

Exploit Fat Mimetic Potential of Different Hydrocolloids in Low Fat Mozzarella Cheese

Muhammad Umair Sattar^{1,*}, Aysha Sameen¹, Nuzhat Huma¹, Muhammad Shahid²

¹National Institute of Food Science and Technology, University of Agriculture-Faisalabad

²Department of Biochemistry, University of Agriculture-Faisalabad

*Corresponding author: umair_nifsat@yahoo.com

Abstract Fat reduction in diet is important as scientific evidence linked the high fat intake with coronary heart diseases and obesity. This association has resulted in increased consumer awareness and demand for low fat dairy products especially cheese. In cheese, fat reduction negatively affects the rheological, sensory and functional characteristics. Mozzarella is a type of pasta filata cheese which contains 30 to 45% milk fat on dry weight basis and about 75% of the Mozzarella cheese produced in the world is used as an ingredient on pizza topping. This work was to exploit fat mimetic potential of different hydrocolloids (guar gum and xanthan gum) by using them at different levels (0.15%, 0.30%, and 0.45% of milk) in low fat Mozzarella cheese. In order to assess the suitability of resulting low fat Mozzarella cheese, it was compared with full fat and half fat cheese manufactured by using milk standardized at 3% and 1.5% fat respectively. Result showed that different levels of hydrocolloids affect cheese composition, functionality, texture, sensory attributes and yield to varying extent. Use of guar gum and xanthan gum at 0.15% level produces low fat Mozzarella cheese comparable with full fat cheese in terms of cheese functionality and yield. This work provides strong evidence for the quality production of low fat Mozzarella cheese by using different hydrocolloids as fat replacer.

Keywords: mozzarella, guar gum, xanthan gum, hydrocolloids, yield

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1. Introduction

In Pakistan about 50 million tons/year milk is produced and most of the milk is consumed as a liquid and a small portion is transformed into different products like pasteurized, sterilized, dried milk, ice cream, butter, yoghurt and cheese. Cheese is considered a substantial dairy invention of economic worth having significant nutritional importance. Hundreds of cheese varieties are being produced in the world which is classified on the basis of their form, manufacturing, ripening and chemical composition [1]. Approximately one third of the total world's milk production is used for cheese manufacturing [2]. 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. Buffalo milk is preferred for Mozzarella cheese due to its high fat, protein and low cholesterol contents [3].

Increased incidence of various chronic diseases such as elevated blood pressure, obesity, atherosclerosis etc. has been found to be associated with high fat intake by people.

This association not only resulted in an increased alertness among consumers but also significantly increases the demand for foods with healthy image [4]. Fat reduction in cheese results in major shift in the compositional balance of cheese when compared with its full fat counterpart. It also adversely affect textural, functional and sensorial attributes such as rubbery texture, poor meltability, bitter taste, lack of flavor and undesirable color [5]. Reduced or low fat food products development is often challenging because functional and sensorial attributes of the foods can be affected after removing fat from numerous food items [6]. In order to increase the acceptability of low fat cheese, several strategies have been proposed. Among them most useful strategy is the use of fat replacers [7].

Fat replacers are ingredients intended to be used in the place of natural fats with the objective of obtaining a reduction in the caloric value. They are categorized as fat substitutes which are fat based and as fat mimetic which are protein and carbohydrate based materials. These are widely recommended because of their ability to mechanically entrap water and their stronger hydrophilic nature. A sense of lubricity and creaminess is created in low fat cheese by use of fat replacers [8]. Hydrocolloids are the most effective carbohydrate based fat replacers and are commonly implied in food processing industry to counter the effects of fat reduction and improvement of its functional properties [9]. These are added to dairy products to stabilize their structure, enhance viscosity and

alter textural characteristics [10]. Keeping in view the unique role of fat in functionality of Mozzarella cheese, dietary guidelines for consumption of low fat products and consumer demand, present study was designed to explore fat mimetic potential of different hydrocolloids in mozzarella cheese development

2. Materials and Methods

In the present study, low fat Mozzarella cheese was manufactured by standardizing milk at 1.5% fat and by adding hydrocolloids (guar gum and xanthan gum) at three levels i.e. 0.15%, 0.30%, 0.45% of milk. Mozzarella cheese manufactured by using milk standardized at 3% fat was used as Control (C) and at 1.5% fat without addition of hydrocolloids was used as negative control (C').

2.1. Mozzarella Cheese Manufacturing

Raw buffalo milk was obtained from the Dairy Farm of University of Agriculture, Faisalabad. Commercially available thermophilic starter culture (*Streptococcus thermophilus* & *Lactobacillus delbrueckii subsp bulgaricus*) and enzyme chymosin (Double strength Chymax, 500000 MCU/mL, Pfizer Inc, Milwaukee, WI, USA) was used for Mozzarella cheese manufacturing. However xanthan gum and guar gum were procured from the local supplier in Faisalabad. Mozzarella cheese was manufactured by following the method of [11] with slight modification in order to incorporate hydrocolloids for development of low fat Mozzarella cheese. Mozzarella cheese was manufactured in different batches as milk standardized at different fat levels were used for manufacturing of Control (C), negative Control (C') and hydrocolloids containing low fat cheese. For each batch, after standardization milk was pasteurized at 65 °C temperature for 30 minutes followed by the addition of calculated quantities of gums (guar gum and xanthan gum) by dissolving in small quantity of hot milk. Cheese milk was cooled to 37°C and inoculated with combined culture *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Chr. Hansen Ireland Ltd.) at 1g/100L of milk. After ripening for 30 minutes, the milk at 37°C was set with Chymosin (50000u/G, Pharm Chemical Co., Ltd, China) @ 1ml/L of milk. Approximately 50 minutes after the addition of rennet, the curd reached the consistency appropriate for cutting into cubes approximately 1 cm³. The mass was then agitated very gently and allowed to heal for 10 minutes. The curd was cooked with gentle stirring in order to avoid matting of curd with gradual increase of temperature to 45 °C. The whey was drained at pH 6.2 and curd was cheddared, milled and stretched at pH 4.9. The cheese was molded and place in the refrigerator.

2.2. Cheese Analysis

2.2.1. Physicochemical Analysis

Moisture of cheese samples were determined by using oven drying method [12]. Titration method was used for acidity calculation [13] and pH meter was used to measure pH value of cheese sample [14]. Protein [12], fat [15], ash

[12] and total calcium contents [16] were also determined by following their respective methods.

2.2.2. Functional Analysis

Meltability

Meltability of all cheese samples were determined by using glass tube of known length and thickness which has one sealed and one unsealed end. Cheese sample was compressed in glass tubes by plunger and length was noted by using verniercaliper. Glass tube was stored at 4°C for 4 hrs and then heated at 110°C for 100 minutes followed by cooling at 22°C. Melting length was noted by verniercaliper [11].

Stretchability

Stretchability was measured by some modifications in tube test method [17]. The distance that cheese strands could be extended was recorded (cm) by insertion of a fork into melted cheese.

2.2.3. Texture Profile of Mozzarella Cheeses

Texture profile of Mozzarella cheeses was determined by performing the texture profile analysis (TPA) of the cheese samples on TA-XT Plus Texture Analyzer (StableMicro Systems, Godalming, Surrey, UK) using compression plate. Cubes of 25mm length x width x height were cut from each sample using a stainless steel wire cutter and equilibrated at 8°C for a further 30 min before analysis. Samples were removed from the incubator and immediately compressed to 30% of the original height in 2 consecutive cycles (i.e., double compression) at a rate of 1 mm/s [11].

2.2.4. Sensory Analysis

Sensory attributes (color and flavor) of hydrocolloids containing low fat Mozzarella cheese was analyzed by panelist using 9 point hedonic scale.

2.3. Cheese Yield

Cheese yield was calculated as the percent weight of the finished cheese divided by weight of the milk used [18].

2.4. Statistical Analysis

The results were subjected to statistical analysis in order to check level of significance [19].

3. Results

3.1. Effect of Hydrocolloids on the Physicochemical Attributes of Low Fat Mozzarella Cheese

Table 1 showed that use of hydrocolloids as fat replacer in low fat Mozzarella cheese significantly affect ($p < 0.01$) all physicochemical attributes. Fat, moisture, protein, ash, pH and acidity of all low fat cheese samples were in the range of 9.4-18.4%, 49.58-53.53%, 25.02-30.85%, 3.25-3.41%, 5.21-5.45 and 0.73-0.93% respectively (Table 2). Results showed that when fat level in cheese milk was reduced from 3 to 1.5%, fat contents in resultant cheese reduced significantly as fat level in C is higher than all

other cheese samples. Use of xanthan gum and guar gum at different levels results in higher fat level in cheese as compared to C' i.e. Mozzarella cheese manufactured without use of any fat replacer. Results also indicated that

as fat level in cheese decrease, moisture, protein and ash content increases however pH and acidity not follow any specific increasing or decreasing pattern.

Table 1. Effect of Hydrocolloids on the Physicochemical Attributes of Low Fat Mozzarella Cheese

| SOV | DF | Fat | Moisture | Protein | Ash | pH | Acidity |
|-----------|----|-----------|-----------|-----------|-----------|----------|-----------|
| Treatment | 7 | 24.8722** | 4.39052** | 9.63639** | 0.01108** | 0.20673* | 0.01768** |
| Error | 16 | 0.5421 | 0.42858 | 0.00920 | 0.00119 | 0.00034 | 0.00080 |
| Total | 23 | | | | | | |

** = Highly significant (p<0.01), * = Significant (p<0.05), NS = Non Significant

Table 2. Mean Values for Physicochemical Attributes

| Treatment | Fat | Moisture | Protein | Ash | pH | Acidity |
|--------------------|---------------|----------------|----------------|---------------|----------------|-----------------|
| C | 18.4 ± 0.02A | 49.58 ± 0.24 B | 25.02 ± 0.12G | 3.25 ± 0.03 D | 5.21 ± 0.015 C | 0.93 ± 0.035 A |
| C' | 9.4 ± 0.75C | 52.51 ± 1.84A | 29.49 ± 0.14C | 3.41 ± 0.08A | 5.23 ± 0.017C | 0.89 ± 0.026ABC |
| GG _{0.15} | 10.6 ± 0.17BC | 52.57 ± 0.41A | 30.62 ± 0.13B | 3.29 ± 0.05CD | 5.31 ± 0.015B | 0.87 ± 0.020 BC |
| GG _{0.30} | 10.1 ± 0.10BC | 52.65 ± 0.75A | 29.46 ± 0.21CD | 3.41 ± 0.02A | 5.24 ± 0.025C | 0.91 ± 0.034AB |
| GG _{0.45} | 10.2 ± 0.25BC | 52.75 ± 0.88A | 28.72 ± 0.12F | 3.36 ± 0.05AB | 5.21 ± 0.020C | 0.92 ± 0.029A |
| XG _{0.15} | 10.5 ± 0.52BC | 53.01 ± 0.65 A | 30.85 ± 0.32A | 3.28 ± 0.03CD | 5.31 ± 0.015B | 0.84 ± 0.025 C |
| XG _{0.30} | 10.8 ± 0.28B | 53.05 ± 0.45A | 29.31 ± 0.30D | 3.33 ± 0.04BC | 5.45 ± 0.015A | 0.73 ± 0.021 D |
| XG _{0.45} | 11.1 ± 0.33B | 53.53 ± 0.77A | 29.08 ± 0.11E | 3.31 ± 0.02BC | 5.44 ± 0.020A | 0.75 ± 0.030D |

C= Control

C'= Negative control

GG_{0.15}= Low fat Mozzarella cheese containing guar gum at 0.15% level

GG_{0.30}= Low fat Mozzarella cheese containing guar gum at 0.30% level

GG_{0.45}= Low fat Mozzarella cheese containing guar gum at 0.45% level

XG_{0.15}= Low fat Mozzarella cheese containing xanthan gum at 0.15% level

XG_{0.30}= Low fat Mozzarella cheese containing xanthan gum at 0.30% level

XG_{0.45}= Low fat Mozzarella cheese containing xanthan gum at 0.45% level.

3.2. Effect of Hydrocolloids on the Functional Attributes of Low Fat Mozzarella Cheese

Table 3. Effect of Hydrocolloids on the Functional Attributes of Low Fat Mozzarella Cheese

| SOV | DF | Meltability | Stretchability |
|-----------|----|-------------|----------------|
| Treatment | 7 | 60.57** | 105.174** |
| Error | 16 | 0.1095 | 0.084 |
| Total | 23 | | |

** = Highly significant (p<0.01), * = Significant (p<0.05), NS = Non Significant.

Table 4. Mean Values for Functionality

| Treatment | Meltability(mm) | Stretchability(cm) |
|--------------------|-----------------|--------------------|
| C | 58.30 ± 0.43A | 48.83 ± 0.15A |
| C' | 46.46 ± 0.30F | 37.20 ± 0.20E |
| GG _{0.15} | 53.16 ± 0.20B | 45.50 ± 0.26B |
| GG _{0.30} | 47.96 ± 0.15E | 38.50 ± 0.43D |
| GG _{0.45} | 44.53 ± 0.50G | 31.46 ± 0.41 G |
| XG _{0.15} | 51.33 ± 0.40C | 41.36 ± 0.25C |
| XG _{0.30} | 48.94 ± 0.22D | 38.30 ± 0.30D |
| XG _{0.45} | 46.23 ± 0.25F | 32.63 ± 0.15 F |

C= Control

C'= Negative control

GG_{0.15}= Low fat Mozzarella cheese containing guar gum at 0.15% level

GG_{0.30}= Low fat Mozzarella cheese containing guar gum at 0.30% level

GG_{0.45}= Low fat Mozzarella cheese containing guar gum at 0.45% level

XG_{0.15}= Low fat Mozzarella cheese containing xanthan gum at 0.15% level

XG_{0.30}= Low fat Mozzarella cheese containing xanthan gum at 0.30% level

XG_{0.45}= Low fat Mozzarella cheese containing xanthan gum at 0.45% level

Stretchability and meltability are most important functional properties of Mozzarella cheese that are directly related to fundamental rheological properties. Fat reduction in cheese milk significantly affect (p<0.01) the meltability and stretchability of low fat cheese (Table 3). Table 4 showed that values for meltability and stretchability were in the range of 44.53-58.30mm and 31.46-48.83cm respectively. Fat reduction in cheese decrease the meltability and stretchability as Mozzarella cheese manufactured from milk standardized at 3% fat (C) showed higher values for these parameters as compared to all other cheese samples. Use of xanthan gum and guar gum at level of 0.15% results in higher meltability and stretchability values as compared to higher levels (i.e. 0.30 %, 0.45%) of these gums as well as negative control (C').

3.3. Effect of Hydrocolloids on Low Fat Mozzarella Cheese Texture

The physical characteristics of Mozzarella cheese like body and texture are altered by the factors like milk composition, starter culture and ripening conditions prevalent during the cheese preparation [20]. The hardness of cheese reflects its chemical composition and the physicochemical state of constituents i.e. solid to fat ratio etc. Hardness also expresses the cheese macrostructure, indicating the existence of heterogeneities like granules connections in curd, fissures and cracks [21]. In the present study, hardness of all cheese samples was significantly affected by fat reduction and was in the range of 1177 to 2944g (Figure 1). Highest hardness value was found in Mozzarella cheese manufactured with milk standardized at 1.5% fat without addition of any hydrocolloids (C') while lowest hardness was in case of

full fat cheese i.e. C. Hardness of all hydrocolloids containing low fat Mozzarella cheese was higher than C i.e. positive control while lower than C' i.e. negative control. Hardness of low fat Mozzarella cheese decreased with increase in levels of hydrocolloids but this decrease

was non-significant. No doubt moisture retention in cheese increased with increase in hydrocolloids levels but on the other hand, there was significant increase in protein contents with fat reduction and it become dominant structural component in the absence of fat.

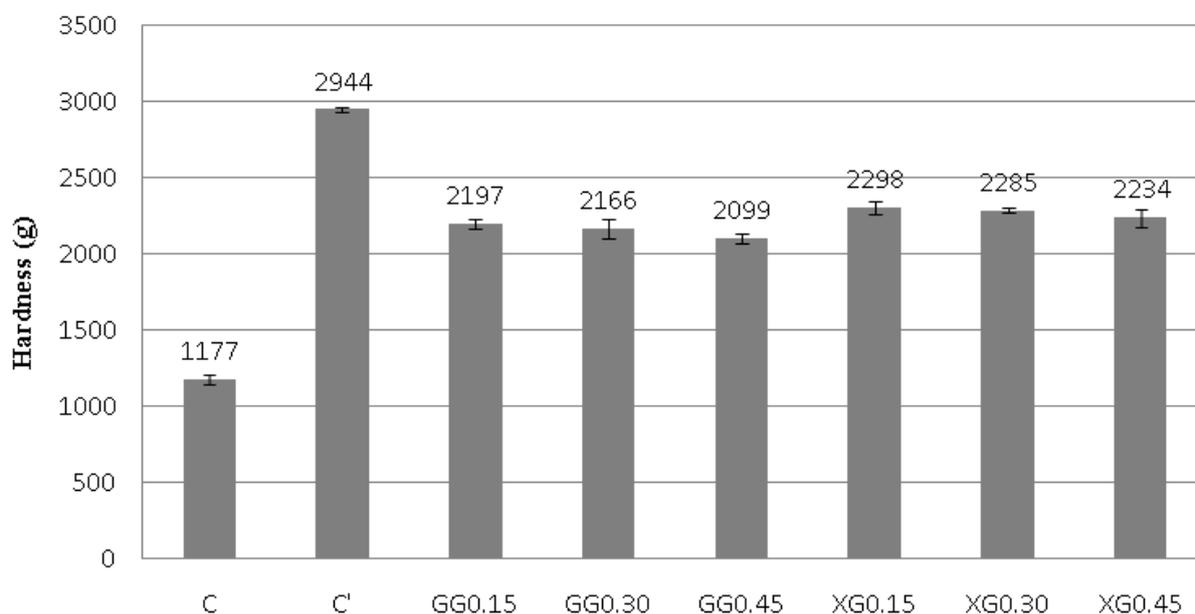


Figure 1. Effect of Hydrocolloids on the Hardness of Low Fat Mozzarella Cheese

Springiness is another most important textural attribute that was significantly affected by reduction of fat in low fat Mozzarella cheese. Springiness values were in the range of 0.84 to 0.983 in all low fat Mozzarella cheese

samples (Figure 2). Addition of hydrocolloids at different levels in low fat Mozzarella cheese decreases the springiness as compared to negative control (C') while these values were higher than positive control (C).

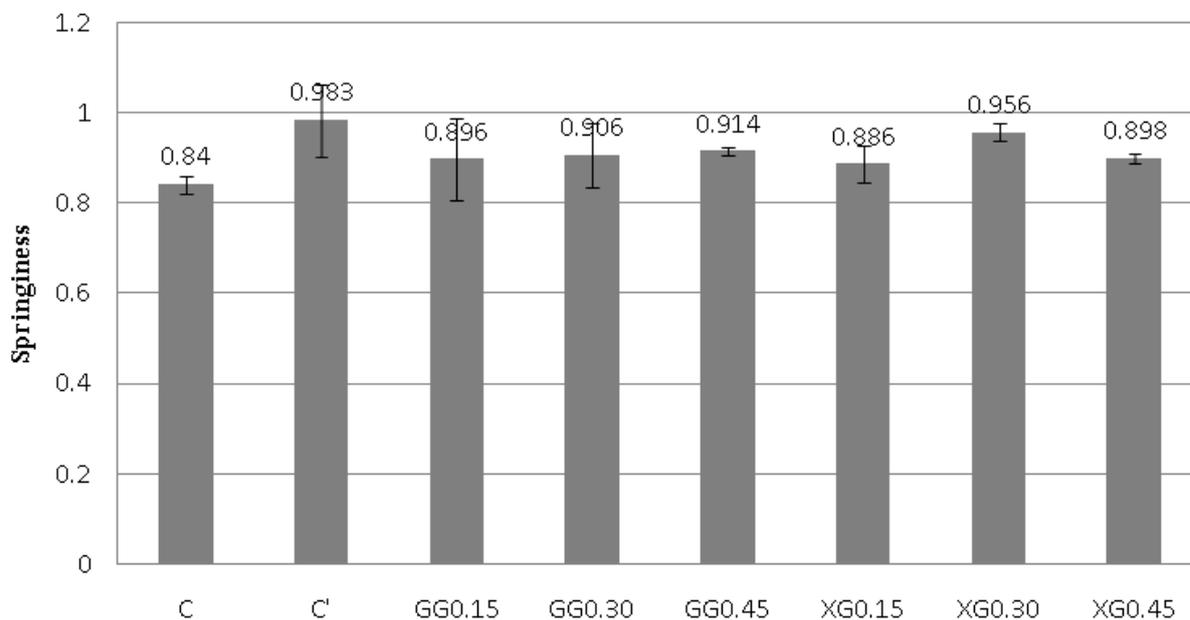


Figure 2. Effect of Hydrocolloids on the Springiness of Low Fat Mozzarella Cheese

3.4. Effect of Hydrocolloids on the Sensory Attributes of Low Fat Mozzarella Cheese

Color and flavor of food not only create expectations that affect what we feel and behave but also influences food identification. The main variation in color of milk and milk products is caused by its fat and protein content.

In this study, color and flavor of all cheese samples were significantly affected by fat reduction (Figure 3). Sensory score of cheese samples was in the range of 5 to 7.5 for color and 5 to 7 for flavor on nine point hedonic scale. Both sensory parameter values were high in positive control (C) as compared to negative control (C') and all hydrocolloids containing low fat Mozzarella cheese.

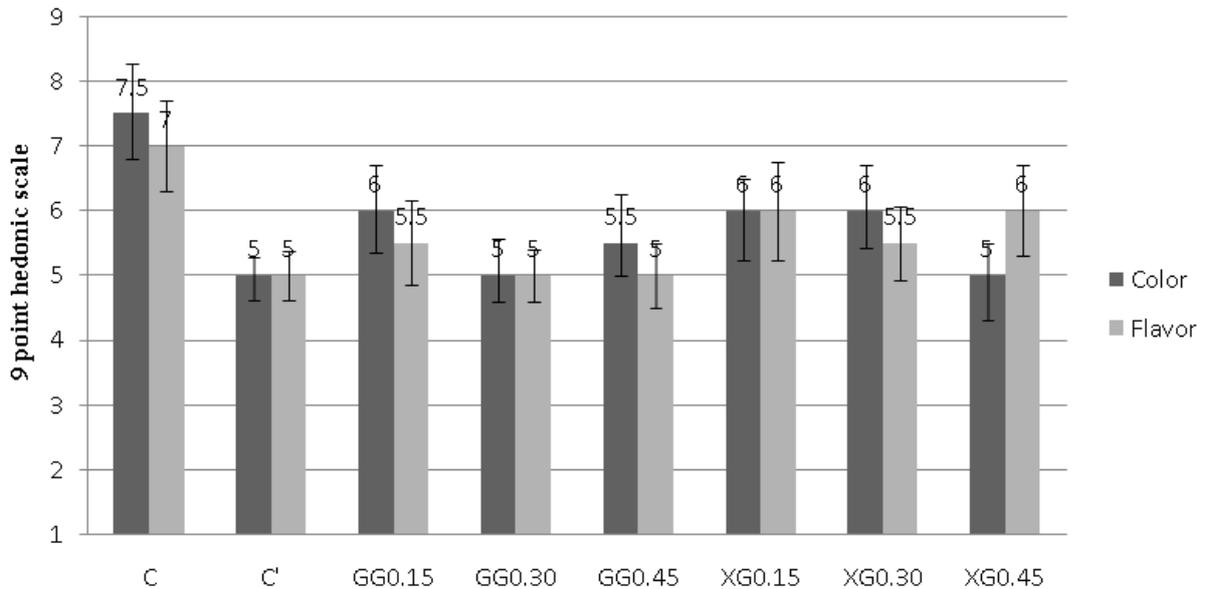


Figure 3. Effect of Hydrocolloids on the Sensory attributes (Color and Flavor) of Low Fat Mozzarella Cheese

3.5. Effect of Hydrocolloids on the Yield of Low Fat Mozzarella Cheese

Cheese yield is very important parameter as higher the recovered percentage of solids, the greater is the amount of cheese obtained and therefore gains in economic terms. When fat level in milk was decreased, cheese yield was significantly affected (Figure 4). In all cheese samples cheese yield was in the range of 10.4 to 14.23%. Results showed that low fat Mozzarella cheese manufactured by using hydrocolloids at different levels has higher cheese

yield as compared to negative control (C'). This is mainly due to the fact that hydrocolloid gums are the most effective carbohydrate-based fat replacers having the ability to control the rheology of water based systems and syneresis inhibition which ultimately increase the cheese yield. In this study, with increase in the level of gums, moisture contents increased non-significantly while protein contents in cheese decreased significantly (Table 2). Therefore cheese yield was not increased proportionally with increase in moisture contents in cheese due to high levels of gums.

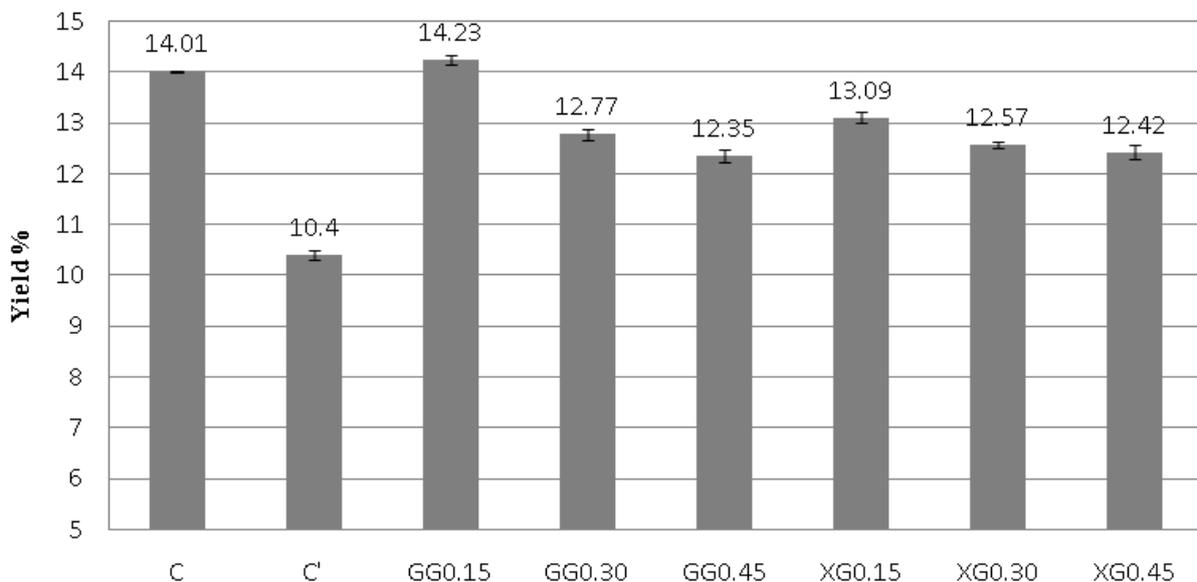


Figure 4. Effect of Hydrocolloids on the Yield of Low Fat Mozzarella Cheese

4. Discussion

Cheese composition, rheology, functionality and yield are dependent on fat level, hence protein to fat ratio of cheese milk [22]. The significant role of fat in cheese is to impart discontinuity to the protein matrix [23], add in the

improvement of texture, flavor and cheese yield [18]. Our data showed significant variation in cheese composition when fat contents in cheese milk were reduced. As fat level in cheese milk was reduced, moisture and protein contents in cheese increased. Increase in the moisture content of the cheese might be due to the reason that low fat level result in increased protein to fat ratio which leads to increase in moisture content of cheese [24]. With

increase in the concentration of gum level, moisture contents in the cheese increase due to higher water retention properties of the gum used [25]. Protein contents of cheese increased with the decrease in fat content of cheese milk [26]. Low fat level in all Mozzarella cheese samples except C is mainly due to fact that cheese milk for C was standardized at 3% fat while milk for all other treatments was standardized at 1.5% fat. In our study, results for the ash content in low fat Mozzarella cheese were in the range i.e. 3.26- 3.56% as reported in the previous study by [27]. pH is a critical factor in several aspects of the manufacturing and ripening of cheese curd [28]. In present study results for pH and acidity also differ significantly with decrease in fat level of cheese milk. Different fat replacers were employed in the development of low-fat white pickled cheese and results showed that fat replacers significantly affect the pH, acidity, total solids and salt in total solids of cheeses [29]. However, in other study no changes in pH had been reported due to different levels of fat in Kefalograviera-type cheese [4].

Functional properties of Mozzarella cheese are dictated by factors such as the degree of proteolysis, fat content, mineral distribution, protein-protein and protein-water interactions [30]. Removal of fat from cheese causes functional and sensory defects such as poor meltability, lack of flavor and undesirable color [18, 31]. All defects in functional attributes of foods resulting from fat reduction are balanced by the use of carbohydrate-based fat replacers [32, 9]. Results of our study also showed significant reduction in Mozzarella cheese meltability and stretchability with fat reduction. Data showed that use of xanthan gum and guar gum as fat replacers in low fat Mozzarella cheese improve these functional properties. Low fat Mozzarella cheeses typically have inferior functional properties such as poor meltability, limited free oil formation during melting and as a result, excessive browning when baked on pizza. All appearance defects were corrected by using fat replacers [7]. Use of xanthan gum and modified corn starch in low fat Mozzarella cheese results in better meltability [33]. Xanthan gum improves melting properties and increases moisture retention in Mozzarella cheese [34].

The textural characteristics of the cheese are determined by the combined structural properties of the protein matrix and the fat droplets immersed in the former [35]. The acceptability of low-fat cheese can be improved by using fat replacers that partially or fully replace fat and simulate the properties of fat [36]. Results of the present study showed inverse relation between fat level and cheese hardness. When fat level is reduced, protein serves as major structural component in cheese. As a result, a high degree of cross-linking of protein molecules occurs which increase the resistance to deformation [37]. In this study use of gums at different levels overcome the problem of cheese hardness to varying extent. Use of water-dispersible fat replacers has been recommended in low-fat cheese formulations to mimic the properties of fat because of their ability to mechanically entrap water that impart lubricity and gives a soft texture product [5]. The low and reduced fat cheeses with higher xanthan gum content resulted in lower hardness. Hardness of reduced fat cheese formulated with fat replacers inversely correlates to moisture content with high moisture resulting in a softer texture and high moisture in non fat substance

i.e. MNFS [38, 39, 40]. The decrease in cheeses hardness containing xanthan gum was probably due to changes in protein matrix compactness since addition of xanthan gum increased water binding capacity of protein matrix [7]. The hardness of cheeses made with hydrocolloid as fat mimetic was very similar to the full-fat cheese [5].

Fat reduction increases cohesiveness and springiness of reduced fat cheese [41, 42]. Springiness values in present study also increased when fat level in cheese milk was decreased from 3% to 1.5%. Use of gums decrease the springiness of low Fat Mozzarella cheese as compared to negative control i.e. C'. Fat reduction in cheese significantly increases the values for various texture attributes such as hardness, gumminess, springiness and chewiness [43]. Use of modified corn starch and combination of xanthan gum and carrageenan in the production of low fat Mozzarella showed relatively higher moisture contents of the cheese and ultimately leading to the improved textural properties of low fat Mozzarella cheese [32]. Use of fat replacers (SimplessesD-100 and Raftilines HP) in reduced fat kashar cheese showed improvement in textural properties [7].

Keeping in view the significance of fat in color and flavor development, these two sensory parameters were analyzed in this study. Results showed significant decrease in sensory score when fat contents in cheese milk decreased from 3 to 1.5%. Use of different types and levels of hydrocolloids for the manufacturing of low fat Mozzarella cheese not compensate role of fat in the development of these sensory attributes. Color of cheese is influenced by intrinsic factors are related to the milk used for cheese making and extrinsic factors such as colorants added to milk, packaging and the storage of the cheese. Light scattering in milk is caused by particles of relatively large size whose refractive index differs from water. In this case, light scattering by fat globules and casein micelles are the primary contributors to the color of milk [44]. In case of cheese, flavor is an important attribute that impacts consumer acceptance and marketing [45]. Fat contributes to the overall flavor quality of cheese [46].

Fat and protein contents are the major milk constituents which directly influence the cheese yield and quality. By decreasing the fat concentrations, yield of the cheese also reduced as fat normally constitutes 50% or more of the dry weight [47]. Results of this study showed significant decrease in cheese yield with fat reduction and use of gums at different levels resulted in more cheese yield as compared to C'. When guar gum was used at 0.15% concentration, cheese yield was even more than C. It has been suggested that water can bind directly to fat replacers and the fat replacers can interfere with the shrinkage of the casein matrix. Therefore, this lowers the driving force involved in expelling water from curd particles [25].

5. Conclusion

Food choices are driven by 3 needs: convenience, taste and health; with health being considered number one marketing driver of the food industry worldwide. Nowadays cheese is essential ingredient in various food formulations but at the same time scientific evidence links the high intake of fatty foods such as cheese with increased incidence of chronic diseases. Reducing the fat

content of cheese without modification of its regular procedure would affect composition, texture, flavor and functional properties with economic consequences. The utilization of carbohydrate based fat replacers such as guar gum and xanthan gum in cheese formulation could solve all problems associated with texture, functionality and yield of low fat Mozzarella cheese.

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