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ORIGINAL ARTICLE

Effects of microbial transglutaminase, fibrimex and alginate on physicochemical properties of cooked ground meat with reduced salt level

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Abstract Effects of microbial transglutaminase (MTGase), fibrin/thrombin combination (fibrimex), alginate or combination of these binding agents on physicochemical parameters of cooked ground beef with reduced salt level were investigated. Seventeen treatments included three control (no binding agent) groups incorporated with varying concentrations of salt (0.5, 1, 2%, w/w) and fourteen treatment groups produced with MTGase or fibrimex or alginate or their combinations at 0.5 or 1% salt levels. The samples were analyzed for cooking loss (CL), pH, color, moisture, fat, protein, ash, salt, texture and TBARS. The results indicated that the use of MTGase or fibrimex or MTGase/fibrimex combination had significant effect on preventing textural deterioration caused by salt reduction. Even though the use of MTGase resulted in higher CL values, formulation of ground beef with fibrimex or alginate or MTGase/fibrimex/alginate combinations reduced CL when compared with the control groups. The use of fibrimex in ground beef resulted in a decrease in TBARS, lightness, redness and pH values. However, the use of alginate caused an increase in pH, lightness and redness values of ground beef. Based on the present study, the use of fibrimex or a combination of fibrimex with MTGase in the product formulation can be an effective strategy to reduce cooking loss, to improve or maintain the textural properties and to extend shelf life of cooked ground beef with reduced salt level.

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Introduction

Salt is one of the most important ingredients used to inhibit microbial growth and to ensure good flavor and texture in meat products. However, it is well established that there is a strong relationship between salt intake and hypertension which is a major risk factor in the development of cardiovascular disease (Askin and Kilic 2009). Therefore reducing dietary salt intake has been recommended to improve the human diet (Desmond 2006). However, there is a concern about quality deteriorations in meat products due to the reduction of salt. Since salt is used to extract myofibrillar proteins which associate into a gel when heated, the elimination or reduction of salt in the manufacture of meat products would negatively impact texture as well as water-holding capacity, fatbinding, flavor, stability and shelf life of meat products (Verma and Banerjee 2012; Desmond 2006). Therefore, new alternatives have been searched for salt substitute in meat products with reduced salt level.

Meat and meat product consumption is more influenced by health and nutritional considerations (da Fonseca and Salay 2008). Efforts to reduce sodium content of meat products are important to the consumers. To meet consumer health demands, meat industry is focusing on the development of strategy to reduce the use of salt in processed meat products without impacting product quality. There are many changes underway in the meat industry and this is reflected in the ingredients area as well. New ingredients applications and ideas are frequently developed in response to new product types, shelf life and consumer

demands. Several cold-set binding systems have been developed to meet the demand for restructured meats or improving the texture of the final products (Bhaskar Reddy et al. 2015). Cold-set binders such as fibrinogen/thrombin systems, alginate, and microbial transglutaminase can be applied in the manufacture of meat products with reduced salt level to avoid quality deterioration due to salt reduction.

Microbial transglutaminase enzyme (MTGase) is a Ca^{++} independent enzyme catalyzes cross-linking between protein molecules (Yokoyama et al. 2004). Microbial transglutaminase catalyses an acyl transfer reaction between the γ-carboxyamide group of peptide bound glutamine residues and a variety of primary amines, including the ε-amino group of lysine residues in certain proteins (Kieliszek and Misiewicz 2014). The enzyme catalyses formation of intermolecular glutamyl lysine cross-linking reactions resulting large polymeric protein molecules from small protein substances (Aktaş and Kılıç 2005). Fibrimex is blood plasma derived product which contains extracted plasma thrombin and fibrinogen (Lennon et al. 2010). The binding mechanism is based on blood clotting process that is conversion of fibrinogen into fibrin monomer by thrombin. When two components are mixed and used on the meat pieces, the thrombin enzyme converts fibrinogen to fibrin. Fibrin molecules become cross-linked by the action of transglutaminase enzyme (present in the partially-purified fibrinogen) which also cross-links fibrin to collagen in the meat (Lennon et al. 2010; Tseng et al. 2006; Boles and Shand 1999). Sodium alginate is an anionic polysaccharide composed of mannuronic and guluronic acid monomer units. It is generally used in combination with a source of divalent cations, e.g. calcium carbonate supplying Ca^{++}, and a weak acidifier, to accelerate the release of calcium. Cross-linking to form a gel occurs between Ca^{++} ions and the guluronic acid moieties of alginate (Lee and Mooney 2012).

The purpose of this study was to investigate effects of microbial transglutaminase, fibrimex, alginate and their combinations on the quality of cooked ground meat with reduced salt level.

Materials and methods

Materials

Fresh skinless, boneless beef (*Musculus longissimus dorsi*) cattle were obtained from a local slaughterhouse (Gülköy Meat Plant, Isparta, Turkey) for each of three replications on separate production days. The age of the meat was controlled among replications and was no more than 5 days postmortem upon receipt. Raw meat was trimmed of visible fat and connective tissue. Each allotment of meat for a given replication was vacuum bagged and frozen (−18 °C) until needed.

MTGase was obtained from Ajinomoto Co. (Hamburg, Germany). The transglutaminase ACTIVA WM was used in a freeze-dried form containing 99 g/100 g maltodextrin and 1 g/100 g MTGase (activity of approx. 100U/g) (Anon. 2002). Fibrimex and sodium alginate were provided by Sonac Co. (Germany) and Sigma-Aldrich Co respectively. Sucrose, Tris, EDTA, propyl gallate, trichloroacetic acid, thiobarbituric acid, KCl, glycerin, sodium dodecyl sulfate (SDS), 2-mercaptoethanol, bromphenol blue, coomassie brilliant blue R-250, methanol and acetic acid were provided by Sigma Chemical Co. (St. Louis, MO, USA). All chemicals used were at least reagent grade.

Sample preparation

After thawing, the meat was ground (9.5 mm), mixed in a bowl mixer and then reground (3.2 mm). All treatments contained 10% added distilled water (meat weight basis). After the first grind and the test ingredients were incorporated using a hand mixer according to the formulations shown in Table 1. Ground meat samples were cooked in capped plastic centrifuge tubes (50 mL). Approximately 45 g of ground meat was placed into each tube and heat processed in a water bath. A cooking endpoint temperature was determined by

Table 1 Formulations of ground beef treatment groups

Groups	Formulation
C1	2% NaCl
C2	1% NaCl
C3	0.5% NaCl
M1	1% NaCl + 1% MTGase
F1	1% NaCl + 5% FB
A1	1% NaCl + 0.5% AL + 0.18% CC
MF1	1% NaCl + 1% MTGase + 5% FB
MA1	1% NaCl + 1% MTGase + 0.5% AL + 0.18% CC
FA1	1% NaCl + 5% FB + 0.5% AL + 0.18% CC
MFA1	1% NaCl + 1% MTGase + 5% FB + 0.5% AL + 0.18% CC
M2	0.5% NaCl + 1% MTGase
F2	0.5% NaCl + 5% FB
A2	0.5% NaCl + 0.5% AL + 0.18% CC
MF2	0.5% NaCl + 1% MTGase + 5% FB
MA2	0.5% NaCl + 1% MTGase + 0.5% AL + 0.18% CC
FA2	0.5% NaCl + 5%FB + 0.5% AL + 0.18% CC
MFA2	0.5% NaCl + 1% MTGase + 5% FB + 0.5% AL + 0.18% CC

NaCl sodium chloride, *MTGase* microbial transglutaminase, *FB* fibrimex, *CC* calcium carbonate, AL alginate

inserting thermocouples (TK100S, Kimo Instruments, France) into the geometric center of extra sample tubes. Samples were cooked to 74 °C. Cooked ground meat samples were stored in tubes at 4 °C for 15 days after decanting of the cookout liquid. Cooking loss, protein, fat, moisture, ash, salt and texture analysis were carried out on production day. Other samples were stored at 4 °C (samples for pH, color and TBARS measurements) and at −28 °C (samples for SDS-PAGE). pH, color and TBARS measurements were performed on days 0, 7 and 15 during storage.

Cooking loss

Cooking loss was determined as described by Kılıç et al. (2014). Cooked ground meat (in triplicate) was removed from the centrifuge tube and rolled over a paper towel to remove any excess liquid. The cooked ground meat weight was determined after the cooked sample was cooled to room temperature (approximately 25 °C). Cooking loss was determined using the following equation:

$$\text{Cooking loss percentage} = \frac{(\text{weight of raw ground meat} - \text{weight of cooked groundmeat})}{(\text{weight of raw ground meat})} \times 100.$$

SDS-polyacrylamide gel electrophoresis

Cooked ground beef samples were analyzed on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli 1970) to confirm the intermolecular covalent cross-link. Sample solutions for SDS-PAGE were prepared as follows. The samples were ground into powder using liquid nitrogen. A 20 mg sample from each group was mixed with 1 ml SDS-urea buffer (8 mol/l urea, 2 mol/l thiourea, 0.05 mol/l Tris (pH 6.8), 75 mmol/l DTT, 3 g/100 g SDS, 0.05 g/100 g bromophenol blue). The mixture was diluted with 1 ml distilled water, sonicated 1 min, heated in a block heater set at 100 °C for 3 min, and stored at −80 °C before use. An 8% (g/l) acrylamide gel was loaded with 7.5 µl sample solution and electrophoresis carried out at a constant current of 20 mA for 1 h. The gel was stained with 0.1 g/100 g Coomassie brilliant blue R-250 in methanol:acetic acid:H_2O (5:1:4 by volume). The gel was destained by soaking in 10 ml/100 ml methanol-7.5 ml/100 ml acetic acid.

Physicochemical composition

The pH was determined using spear electrode (FC 200, Hanna Instruments, Germany) attached to a portable pH meter (HI 9024, Hanna Instruments, Germany). Meter was calibrated against 4 and 7 pH buffer standards. The pH of cooked samples was measured. Color measurement (in triplicate) was taken with a Hunterlab model Precise Color Reader TCR 200 (BAMR Ltd, Claremont, South Africa) colorimeter. L*, a*, b* values were determined at manufacturing day, during 7 and 15 days of storage. The moisture (AOAC 950.46), ash (AOAC 920.153), protein (AOAC 992.15), fat (AOAC 991.36) and salt (AOAC 935.47) contents in all the samples were determined according to the AOAC (1995) methods. Moisture, ash, protein, fat and salt measurements were repeated three times for each group. For texture profile analysis (TPA), the samples were cut into cylinders with height 10 ± 0.5 mm, wrapped with plastic, and held for equilibration to room temperature (20 °C). TPA tests were performed using a TA.XC1 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, UK) to determine hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience. Test conditions were: aluminium rectangular probe (5 cm × 4 cm); test speed 5 mm/s; pre-test speed 2 mm/s, post-test speed 2 mm/s; compression 70%; and 50 kg load cell.

Thiobarbituric acid reactive substances (TBARS) analysis

TBARS were determined in duplicate from each sample using the muscle extraction procedure of Lemon (1975). This method requires addition of EDTA and propyl gallate to the trichloroacetic acid (TCA) extraction solution to prevent the development of TBARS during the analytical procedure. A meat sample (1 g) was blended into 6 mL of extraction solution. The samples were homogenized with the polytron homogenizer for 15 s at high speed. The homogenate was filtered through Whatman 1 filter paper. Filtrate (1 mL) was mixed with 1 mL of thiobarbituric acid (TBA) and vortexed. The mixture was heated at 100 °C for 40 min. After cooling, the sample was centrifuged at 2000×*g* for 5 min. Absorbance was determined at 532 nm against a blank containing 1 mL TCA extraction solution and 1 mL TBA solution. The TBARS values were expressed as mg malondialdehyde (MDA) per kg meat.

Statistical analysis

The entire experiment was replicated three times on separate production days. Data collected for TBARS and physicochemical properties were analyzed by the statistical analysis system. The statistical evaluation of the results was performed using the SPSS 18.0.0 (SPSS Inc., Chicago, USA). The generated data were analyzed by analysis of variance (ANOVA). Differences among mean values were established using the Duncan test and were considered significant when $p < 0.05$.

Results and discussion

Cooking loss

The results (Table 2) showed that the highest ($p < 0.05$) cooking loss was observed in the samples manufactured with only microbial transglutaminase (M1, M2) for all tested salt levels while the formulation of ground beef with a combination of fibrimex and alginate at 0.5% salt level (FA2) resulted in the lowest ($p < 0.05$) cooking loss values. Increased cooking loss was observed in control groups (C2, C3) with lower salt levels (1.0 and 0.5% salt) compared to control group (C1) with 2.0% salt ($p < 0.05$). Increased cooking loss has also been reported previously when reducing salt level from 2 to 1% in meat and meat products (Jimenez-Colmenero et al. 2010; Dimitrakopoulou et al. 2005; Ruusunen et al. 2001). Determination of higher cooking loss values in the samples formulated with microbial trasglutaminase (M1, M2) at 0.5 and 1.0% salt levels compared to their control counterparts (C1, C2) indicated that the use of microbial transglutaminase had negative effect on water binding capacity of meat. In contrast, Uran et al. (2013) reported that microbial transglutaminase did not create any significant difference on cooking loss. On the other hand, other previous studies suggested that the use a combination of microbial transglutaminase and sodium caseinate decreased cooking loss (Askin and Kilic 2009; Flores et al. 2007; Colmenero et al. 2005). Even though there is a controversy regarding the effects of microbial transglutaminase on cooking loss, the results of our present study are in agreement with those of some researchers who also found an increase in cooking loss in muscle foods formulated with microbial transglutaminase (Lennon et al. 2010; Flores et al. 2007; Dimitrakopoulou et al. 2005; Tseng et al. 2000). The results of present study revealed that the combination of microbial transglutaminase with alginate (MA0.5, MA1) or fibrimex (MF0.5, MF1) resulted in lower ($p < 0.05$) cooking loss in cooked ground beef incorporated with 0.5 or 1.0% salt than those formulated with microbial transglutaminase only (M1, M2). Indeed, cooking loss values determined in MA0.5, MA1, MF0.5, MF1 were even lower than those of all control groups (C1, C2, C3) containing various salt levels ($p < 0.05$). Moreover, the combination of microbial transglutaminase with fibrimex and alginate (MFA0.5, MFA1) had no effect on advancing cooking loss reduction level obtained by addition of fibrimex (MF0.5, MF1) or

Table 2 The results of fat, ash, moisture, protein, salt, and cooking loss of cooked ground beef

Treatments	Fat (%)	Ash (%)	Moisture (%)	Protein (%)	Salt (%)	Cooking loss (%)
C1	6.04 ± 0.69^{bcde}	2.59 ± 0.021^{a}	67.44 ± 0.10^{bc}	22.93 ± 0.64^{d}	2.03 ± 0.09^{a}	19.76 ± 0.54^{c}
C2	6.53 ± 0.35^{bc}	1.67 ± 0.028^{d}	66.02 ± 0.18^{ef}	24.78 ± 0.71^{abcd}	1.13 ± 0.06^{bc}	25.29 ± 0.63^{b}
C3	7.05 ± 0.47^{ab}	1.45 ± 0.028^{e}	64.90 ± 0.30^{gh}	25.60 ± 0.44^{abcd}	0.66 ± 0.03^{gh}	26.92 ± 0.60^{b}
M1	8.03 ± 0.10^{a}	1.58 ± 0.007^{de}	63.69 ± 0.3^{1i}	25.70 ± 0.72^{abcd}	1.09 ± 0.09^{bc}	30.37 ± 0.40^{a}
F1	5.91 ± 0.40^{bcdef}	2.47 ± 0.014^{ab}	65.83 ± 0.47^{efg}	24.79 ± 0.33^{abcd}	1.09 ± 0.03^{bc}	14.16 ± 0.01^{ef}
A1	5.65 ± 0.32^{bcdefg}	1.99 ± 0.035^{c}	68.03 ± 0.54^{b}	23.33 ± 0.41^{cd}	0.99 ± 0.06^{cde}	16.94 ± 0.11^{d}
MF1	3.99 ± 0.08^{h}	2.43 ± 0.028^{b}	65.95 ± 0.08^{ef}	26.63 ± 0.53^{ab}	1.09 ± 0.09^{bc}	14.03 ± 0.30^{ef}
MA1	4.63 ± 0.33^{efgh}	1.93 ± 0.001^{c}	67.14 ± 0.18^{bcd}	25.30 ± 0.34^{abcd}	0.70 ± 0.06^{fgh}	16.25 ± 0.08^{de}
FA1	4.44 ± 0.13^{gh}	2.39 ± 0.064^{b}	67.66 ± 0.15^{bc}	24.51 ± 0.65^{abcd}	1.25 ± 0.09^{b}	13.81 ± 0.07^{f}
MFA1	4.28 ± 0.29^{gh}	2.41 ± 0.021^{b}	66.15 ± 0.20^{def}	26.16 ± 0.63^{abc}	1.05 ± 0.06^{cd}	15.58 ± 1.05^{def}
M2	6.16 ± 0.63^{bcd}	1.30 ± 0.085^{f}	64.05 ± 0.24^{hi}	27.49 ± 0.71^{a}	0.58 ± 0.06^{h}	30.48 ± 0.21^{a}
F2	4.57 ± 0.33^{fgh}	1.91 ± 0.021^{c}	66.69 ± 0.12^{cde}	25.83 ± 0.44^{abcd}	0.88 ± 0.06^{def}	13.73 ± 1.16^{f}
A2	4.43 ± 0.47^{gh}	1.56 ± 0.014^{de}	69.23 ± 0.11^{a}	23.78 ± 0.39^{bcd}	0.78 ± 0.03^{fg}	14.12 ± 0.03^{ef}
MF2	4.64 ± 0.09^{efgh}	1.93 ± 0.007^{c}	66.22 ± 0.12^{def}	26.21 ± 0.55^{abc}	0.82 ± 0.06^{efg}	15.33 ± 0.88^{def}
MA2	4.56 ± 0.08^{fgh}	1.53 ± 0.007^{e}	68.03 ± 0.01^{b}	24.88 ± 0.58^{abcd}	0.55 ± 0.03^{h}	16.83 ± 0.39^{d}
FA2	4.78 ± 0.16^{defgh}	1.97 ± 0.042^{c}	65.99 ± 0.44^{ef}	26.32 ± 0.34^{abc}	0.64 ± 0.06^{gh}	10.19 ± 0.24^{g}
MFA2	5.59 ± 0.42^{cdefg}	1.99 ± 0.001^{c}	65.17 ± 0.14^{fg}	26.25 ± 0.48^{abc}	1.05 ± 0.06^{cd}	15.18 ± 1.07^{def}

$^{a-i}$ (↓) Different letters within a column are significantly different ($p < 0.05$)

alginate (MA0.5, MA1) at both 0.5 and 1.0% salt levels. Results indicated that the use of fibrimex (F0.5, F1) or alginate (A0.5, A1) alone resulted in significant reduction in cooking loss values compared to control groups at both salt levels ($p < 0.05$). Fibrimex was more effective in reducing cooking loss in samples containing 1% salt compared to alginate ($p < 0.05$), dissimilar to the samples containing 0.5% salt, where the use of fibrimex or alginate resulted in the same level of cooking loss in cooked ground beef samples. The effect of fibrin/thrombin combination on cooking loss reduction in meat system was previously reported (Lennon et al. 2010; Flores et al. 2007; Pietrasik et al. 2007; Boles and Shand 1999). However, the higher cook yield for alginate was observed in restructured beef compared to fibrin/thrombin combination by Boles and Shand (1999). The authors reported that high cook yield for alginate could be attributed to the increased water binding properties of the hydrocolloid system (Clarke et al. 1988). In addition, the same level of cooking loss values were reported for fibrin/thrombin combination and alginate in restructured beef steaks (Lennon et al. 2010).

SDS-page analysis

SDS–PAGE was used as a separation technique to diversify and disassociate the meat proteins according to their size (Ahhmed et al. 2007). The pattern shows how cold-set binders used in this study affected the protein bands in ground beef samples (Fig. 1). SDS-PAGE results showed that microbial transglutaminase catalyzed the formation of polymer. Some of the large molecular size components formed by microbial transglutaminase did not enter the gel and were removed during destaining procedure. The analysis of proteins illustrated the density variation in the bands of myosin and actin, especially for samples treated with combination of MTGase and fibrimex. Myosin heavy chain bands (MHC) were less dense in the samples treated with MTGase or combination of MTGase with fibrimex (Fig. 1). This may suggest that there were some cross-linking reactions between myosin, other meat and plasma proteins. It has been shown that myosin heavy chain decreased and polymer content increased as a function of setting time or MTGase level (Lee et al. 1997; Kumazawa et al. 1993). Nishimoto et al. (1987) hypothesized that a reduction in myosin content was evidence of cross-linking of myosin. This may explain the changes in myosin band in our study. A slight decrease in the density of the actin band was observed in samples treated with MTGase or combination of MTGase with fibrimex. On the other hand, the use of fibrimex or alginate or combination of these two binding agents created similar protein band patterns with control groups. The results of SDS-PAGE analysis in the present study are in agreement with the findings of previous studies

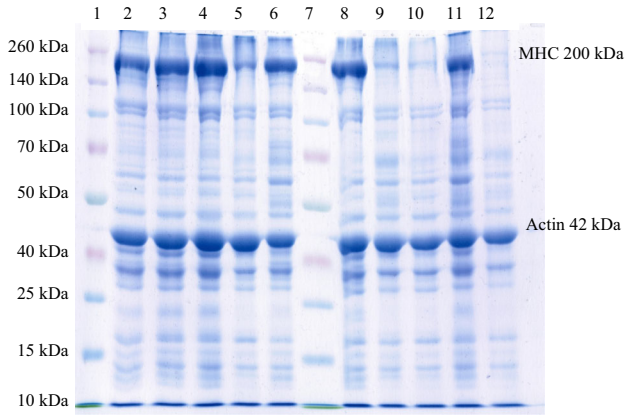

Fig. 1 SDS-PAGE of ground beef samples with 1% salt. *1* and *7* thermo scientific SDS-PAGE multicolor broad range protein standard, *2* 2% NaCl (C1), *3* 1% NaCl (C2), *4* 0.5% NaCl (C3), *5* 1% NaCl + 1% MTGase (M1), *6* 1% NaCl + 5% FB (F1), *8* 1% NaCl + 0.5% AL + 0.18% CC (A1), *9* 1% NaCl + 1% MTGase + 5% FB (MF1), *10* 1% NaCl + 1% MTGase + 0.5% AL + 0.18% CC (MA1), *11* 1% NaCl + 5%FB + 0.5% AL + 0.18% CC (FA1), and *12* 1% NaCl + 1% MTGase + 5% FB + 0.5% AL + 0.18% CC (MFA1)

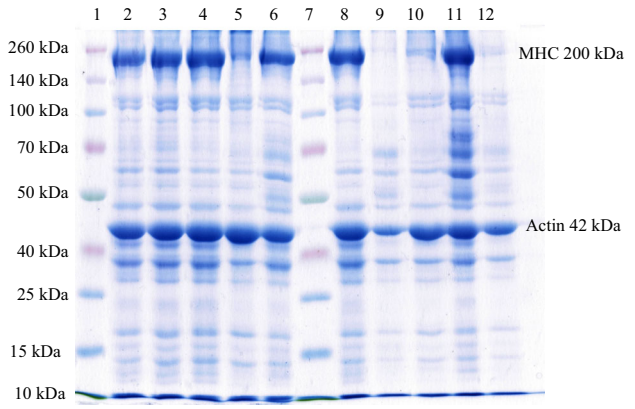

Fig. 2 SDS-PAGE of ground beef samples with 0.5% salt. *1* and *7* thermo scientific SDS-PAGE multicolor broad range protein standard, *2* 2% NaCl (C1), *3* 1% NaCl (C2), *4* 0.5% NaCl (C3), *5* 0.5% NaCl + 1% MTGase (M2), *6* 0.5% NaCl + 5% FB (F2), *8* 0.5% NaCl + 0.5% AL + 0.18% CC (A2), *9* 0.5% NaCl + 1% MTGase + 5% FB (MF2), *10* 0.5% NaCl + 1% MTGase + 0.5% AL + 0.18% CC (MA2), *11* 0.5% NaCl + 5%FB + 0.5% AL + 0.18% CC (FA2), and *12* 0.5% NaCl + 1% MTGase + 5% FB + 0.5% AL + 0.18% CC (MFA2)

(Askin and Kilic 2009; Aktaş and Kılıç 2005; Kilic 2003) (Fig. 2).

Physicochemical composition

As shown in Table 4, the highest pH values were determined in the samples formulated with combination of alginate with microbial transglutaminase (MA1) or fibrimex (FA1) on production day and in the samples with

fibrimex (FA1) or combination of fibrimex, microbial transglutaminase and alginate (MFA1) at the end of storage compared to the other treatment groups ($p < 0.05$). On the other hand, the lowest pH values were determined in the samples formulated with combination of microbial transglutaminase and fibrimex (MF1, MF2) or fibrimex (F2) on production day ($p < 0.05$). The samples with 0.5% salt addition and formulated with microbial transglutaminase (M2) had lower ($p < 0.05$) pH levels compared to other groups except MF1 and F2. In general, the results showed that addition of alginate tended to increase pH values of the samples, while using fibrimex or combination of fibrimex and microbial transglutaminase caused a decrease in pH ($p < 0.05$). Since the samples with alginate were also incorporated with calcium carbonate, an increased pH in the samples with alginate was thought to be associated with calcium carbonate which naturally increases pH. Similar findings were reported by Hong and Chin (2009) who indicated that a calcium carbonate caused an increase in the pH of myofibrillar protein gel.

Color analysis results (data is not presented) indicated that the highest ($p < 0.05$) L* values on day 0 were determined in the samples with 0.5% salt and alginate (A2), which had similar values with C2 and M2 groups. On the other hand, the lowest ($p < 0.05$) L* values were obtained in the samples with 0.5% salt, microbial transglutaminase, fibrimex and alginate (MFA2) that had similar values with control group included 2% salt (C1) and the samples with 1% salt, fibrimex and alginate combination (FA1). Among control groups, the higher L* values were obtained for C2 and C3 compared to C1 at the end of storage ($p < 0.05$). The results revealed that the use of fibrimex in both salt levels generally resulted in a lower L* values compared to control group ($p < 0.05$). Results of a* values indicated that the highest ($p < 0.05$) redness values on day 0 was obtained in the samples with 0.5% salt, microbial transglutaminase and alginate (MA2). There was no significant differences among control groups (C, C1, C2) regarding redness values. Dimitrakopoulou et al. (2005) showed that reduction in salt level did not affect redness but caused an increase in lightness and yellowness. The results of our study revealed that the use of alginate in both salt levels generally resulted in a higher a* values compared to control group ($p < 0.05$). But, the use of fibrimex caused a decrease in a* values of the samples at 0.5% salt level (F2, MF2, FA2, MFA2) compared to control (C2) group ($p < 0.05$). At the end of storage, a higher a* values were determined in MA1, FA1 and MFA1 groups at 1% salt level and M2, MA2, FA2 and MFA2 groups at 0.5% salt level compared to corresponding control groups respectively ($p < 0.05$). In general, redness values decreased during storage period ($p < 0.05$). Study results indicated that F1 and MA1 groups with 1% salt had lower b* values compared to C1. As far as the samples incorporated with 0.5% salt are concerned, M2, F2, FA2 and MFA2 groups had lower and A2 group had higher b* values compared to C2 ($p < 0.05$). Lennon et al. (2010) reported that the use of microbial transglutaminase caused a decrease in lightness while fibrin/thrombin combination or alginate did not show any significant effect on lightness. The authors also reported that there was no difference among the groups in terms of redness and yellowness due to use of microbial transglutaminase or fibrin/thrombin combination or alginate in the same study. Boles and Shand (1999) reported that alginate or fibrin/thrombin combination did not affect the lightness, but the use of fibrin/thrombin combination resulted in an increase redness and yellowness. In addition, Moreno et al. (2010) stated that use of microbial transglutaminase caused an increase in lightness but did not affect redness.

The comparison of three control groups for moisture levels (Table 2) showed that the moisture level determined in the samples increased with increasing salt addition ($p < 0.05$). Effect of salt level on water holding capacity of meat is well established. Dimitrakopoulou et al. (2005) indicated that increasing the amount of added salt into meat system caused an increase in moisture content, enhanced water holding capacity and binding properties of pork. The results indicated that the highest ($p < 0.05$) moisture level was obtained in alginate added samples with 0.5% salt (A2). On the other hand the use of microbial transglutaminase resulted in the lowest ($p < 0.05$) moisture level in the samples with 1% salt addition (M1) compared to other treatment groups except the group containing microbial transglutaminase and 0.5% salt (M2). The use of alginate or combination of alginate with microbial transglutaminase or fibrimex (A1, MA1, FA1) resulted in higher moisture level in the samples containing 1% salt compared to the control (C1) ($p < 0.05$). On the other hand, the lower moisture level was determined in the samples with 1% salt and microbial transglutaminase (M1) compared to the control (C2). In the samples with 0.5% salt, the higher moisture levels were obtained in F2, A2, MF2, MA2, and FA2 compared to the control (C3, $p < 0.05$). In both salt levels, the use of microbial transglutaminase, fibrimex and alginate combination did not have significant effect on moisture level compared to responsible control groups due to negative effect of microbial transglutaminase on moisture level. In general, the results revealed that increased moisture level in ground beef samples with reduced salt level can be achieved with incorporation of fibrimex or alginate or combination of these two binding agents.

The highest ash level (see Table 2) was determined in C1 compared to other treatment groups except F1 which was similar to C1 ($p < 0.05$). The lowest ($p < 0.05$) ash level was obtained in the sample with 0.5% salt and

Table 3 The results of texture analysis of cooked ground beef

Treatments	Hardness (N)	Cohesiveness	Springiness	Gumminess (N)	Chewiness	Adhesiveness (N sec)	Resilience
C1	6.07 ± 0.46^{bcd}	0.56 ± 0.03^{ab}	0.91 ± 0.07^{ab}	2.46 ± 0.35^{def}	2.73 ± 0.20^{cd}	0.70 ± 0.14^{b}	0.11 ± 0.02^{abc}
C2	5.11 ± 0.41^{def}	0.43 ± 0.05^{bc}	0.83 ± 0.02^{ab}	2.45 ± 0.32^{def}	2.40 ± 0.18^{de}	0.40 ± 0.15^{bc}	0.10 ± 0.01^{bc}
C3	3.47 ± 0.29^{efg}	0.38 ± 0.03^{c}	0.75 ± 0.04^{b}	1.63 ± 0.26^{ef}	1.44 ± 0.22^{ef}	0.35 ± 0.18^{bc}	0.07 ± 0.02^{c}
M1	6.88 ± 1.22^{bcd}	0.53 ± 0.04^{ab}	0.84 ± 0.02^{ab}	3.61 ± 0.65^{bcd}	3.05 ± 0.59^{bcd}	0.35 ± 0.15^{bc}	0.13 ± 0.01^{ab}
F1	7.60 ± 1.52^{bc}	0.56 ± 0.05^{ab}	0.96 ± 0.19^{a}	4.31 ± 1.16^{ab}	4.00 ± 0.79^{ab}	0.53 ± 0.23^{bc}	0.14 ± 0.03^{ab}
A1	2.23 ± 0.73^{g}	0.49 ± 0.04^{abc}	0.92 ± 0.27^{ab}	1.17 ± 0.37^{f}	1.10 ± 0.51^{f}	0.27 ± 0.29^{bc}	0.11 ± 0.03^{abc}
MF1	8.04 ± 0.67^{ab}	0.55 ± 0.03^{ab}	0.89 ± 0.02^{ab}	4.38 ± 0.49^{ab}	3.89 ± 0.43^{abc}	0.32 ± 0.10^{bc}	0.14 ± 0.02^{ab}
MA1	3.30 ± 1.14^{fg}	0.51 ± 0.07^{abc}	0.79 ± 0.03^{ab}	1.82 ± 0.83^{ef}	1.44 ± 0.70^{ef}	0.18 ± 0.08^{bc}	0.13 ± 0.03^{ab}
FA1	6.03 ± 1.35^{bcd}	0.55 ± 0.03^{ab}	0.86 ± 0.03^{ab}	3.25 ± 0.74^{bcd}	2.79 ± 0.65^{cd}	0.30 ± 0.19^{bc}	0.14 ± 0.02^{ab}
MFA1	6.63 ± 0.83^{bcd}	0.56 ± 0.06^{ab}	0.89 ± 0.03^{ab}	3.68 ± 0.46^{bcd}	3.27 ± 0.38^{bcd}	0.40 ± 0.09^{bc}	0.14 ± 0.03^{ab}
M2	5.54 ± 1.72^{cde}	0.51 ± 0.05^{abc}	0.81 ± 0.03^{ab}	2.82 ± 0.89^{cde}	2.29 ± 0.77^{de}	0.20 ± 0.11^{bc}	0.15 ± 0.03^{ab}
F2	6.90 ± 0.87^{bcd}	0.58 ± 0.04^{a}	0.89 ± 0.04^{ab}	3.90 ± 0.84^{abc}	3.46 ± 0.65^{bcd}	0.37 ± 0.14^{bc}	0.15 ± 0.04^{ab}
A2	2.73 ± 0.27^{g}	0.47 ± 0.04^{abc}	0.79 ± 0.02^{ab}	1.29 ± 0.22^{f}	1.03 ± 0.20^{f}	0.23 ± 0.10^{bc}	0.11 ± 0.02^{abc}
MF2	9.87 ± 1.26^{a}	0.54 ± 0.03^{ab}	0.88 ± 0.02^{ab}	5.27 ± 0.53^{a}	4.65 ± 0.45^{a}	0.45 ± 0.16^{bc}	0.16 ± 0.02^{a}
MA2	2.46 ± 0.59^{g}	0.46 ± 0.06^{abc}	0.82 ± 0.06^{ab}	1.14 ± 0.40^{f}	0.93 ± 0.30^{f}	0.14 ± 0.10^{c}	0.12 ± 0.04^{abc}
FA2	6.87 ± 0.57^{bcd}	0.50 ± 0.22^{abc}	0.96 ± 0.11^{a}	4.02 ± 0.37^{abc}	3.85 ± 0.70^{abc}	1.27 ± 0.86^{a}	0.12 ± 0.03^{abc}
MFA2	7.14 ± 1.73^{bcd}	0.55 ± 0.05^{ab}	0.93 ± 0.04^{ab}	3.98 ± 1.30^{abc}	3.66 ± 1.14^{abc}	0.55 ± 0.38^{bc}	0.13 ± 0.04^{abc}

[a–g] (↓) Different letters within a column are significantly different ($p < 0.05$)

microbial transglutaminase (M2). In general the use of fibrimex or combination of fibrimex with alginate or microbial transglutaminase tend to increase ash level ($p < 0.05$) in the samples containing 0.5 or 1% salt. In addition, increasing the level of added salt resulted in an increase in ash level ($p < 0.05$) when control groups were compared with each other.

As far as fat level (Table 2) is concerned, the highest fat level was obtained in the samples formulated with 1% salt and microbial transglutaminase (M1) compared to the rest of treatments groups except C2 which had similar fat level with M1 ($p < 0.05$). The reason for this may be associated with higher cooking loss in these groups compared with others. In general, no significant differences were obtained among other groups regarding fat level. Protein analysis results showed that the use of different binding agents or salt levels generally did not create significant differences in protein level among groups.

Study results indicated that the highest salt level (Table 2) was determined in the control formulated with 2% salt. Increasing the amount of added salt led to have an increase in determined salt level ($p < 0.05$). The results also showed that the use of tested binding agents generally did not have an effect on determined salt level.

The texture profile analysis results (Table 3) indicated that hardness values generally decreased with increasing the amount of salt incorporation ($p < 0.05$). MF2 had the highest hardness values compared to other groups except MF1 which had similar hardness values with MF2. The results revealed that the use of alginate generally resulted in a decrease in hardness values in both salt levels ($p < 0.05$). This may be results of increased moisture level due to the effect of alginate. It was also determined that using fibrimex or combination of fibrimex with other binding agents generally increased hardness values in both salt levels ($p < 0.05$). Even though the use of microbial transglutaminase did not have significant effect on hardness values, combination of microbial transglutaminase and fibrimex (MF1, MF2) resulted in higher ($p < 0.05$) hardness values in both salt levels due to possible synergic effects between microbial transglutaminase and fibrimex. Cohesiveness results indicated that the use of 0.5% salt decreased cohesiveness compared to 2% salt in control samples ($p < 0.05$). It was also determined that the use of fibrimex in the samples with 0.5% salt (F2, MF2, MFA2) resulted in an increase in cohesiveness values ($p < 0.05$), however, this was not a case in the samples formulated with 1% salt. The results showed that salt level had no effect on gumminess values among control groups. However, it was determined that fibrimex addition generally caused an increase in gumminess in the samples (F1, MF1, F2, MF2, FA2, MFA2) compared to corresponding control groups (C2, C3) respectively ($p < 0.05$). The results indicated that samples with 0.5% salt (C3) has lower ($p < 0.05$) chewiness values compared to those with 2% salt (C1). Furthermore, chewiness values were increased with addition of fibrimex or combination of fibrimex with microbial transglutaminase or alginate ($p < 0.05$). Regarding adhesiveness, even though

Table 4 pH and TBARS values of cooked ground beef samples during storage

Treatments	pH			TBARS		
	0	7	15	0	7	15
C1	5.86 ± 0.004^{fZ}	5.93 ± 0.008^{fX}	5.88 ± 0.012^{efY}	2.49 ± 0.17^{defghZ}	19.79 ± 1.47^{bcY}	26.89 ± 0.74^{bX}
C2	5.90 ± 0.008^{eX}	5.92 ± 0.005^{fghX}	5.90 ± 0.010^{deX}	3.74 ± 0.08^{abcZ}	22.09 ± 0.44^{abY}	26.49 ± 0.67^{bcX}
C3	5.92 ± 0.005^{deXY}	5.90 ± 0.008^{hY}	5.97 ± 0.066^{cX}	4.63 ± 0.19^{aZ}	19.97 ± 2.31^{bcY}	27.38 ± 0.36^{bX}
M1	5.91 ± 0.006^{eX}	5.91 ± 0.008^{ghX}	5.91 ± 0.012^{deX}	3.67 ± 0.36^{abcZ}	18.84 ± 1.25^{cY}	26.58 ± 0.93^{bcX}
F1	5.83 ± 0.017^{gY}	5.91 ± 0.010^{hX}	5.90 ± 0.012^{deX}	1.87 ± 0.28^{ghZ}	2.41 ± 0.30^{eY}	4.21 ± 0.16^{gX}
A1	5.96 ± 0.005^{bcY}	5.99 ± 0.011^{deX}	5.91 ± 0.021^{deZ}	2.62 ± 0.17^{defgZ}	18.89 ± 1.28^{cY}	29.54 ± 1.18^{aX}
MF1	5.79 ± 0.010^{hY}	5.83 ± 0.025^{jX}	5.84 ± 0.015^{fgX}	2.18 ± 0.63^{efghY}	2.75 ± 0.69^{deY}	4.03 ± 0.35^{gX}
MA1	6.00 ± 0.008^{aY}	6.01 ± 0.008^{cdXY}	6.02 ± 0.012^{bX}	2.02 ± 0.03^{fghZ}	19.17 ± 0.91^{cY}	25.23 ± 0.59^{cdX}
FA1	6.00 ± 0.008^{aY}	6.11 ± 0.008^{aX}	6.11 ± 0.021^{aX}	1.72 ± 0.18^{ghZ}	2.58 ± 0.24^{deY}	4.35 ± 0.18^{fgX}
MFA1	5.96 ± 0.015^{bcZ}	6.03 ± 0.012^{bY}	6.07 ± 0.010^{aX}	1.49 ± 0.26^{hZ}	2.90 ± 0.03^{deY}	4.13 ± 0.21^{gX}
M2	5.86 ± 0.004^{fX}	5.87 ± 0.010^{iX}	5.82 ± 0.015^{gY}	4.40 ± 0.55^{aZ}	20.39 ± 0.40^{bcY}	26.47 ± 1.12^{bcX}
F2	5.85 ± 0.008^{hY}	5.80 ± 0.005^{ijX}	5.85 ± 0.016^{fgX}	4.17 ± 0.90^{abY}	3.24 ± 0.28^{deY}	5.32 ± 0.13^{efgX}
A2	5.93 ± 0.010^{cdX}	5.94 ± 0.014^{fgX}	5.95 ± 0.014^{cdX}	3.00 ± 0.26^{cdefZ}	23.88 ± 1.22^{aY}	28.98 ± 0.34^{aX}
MF2	5.86 ± 0.006^{hY}	5.80 ± 0.030^{iX}	5.87 ± 0.009^{efX}	3.09 ± 0.83^{cdeY}	3.00 ± 0.44^{deY}	5.96 ± 0.45^{eX}
MA2	6.03 ± 0.005^{bY}	5.98 ± 0.008^{bcX}	6.02 ± 0.012^{bX}	3.92 ± 0.15^{abcZ}	19.33 ± 0.53^{cY}	24.78 ± 0.69^{dX}
FA2	5.98 ± 0.010^{deZ}	5.92 ± 0.012^{deY}	6.02 ± 0.021^{bX}	3.11 ± 0.17^{cdeZ}	4.42 ± 0.19^{deY}	5.88 ± 0.08^{efX}
MFA2	5.98 ± 0.011^{fY}	5.86 ± 0.017^{eX}	5.97 ± 0.023^{cX}	3.35 ± 0.15^{bcdZ}	4.94 ± 0.45^{dY}	6.22 ± 0.22^{eX}

$^{a–j}$ (↓) Different letters within a column are significantly different ($p < 0.05$)

^{XYZ} (→) Different letters within a row are significantly different ($p < 0.05$)

the highest ($p < 0.05$) adhesiveness value was determined in FA2 group, there was no significant differences among the rest of the other treatment groups. No significant differences were also determined among all treatment groups for elasticity and springiness. Moreno et al. (2010) reported that the use of microbial transglutaminase resulted in an increase in the hardness and adhesiveness in muscle foods. Another previous study indicated that the use of microbial transglutaminase increased hardness and chewiness but decreased springiness and cohesiveness (Pietrasik and Li-Chan 2002). Fibrimex has been reported to produce less binding in both raw and cooked restructured pork compared to MTGase (Flores et al. 2007). Previous studies reported that restructured beef steaks formulated with fibrimex produced a weaker bind compared with alginate. However, once cooked, fibrimex steaks were found to have a binding strength equal or stronger than that of steaks restructured with alginate (Boles and Shand 1999). On the other hand, the stronger binding strength was reported for both raw and cooked steaks formulated with fibrimex compared with alginate (Lennon et al. 2010).

Thiobarbituric acid reactive substances (TBARS)

Results of TBARS analysis (Table 4) illustrated that the TBARS values in all treatment groups increased gradually during storage period ($p < 0.05$). The highest ($p < 0.05$) TBARS values were determined in the samples formulated with alginate (A1, A2). The use of alginate in coating materials was found to be effective to control lipid oxidation and the formation of warmed-over flavor in precooked meat and meat products (Wu et al. 2001; Handley et al. 1996). However, there is a lack of information about the effect of alginate on lipid oxidation in muscle foods. In general, the results of present study revealed that the use of fibrimex or combination of fibrimex with microbial transglutaminase or alginate resulted in lower TBARS levels compared to the groups formulated without fibrimex ($p < 0.05$). Thus, the results of this study showed that fibrimex was effective in reducing oxidative reactions in cooked ground beef during refrigerated storage. It was previously reported that the cold-set methods for manufacture of restructured meats reduces oxidative rancidity compared to hot-set methods (Bhaskar Reddy et al. 2015). Even though Tseng et al. (2006) reported that addition of different percentages of binder solution containing transglutaminase, thrombin and fibrinogen did not inhibit lipid oxidation in restructured meat, there is no information about effect of fibrimex on lipid oxidation inhibition in meat and meat products.

Conclusion

Study results indicated that the addition of fibrimex alone or in combined with MTGase was effective for improving or maintaining textural properties of low-salt cooked ground beef without any significant adverse effect on

cooking loss, color and pH. The use of fibrimex was capable of reducing the formation of TBARS in cooked ground beef during refrigerated storage. In addition, incorporation of fibrimex or alginate or fibrimex/alginate combination to low-salt ground beef formulation significantly reduced cooking loss. It is suggested that the meat industry may achieve the benefits of reduced cooking loss, improved textural properties and extended shelf life in low-salt meat products by using fibrimex or a combination of fibrimex with MTGase in their product formulations.

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