J Food Sci Technol (June 2016) 53(6):2876–2886
DOI 10.1007/s13197-016-2270-4

ORIGINAL ARTICLE

Application of guar–xanthan gum mixture as a partial fat replacer in meat emulsions

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Revised: 26 May 2016 / Accepted: 29 May 2016 / Published online: 20 June 2016

Abstract The physicochemical, oxidative, texture and microstructure properties were evaluated for low fat meat emulsions containing varying levels of guar/xanthan gum mixture (1:1 ratio) as a fat substitute. Partial replacement of fat with guar/xanthan gum resulted in higher emulsion stability and cooking yield but lower penetration force. Proximate composition revealed that high fat control had significantly higher fat and lower moisture content due to the difference in basic formulation. Colour evaluation revealed that low fat formulations containing gum mixture had significantly lower lightness and higher yellowness values than high fat control formulation. However non-significant difference was observed in redness values between low fat formulations and the high fat control. The pH values of the low fat formulations containing gum mixture were lower than the control formulations (T0 and TC). The MetMb% of the high fat emulsion formulation was higher than low fat formulations. The significant increase of TBARS value, protein carbonyl groups and loss of protein sulphydryl groups in high fat formulation reflect the more oxidative degradation of lipids and muscle proteins during the preparation of meat emulsion than low fat formulations. The SEM showed a porous matrix in the treatments containing gum mixture. Thus, the guar/xanthan gum mixture improved the physicochemical and oxidative quality of low fat meat emulsions than the control formulations.

Keywords Low fat · High fat · Emulsions · Guar/xanthan gum · Oxidative stability

Introduction

The development of meat products with health promoting properties and low caloric content has become a key target for the meat industry. Animal fat contributes to essential quality attributes in reconstituted and comminuted meat products. However, current market demands products with low fat content as the high fat intake mainly saturated fat and cholesterol has been correlated with chronic disease events like cardiovascular diseases, cancer, type 2 diabetes, and others related to obesity (Felisberto et al. 2015; Decker and Park 2010; Rather et al. 2016; Santarelli et al. 2008; Schonfeldt and Gibson 2008). Some restructured meat products contain higher fat contents ranging from 20 to 30 %, revealing an interesting opportunity for reduction of this component (Calliari et al. 2015; Rather et al. 2015b; Campagnol et al. 2012). In addition to serious health concerns, onset of oxidative reactions in lipids initiates the oxidation of proteins, constitutes a major threat to meat quality. Because it can lead to organoleptic degradation of meat products and thus affect flavour, colour and cause serious health concerns (Hęś et al. 2012; Ganhão et al. 2010). However, the decrease in fat content generally implies deterioration of some important quality attributes in meat products, such as tenderness, succulence, flavour and yield (Brewer 2012; Pietrasik and Duda 2000; Carballo et al. 1995). Increasing demand for meat products with low and/or reduced fat levels has led to the development of new

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products to replace the fat in traditional formulation. Three main approaches can be applied in meat products segment to reduce the content of fat in meat products such as: (1) modifying carcass chemical composition of meat with lower fat content, (2) use of lean meat as a raw material to decrease the amount of fat in the formulation, and/or (3) replacing the fat with lower caloric ingredients and/or water (Schmiele et al. 2015; Gibis and Weiss 2015; Schuh et al. 2013; Verma and Banerjee 2010; Jiménez-Colmenero 2000). This latter depicts an alternative opportunity to add value to restructured meat products by the addition of new ingredients, aiming for development of healthier products (Bernardi et al. 2008; Huda et al. 2014; Mendoza et al. 2001). There are reports in literature on the use of a number of hydrocolloid systems in meat products as fat replacers. For example, Konjac gel (Salcedo-Sandoval et al. 2013), carrageenan (Modi et al. 2009), carrageenan and locust bean gum (Garcia-Garcia and Totosaus 2008), inulin and β-glucan (Álvarez and Barbut 2013), oat's β-glucan (Piñero et al. 2008), amorphous cellulose (Campagnol et al. 2012), olive pectin (Galanakis 2011; Galanakis et al. 2010) etc. for improving the quality of low fat meat products. In this context, industries and researchers search for new ingredients to solve the problems related to the quality of low fat meat products.

In India large number of traditional meat products like kabab, rista, goshtaba, etc. are prepared from emulsion type ground meat and are usually prepared by using mutton or beef along with salt, spices and condiments. Considerable amount of animal fat (20–30 %) is used in their formulation to achieve a stable emulsion, and also to impart a special taste and flavour to the products (Rather et al. 2015b, c; Samoon and Sharma 1991). Thus, there is great scope and need for improvements over the traditional practices followed in the formulation and preparation of meat emulsions for product development, so as to reduce the fat content to safeguard the health of consumers without altering the quality attributes and stability of the products. For effective use of ingredients as fat substitute in meat products, it is necessary to analyze the interaction of these ingredients with the meat matrix components. Use of guar and xanthan gum has been reported as a suitable component to replace fat in restructured meat products (Ulu 2006; Luruena-Martınez et al. 2004). Guar gum, (galactomannan) extracted from the seeds of the leguminous plant *Cyamopsis tetragonoloba* is a widely used polysaccharide in food industry (Mudgil et al. 2014). Due to plethora uses of guar gum such as gelling, thickening, firming and emulsifying agent, it is considered the most significant galactomannan employed industrially (Butt et al. 2007). Guar gum is also used as a source of soluble dietary fibre in food products (Roberts 2011). In addition it has some health benefits such as reduction in postprandial glycemia and insulinemia (Butt et al. 2007). Guar gum imparts a slick creamy mouth feel to meat products, which mimics fat and is being used in low fat reformulated meat products (Pearson and Gillett 1997).

Xanthan gum is an anionic microbial hydrocolloid produced through anaerobic fermentation by bacterium *Xanthomonas campestris*. Xanthan gum is completely soluble in hot or cold water, hydrates quickly once dispersed and provides water binding resulting in very high viscosity solutions at low concentration (Demirci et al. 2014; Pettit 1979). Its rheological behaviour enables to contribute to good sensory qualities, including mouth-feel and flavour release in foods and solution and is stable even during variation in pH and temperature. Recently, the potential antioxidant activity of xanthan gum attracted attention of researchers to use it in lipid model system (Trommer and Reinhard 2005). It has been reported that synergistic interaction occurs between xanthan gum and galactomannans, such as guar and locust bean gum in solutions and results in enhanced gelation (Khouryieh et al. 2015; Long et al. 2013). Therefore, the present study aimed at evaluating the effect of addition of guar/xanthan gum mixture on some important quality parameters of meat emulsions with a simultaneous reduction of fat content.

Materials and methods

Meat emulsion formulation

Fresh lean mutton and mutton fat were purchased from a selected retail meat shop located at Hazratbal Srinagar, India. All subcutaneous fat and visible connective tissues were removed from fresh muscles by a butcher knife. The meat was initially analyzed for fat content (2.08 %) prior to the manufacture of the emulsion. Lean meat and mutton fat were initially ground separately in a mincer (SIRMAN, TC 22, Italy) through an 8-mm plate and divided into five parts for various formulations, which differed in composition with respect to fat and gum concentration (Hi-Media Pvt. Ltd., Mumbai, India) (Table 1). The first part was used as high fat control and fat content was adjusted to 20 % (T0). The second part was used as low fat control (TC) and the fat content was adjusted to 10 %. The other parts were supplemented with 0.5 % (T1), 1 % (T2), and 1.5 % (T3) gum levels (guar and xanthan gum mixture 1:1 ratio) and the fat content was adjusted to 10 % with the addition of mutton fat. The lean mutton and fat were homogenized separately in a blender for 2 min and mixed uniformly with gum mixture. The remaining non-meat ingredients (salt 2.5 %, large cardamom seeds 0.2 % and cumin 0.1 %) were then added to the mixture and mixed for additional 8 min to make homogenous viscoelastic mass. The

Table 1 Composition of low fat meat emulsion formulations

Ingredients (g/1000 g)	Treatments				
	T0	TC	T1	T2	T3
Sheep meat	672	672	672	672	672
Mutton fat	200	100	100	100	100
Ice water	100	200	195	190	185
Gum mixture powder	0	0	5	10	15
Common salt (NaCl)	25	25	25	25	25
Large cardamom seeds	2	2	2	2	2
Cumin	1	1	1	1	1
Total	1000	1000	1000	1000	1000

T0: control containing fat (20 %); TC: fat (10 %); T1: fat (10 %) + gum mixture (0.5 %); T2: fat (10 %) + gum mixture (1.0 %); T3: fat (10 %) + gum mixture (1.5 %)

mixtures were reground separately in a mincer (SIRMAN, TC 22, Italy) through a 6-mm plate. The emulsion formulations were then packaged and stored at 4 °C before analysis.

Emulsion stability

The emulsion stability was performed according to Felisberto et al. (2015) with some modifications to the cooking process. The 35 g emulsion samples were stuffed into 50 ml polypropylene tubes and cooked in a water-bath from 22 to 72 °C for 1.5 h. The total amount of liquid released was expressed as a percentage of the sample weight.

Cooking yield

The emulsions were shaped into meat balls weighing 50–60 g and diameter of about 60 mm. The balls were rolled between the palms to achieve their sphericity. The meat balls from each batch were then processed in boiling water separately at 85 °C for 30 min. Cooking yield was determined by measuring weight of meat balls before and after cooking multiplied by 100 for each treatment in five replicates using electronic weighing balance.

Instrumental texture

The instrumental texture was measured using a TA-XT2 texture analyzer equipped with a 5 kg load cell, using Texture Expert V1.19 software (Stable Micro Systems, UK). The meat emulsions were stored at 4 °C for 24 h in a container (5 cm × 7 cm) for stabilization, and the force required to penetrate 2.5 cm material was measured at 2 mm/s using a 60 °C conical probe.

Proximate composition

Moisture, protein, fat and ash contents were determined according to Association of Official Analytical Chemists (AOAC 1990). The samples were analyzed in triplicate for each component.

pH value

The pH value of emulsions were measured in a homogenate prepared with 5 g of sample and distilled water (20 ml) using a pH meter (HANNA pH2). All determinations were performed in triplicate.

Instrumental colour

The colour values of the samples were determined using a Hunter Colour Lab (Mini Scan XE Plus, Model No. 45/0-L, Hunter Associate Laboratory Reston, USA). The instrument was calibrated with black and white tiles before colour measurement. The 'L*' value indicates the lightness, 0–100 representing dark to light. The 'a*' value gives the degree of the red–green colour, with a higher positive 'a*' value indicating more red. The 'b*' value indicates the degree of the yellow–blue colour, with a higher positive 'b*' value indicating more yellow. The average value of three replicates was reported.

Metmyoglobin (%)

Meat samples (5 g) were homogenized in 25 ml ice-cold 40 mM phosphate buffer (pH 6.8) for 10 s using a Virtis homogenizer (The Virtis Co., Gardiner, NY). The homogenate was allowed to stand for 1 h at 4 °C and centrifuged at 4500×*g* for 30 min at 4 °C. The supernatant was filtered through Whatman No. 1 filter paper and absorbance measured at 572, 565, 545 and 525 nm using an UV–Vis scanning spectrophotometer (UV-Spectrophotometer, Model U-2900 2JI-0003, Hitachi, Japan). The metmyoglobin percent was calculated as described by Bekhit et al. (2003) using the formula:

$$\begin{aligned}\text{MetMb}\% = \{&-2.51 * (A_{572}/A_{525}) + 0.777 * (A_{565}/A_{525}) \\ &+ 0.8(A_{545}/A_{525}) + 1.098\} * 100\end{aligned}$$

Lipid oxidation (TBARS)

Oxidative stability was evaluated by changes in thiobarbituric acid reactive substances (TBARS). The procedure for measurement of TBARS was based on methods used by Serrano et al. (2006). Five gram sample was homogenized in 35 ml of 7.5 % trichloroacetic acid. The homogenized

sample was centrifuged (3000×*g*, 2 min) and 5 ml of the supernatant was mixed with 5 ml of 20 mM thiobarbituric acid and kept in dark for 20 h at 20 ± 1.5 °C. The pink color that formed was measured spectrophotometrically (UV-Spectrophotometer, Model U-2900 2JI-0003, Hitachi, Japan) at 532 nm. A calibration curve was plotted with 1, 1, 3, 3-tetraethoxypropane to obtain the malonaldehyde (MDA) concentration and results were expressed as mg MDA/kg of sample. TBARS determinations for each sample were performed in triplicate.

Protein oxidation measurement

Protein carbonyls

Protein carbonyls were measured by estimation of total carbonyl groups according to the method of Levine et al. (1990) with some modifications as described by Srinivasan and Hultin (1995). From two fractions of 50 µl protein samples, one aliquot was treated with 2 ml of 2.0 N HCl (control) and the other was treated with 2.0 ml of 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2.0 N HCl for 1 h at room temperature. After incubation, the two fractions were then precipitated with 2.0 ml of 20 % trichloroacetic acid. The precipitate was washed twice with 4.0 ml of ethanol: ethylacetate (1:1, v/v) solution to remove unreacted DNPH and blow-dried. The pellet was then dissolved in 1.5 ml of 6.0 M guanidine hydrochloride with 20 mM potassium phosphate buffer (pH 2.3). Absorbance was measured spectrophotometrically at 370 nm. The amount of protein carbonyl content was expressed as nmol of mg protein using an absorption coefficient of 2.2×10^4 M^{-1} cm^{-1} for protein hydrazones.

Sulphydryl groups

Sulphydryl groups (thiol content) were determined according to the method described by Srinivasan and Hultin (1997). Total free sulphydryl groups were determined by reacting with 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB). One gram of meat was blended with 50 ml of cold distilled water and homogenized. Protein concentration of homogenate was diluted to 2 mg/ml with 0.1 M phosphate buffer (pH 7.4) and protein content was determined using the Kjeldhal method. A 0.5 ml of homogenate was transferred to a tube and dissolved in urea buffer (1:1). After incubation with 0.5 ml DTNB reagent at room temperature for 15 min, absorbance was measured at 412 nm. Sample blanks with 0.5 ml phosphate buffer without DTNB and reagent blanks with only water were prepared. Sulphydryl content was calculated using a molar extinction coefficient of 11,400 M^{-1} cm^{-1} for 5, 5′-dithiobis at this wavelength. Results were expressed as nmol of total free sulphydryl groups per milligram of protein.

Microstructure

Microstructure was analyzed by scanning electron microscopy (SEM) as reported by Jiménez-Colmenero et al. (1995). The samples were fixed with a mixture (1:1 v/v) of paraformaldehyde (4 g/100 g) and glutaraldehyde (0.2 g/100 g) in 0.1 M phosphate buffer pH 7.2, post-fixed with OsO_4, washed, dehydrated in increasing concentrations of acetone, critical point-dried, sputter-coated with gold/palladium in a metallizer (Blazer, SCD004) and scanned by SEM (S-3000 H, Hitachi, Japan) at 5.0 kV. A large number of micrographs were taken in order to select the most representative ones.

Statistical analysis

Mean values, standard deviation, analysis of variance were computed using a commercial statistical package SPSS 16 (USA). The data were then compared using Duncan's multiple range tests at 5 % significance level.

Results and discussion

Emulsion stability

Stability of meat emulsions is an important parameter that influences the quality of restructured meat products and percent fluid release is considered as an important index of emulsion stability. The percent fluid release of the emulsions with varying levels of fat and gum mixture is presented in Table 2. The lowest emulsion stability with high levels of fluid release was observed in the low fat control (TC) formulation ($p < 0.05$). The higher fluid release can be attributed to the larger amounts of water being added, coupled with more compact gel formation thus, contributing to a greater liquid release after cooking (Felisberto et al. 2015). The high fat control formulation showed a non-significant difference with that of low fat formulations containing 0.5 % (T1) and 1.0 % (T2) gum mixture ($p > 0.05$). Gum mixture at 1.5 % level (T3) significantly improved the emulsion stability by a less fluid release than other formulations including high fat control ($p < 0.05$). This may be due to the higher moisture and fat retention capacity of gums during cooking. This observation leads to conclude that gum mixture performs a role in stability of low fat emulsions.

Cooking yield (%)

The cooking yield of the emulsion formulations is shown in Table 2. The low fat formulation containing 1.5 % gum mixture showed significantly higher cooking yield than all

Table 2 Effect of gum mixture on fluid release (%), penetration force (N) and cooking yield (%) of low fat meat emulsions

Treatments	Fluid release (%)	Penetration force (N)	Cooking yield (%)
T0	24.48 ± 0.51^{b}	14.44 ± 0.36^{c}	93.94 ± 0.54^{c}
TC	27.43 ± 0.43^{c}	14.08 ± 0.43^{c}	87.17 ± 1.12^{a}
T1	25.03 ± 0.54^{b}	13.21 ± 0.02^{b}	91.18 ± 0.32^{b}
T2	24.09 ± 0.09^{ab}	12.72 ± 0.20^{ab}	95.04 ± 0.20^{c}
T3	23.00 ± 1.79^{a}	11.79 ± 0.98^{a}	97.33 ± 0.21^{d}

All values are mean ± standard deviation of three replicates

T0: control containing fat (20 %); TC: fat (10 %); T1: fat (10 %) + gum mixture (0.5 %); T2: fat (10 %) + gum mixture (1.0 %); T3: fat (10 %) + gum mixture (1.5 %)

Means in the same column with different superscripts differ significantly: * $p < 0.05$

other formulations ($p < 0.05$). Non-significant difference was observed between low fat formulation containing 1 % gum mixture and high fat control ($p > 0.05$). The low fat control formulation showed significantly lower cooking yield than the high fat control and low fat formulations containing varying levels of gum mixture ($p < 0.05$). The lower cooking yield in low fat control could be due to the excessive fat separation and water release during cooking (Pietrasik and Janz 2010). Cooking yield increased significantly with the increasing concentrations of gum mixture ($p < 0.05$). Increase in cooking yield occurred due to the high water and fat holding and binding capacity of gum mixture, thus retaining moisture and fat in the matrix (Rather et al. 2015c). Similar results were also observed by earlier workers (Galanakis et al. 2010) wherein, olive pectin was used to improve the cooking properties of meat balls. With respect to this property, partial substitution of fat by guar/xanthan gum mixture is a possible strategy in low fat restructured meat products to retain their quality. The higher cooking yields could thus create economically profitable products.

Instrumental texture

Texture is an important organoleptic attribute of restructured meat products and is related to their ability to bind water and fat during preparation of emulsions and retention of water during cooking. The addition of gum mixture results in significant changes in texture of meat emulsions (Table 2). The results indicate that control formulations (T0 and TC) showed significantly higher penetration force than the low fat formulations with added gum mixture ($p < 0.05$). Formulations containing 0.5 % (T1) and 1 % (T2) gum mixture exhibited non-significant difference in penetration force ($p > 0.05$). The low fat formulation containing 1.5 % (T3) gum mixture exhibited significantly lower penetration force than all other formulations including high fat control ($p < 0.05$). The variation in penetration force in meat emulsions may be related to the water-holding capacity of gum mixture (Horita et al. 2011). The low fat formulation without gum mixture exhibited higher penetration force than low fat formulations with added gum mixture ($p < 0.05$). The higher penetration force of low fat control is thus correlated with lower cooking yield and higher liquid release. These results also highlight the role of fat in the texture of an emulsified meat network. The decreased penetration force of the low-fat formulations may be due to reduction in fat content, high water content and high water holding capacity of gum mixture while keeping protein level virtually constant (Pietrasik and Janz 2010).

Proximate composition

The proximate composition of the emulsions is presented in Table 3. The moisture content of high fat emulsion was significantly lower than its low fat counterparts. The variation in the moisture content is due to initial substitution of fat with water while formulating the emulsion (Table 1). Whereas, non-significant difference was observed in the moisture content between low fat control and formulations containing varying concentrations of gum mixture ($p > 0.05$). The low moisture content of high fat control is because of variation in original composition of emulsion. The moisture content was inversely proportional to the fat content and this is due to substitution of fat by moisture in the low fat products (Pietrasik and Janz 2010; Pietrasik and Duda 2000). The protein content of the formulations ranged from 10.97 to 11.48 % and non-significant difference was observed between high fat control and low fat formulations with and without gum mixture ($p > 0.05$). This is because there is no variation in the initial meat protein content of the formulations. The fat content of high fat control (T0) formulation was significantly higher than low fat formulations ($p < 0.05$), because fat was replaced by water and gum in the original formulation. The ash content of the emulsion samples ranged from 3.01 to 3.10 % and did not show any significant difference between high fat control and low fat counterparts ($p > 0.05$).

Table 3 Proximate composition of low fat meat emulsions formulated with varying levels of gum mixture

Treatments	Protein content (%)	Moisture content (%)	Fat content (%)	Ash content (%)
T0	11.33 ± 0.74[a]	67.02 ± 1.02[a]	19.11 ± 0.89[b]	3.09 ± 0.09[a]
TC	11.48 ± 1.04[a]	76.54 ± 0.545[b]	9.23 ± 0.3[a]	3.03 ± 0.01[a]
T1	11.43 ± 0.59[a]	76.82 ± 0.421[b]	9.21 ± 0.24[a]	3.10 ± 0.14[a]
T2	11.48 ± 0.43[a]	76.35 ± 1.01[b]	9.2 ± 0.242[a]	3.02 ± 0.02[a]
T3	10.97 ± 0.54[a]	76.47 ± 0.53[b]	9.2 ± 0.14[a]	3.01 ± 0.05[a]

All values are mean ± standard deviation of three replicates

T0: control containing fat (20 %); TC: fat (10 %); T1: fat (10 %) + gum mixture (0.5 %); T2: fat (10 %) + gum mixture (1.0 %); T3: fat (10 %) + gum mixture (1.5 %)

Means in the same column with different superscripts differ significantly: * $p < 0.05$

Instrumental colour and pH

It is very important to study the colour behaviour in the meat emulsions, because the colour parameter significantly influences consumer acceptance and facilitates prediction of changes occurring in the final product. The results for colour determination are given in Table 4. The high fat control (T0) had exhibited significantly higher lightness value (L*) than the low fat formulations ($p < 0.05$). However, no significant difference was observed in low fat formulations with different concentrations of gum mixture ($p > 0.05$). Decrease in lightness value of the low fat formulations could be due to the reduction in fat content as compared with the high fat formulation (Salcedo-Sandoval et al. 2013). Significantly higher redness (a^*) value was observed for the low fat control (TC) formulation than the other formulations including high fat control ($p < 0.05$). The low fat formulations containing gum mixture did not differ significantly in redness from the high fat control ($p > 0.05$). Low fat control formulation was expected to be more red because of the concentration of lean meat (Rather et al. 2015a). This difference may also be explained by the different physico-chemical characteristics (fat and moisture) of the meat emulsions. The yellowness value (b^*) was higher in low fat emulsions with varying concentrations of gum mixture (T1, T2 and T3) whereas lower in low fat control formulation ($p < 0.05$).

The pH value of the meat emulsions is presented in Table 4. The pH value of the low fat formulations decreased with the increased concentration of gum mixture and was significantly lower in the formulation containing 1.5 % gum mixture ($p < 0.05$). The highest pH value was observed in high fat control formulation ($p < 0.05$). The decrease in pH of the low fat formulations containing gum mixture might be due to the presence of pyruvic acid residue in xanthan polysaccharide thus results in reduction of pH (Khouryieh et al. 2015; Sharma et al. 2006).

Metmyoglobin content

The accumulation of metmyoglobin (MetMb) at the meat surface is the major factor that results in a gradual discolouration (red to brown) of uncooked meat. Reduction of MetMb has been shown in vitro to occur under specific conditions and depends upon MetMb reducing activity and the availability of cofactors (Arihara et al. 1995; Hagler et al. 1979). In fresh meat, Mb exists in three forms—deoxymyoglobin [deoxy (Fe^{2+}) Mb], oxymyoglobin [oxy (Fe^{2+}) Mb], and metmyoglobin [met (Fe^{3+}) Mb]. The addition of antioxidant compounds to uncooked meat

Table 4 Effect of gum mixture on colour and pH of low fat meat emulsions

Treatments	Colour			pH
	L*	a*	b*	
T0	51.67 ± 0.580[c]	13.49 ± 0.255[a]	8.22 ± 0.225[b]	5.90 ± 0.35[e]
TC	40.41 ± 0.771[a]	15.47 ± 0.561[b]	7.08 ± 0.132[a]	5.88 ± 0.08[d]
T1	45.42 ± 2.535[b]	14.23 ± 0.677[a]	9.19 ± 0.076[c]	5.64 ± 0.046[c]
T2	45.08 ± 1.646[b]	14.10 ± 0.364[a]	9.24 ± 0.837[c]	5.52 ± 0.026[b]
T3	44.31 ± 0.957[b]	14.00 ± 0.300[a]	9.32 ± 0.629[c]	5.39 ± 0.050[a]

All values are mean ± standard deviation of three replicates

T0: control containing fat (20 %); TC: fat (10 %); T1: fat (10 %) + gum mixture (0.5 %); T2: fat (10 %) + gum mixture (1.0 %); T3: fat (10 %) + gum mixture (1.5 %)

Means in the same column with different superscripts differ significantly: * $p < 0.05$

retards MetMb formation. The MetMb% of the uncooked meat emulsions is presented in Table 5. The reduction of fat content in meat emulsions significantly affected the percent MetMb formation during processing ($p < 0.05$). The high fat control (T0) exhibited significantly higher MetMb% than the low fat formulations ($p < 0.05$). The MetMb% of low fat control (TC) and formulations containing 0.5 and 1 % gum mixture (T1 and T2) showed non-significant difference ($p > 0.05$). However, the low fat formulation containing 1.5 % gum mixture showed significantly lower MetMb% ($p < 0.05$) than other formulations. Although the processing of the emulsions was conducted with good practice, the higher MetMb content of the high fat control might be due to the more oxidative reactions between lipids and Mb during processing. The lower MetMb% of low fat formulations could be due to the lower fat content used in the original formulation (Table 1) and the antioxidant activity of gum mixture (Khouryieh et al. 2015; Chen et al. 2010; Wanga et al. 2012). The discolouration of meat emulsions is generally attributed to the oxidation of ferrous heme–iron (Fe^{2+}) into its ferric form (Fe^{3+}) induced by lipid oxidation products (Yin and Faustman 1993). Under these circumstances, oxymyoglobin is transformed into MetMb and the colour shifts from a pleasant bright red to an undesirable brownish colour.

TBARS value

Lipid oxidation was evaluated by the levels of TBARS that developed during processing of meat emulsions (Table 5). The high fat control formulation (T0) exhibited significantly higher TBARS value ($p < 0.05$) which might be probably due to the higher fat content used in the original formulation of emulsion preparation. This increase in TBARS indicated formation of secondary lipid oxidation products which will contribute to the off-odour development in the meat (Rather et al. 2015a). The low fat formulation containing 1.5 % gum mixture (T3) showed lower TBARS value ($p < 0.05$) whereas, the low fat formulations containing 0.5 % and 1.0 % gum mixture (T1 and T2) did not show any significant difference in TBARS value ($p > 0.05$). The low fat control formulation showed significantly higher TBARS value than the formulations containing varying concentrations of gum mixture ($p < 0.05$). The lower TBARS values of the low fat emulsions formulated with gum mixture may be due to the ability of gums to inhibit the inactive peroxyl radicals by chelation of transition metals like iron in the products (Khouryieh et al. 2015; Chen et al. 2010). Several researchers reported that xanthan gum suppress the lipid oxidation by chelation of iron between two side chains with a pyruvate residue and therefore inactivating the peroxyl radicals (Faraji et al. 2004; Donelly et al. 1998; Mei et al. 1998). The initial TBARS values suggesting that lipid oxidation had occurred during postmortem handling and processing of meat emulsions to some extent.

Protein oxidation measurement

Protein carbonyls

Carbonyl content was measured to evaluate the degree of protein oxidation during postmortem handling and processing of meat emulsions. Carbonyl compounds are formed by the preferential attack of reactive oxygen species on the side chain of amino acid residues, cause proteins to have a loss of catalytic activity and increased susceptibility to proteolytic degradation (Stadtman 1990). Oxidation of proteins in fresh meat results in reduced functionality of myofibrillar proteins and affect water binding capacity and thereby reduce juiciness of meat and meat products (Huff-Lonergan and Lonergan 2005). The most abundant meat protein, myosin, is highly susceptible to oxidation and is known to polymerize through intermolecular cross-linking in model systems (Ooizumi and

Table 5 Effect of gum mixture on metmyoglobin (%), TBARS, carbonyl content and sulphydryl content of low fat meat emulsions

Treatments	Metmyoglobin (%)	TBARS (mg MDA/kg)	Carbonyl content (nmol/mg protein)	Sulphydryl content (nmol/mg protein)
T0	38.20 ± 0.93^{c}	0.27 ± 0.015^{d}	0.90 ± 0.02^{c}	59.00 ± 2.00^{a}
TC	36.48 ± 0.74^{b}	0.23 ± 0.011^{c}	0.86 ± 0.01^{b}	60.20 ± 1.74^{ab}
T1	35.02 ± 1.245^{b}	0.20 ± 0.015^{b}	0.84 ± 0.005^{b}	62.01 ± 1.755^{bc}
T2	35.00 ± 0.273^{b}	0.19 ± 0.011^{b}	0.80 ± 0.020^{a}	62.64 ± 0.481^{bc}
T3	33.00 ± 0.366^{a}	0.16 ± 0.015^{a}	0.77 ± 0.023^{a}	63.78 ± 1.475^{c}

All values are mean ± standard deviation of three replicates

T0: control containing fat (20 %); TC: fat (10 %); T1: fat (10 %) + gum mixture (0.5 %); T2: fat (10 %) + gum mixture (1.0 %); T3: fat (10 %) + gum mixture (1.5 %)

Means in the same column with different superscripts differ significantly: * $p < 0.05$

Xiong 2004; Hanan and Shaklai 1995; Decker et al. 1993; Bhoite-Solomon et al. 1992) and influence meat quality negatively. The protein oxidation by carbonyl group measurements of emulsion formulations are presented in Table 5. The high fat control exhibited significantly higher carbonyl group content than its low fat counterparts with or without gum mixture ($p < 0.05$). The higher carbonyl content in high fat control emulsion could be due to the high fat content used and protein carbonyl group formation is initiated during the processing of meat emulsions. Estévez and Cava (2004) reported that the protein carbonylation is closely related to the occurrence of other biochemical changes such as the increase of lipid oxidation. According to Soyer et al. (2010) the primary and secondary lipid oxidation products can act as substrates for protein oxidation, so once the oxidation of lipids starts, the oxidation of proteins will also occur. Among the low fat emulsions the carbonyl groups decreased with the increased concentration of gums (0.5–1.5 %) and low fat formulations containing 1 and 1.5 % gum mixture had significantly lower carbonyl contents ($p < 0.05$). The decrease in carbonyl content with increasing levels of gum mixture levels (0.5–1.5 %) might be due to the antioxidant activity of gums (Khouryieh et al. 2015; Wanga et al. 2012). This was probably due to the chelation of heme and non-heme iron as well as suppression of lipid oxidation by pyruvate residues of xanthan gum (Khouryieh et al. 2015; Chen et al. 2010; Ganhão et al. 2010; Estévez et al. 2008a, b; Salminen et al. 2008; Kroger-Ohlsen et al. 2003).

Sulphydryl groups

Sulphydryl groups are one of the most reactive functional groups in proteins and oxidation of proteins is associated with a decrease in sulphydryl groups (Soyer et al. 2010). The sulphydryl group of cysteine (RSH) is highly susceptible to oxidation. The oxidation of sulphydryl groups leads to a series of complex reactions resulting in formation of various oxidized products such as sulfenic acid (RSOH), sulfinic acid (RSOOH), and disulfide cross-links (RSSR). The protein oxidation measurements by loss of sulphydryls of meat emulsions are presented in Table 5. The high fat control emulsion had significantly highest loss of sulphydryls ($p < 0.05$), which can be due to more reactive oxygen species generation during processing of emulsions due to high fat content (20 %) used in the formulation, and proteins are targeted by reactive oxygen species. This interaction leads to the loss of sulphydryl groups from the proteins (Soyer and Hultin 2000; Xiong 2000; Stadtman 1990). Loss of total sulphydryl groups decreased with the incorporation of gums (0.5–1.5 %) and the formulation containing 1.5 % gum mixture showed lowest loss of sulphydryl groups. The decrease in loss of sulphydryl groups with the addition of guar/xanthan gum can be due to the antioxidant activity of gums and thus results in the suppression of protein oxidation (Xiong et al. 2013; Wanga et al. 2012).

Scanning electron microscopy

Scanning electron microscopy of meat emulsions provides high-resolution imaging of fine surface morphology and is an important determinant of its functional properties, such as WHC and to support texture results. The morphology of the high fat control emulsion showed the formation of cavities of different sizes producing structures with a honeycomb-like appearance and with spheres suspended in the protein matrix (Fig. 1a). These spheres may be fat globules and the cavities may have been due to the expansion of water, fat and air (Felisberto et al. 2015; Salcedo-Sandoval et al. 2013). The morphology of low fat control emulsion showed more compact structure without spongy appearance and may be due to the lower fat content added and formation of more dense protein network (Fig. 1b). The morphological characteristics observed in the low fat control conferred textural changes, so that it showed higher penetration force than low fat formulations added with gum mixture. The formulations containing varying levels of gum mixture showed less dense and less compact structures with similar porosity to the high fat control formulation and with the presence of empty spaces than low fat control emulsion (Fig. 1c–e). The high proportion of empty spaces can be interpreted as additional water retained due to gum mixture addition (Felisberto et al. 2015). Such an evolution of microstructure can explain the change in the water holding capacity, textural properties (penetration force) of meat emulsions. As the concentration of gum mixture increased (0.5–1.5 %) the gel network increased and this resulted in increase in binding ability and water holding capacity of low fat emulsion formulations (Ayadi et al. 2009). Significant decrease was observed in fluid release and increase in cooking yield at high levels of gum mixture addition (1.5 %) due to the high water and fat holding capacity.

Conclusion

In conclusion the partial replacement of fat by gum mixture in meat emulsions could be used to develop healthier restructured meat products with reduced fat content. Guar/xanthan gum incorporation in low fat emulsion formulations resulted in higher emulsion stability and cooking yield, thus creating economically profitable products. Proximate composition varied only due to the difference in basic formulation. The addition of guar/xanthan gum

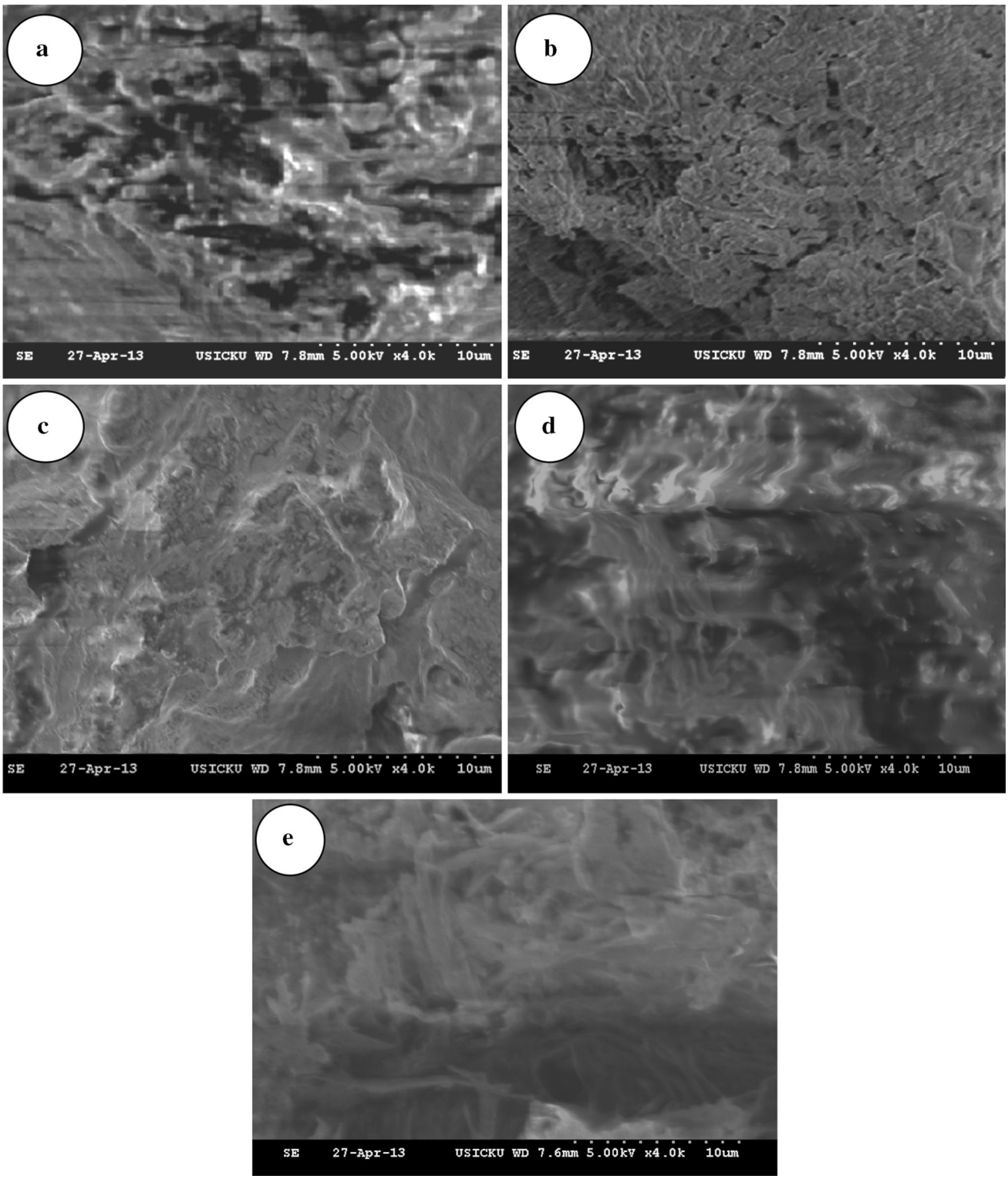

Fig. 1 Scanning electron micrographs (SEM) of low fat meat emulsions formulated with varying levels of gum mixture: **a** T0: control containing fat (20 %); **b** TC: low fat control (10 %); **c** T1: fat (10 %) + gum mixture (0.5 %); **d** T2: fat (10 %) + gum mixture (1.0 %); **e** T3: fat (10 %) + gum mixture (1.5 %)

mixture resulted in lower MetMb%, TBARS value, protein carbonyls and loss of protein sulphydryl groups during meat emulsion processing. The SEM showed a porous matrix in the treatments containing gum mixture resulting in higher water holding capacity. The study concluded that addition of guar/xanthan gum mixture improved the

stability of meat emulsions on the basis of technological and oxidative properties, thus, affecting the organoleptic properties of the products and the consumer acceptability.

Acknowledgments The authors are thankful to Department of Biotechnology, Govt. of India, for their financial support.

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