

Research Article

Impact of different casein to fat ratios on the physicochemical composition, functionality and sensory quality of mozzarella cheese

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Abstract

Mozzarella cheese is pasta filata product, manufactured in various shapes and used as an ingredient in prepared foods to add taste, texture and nutritional quality. Milk composition especially casein to fat ratio (C/F) is prime factor that dictate the functionality and sensory properties of Mozzarella cheese. Large restaurants do not purchase cheese from local manufacturer due to their non-standardized manufacturing conditions which imparts variability in product quality. The study was aimed to standardize the Mozzarella cheese manufacturing conditions with special reference to C/F and investigate its impact on the Mozzarella cheese quality during storage. Cheese milk was standardized at three different C/F (1.6, 0.8 and 0.4) and cheese so produced was analyzed for physicochemical composition, functionality and sensory attributes at 0, 30 and 60 days of storage. Results showed significant ($p < 0.01$) increase in cheese protein and stretchability with increase in C/F of cheese milk. However, moisture, fat and meltability are inversely related with C/F. High C/F results in fat reduction in cheese which adversely affect the Mozzarella cheese sensory attributes. Casein to fat ratio (C/F) 0.8 was found to produce Mozzarella cheese with good stretchability (35.61cm), optimum melt time (118.33 sec.) and excellent sensory properties.

1. Introduction

Pakistan is the 4th largest milk producing country in the world with annual production of about 52.6 million tons (GOP, 2015). Buffalo milk is at the top in Pakistan's milk production with annual value of 31.25 million tons (GOP, 2014) and has promising research interests because of its nutritional profile and buffering capacity that offers remarkable prospects for fermented products like cheese (Murtaza et al., 2015; Tahira et al., 2014). A wide range of cheese varieties exists in the world, each with unique characteristics (Falegan & Akere, 2014). Cheese is a complex food made from few simple ingredients. It is the coagulated and concentrated form of milk that is manufactured at appropriate temperature and humidity (Murtaza et al., 2014). Cheese varieties are classified on the basis of origin of milk sources, chemical composition, manufacturing procedure, shape, texture and ripening (Walstra et al., 2006; Manish & Srivastava, 2002).

Mozzarella cheese represents about 80% of all Italian style cheeses and 32% of total cheese produced in the world. It has become one of the most popular cheese

varieties in world because of its primary use for pizza toppings due to its exceptional properties of meltability, stretchability and elasticity. Its usage is expected to grow due to increased global interest for pizza and other foods that use Mozzarella cheese as ingredient. The quality of locally manufactured Mozzarella is not compatible with the imported cheese due to lack of non standardized processing conditions. The macro-constituents of milk especially fat and protein along with cheese pH are the major determinant for technological properties (Fox et al., 2000). Differences in fat level and hence protein to fat ratio, that occur in milks have marked influences on composition, functionality, and sensory attributes of cheese (Guinee et al., 2002). Keeping in view the production and prospects of buffalo milk as well industrial demand for optimum conditions for the manufacturing of good quality Mozzarella cheese, the study was aimed to assess the impact of different casein to fat ratios (C/F) on composition, functionality and sensory attributes of Mozzarella cheese.

2. Materials and Methods

2.1 Raw materials

Raw buffalo milk was procured from dairy farm, University of Agriculture, Faisalabad. Freeze dried thermophilic Mozzarella cheese culture (YH 092E from SACCO International) and rennet (Double strength Chy-max, 500000 MCU/mL, Pifzer Inc, Milwaukee, WI, USA) were purchased from local market.

2.2 Standardization of milk at different casein to fat ratios

Buffalo milk was subjected to cream separation followed by standardized at different casein to fat ratios (C/F) by increasing fat level in milk (with constant level of casein) to adjust C/F at 1.6, 0.8 and 0.4 for different treatments.

2.3 Cheese milk analysis

The milk samples were analyzed for pH value by using pH meter, acidity by titration method (Method No. 947.05; AOAC, 2000), fat by Gerber method (Marshall, 1993), protein by Kjeldahl's method (Method No. 991.20; AOAC, 2000) and moisture by oven drying method (AOAC, 2000). Casein content in milk was determined (Method No. 998.06; AOAC, 2000) after precipitation of casein at pH 4.6. The acidified solution, which contains the non-casein N components of the test portion, was separated from casein precipitate by filtration. The filter paper along with residue was placed in hot air oven for drying. After drying filter paper wrapped casein was subjected to Kjeldahl's method to determine casein nitrogen and then multiplied with 6.38 to get casein % in the sample.

2.4 Mozzarella cheese manufacturing

Mozzarella cheeses were manufactured by following the method of Zisu & Shah (2007). Mozzarella cheese so produced were stored at 4 °C for 60 days to perform various analysis.

The detail of various cheese samples prepared for the study is as given below;

MC₁= Casein to fat ratio 1.6

MC₂= Casein to fat ratio 0.8

MC₃= Casein to fat ratio 0.4

2.5 Mozzarella cheese analysis

2.5.1 Physicochemical analysis

The cheese samples were analyzed for moisture by oven drying (AOAC, No. 926.08, 2000), protein by Kjeldhal method (IDF, 2006), fat by Gerber method (Marshall, 1993), ash content by igniting the cheese samples (AOAC, No. 935.42, 2000), acidity by titration method (AOAC, No. 920.124, 2000), and pH using pH meter (Ong et al., 2007). Total calcium was determined by running Mozzarella cheese samples on atomic absorption spectrophotometer calibrated with reference standards prepared from calcium reference solution (1000 mg/L Ca, 514870, Sherwood Scientific Ltd, Cambridge, UK) by following the method of Metzger et al. (2000).

2.5.2 Functional analysis

Stretchability was measured by some modifications in tube test method (McMahon et al., 1999). The distance that cheese strands could be extended was recorded (cm) by insertion of a fork into melted cheese. The time required for a fixed weight of shredded cheese (1.73 kg /m²) to melt down and fuse into a molten mass free of shred identity was determined by adopting the method described by Guinee et al. (2002).

2.5.3 Sensory evaluation

Mozzarella cheese samples were evaluated for sensory perception at 0 day on a hedonic rating scale (0–9) by a panel of judges drawn from faculty members and post-graduate students to assess the influence of casein to fat ratio on sensory parameters as described by Awad et al. (2004).

2.6 Statistical analysis

The results obtained were subjected to statistical analysis using completely randomized design and least significant difference (LSD) test was used for multiple comparisons ($\alpha= 0.05$) between means (Montgomery, 2013).

3. Results and Discussions

3.1 Cheese milk analysis

Mean values for physicochemical composition and casein to fat ratio (C/F) in cheese milk standardized for different treatments is presented in the Table 1. Results showed that mean values for protein, casein, acidity, pH

Table 1: Physicochemical composition and casein to fat ratio (C/F) in cheese milk for different treatments

| Treatments | MC ₁ | MC ₂ | MC ₃ |
|----------------------------------|-------------------------|-------------------------|-------------------------|
| Moisture % | 89.18±0.44 ^a | 87.18±0.44 ^b | 85.23±0.51 ^c |
| Fat % | 1.5±0.08 ^c | 3.0±0.11 ^b | 6.0±0.53 ^a |
| Protein % | 3.94±0.06 | 3.94±0.07 | 3.95±0.04 |
| Casein % | 2.40±0.09 | 2.40±0.04 | 2.40±0.02 |
| Casein to fat ratio (C/F) | 1.6 | 0.8 | 0.4 |
| Acidity % | 0.14±0.02 | 0.14±0.02 | 0.15±0.03 |
| pH | 6.66±0.07 | 6.66±0.07 | 6.67±0.05 |

Superscripts a-c describe significance among treatments

remains same with increase in fat level in cheese milk for standardizing milk at different C/F ratios. However, there was significant decrease in moisture contents with increase in fat level of milk. This might be due to increase in total solids of milk with the addition of fat.

3.2 Mozzarella cheese analysis

3.2.1 Physicochemical properties

Results showed significant ($p < 0.01$) increase in moisture contents with decrease in casein to fat ratio (C/F) in cheese milk (Table 2). Highest moisture contents were found in MC₃ (50.56%) and lowest in MC₁ (49.29%). However, significant ($p < 0.01$) decrease in moisture contents was observed in all treatments during storage. Contrarily to moisture contents, the protein contents were found to be directly related with C/F ratio as highest protein contents were found in MC₁ (26.28%). Fat contents in cheese samples at 0 days varied significantly ($p < 0.01$) between 16.13 to 21.58% and were found to be inversely related with C/F ratio. Non-significant ($p > 0.05$) decreased in fat contents were observed during storage in all treatments. Similarly, a significant difference was noted in ash contents (3.40-3.71%) of different Mozzarella cheese samples and highest ash contents were found in MC₃ (3.71%) followed by MC₂ (3.54%). However, in all treatments gradual increase in ash contents was observed during storage.

Among different treatments, acidity and pH varied from 0.72-0.73% and 5.18-5.19 respectively at 0 day (Table 2). Change in casein to fat level of cheese milk did not influence the both acidity and pH of cheese. During ripening significant ($p < 0.01$) increase in acidity while significant ($p < 0.01$) decrease in pH was found in all

treatments. Calcium contents differed significantly ($p < 0.01$) in cheese samples and varied from 608.6 (MC₃) to 628.3 mg 100g⁻¹ (MC₁) at 0 day. However, non-significant ($p > 0.05$) effect of storage on calcium contents was observed during study.

When milk is standardized at different fat levels, there is always a change in the protein to fat ratio in the milk that is depicted in cheese composition (Guinee & Law, 2002). Decrease in moisture contents during storage is due to involvement of water in various biochemical activities in the curd such as hydrolysis of fat and protein during ripening, increased hydration of casein and curd syneresis (Buriti et al., 2005). With increase in C/F, protein contents of cheese increased due to decrease in fat content of cheese milk (Guinee et al., 2007). Protein contents increased during storage in all treatments. During storage, gradual increase in protein contents is due to the progressive loss of moisture during storage which increased the relative proportion of protein in the cheese (Abbas, 2003). Variation in fat contents of different Mozzarella cheese samples with respect to treatments is mainly due to standardization of cheese milk at different C/F as its higher values decrease the fat contents of the cheese (Jaros et al., 2001). Results of the present study regarding the non-significant effect of ripening days on the fat contents of the Mozzarella cheese samples agree with the Lopez et al. (2006) as they also reported that the fat content in Emmental cheese did not change during ripening. The gradual increase in ash contents of cheese is due to the progressive loss of moisture that occurred during storage. Results regarding increase in ash content during cheese ripening are in line with the finding of Abbas (2003) who reported that as storage period progressed, ash contents of cheese increased. However, results are in contrary to the findings of Guo & Kindstedt (1995) who reported that ripening period did not affect the concentration of any mineral content in Mozzarella cheese and consequently the ash also remained unaffected during storage of cheese. In the similar way, no changes in acidity and pH had been reported due to standardizing the milk at different fat levels in Kefalograviera-type cheese (Katsiari et al., 2002). Decrease in pH value and corresponding increase in acidity of MCs during 60 days storage is due to conversion of lactose into lactic acid and loss of buffering capacity of milk. The lactate that produced in

Table 2: Effect of different casein to fat ratio (C/F) on moisture, protein, fat, ash, acidity, pH and calcium (Ca) contents of Mozzarella cheeses

| Analysis | MC ₁ | | | MC ₂ | | | MC ₃ | | |
|------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | 0 | 30 | 60 | 0 | 30 | 60 | 0 | 30 | 60 |
| Moisture % | 49.29 ^{Da} | 48.68 ^{Eb} | 48.13 ^{Fc} | 49.91 ^{Ba} | 49.11 ^{Db} | 48.54 ^{Ec} | 50.56 ^{Aa} | 50.04 ^{Bb} | 49.49 ^{Cc} |
| Protein % | 26.28 ^{Bc} | 26.40 ^{Ab} | 26.53 ^{Aa} | 24.06 ^{Dc} | 24.18 ^{Cb} | 24.31 ^{Ca} | 21.54 ^{Fc} | 21.67 ^{Eb} | 21.78 ^{Ea} |
| Fat % | 16.13 ^D | 16.11 ^D | 16.08 ^D | 18.45 ^C | 18.42 ^C | 18.37 ^C | 21.58 ^A | 21.51 ^A | 21.44 ^B |
| Ash % | 3.40 ^{Ec} | 3.47 ^{Db} | 3.53 ^{Ca} | 3.54 ^{Cb} | 3.61 ^{Ba} | 3.68 ^{Ba} | 3.71 ^{Ab} | 3.75 ^{Aa} | 3.79 ^{Aa} |
| Acidity % | 0.72 ^{Dc} | 0.78 ^{Cb} | 0.84 ^{Ba} | 0.73 ^{Dc} | 0.79 ^{Cb} | 0.83 ^{Ba} | 0.73 ^{Dc} | 0.80 ^{Cb} | 0.87 ^{Aa} |
| pH | 5.19 ^{Aa} | 5.07 ^{Bb} | 5.01 ^{Cc} | 5.19 ^{Aa} | 5.06 ^{Bb} | 5.03 ^{Cc} | 5.18 ^{Aa} | 5.08 ^{Bb} | 5.03 ^{Cc} |
| Ca mg/100g | 628.3 ^A | 628.9 ^A | 629.1 ^A | 618.6 ^B | 619.1 ^B | 619.8 ^B | 608.6 ^C | 609.2 ^C | 609.9 ^C |

Table 3: Effect of different casein to fat ratio (C/F) on stretching and melt time of Mozzarella cheeses

| Analysis | MC ₁ | | | MC ₂ | | | MC ₃ | | |
|-----------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | 0 | 30 | 60 | 0 | 30 | 60 | 0 | 30 | 60 |
| Stretching (cm) | 39.66 ^{Dc} | 43.51 ^{Bb} | 47.11 ^{Aa} | 35.61 ^{Fc} | 40.81 ^{Cb} | 47.01 ^{Aa} | 29.64 ^{Ac} | 35.51 ^{Fb} | 37.23 ^{Ea} |
| Melt time (Sec) | 151.3 ^{Aa} | 139.2 ^{Bb} | 120.5 ^{Cc} | 118.3 ^{Da} | 110.4 ^{Eb} | 102.3 ^{Fc} | 98.3 ^{Ga} | 92.4 ^{Hb} | 84.5 ^{lc} |

Superscripts A-F describe significance among treatments and Superscripts a-c describe significance among storage days within same treatment
MC₁= Casein to fat ratio 1.6; MC₂= Casein to fat ratio 0.8; MC₃= Casein to fat ratio 0.4

early stages of ripening is an important precursor for different reactions such as oxidation or microbial metabolism that leads to variation of acidity in the later stages of cheese ripening (Ong et al., 2007). Results of the present study regarding increase in calcium contents with decrease in fat level due to high C/F are well supported by findings of Rudan et al. (1999) who found that the calcium contents of Mozzarella cheese increased progressively as fat content was reduced.

3.2.2 Functional Properties

Stretchability is the tendency of an object to form elongated fibrous strands that do not break when pulled. Different casein to fat ratio in milk (Table 3) showed a significant ($p < 0.01$) effect on the stretchability of the Mozzarella cheese samples. Results showed that Mozzarella cheese stretchability is directly related to C/F as cheese with high C/F (MC₁) showed more stretchability (39.66 cm) as compared to others. However, a significant ($p < 0.01$) increase in stretchability was observed in all Mozzarella cheese samples during storage. Melt time is defined as the time required for shredded cheese, loaded at a rate of 0.173 g cm⁻² onto a polished stainless steel tray, to melt into a uniform mass free of shred identity (Guinee et al., 1998). The results showed that C/F had significant ($p < 0.01$) effect on melt time and it varied between 98.3 to 151.3 sec. (Table 3). During storage there was significant ($p < 0.01$) decrease in melt time in all

treatments. Findings of this study regarding linear relationship between cheese stretchability and C/F are in line with Kindsted (1993) who reported that stretchability is negatively correlated with the levels of fat. Fife et al. (2002) found that low fat cheese stretched to a greater degree but the stretch quality in low fat cheese appeared to be more fibrous, thin strands, less pliable and more brittle than those formed by the high fat cheese. Mozzarella cheese stretchability increased during storage because as a result of aging, the casein matrix porosity increased due to proteolysis and thereof exhibits less resistance to stretching (Guinee, 2003). Cheese melt time is directly related with fat content and amount of intact casein in cheese. As C/F increases, the fat level in cheese decreased which increased the melt time (Guinee, 2003). Melt time decreased with increase in ripening days due to proteolysis of CN-matrix and more hydration of protein network as a result of which the ability of the cheese to maintain its structure during heating decreased (Zisu & Shah, 2007).

3.2.3 Sensory properties

Mozzarella cheese prepared by using buffalo milk with different casein to fat ratio was evaluated for different sensory parameters by using 9 point hedonic scale. The appearance of cheese has great value in its acceptability. Cheese prepared from milk with C/F=0.8 (MC₂) proved good in appearance followed by MC₃, while in case of

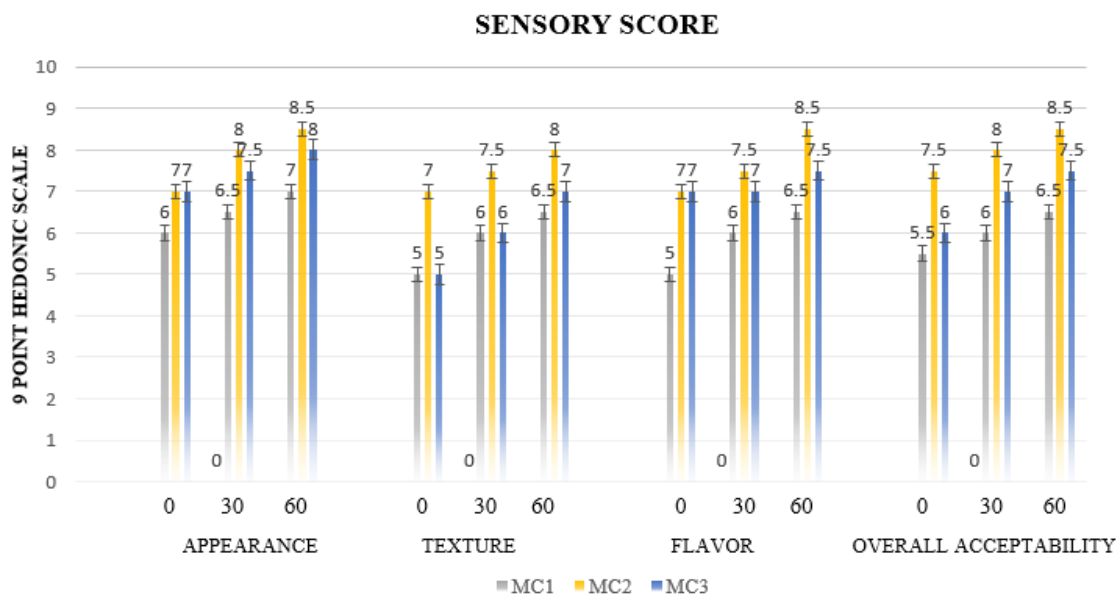


Figure. 1: Effect of different casein to fat ratio (C/F) on sensory attributes of Mozzarella cheeses

cheese with high C/F (MC₁), the appearance was poor, rough and lacking lust (Fig. 1). Fat along with protein plays key role in the development of texture. Among the cheese samples, MC₂ and MC₃ showed better texture as compared to low fat cheese i.e. MC₁ (Fig. 1). In case of cheese, flavor is an important attribute that impacts consumer acceptance and marketing (Yates & Drake, 2007). Overall, highest scores for flavor were awarded to MC₂ (C/F=0.8) and MC₃ (C/F=0.4) while MC₁ exhibited minimum score for flavor as compare to all other cheese samples (Fig. 1). So keeping in view the score of sensory parameters, cheese with C/F=0.8 is considered good as compared to other two treatments. Results for all sensory parameters increased during storage in all cheese samples.

In this study, cheeses with high fat contents or low C/F (MC₂ and MC₃) were more whitish in appearance which might be due to more light scattering phenomenon in high fat cheeses. Reduction in appearance score with fat reduction in cheese are in agreement with the findings of Rudan et al. (1999) who found that fat contents significantly affected the appearance of the cheese because fat reduction made the cheese less white and more translucent. Major role of fat in development of cheese texture is that it imparts discontinuity in cheese matrix so removing fat from cheese milk make the end product a dense, homogenous protein network (Rogers et al., 2010). Fat contributes to the overall flavor quality of cheese (Kucukoner &

Haque, 2006). Variation in flavor score among different treatments is due to their different fat contents as a result of standardization of cheese milk at different C/F. Though Mozzarella cheese is not considered as ripened cheese but a small degree of breakdown (proteolysis, lipolysis etc.) is required for the development of optimum sensory properties (Mistry, 2001).

4. Conclusion:

Casein to fat ratio (C/F) of cheese milk significantly affected the moisture, protein, fat and calcium contents of Mozzarella cheese which ultimately influenced its functionality and sensory characteristics. Mozzarella cheese storage for 60 days improve its functionality, appearance, texture and flavor. Among different casein to fat ratios (C/F), C/F=0.8 was found to be good in terms of its stretchability, melt time and sensory acceptability.

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6. Conflict of interest statement

The authors have no conflict of interest to declare.

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