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Synergistic Impact of Fat Mimetic and Homogenization on Functionality, Texture and Proteolysis of Low Fat Buffalo Mozzarella

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MUS designed the project, performed the experimental work and wrote the article; AS and NH provide technical guidance regarding data collection, analysis and results interpretation MS helped in data arrangement for statistical analysis

Key words
Buffalo Mozzarella, Fat mimetics, homogenization, Functionality, Texture.

ABSTRACT

Low fat cheeses have increasing demand globally but fat reduction in cheeses has negative impact on the composition, functionality and texture of cheese. The study was planned to investigate combined impact of fat mimetic (guar gum) and homogenization as fat reduction strategies on the quality of low fat Mozzarella cheeses (LFMCs). Guar gum was used at level of 0.15, 0.30 and 0.45% with and without cheese milk homogenization (2000 psi at 45°C) for LFMCs production. LFMCs produced were subjected to analysis for physicochemical parameters, functional attributes, texture (hardness), appearance (L* value) and proteolysis by RP-HPLC. Use of guar gum as fat mimetic and cheese milk homogenization significantly ($p<0.01$) affected the composition, functionality, texture and appearance of LFMCs. LFMCs functionality and texture also improved during ripening but appearance was negatively affected. Reverse phase HPLC results indicated that level of intact casein in LFMCs decreased with increase in ripening period. Use of guar gum at 0.15% level ($G_{0.15}$) was found to be more effective as it act synergistically with cheese milk homogenization. They collectively increased the fat retention (10.67%) which inturn improve meltability (59.72 mm), stretchability (52.63 cm), texture (1650.7 g) and appearance (L*= 63.30) of $G_{0.15}$. In conclusion, use of guar gum at 0.15% level along with cheese milk homogenization offers a perspective to reduce fat in Mozzarella cheese without compromising its quality.

INTRODUCTION

Pakistan ranks the second largest buffalo milk producing country in the world with annual production of 32.18 million tons contributing 61% in total milk production (GoP, 2015-16). The buffalo milk owing to its unique nutritional profile receives an increasing research attention and investment in many countries (Murtaza *et al*., 2014). The dairy products especially cheeses prepared from buffalo milk are becoming increasingly popular throughout the world (Hofi, 2013). Cheese is an important dairy invention of economic worth along with high nutritional significance. Mozzarella cheese represents about 80% of all Italian style cheeses and 32% of total cheese produced in the world. Mozzarella cheese has gained tremendous popularity all over the world because of its exceptional functional properties making it ideal choices for pizza toppings, salads, sandwiches and stuffing. High fat intake has been associated with an increased risk of obesity, atherosclerosis, coronary heart disease and elevated blood pressure which increased the demand for low fat foods, including cheese (Katsiari *et al*., 2002).

The term low fat cheese refers to cheese whose fat contents are lower than its corresponding full fat variety. Fat reduction in cheese causes major shift in the compositional balance which in turn adversely affect the cheese functionality, textural (Guinee and Law, 2002) and sensorial attributes. Such cheese is characterized by poor meltability, rubbery texture, lack of flavor (Johnson *et al*., 2010), bitter taste and undesirable color (Romeih *et al*., 2002). Successful production of low fat cheese requires mimicking the role of fat in texture, functional performance, flavor and color of cheese (McMahon, 2010). Strategies like procedure modification and use of fat replacers might be helpful in improving the acceptability of low fat cheeses. Procedure modification involves incorporation of additional steps like homogenization in cheese manufacturing process. Homogenization reduce the size of fat globule ≤ 1mm and there is more uniform

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distribution of fat in cheese (Nair *et al.*, 2000), due to which, hardness of the cheese is reduced (Brito *et al.*, 2006).

Fat replacers are ingredients intended to be used in the place of natural fats for reduction in the caloric value. Hydrocolloids such as starch and gums are among the ingredients that have been used in formulation of low fat natural and processed cheeses (Hosseini-Parvar *et al.*, 2014). Gums are considered as an efficient carbohydrate based fat replacers and are frequently used in food processing industry to offset the effects of fat reduction and for improving functionality (Totosaus and Gumes-Vera, 2008). These are strongly hydrophilic in nature and have the ability to mechanically entrap the water. Therefore, these are widely recommended for use in the development of low fat cheeses.

Guar gum, galactomannan, is a non-gelling neutral polysaccharide composed of a linear (1→ 4) β-D-mannan backbone with varying amounts of side chains. These consist of a single D-galactose unit, linked by (1→6)-α-glycosidic bonds to the backbone. It has been used also as a versatile fat mimetic for different dairy products including cheese (Srivastava, 2002). Oliveira *et al.* (2010) used guar gum in the development of low fat green Edam cheese and reported significant improvement in its physicochemical, thermal, rheological and textural properties. This study was planned to investigate synergistic impact of guar gum and cheese milk homogenization in reducing fat of Mozzarella cheese.

MATERIALS AND METHODS

Raw materials

Raw buffalo milk was obtained from the Dairy Farm, University of Agriculture, Faisalabad. Guar gum (G4129) was procured from Sigma-Aldrich (USA) and commercially available thermophilic starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) and enzyme chymosin (Double strength Chy-max, 500000 MCU/mL, Pifzer Inc, Milwaukee, WI, USA) were used for Mozzarella cheese manufacturing.

Low fat Mozzarella cheese (LFMC) manufacturing

Buffalo milk was standardized at 1.5% fat and guar gum was added at 0.15%, 0.30%, 0.45% levels with and without cheese milk homogenization. LFMC was manufactured by following the method of Zisu and Shah (2007) with slight modification in order to incorporate guar gum and cheese milk homogenization (2000 psi at 45°C).

Physicochemical analysis of LFMC

Fat content in cheese was measured according to Method No. 933.05 (AOAC, 2000). The moisture content in LFMCs were determined by drying samples in oven at 103 ± 5°C till the constant weight is obtained (Method No. 926.08; AOAC, 2000). The protein (nitrogen) contents were determined by Kjeldahl method using kjeltec system (D-40599, Behr Labor Technik, Germany) as given in FIL-IDF (1993). Acidity percentage was calculated by titration (Method No. 920.124; AOAC, 2000). Total calcium was determined by running LFMC sample on atomic absorption spectrophotometer calibrated with reference standards prepared from calcium reference solution (1000 mg/L Ca, 514870, Sherwood Scientific Ltd., Cambride, UK) by following the method of Metzger *et al.* (2000).

Functional analysis of LFMC

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^{0}C for 4 hrs and then heated at 110^{o}C for 100 minfollowed by cooling at 22^{o}C. Melting length was noted by verniercaliper (Zisu and Shah, 2007). 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.

Texture (hardness) analysis

Texture in terms of hardness of LFMCs were determined on TA-XT Plus Texture Analyzer (Stable Micro 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 at a rate of 1 mm/s (Zisu and Shah, 2007).

Appearance (L value)*

Appearance of LFMCs were assessed on the basis of Hunter L* value (white to black) by using Minolta colorimeter (Model CR-300, Minolta Camera Co., Tokyo, Japan). Each index was measured at 3 points around the circumference of the cheese (Shaker *et al.*, 2012).

Proteolysis by RP-HPLC

Proteolysis in freeze dried soluble fraction (pH 4.6) of LFMCs were determined on an RP-HPLC system (Shimadzu, Japan) consisted of C18 column (Shim-pack CLC-ODS, 250 cm × 4.6 mm, 5 µm) equipped with pump (LC-10AT, Shimadzu, Japan), an auto-sampler and UV detector (SPD-10AV, Shimadzu, Japan). Purposely, 10 mg freeze dried sample was dissolved in 0.5 mL urea solution (48 g Urea, 2 g Tris, 1.3 g sodium citrate and 300 µl mercaptoethanol/100 mL) and 1.5 mL TFA/water solution. The solution was mixed and filtered through 0.2 µm filter (Fisher Scientific, Ireland) and injected (20 µL) into the HPLC system (Shimadzu, Japan). Gradient elution was used with solvent A: 0.1% trifluoroacetic acid (TFA) in water and solvent B: 0.1% TFA in acetonitrile and chromatographic separation was conducted at room temperature, 1.0 mL/min flow rate and 214 nm wavelength (Costabel *et al*., 2007).

Statistical analysis

The analyses were performed in triplicate to investigate the impact of guar gum levels, cheese milk homogenization and ripening time on biochemical changes and proteolysis in LFMCs. The resultant data analyzed statistically by ANOVA using Minitab statistical package and least significant difference (LSD) test was used for multiple comparisons ($\alpha= 0.05$) between means.

RESULTS AND DISCUSSION

Physicochemical composition of LFMCs

Fat contents in LFMCs was significantly ($p<0.01$) affected by treatments and processing (homogenization) while cheese ripening showed non-significant ($p>0.05$) decrease in LFMCs fat as shown in Table I. The highest fat contents (10.67%) were found in Mozzarella cheese containing guar gum at 0.15% level while HF cheese contain the lowest fat contents (8.46%). Supplementation of cheese milk with GG retards the curd syneresis during whey expulsion leading to retention of more milk fat globules in cheese matrix (Lashkari *et al*., 2014). Homogenization of the cheese milk increased the fat contents in LFMCs because it results in small disrupted fat droplets covered with casein and whey proteins, thereby promoting their interaction with casein matrix which ultimately reduces the fat loss in whey (Michalski *et al*., 2003). Karaman and Akalin (2013) also reported higher fat (14.07%) in homogenized cream low fat Turkish white cheese as compared to non-homogenized cream cheese (12.12%)

Guar gum addition and cheese ripening significantly ($p<0.01$) increased the protein contents whereas processing of cheese milk decreased the protein contents of LFMCs. Guar gum containing LFMCs showed higher protein contents (30.37 to 32.45%) as compared to HF (29.86%). Lower protein contents in homogenized milk LFMCs are mainly due to the fact that homogenization results in lower protein to fat ratio in cheese milk and ultimately less protein in homogenized milk Mozzarella cheeses as compared to non-homogenized milk cheeses. Rowney *et al*. (2003) also reported lower protein content (20.2%) in homogenized milk Mozzarella cheese compared to non-homogenized milk cheese (23.7%). 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).

Moisture contents of LFMCs varied significantly ($p<0.01$) from 50.35 to 53.72% for treatments, 51.90 to 52.20% for processing and from 51.47 to 52.73% for ripening days as shown in Table I. Highest moisture contents were found in $G_{0.15}$ (53.72%) followed by $G_{0.30}$ and HF (52.06%). The gums are polysaccharides of linear chains with side chains residues so they increased moisture contents of the product because of their higher moisture retention (Santos, 2009). Comparatively lower moisture contents in homogenized milk LFMCs is mainly due to the reason that homogenization results in smaller fat globules than non-homogenized milk. These smaller globules have greater casein deposition on their surface, this casein establish strong interactions with each other. These strong protein-protein interactions lead to less moisture retention and higher fat recovery in homogenized milk cheeses (Madadlou *et al*., 2007). 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 synersis (Buriti *et al*., 2005).

Table I.- Effect of treatments, processing and ripening days on the physicochemical composition of LFMCs.

Analysis	Treatments				SEM	Processing		SEM	Ripening days					SEM
	HF	$G_{0.15}$	$G_{0.30}$	$G_{0.45}$		NH	H		0	15	30	45	60	
Fat (%)	8.46^C	10.67^A	10.52^A	10.25^B	0.14	9.67^x	10.28^y	0.11	10.06	10.01	9.96	9.94	9.90	0.18
Protein (%)	29.86^D	30.37^C	31.26^B	32.45^A	0.10	31.47^x	30.50^y	0.08	30.82^{ab}	30.58^b	31.06^{ab}	31.16^a	31.31^a	0.09
Moisture (%)	52.06^B	53.72^A	52.06^B	50.35^C	0.15	52.20^x	51.90^y	0.12	52.73^a	52.26^b	52.01^{bc}	51.76^{cd}	51.47^d	0.14
Acidity (%)	0.94	0.94	0.94	0.95	0.01	0.94	0.94	0.01	0.92^e	0.93^d	0.94^c	0.95^b	0.97^a	0.01
Ca (mg/100g)	1033.3^A	912.6^D	933.4^C	948.4^B	4.03	964.7^x	949.1^y	3.28	949.9^c	953.5^{bc}	956.3^{bc}	960.0^{ab}	964.8^a	5.91

Analyses were performed in triplicate and results are expressed as means with standard error of mean (*SEM*). Superscripts A-D and a-e describe significance among treatments and ripening days; x, y used for process comparison.

Addition of guar gum and cheese milk homogenization non-significantly ($p>0.5$) affected the LFMCs acidity while significant ($p<0.01$) increase in acidity (%) of LFMCs were observed during ripening as shown in Table I. Acidity increased from 0.92 to 0.97% in all LFMCs during 60 days storage due to conversion of lactose into lactic acid and loss of buffering capacity of milk. The lactate that produced in 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).

Calcium (Ca) contents in LFMCs varied significantly ($p<0.01$) with respect to treatments, processing and cheese ripening. Highest Ca contents were found in HF (1033.3 mg/100g) and cheese milk supplementation with gaur gum resulted in lower Ca contents (912.6 to 948.4mg/100g) of LFMCs. Homogenization of cheese milk increased the fat level in LFMCs which ultimately resulted in relatively lower calcium when compared with non-homogenized milk cheeses. Variation in Ca contents during ripening is due to significant changes in the proportions of Ca in the insoluble and soluble phase *i.e.* shift in the mineral equilibrium during ripening (Hassan *et al*., 2004).

Functional properties of LFMCs

Meltability and stretchability of LFMCs were determined and results are presented in Table II. Results showed that all guar gum containing LFMCs showed significantly ($p<0.01$) higher meltability (51.93 to 59.72 mm) as compared to HF (51.08 mm). Cheese meltability decreased when guar gum level increased from 0.15 to 0.45%. Higher meltability in guar gum containing LFMCs as compared to HF is due to the fact that it acted as a surface film on casein micelle thereby prevented it from becoming a part of protein matrix and facilitate cheese meltability. $G_{0.15}$ increased the fat retention in cheese as compared to other treatments so it resulted in more meltability. Oliveira *et al*. (2010) found that when level of guar gum increased in green Edam cheese, intermolecular interaction also increased, thereby creating a more compact three-dimensional matrix that decreased the cheese meltability. Results showed that homogenized (H) milk LFMCs showed higher meltability (55.80 mm) as compared to non-homogenized milk LFMCs (53.26 mm). Meltability increases with homogenization due to more uniform distribution of fat in cheese that represent a uniform flow upon heating. This collapse occurs faster in small fat globule (homogenized milk) cheese because the inter-fat inclusion distance is smaller and casein strands are thinner (Michalski *et al*., 2003). Meltability of LFMCs increased significantly ($p<0.01$) during ripening from 48.65 to 59.09 mm. Meltability increased 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 and Shah, 2007).

Stretchability varied significantly ($p<0.01$) from 38.78 to 52.63 cm for treatments, 44.94 to 45.71 cm for processing and from 37.86 to 49.99 cm for ripening (Table II). It is evident from the results that stretchability decreased with increase in the levels of guar gum as $G_{0.15}$ showed highest stretchability (52.63 cm) while lowest stretchability was found in $G_{0.45}$ (38.78 cm). Different stretching behavior in LFMCs manufactured by using different levels of guar gum is due to the reason that cheese stretching involves fusion of protein matrix. Any material that is incompatible with protein such as gums when present in sufficient quantity; they interfere the protein fusion process during cheese stretching and ultimately decreased its values. Homogenization (processing) of cheese milk resulted in higher stretchability (45.71 cm) as compared to non-homogenized milk LFMCs (44.94 cm). More stretchability of homogenized milk LFMCs is due to the fact that homogenization results in small disrupted fat droplets covered with casein. The casein adsorbed onto the fat globule surface after homogenization form protein-protein bond and increase stretchability (Michalski *et al*., 2002). Stretchability depends on amount of intact casein, arrangement of the protein within network and extent of proteolysis. Its value increased significantly 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).

Table II.- Effect of treatments, processing and ripening days on the functionality of LFMCs.

Analysis	Treatments				SEM	Processing		SEM	Ripening days					SEM
	HF	$G_{0.15}$	$G_{0.30}$	$G_{0.45}$		NH	H		0	15	30	45	60	
Meltability (mm)	51.08^{D}	59.72^{A}	55.40^{B}	51.93^{C}	0.22	53.26^{x}	55.80^{y}	0.28	48.65^{e}	52.85^{d}	55.10^{c}	56.97^{b}	59.09^{a}	0.30
Stretchability (cm)	44.21^{C}	52.63^{A}	45.69^{B}	38.78^{D}	0.16	44.94^{x}	45.71^{y}	0.21	37.86^{e}	42.47^{d}	46.71^{c}	49.99^{a}	49.60^{b}	0.18

Analyses were performed in triplicate and results are expressed as means with standard error of mean (*SEM*). Superscripts A-D and a-e describe significance among treatments and ripening days; x, y used for process comparison.

Table III.- Effect of treatments, processing and ripening days on the texture and appearance of LFMCs.

Analysis	Treatments				SEM	Processing		SEM	Ripening days					SEM
	HF	$G_{0.15}$	$G_{0.30}$	$G_{0.45}$		NH	H		0	15	30	45	60	
Hardness (g)	2768.2^{A}	1650.7^{D}	1713.0^{C}	1730.7^{B}	9.73	1991.4^{x}	1939.8^{y}	5.82	2142.8^{a}	2045.1^{b}	1971.6^{c}	1869.2^{d}	1799.5^{e}	8.36
L* value	59.78^{C}	63.30^{A}	62.13^{B}	61.74^{B}	1.60	61.16^{x}	62.32^{y}	1.81	63.35^{a}	62.39^{ab}	61.74^{bc}	60.97^{cd}	60.24^{d}	1.93

Analyses were performed in triplicate and results are expressed as means with standard error of mean (*SEM*). Superscripts A-D and a-e describe significance among treatments and ripening days; x, y used for process comparison.

LFMCs texture (hardness)

Texture (hardness) of LFMCs manufactured by using different levels of guar gum showed significant variation ($p<0.01$) with respect to treatments, processing and ripening as shown in Table III. Use of guar gum significantly ($p<0.01$) decreased the LFMCs hardness (1650.7 to 1730.7 g) as compared to HF (2768.2 g). Addition of guar gum decreased the cheese hardness by increasing the total filler volume that is composed of fat and moisture contents. Guar gum decreased the protein matrix compactness by increasing its water holding capacity thereby decreasing LFMCs hardness. It has been frequently reported that use of different fat replacers had decreased the hardness of cheeses (Bhaskaracharya and Shah, 2001; Romeih *et al*., 2002; Zisu and Shah, 2005). Processing (homogenization) resulted in decreased hardness of LFMCs. The higher FDM in cheese as a result of homogenization can account for their decreased hardness (Madadlou *et al*., 2007). Results regarding decrease in cheese hardness by homogenization (processing) are in line with the findings of Karaman and Akalın (2013) who also reported decreased hardness in homogenized cream low fat Turkish white cheeses. Decrease in LFMCs hardness from 2142.8 to 1799.5 g is due to proteolysis. The casein network weakens as proteolysis continues under the combined influence of the coagulant, starter and non-starter bacterial enzymes. Consequently, an increased ripening index made all cheeses softer (Attaie, 2005). Hardness decreased significantly between 7 to 90 days in all cheese types due to the hydration of the protein matrix and as a result of proteolysis (Zisu and Shah, 2007).

LFMCs Appearance (L value)*

Mean values for appearance showed (Table III) that use of guar gum in LFMC significantly ($p<0.01$) improved the appearance. $G_{0.15}$ was found to be more whitish (L* = 63.30) in appearance followed by $G_{0.30}$ (L* = 62.13). Cheese milk processing also improved the LFMCs appearance as H milk LFMCs were more whitish (L*= 62.32) as compared to NH milk LFMCs (L*= 61.16). Rudan *et al*. (1998) reported that the largest effect of fat type was on the appearance of cheese and homogenization cause change in the fat dispersion of the cheese through reduction of fat globules size which makes the cheese more whitish in appearance. Results showed that LFMCs whiteness significantly ($p<0.01$) decreased from 63.35 to 60.24 during 60 days ripening. The intact casein and calcium level are responsible for the white color of cheeses and decrease in the intensity of whiteness during ripening may be associated with their decreased levels. Zisu and Shah (2005) also reported significant decrease in color L* value of low fat Mozzarella cheese during storage period of 45 days.

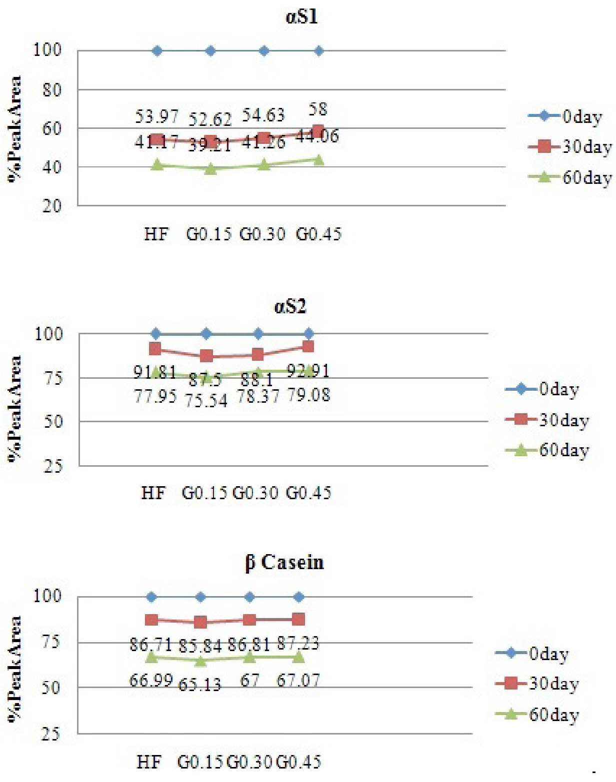

Fig. 1. Percentage reduction in peak areas of different casein fractions in different treatments

Proteolysis in LFMCs

Proteolysis is the most important chemical and biochemical change occur during cheese ripening (Rafiq *et al*., 2016). In Mozzarella cheese αS_1-, αS_2- and β-casein hydrolysis is an important event of casein degradation. Significant inter-species differences in the concentration and types of caseins contents of milk are reflected in the characteristics of the cheeses produced from them (Tahira *et al*., 2014). In this study, amount of casein breakdown in LFMCs was monitored by using water soluble fractions through RP-HPLC. In all LFMCs, three peaks (αS_1-, αS_2- and β-casein) were identified by comparing their retention time with standard. Results for the αS_1-, αS_2- and β-casein degradation during ripening are explained on the basis % of remaining peak areas in different LFMCs as shown in Figure 1. It is evident from the result that when peak area for αS_1-, αS_2- and β-casein fraction was considered as 100% at zero days, percentage of remaining peak area of all fractions decreased in all LFMCs during ripening. Major factors that determine the rate of proteolysis in cheese are residual starter culture, coagulating enzymes and moisture to protein ratio (M/P). The starter culture and coagulating enzymes remained constant in this study so difference in % of remaining peak area of casein fractions among different LFMCs is possibly due to difference in their M/P as cheese with higher M/P showed more proteolysis rate as compared to LFMCs having low M/P as shown in Figure 2. Processing not alters the M/P of different LFMCs therefore its effect was found to be non-significant ($p>0.05$) on the rate of proteolysis. Results of the present study regarding decrease in αS- and β-casein fractions peak area as a result of proteolysis during Mozzarella cheese maturation are well supported by the finding of Dave *et al*. (2003). They also expressed the extent of proteolysis in Mozzarella cheese during storage on the basis of % of remaining peak areas and reported that as cheese ripening proceeds there was corresponding decrease in the peak areas for αS- and β-casein. It might be due to formation of water soluble peptides and amino acids from casein that reduced the intact casein fractions.

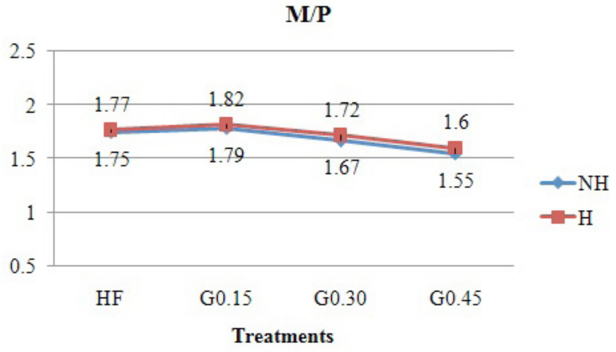

Fig. 2. Effect of cheese milk homogenization on moisture to protein ratio (M/P) of LFMCs.

CONCLUSIONS

Low fat Mozzarella cheese can be manufactured by using guar gum as fat mimetic. Use of guar gum at 0.15% level was found to be more effective for development of LFMC. In addition cheese milk homogenization act synergistically in improving the LFMC functionality, appearance and texture.

Statement of conflict of interest

Authors have declared no conflict of interest.

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