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RESEARCH PAPER

Process Optimization for Mozzarella Cheese from Cow and Buffalo Milk

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Abstract

Mozzarella cheese is soft, unripened cheese variety of the pasta filata family which had its origin in the Battipaglia region in Italy and was traditionally made from buffalo milk. In the present work, it is prepared by admixing of two different kinds of milk viz., Cow and Buffalo milk. The samples were prepared as To to T5 and the sample T1 and T2 proved to be the best samples on the basis of sensory evaluation. The protein content of sample T1 and T2 was observed to be 22.95% and 22.87%, respectively, whereas, the fat content of sample T1 and T2 was 22.77% and 20.61%, respectively. The observation recorded shows that yield and moisture content of mozzarella cheese depend on total solid content of buffalo or cow milk. The cheeses were subjected to microbiological analysis and sensory evaluation at storage refrigerated temperature (40°F) and at intervals of 15, 30, 45 and 60 days. New imitated process cheese, similar to stretched cheese, was used successfully as a topping material for pizza instead of Mozzarella cheese.

Keyword: - Mozzarella cheese, buffalo milk, protein, fat, pizza

Cheese manufacturing is one of the classical example of food preservation, dating from 6000-7000 BC. Preservation of the most important constituents of milk (*i.e.* fat and protein) as cheese exploits two of the classical principles of food preservation, *i.e.* lactic acid fermentation and reduction of water activity through removal of water and addition of salt. The annual world trade in milk products (excluding intra-EU) amounts to 33 million tonnes, valued at US$ 10n billion. Barely 6 to 7% of the world milk production is traded internationally. Bulk of the world dairy trade is in cheese, butter and powders. A growing shift towards cheese is expected in the near future.

The Indian dairy industry achieved substantial growth during the 8th Five Year Plan, achieving an annual output of over 60 million tonnes of milk. This not only places our industry second in the world after the United States, represented sustained growth in real availability of milk and milk products for our burgeoning population. Most important, dairying has become an important secondary source of income for millions of rural families. The Cheese industry growth rate is estimated at about 10%-12% per year in terms of volume and 16%-17% per year in value terms. Current household cheese penetration is 5%, with about 50% of consumption being limited to cities. Mumbai and Delhi together capture half of the cheese market. Within cheese products, around 60% of the market is dominated by processed cheese, 30% by cheese spreads and the remaining 10% by flavoured and specialty cheese.

Mozzarella cheese is an excellent source of protein, calcium, phosphorus, fat soluble vitamins and is palatable and easily digestible. A concentrated form of cheese gives about 400 calories/100g. Day-by-day demand of cheese increases in food market because of its own importance concerned with taste, nutrition, health benefits, etc. Hence, selected this cheese for optimization.

Mozzarella cheese is classified by standards of identity into four separate categories based on moisture content and fat in dry matter. Several recent investigations have shown a significant correlation between milk fat intake and coronary heart diseases. The pizza industry has played a major role in the increased production of mozzarella cheese. The majority of mozzarella cheese produced must have functional properties that are suitable for pizza production. Mozzarella cheese should exhibit good shredding, melting and stretching properties and be free of off-flavours or textural defects for pizza production. The study carried out on preparation Mozzarella cheese from cow and buffalo milk and its evaluation.

MATERIALS AND METHODOLOGY

Raw material: Milk, Cultures, Coagulating enzyme, Salt, Packaging material.

Methods: The methods used for manufacturing of mozzarella cheese was used as described here.

Standardization of milk: The procedure followed for manufacture of cultured Mozzarella cheese was as described by Wak (2000) with slight modification.

The cow and buffalo milk used for manufacturing mozzarella cheese were standardized at 3.5 and 6.0% fat content respectively with the skim milk of particular milk. Cow and buffalo milk were mixed in individual combination as shown in Table 1 by standardizing cow and buffalo milk to 3.5 and 6.0 fat per cent, respectively and pasteurized in an open pan for 15 minutes on a gas burner at 61°C. Pasteurized milk was then, cooled to 31°C. The Lactic acid starter culture designed for Mozzarella cheese was added to the cheese milk @ of 0.04%, followed by addition of $CaCl_2$ 0.03%. After 15 min of ripening, Rennet (Microbial Rennet 2180 IMCU 100% pure chymosin produced from *Rhizo mucormiehei*) was added with 0.019%. The curd was formed in 20-25 min and then, cut with wire knife horizontally and vertically in a cube form and allowed to heal for 15 min. It was then cooked at 45°C for 2 hours and developed acidity of whey with 0.18 to 0.19%. The curd was piled in the center of vat. The curd slab were turned every 15 min until the curd reached at desired pH 5.2 to 5.6. The whey was then, drained and the curd slabs were heated in tap water up to 70°C-80°C, stretched manually for 7 min and molded. The molded cheese was cooled in cold water at 4°C for 2 hour and brined in 23% brine solution for 2 hr. The cheese was packaged in a barrier HDPE pouch and stored at 4°C.

Table 1: Combinations of cow and buffalo milk for Mozzarella cheese

Sample	Cow milk (%)	Buffalo milk (%)
T_0 (Control)	—	100
T_1	20	80
T_2	40	60
T_3	60	40
T_4	80	20
T_5	100	0

Direct acidification method

A typical flowchart for manufacture of Mozzarella cheese by Direct Acidification method (Breene *et al.*, 1964) is given below:

Milk/Part skimmed
↓
Pasteurize (61°C)
↓
Cool (5°C)
↓
Acidify to pH 5.6 (Citric/Acetic/Lactic/Hcl)
↓
Warming (31°C)
↓
Renneting (1 gm/100 lit)
↓
Cutting
↓
Curd healing

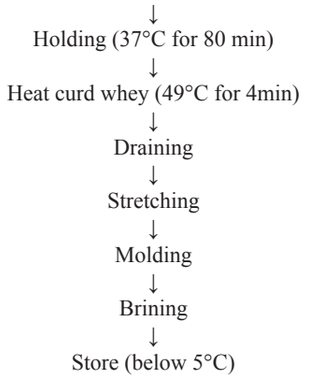

Fig. 1: Process flow chart for manufacturing of Mozzarella cheese by direct acidification method

Analysis

Moisture content was determined by using hot air oven drying method (Ranganna, 1986). The pH of milk was measured through electronic digital pH meter (Inolab WTW Series 720) (Ranganna, 1986). The acidity in milk samples was determined by the method (No. 947.05) given in AOAC (2000). Ash content was measured by the use of muffle furnace (Ranganna, 1986). The fat estimation is done by Gerber method. The determination of protein content was carried out by Kjeldahl's method (Ranganna, 1986). Total solids of milk were determined according to the method described in AOAC 925.23 (1990).

Rheological properties of Mozzarella Cheese

Texture profile analysis: Prepared Mozzarella cheese of various treatments were analyzed for its hardness, adhesiveness, cohesiveness and gumminess by using probe P-75 Texture analyzer (Model TA-XT plus). Cylindrical samples of each 1.54 cm2 cross-sectional area and 1.5 cm height were obtained from a cheese sample with help of a cork borer and knife/ wire cutting device. Each cylindrical sample was compressed to 50% of its distance and two consecutive bites were taken at 21°C. Hardness - H1 is Height of peak during first compression. Adhesiveness - A is height of negative peak during first compression. Cohesiveness - Ratio of positive area under second peak to that of the first peak (A2/A1). Gumminess - Product of hardness and cohesiveness.

Stretchability: The stretch quality of mozzarella cheese was evaluated by Sabhiki (2012).

Meltability: The method adopted was similar to that described by (Poduval and Mistry, 1999). Meltability of Mozzarella cheeses was determined (Zisu, 2005).

Sensory evaluation

The prepared samples were evaluated for sensory parameters such as color, flavour texture, taste and overall acceptability using 9 point hedonic scale by a panel of 10 semi trained panel members. Sensory evaluation of mozzarella cheese presented in Table 8 was done for the attributes namely color & appearance, flavor, taste and texture of the mozzarella cheese prepared. Evaluation was done on the basis of 9 point hedonic scale. Prepared market sample taken as reference sample.

Shelf-life study

Shelf-life study was carried out to determine the keeping quality of product. Different packaging materials and storage conditions were used and their effect on sensory quality parameters was determined.

Microbiological test of Mozzarella cheese

Total viable count: Total viable count was carried out using the plate method described by Harrigan and MacCance (1966).

Yeast and mould count: Yeast and mould count was carried out using the plate method described by Sulieman (2013).

Coliform count: Coliform count was carried out using the plate method described by Sulieman (2013).

Storage studies

Mozzarella cheese sample were prepared as per combinations at different levels of cow and buffalo milk and stored at refrigerator condition in HDPE packaging. Stored cheese was examined at 0, 15, 30, 45 and 60 days for microbial tests as follows.

RESULTS AND DISCUSSION

Quality of milk

The physico-chemical analysis of cow and buffalo milk is presented in Table 2.

The protein, fat and total solids (TS) contents of cow milk with an average of 3.50%, 4.3% and 14.47%, respectively. The average acidity of cow milk was 0.14% and pH was 6.7.

Table 2: Physico-chemical analysis of raw milk

Parameters	Cow milk	Buffalo milk
Protein %	3.50	4.37
Fat %	4.3	6.8
Total solids%	14.47	17.92
Ash %	0.67	0.79
Acidity %	0.14	0.15
P^H	6.7	6.8
Color	Yellowish white	White

Buffalo milk had an average protein, fat and total solids (TS) of 4.37%, 6.8% and 17.92%, respectively, the fat per cent ranged from 5.8 to 8.4. The average acidity and pH of buffalo milk was 0.15% and 6.8.

In milk quality, the buffalo milk was better with fat, protein and Stretchability than cow milk.

Physico-chemical properties of mozzarella cheese

The physico-chemical analysis of individually prepared mozzarella cheese presented in Table 3.

Individually prepared Mozzarella cheese from cow and buffalo milk was analyzed. The proximate composition of mozzarella cheese sample T1 was more effective than sample T2, T3, T4 and T5. Sample T1 showed good hardness, Stretchability and Meltability. It was concluded that the Mozzarella cheese from Buffalo milk was better quality in chemical composition.

Moisture content: The moisture content of mozzarella cheese is presented in Table 4. The moisture content consistently increased, with the increasing the proportion of cow milk in the combination. The lowest moisture content 50.08 and 50.30% was in T1 and T2 which were having high proportion of buffalo milk in a combination and T4 and T5 having 52.99 and 53.12% respectively which having high proportion of cow milk. The moisture content of T3 is having 50.39%. The lower yield despite the higher moisture retention may be due to decreasing total solid in cheese milk as a consequence of cow milk addition. Thus, the yield of cheese is directly proportional to the total solids (TS) of milk used for cheese manufacturing.

Table 3: Analysis of Mozzarella cheese individually from cow and buffalo milk

Parameters	Cow milk	Buffalo milk
Moisture (%)	53.00	50.28
Fat (%)	21.28	23.11
Protein (%)	19.91	20.13
Ash (%)	2.80	3.17
pH	5.51	5.60
Acidity (%)	0.37	0.36
Yield (%)	14.09	17.85
Stretchability (cm)	62.36	67.04
Meltability (cm)	11.19	11.50

Table 4: Proximate analysis of mozzarella cheese

Parameters	Samples					
	T_0	T_1	T_2	T_3	T_4	T_5
Moisture (%)	50.28	50.08	50.30	50.39	52.99	53.12
Fat (%)	23.10	22.77	20.61	20.60	20.59	20.46
Protein (%)	22.81	22.95	22.87	21.74	20.95	20.69
Ash (%)	03.18	02.94	02.89	02.69	02.67	02.60
pH	05.66	05.61	05.60	05.60	05.53	05.42
Acidity (%)	0.360	0.363	0.362	0.350	0.350	0.348

Fat content: The fat content of mozzarella cheese is presented in Table 4. The fat content of all milk cheese is about 15-23% however the fat recovery was increased with increased in proportion of buffalo milk. This again reason was of decrease in yield as

the cow milk was higher in proportion. The yield is slightly increased and if buffalo milk is more the yield is higher than other combinations.

Protein content: The protein content of mozzarella cheese is presented in Table 4. The protein content of 22.81% was found in sample T0 fallowed by 22.95% in T1 which had high amount of buffalo milk and in other blend it was 22.87% in sample T2 high proportion of buffalo milk while 21.74, 20.59 and 20.46% in sample T3, T4 and T5 having high proportion of cow milk. The protein content showed decrease as the cow milk proportion was increased in the combination, so that the protein content was inversely proportional to the moisture content of cheese, as reported by Rao, (1990) also.

Ash content: As the cow milk proportion was increased in the combination, ash percentage in cheese was decreased as shown in Table 4. This may be due to lower total solid content. The highest ash was found in sample T1 and T2 however the buffalo milk proportion was increased in these samples. It was concluded that the ash content is directly proportional to the solid content of Milk.

pH: The results of current study shown in Table 4. The pH of prepared Mozzarella cheese was in between 5.2 to 5.7. The highest pH was in sample T1 while T2 having pH 5.61 and 5.60 respectively T3 having 5.60. High pH results into more stretchablity and meltability of mozzarella cheese, as reported by Guinee, (2002).

Acidity: The titrable acidity of mozzarella cheese was showed in the Table 4, which was ranged from 0.360 to 0.428 % respectively.

Yield: The yield of Mozzarella cheese is shown in Table 5. It is apparent that the yield of cheese is decreasing with increase in the proportion of cow milk in the combination, varying the highest yield was 17.67% and 17.39% in sample T1 and T2 in high per cent of buffalo and low percent of cow milk in combination. The lowest yield is 15.11, 14.72% and 13.82% in sample T3, T4 and T5 which having low proportion of buffalo and high per cent of cow milk in cheese. Sample T0 which is made from 100 % buffalo milk gives highest yield which was control sample. Higher the amount of total solids of milk, higher was the yield of mozzarella cheese as reported by Mona A.M, (2011); Owni (2009).

Table 5: Yield (%) of mozzarella cheese

Sample	R_1(%)	R_2(%)	R_3(%)	Mean	SD
T_0	17.86	17.86	17.86	17.86	
T_1	17.61	18.07	17.35	17.67	0.3646
T_2	17.07	16.90	18.20	17.39	0.7066
T_3	15.17	14.90	15.27	15.11	0.1913
T_4	14.45	15.07	14.66	14.72	0.3153
T_5	13.07	14.30	14.11	13.82	0.6621

Rheological properties of Mozzarella cheese

Texture profile analysis for Mozzarella cheese

Hardness is the force required attaining a given deformation, Cohesiveness is defined as the quantity simulating the strength of the internal bonds making up the body of the product; gumminess deals with the stickiness of the product. The property of sticking together is known as adhesiveness of that product. As given in Table 6, the texture analysis of all the samples of cheese was carried out by using TA XT Texture Analyzer.

Table 6: Texture profile analysis of Mozzarella cheese

Sample	Hardness (kg)	Adhesiveness (kg)	Cohesive-ness	Gumminess (kg)
T_0	1.804	-1.447	0.636	1.147
T_1	1.617	-1.290	0.600	0.970
T_2	1.593	-1.135	0.556	0.885
T_3	0.722	-0.748	0.621	0.448
T_4	0.694	-0.644	0.619	0.429
T_5	0.432	-0.347	0.492	0.213

Texture profile was analyzed by using probe P 75. Hardness of the prepared cheese increased when the buffalo milk in the sample was more. Sample T1 showed the highest hardness of 1.617 that contained 20% cow milk and 80% buffalo milk. Sample T4

showed the lowest hardness of 0.432 and was containing 80% cow milk and 20% buffalo milk. The control sample had the hardness of 1.804.

Stretchability of Mozzarella cheese

From Table 7 it is evident that stretchablity increased with increased proportion of buffalo milk and decreased proportion of cow milk. Since the buffalo milk content in sample T1 and T2 was higher, the cheese showed good stretchability. The sample T1 containing 20% cow milk and 80% buffalo milk showed stretchability of 68.30 cm which was higher. The sample T2 having 40% cow milk and 60% buffalo milk had stretchability of 67.29 cm. Sample T1 was the best sample in terms of stretchability. Sample T3, T4 and T5 containing least stretchability of 66.00, 65.66 & 63.30 cm as it had higher proportion of cow milk Hayam, (2014).

Table 7: Stretchablity and Meltability of mozzarella cheese

Sample	Parameters	
	Stretchablity (cm)	Meltablity (cm)
T_0	68.88	12.00
T_1	68.30	11.58
T_2	67.29	11.50
T_3	66.00	11.10
T_4	65.66	10.76
T_5	63.00	10.30

Meltablity of Mozzarella cheese

Meltability is an important character, which determines to a great extent the quality of process cheese as well as Mozzarella cheese. Meltability of the processed cheese was expressed as the distance of cheese flow in centimeters. Lawrence, (1968) gives a wide explanation for the factors that affect the melting quality of the processed cheese. These are the heat induced interaction of casein and whey proteins resulting in reduced capacity to flow, binding of free water by denatured protein or casein complex, calcium binding by denatured lactoglobulin and reduced proteolysis. Process cheese made by acidified milk with lemon juice had a lowest pH and high acidity; which lead to weaken of protein bonds and de-emulsion of fat as reported by Hayam (2014).

Decreased calcium content causes increase in interaction between protein and moisture. The protein matrix expands and becomes more hydrated and increases meltability as reported by Tahra, (2008). From Table 7, it is apparent that the control sample had meltability of 12.00cm. Sample T1 showed higher amount of meltability of 11.58 cm. Sample T5 showed least amount of meltability of 10.30 cm. which was comparable with control sample El-Tahra, (2008).

Sensory evaluation of mozzarella cheese

The control sample scored highest with score 9 of overall acceptability. Scores for all the parameters of prepared mozzarella cheese were increased as the buffalo and cow milk proportion in the combination was increased. The overall acceptability of mozzarella cheese was 8.5, 8.31 (T1, T2) and 7.87, 8.06 (T5, T4) respectively made from higher concentration of buffalo milk and cow milk similar observations wre made by Tahara, (2008) and Upadhyay, (2006).

Table 8: Sensory evaluation of Mozzarella cheese

Sample	Color and appearance	Flavor	Taste	Texture	Overall acceptability
$T_{0(Control)}$	9.0	8.0	9.0	9.0	9.0
T_1	8.25	8.5	8.25	8.5	8.37
T_2	8.5	8.0	8.25	8.5	8.31
T_3	8.25	7.25	7.0	8.5	7.75
T_4	8.0	8.25	8.0	8.0	8.06
T_5	8.5	7.5	7.5	8.0	7.87

The mean sensory scores of the cheese made from all compositions. The control sample scored highest with score 9 of overall acceptability. Sample T1 and T2 was more overall acceptability.

Shelf-life of Mozzarella cheese

Effect on weight of Mozzarella cheese at refrigerated storage

Resulting in Table 9 show the weight losses of

mozzarella cheese were increased with increasing in the proportion in the buffalo milk in the combination i.e. T3 and T4 having high proportion of buffalo milk i.e. 60%. The minimum weight loss occurred in sample T1 and T2 sample having high proportion of buffalo milk. The samples were prepared with higher quantity of cow milk in the combination the losses were found in the intermediate range as compared to buffalo milk. Cheese weight loss during storage was due to the loss of its moisture content as a result of water expulsion from the cheese as drip loss due to curd contraction as reported by Owni (2009).

Table 9: Effect of refrigerated storage on weight of mozzarella cheese

Sample / Days	Weight loss (%)				
	0	15	30	45	60
T_0	Nil	0.65	0.97	1.21	1.31
T_1	Nil	0.61	1.16	1.44	1.67
T_2	Nil	0.79	1.21	1.54	1.80
T_3	Nil	0.91	1.93	2.51	2.71
T_4	Nil	1.90	2.89	3.67	4.12
T_5	Nil	1.01	1.96	2.47	2.69

Storage of cheese

Total plate count of Mozzarella cheese

Changes in total plate counts are presented in Table 10. Total plate count in fresh mozzarella cheese was not detected. After 15 days the TPC were detected in all the samples at 10^2 dilutions. After final microbial test of 60 days the highest TPC counts were found 28×10^3 respectively in T3 sample having more proportion of cow milk which leads to high moisture content in mozzarella. So, due to the high moisture content the microbial growth was higher than other samples. In the other side least TPC counts were found 17×10^3 and 15×10^3 in T1 and T2 which had higher proportion of buffalo milk mozzarella having low moisture content than others which affects the growth rate of microbes. The maximum limit for TPC in mozzarella cheese was 50000 cfu/ml as reported in food safety standards, (2011).

Table 10: Total plate count (cfu/ml) of mozzarella cheese

Sample	Fresh	15 days	30 days	45 days	60 days
T_0	ND	3×10^2	8×10^2	17×10^2	16×10^3
T_1	ND	1×10^2	10×10^2	21×10^2	17×10^3
T_2	ND	2×10^2	6×10^2	19×10^2	15×10^3
T_3	ND	6×10^2	15×10^2	29×10^2	28×10^3
T_4	ND	4×10^2	11×10^2	24×10^2	22×10^3
T_5	ND	5×10^2	13×10^2	25×10^2	21×10^3

Yeast and mold count (cfu /ml) of mozzarella cheese

The yeast and mold count is presented in Table 11 initially in first 45 days the yeast mold count was not detected. After 60 days, the growth count ranged from 1 to 2.3 cfu/ml the yeast and mold count of less than 10cfu/ml in mozzarella cheese is acceptable Table food safety standards (2011)

Table 11: Yeast and mold (cfu/ml) count of mozzarella cheese

Sample	Fresh	15 days	30 days	45 days	60 days
T_0	ND	ND	ND	ND	1
T_1	ND	ND	ND	ND	1
T_2	ND	ND	ND	ND	1
T_3	ND	ND	ND	ND	2.3
T_4	ND	ND	ND	ND	1.8
T_5	ND	ND	ND	ND	1.9

Coliform Count of mozzarella cheese

Coliform count was not detected in any of the sample even after 60 days.

Sensory evaluation of Mozzarella cheese

Control sample used for sensory evaluation was market sample. The sensory evaluation of mozzarella cheese during storage was done for over 60 day's. On the basis of sensory evaluation of initial product, microbial analysis and textural analysis; sample T1 and T2 were considered as the best.

Table 12: Sensory evaluation of Mozzarella cheese during shelf-life study

Sample	Days	Color and Appearance	Flavor	Taste	Texture	Overall Acceptability
Control	0	9.0	9.0	9.0	9.0	9.0
	15	9.0	8.0	9.0	9.0	8.75
	30	9.0	7.75	8.75	9.0	8.62
	45	9.0	7.5	8.5	8.5	8.37
	60	8.5	7.5	8.5	8.25	8.18
T_1	0	8.25	8.5	8.25	8.5	8.37
	15	8.25	8.25	8.0	8.5	8.25
	30	8.25	8.25	8.0	8.5	8.25
	45	8.25	8.0	8.0	8.25	8.12
	60	8.0	7.75	7.75	8.0	7.87
T_2	0	8.5	8.0	8.25	8.5	8.31
	15	8.5	8.0	8.0	8.5	8.25
	30	8.5	7.75	8.0	8.25	8.12
	45	8.0	7.75	8.0	8.0	7.93
	60	7.75	7.5	7.5	7.75	7.62

CONCLUSION

The yield and moisture content of mozzarella cheese depends on total solid content of buffalo and cow milk used. The protein content of samples T1 and T2 were 22.95% and 22.87%, respectively whereas the fat content of sample T1 and T2 was 22.77% and 20.61%, respectively. The sample T1 and T2 proved to be the best samples as it was high in terms of nutrient contents. In preparation of mozzarella cheese, use of cow milk up to 20 and 40% was acceptable as represented in sample T1 and T2.

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