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CHEMICAL COMPOSITION AND SENSORY EVALUATION OF MOZZARELLA CHEESE: INFLUENCE BY MILK SOURCES, FAT LEVELS, STARTER CULTURES AND RIPENING PERIOD

Ayesha Sameen[1]*, Faqir Muhammad Anjum[1], Nuzhat Huma[1] and Haq Nawaz[2]
[1]National Institute of Food Science and Technology, University of Agriculture, Faisalabad
[2]Institute of Animal Nutrition and Feed Technology, University of Agriculture Faisalabad
*Corresponding author's e-mail: ash_sameen@yahoo.com

Mozzarella cheese samples were prepared from buffalo milk alone and mixture of cow and buffalo (1:1) milk standardized at 1.5 and 2.5% fat level. Indigenous and commercial starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*) were used in cheese manufacturing for comparison and cheese samples were stored at 4°C and were analyzed for moisture, protein, fat, pH and acidity at specified intervals (0, 20, 40, 60 days). Moisture and protein content significantly affected due to difference in milk sources, fat level, starter cultures during ripening. The pH and acidity also influenced by all variables except fat level whereas the fat content of cheese only influenced by fat levels of milk. Cheese samples were also evaluated for organic acids production (lactic and citric). Ripening days, fat levels and cultures significantly affected the concentration of both acids. Sensory evaluation of cheese texture revealed that only fat level and ripening affect the texture significantly.

Keywords: Chemical composition, fat, sensory evaluation, starter culture, ripening, Mozzarella

INTRODUCTION

Pakistan ranks at 5th position among the top milk producing countries of the world. In Pakistan about 42.199 million tons/year milk is produced (GOP, 2008). In total milk production of country, buffalo contribute 58%, while cow's share is 35% (GOP,2008).Milk is used as a liquid and can be transformed into different products like pasteurized, sterilized, dried and concentrated milk, ice cream, butter, yoghurt and cheese etc. Among all of these milk products about one third is used in cheese manufacturing at global level (Farkye, 2004).

The basic technology for the production of all types of cheese is same with relatively small changes resulting significant differences in the final cheese. The art or science of cheese making comprises five key factors: milk composition, rate and extent of acid development, moisture content, curd manipulation and ripening conditions (Lucey *et al.,* 2003). Other factors that may influence the quality of different varieties of cheese are: composition of milk, types of milk, starter cultures and manufacturing technology (Varnam and Sutherland, 1994).

More than 900 individual varieties of cheeses are being produced in the world which is classified on the basis of their form, manufacturing, ripening and chemical composition (Walstra *et al.*, 2006).

Mozzarella cheese has become one of the most popular cheese varieties in world because of its primary use is in pizza preparation. Its usage is expected to grow as global interest due to the ever increasing demand for pizza and other foods that use mozzarella. Buffalo milk is preferred for Mozzarella due to high fat, vitamin A, protein and low cholesterol (Zicarelli, 2004). Differences in fat level and hence protein to fat ratio, that occur in milks have marked influences on composition, yield, rheology, flavour and sensory attributes of cheese (Guinee *et al*., 2002).

Starter cultures are used in cheese making to promote acid development during curd manufacturing and also to confer distinct textural and flavor properties (Varnam and Sutherland, 1994). The rate of acid production is critical in determining cheese quality. The acidification also contributes to a preservative effect with the result that many pathogenic and spoilage bacteria are inhibited (Banks, 2004). Various types of cultures such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp bulgaricus* or *Lactobacillus helveticus* can be used in combination or alone to obtain desirable characteristics of mozarrella Cheese (Coppola *et al*., 2001).

In Pakistan imported commercial cultures are generally used for these fermented dairy products because still there is no production of commercial culture at local level. Recently an attempt was made in Food Microbiology Laboratory of National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad for the development of few dairy cultures.

Pakistan is producing large quantity of milk but due to lack of knowledge and expertise for the production of cheese varieties very little work is reported at local level. Considering these problems, this research project was planned to study the manufacturing parameters for mozzarella (effect of milk source, cultures and milk fat) on quality of cheese.

MATERIALS AND METHODS

Raw milk

Raw buffalo and cow milk was obtained from Dairy Farm, University of Agriculture, Faisalabad, Pakistan and used in the preparation of cheese in Dairy Laboratory, National Institute of Food Science and Technology.

Chemical analysis of milk

Raw milk was analyzed for moisture, pH, acidity, total protein, casein, lactose, Ca content (AOAC, 2000) and fat content (Marshal, 1993) prior to use in cheese preparation.

Table 1. Detail of treatments

Treatments	Milk sources		Fat levels		Cultures	
	Buffalo	Cow+ Buffalo	1.5 %	2.5 %	IC	CC
T1	+		+		+	
T2	+		+			+
T3	+			+	+	
T4	+			+		+
T5		+	+		+	
T6		+	+			+
T7		+		+	+	
T8		+		+		+

IC= Indigenous culture CC= Commercial culture

Two types of milk (buffalo and mixture of cow and buffalo), were standardized at two fat levels (1.5 and 2.5%) and two types culture (indigenous and commercial) were used in cheese production. All the cheese samples were analyzed at 0, 20, 40 and 60 days of ripening.

Cheese preparation

The samples of milk were first pasteurized at a temperature of 65°C for 30 minutes. After this the standardized milks were divided into two parts. Then cooled to 37°C subsequent to this one part of each milk was inoculated with (combined culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* @ 2%) indigenous culture and other part with commercial culture. After ripening for 30 minutes at 37°C curd was set with Chymosin (Double Strength Chy-max, 500000 MCU/mL, Pifzer Inc, Milwaukee, WI, USA) @ 0.077mL/kg of milk. Approximately 40 minutes after chymosin addition, the curd was cut with 1.9 cm wire knives and then allowed to heal in the whey for 10 minutes with periodic gentle agitation to prevent curd matting. The temperature was increased gradually to 42°C during cooking and stirring of the curd. The whey was drained at pH 6.2 and curd was matted, then cutting of matted curd into pieces and turned every 15-20 minutes and milled at pH 5.2. Then the salt was added at the rate of 1.5% of the curd. The salted curd was then hand stretched in hot water of 70°C until the uniform and elastic cheese consistency was achieved. The volume of stretching water was 2.5 times of the weight of curd and had 3% of salt w/w. The salted curd was then molded and vacuums packed and stored at 4°C for ripening.

Compositional analysis of cheese

Grated cheese was analyzed for moisture, fat, protein, pH and acidity (AOAC, 1990) at 0, 20, 40 and 60 days of ripening. Organic acids content was determined with high performance liquid chromatography (HPLC) during ripening as described by Akalin *et al.* (2002). Samples were run on HPLC (Perkin Elmer 200 series). Operating conditions were: mobile phase: 0.5% (wt/vol) $(NH4)_2HPO_4$ (0.038 M)−0.2% (vol/vol) acetonitrile (0.049 M) adjusted to pH 2.24 with H_3PO_4; flow rate 0.5 mL/min.

Sensory evaluation of cheese texture

The cheese was evaluated for different sensory texture parameter (hand firmness, first bite firmness, chew down degree of break down and chew down degree of smoothness) in terms of intensity on nine point equidistant scale according to Brown *et al.* (2003).

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.* (1996) to evaluate the influence of different parameter on quality and acceptability of Mozzarella cheese using minitab V 11.1.Duncan's multiple range (DMR) test was applied to find the difference between means.

RESULTS AND DISCUSSION

Cheese milk composition

Chemical composition of raw milk is shown in Table 2. The protein content of standardized buffalo milk was higher (3.71% and 3.68%) than the mixture (cow + buffalo) milk (3.53% and 3.50%). The pH and acidity of all milk samples were found in the range of 6.62-6.64 and 0.13-0.14%, respectively. The calcium content of buffalo milk was 1120 mg/100g which was higher than that of mixture (cow + buffalo) milk. The results of the present investigation are in agreement with the findings of various researchers (Ganguli, 1992; Patino, 2004). The comparison of cow and buffalo milk showed that composition varies significantly among them and most of the components were higher for buffalo than that of mixed cow+buffalo milk which is supported by the results of Ahmad *et al.* (2008).

Table 2. Physico-chemical composition of cheese milk

	Buffalo		Cow+ Buffalo	
	F_1	F_2	F_1	F_2
Moisture %	85.07	84.45	87.55	86.29
Fat %	1.5	2.5	1.5	2.5
Protein %	3.71	3.68	3.53	3.50
Casein %	2.85	2.82	2.79	2.76
Lactose %	4.88	4.88	4.79	4.79
pH	6.62	6.63	6.63	6.64
Acidity %	0.14	0.13	0.14	0.14
Calcium mg/100g	1120	1120	1025	1025

F_1 = Milk with 1.5% fat F_2 = Milk with 2.5% fat

Cheese composition

The chemical composition of cheese shown in Table 3 with respect to milk source indicated that moisture, protein, and pH of cheese differ significantly. The moisture content 47.49% and 48.76%, protein content 28.56% and 27.89% and pH 5.26 and 5.18 was observed for buffalo and mixture of cow and buffalo milk cheese, respectively. It is revealed from result (Table 4) that with an increase in fat content the protein and moisture content decreased significantly. The starter culture also significantly influenced the moisture, protein, acidity and pH (Table 5). Indigenous culture cheese contained more moisture (48.40%), acidity (0.97%) and low protein (28.07%) and pH (5.19) than the cheese from commercial culture which showed 47.85%, 28.39%, 0.97%, 5.25 values for moisture, protein, acidity and pH, respectively. Fat content did not change with ripening (Table 6), while the other parameters were affected significantly. Moisture decreased from 48.63 to 47.63%, protein increased from 27.88 to 28.48% and acidity increase from 0.92 to 0.98% during ripening. There was increasing and decreasing trend through out the ripening in pH.

The significant difference in moisture and protein content of buffalo milk cheese and mixture of cow and buffalo milk cheese samples may be due to variation in initial composition of milk as reported by Ahmad *et al.* (2008).The variation in moisture may be related to the faster acid production rate in the indigenous cultured cheese as compared to the commercial culture cheese which contained less moisture content (Dave *et al.,* 2003). Fife *et al.* (1996) also reported an increase in the moisture content of the cheese due to lowering the fat level resulted in increased protein to fat ratio which leads to increase in moisture content of cheese. Other researchers claimed that these differences in the composition and types of caseins might be due to difference in species which can be perceived in the cheese produced from them (Fox and McSweeney, 2004). The decrease in pH during first 20 days of storage was attributed to the increase in lactic acid production due to the activity of starter culture and later on culture and coagulant loss their activities. Several researchers maintained that this increase in pH of cheese may be associated with a reduction in the lactate-to-protein ratio (Huffman and Kristofferson, 1984; Fox and Wallace, 1997) and a loss of buffering capacity of the curd (Czulak *et al.,* 1969).

The lactose is converted into lactic acid during cheese making by the starter culture (Azarnia *et al.*, 2006) therefore lactic acid is the most abundant organic acid in all type of cheese (Izco *et al.*, 2002). Citric acid is naturally present in milk in small amount and can also be produced during ripening.

The milk sources had non significant (data not shown) affect on citric and lactic acid production. The cheese prepared from milk with 1.5% fat possessed more concentration (Fig. 1 and 3) of lactic acid (13783.19 ppm) and citric acid (2418.75ppm) as compared to cheese prepared from milk with 2.5% fat (11770.69 ppm) and (2297.13ppm), respectively. This could be attributed to higher moisture content of the low-fat cheese (Katsiari *et al.*, 2002) which can enhance the rate of glycolysis. The rate of lactose fermentation also

Table 3. Effect of milk source on moisture protein, fat, acidity and pH

Milk source	Moisture %	Protein %	Fat %	Acidity %	pH
Buffalo	47.49 b	28.56 a	17.91 a	0.95 a	5.26 a
Cow Buffalo (1:1)	48.76 a	27.89 b	17.37 a	0.95 a	5.18 b

Mean carrying same letters in a column are not significantly different

Table 4. Effect of fat level on moisture protein, fat, acidity and pH

Fat level	Moisture %	Protein %	Fat %	Acidity %	pH
1.5% Fat in milk	49.95 a	29.61 a	13.43 b	0.95 a	5.21 a
2.5% Fat in milk	46.30 b	26.84 b	21.59 a	0.95 a	5.22 a

Mean carrying same letters in a column are not significantly different

Table 5. Effect of culture on moisture protein, fat, acidity and pH

Culture	Moisture %	Protein %	Fat %	Acidity %	pH
Indigenous culture	48.40 a	28.07 b	17.47 a	0.97 a	5.19 b
Commercial culture	47.85 b	28.39 a	17.56 a	0.92 b	5.25 a

Mean carrying same letters in a column are not significantly different

Table 6. Effect of ripening days on moisture protein, fat, acidity and pH

Ripening days	Moisture %	Protein %	Fat %	Acidity %	pH
0	48.63 a	27.88 b	17.91 a	0.92 c	5.24 ab
20	48.16 ab	28.20 ab	17.74 a	0.93 bc	5.16 b
40	48.09 ab	28.37 a	17.54 a	0.95 b	5.27 a
60	47.63 b	28.48 a	17.32 a	0.98 a	5.20 ab

Mean carrying same letters in a column are not significantly different

depends on the percentage of salt-in moisture content of the curd. At low salt-in-moisture concentrations, residual lactose is converted mainly to L (+) lactate by the starter bacteria (Choisy *et al.*, 2000).

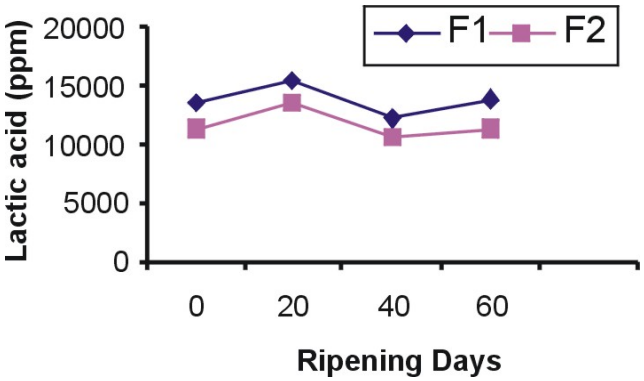

Fig. 1. Effect of fat level on the lactic acid during ripening

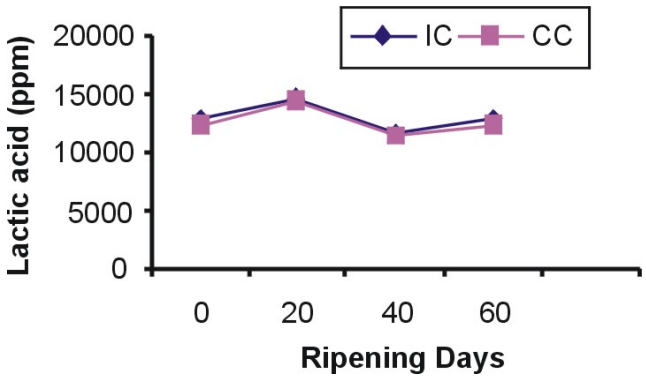

Fig. 2. Effect of starter culture on the lactic acid during ripening

It is evident from results (Fig. 2 and 4) that the lactic acid and citric acid content of mozzarella cheese were significantly higher from indigenous culture. This showed that the indigenous culture possessed more capacity towards citric acid production. The acid

production in the early stages of mozzarella cheese making is largely due to *Streptococcus thermophilus*, where as *Lactobacillus bulgaricus* becomes more important towards the end of cheese manufacture process (Kindstedt, 1993; Wong *et al*., 1974). The conversion of lactic acid to citric acid is the result of activity of non starter lactic acid bacteria which are added to cheese during manufacturing from the environment. The growth of starter culture decreased to 10^2 few days after manufacturing, while the non starter lactic acid bacteria increased up to 10^6-10^8 (Fox *et al.,* 2000).

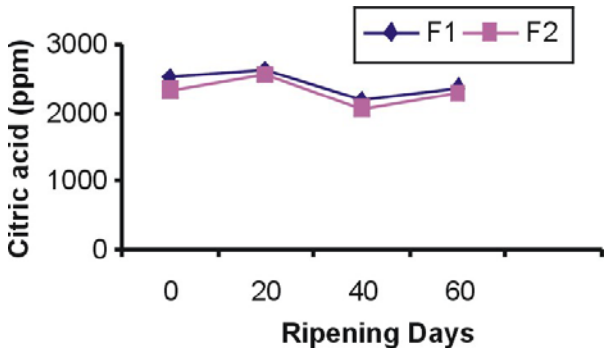

Fig. 3. Effect of fat level on the citric acid during ripening

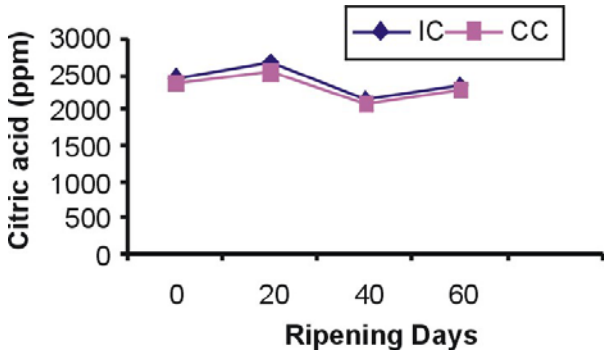

Fig. 4. Effect of starter culture on the citric acid during ripening

Sensory evaluation of cheese texture

All the cheese samples became significantly less firm over time as considered by sensory measurement. The scores of sensory parameter (Table 7) such as hand firmness and first bite firmness decreased with the ripening, while scores for chewdown degree of break down, and chew down smoothness increased with ripening. The fat level in milk affected the sensory attributes of cheese significantly. The cheese samples which were manufactured from milk with 1.5% fat level possessed higher scores 6.73, 7.13, and 6.60 for hand firmness, first bite firmness and chewdown degree of breakdown, respectively and lower scores for chewdown degree of smoothness. Other factor such as milk source and the starter culture did not significantly affect scores for different sensory parameters of cheese (data not shown). The smoothness of all the cheese samples increased with the progressive ripening days and it was highly desirable sensory attribute especially for cheese. The increase in smoothness might be due to change in average size of fat globules, distance between fat globules and the variation in the size of globules (Richardson and Booth, 1993).

CONCLUSION

The differences in milk source and milk fat level affect the cheese composition. The variation in fat levels also influences the moisture content of cheese that ultimately affects the organic acids composition. Significantly high concentration of lactic and citric acid was observed in cheese prepared with low fat milk. The starter cultures also influence the production of citric acid in cheese. The sensory attribute significantly affected due to difference in fat level and ripening period. Indigenous resources for value addition of local product especially in the dairy sector could be worthwhile. So there is need to explore to these sources for the nourishment of cheese industry

Table 7. Effect of ripening days and fat level on sensory scores of different attributes

Ripening days	Hand firm ness	First bite firmness	Chewdown degree of breakdown	Chewdown degree of smoothness
0	7.90 a	7.85 a	4.04 d	4.98 c
20	7.08 b	7.40 b	5.44 c	6.13 c
40	6.25 c	6.60 c	7.06 b	6.94 b
60	5.02 d	5.15 d	7.90 a	7.44 a
Fat levels				
1.5% Fat in milk	6.73 a	7.13 a	6.60 a	5.81 b
2.5% Fat in milk	6.50 b	6.96 b	6.45 b	5.99 a

Mean carrying same letters in a column are not significantly different

because the prepared from indigenous culture has comparable quality with that cheese which is prepared from commercial culture.

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