EFFECT OF FAT LEVELS, STARTER CULTURES AND MILK SOURCE ON THE EXPRESSIBLE SERUM (G/100G CHEESE MOISTURE) OF MOZZARELLA CHEESE DURING RIPENING PERIOD

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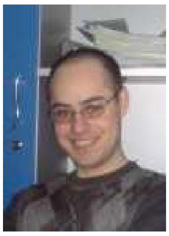

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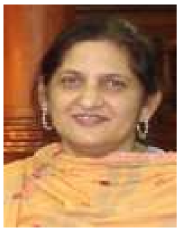

REZUMAT. Cererea pe piață și consumul de brânză de tip Mozzarella a crescut considerabil în ultimii ani, în principal datorită creșterii consumului de pizza și alimente fast-food. Consumul de brânzã în diferite forme (creme pentru pizza, amestecuri, în salate, în sandwichuri, ca ºi umpluturi) a crescut datoritã preferinþelor dietetice, dar ºi datoritã uºurinþei ºi a flexibilitãþii în folosire. În acest context, a existat un efort continuu de a menþine nivelul de umiditate ºi nivelul seric pentru a împiedica fie uscarea suprafeþei, fie umezirea în exces. Un anumit nivel seric asigurã calitãþile potrivite de conservare în timpul procesului de maturare. Studiul de faþã a încercat prepararea brânzei din lapte de vacã ºi de bivoliþã, analizându-se efectul nivelurilor de grãsime ºi al culturilor de început asupra serului (g/100g umezealã) din brânza Mozzarella în procesul de maturare. Nivelul de grãsime al laptelui a afectat în mod semnificativ compoziția chimicã a brânzei. Nivelul de umiditate al brânzei fost mai ridicat în cazul laptelui cu un conținut de grãsime mai scãut față de laptele cu un conținut de grãsime mai ridicat. Brânza Mozzarela preparatã din culturi de început izolate a avut o umiditate semnificativ mai mare decât cea din culture comerciale. Nivelul serului a fost deasemenea afectat semnificativ de perioada de maturare și de nivelurile de grãsime ale laptelui. S-a constatat o scãdere a serului de le 22.63g/100g la 3.85g/100g în perioada de maturare. Nivelul serului a fost mai ridicat în cazul brânzei cu o concentrație de grãsime mai mare, comparative cu cea cu o concentrație de grãsime mai micã.

Cuvinte cheie: brânzã Mozzarella, compoziție chimicã, randament

ABSTRACT.The mozzarella cheese consumption and market demand has been increasing considerably in recent years, due to increasing demand for pizzas and fast foods. The consumption of cheese in the form of pizza toppings, cheese blends, salads, sandwiches, stuffing, has increased due to dietary likeness, ease and flexibility in use. There has been a continuous effort to maintain the appropriate moisture level or serum level to overcome the surface dryness or on the other side excessive moisture in the cheese matrix. The desired serum level ensures the best keeping qualities during cheese ripening process.The current study attempted to prepare cheese from cow and buffalo milk sources and was analyzed for effect of fat levels and starter cultures on the expressible serum (g/100g cheese moisture) of Mozzarella cheese during ripening. The milk fat level significantly affected the cheese chemical composition. The moisture content was observed higher in low fat milk cheese as compared to high fat milk cheese. Mozzarella cheese prepared from locally isolated starter cultures possessed significantly higher moisture content, as compared to commercial culture cheese. The expressible serum was significantly affected by the ripening period and fat levels. There was a decrease in expressible serum from 22.63g/100g to 3.85g/100g during ripening. The serum was recorded more in high fat cheese as compared to low fat cheese.

Keywords: Mozzarella cheese, chemical composition, yield

1. INTRODUCTION

Mozzarella cheese demand and production level has grown considerably in recent years. The impetus for the dynamic growth of mozzarella usage has been the growing demand for pizzas. Cheese is one of the most diverse groups of the dairy products and perhaps the most interesting and challenging one. More than 900 individual varieties of cheese are being produced in the world (Banks, 1998) which are classified on the basis of their form, manufacturing, ripening and chemical composition (Walstra *et al.*, 2006). 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).

In cheese manufacturing preservation of the most important

constituents of milk (i.e. fat and protein) are exploited by two classical principles of preservation, i.e. lactic acid fermentation, and reduction of water activity through removal of water and addition of salt. Establishment of low redox potential and secretion of antibiotic by starter culture contribute to storage stability of cheese (McSweeney, 2004a). Mozzarella cheese includes in the group of “Pasta filata” or stretched cheese. In the pasta filata cheese first the curd is produced as normal process of cheese making then kept in hot water or whey to consolidate into a solid mass, subsequently, stretching of this mass convert the curd into a uniform and elastic cheese consistency. Stretching is a treatment that renders the curd elastics which is a unique quality attribute of the Mozzarella cheese (Luecy *et al.*, 2003).The increase in production of cheese is about 4% per year from the last thirty years in the world (Fox *et al*., 2000).

Traditionally Mozzarella was made from buffalo milk which was preferred due to its characteristic flavor. The flavor and texture of fresh Mozzarella is different from processed sliced or shredded Mozzarella, as fresh Mozzarella is moist, soft, quick in melt and delicate in taste. However, 'real' Mozzarella does not maintain ideal freshness beyond 12-24 hours (Rowney *et al*., 2003). Guidelines set by the USDA indicate that a low moisture Mozzarella cheese shall contain moisture 45% to 52% and milk fat 30% to 45% on dry weight basis. Low moisture part skim Mozzarella is mostly utilized in the pizza industry due to exceptional properties of meltability, stretchability and elasticity (USDA, 1980).The functionality of Mozzarella cheese is important because about 75% of the total Mozzarella produced is used as an ingredient for pizza. The factors for example composition of cheese, especially fat contents and moisture, starter culture, coagulating enzymes, cooking and stretching, salt concentration, and the process occur during ripening influence the functionality of Mozzarella cheese (Kindstedt, 1993). Fresh manufactured Mozzarella cheese is unacceptable as a pizza ingredient because it melts to tough, very elastic possessing somewhat granular consistency with poor water holding capacity and limited stretch. However, with too much aging the cheese becomes excessively soft and fluid when melted and is no longer acceptable for pizza. So a precise ripening period is necessary to achieve desirable functionality (Rowney, 1999).

The choice of the starter culture is important for proper acid production and texture and flavor profile in high-fat and reduced-fat cheeses which are vulnerable to development of off-flavour (Banks, 2004). Mozzarella is produced using a paired lactic acid bacteria starter culture comprised of *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp bulgaricus* or *Lactobacillus helveticus* (Coppola *et al*., 2001).There is a potential for production of novel low fat cheeses which gave added health benefits to the marketplace (Guinee *et al.*, 2000). In addition to nutritional significance fat also adds sensory and functional characteristics to cheese. Low moisture part skim (LMPS) Mozzarella cheese is commonly used for pizza because of its desirable functionality (Rudan and Michael, 2002).

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 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.

The objective of the current study was to assess the effect of milk sources (cow and buffalo), starter cultures (indigenous and commercial) and milk fat levels (1.5% and 2.5%) and ripening period on expressible serum content of Mozzarella cheese. Standardized buffalo milk contained more protein (3.71%), casein (2.85) and lactose (4.88%) content as compared to the mixture of cow and buffalo milk protein (3.53), casein (2.79) and lactose (4.79%). The pH and acidity of all milk samples varied from 6.62 to 6.64 and 0.13 to 0.14%, respectively. Cheese was prepared from these milk sources and was analyzed for effect of fat levels and starter cultures on the expressible serum (g/100g cheese moisture) of Mozzarella cheese during ripening. The milk fat level significantly affected the cheese chemical composition. The expressible serum was significantly affected by the ripening period and fat levels. The understanding gained of the interplay among milk source, fat concentration and type of culture used may help in the effective design of a food matrix with desirable characteristics.

2. MATERIALS AND METHODS

Mozarella cheese was prepared by cow and buffalo milk (1:1) collected from livestock farm house. The compositions, microbial load, indigenous enzymes of milk are the major factors that influence different characteristics of the Mozzarella cheese. Pasteurization, through the use of plate heat exchanger and holding tubes with typical time temperature relations of 72°C/15 sec, is standard practice to kill pathogens (Maubois, 2002). Pasteurization >72°C have generally not been used in milk for cheese making practices because of their adverse effects on curd formation (Guinee *et al.*, 1997; Singh and Waungana, 2001) and curd syneresis (Pearse *et al.*, 1985; Pears and MacKinlay, 1989).

Indigenous starter cultures *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* cultures isolated, identified and purified in the Food Microbiology and Biotechnology laboratory were used for cheese manufacturing as indigenous cultures. Commercially available culture for Mozzarella cheese (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp bulgaricus*) from Chr. Hansen's Laboratory (Ireland) Ltd, Cork, Ireland was also used for cheese manufacturing for comparison with locally isolated culture. For mother cultures indegenous cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp bulgaricus* were propagated and tested for curd formation individually and in different combinations. The best combination (2:1) was selected and used to prepare mother culture using UHT skim milk as a substrate medium. Skim milk was inoculated with the propagated culture and incubated at 37°C for 6 hours to prepare the mother culture. Similarly commercially available cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were propagated in the same combination (2:1) as for indigenous cultures to prepare the mother culture. Rennet; the enzyme chymosin (Double strength Chy-max, 500000 MCU/mL, Pifzer Inc, Milwaukee, WI, USA) was used to coagulate the milk in the present study. The fat level was standardized in milk by using the Pearson's square method at a level of 1.5 and 2.5%.

The expressible serum was determined by centrifugation of cheese at 12500 x g for 75 minute at 25oC as described by Guo and Kindstedt (1995). Pre-weighed graduated cylinder was used to collect expressed serum and fat then it was stored at 4 oC for the solidification of surface layer. The fat layer was pierced to get the supernatant serum. The weight of serum was taken as expressible serum and expressed as cheese moisture in g /100g.

Table 1: *Treatments with milk source, fat levels and type of culture used*

Samples	Milk sources		Fat levels		Cultures	
	Buffalo	Cow+ Buffalo(50:50)	F1	F_2	IC	CC
C_1	+		+		+	
C_2	+		+			+
C_3	+			+	+	
C_4	+			+		+
C_5		+	+		+	
C_6		+	+			+
C_7		+		+	+	
C_8		+		+		+

Statistical analysis

Results obtained from different parameters were subjected to statistical analysis using Analysis of Variance Technique (ANOVA) under four factor factorial completely randomized designs (CRD) as described by Steel *et al.* (1997) to evaluate the influence of different parameter on quality and acceptability of Mozzarella cheese. Duncon's multiple range (DMR) test was applied to find the difference between means.

3. RESULTS AND DISCUSSION

The level of expressible serum has been used as an index of the water-binding capacity of the cheese para-casein, with a low level indicating a high water-binding capacity (Kindstedt, 1995). The water-binding capacity of the paracasein affects the ability of the curd to retain moisture and hence its ability to flow, stretch and remain juicy during baking. Bound moisture exerts an equilibrium water vapour pressure lower than that of pure (Guinee *et al.*, 2000) and is thus less susceptible to being lost from the cheese during baking. Hence, the increase in the water-binding capacity of Mozzarella on ageing coincides with an improvement in functionality and a lessened tendency for the cheese to dry out, blister and crust during baking (Kindstedt, 1993). Mozzarella cheese normally gives off free moisture at the surfaces that make the cheese unsuitable for shredding and melting during first few days after manufacture (Guo and Kindstedt, 1995). The amount of expressible serum is obtained by centrifuging the Mozzarella cheese. It is an indicative for water holding capacity of cheese, may be pinpointing for structural and physico-chemical changes in cheese that could dictate functionalcharacteristics (Kindstedt, 1995). The mean squares regarding the expressible serum of different Mozzarella cheese are given in Table 2.

The result showed that ripening (*P*<0.01), fat levels (*P*<0.01) and interaction of ripening x fat level showed significant effect on the amount of expressible serum of different Mozzarella cheese. The statistical results further showed that milk sources, starter culture and the interaction of all other variables did not significantly affect the amount of expressible serum. The result in Table2 indicated that the amount of expressible serum decreased significantly as the ripening days of cheese progressed. The moisture distribution is different in Mozzarella cheese from most of other cheese because of extraordinary microstructure caused by stretching. As aging in Mozzarella cheese progresses the microstructure swell and large open columns of loosely held serum that are unique to stretched cheese are progressively transformed to a continuous hydrates protein gel that increase the water holding capacity of Mozzarella cheese. More over expansion of the protein matrix occurred over the same duration as the decrease in expressible water and indicated that the protein matrix is adsorbing water originally located in fat-serum channels (Guo and Kindstedt, 1995; Guo *et al.*, 1997 and McMahon *et al.*, 1999). Additionally, meltability of Mozzarella increased during storage while the percentage of entrapped water increased, suggesting that the improvement in the meltability occurs concomitantly with the protein matrix becoming more hydrated (McMahon *et al.*, 1999). The Table 2 indicates that the amount of expressible serum did not differ significantly due to milk sources and the amount of expressible serum varies from 12.83 to13.39 g/100g for buffalo and mixture milk, respectively.

Table 2. *Effect of milk sources, fat levels and starter cultures on the expressible serum (g/100g cheese moisture) of Mozzarella cheese during ripening*

Ripening	Buffalo milk				Cow +Buffalo milk				Mean
	F1		F2		F1		F2		
	IC	CC	IC	CC	IC	CC	IC	CC	
0	19.25	19.75	24.13	25.32	20.01	21.32	25.00	26.23	22.63a
5	15.22	15.67	19.18	18.39	15.27	18.45	19.75	19.26	17.65b
10	7.34	6.09	10.25	9.23	7.27	8.25	8.24	9.85	8.32c
20	3.12	2.33	5.22	4.75	2.56	2.5	5.22	5.11	3.85d
Mean	12.83 a				13.39a				

The (Table 3) showed that the mean vale for expressible serum was more in cheese that are prepared from 2.5% fat level in milk as compare to cheese that were prepared by the 1.5% fat level in milk. Although no satisfactory explanation can be offered for the differences in the serum expressed but it could be possible that serum associated in some manner with the fat globules (McMahon *et al.*, 1999), so higher the fat in cheese, higher will be expressible serum in cheese. It also shows that the ES in cheese either prepared from high fat and low fat, decrease during ripening. The Table3 indicates that the amount of expressible serum did not differ significantly due to starter culture and the amount of expressible serum varies from 12.94 to 13.38 g/100g for indigenous and commercial culture, respectively.

Mean carrying same letters are statistically non-significant

IC= Indigenous culture CC= Commercial culture

F1= 1.5% Fat in milk F2= 2.5% Fat in milk

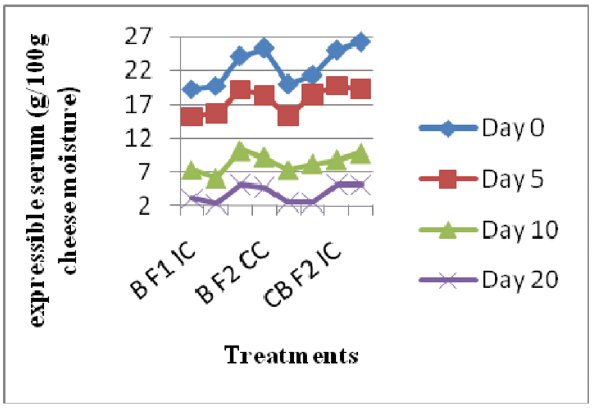

Fig. 1. *Effect of milk sources, fat levels and starter cultures onthe expressible serum (g/100g cheese moisture) of Mozzarellacheese during ripening*

BF1 IC – buffalo milk, fat 1.5%, indigenous culture
BF1 CC – buffalo milk, fat 1.5%, commercial culture

BF2 IC – buffalo milk, fat 2.5%, indigenous culture
BF2 CC – buffalo milk, fat 2.5%, commercial culture
CBF1 IC – cow + buffalo milk, fat1.5%, indigenous culture
CBF1 CC – cow + buffalo milk, fat1.5%, commercial culture
CBF2 IC – cow + buffalo milk, fat 2.5%, indigenous culture
CBF2 CC – cow + buffalo milk, fat 2.5%, commercial culture

Table 3. *Effect of milk sources, fat levels and starter cultures on the expressible serum (g/100g cheese moisture) of Mozzarella cheese*

Cultures	F1		F2		Mean
	Buffalo	Cow + Buffalo	Buffalo	Cow + Buffalo	
IC	11.23	11.28	14.70	14.55	12.94a
CC	10.96	12.63	14.42	15.11	13.28a
Mean	11.53b		14.70a		

IC= Indigenous culture CC= Commercial culture
F1= 1.5% Fat in milk F2= 2.5% Fat in milk

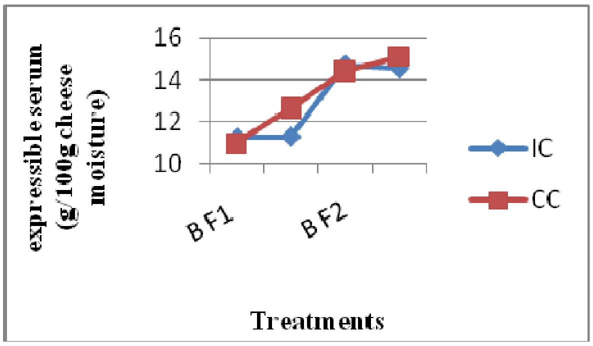

Fig. 2. *Effect of milk sources, fat levels and starter cultures on the expressible serum (g/100g cheese moisture) of Mozzarella cheese*

BF1 – buffalo milk, fat 1.5%
CBF1 – cow + buffalo milk, fat 1.5%
BF2 – buffalo milk, fat 2.5%
CBF2 – cow + buffalo milk, fat 2.5%

4. CONCLUSIONS

The result showed that ripening , fat levels and interaction of ripening x fat level showed significant effect on the amount of expressible serum of different Mozzarella cheese, while milk sources, starter culture and the interaction of all other variables did not significantly affect the amount of expressible serum. The results obtained indicated that the amount of expressible serum decreased significantly as the ripening days of cheese progressed. More over expansion of the protein matrix occurred over the same duration as the decrease in expressible water and indicated that the protein matrix is adsorbing water originally located in fat-serum channels. The percentage of entrapped water increased, suggesting that the improvement in the meltability occurs concomitantly with the protein matrix becoming more hydrated.

The results show that the amount of expressible serum did not differ significantly due to milk sources and the amount of expressible serum varies from 12.83 to13.39 g/100g for buffalo and mixture milk, respectively. Moreover the mean vale for expressible serum was more in cheese that are prepared from 2.5% fat level in milk as compare to cheese that were prepared by the 1.5% fat level in milk. Although no satisfactory explanation can be offered for the differences in the serum expressed but it could be possible that serum associated in some manner with the fat globules, so higher the fat in cheese, higher will be expressible serum in cheese. The results also show that the Expressible Serum in cheese either prepared from high fat and low fat, decrease during ripening. While the amount of expressible serum did not differ significantly due to starter culture and the amount of expressible serum varies from 12.94 to 13.38 g/100g for indigenous and commercial culture, respectively.

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