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Efficacy of Carboxymethyl Cellulose as Fat Replacer on the Processing and Storage Quality of Buffalo Mozzarella Cheese

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ABSTRACT

Processing and storage (4 ± 1°C) quality of Low-fat buffalo mozzarella cheese (BMC) was evaluated with carboxymethyl cellulose (CMC) as fat replacer. Five treatments of BMC viz. Control full-fat cheese (6.0% milk fat; CFFC), Control low-fat cheese (<0.5% MF) without CMC (CLFC), Low-fat cheese with 0.2% CMC (LFC-1), 0.4% CMC (LFC-2) and 0.6% CMC (LFC-3) were comparatively evaluated. Melting time increased, whereas hardness and chewiness decreased with CMC in BMC. Sensory panellists awarded LFC-2 highest and lowest to LFC-3, however treated products at all selected levels were superior than CLFC. Oxidative and microbial stability was improved in LFC-2 than CFFC during storage. Results concluded that 0.4% CMC is optimum for the development of extended shelf life functional BMC.

Keywords: Mozzarella cheese, carboxymethyl cellulose, full-fat cheese, low-fat cheese, physico-chemical quality, storage quality

Rheological, processing, sensory and storage quality of milk products is directed by the amount of fat content. The reduction of fat leads to low yield and deterioration in quality such as textural, functional and sensory characters in cheese (Sipahioglu *et al.* 1999). Low-fat cheese has bland taste, firm and rubbery texture, and develops colour changes and off-flavours (Rodriguez, 1998). However, the risk associated with the consumption of high dietary fat such as obesity, hypertension and cardiovascular diseases lead to increase in the demand for low-fat dairy products during the last few years. Various approaches have been employed so far to combat the effects of fat reduction in low-fat cheese products, however the active approach is modification in cheese making procedure, use of adjunct starter culture and use of fat replacers (Mistry, 2001).

Fat replacers are water-dispersible substances, which improve the sensory and functional properties of low-fat cheese by bulking effect associated with moisture retention as well as providing a sense of lubricity and creaminess (Sahan *et al.* 2008). However, they cannot positively impact the flavour defects in cheese (Dubey, 2011). Mainly two types of fat replacers have been recommended for use in cheese products viz. microparticulated protein based and microparticulated carbohydrate based (Romeih *et al.* 2002). The use of a particular type of fat replacer in a food depends on the composition and characteristics of that food (Sandrou and Arvanitoyannis, 2000). Carboxymethyl cellulose (CMC) is a carbohydrate based fat replacer, which acts as thickening agent, stabilizer and suspending agent, and used in a variety of dairy products due to its technological and nutritional advantages (Jellema *et al.* 2005).

Mozzarella cheese is a soft cheese, with characteristic fibrous structure along with unique melting and stretching properties (Sandrou and Arvanitoyannis, 2000). Traditionally, it is prepared from buffalo milk. Moreover, due to higher production of buffalo milk (>50 MT) in India, the majority of mozzarella cheese is prepared from buffalo milk. The shelf-life of Mozzarella cheese under refrigeration conditions is limited due to proteolysis (Melachouris and Tuckey, 1967), lipid oxidation and fast growth of microbes. Abd Elhamid (2013) studied that as the carboxymethyl cellulose content of cheese milk increased, cheese yield and moisture content of low-fat Domiati cheese increased significantly, but the protein, salt and fat values decreased significantly. Ghosh and Kulkarni (1996) developed low cholesterol Mozzarella cheese using groundnut oil, sunflower oil and hydrogenated oil as fat replacers and they observed that the cheese packed in polyethylene pouches kept well for 8-10 days at 8-10°C and for about 90 days in freezer. Though some information has been published regarding the effect of carboxymethyl cellulose concentration on flavour and textural properties of custard desserts, studies have paid less attention to the processing, sensory and rheological properties of carboxymethyl cellulose treated milk systems than to the starch treated milk systems. In lieu of that, the present study was envisaged to optimize the level of incorporation of carboxymethyl cellulose as fat replacer in buffalo Mozzarella cheese on the basis of processing, nutritional, textural, sensory and storage quality attributes and to compare between the low-fat cheese with selected levels of carboxymethyl cellulose and full-fat cheese as control for its storage stability at refrigeration temperature (4 ± 1°C) under aerobic conditions.

MATERIALS AND METHODS

Fresh six litres of buffalo milk was procured from the Dairy farm of Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) Ludhiana. Citric acid (5%) was used as acidulant and freeze dried microbial rennet from *Mucor miehei* Type II was used as coagulant (Danlac, Canada). Carboxymethyl cellulose (Sodium salt High Viscosity carboxymethyl, S D Fine-CHEM Ltd., Mumbai, India; Code No. 56095, CMC) was used as fat replacer.

Preparation of Mozzarella cheese

Mozzarella cheese was prepared by direct acidification method standardized in our laboratory with citric acid 5% as acidulant and microbial rennet for the coagulation process. Six litres of buffalo milk was separated to cream and skim milk using a cream separator. Two lots of milk samples were prepared - full-fat milk (standardized at 6.0% fat and 8.5% SNF) and skim milk (<0.5% fat). Finally, five different batches were prepared viz. Control full-fat cheese with 6.0% fat (CFFC), Control low-fat cheese with <0.5% fat and without CMC (CLFC), Low-fat cheese with 0.2% CMC (LFC-1), Low-fat cheese with 0.4% CMC (LFC-2) and Low-fat cheese with 0.6% CMC (LFC-3). For CFFC, 1 litre of full-fat milk and for each of all the other samples (CLFC, LFC-1, LFC-2 and LFC-3), 1 litre of skim milk was used. The fat replacer was added to the cheese milk at 30 °C and mixed properly, followed by the addition of 5% citric acid slowly, so as to decrease the pH of milk to 5.40 ± 0.02. Then, microbial rennet (1% solution, 1.5 ml/litre) was added and incubated for 45 minutes at 35°C. The settled curd was cut into 1 cm cubes. The curd was gradually heated to a temperature of 42°C using hot water. The cheese whey was drained and the curd was scalded at 80°C in water and shaped manually. The resultant cheese was packed in Low Density Polyethylene (LDPE) bags, and was subjected to analysis for proximate composition, texture, colour and sensory attributes.

Physico-chemical analysis

The average yield of buffalo mozzarella cheese (BMC) was calculated simply by dividing the weight of cheese (grams) with amount of milk (grams) multiplied by 100.

Melt time of cheese was determined as per the method of Guinee *et al.* (2002) and it was recorded as the time taken in melting 100 gm of cheese over hot water bath maintained at temperature of 82°C.

Water activity (a_w) was determined using hand held portable digital water activity meter (Rotonix HYGRO Palm AW1 Set/40, 60146499). Finely grounded cheese sample was filled up (80%) in a moisture free sample cup over which the sensor was placed for five min and reading was noted.

The pH was determined (Trout *et al.* 1992) with digital pH meter (SAB 5000, LABINDIA, New Delhi, India). For this, 10 g of sample was homogenized with 50 ml of distilled water and the electrode was dipped into the suspension to note down the pH.

For determination of titratable acidity (Shelef and Jay, 1970), 10 g of cheese sample was blended with 200 ml of distilled water and made the volume 250 ml in a volumetric flask. The slurry was filtered through Whatman filter paper No.1 and 25 ml of this filtrate was added with 75 ml distilled water with three drops of 1% phenolphathalein indicator solution and titrated against 0.1 N NaOH to get the end point (pink colour). Titrable acidity was calculated as:

Titratable acidity (% lactic acid) = [(ml of 0.1N NaOH × 0.1 × meq wt. of lactic acid) / weight of sample (g)] × 100

Proximate Composition

Proximate composition of cheese sample was determined as per the procedures of AOAC (2000). The moisture content was determined by using hot air oven (Macro Scientific Works MAC 10A/UA, New Delhi) at 100°C, fat by ether extraction method using Socs Plus (SCS-6-AS, Pelican Industries, Chennai, India) and protein by using automatic digestion and distillation unit (Kel Plus-KES 12L, Pelican Industries, Chennai). For ash estimation, moisture free sample was ashed at 550°C in muffle furnace for about 7-8 hours. Estimates of total calories in the cheese samples were calculated on the basis of 100 g portion using Atwater values for fat (9 kcal/g), protein (4.02 kcal/g) and carbohydrate (4 kcal/g). Therefore, the calorie values were estimates and not actual values.

Texture profile

The texture Profile Analysis (TPA) was performed as per the procedure outlined by Bourne (1978) and was determined using Texture analyzer (TMS-PRO, Food Technology Corporation, USA). Sample size of 1.0 cm × 1.0 cm × 1.0 cm was subjected to pretest speed (30 mm/sec), post test speed (100 mm/sec) and test speed (100 mm/sec) to a double compression cycle with a load cell of 2500 N. A compression platform of 25 mm was used as a probe. Parameters such as hardness (N), adhesiveness (mJ), springiness (mm), stringiness (mm), cohesiveness (ratio), chewiness (J), gumminess (N) and resilience (ratio) were calculated automatically by the preloaded software in the equipment from the force-time plot.

Colour profile

In colour profile analysis, CIE L^* (lightness), a^* (redness) and b^* (yellowness) values were measured at three random locations on each cheese sample using Lovibond Tintometer (Lovibond RT-300, Reflactance Tintometer, United Kingdom) set at 2° of cool white light (D_{65}) and 2.54-cm diameter aperture (AMSA 1991).

Oxidative Stability parameters

For Free fatty acids (FFA) (Koniecko, 1979) estimation, 5 g of cheese sample was blended with 30 ml of chloroform in the presence of anhydrous sodium sulphate. The filtrate (Whatman filter paper No. 1) was added with 2 drops of 0.2 percent phenolphthalein indicator and titrated against 0.1N alcoholic KOH to get the end point (pink colour). Percent FFA content was calculated as:

FFA (%) = [(0.1 × ml of 0.1N alc. KOH × 0.282) / sample weight (g)] × 100

Peroxide value (PV) (Koniecko, 1979) was determined by blending 5 g of cheese sample with 30 ml chloroform in the presence of anhydrous sodium sulphate. The filtrate (Whatman filter paper No. 1) was added with 30 ml of glacial acetic acid and 2 ml of saturated KI solution and left for 2 min with occasional shaking after which 100 ml of distilled water and 2 ml of fresh 1 percent starch solution were added. The content was titrated against 0.1N sodium thiosulphate to get the end point (non-aqueous layer turned to colourless). The PV was calculated as:

PV (meq/kg sample) = [(0.1 × ml of 0.1N sodium thiosulphate) / sample weight (g)] × 1000

TBARS (Thiobarbituric acid reactive substances) value was determined as per the extraction method described by Witte *et al.* (1970). Briefly, 10g of cheese sample was triturated with 25 ml of pre-cooled 20% trichloroacetic acid (TCA) in 2 M orthophosphoric acid solution for 2 min. The content was filtered through Whatman filter paper No. 1 to get TCA extract. 3 ml of this TCA extract was mixed with 3 ml of TBA reagent (0.005 M) in test tubes and placed in a dark room for 16 hrs. A blank sample was prepared by mixing 1.5 ml of 20% TCA, 1.5 ml distilled water and 3 ml of 0.005 M TBA reagent. Absorbance (O.D.) was measured at fixed wavelength of 532 nm with a scanning range of 531 nm to 533 nm using UV-VIS spectrophotometer (Elico SL-159, Mumbai, India). TBARS was calculated as mg malonaldehyde per kg of sample by multiplying O.D. value with a factor 5.2.

Microbiological analysis

Microbiological quality parameters were estimated by pour plate method using serial dilutions. Standard plate

Table 1: Effect of different levels of carboxymethyl cellulose on proximate composition and physico-chemical parameters of Mozzarella cheese

Parameters	CFFC	CLFC	LFC-1	LFC-2	LFC-3
Moisture (%)	50.78±1.44^{a}	57.15±0.84^{b}	61.19±0.99^{c}	62.26±0.78^{c}	63.13±0.41^{c}
Protein (%)	22.94±0.84^{a}	32.76±1.08^{c}	30.33±1.70^{b}	32.70±1.13^{c}	30.72±1.89^{b}
Fat (%)	16.18±0.79^{b}	0.37±0.07^{a}	0.37±0.16^{a}	0.29±0.05^{a}	0.21±0.03^{a}
Ash (%)	1.09±0.17^{a}	1.96±0.23^{b}	1.99±0.19^{b}	2.27±0.19^{b}	1.80±0.16^{b}
Energy (Kcal/100g)	245.39±8.36^{c}	142.42±4.84^{b}	132.60±7.16^{ab}	141.41±4.37^{ab}	132.77±7.57^{a}
Melt time (minutes)	6.33±0.33^{a}	9.17±0.31^{b}	9.17±0.31^{b}	9.17±0.31^{b}	9.17±0.31^{b}
Average yield (%)	13.12±0.58^{c}	8.90±0.43^{a}	9.64±0.38^{ab}	10.18±0.27^{b}	10.67±0.068^{b}

n=6, Mean ± SE with different superscripts in the same row differ significantly ($p<0.05$). CFFC - Control full-fat cheese, CLFC - Control low-fat cheese without CMC, LFC-1 - Low-fat cheese with 0.2% CMC, LFC-2 - Low-fat cheese with 0.4% CMC and LFC-3 - Low-fat cheese with 0.6% CMC.

Table 2: Effect of different levels of carboxymethyl cellulose on texture and colour profile of Mozzarella cheese

Parameters	CFFC	CLFC	LFC-1	LFC-2	LFC-3
Texture profile					
Hardness (N)	16.01±0.54^{b}	27.46±1.74^{c}	19.16±1.14^{b}	16.91±1.65^{b}	11.24±0.16^{a}
Adhesiveness (mJ)	0.22±0.01	0.15±0.01	0.16±0.03	0.22±0.04	0.17±0.03
Springiness (mm)	6.04±0.13^{a}	7.16±0.38^{b}	7.07±0.47^{b}	6.41±0.28^{ab}	6.28±0.23^{ab}
Stringiness (mm)	0.88±0.11^{a}	14.28±0.81^{b}	21.56±1.98^{c}	19.91±1.99^{c}	23.14±1.93^{c}
Cohesiveness (ratio)	0.71±0.02^{a}	0.72±0.01^{a}	0.77±0.04^{b}	0.77±0.03^{b}	0.78±0.01^{b}
Chewiness (J)	104.02±14.68^{bc}	128.06±5.26^{c}	124.44±4.12^{c}	87.78±8.64^{b}	46.88±1.54^{a}
Gumminess (N)	11.16±0.58^{a}	22.59±1.08^{c}	16.56±0.65^{c}	13.25±1.72^{b}	15.03±0.84^{b}
Resilience (ratio)	3.19±1.03^{a}	5.35±0.61^{b}	5.81±0.02^{b}	6.74±0.31^{c}	6.05±0.23^{c}
Colour profile					
L*	82.83±1.98^{b}	72.60±4.62^{ab}	75.78±2.44^{ab}	76.19±2.87^{ab}	65.42±5.17^{a}
a*(-)	0.94±0.19^{a}	2.87±0.06^{bc}	3.24±0.17^{c}	2.95±0.03^{bc}	2.64±0.12^{b}
b*	16.44±1.11^{b}	15.97±1.61^{b}	12.40±1.02^{ab}	13.09±1.52^{ab}	10.90±1.64^{a}

n=9, Mean ± SE with different superscripts in the same row differ significantly ($p<0.05$). CFFC - Control full-fat cheese, CLFC - Control low-fat cheese without CMC, LFC-1 - Low-fat cheese with 0.2% CMC, LFC-2 - Low-fat cheese with 0.4% CMC and LFC-3 - Low-fat cheese with 0.6% CMC.

counts and psychrophilic counts were measured on plate count agar media (M091; HiMedia Laboratories Pvt. Ltd., Mumbai, India) incubated for 48 hrs at 35°C and 10 days at 4°C respectively. Whereas, total coliform count was estimated on violet red bile glucose agar (VRBGA, ME581; HiMedia Laboratories Pvt. Ltd., Mumbai, India) after incubation at 35°C for 24 hrs and yeast and mould count was carried out on potato dextrose agar (M096; HiMedia Laboratories Pvt. Ltd., Mumbai, India) at 25°C for 5 days. The average number of colonies were multiplied by reciprocal of the respective dilution and expressed as $\log_{10}$cfu/g of sample (APHA, 1984).

Sensory evaluation

A seven member experienced panel of judges consisting of teachers and postgraduate students of College of Veterinary Science, GADVASU evaluated the samples

Table 3: Effect of carboxymethyl cellulose on the physico-chemical, oxidative and microbiological parameters of Mozzarella cheese stored at 4±1°C for 10 days

Treatment/Days	Day 1	Day 4	Day 7	Day 10
Physico-chemical parameters				
Water activity (a_w)				
CFFC	$0.94±0.01^{wa}$	$0.96±0.01^{xa}$	$0.98±0.01^{xa}$	$0.97±0.01^{xa}$
LFC-2	$0.96±0.01^{wa}$	$0.97±0.01^{wa}$	$0.99±0.01^{xb}$	$0.98±0.01^{xb}$
pH				
CFFC	5.19±0.10	5.17±0.07	5.12±0.08	5.09±0.01
LFC-2	5.11±0.03	5.10±0.01	5.05±0.03	5.05±0.02
Titratable acidity (% lactic acid)				
CFFC	$0.04±0.01^{wa}$	$0.05±0.01^{xa}$	$0.05±0.01^{xa}$	$0.06±0.01^{ya}$
LFC-2	0.05±0.01	0.05±0.01	0.06±0.01	0.07±0.02
Oxidative stability parameters				
Free Fatty acids (%)				
CFFC	$0.12±0.01^{wb}$	$0.18±0.14^{wb}$	$0.24±0.05^{xb}$	$0.48±0.01^{yb}$
LFC-2	$0.08±0.01^{wa}$	$0.08±0.01^{wa}$	$0.14±0.01^{xa}$	$0.18±0.01^{xa}$
Peroxide Value (meq/kg)				
CFFC	$0.59±0.01^{wb}$	$0.79±0.01^{xb}$	$2.40±0.31^{yb}$	$3.49±0.26^{zb}$
LFC-2	$0.39±0.01^{wa}$	$0.59±0.07^{xa}$	$1.08±0.28^{ya}$	$2.49±0.32^{za}$
TBARS value (mg MDA/kg)				
CFFC	$0.31±0.01^{wb}$	$0.44±0.02^{xb}$	$0.59±0.20^{xyb}$	$0.95±0.23^{yb}$
LFC-2	$0.04±0.01^{wa}$	$0.16±0.04^{xa}$	$0.17±0.07^{xa}$	$0.41±0.09^{ya}$
Microbiological parameters				
Standard plate count (log_{10} cfu/g)				
CFFC	$3.96±0.01^{wb}$	$4.20±0.01^{xb}$	$4.31±0.01^{yb}$	$4.44±0.01^{zb}$
LFC-2	$3.80±0.01^{wa}$	$4.04±0.01^{xa}$	$4.09±0.08^{xa}$	$4.35±0.01^{ya}$
Coliform count (log_{10} cfu/g)				
CFFC	$3.47±0.01^{wb}$	$3.83±0.01^{xb}$	$4.04±0.01^{yb}$	$4.16±0.01^{zb}$
LFC-2	$3.19±0.03^{wa}$	$3.74±0.01^{xa}$	$3.94±0.01^{ya}$	$4.08±0.01^{za}$
Yeast and mould count (log_{10} cfu/g)				
CFFC	$1.05±0.03^{wa}$	$2.83±0.05^{xa}$	$3.04±0.05^{yb}$	$3.31±0.02^{zb}$
LFC-2	$1.05±0.03^{wa}$	$2.20±0.32^{xa}$	$2.99±0.04^{yab}$	$3.17±0.02^{za}$
Psychrophiles count (log_{10} cfu/g)				
CFFC	$4.02±0.01^{wb}$	$4.25±0.01^{xb}$	$4.38±0.01^{xb}$	$4.51±0.07^{yb}$
LFC-2	$3.90±0.01^{wa}$	$4.15±0.01^{xa}$	$4.32±0.01^{ya}$	$4.41±0.08^{ya}$

n=6, Mean±S.E. with different superscripts row wise (w-z) and column wise (a-b) differ significantly ($p < 0.05$). CFFC - Control full-fat cheese, LFC-2 - Low-fat cheese with 0.4% CMC

for different attributes viz. Appearance / colour, texture, flavour, juiciness and overall acceptability using an 8-point descriptive scale (Keeton, 1983), where 8=extremely desirable and 1=extremely undesirable. The members selected were having sufficient experience and knowledge about the quality characteristics of the mozzarella cheese. Two sessions were conducted and all the panellists were detailed about the descriptive scale and product characteristics. The panellists carried out evaluation in a room free of noise and odours and suitably illuminated with natural light. The coded samples, tempered at room temperature were presented to the panellists. Drinking water was provided to the panellist for rinsing the mouth intermittently.

Statistical analysis

Data was analyzed statistically using SPSS-16.0 (SPSS Inc. Chicago IL, USA) software package as per standard methods (Snedecor and Cochran, 1994). The experiment was repeated three times and duplicate samples (n=6) were drawn for each parameter except colour and texture profile (n=9), carried out in triplicate. Sensory evaluation was performed by a panel of seven judges (n=21). Data were subjected to two-way analysis of variance, homogeneity test and Duncan's Multiple Range Test for comparing the means to find the effects between treatment, between storage periods and their interactions. The statistical significance was expressed at $p<0.05$.

RESULTS AND DISCUSSION

Proximate composition and physico-chemical parameters

Reduction of fat level in cheese or the use of fat replacers in the manufacture of BMC significantly ($p<0.05$) influenced gross composition and yield (Table 1). Moisture, protein and ash % were significantly higher ($p<0.05$) in all the low-fat cheeses than CFFC samples. The moisture content increased with the increase in level of incorporation of CMC in cheese. It might be due to the water-binding capacity of carboxymethyl cellulose. Similar observations were recorded by various scientists in different varieties of cheese (Katsiari and Voutsinas, 1994; Drake *et al.* 1996; Rudan *et al.* 1998; Sipahioglu *et al.* 1999; Romeih *et al.* 2002; Zalazar *et al.* 2002; Kavas *et al.* 2004; Koca and Metin, 2004; Shendi *et al.* 2010). Significantly lower ($p<0.05$) fat values in low-fat cheese samples (CLFC, LFC-1, LFC-2 and LFC-3) were due to the use of skim milk for their preparation. Similarly, the energy content of low-fat cheeses was decreased by 42-46% mainly due to absence of fat than CFFC. Among the physico-chemical attributes, the melting time was non-significant with CMC or without CMC (CLFC) but significant between CFFC and all low fat cheese combinations. Higher melt time in low-fat cheeses is in accordance with the statement of Mc Mahon and Oberg (1998) who reported that meltability of buffalo Mozzarella cheese was directly related to fat content of cheese and as the fat content decreased, the melt time increased. The average yield was significantly lower ($p<0.05$) in CLFC (8.90%) than CFFC (13.12%). It is attributed to lower total solid content. During cheese preparation, fat gets entrapped in casein matrix (Rudan *et al.* 1999). Though fat is replaced by moisture, yet there is an overall reduction in yield of low-fat cheese because the total amount of fat removed is not equal to the amount of moisture added (Mistry, 2001). Therefore, the sum of the casein and fat contents of the milk, which are the principal components determining cheese yield, are reduced (Romeih *et al.* 2002). However, among the CMC added groups, cheese yield increased non-significantly with the increase in the concentration of CMC attributed to the water-binding property of the CMC and the increasing moisture content.

Texture and colour profile

Texture profile of the samples revealed that the fat content of cheese milk played a crucial role in the development of texture (Table 2). The hardness was significantly higher ($p<0.05$) in CLFC than all the other variants. This can be attributed to its lower moisture content and formation of a relatively denser network of elastic proteins due to reduction of fat (Tunick *et al.* 1995). Amongst treatments, hardness of LFC-3 was significantly ($p<0.05$) lower than all the low-fat products. In general, reduction of fat in cheese lead to a harder texture, however, hardness decreased with the addition of fat replacers. Zisu and Shah (2005) also observed that low-fat mozzarella cheese appeared harder, brittle and less pliable than high-fat cheese. Similar results were reported in Feta and White-brined cheeses (Sipahioglu *et al.* 1999; Romeih *et al.* 2002; Volikakis *et al.* 2004), fresh Kashar (Koca and Metin, 2004) and Cheddar cheese (Drake *et al.* 1996; Fenelon and Guinee, 1997; Konuklar *et al.* 2004). Adhesiveness was found to be non-significant between full-fat and low-fat cheese samples. Springiness was significantly higher ($p<0.05$) in low-fat cheese samples i.e CLFC and LFC-1, than CFFC, LFC-2 and LFC-3 batches. Reduction of fat in cheese lead to a denser and more elastic protein network, which increased the springiness values in Mozzarella cheese (Tunick *et al.* 1995). The values for springiness in cheese correlated well with the hardness values. Similar correlation was found in Gaziantep cheese by Kahyaoglu *et al.* (2005). Stringiness and cohesiveness were significantly higher ($p<0.05$) in all the CMC added cheese (LFC-1, LFC-2 and LFC-3) than CFFC and CLFC samples. It seems that addition of fat replacers made a positive contribution towards the cohesiveness and stringiness values of cheese samples. Also due to gelling properties of CMC, which provided comparable and superior cohesiveness to LFC-1, LFC-2

Table 4: Effect of carboxymethyl cellulose on the texture and colour profile analysis of Mozzarella cheese stored at 4±1°C for 10 days

Treatment/Days	Day 1	Day 4	Day 7	Day 10
Texture profile analysis				
Hardness (N)				
CFFC	9.32±0.57^{xa}	5.77±0.17^{wa}	6.14±0.29^{wa}	6.59±0.26^{wa}
LFC-2	14.93±1.17^{xb}	12.94±0.52^{wb}	15.31±0.70^{xb}	12.91±0.62^{wb}
Adhesiveness (mJ)				
CFFC	0.14±0.01^{xb}	0.13±0.00^{wa}	0.11±0.01^{wxa}	0.11±0.00^{wa}
LFC-2	0.16±0.01^{wb}	0.13±0.00^{wab}	0.12±0.01^{wa}	0.12±0.00^{wa}
Springiness (mm)				
CFFC	27.80±0.10^{wab}	28.23±0.10^{wb}	28.22±0.23^{wb}	28.34±0.24^{wb}
LFC-2	27.51±0.05^{wxa}	27.43±0.05^{wxa}	27.38±0.03^{wa}	27.53±0.03^{xa}
Stringiness (mm)				
CFFC	24.38±1.56^{yb}	18.01±0.62^{wa}	16.11±1.42^{wa}	21.83±1.15^{xa}
LFC-2	22.20±1.28^{wa}	22.78±1.97^{wb}	22.80±1.47^{wb}	23.05±2.03^{wb}
Cohesiveness (ratio)				
CFFC	0.74±0.02^{xa}	0.59±0.02^{wxa}	0.56±0.08^{wa}	0.51±0.07^{wxa}
LFC-2	0.72±0.03^{wa}	0.75±0.01^{wb}	0.72±0.02^{wb}	0.69±0.02^{wa}
Chewiness (J)				
CFFC	175.80±17.76^{xa}	113.60±7.62^{wa}	105.50±9.46^{wa}	103.40±12.43^{wa}
LFC-2	288.53±32.94^{wb}	254.00±19.25^{wb}	234.39±8.74^{wb}	229.44±14.27^{wb}
Gumminess (N)				
CFFC	7.48±0.49^{xa}	3.79±0.26^{wa}	3.60±0.49^{wa}	3.09±0.54^{wa}
LFC-2	10.53±1.20^{xa}	9.29±0.69^{wxb}	8.52±0.33^{wb}	8.33±0.52^{wb}
Resilience (ratio)				
CFFC	0.52±0.07^{wa}	0.69±0.05^{xa}	0.70±0.36^{xa}	1.06±0.08^{yb}
LFC-2	0.58±0.08^{wa}	0.64±0.09^{wa}	0.66±0.05^{wa}	0.71±0.02^{wa}
Colour profile analysis				
L^* value				
CFFC	77.38±5.12^{wa}	93.41±1.19^{xb}	89.96±0.32^{xb}	90.65±0.21^{xb}
LFC-2	75.39±3.84^{wa}	84.80±1.95^{xa}	83.40±0.68^{xa}	84.00±0.27^{xa}
a^*(-) value				
CFFC	0.12±0.03^{wa}	0.16±0.04^{wa}	0.17±0.03^{wa}	0.22±0.02^{xa}
LFC-2	2.43±0.13^{wb}	2.78±0.13^{xb}	2.70±0.08^{wxb}	2.56±0.11^{wb}
b^* value				
CFFC	15.04±0.76^{wb}	16.64±0.86^{xb}	15.10±0.28^{wb}	15.10±0.18^{wb}
LFC-2	12.72±0.47^{wa}	14.32±0.65^{xa}	13.26±0.22^{wxa}	12.96±0.34^{wa}

n=9, Mean±S.E. with different superscripts row wise (w-z) and column wise (a-b) differ significantly ($p < 0.05$). CFFC - Control full-fat cheese, LFC-2 - Low-fat cheese with 0.4% CMC.

and LFC-3. Chewiness was significantly lower ($p<0.05$) in LFC-3 sample than CFFC and other variants. Gumminess and resilience were significantly lower ($p<0.05$) in CFFC than all the other samples. Removing fat from cheese resulted in an increase in gumminess values. Koca & Metin (2004) also observed that use of fat replacers decreased the hardness, springiness, gumminess and chewiness and increased cohesiveness in low-fat fresh kashar cheese. In colour profile, L^* and b^* values did not vary significantly among full-fat and low-fat cheese samples. However, a^* (-) value was significantly lower ($p<0.05$) in all the low-fat cheeses than CFFC samples. Shendi *et al.* (2010) also observed similar trends in b^* values of low-fat Iranian cheese.

Sensory evaluation

Figure 1 shows the effect of different levels of CMC on 8-point descriptive scale for sensory attributes. Among these, colour/appearance and overall acceptability were significantly higher ($p<0.05$) in CFFC than all the other cheese samples. Lower scores for colour/appearance in low-fat cheeses than CFFC counterpart might be related to transparent surface due to lack of fat. Sameen *et al.* (2008) also proved that high-fat cheeses possessed good appearance, whereas the appearance of low-fat cheeses was poor, rough and lacking lust. Similar observations were reported by Rudan *et al.* (1999) for Mozzarella and Koca and Metin (2004) for fresh Kashar cheeses. Also flavour, texture/tenderness and juiciness were significantly higher ($p<0.05$) in CFFC than all other low fat combinations. CFFC cheese samples got highest, whereas CLFC samples got lowest sensory scores for all the attributes. The reduction in fat content adversely affected all the sensory attributes, because fat in cheese carries much of the flavour, and when fat is reduced, there is less pronounced flavour probably due to flavour dilution and excessive moisture retention (Sipahioglu *et al.* 1999). However, supplementation of low-fat milk with CMC led to improvement of scores for the evaluated attributes. Among the CMC added samples (LFC-1, LFC-2 and LFC-3), the scores for all the sensory attributes (except colour/appearance) for LFC-2 (0.4% CMC) cheese samples were comparatively higher than LFC-1 and LFC-3 and were relatively closer to full-fat cheese i.e. control samples, so overall LFC-2 was graded as best.

Effect of carboxymethyl cellulose on the physico-chemical, oxidative stability and microbiological parameters of Mozzarella cheese stored at 4 ± 1°C for 10 days

From the above findings, it was clear that various physico-chemical, textural, colour and almost all the sensory attributes were most favourable for LFC-2 cheese sample in which 0.4% CMC was added. Hence CFFC and LFC-2 samples were selected for further storage studies. The two groups of cheese samples after packing in LDPE bags, were stored at 4 ± 1°C for 10 days. The sample was drawn on every alternate day i.e. 1, 4, 7 and 10 days and analyzed for different physico-chemical, oxidative stability, microbiological, textural, colour and sensory attributes.

Physico-chemical and oxidative stability

Perusal of Table 3 revealed that, on day 1 and 4, water activity did not vary significantly between full-fat and low-fat cheeses. However, at the end of storage (day 7 and 10), it was significantly higher ($p<0.05$) in LFC-2 than CFFC. With increase in the storage period, water activity of CFFC and LFC-2 first increased significantly till day 7, but again decreased non-significantly on day 10. There was no significant difference in pH and titratable acidity of LFC-2 than CFFC during the entire storage period. But as the storage period advanced, pH decreased and titratable acidity increased. Patel *et al.* (1986) also reported lower acidity and higher pH values in high-fat Mozzarella cheese than those from low-fat cheese. Ahmed *et al.* (2011) also observed decrease in pH and increase in titratable acidity of buffalo Mozzarella cheese during storage at 4°C for 4 weeks. Similar results regarding pH and titratable acidity were reported by Mohammed Ali and Abdel-Razig (2011) during storage of Mozzarella cheese at 4°C for 30 days. All the oxidative stability parameters i.e. free fatty acids, peroxide and TBARS values were significantly lower ($p<0.05$) in LFC-2 than CFFC samples on all the days of analysis. Moreover as the storage period advanced, there was a significant increase in the values of these parameters in both CFFC and LFC-2 samples. On day 10, the values of free fatty acids, peroxide and TBARS were 0.18%, 2.49 meq/kg and 0.41 mg MDA/kg respectively for CMC treated Mozzarella cheese. These may be due to extensive lipolysis in cheese fat. Similar relationship between fat replacer and full-fat or low-fat cheeses was observed in low-fat Feta (Katsiari and Voutsinas, 1994) and low-fat white-brined cheeses (Romeih *et al.* 2002; Kavas *et al.* 2004).

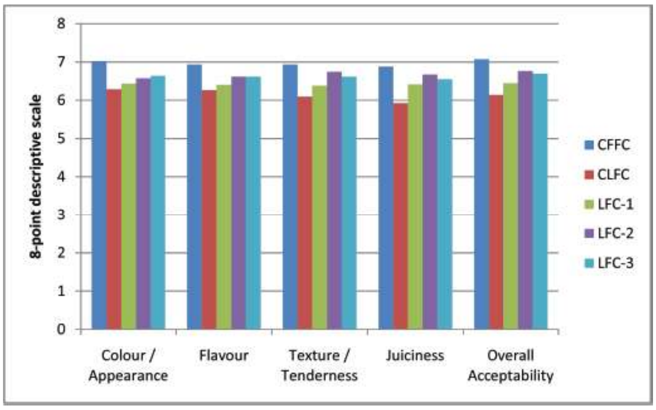

Fig. 1: Effect of different levels of carboxymethyl cellulose on sensory attributes of Mozzarella cheese. CFFC - Control full-fat cheese, CLFC - Control low-fat cheese without CMC, LFC- Low-fat cheese with 0.2% CMC, LFC-2 - Low-fat cheese with 0.4% CMC and LFC-3 - Low-fat cheese with 0.6% CMC.

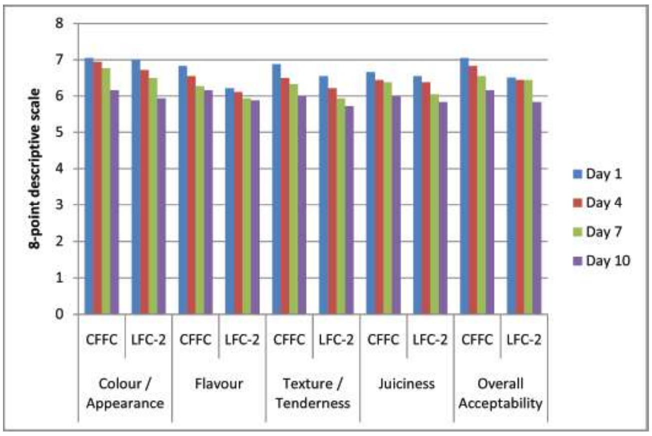

Fig. 2: Effect of carboxymethyl cellulose on the sensory attributes of Mozzarella cheese during storage at 4±1°C for 10 days. CFFC - Control full-fat cheese, CLFC - Control low-fat cheese without CMC, LFC- Low-fat cheese with 0.2% CMC, LFC-2 - Low-fat cheese with 0.4% CMC and LFC-3 - Low-fat cheese with 0.6% CMC.

Microbiological Quality

In microbiological profile, standard plate count, coliform count and psychrophile counts were significantly lower ($p<0.05$) in LFC-2 than CFFC samples on all the storage intervals (Table 3). On day 1, 4 and 7, yeast and mould counts did not vary significantly between CFFC and LFC-2 samples, but were significantly lower ($p<0.05$) in LFC-2 than CFFC on day 10. Also as the storage period progressed, there was progressive and significant increase in the values of all the microbiological counts. However, the relative increase in the values was less in LFC-2 than for CFFC batch. Coppola *et al.* (1995) also observed increase in total viable count, psychrotrophic bacteria, enterococci during storage of Mozzarella cheese. Fleet and Mian (1987) reported that increase in yeast and mould counts during storage was due to low pH, low moisture content and low temperature of cheese, all of which lead to its rapid growth. Voigt *et al.* (2010) also observed increase in yeast and mould counts of blue-veined cheese during storage at 4°C for 28 days.

Instrumental texture and colour profile

In texture profile (Table 4), hardness was found to be significantly higher ($p<0.05$) in LFC-2 batch than CFFC on all the days of storage. A significant ($p<0.05$) decrease in hardness at the end of storage (day 10) was observed than the beginning of the storage (day 1), which might be due to breakdown of α_{s1}-casein into lower molecular weight peptides in cheese and hydration of the protein matrix (Tunick *et al.* 1993). These decreases were also observed by other researchers (Lawrence *et al.* 1987; Fenelon and Guinee, 1997; Zisu and Shah, 2005). Adhesiveness did not vary significantly between CFFC and LFC-2 batches on all the storage days. Springiness of LFC-2 batch was significantly lower ($p<0.05$) than CFFC on day 4, 7 and 10, but storage was having no significant effect on springiness of Mozzarella cheese. Van Hekken *et al.* (2007) also did not observed significant difference of springiness in Mozzarella cheese, but they observed a slight increase in springiness values during storage. However, stringiness was significantly higher ($p<0.05$) in LFC-2 batch than CFFC on day 4, 7 and 10. Cohesiveness of CMC treated batch remained insignificant between different days of storage, but it was significantly higher ($p<0.05$) in LFC-2 batch than CFFC on day 4 and 7. With storage, cohesiveness and gumminess values decreased which may be due to proteolysis caused during storage. Chewiness and gumminess of LFC-2 batch was significantly higher ($p<0.05$) than CFFC on all the days of storage. There was a non-significant increase in resilience with storage, upto

7th day in CFFC and upto 10th day in LFC-2.

In colour profile (Table 4), L^* values were significantly higher ($p<0.05$) in CFFC and LFC-2 samples at the end of storage i.e. day 10 as compared to day 1. Significantly lower ($p<0.05$) L^* values were observed for LFC-2 batch than CFFC on day 4, 7 and 10. Ayyash and Shah (2011) also reported increased L^* values of low-moisture Mozzarella cheese during storage at 4°C for 27 days. a^* (-) values were significantly lower ($p<0.05$) for LFC-2 batch than CFFC on all the days of storage. b^* values were significantly lower ($p<0.05$) in LFC-2 than CFFC samples on all the storage days.

Sensory quality attributes

Among the sensory attributes on 8-point descriptive scale (Fig 2), colour/appearance did not vary significantly between CFFC and LFC-2 batches. Flavour scores were significantly higher ($p<0.05$) in CFFC than LFC-2 on all days except for day 10, where these were non-significant. Texture/tenderness scores were significantly higher ($p<0.05$) in CFFC than LFC-2 only on day 10, otherwise they were non-significant. Juiciness scores were significantly higher ($p<0.05$) in CFFC than LFC-2 only on day 7, and were non-significant on rest of the days of analysis. Overall acceptability was significantly higher ($p<0.05$) in CFFC than LFC-2 on all the days of analysis, except for day 7. As the storage period advanced, the scores for all the sensory attributes for CFFC and LFC-2 were significantly lower ($p<0.05$) on day 10 than on day 1. In general, the scores for all the sensory attributes decreased with increase in storage period. Sulieman *et al.* (2013) also observed continuous decrease in sensory scores of all the sensory attributes of Mozzarella cheese during storage at 5°C for 30 days. Though in storage studies all the parameters i.e. physico-chemical, oxidative stability, microbiological, textural and colour were favourable in case of LFC-2 batch, yet its sensory scores were comparatively less than CFFC batch which might be due to the presence of more and natural fat in full-fat cheese than CMC treated sample.

CONCLUSION

Results concluded that low-fat fresh buffalo mozzarella can be successfully manufactured with the incorporation of 0.4% carboxymethyl cellulose. The developed product has comparable physico-chemical, textural, colour and sensory attributes, and almost 45% lower calories, 58% lower TBARS on 10th day, better oxidative stability and microbiological quality than high-fat control product throughout storage.

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