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Effects of different stretching temperatures on the quality of buffalo milk mozzarella cheese

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Abstract: Mozzarella cheese is a multifunctional food ingredient that has been used in pizza, soups, and bakery products to impart unique flavor and texture. Many factors, i.e., milk quality, culture, coagulation, whey drainage, milling, pH, and stretching water temperature, affect cheese's various functional attributes. In this study mozzarella cheese curd was prepared by following the traditional method and stretched at 65, 70, 75, and 80 C. Afterward, it was stored at 4 C for 60 days and the effect of storage and different temperatures was observed on rheological (texture, stretch-ability), physicochemical (pH, acidity) and compositional properties (total nitrogen and mineral content) during 0, 15, 30, 45 and 60 days. It was revealed that stretchability, texture, and acidity increased with the passage of storage time. It was also revealed that the curd stretched at 80 C showed higher stretchability and the best textural properties during the storage period.

Keywords: Buffalo milk, Stretch-ability, Mozzarella Cheese, Temperature

1. Introduction

There are many ways of milk preservation among them the most promising method is cheese manufacturing. It is one of the typical and classical examples of milk preservation, dating from 6000~8000 BC. Cheese is one of the most demanding milk products, used for garnishing and improving the properties of other food products (Jeewanthi et al. 2015). Cheese and various other milk-fermented products are among the most demanding and consumable foods in the world. These are also considered as one of the wonders because they are found in a huge diversity of flavors, and shapes, and are enormously nutritious. Cheese is one of the most distinctive groups of dairy products and perhaps the most fascinating one. It is reported that currently more than 1400 individual cheese varieties are being produced in the world and this number is most prominent in France. These are grouped based on their manufacturing technique, ripening, chemical composition, and various shapes or forms of cheeses (Walstra et al. 2006).

It is reported that almost one-third of the milk produced in the world is used for cheese manufacturing to preserve the most desirable milk components such as casein, fat, calcium, and phosphorus (Adda et al. 1982). About thirty percent of total dairy product sales represent the global sale value of cheese. Mozzarella cheese comprises eighty percent of all Italian-style cheeses and thirty-two percent of the total cheese produced in the world. It is reported that every year there has been a 4% increase in cheese production in the last three decades in the world (FAO, 2016).

It is reported that 5000 tons of cheese is consumed annually in Pakistan whereas its production per annum in Pakistan is 2500 tons. Whereas, only 90-100 tons of mozzarella cheese is being produced in Pakistan (PDDC 2006). Demand for mozzarella cheese is increasing day by day due to the great popularity and consumption of pizza in Pakistan and the opening of multinational pizza restaurants in Pakistan also increased its demand. The quality of locally produced mozzarella cheese is not compatible with the imported cheese.

Mozzarella cheese is one of the most popular cheese types in the world due to its major and primary use as a pizza topping (Kindstedt 2004). It is included in the group of "Pasta filata" or stretched cheese. The various physical attributes of mozzarella cheese like texture, body, meltability, stretch-ability, and color depend on

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different factors like milk composition, starter culture, and ripening conditions during the cheese production (Lucey et al. 2003).
The functionality of mozzarella cheese is significantly important due to its use in pizza, and it is reported that 75% of the total produced mozzarella cheese is currently being used as an ingredient for pizza especially topping (Kindstedt 1993). Different factors such as the extent of proteolysis, fat content, protein-protein, and protein-water interactions influence the characteristics of cheese. During storage, proteolytic activity plays a vital role in the development of functional properties i.e. flavor and texture in mozzarella cheese (Rowney et al. 2004).
The temperature of curd throughout the manufacturing process is influenced by water temperature and time duration. It may affect the functional properties of mozzarella cheese such as melting and shredding. It was observed that an increase in the stretching temperature of the curd from 57 to 75 C caused the loss of elastic properties of the cheese. The temperature is a paramount factor in influencing free oil formation. A higher cooking temperature will make the protein network less stretchable (Mulvaney et al. 1997).
The current study was designed to evaluate the effects of different stretching water temperatures on the quality of mozzarella cheese made from buffalo milk particularly its impact on
texture, stretchability, and physicochemical properties of cheese.

2. Materials and methods

The research work was conducted in the Laboratories of the National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad, Pakistan. The research work was carried out to manufacture mozzarella cheese from buffalo milk and evaluated for compositional, physico-chemical, rheological, and other characteristics.
The treatment plan that was adopted during this research had four treatments, T1: 65 oC; T2: 70 oC; T3: 75 oC; and T4: 80 oC, respectively. Various rheological and physic-chemical analyses were performed at every temperature treatment of mozzarella cheese.

2.1 Procurement of Raw Materials Milk

Raw milk buffalo was procured just after milking from a dairy farm at, the University of Agriculture, Faisalabad for the preparation of mozzarella cheese.

2.2 Cultures

Only commercially available culture for Mozzarella cheese was used for the manufacturing of cheese. These cultures are (Lactococcus lactis subspp. Lactis, Lactobacillus delbrueckii subspp. Lactis, Lactobacillus helveticus, and Streptococcus thermophilus).

2.3 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 which was provided by Noon Dairies, Bhalwal, Sargodha, Pakistan.

2.4 Preparation of cheese milk Fat standardization

After the collection of milk from both buffalo and cow, it was de-creamed in the maximum possible hygienic conditions and then stored at 4 C. Both buffalo milk and cow milk were standardized for the fat content before manufacturing Mozzarella cheese. The Gerber method determined the milk samples (buffalo and cow milk) and the content of both cream and milk. The fat level was standardized in milk by using Pearson's square method at a level of 3.5%.

2.5 Mozzarella cheese manufacturing

Buffalo milk was standardized at a 3.5% fat level and homogenized. Buffalo milk was pasteurized at 65 C for 30 min and cooled to 37 C. It was inoculated with 2% combined culture (Lactobacillus bulgaricus and Streptococcus thermophilus). After ripening for 30 min, the milk at 37 C was set with 0.077ml/kg Chymosin (Double Strength Chy-max,). After 40 min of rennet addition, the curd was cut with 1.9 cm wire knives and allowed to heal in the whey for 10 min. Then with periodic gentle agitation of curd, it was heated from 37 oC to 42 oC over 30 min. The temperature was increased gradually to 42 °C during the cooking of curd. The whey was drained at pH 6.2 and the curd was turned every 15-20 min and milled at pH 5.2. Then 1.5% salt was added. The stretching of salted curd was done utilizing hand in water at a temperature of 65 C, 70 C, 75 C, and 80 C until the uniform and elastic cheese consistency was achieved. The quantity of water used for stretching was 2.5 times the weight of curd and had 3% of salt w/w. The salted curd was then molded vacuum packed and stored at 4 C for ripening.

2.6 Rheological Analysis Stretch-ability

After molding it was stretched at different temperatures such as 65, 70, 75, and 80 C. Then after every temperature treatment, it was applied as a pizza topping to check its stretch-ability which was measured by the method of Catherine and coworkers (1998).

2.7 Cheese texture and firmness

Cheese samples were prepared by cutting the cheese cubes from each treatment at prescribed intervals and stored in sealed plastic bags at 8 0C for twelve hours. Afterward, these cubes were compressed at the rate of 20 mm/min on a texture profile analyzer (Stable Micro System) at room temperature. The result was recorded as the force used to compress the cheese sample as described by Guinee and coworkers (2000).
Physicochemical analysis of cheese
The standardized cheese samples were analyzed for physico-chemical composition such as pH, acidity, total nitrogen, non-protein nitrogen, and mineral content.

2.8 pH

Twenty grams of shredded mozzarella cheese was blended with 12 mL water to prepare the cheese slurry and pH was measured by a pH meter (Inolab WTW Series 720) after calibrating it with fresh pH 7.0 and 4.0 standard buffers.

2.9 Acidity

Acidity in mozzarella cheese was estimated by titration method AOAC (2000). 5 g of crushed sample was taken in a conical flask, 50 ml of water was added and kept at room temperature (18 0C -20 0C) for 4-5 h the water bath for 30 min. It was mixed well and filtered through dry filter paper. The phenolphthalein was used as an indicator and the filtrate was titrated with 0.1 N NaOH.

2.10 Compositional Analysis Nitrogenous fractions Total Nitrogen (TN)

The total protein content in cheese samples was measured by Kjeldahl's method IDFA (2006). A sample of cheese (3g) was taken and digested in a Kjeldhal digestion flask with 20 mL of concentrated sulfuric acid and digestion tablets until a clear solution was obtained. The solution was distilled by adding 40% NaOH, and the produced gas was trapped in 10 ml of 4% boric acid. It was then titrated against 0.1N acid solution. Methyl red was used as an indicator. The protein content in Mozzarella cheese was determined by multiplying N% with a factor of 6.38. The results of total nitrogen are elaborated as total protein in the results and discussion.

2.11 Minerals content in Mozzarella cheese

Calcium was determined using an atomic absorption spectrophotometer as described by Fuente and colleagues (1997). Concentrated nitric acid (2 ml) and 2 ml H_2O_2 were added to 100 mg of cheese sample in a vessel flask and rapidly heated for 5 min. afterwards, it was heated at lower heat for 10 min or up to colorless. After digestion distilled water was used to make the volume of the sample 100 ml. Standard solutions of known concentration were prepared for calcium separately and run on an atomic absorption spectrophotometer followed by the test samples. The standard curves were obtained by plotting values of standards against appropriate concentrations of elements.

2.12 Statistics

All results were taken in three replicates and were expressed as mean. These results were statistically compared and analyzed with the help of Statistics Version 8.1.1 software. Differences between means were assessed using Tukey's test. Significant differences were defined as $p < .05$.

3. Results and Discussion Rheological Analysis Stretch-ability

Stretchability refers to the capacity of melted cheese to form a fibrous stand that extends under tension. It is therefore one of the most important and fascinating characteristics of pizza cheese but the most difficult parameter to measure is the stretch when the cheese is melted (Kindsted et al. 1995). Stretch-ability is the tendency of a casein molecule to sustain its integrity when constant stress is applied to the cheese. When the cheese is stressed, casein molecules interact release stress, and become pliable (Lucey et al. 2003).
The mean values for stretchability are shown in Fig 1. It was revealed that the stretchability in pizza cheese increased progressively during ripening. The results of treatments indicated a significant difference, a high value was observed in T3 and T4 among all treatments during 1st day of storage highest value (31.67cm) in T3 and the lowest value of 27.32cm in T1 was observed. At the end of storage, the highest value (42.32cm) in T4 and Tl showed a 36.33 cm stretch. There was an increase in stretch-ability from 1st to the 60th day of pizza cheese and T4 showed the highest results.

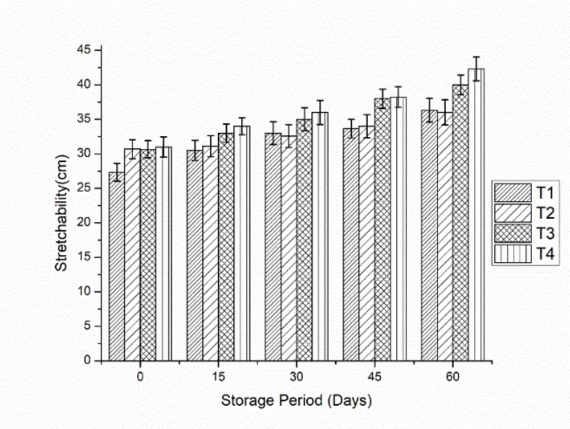

Fig 1. Effect of various temperatures and storage on Stretch-ability

These results were in corroboration with the earlier studies on mozzarella cheese conducted by (Guinee et al. 2003). The stretchability of cheese increased significantly during storage. This increase in stretch-ability may be due to the increase in proteolysis, fat coalescence, and water binding capacity; of the casein matrix, which increased the heat-induced displacement of adjoining layers of the casein matrix on heating. Because of aging, the casein matrix porosity increases due to proteolysis and therefore exhibits less resistance to stretching (Thunick et al. 1991). Stretch-ability values in our study were in agreement with the findings of Fife and coworkers who studied the stretchability of various melted cheeses (Fife et al. 2002).

3.1 Cheese texture and firmness

Firmness is an attribute related to the rheological properties of cheese which is crucial for manufacturers, packagers, distributors, retailers, industrial users, and ultimately the consumer. Firmness is a physical parameter used to categorize the cheese into a specific cheese group such as very hard, hard, semi-hard, semi-soft, soft, and fresh cheeses (Fox et al. 2000).

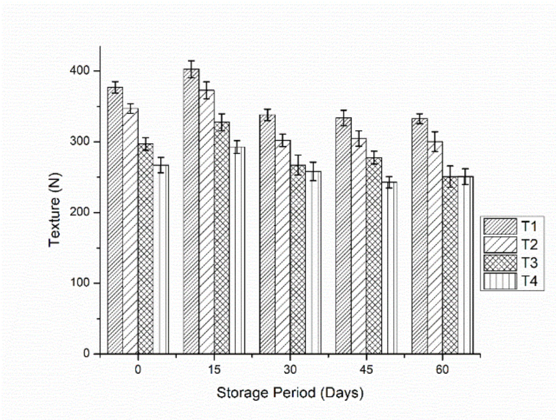

Fig 2. Effect of various temperatures and storage on Texture

The results in Fig 2 revealed that the firmness of cheese decreased during ripening from 0 to 60 days. Our results were in agreement with the findings of Kindstedt (1995) who previously conducted experiments on Mozzarella cheese. The reason for this decrease was the cleavage of αs1-casein at Phe23-Phe24 by residual chymosin activity which may be associated with a decrease in firmness during the ripening.
Physicochemical analysis of cheese.

3.2 Acidity

Acidity and pH have an inverse relation i.e. if pH is high then acidity will be lower. Acidity in cheese increased during storage due to several integrated factors the most important are the culture activity by lactose utilization, proteolysis, and lipolytic actions resulting in the production of lactic acid, amino acid, and fatty acid respectively. Autolysis of food may also take its part in the breakdown of the different food components causing an increase in its acid content e.g. rancidity of fat. The results of acidity with storage days are represented in Fig 3. The behavior of the data taken from all samples during storage unraveled a gradual increase with time. The unusual increase might be due to the production of some acidic bacteria or more chemical reactions. The initial highest value of acidity at 0 days is 0.75 in T3 and the lowest at different values is 0.71 in T4. At the end of the storage, the highest acidity (0.80) was observed in T2 and T4. These results were the findings of Chaves and his colleagues they revealed a significant increase in acidity during the ripening process of Mozzarella cheese (Chaves et al. 1999). These values of acidity observed in this study are the values of acidity described by Vernam and Sutherland (1994) in their study on the technological, microbiological, and functional properties of different cheeses.

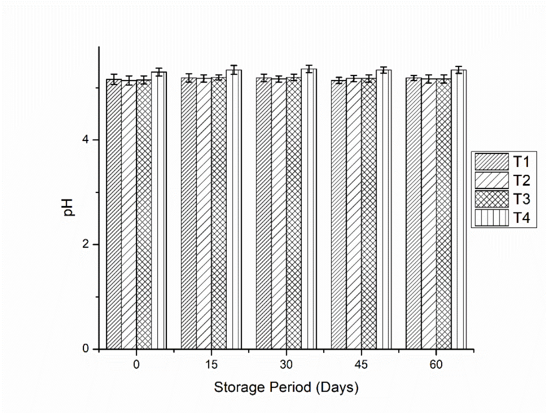

Fig. 3 Effect of various temperatures and storage on Acidity

3.3 pH of pizza cheese

pH is a critical factor in several aspects of the manufacture and ripening of cheese curd (McSweeney 2007). The actual pH attained is strongly affected by the buffering capacity of the milk and curd (Guinee and Law 2002). The pH of pizza cheese increased gradually with storage time and maximum at the end of the storage period.

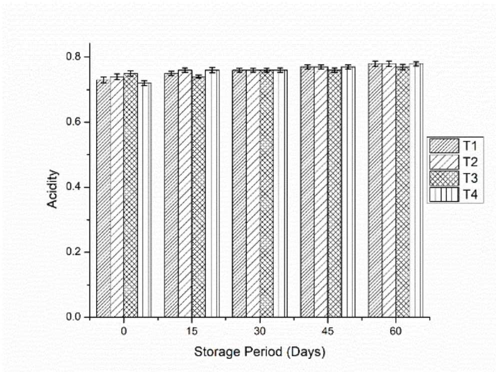

Fig 4. Effect of various temperatures and storage on pH

Fig 4 shows the results of pH for different treatments at various storage days, maximum pH was observed in T1 and T4 and minimum in T2 at the 0 days of storage. The maximum pH for cheese was on the 0 day for all treatments and minimum at the end of the 6th week but in the later weeks, the pH value was not decreased. At

the end of the 60th day of storage, the highest value of pH was observed. The maximum pH value of 5.41 was observed in T4 and a minimum in T1 (5.14) was observed.
These findings were the results of Watkinson and coworkers who studied the effect of cheese pH and ripening time on model cheese textural properties and proteolysis (Watkinson et al. 2001).

3.4 Nitrogenous fractions

Protein in cheese was determined by the nitrogen content of the sample. It may be non-protein nitrogen (NPN) due to the presence of other compounds that consist of nitrogen i.e. urea ammonia and nitrogen from protein which is called true protein in milk.

3.5 Total Nitrogen (TN)

Protein contributes to the flavor, body, and texture of the final products. It is also essential for the formation of small stable air cells (Web et al. 1974). After fat and carbohydrates, protein occupies a third place as a source of calories.

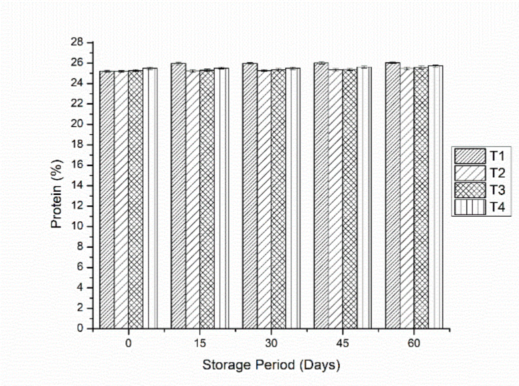

Fig 5. Effect of various temperatures and storage on Total Protein

The means of the data contents are shown in Fig 5. The average protein content for T1, T2, T3, and T4 are 25.97, 25.31, 25.37, and 25.60%, respectively. The highest protein content (25.97%) was found in T1 and the least in T2. A slight increase in protein content was observed in all cheese samples. The maximum increase was found in T4 (0.29%) followed by T2 (0.27%), T3 (0.23%), and T1 (0.11%). It was evident from the results that there was a slight increase in the protein content of cheese during storage. It was observed that protein contents increased from 25.62% to 25.88% after the storage period of 60 days. This slight increase could be due to the loss of moisture and conversion of amino acid to proteins by the microbial action.

3.6 Minerals content in Mozzarella cheese

The amount of calcium in cheese associated with casein is described as insoluble calcium. The main role of calcium is as a buffering agent in cheese. Calcium chloride was added to milk to reduce the amount of rennet needed for the optimum curd-making properties under certain conditions and may increase the yield of cheese (Lucey and Fox 1992).

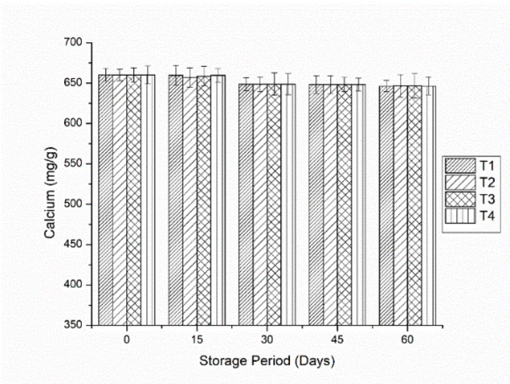

Fig 6. Effect of various temperatures and storage on mineral content

The Fig 6 revealed that calcium was decreased throughout the storage. There was a little decrease in calcium values, T1 contains the highest 660.31 mg amount of calcium at the 0 days of storage, and T4 lowest 660.14 mg calcium value. At the 60 days of storage, T3 showed a high 646.68 mg of calcium while T2 646.29 mg showed the lowest value of calcium. The mean of the mean of treatment showed a significant and nonsignificant relation. T1 and T4 showed nonsignificantness to each other because of the same value of calcium, similarly, T3 and T2 were nonsignificant to each other. The mean of the mean storage indicated the significant relationship expects 30, and 45 days which contain the same value. The same results were also observed in the study of the Addition of calcium cheese affects the textural and melting properties of cheese (Dolby et al. 1941).

Conclusion

This study aimed to introduce local cheese producers to international standards of quality, which should be adopted to produce good-quality cheese under local conditions. It is concluded from the study that cheese stretched at 80 C possessed better quality and had higher stretchability and firmness as compared with other stretching water temperatures. During storage, the texture was decreased with time in all treatments but treatment T4 i.e. cheese stretched at 80 C revealed the best texture. The optimum pH and acidity values in this experiment were 5.41 and 0.8, respectively. Cheese stretch-ability was affected by the milling pH of the curd and especially stretching water temperature at the end the most important point is stretching water temperature and 80 C was best for the stretching of cheese.

It could be concluded from the study that the milk should be analyzed for its pH, acidity, fat, Lactose, SNF, and clots on boiling (COB) immediately after milk reception. Casein to fat ratio should be maintained at 0.91, appropriate amount and ratio of defined cultures and rennet should be used for mozzarella cheese production. The control of processing factors mainly temperature, starter cultures and elapsed times for each step is necessary for the industry to obtain a more uniform efficiency in the production of mozzarella cheese.

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