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Properties and Mechanism of Melted Cheeses and Non-melted Cheeses: A Case Study Mozzarella and Bread Cheese (Juustoleipa)

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Abstract This study compares the characteristics of Melted cheeses (Mozzarella cheese) and Non-melted cheeses (Bread cheese). The results of the physicochemical parameters of the cheeses showed that Mozzarella cheese had high protein, moisture and acidity whereas Bread had high pH, total solids and ash. In addition Bread cheese had slightly higher calcium and fat content than Mozzarella cheese. The texture analysis revealed that Bread cheese milk gel hardness was slightly high compared with Mozzarella cheese milk gel as well as the hardness of the Bread cheese. However, Bread cheese was less gummy, springy, adhesive and cohesive compared with Mozzarella cheese. The microstructure showed a difference in the gels and cheeses matrices. The Mozzarella cheese matrix was porous whereas the Bread cheese matrix was compact and dense.

Keywords: *mozzarella, bread cheese, melting, protein, microstructure, texture*

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1. Introduction

Cheese is one of the most dairy products consumed worldwide. There are two categories of cheese on the basis of meltability. The melted cheeses category includes Mozzarella cheese, Cheddar cheese etc. Mozzarella is one of the mostly consumed because of its use in pizza baking. Therefore, quality improvement is important for cheese makers. The role of cheese as an ingredient in the dairy industry has considerably increased during the last few years [1].

In fact, meltability is defined as the ease with which cheese flows or spreads upon heating **[2]**. It remains one of the important characteristics of Mozzarella that increases its acceptability by consumers. Meltability and stretchability are functional properties which are essential for the use of cheeses in cooking meals [3]. Mozzarella cheese is expected to exhibit good functional properties, particularly meltability [4].

In a survey, over 50% of pizza restaurants reported occasional as well as frequent problems with the quality of cheese of which 67% were related to the melting of Mozzarella cheese [5]. On the other hand, the non-melted category composed of Juustoleipa or Bread cheese, Halloumi, Paneer etc. "Bread cheese" in Finnish, Juustoleipa has been produced for more than 200 years in northern Finland and Sweden. This cheese is unusual in that it is baked during the cheese making process. Leipäjuusto is a fresh cheese made from cow's beestings, rich milk from a cow that has recently calved. It can be made also from reindeer or even goat's milk. By knowing the mechanism of melting and non-melting of the two types of cheese we may be able to improve manufacturing process for desirable melting or non-melting properties. Variations in the manufacturing procedures offer an opportunity for the cheese-maker to improve the functionality of the finished cheese [6].

This study was undertaken to compare the physicochemical, textural and microstructural characteristics of Mozzarella and Bread cheese to find out the different factors and parameters involved in the melting and non-melting properties of the two cheeses.

2. Materials and Methods

2.1. Materials

Cow milk containing 3.21% of fat, 2.72% of protein, and 3.90% of lactose was obtained from a commercial farm near Beijing. Rennet powder (Chy-Max Powder Extra NB, 2235 IMCU/mL, Chr. Hansen A/S, Hoersholm, Denmark), starter culture (Streptococcus thermophiles Chr. Hansen A/S, Hoersholm, Denmark), salt was purchased from a supermarket in Beijing.

2.2. Manufacture of Mozzarella and Bread Cheese (Juustoleipa)

2.2.1. Mozzarella Cheese

Mozzarella Cheese was manufactured using the process proposed by the Wisconsin Centre for Dairy Research with slight modifications. Cow milk (20 L) was obtained from a commercial farm, divided into two vats was pasteurized (65°C) for 30 min followed by cooling to 34°C. 0.21 g/L of milk of starter culture was added. When the pH decreased to 6.56, rennet solution 0.2 mL/L of milk diluted before at 2g/100mL in distilled water was added. After 35 min the coagulum was cut and cooked (34 to 41°C) for 30 min. After whey draining; the curd was milled, salted at 2% (wt. /wt.) and stretched (at 65°C). After stretching, the cheese was kept in cold water (7°C) for one hour and brined (20% salt). At the last step the cheese was vacuum-packed and stored at 4°C for analysis.

2.1.2. Bread Cheese (Juustoleipa)

Bread cheese was manufactured using the method described by Susan **[7]**. Cow milk (20L) was divided into three vats warmed up to 36°C. When the desired temperature was reached, 14.8g of salt and rennet solution diluted before at 2 g/100mL in distilled water was added. Afterwards, milk was stirred and incubated in the water bath to 36°C. After 20 min, the cheese curd was cut and pre-cooked for 30 min at 36°C and the whey was drained. The resulting curd was poured in an aluminium mould and cooked in oven at 200°C for 1 h. Cheese was removed cold at room temperature for 20 min vacuum packed and storage at 4°C for analysis. The cheeses processing trials was repeated twice for each type of cheese.

2.2. Physicochemical Analysis

Cheese samples were analysed for fat using Soxcelet method, protein (total percentage N × 6.38) by using Kjeldahl, moisture content by using oven drying [8], The total solid content of the different cheese samples was determined as described by Sulieman et al. [9], three grams of samples were weighed into a dry clean crucible, and then heated in water bath for 10-15 minutes. The dishes were then placed in the oven at 80°C overnight for 16 hours, cooled down in a desiccator and weighed. The total solids content was calculated from the following equation:

$$Total\ solids\ content\,(\%) = \frac{W1}{W2} \times 100$$

Where: W_1 = weight of sample after drying W_2 = original weight of the sample

Titratable acidity was determined using the titrimetric method, ash content was estimated by incineration with the furnace (Foss Tecator CB160, Bibby Sterilin LTD, Stone UK.) at 550°C [9] water activity was determined using a Water Activity Meter (Series 4TE, AQUA LAB USA), the cavity was filled in half by the grated cheese sample and the result was read on the screen after stability. The pH was determined using a pH-meter (Mettler Teledo, Delta 320 Shanghai Co. Ltd) directly into a small block of cheese, equilibrated at 22°C **[10]** The total calcium was measured using inductively coupled plasma mass spectroscopy (Vista-MPX Simultaneous ICP-MS; Varian Inc., Palo Alto, CA) [11]. All physicochemical analysis were carried out in triplicate.

2.3. Texture Profile Analysis (TPA) of Mozzarella Cheese and Bread Cheese

Texture profile analysis (TPA) of the cheeses and cheese milk gels was performed using a TA-XT2i texture analyser (Stable Micro Systems, Godalming, and Surrey, UK) according to the method of O'Mahony, Lucey, & McSweeney [12] with slight modifications.

Cheese sample preparation: Three cylindrical samples of height 10 mm, and diameter 25 mm were cut from the core of the cheese and equilibrated at 6°C for further 30 min before the analysis. The parameters measured were hardness, cohesiveness, springiness, chewiness, gumminess, fracturability, and resilience. Samples were analysed in triplicate for each type of cheese.

2.4. Microstructure Analysis of Mozzarella Cheese and Bread Cheese

Gel sample preparation: The gel sample was prepared according to the method proposed by Lydia et al. [13] with slight modifications. 40 mL of renneted milk was poured into 50 mL graduated glass beaker and incubated at 34°C for 35 min and the milk was allowed to coagulate within the 35 minutes.

Cheese sample preparation: The cheese samples four days after manufacture were cut into thin strips of approximately (10×10×1) mm in the core of the cheese and placed on a cavity slide. 20 µL of the staining solution Nile red (0.005 gL^{-1}) and fast green FCF (0.012 gL^{-1}) were poured on the surface of the samples and stained for 30 min.

Microstructure of the gel samples and cheese samples were analysed in random order using a confocal scanning laser microscope (Olympus, Fluoview FV1000, Bx61, Japan) powered by Ar/Kr and He/Ne lasers. The samples were viewed using an oil immersion 63 x lens microscope (1.32 Numerical Aperture) and the pin-hole diameter was maintained at 1 Airy Unit. Nile red or WGA 488 was excited at a wavelength of 488 nm and fast green FCF at 633 nm. The emission filters were set at 555 to 620 nm for Nile red or WGA 488 and 660 to 710 nm for fast green FCF. These individual images were then combined in an overlay image in which the Nile red stained fat appears red and the fast green FCF stained protein appears green. In contrast, the aqueous phase and air voids appear black in these images. Images were recorded at a depth of 10 µM from the surface of the glass coverslip. Olympus confocal software was used to acquire digital images at 1024×1024 pixels. Three different samples of each type of cheese were analysed three times (different days) and the best pictures were selected.

2.5. Statistical Analysis

The data collected were statistically analysed by t test (paired test) using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com" to see the significance between the quantitative parameters.

3. Results and Discussion

3.1. Physicochemical Parameters

The physicochemical parameters of Mozzarella and Bread cheese are showed in the Table 1. There was a significant difference ($p<0.001$) in the pH between the two cheeses. Bread cheese pH was higher than that of Mozzarella cheese. Moreover, Bread cheese calcium content was high compared with Mozzarella cheese even though there was no significantly difference. There was also a significant difference ($p<0.05$) for protein and total solids content. Furthermore, the results showed a significant difference ($p<0.01$) in moisture and ash content, with lower moisture content of Bread cheese than Mozzarella cheese. However no significant difference was observed in the fat content and the water activity. The high protein, moisture and Titratable acidity of Mozzarella cheese (Table 1) were expected since, it known that these parameters are favourable for cheese melting property. The low moisture content of Bread cheese was probably due to the low acidity and the baking of the curd in the oven. Furthermore Bread cheese had high pH value, fat, calcium, and ash (Table 1) content compared with Mozzarella cheese. The high pH value and calcium content of Bread cheese was probably due to the lack of acidification of the cheese milk during the cheese manufacturing process. Calcium, protein and pH have relationship with cheese structure and its functional properties. Low pH values result in dissolution of colloidal calcium phosphate and dissociation of casein micelles. Lactic acid may change the calcium phosphate balance in the cheese by dropping the pH which allows the dissolution of the calcium improving the functionality such as melt and stretch ability. A cheese with insufficient acidity or high pH will be too curdy and will have a poor strechtability and meltability due to the excessive remaining calcium in the curd, cross-linked in the protein matrix [14] (Figure 2). The Figure 2a showed much interconnected cheese protein matrix with high insoluble calcium content and Figure 2b shows a less interconnected cheese protein matrix with low insoluble calcium content. Tunick, Hekken, Iandola, & Tomasul [15] studied Queso Fresco cheese reported that the high pH level ensured that colloidal $CaPO_4$ would remain in the case in matrix preventing melting.

Table 1. The physicochemical parameters of Mozzarella Cheese and Bread cheese#.

Composition	Mozzarella cheese	Bread cheese	Sig.
Protein (%)	28.42 ± 2.06	22.46 ± 0.67	*
Fat (%)	20.98 ± 0.18	25.30 ± 0.13	NS
pH	5.23 ± 0.02	6.11 ± 0.01	***
Titratable acidity	0.65 ± 0.04	0.43 ± 0.05	***
Moisture (%)	49.67 ± 0.54	41.30 ± 0.74	**
Total solids (%)	49.57 ± 1.21	65.75 ± 3.88	*
Calcium (mg/100g)	656.47 ± 3.66	680.17 ± 5.09	NS
Ash (%)	1.38 ± 0.02	2.17 ± 0.02	**
Water Activity (%)	0.99 ± 0.00	98 ± 0.00	NS

#Results are expressed as mean ± standard deviation (n = 6) Significance: *P< 0.05; **P< 0.01. NS: Non significant difference.

3.2. Texture Profile Analysis (TPA) of Mozzarella and Bread Cheese

The texture is an important attribute of food quality which influences the product acceptability and perception by consumers.

Table 2 shows the TPA results of mozzarella cheese milk gel and Bread cheese milk gel.

The results showed a significant difference ($p<0.05$), of the textural characteristics between the cheeses gels, especially the adhesiveness, gumminess and fracturability (brittleness).

Mozzarella cheese gel was more adhesive (-34.69 ± 1.17) and resilient (0.13 ± 0.01) than Bread cheese gel (Table 2). On the other hand, Bread cheese gel was gummier and fracturable than Mozzarella cheese gel respectively (Table 2). However, there was no significant difference ($p<0.05$) in the hardness, springiness, chewiness, and cohesiveness. Nevertheless, Bread cheese gel had higher hardness (147.36 ± 13.85) and chewiness (54.33 ± 6.33) compared with Mozzarella cheese gel (Table 2), respectively. The higher hardness exhibited by the Bread cheese milk gel could be due to the low acidification of the milk. It is know that high pH maintain the colloidal calcium in the cheese curd which maintains the casein molecules together resulting in a low water-hold capacity.

Table 2. Texture parameters of Mozzarella and Bread cheese milk gel#

Parameters	Mozzarella gel	Bread cheese gel	Sig.
Hardness (g)	111.67 ± 6.74	147.36 ± 13.85	NS
Adhesiveness (g.s)	-34.69 ± 1.17	-66.78 ± 8.20	*
Springiness	0.94 ± 0.02	0.84 ± 0.05	NS
Chewiness	51.91 ± 6.30	54.33 ± 6.33	NS
Cohesiveness	0.48 ± 0.02	0.43 ± 0.01	NS
Gumminess	54.23 ± 5.64	64.07 ± 3.45	*
Resilience	0.13 ± 0.01	0.05 ± 0.00	*
Fracturability(g)	83.88 ± 5.79	122.08 ± 12.82	*

#Results are expressed as mean ± standard deviation (n = 3) Significance: *P< 0.05 NS: Non significance difference.

In Table 3, the results of the textural characteristics of cheeses showed a significant difference ($p<0.05$), ($p<0.01$) and ($p<0.001$) for most of characteristics except hardness. Mozzarella cheese was more adhesive ($p<0.05$), cohesive ($p<0.01$), gummy ($p<0.05$) and springy ($p<0.01$), and had higher chewiness ($p<0.01$) than the Bread cheese (Table 3). Moreover the results showed a significantly higher gumminess of Mozzarella cheese. Even though the different was not significant, Bread cheese had high hardness compared with Mozzarella (Table 3). The hardness value of Bread cheese reported in this study was higher than that of Queso Fresco [15,16]. The higher hardness value of Bread cheese compared with Mozzarella cheese may be attributed to the lower moisture content. It is known that moisture content and extent of primary proteolysis influence the rheological properties of cheese [17]. The hardness was due to the denseness and compact structure of the sample due to the shrinkage of the caseins in the last step of the processing (baking in oven at 200°C). The Bread cheese texture was also probably due to the inactivation of the chymosin during the cheese curd cooking process. During the ripening of cheese, proteolysis continues to take place by the action of the chymosin on the casein. Chymosin is involved in cheese ripening, and is believed to be responsible for the initial softening of cheese texture, via hydrolysis of α_{s1}-casein [17]. Chymosin is active at a certain range of temperature; beyond this temperature, it is inactive. Most milk coagulant enzymes are optimally active between 30±50°C, but they lose activity at higher temperatures [18].

Table 3. Texture parameters of Mozzarella cheese and Bread cheese

Parameters	Mozzarella cheese	Bread cheese	Sig.
Hardness (g)	2747.38 ± 264.79	2841.06 ± 335.44	NS
Adhesiveness (g.s)	-52.29 ± 2.50	-90.88 ± 16.99	*
Springiness	0.86 ± 0.11	0.75 ± 0.09	**
Chewiness	1756.17 ± 455.22	266.09 ± 336.95	**
Cohesiveness	0.73± 0.03	0.58 ± 0.02	**
Gumminess	2014.74 ± 263.41	1670.59 ± 246.69	*
Resilience	0.43 ± 0.00	0.25 ±0.01	***
Fracturability (g)	2580.82 ± 283.85	2446.85 ± 318.39	*

#Results are expressed as mean ± standard deviation (n = 3) Significance: P< 0.05; **P< 0.01 ***P<0.001. NS: Non significance difference.

3.3. Microstructure using Confocal Laser Scanning Microscopy

Figure 1 shows on the one hand the microstructure of Bread cheese and Mozzarella cheese milk gels (Figure 1a, b) on the other hand the microstructure of Bread cheese and Mozzarella cheese (Figure 1c, d).

There was a difference in cheese milk gel microstructure. Each gel has a unique microstructure features which reflect the chemical and biological changes occurred during gel formation. Mozzarella cheese gel presented an organized casein network; caseins were cross-linked in the matrix forming clusters and more porous. It can be predicted that Mozzarella cheese gel would have the high water holding capacity and less syneresis which justify the higher moisture of the cheese. On the contrary, Bread cheese milk gel showed casein micelles with a circular shape aggregated in the casein matrix. This structure is probably due to the lack of starter culture in the Bread cheese milk to allow the cheese milk acidification during the coagulation process. Microstructure of Bread cheese and Mozzarella cheeses showed different shapes of proteins and fat. Mozzarella cheese had protein fibres that were arranged in parallel and non-globular fat and serum was present within the protein channels. More specifically for Mozzarella, the protein fibres was aligned because the cheese was stretched and moulded during manufacture [1]. The non-globular fat in Mozzarella was probably due to the damage the fat globules membrane during curd manufacture (stretching step). Unlike Mozzarella cheese Bread cheese protein matrix was more compact, clumped, almost no spaces in the casein matrix; this probably explains the low moisture content and the harder texture observed in the texture profile. The coalesced fat globules with was dispersed in the casein matrix. A high protein concentration lowers the volume fraction of the aqueous phase, which consequently lowers the mean distance between the casein micelles as well as increases the extent of the aggregation of the casein micelles [19]. Our results were in disagreement with their study because Bread cheese protein content is lower than that of Mozzarella cheese. Therefore, the Bread cheese microstructure probably was influenced by other factors during the process.

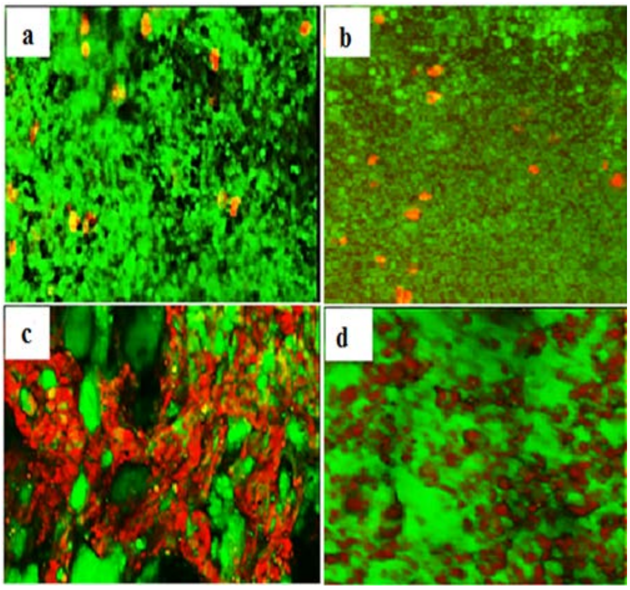

Figure 1. Confocal micrographs showing the microstructure of (a), (b) Mozzarella and Bread cheese milk gels respectively (c) Mozzarella cheese and (d) Bread cheese. The Nile red stained fat appears red and the fast green FCF stained protein appears green in these images. All the scale bars are 10 µM in length.

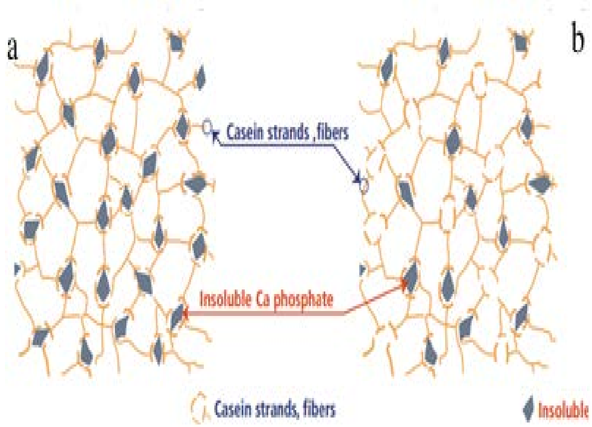

Figure 2. Matrix of cheese curd (a) apt to melt and (b) not apt to melt

4. Conclusion

The differences between Mozzarella cheese and Bread cheese was observed from the physicochemical to the microstructure characteristics. The comparison between Mozzarella cheese and Bread cheese revealed a difference in the acidity, the pH, the moisture and calcium content which are the main parameters involved in the melting property. These parameters were higher in the Bread cheese because of the non-use of starter culture during the processing which affected the textural and microstructural characteristics. In addition the high temperature used for the Bread cheese curd cooking had played a role by the inactivation of the remaining chymosin in the curd. The differences between Mozzarella cheese and Bread cheese observed in the present study can be used as maker for the improvement of Mozzarella cheese the melting property

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