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The effects of incorporating chitosan on the functional and physical characteristics of ground beef patties

Die Auswirkungen der Einarbeitung von Chitosan auf die funktionellen und physikalischen Eigenschaften von Rinderhackfleischpasteten

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Summary

This study investigated the effects of adding chitosan at 0% (control), 0.5% (0.5-C), 1.0% (1-C) and 1.5% (1.5-C) on some quality traits of ground beef patties during refrigerated storage at 4°C for 4 days. Incorporating chitosan resulted in higher pH and lower thiobarbituric acid (TBA) values in comparison to the control ($p<0.05$). The addition of chitosan resulted in a significant color stabilizing effect with higher CIE redness (a*) and a lower percentage of metmyoglobin (MetMb). At the end of the storage period, the MetMb percentage increased significantly in all sample groups and reached 61.26%, 33.09%, 24.55% and 29.2% in C, 0.5-C, 1-C and 1.5-C, respectively. After cooking, the diameter reduction of ground beef patties decreased, while cooking yield and moisture retention increased with an increasing concentration of chitosan ($p<0.05$). In texture profile analyses, the incorporation of chitosan resulted in a higher hardness in raw samples and higher cohesiveness, springiness and chewiness in cooked samples ($p<0.05$). Thus, chitosan was shown to be an effective agent in providing enhanced color, oxidative stability and yield in ground meat patties due to its promising antioxidant activity and water-binding ability.

Keywords: Ground beef patties, chitosan, color, lipid oxidation

Introduction

Besides being basic components of a balanced diet, meat and meat products are prone to oxidative and microbiological changes and have a perishable nature due to their favorable nutritional composition (Lawrie and Ledward 2006). Alterations in lipids and proteins as a result of oxidative processes contribute to a deterioration in the flavor, texture and color of meat products. Consequently, several preservation methods have been proposed to inhibit oxidative deteriorations, such as modified atmosphere packaging, active packaging with oxygen scavengers, and the use of synthetic antioxidant compounds (Zhou et al. 2010). Whilst synthetic food additives have proved to be efficient antioxidant agents, their potentially hazardous effects on health have put consumers off (Kodal-Coşkun et al. 2014). Thus, considerable attention has been given to safer, alternative natural substances including animal by-products (Genskowsky et al. 2015, Lekjing 2016, Şahin et al. 2017) and plant extracts (Kodal-Coşkun et al. 2014) to replace synthetic antioxidant additives like BHA, BHT and TBHQ (Kashanian and Dolatabadi 2009). Within animal by-products, chitin and chitosan are well recognized as promising biopolymers for various food applications and have been reported to have antioxidative properties (Gutierrez 2017).

Chitosan (2-deoxy-2-amino glucose polymer) is a polysaccharide obtained by the deacetylation of chitin, which originates from crustaceans, fungi and insects (Knorr, 1982). Since it possesses non-toxic, biodegradable and biocompatible properties and is inexpensive, the use of chitosan as a versatile biopolymer has been evaluated for use in a broad range of industrial and biomedical applications (Dutta et al. 2004). Being accepted as "GRAS" (Generally Recognized As Safe) by the FDA (Food and Drug Administration 2005), it has been employed as dietary fiber (Gades and Stern 2005), for moisture retention (Chen et al 2003; Abdallah et al. 2017), as an emulsifier (Klinkesorn 2013), for antimicrobial use (Genskowsky et al. 2015, Costa et al. 2017), and as an antioxidant (Liu et al. 2017, Kurniasih et al. 2018) agent for food applications. The use of chitosan has also been suggested, on its own or in combination with other methods, to improve the quality of fruits, vegetables and muscle building foods in the form of edible films or coatings (Romanazzi et al. 2017; Şahin et al. 2017). In the case of muscle foods, a chitosan coating resulted in a lower aerobic plate count and reduced total volatile basic nitrogen (TVBN) and trimethylamine (TMAN) content during refrigerated storage of shrimps *(Parapenaeus longirostris)* (Aşık and Candoğan 2014), as well as lower thiobarbituric acid reactive substance (TBARS) values and moisture loss in pastirma compared to uncoated samples (Abdallah et al. 2017). Chang et al. (2018) found lower drip loss combined with extended chemical and microbial quality in chilled meat packaged with chitosan films. However, there have only been limited studies on the direct incorporation of chitosan into meat formulations (Sayas-Barbera et al. 2011; Chounou et al. 2013). Consequently, this study was designed to assess the potential of chitosan as a natural food additive. This study evaluated the effects on some chemical and physical characteristics caused by adding chitosan at different concentrations to ground beef patties.

Materials and Methods

Preparation of ground beef patties

Ground beef was purchased from a local retailer 24 hours after slaughter, put immediately into an ice-box and transported to the laboratory. It was analyzed immediately. This process was repeated twice. The ground beef had 59.7 ± 0.59% moisture, 21.98 ± 0.57% fat, 17.88 ± 0.30% protein and 0.79 ± 0.01% ash content (mean ± standard deviation). The initial chemical composition of the ground meat was determined by AOAC (1990) standard methods.

Chitosan was kindly supplied by Primex Ingredients ASA (Norway) as Chitoclear®, which had a 91% degree of deacetylation, and an average viscometric molecular weight of 236 kDa (according to the manufacturer). It was incorporated into the ground beef in powder form at concentrations of 0% (Control), 0.5% (0.5-C), 1.0% (1-C) and 1.5% (1.5-C) (w/w). For each sample group, approximately 600 g of mixture was prepared and cut into 20 g (5×5×0.5 cm) size patties (30 patties in each group) after manually kneading for 5 minutes for a homogenous distribution of the chitosan. Three patties were placed in polystyrene foam trays (a total of 10 trays for each group), covered with low-density polyethylene (LLDPE) film, and stored at 4 °C for 4 days. The analyses were carried out every day to evaluate their chemical and physical characteristics. Cooking traits and texture profiles were analyzed on the initial day of the study. All analyses were handled in parallel and duplicated.

Cooking yields, moisture retention and diameter reductions

Ground beef patties were placed in a Teflon® pan and cooked for five minutes each side at 180 °C. Cooking traits were obtained by the difference between raw and cooked ground beef patties. Cooking yields, moisture retention and diameter reductions were calculated according to the following equations (Ulu 2004):

$$Cooking\ yield\ (\%) = \frac{(cooked\ weight)}{(raw\ weight)} \times 100 \quad (1)$$

$$Moisture\ retention\ (\%) = \frac{(\%yield)\times(\%moisture\ of\ the\ cooked\ sample)}{100} \quad (2)$$

$$Diameter\ reduction\ (\%) = \frac{(raw\ sample\ diameter)-(cooked\ sample\ diameter)}{(raw\ sample\ diameter)} \times 100 \quad (3)$$

Texture profile analysis

Texture profile analysis (TPA) was conducted with a TA1 texture analyzer (Lloyd, Materials Testing, Ametek, USA) for raw and cooked ground beef patties to measure hardness (N), springiness (mm), cohesiveness and chewiness (Nmm). The average data from 6 measurements per group were used.

The pH value

Ten grams of ground meat were homogenized with 100 mL of distilled water for one minute using an Ultra Turrax® T25 dispersing instrument (IKA Labortechnik, Staufen, Germany). Then, the pH value of the homogenate was measured by a Hanna, HI 221 pH meter (Romania).

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Thiobarbituric acid (TBA) value

The TBA value of the sample groups were evaluated spectrophotometrically (Tarladgis et al. 1960) and calculated according to the following equation:

$$TBA\ value\ \left(\frac{MDA\ (mg)}{sample\ (kg)}\right) = A \times 7.8 \quad (4)$$

where A and MDA refer to the absorbance and the malondialdehyde, respectively

Metmyoglobin (MetMb) percentage

Ground beef samples were blended with a K-phosphate solution (pH 6.8) by an Ultra Turrax® T25 dispersing instrument (IKA Labortechnik, Staufen, Germany) and stored in a refrigerator for 1 hour. The homogenate was centrifuged at 5000 rpm for 30 minutes at 4°C. The supernatant was filtered through a Whatman® No 1 filter paper (Whatman International, UK) and the absorbance of the filtrate was measured at wavelengths of 700, 572 and 525 nm using a spectrophotometer (Shimadzu UV-2401 PC). The metmyoglobin percentage was calculated according to the following equation (Krzywicki 1982, Kannan et al. 2001):

$$\%\ MetMb = \left\{1{,}395 - \frac{(A572-A700)}{(A525-A700)}\right\} \times 100 \quad (5)$$

Instrumental color

Instrumental color parameters were obtained randomly from 6 areas on the surfaces of the ground beef patties by a Minolta Chroma meter CR300 (Minolta Inc., Osaka, Japan). CIE L* (lightness), a* (redness) and b* (yellowness) values were determined. The instrument was calibrated against a white reference tile before the color measurements.

Statistical analysis

Data from the two replications were analyzed by one-way analysis of variance (ANOVA) using SAS analysis of variance procedures (ANOVA) (SAS 1996). Comparisons among means were carried out using the least significant difference (LSD) procedure at p<0.05.

Results and Discussion

Cooking yields, moisture retention and diameter reduction

Cooking yield, moisture retention and diameter reduction of the ground beef patties are shown in Figure 1. Cooking yield is an important criterion that impacts both the textural and nutritional characteristics of meat (Bombrun et al. 2012). The control group had a cooking yield of 69.3% that increased (p<0.05) with the addition of chitosan to 74.7%, 77.8% and 78.5% in the 0.5-C, 1.0-C and 1.5-C groups, respectively, as a result of its moisture retention effect. While the effect of chitosan on cooking yield and moisture retention was concentration dependent (p<0.05), no difference was observed in the diameter reduction in chitosan-incorporated groups (p>0.05). The improved cooking yield and moisture retention, and lower diameter reduction by adding chitosan could be attributed to the water-holding and fat-binding ability of chitosan, which was also noted as the reason for increased cooking yield in chitosan burgers by Sayas-Barbera et al. (2011).

Texture profile of ground beef patties

Texture profile analyses results are given in Table 1. The effect of chitosan addition on the textural attributes in raw ground beef patties was found to be insignificant with the exception of hardness, which exhibited a significant increase (p<0.05) with the addition of 1.5% chitosan in comparison to the control group. Hardness showed increases with cooking. Although groups with added chitosan had lower hardness values, this was significant (p<0.05) only for the 0.5-C group. This may be explained by the lowest hardness value measured for the raw sample group of 0.5-C; however, there was no significant difference between cooked samples with added chitosan. Verma et al. (2015)

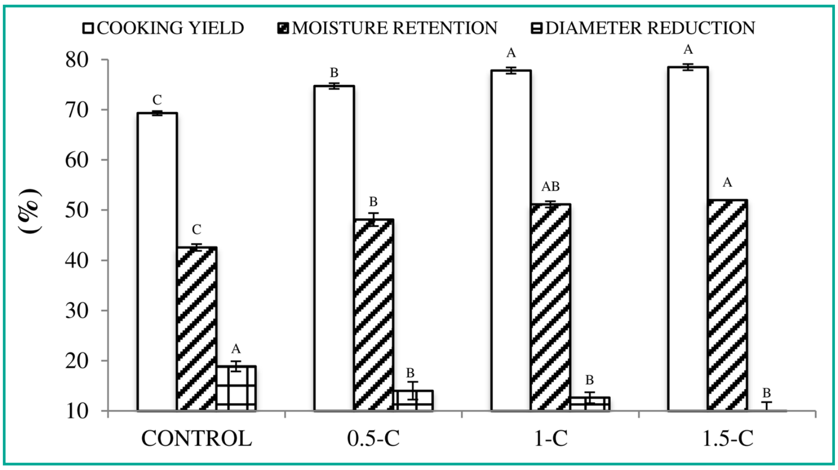

FIGURE 1: *Cooking yield, diameter reduction and moisture retention (%) of chitosan incorporated ground beef patties after cooking. 0.5-C, 1-C, and 1.5-C are 0.5%, 1%, 1.5% chitosan incorporated groups, respectively. Different upper case (A-C) is significantly different within columns (p<0.05).*

TABLE 1: *Instrumental CIE (L*,a*,b*) values of chitosan incorporated ground beef patties during refrigerated storage.*

CIE color values	Days	Control	0.5-C	1-C	1.5-C
L*	0	49.14±0.52*^{ABa}	47.83±0.66^{Aab}	45.68±0.54^{ABc}	46.67±0.60^{BCbc}
	1	47.85±0.93^{Bb}	47.70±0.42^{Ab}	47.21±0.82^{Ab}	50.88±0.32^{Aa}
	2	48.95±0.98^{Ba}	47.15±0.76^{ABb}	45.42±0.40^{Bbc}	45.31±0.16^{Cc}
	3	49.09±0.51^{ABa}	45.94±0.38^{Bb}	45.31±0.51^{Bb}	44.50±0.66^{Cb}
	4	51.11±0.34^{Aa}	46.86±0.61^{ABb}	46.74±0.47^{ABb}	48.19±0.60^{Bb}
a*	0	23.22±0.60^{Aa}	23.60±0.19^{Aa}	23.96±0.28^{Aa}	23.08±0.25^{ABa}
	1	23.07±0.35^{Aa}	24.46±0.39^{Aa}	24.14±0.78^{Aa}	24.14±0.44^{Aa}
	2	20.76±0.46^{Bb}	24.09±0.49^{Aa}	24.04±0.89^{Aa}	23.96±0.79^{Aa}
	3	16.87±0.14^{Cb}	20.25±0.36^{Bb}	22.00±0.55^{Ba}	22.15±0.21^{Ba}
	4	13.39±0.69^{Dc}	15.48±0.17^{Cb}	17.21±0.21^{Ca}	18.01±0.58^{Ca}
b*	0	11.33±0.27^{ABa}	11.11±0.21^{Bab}	10.73±0.14^{Bb}	10.59±0.20^{Cb}
	1	11.95±0.31^{Aab}	11.94±0.26^{Aab}	11.38±0.35^{ABb}	12.26±0.23^{Aa}
	2	11.65±0.16^{ABa}	11.48±0.23^{ABa}	11.61±0.40^{Aa}	11.64±0.11^{Ba}
	3	9.62±0.17^{Ba}	11.24±0.18^{Ba}	11.22±0.24^{ABa}	11.53±0.01^{Ba}
	4	11.60±0.23^{ABa}	11.07±0.20^{Bab}	10.84±0.26^{ABb}	11.27±0.24^{Bab}

* Mean ± SD. 0.5-C, 1-C, and 1.5-C are 0.5%, 1%, 1.5% chitosan incorporated groups, respectively. $^{(a-c)}$ Values with different superscripts within the same column are significantly (P<0.05) different. $^{(A-D)}$ Values with different superscripts within the same raw are significantly (P<0.05) different.

and Cho et al. (2017) reported reduced hardness values for hamburger patties with added soy flour and for pork patties with added sweet potato flour, respectively. Both studies attributed these results to the fat and moisture retention ability of the incorporated ingredients, which had already been attributed to chitosan. In contrast, Amaral et al. (2015), detailed higher hardness values for cooked goat sausage including chitosan that was explained by the formation of a stronger gel and a more stable structure.

After cooking, higher springiness values in 1-C and 1.5-C, and lower chewiness values in 1.5-C, were obtained in comparison to the control group ($p<0.05$). Chitosan incorporation also resulted in a difference in the cohesiveness of cooked ground beef samples that were independent of the concentration. In contrast, Sayas-Barbera et al. (2011) reported increased cohesiveness that was determined by sensory evaluation for cooked pork burgers containing 1% chitosan. The effect of cooking on the texture profile could be explained by the changes in the connective tissue proteins, soluble proteins and myofibrillar proteins of meat (Murphy and Marks 2000). Toughening and softening of muscle tissue during cooking are caused mainly by denaturation and dissociation of myofibrillar proteins, and the transformation of connective tissue collagen into gelatin, respectively. Nevertheless, cooking loss, shrinkage of meat fibers, cooking temperature and time also contribute to meat texture transitions (Chang et al. 2011). The chitosan addition enhanced textural characteristic of ground beef by increasing water-holding capacity which was adversely affected by the denaturation of myofibrillar proteins due to the heat increase (Akwetey and Knipe 2012).

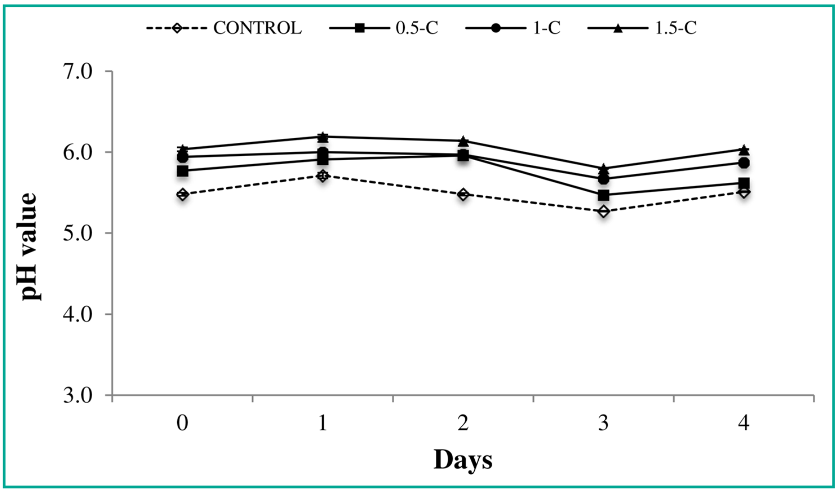

FIGURE 2: *pH-values of chitosan incorporated ground beef patties during refrigerated storage. 0.5-C, 1-C, and 1.5-C are 0.5%, 1%, 1.5% chitosan incorporated groups, respectively.*

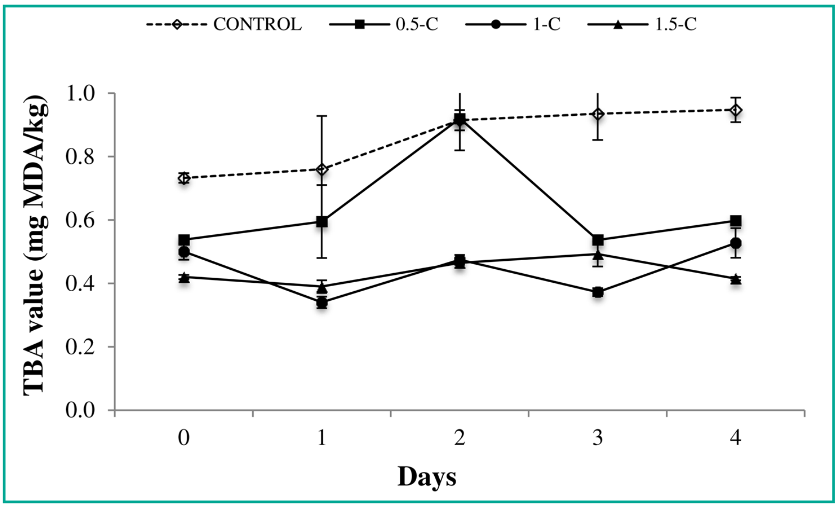

FIGURE 3: *TBA values (mg MA/kg) of chitosan incorporated ground beef patties during refrigerated storage. 0.5-C, 1-C, and 1.5-C are 0.5%, 1%, 1.5% chitosan incorporated groups, respectively.*

The pH value

The pH values of ground beef patties resulting from different levels of chitosan during refrigerated storage are given in Figure 2. Higher initial pH values ($p<0.05$) were determined in the 0.5-C, 1-C and 1.5-C groups with pH values of 5.77, 5.94, 6.04, respectively, compared to the control (pH 5.48) owing to the amino group in the chitosan structure (Amaral et al. 2015), and this difference persisted until the end of the storage period. Similar results were reported by Suman et al. (2011) in ground beef patties incorporating 1% chitosan stored in different packaging systems at 1°C with an increased pH due to the addition of chitosan. This was also reported by Sayas-Barbera et al. (2011) in pork model burgers with added chitosan at a concentration of 0%, 0.25%, 0.5% and 1%, exhibiting both molecular weight and concentration-dependent increases in the pH value caused by chitosan.

TBA value

TBA value is a crucial parameter for animal-derived foods indicating oxidative rancidity due to lipid oxidation. TBA values of ground beef patties during refrigerated storage are shown in Figure 3. The control samples had significantly higher TBA values compared to those with added chitosan ($p<0.05$). At the end of the storage period, TBA values were 0.95, 0.59, 0.53 and 0.41 mg of MDA/kg decreasing in order from the control group to the 1.5-C group. The 1.5-C group possessed notably lower TBA values than the control and 0.5-C groups. Chitosan and its derivatives might have retarded lipid oxidation in the ground beef matrix due to their amine groups which act as a scavenger of hydroxyl radicals and a chelator of ferrous ions (Kim and Thomas 2007, Yen et al. 2008). The findings of this present study are in agreement with Soultos et al. (2008) who found a decreased lipid oxidation rate in pork sausages with 1% and 0.5% added chitosan at 4°C. Furthermore, Abdallah et al. (2017) found a significant reduction in the TBARS values from 45 to 48% for pastirma coated with chitosan. However, Genskowsky et al. (2015) and Cai et al. (2014) stated that there was no significant contribution from chitosan to antioxidant activity.

Metmyoglobin percentage

Acceptability of fresh meat is highly related to oxymyoglobin (OMb), which reflects a bright red color, while metmyoglobin (MetMb) is the major cause of fresh meat browning (Livingston and Brown 1981). The MetMb percentages of ground beef patty samples are shown in Figure 4. In general, the color stabilizing effect of chitosan was observed with a lower MetMb content in comparison to the control group over the refrigerated storage period ($p<0.05$). At day 4, the MetMb contents of C, 0.5-C, 1-C, and 1.5-C reached 61.26%, 33.09%, 24.55% and 29.02%, respectively, indicating that increasing the chitosan concentration resulted in less MetMb formation in the ground beef. Color retention could be accomplished by the chitosan reducing the MetMb concentration due to its antioxidative activity (Qin et al. 2013). The control group exhibited a remarkable ($p<0.05$) increase in the MetMb percentage during storage that exceeded the rejection level (40%) reported by Greene et al. (1971) at day 1, whereas other groups were found to be significantly ($p<0.05$) lower than the C group during storage, independent of chitosan concentration. These results are consistent with those of Qin et al. (2013), who noted retarded MetMb levels for pork meat patties with chitosan films at 4°C. Cardoso et al. (2016) also showed a reduction from 50% to 33% in the MetMb percentage of beef coated with a chitosan-gelatin combination during storage that was reported to be due to the ability of chitosan to act as a chelating agent.

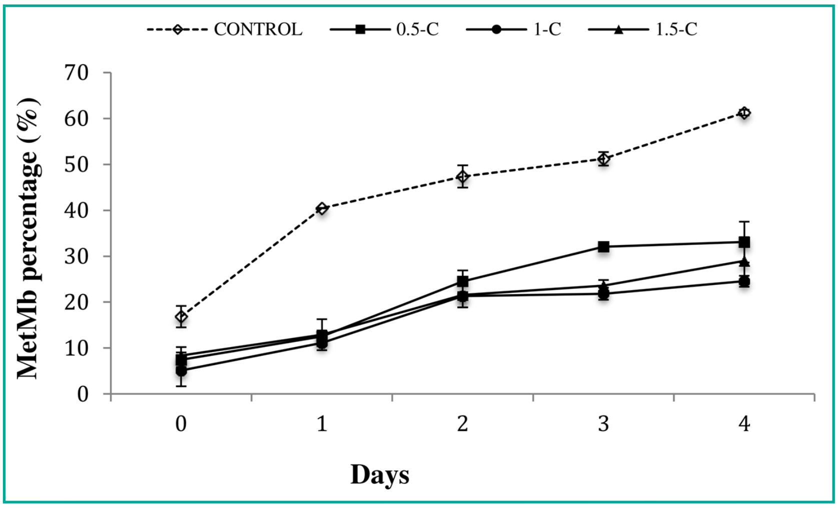

FIGURE 4: *MetMb content of of chitosan incorporated ground beef patties during refrigerated storage. 0.5-C, 1-C, and 1.5-C are 0.5%, 1%, 1.5% chitosan incorporated groups, respectively.*

TABLE 2: *Texture profile of chitosan incorporated ground beef patties before and after cooking.*

Texture parameter	Control	0.5-C	1-C	1.5-C
Raw				
Chewiness	1.76±0.21*[a]	1.67±0.23[a]	1.78±0.09[a]	1.82±0.09[a]
Springeness	2.31±0.09[a]	2.24±0.04[a]	2.16±0.04[a]	2.20±0.05[a]
Cohesiveness	0.26±0.02[a]	0.25±0.01[a]	0.24±0.02[a]	0.26±0.01[a]
Hardness 1	2.94±0.23[a]	2.97±0.16[a]	2.99±0.22[a]	3.23±0.13[a]
Hardness 2	1.81±0.11[b]	1.91±0.08[ab]	2.02±0.09[ab]	2.15±0.09[a]
Cooked				
Chewiness	9.15±0.51[b]	9.76±0.32[ab]	10.51±0.78[ab]	11.12±0.62[a]
Springeness	2.90±0.02[c]	2.96±0.03[bc]	3.01±0.02[ab]	3.04±0.02[a]
Cohesiveness	0.31±0.01[b]	0.36±0.01[a]	0.38±0.02[a]	0.39±0.01[a]
Hardness 1	11.24±0.74[a]	8.72±0.50[b]	9.21±0.65[b]	10.12±0.54[ab]
Hardess 2	8.34±0.53[a]	6.59±0.38[b]	7.10±0.55[ab]	7.51±0.36[ab]

* Mean ± SD. 0.5-C, 1-C, and 1.5-C are 0.5%, 1%, 1.5% chitosan incorporated groups, respectively. [(a–c)] Values with different superscripts within the same column are significantly ($P<0.05$) different.

3.6. Instrumental color

CIE L*, a* and b* values are shown in Table 2. The a* values decreased during storage possibly due to spoilage and MetMb formation. The 1-C and 1.5-C groups possessed significantly ($p<0.05$) higher a* values than the control group, whereas groups with added chitosan had lower L* value as of the second day of storage ($p<0.05$). No significant difference in L* value was determined in samples with added chitosan. The b* value was not affected by the addition of chitosan either ($p>0.05$). Thus, it can be concluded that the addition of chitosan maintained a fresh appearance in raw ground meat that meets consumer demand. Similar to the findings of this present study in terms of the effect on color of added chitosan, Suman et al. (2010) obtained decreasing L* values for both added chitosan (1%) and control ground beef stored under modified atmosphere systems in which aerobic and carbon monoxide packaging stabilized the red color. Furthermore, Jo et al. (2001) determined an increase in L* and b* values on the surface of pork sausage packaged under aerobic or vacuum conditions with an added chitosan oligomer (0.2%). Nevertheless, a decrease in L* values for ground up meat with added chitosan was found by Chounou et al. (2013) with no effect on the a* values, while no significant effect on color attributes due to the use of chitosan was reported by Latou et al. (2014) and Lekjing (2016) for chicken breast fillets and cooked pork sausages, respectively.

Conclusion

The results of this present study provided evidence that chitosan might improve the functional and physical quality characteristics of meat products with an inhibitory effect on oxidative deteriorations in lipids and in myoglobin pigment. Added chitosan maintained the fresh appearance in raw ground meat with lower MetMb content and higher a* values, while high concentrations of chitosan prevented rancidity produced by lipid oxidation. Also, the advantage of production cost savings due to higher cooking yield was provided by the water-binding ability of chitosan in cooked ground meat. Thus, chitosan, as a natural additive, could be recommended for incorporation into ground beef or ready-to-eat, meat-product formulations to maintain color and retard oxidative changes.

Conflict of interest

The authors declare that there is no conflict of interest.

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