temperatures. The higher coagulation temperature may have stiffened the junctions, leading to dense aggregates. In addition, after a gel has been formed, collisions between casein micelle aggregates and network strands lead to further aggregation and a coarsening of the network.[17]

pH	$CaCl_2$(%)		
	0.025	0.050	0.075
4.2			
4.6			

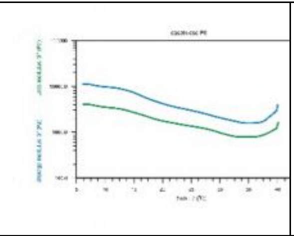
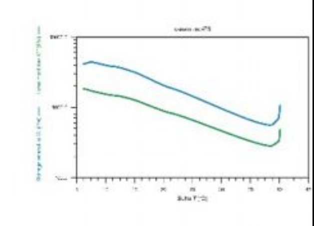
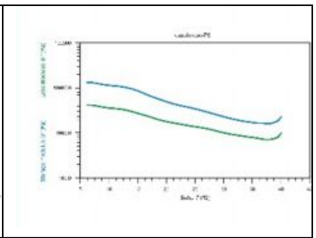
5.0			

Figure 1.A, B and C. The effect of pH value and levels of $CaCl_2$ on rheology of modified casein

Effect of pH value and levels of $CaCl_2$ on protein solubility of modified casein.

As shown in Table 1, the main effect namely pH value and levels of $CaCl_2$ was considered insignificant statistically ($p>0.05$) on protein solubility. Casein proteins are unstable when the pH value of an aqueous phase is ≤ 4.6. However, micelles aggregate and precipitate at pH values at or below the isoelectric point of casein (4.6), resulting in hydrophobic interactions between protein molecules. The addition of $CaCl_2$ resulted in the formation of intra and intermolecular crosslink casein micelles. Combination of pH value and levels of $CaCl_2$ generate more intensive of interaction intra and intermolecular casein micelles, leading to attraction of casein micelles are more powerful and it was probably because the wrinkle casein and hydrophilic proteins trapped in the casein micelles will be dissolved into a solution of hydrophilic.

Under neutral pH conditions, the milk protein casein can provide excellent steric stabilizing properties to dispersed protein particles and oil/fat droplets.[18]This same protein has a strong tendency to aggregate in the presence of calcium ions and upon lowering the pH value. The extent of aggregation of sodium caseinate depends on the relative proportions of the different monomeric casein. Temperature also important affects the strength of the hydrophobic interactions, pH value and ionic strength, which strongly affected the electrostatic interactions.

Table 1. Protein and calcium solubility of modified casein.

pH	Level of$CaCl_2$	Protein Solubility (%)	Calcium Solubility (mg/L)
4.2	0.025	1.14	4.61
	0.050	1.25	1.44
	0.075	1.20	1.67
4.6	0.025	1.15	1.00
	0.050	1.17	3.33
	0.075	1.23	2.53
	0.025	1.21	2.73
	0.050	1.29	2.50
5.0	0.075	1.21	2.47

Effect of pH and Levels of $CaCl_2$ on Calcium Solubility of Modified Casein.

Table 1 showed that pH values, levels of $CaCl_2$ and combination of pH valuesand levels of $CaCl_2$ were considered significant statistically ($p<0.05$). It should also be noted that $CaCl_2$ as a cross linking agent has affinity to different casein. The addition of $CaCl_2$ resulted in the formation of intra and intermolecular crosslink casein micelles. $CaCl_2$ also reduces the pH value of milk and resulting in an increased protein aggregation rate. The influence of increasing the concentration of Ca^{2+} on milk increased the rennetted gel strength. Also, addition of higher concentrations of $CaCl_2$ may enhance aggregation while inhibiting gelation.

Effect of pH and Levels of $CaCl_2$ on Protein Profile of Modified Casein.

SDS-PAGE confirmed the aggregate formation attained in chromatography. It also showed that despite the formation of dimers and trimers, it is not always possible to see their existence by chromatography because they elute in a non-disrupted form, which means at the same elution time.

The results obtained using SDS-PAGE shown in Figure 2, and the treatments of pH values of 4.2 and 4.6 showed thicker band than the treatment of pH value of 5.0. These results indicated that the interaction between casein micelles at pH 4.2 and 4.6 was more intensive than at pH 5.0. One of the interactions between micelles casein was the formation of crosslink between casein by Ca^{2+} ions from $CaCl_2$. Appeal conditions and attraction between the isoelectric casein micelles greatly affected by ability of Ca^{2+} ions to form crosslink. $CaCl_2$ treatment may be influenced by pH value, where treatment of pH 4.2 and 4.6 showed that all treatments $CaCl_2$ at acidic pH generating thicker band compared to pH 5.0. Thinness band at pH 5.0 showed that the condition of the electrostatic repulsion that was still high and a weak electrostatic attraction resulting casein micelle, which difficult to form crosslink $CaCl_2$, whereas at pH values of 4.2 and 4.6 the casein micelles have low electrostatic repulsion so, in pH value of 4.2 the casein micelles have electrostatic attraction which tends to increase. Most conditions allow to the formation of crosslink that is on condition of thrust and a low electrostatic attraction at pH value of 4.6.

The addition of $CaCl_2$ also reduces the pH value of milk, resulting in an increased protein aggregation rate. The influence of pH value on clotting time is very strong; the decrease in the pH of milk from 7.0 to 5.2 causes decreases in the clotting time. The pH optimum value for the hydrolysis of κ-casein being 5.1 – 5.3, so, the most important effects of lowering the pH of the milk are the solubilization of micellar calcium phosphate.[19]

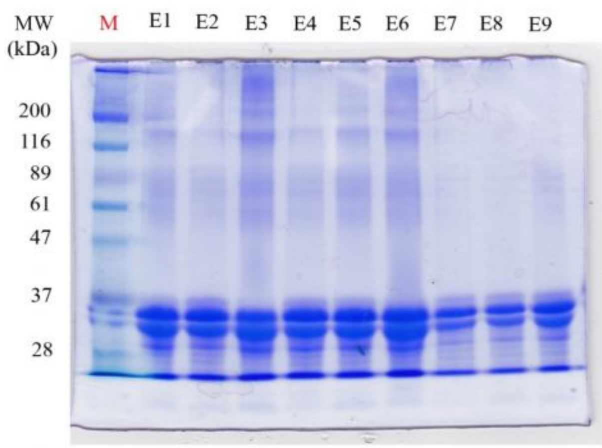


Figure 2. SDS Page of the samples of modified casein

MW (kDa): molecular weight

M: protein marker E1: Casein pH 4.2, $CaCl_2$ 0.025 g/ml E2: Casein pH 4.2, $CaCl_2$ 0.05 g/ml E3: Casein pH 4.2, $CaCl_2$ 0.075 g/ml E4: Casein pH 4.6, $CaCl_2$ 0.025 gr/mL	E5: Casein pH 4.6, $CaCl_2$ 0.05 g/ml E6: Casein pH 4.6, $CaCl_2$ 0.075 g/ml E7: Casein pH 5, $CaCl_2$ 0.025 g/ml E8: Casein pH 5, $CaCl_2$ 0.05 g/ml E9: Casein pH 5, $CaCl_2$ 0.075 gr/mL

Influence of casein modified and inulin addition on meltability of Mozzarella cheese.

Ratio of casein modified and inulin was considered insignificant statistically ($p>0.05$) on meltability of Mozzarella cheese. As shown in Table 2 ratio of casein modified and inulin 80: 20 gave the lowest meltability of Mozzarella cheese. Whilst when the ratio of casein modified (60): inulin (40), the highest meltability was achieved.

Melting of cheese reflects the ability of cheese particles to flow past one another when heated. For good meltaility, a strong interaction between protein and moisture in the cheese structure is required. Lowering of calcium causes increased interaction between proteins and surrounding water. The protein matrix expands and becomes more hydrated, resulting in increased melting.[20]

The results presented in Table 2 indicated that increasing the ratio of casein modified reduced the meltability of Mozzarella cheese. This could have affected the strength of bond created between casein and inulin. Inulin is a carbohydrate found in many types of plants, but especially in roots and rhizomes. [21] The presence of inulin as a substitute of fat cheese causes similar disruptions like those caused by the original fat milk to casein network during coagulation, resulting in a smooth and creamy cheese.[22]Fat content also plays a significant factor in meltability and an increased fat content prevents casein aggregation from interacting with each other thereby producing a weaker protein matrix.[23]

Table 2. The effect of casein and inulin ratio on meltability, fracture point and stretchability of Mozzarella cheese.

Ratio casein:inulin	Meltability	Fracture Point	Stretchability
80:20	4.97^{a}	1.1206^{b}	0.0367^{a}
70:30	5.06^{a}	0.8696^{b}	0.0321^{a}
60:40	5.22^{a}	0.5054^{a}	0.0266^{a}
50:50	5.07^{a}	0.8625^{b}	0.0209^{a}

Influence of casein modified and inulin addition on fracture point of Mozzarella cheese.

Statistical analysis revealed that ratio of casein modified and inulin was significantly difference effect ($p<0.05$) on fracture point of Mozzarella cheese. As shown in Table 2, the highest fracture point approximately 3.36 was achieved when the ratio of casein and inulin was 80:20. Where the lowest fracture point was attained when the ratio of casein and inulin was 60:40 of approximately 1.52.

This may be due to the fact that proteolysis is a major determinant of the intact casein content which has a large impact on the functionality of cheese. Hydrolysis of α casein at peptide bond by residual chymosin results in a marked weakening of para casein matrix and decreases in fracture stress and firmness.[6]

Influence of Casein Modified and Inulin Addition on Stretchability of Mozzarella Cheese

Statistical analysis showed that ratio of casein modified and inulin gave significantly difference effect ($p<0.05$) on the stretchability of Mozzarella cheese. As shown in Table 2, the highest stretchability was achieved when the ratio casein and inulin 80:20, approximately 0.11. The lowest stretchability was attained when the ratio of casein and inulin 50:50 of approximately 0.06, respectively.

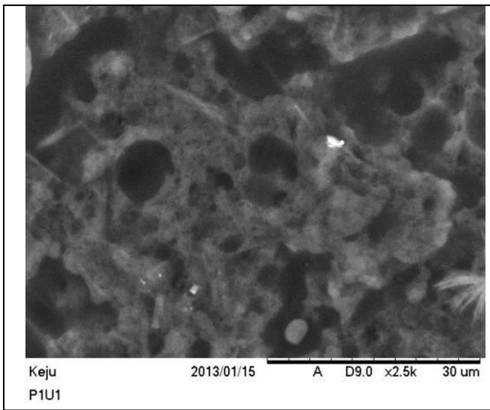
Stretch is the ability of the casein network to maintain its integrity and not break when pressure is applied to the cheese. The stretch properties of cheese are based on the number of interactions between casein molecules. Stretch requires an intact, interconnected casein network and is lost as the interactions between casein molecules or aggregates decrease. Stretch is the results of casein–casein interactions that are broken easily but also readily reform at different locations in the casein network. However, if the pH falls too low, the stretch characteristic can be lost. At a low pH, the caseins aggregate into large masses but there are a few contacts or interactions between the masses to allow stretch.[17]

Influence of casein modified and inulin addition on microstructure of Mozzarella cheese.

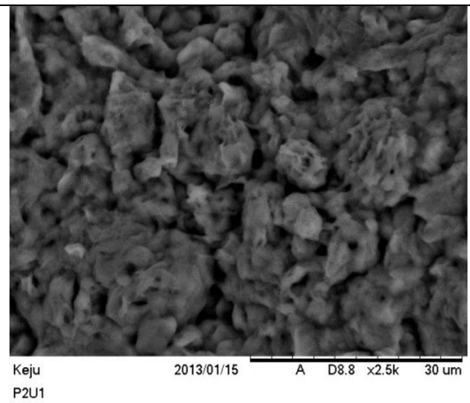
Casein modified and inulin addition on microstructure of Mozzarella cheese shown in Figure 3. The porosity structure of Mozzarella cheese was attained when ratio of casein modified:inulin was 80:20, while for the ratio of 70:30; 60:40 and 50:50,respectively the size and porosity structure was less and more swollen.

Microstructure is the structural arrangement of the components. Hydrophobic interactions, which influence the aggregation of casein particles, are favored at higher temperatures. Porous structure of Mozzarella cheese may be affected by inulin addition. The increasing of inulin addition in Mozzarella cheese could reduce the cavity structure of Mozzarella cheese. Inulin can absorb water from the curd and casein modified.

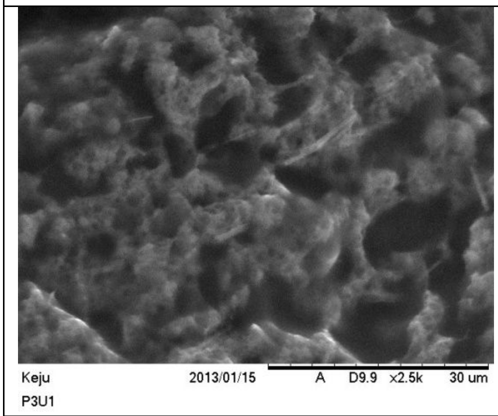
Therefore, the water content of Mozzarella cheese will be decrease and affected the porosity of Mozzarella cheese.



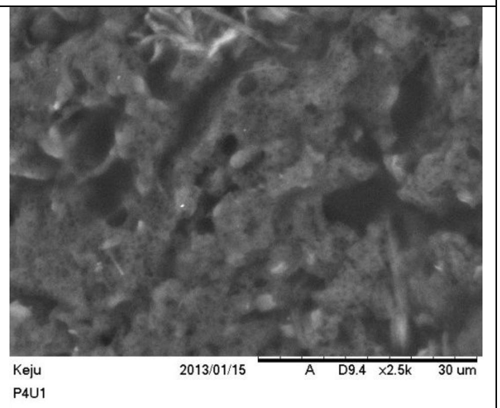
Ratio Casein modified and inulin 80:20



Ratio Casein modified and inulin 70:30



Ratio Casein modified and inulin 60:40



Ratio Casein modified and inulin 50:50

Figure 2. Microstructure (SEM) Mozzarella cheese produced using different casein modified and inulin ratio.

Influence of microwave power level on water activity of Mozzarella cheese.

Statistical analysis at Table 3 revealed that microwave power level was significantly difference effect ($P>0.05$) on water activity of Mozzarella cheese. The differences water activity of Mozzarella cheese after microwave treatment which may be due possibly to the differences in the component of dry matter and water content, when the microwave power at high level, was resulted a relatively low dry matter of Mozzarella cheese, which produce a lower water activity.

Table 3. Water activity values, color, fracture point and expansion of microwavable Mozzarella cheese.

Treatment	**Water Activity**	**Colour**			**Fracture Point**	**Expansion**
		L	**A**	**b**		
Low	0.924^{d}	56.57^{c}	9.93^{a}	23.30^{a}	0.73^{a}	
Med Low	0.882^{c}	56.37^{c}	9.97^{a}	25.83^{bc}	1.17^{b}	69.54^{a}
Medium	0.773^{b}	57.07^{c}	11.13^{b}	25.83^{bc}	1.73^{d}	142.55^{b}
Med High	0.582^{a}	52.73^{b}	13.40^{c}	25.07^{ab}	1.23^{b}	90.77^{ab}
High	0.576^{a}	50.07^{a}	16.30^{d}	27.23^{c}	1.63^{c}	50.43^{a}

Influence of microwave power level on lightness (L), redness (a), and yellowness (b) of Mozzarella cheese.

Statistical analysis at Table 3 showed that the microwave power levels were significantly difference effect ($p<0.05$) on color of L*a*b* of Mozzarella cheese. Additionally, it was found that the lowest lightness, highest redness and yellowness of Mozzarella cheese were attained when the level of microwave power was increased, while the microwave power was provided at low level; the highest lightness, lowest redness and yellowness of Mozzarella cheese were achieved.

Color is another key factor in food quality assessment and may be evaluated visually or objectively by instrumental measuring methods, using color scales to determine the color differences.[17] Several factors related to the raw materials and their processing may alter the colors of processed cheeses, which typically vary from creamy white to white. Cheese color defects, such as the development of a pale brownish yellow color may be caused by the Maillard reaction, in which amino groups of proteins react slowly with reducing sugars to form brown pigments and aromatic compounds. Maillard reactions and caramelization are common defects that may occur when inadequate processing conditions or raw materials containing excessive levels of lactose are used.[24]

Influence of microwave power level on fracture point of Mozzarella cheese.

Level power of microwave gave significantly difference effect ($p<0.05$) on fracture point of Mozzarella cheese. As shown in Table3, that at low power level of microwave, the lowest of fracture point was achieved, approximately 2.2. Additionally, power of microwave at medium level, approximately 5.2 of fracture point, was accomplished. Moreover, even though power of microwave at high level, the fracture point of Mozzarella cheese was on the contrary low.

Fracture properties of many types of cheese change significantly mostly because of protein hydrolysis. The extent of proteolysis in Mozzarella is lower than in Cheddar or Gouda cheese, approximately 20 to 40% decrease in α casein and β casein during 2 to 4 weeks of storage at refrigerator. Temperature could also affect the fracture properties and results from melting of fat globules and softening of protein matrix.[25, 26]

Influence of microwave power level on expansion of Mozzarella cheese.

Statistical analysis showed that levels of microwave gave the significant effect ($p<0.05$) on expansion of Mozzarella cheese. The lowest expansion of Mozzarella cheese was attained when the power of microwave at high level, while when the power of microwave at medium level, the highest expansion of Mozzarella cheese was achieved (Table 3).

Microwave expansion of imitation cheese decreased with decreasing moisture content and increased with prolonging storage time prior to microwaving.[14]Moisture content influences microwave expansion of imitation cheese by providing the driving force for expansion and plasticizing the cheese matrix.

Conclusion

pH values and $CaCl_2$levels could affect the formation of crosslink in the structure of casein. pH value of 4.6 with $CaCl_2$level of 0.025 g/ml was resulted in great crosslink with the highest levels of Ca, but did not gave significant effect on protein and calcium solubility ($P>0.05$).

Ratio of casein modified and inulin did not give significanly difference effect ($P>0.05$) on meltability and gave significantly differenece effect ($P<0.05$) on fracture point and stretchability of Mozzarella cheese. The porosity structure of Mozzarella cheese was attained when ratio of casein modified:inulin 80:20, while for the ratio 70:30; 60:40 and 50:50, the size and porosity structure was less and more swollen.

Statistical analysis revealed that microwave power level wasgave significantly difference effect ($P<0.05$) on water activity, color L*a*b, fracture point, and expansion of Mozzarella cheese.

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