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Effect of chitosan on quality and shelf life of beef at refrigerated storage

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Abstract

In this study, the microbiological quality and shelf life of beef treated with different concentrations of chitosan (CHI) was investigated. Beef samples obtained from a local market were dipped into 1%, 1.5% and 2% chitosan solutions prepared with 1% acetic acid. The samples were drained, vacuum packed and stored at 4°C for a period of 12 days. The samples were evaluated for sensorial properties (color, odor and overall acceptability) and microbial counts (TVC, TCC and TYMC) on 0, 4, 8 and 12 days of storage. Chitosan treated samples having 1%, 1.5%, 2% chitosan solution and control which were expressed as T1, T2, T3 and T0 respectively. The obtained results showed that addition of chitosan solution, significantly ($p<0.05$) affected on physicochemical (pH, CP, POV, Cooking Loss), microbiological (TVC, TCC, TYMC) and sensory attributes (color, odor, overall acceptance) compared to control samples at refrigerated temperature. The pH and POV of all the treatment groups increase significantly ($p<0.05$) compared to control group at different days of interval during storage. The CP and cooking loss of different treatment groups decrease significantly ($p<0.05$) compared to control group at different days of interval during storage. The results also revealed that the samples were dipped in chitosan solution (1%, 1.5%, 2%) significantly ($p<0.05$) improved the microbiological quality, sensory attributes and reduced lipid oxidation in beef samples compared to the control samples at different days interval. However, abnormal changes were not determined on the samples treated with chitosan, even on the last day of storage. In beef, storage at 4°C for 12 days, chitosan inhibited the growth of spoilage bacteria, reduce lipid oxidation, putrefaction and resulted in better sensory test. The results indicated that the application of chitosan on the beef samples improve the microbiological quality and extends the shelf life usually 5-8 days, which could an alternative to chemical protective additives.

Key words: antimicrobial effect, sensory, storage, shelf life, chitosan

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Introduction

Meat refers to skeletal muscle and associated fat and other tissues, but it may also describe other edible tissues such as offal (Lawrie and Ledward, 2006). Beef is defined as the meat of cattle used as food. The nutritional attributes of meat, which provide a major proportion of consumer requirements for protein, some vitamins and certain minerals, are highlighted in work on the nutritional value of meat in other countries (Breidenstein, 1987; Johnson, 1987; Robinson, 2001). A portion of (10-12%) of total beef comes from growing animals during the Muslim religious festival, Eid-ul-Azha (Begum *et al*., 2007) in Bangladesh. Microbial growth and lipid oxidation are the two leading factors for quality deterioration of meat. Consumers demand high quality and convenient meat products, with natural flavour and taste, and they appreciate the fresh appearance of beef (Hugas *et al*., 2002). Colour is an important parameter that consumers use to judge the freshness and wholesomeness of beef. It has substantial influence on acceptability and purchasing decision at retail points (Eikelenboom *et al.,* 2000).

Oxidative processes, which occur during raw material storage, processing, heat treatment and further storage of final products, are major non-microbiological factors involved in quality deterioration of meat during refrigerated storage. Oxidation induces modifications of muscle lipids and proteins and, therefore, affects the organoleptic and nutritional properties of meat and meat products. This is reflected in economic losses and health disorders (Insani *et al.,* 2008 and Karpinska *et al.*, 2001). Chitosan, which is mainly made from crustacean shells, is the second most abundant natural polymer in nature after cellulose (Shahidi *et al.,* 1999). Chitosan is insoluble in most organic solvents and in water at neutral pH, but dissolves in dilute solutions of organic acids such as acetic, formic, tartaric, valeric, lactic, glycolytic and citric acids and also dissolves in dilute inorganic acids such as hydrochloric and sulfuric acids. Water-insolubility of chitosan is disadvantageous for its wide application as an antibacterial agent (Sashiwa and Aiba, ,,2004). In the recent decades, extensive investigations have been carried out to prepare functional chitosan and to increase its solubility in water in order to broaden its application. It has been widely used

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as a natural food additive in the food industry due to its nontoxic nature, biocompatibility, antibacterial and film forming properties (Majeti and Ravi, 2000). Function of chitosan differs from its molecular weight and degree of de-acetylation. The antimicrobial activity of chitosan with high molecular weight and high degree of de-acetylation was well documented against a number of food spoilage and pathogenic microorganisms with concentration varying from 0.5% to 1.5% (No *et al.,* 2002). In meat industry one of the most important scientific areas for research and application of chitosan is the study of its antibacterial and antifungal properties and the development of protective coatings on the basis of this polysaccharide with myco-bacteriostatic or myco-bactericidal properties. Analysis of the properties of various chitosan grades has resulted in a working hypothesis that chitosan can be used as part of protective film-forming coatings for meat and meat products. Recently, interest has considerably increased in finding naturally occurring antioxidant for usage in foods in order to replace the synthetic antioxidants which are being restricted legitimately due to their side effects (Guilcin *et al.*, 2003). In this research, chitosan as natural antioxidant will be used instead of synthetic antioxidant (BHA). Actually, chitosan has antioxidant and antimicrobial agents as well as to prolong the shelf life of meat and meat products. So far, we know that there is no research on preservation technique of beef at refrigerated temperature using chitosan in Bangladesh context. That's why the present work was conducted to fulfill the following objectives: i) to investigate the quality changes of beef at refrigerated temperature, ii) to evaluate the effect of chitosan on delaying lipid oxidation and iii) to evaluate the effect of chitosan on inhibiting microbial growth and extend the shelf life of beef.

Materials and Methods

Source of chitosan

Chitosan was collected from agricultural chemistry laboratory, Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh.

Preparation of Beef Sample

All visible fat and connective tissue were trimmed off as far as possible with the help of knife and the sample was cut into small pieces. Then whole sample was soaked into 3000 ml distilled water mixed with 30 g salt.

Preparation of Chitosan Solution

To prepare 1%, 1.5% and 2% chitosan solution respectively 4, 6 and 8 gram chitosan was mixed with 4 ml glacial acetic acid and stirred until dissolved it. Then 150 ml distilled water was added with the mixture and stirred again until mixed properly. Finally the solution was made up to 300 ml with distilled water.

Sensory properties of beef

Sensory evaluation

Each meat sample was evaluated by a trained 6-member panel. The sensory questionnaires measured intensity on a 5- point balanced semantic scale (weak to strong) for the following attributes color, off-odor, and overall acceptability. The judges evaluated the samples based on the above criterions. Panelists were selected among department staff and students and trained according to the American Meat Science Association guidelines (AMSA, 1995). Sensory evaluation was accomplished at 0 day and repeated at 4 day, 8 day and 12 day; up to the end of refrigerated storage at 4°C.

Physicochemical properties of beef

pH measurement

Samples (5 g) were homogenized in 45 ml of distilled water using a grinder (SFM1500NM, Shinil Co. China) for 1 min. Sample solutions were centrifuged for 15 min at 2000 x*g* and the pH was measured using a pH meter (Seven Easy pH, Mettler-Toledo GmbH, Switzerland).

Peroxide value

Peroxide value (POV) was determined according to (Sallam *et al*., 2004).

POV was calculated and expressed as mili-equivalent peroxide per kilogram of sample:

$$\text{POV (meq/kg)} = \frac{S \times N}{W} \times 1000$$

Where S is the volume of titration (mL), N is the normality of sodium thiosulfate solution (*n* = 0.01) and W is the sample weight (g).

Crude Protein

Crude protein was determined by micro kjeldahl method. The calculation is as follows:

$$\% \text{ Nitrogen} = \frac{\text{Titrate required (ml)} \times .014 \text{ (milliequivalent of N2)} \times \text{Strength of HCl}}{\text{weight of sample}} \times 100$$

% of CP = % of nitrogen × conversion factor (6.25)

Cooking loss

Cooking loss was calculated after draining the drip coming from the cooked meat as follows:

Cooking loss (%) = $[(w_2-w_3) \div w_2] \times 100$;

Where, w_2 = meat weight before cooking and w_3= meat weight after cooking.

Table 1. Effect of chitosan on physicochemical parameters (Mean ± SE) in beef at 4°C temperatures

Parameters	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat.	DI	T*DI
pH	0	5.77± 0.0088	5.806± 0.012	5.80± 0.0057	5.85± 0.0057	5.80^{a}± 0.0080	<.0001	<.0001	<.0001
	4	5.66± 0.0057	5.36± 0.0057	5.40± 0.0066	5.47± 0.0057	5.47^{b}± 0.0057			
	8	5.31± 0.0057	5.48± 0.0057	5.53± 0.0057	5.58± 0.0057	5.47^{bb}± 0.0057			
	12	4.91± 0.0057	5.63± 0.0057	5.67± 0.0057	5.70± 0.0057	5.47^{bb}± 0.0064			
	Mean	**5.43^{d}± 0.0064**	**5.56^{c}± 0.0072**	**5.59^{b}± 0.0059**	**5.63^{a}± 0.0057**				
CP	0	24.39± 0.0057	23.58± 0.0057	23.86± 0.0057	24.28± 0.0057	23.78^{a}± 0.0057	<.0001	<.0001	<.0001
	4	23.003± 0.0033	23.10± 0.0057	23.34± 0.0057	24.11± 0.0057	23.39^{b}± 0.0051			
	8	22.57± 0.0057	22.58± 0.0057	22.84± 0.0057	23.85± 0.0057	22.96^{c}± 0.0057			
	12	21.37± 0.0057	21.976± 0.0066	22.31± 0.0057	23.48± 0.0057	22.28^{d}± 0.0064			
	Mean	**22.56^{d}± 0.0051**	**22.81^{c}± 0.0064**	**23.09^{b}±0. 0057**	**23.96^{a}± 0.0057**				
POV	0	1.86± 0.0057	1.46± 0.0057	1.37± 0.0057	1.29± 0.0057	1.49^{d}± 0.0057	<.0001	<.0001	<.0001
	4	1.95± 0.0057	1.51± 0.0057	1.48± 0.0057	1.40± 0.0057	1.59^{c}± 0.0057			
	8	2.10± 0.0057	1.63± 0.0057	1.54± 0.0057	1.46± 0.0057	1.68^{b}± 0.0057			
	12	2.31± 0.0057	1.78± 0.0057	1.69± 0.0057	1.57± 0.0057	1.84^{a}± 0.0057			
	Mean	**2.06^{a}± 0.0057**	**1.59^{b}± 0.0057**	**1.52^{c}±0.0 057**	**1.43^{d}±0. 0057**				
Cooking Loss	0	27.83± 0.0057	25.17± 0.0057	26.38± 0.0057	26.98± 0.0057	26.59^{a}± 0.0057	<.0001	<.0001	<.0001
	4	26.34± 0.0057	23.82± 0.0057	25.02± 0.0057	25.31± 0.0057	25.12^{b}± 0.0057			
	8	24.83± 0.0057	22.13± 0.0057	23.52± 0.0088	23.67± 0.0057	23.54^{c}± 0.0065			
	12	23.11± 0.0057	21.74± 0.0057	22.03± 0.0057	22.23± 0.0057	22.28^{d}± 0.0057			
	Mean	**25.53^{a}± 0.0057**	**23.22^{d}± 0.0057**	**24.24^{c}±0. 0065**	**24.55^{b}± 0.0057**				

Mean in each row having different superscript varies significantly at values *P < 0.05. Again, mean values having same superscript in each row did not differ significantly at P > 0.05. T0, Control group; T1, 1% chitosan treated samples; T2, 1.5% chitosan treated samples; T3, 2% chitosan treated samples; DI, Days of Interval; Treat, Treatment, T*DI=Interaction of Treatment and Days of Intervals, CP, Crude Protein; POV, Peroxide Value

Table 2. Effect of chitosan on different microbial population (Mean ± SE) in beef at 4°C temperatures

Parameters	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat.	DI	T*DI
TVC (logCFU/g)	0	5.67± 0.0088	5.59± 0.0057	5.61± 0.0088	5.53± 0.0057	5.60^{d}± 0.0073	<.0001	<.0001	<.0001
	4	5.91± 0.0088	5.84± 0.0066	5.86± 0.0057	5.74± 0.0057	5.84^{c}± 0.0067			
	8	6.47± 0.0057	6.26± 0.0088	6.23± 0.0057	6.09± 0.0057	6.26^{b}± 0.0065			
	12	7.19± 0.0057	6.63± 0.0057	6.56± 0.0057	6.40± 0.0057	6.69^{a}± 0.0057			
	Mean	**6.31^{a}± 0.0073**	**6.08^{b}± 0.0067**	**6.07^{c}± 0.0065**	**5.94^{d}± 0.0057**				
TCC (logCFU/g)	0	1.44± 0.0088	1.39± 0.0057	1.24± 0.0057	1.26± 0.0057	1.33^{d}± 0.0065	<.0001	<.0001	<.0001
	4	1.58± 0.0057	1.54± 0.0057	1.40± 0.0057	1.36± 0.0057	1.47^{c}± 0.0057			
	8	1.78± 0.0057	1.67± 0.0057	1.55± 0.0057	1.49± 0.0057	1.62^{b}± 0.0057			
	12	2.06± 0.0057	1.826± 0.0033	1.72± 0.0057	1.63± 0.0057	1.81^{a}± 0.0051			
	Mean	**1.72^{a}± 0.0065**	**1.61^{b}± 0.0051**	**1.48^{c}± 0.0057**	**1.44^{d}± 0.0057**				
TYMC (logCFU/g)	0	1.87± 0.0057	1.51± 0.0057	1.496± 0.0088	1.48± 0.0087	1.59^{d}± 0.0067	<.0001	<.0001	<.0001
	4	1.96± 0.0057	1.65± 0.0057	1.62± 0.0057	1.59± 0.0057	1.71^{c}± 0.0057			
	8	2.07± 0.0057	1.76± 0.0057	1.71± 0.0057	1.67± 0.0057	1.80^{b}± 0.0057			
	12	2.21± 0.0057	1.90± 0.0057	1.81± 0.0057	1.75± 0.0066	1.92^{a}± 0.0059			
	Mean	**2.03^{a}± 0.0057**	**1.71^{b}± 0.0057**	**1.66^{c}± 0.0065**	**1.63^{d}± 0.0067**				

Mean in each row having different superscript varies significantly at values *P < 0.05. Again, mean values having same superscript in each row did not differ significantly at P > 0.05. T0, Control group; T1, 1% chitosan treated samples; T2, 1.5% chitosan treated samples; T3, 2% chitosan treated samples; DI, Days of Interval; Treat, Treatment, T*DI=Interaction of Treatment and Days of Intervals, TVC, Total Viable Count; TCC, Total Coliform Count, TYMC, Total Yeast Mold Count.

Table 3. Effect of chitosan on different microbial population (Mean ± SE) in beef at 4°C temperatures

Parameters	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat.	DI	T*DI
TVC (logCFU/g)	0	5.67± 0.0088	5.59± 0.0057	5.61± 0.0088	5.53± 0.0057	5.60^{d}± 0.0073	<.0001	<.0001	<.0001
	4	5.91± 0.0088	5.84± 0.0066	5.86± 0.0057	5.74± 0.0057	5.84^{c}± 0.0067			
	8	6.47± 0.0057	6.26± 0.0088	6.23± 0.0057	6.09± 0.0057	6.26^{b}± 0.0065			
	12	7.19± 0.0057	6.63± 0.0057	6.56± 0.0057	6.40± 0.0057	6.69^{a}± 0.0057			
	Mean	**6.31^{a}± 0.0073**	**6.08^{b}± 0.0067**	**6.07^{c}± 0.0065**	**5.94^{d}± 0.0057**				
TCC (logCFU/g)	0	1.44± 0.0088	1.39± 0.0057	1.24± 0.0057	1.26± 0.0057	1.33^{d}± 0.0065	<.0001	<.0001	<.0001
	4	1.58± 0.0057	1.54± 0.0057	1.40± 0.0057	1.36± 0.0057	1.47^{c}± 0.0057			
	8	1.78± 0.0057	1.67± 0.0057	1.55± 0.0057	1.49± 0.0057	1.62^{b}± 0.0057			
	12	2.06± 0.0057	1.826± 0.0033	1.72± 0.0057	1.63± 0.0057	1.81^{a}± 0.0051			
	Mean	**1.72^{a}± 0.0065**	**1.61^{b}± 0.0051**	**1.48^{c}± 0.0057**	**1.44^{d}± 0.0057**				
TYMC (logCFU/g)	0	1.87± 0.0057	1.51± 0.0057	1.496± 0.0088	1.48± 0.0087	1.59^{d}± 0.0067	<.0001	<.0001	<.0001
	4	1.96± 0.0057	1.65± 0.0057	1.62± 0.0057	1.59± 0.0057	1.71^{c}± 0.0057			
	8	2.07± 0.0057	1.76± 0.0057	1.71± 0.0057	1.67± 0.0057	1.80^{b}± 0.0057			
	12	2.21± 0.0057	1.90± 0.0057	1.81± 0.0057	1.75± 0.0066	1.92^{a}± 0.0059			
	Mean	**2.03^{a}± 0.0057**	**1.71^{b}± 0.0057**	**1.66^{c}± 0.0065**	**1.63^{d}± 0.0067**				

Mean in each row having different superscript varies significantly at values *P < 0.05. Again, mean values having same superscript in each row did not differ significantly at P > 0.05. T0, Control group; T1, 1% chitosan treated samples; T2, 1.5% chitosan treated samples; T3, 2% chitosan treated samples; DI, Days of Interval; Treat, Treatment, T*DI=Interaction of Treatment and Days of Intervals, TVC, Total Viable Count; TCC, Total Coliform Count, TYMC, Total Yeast Mold Count.

Microbial assessment

For microbial assessment total viable count, total coliform count and total yeast-mould count were undertaken. A quantity of 10 g of beef meat sample was aseptically excised from stored stock sample. Each of the stored beef meat samples was thoroughly and uniformly macerated in a mechanical blender using a sterile diluent (0.1% peptone water) as per the recommendation of International Organization for Standardization (ISO, 1995). A quantity of ten (10) grams of the minced meat sample was taken aseptically transferred into a sterile container containing 90 ml of

0.1% peptone water. A homogenized suspension was made in a sterile blender. Thus 1:10 dilution of the samples was obtained. Later on using whirly mixture machine different

serial dilutions ranging from 10-2 to 10-6 were prepared according to the instruction of the standard method (ISO, 1995).

CFU/gm = (number of colonies / (volume plated × total dilution)

Statistical model and analysis

The proposed model for the planned experiment was factorial experiment with two factors A *(Treatments)* and *B (Days of Intervals)* is:

$yijk = \mu + Ai + Bj + (AB)ij + \varepsilon ijk$ $i = 1,\ldots,a$; $j = 1,\ldots,b$; $k = 1,\ldots,n$

Where, *yijk*= observation *k* in level *i* of factor *A* and level *j* of factor *B*

B μ = the overall mean

Ai = the effect of level *i* of factor *A*

Bj= the effect of level *j* of factor *B*

Data were statistically analyzed using SAS statistical discovery software, NC, USA. DMRT test was used to determine the significance of differences among treatments means.

Results and Discussion

Physicochemical Analysis

pH Value

The pH values of beef samples prepared with chitosan solution were significantly (P<0.05) higher compared to control. pH of beef samples showed a significant difference (P<0.05) among treatments throughout the storage periods. The different superscript was observed from 0, 4^{th}, 8^{th} and 12^{th} days of observation indicates there were significant difference among these fourth days observation. The pH value of all beef samples slightly decreased during the first 4 days of storage, whereas after 3 days there was a gradual increase. This decrease indicates that some fermentation occurs during storage. The last pH values increase might have been due to the liberation of ammonia compounds as a result of endoprotease activity or the proteolytic microbial flora present in the raw meat (Mokhtar *et al.,* 2012).There was a gradual increase in pH in all samples during storage, probably due to the accumulation of basic compounds such as ammonia, derived from microbial action (Nychas *et al*., 1998).

Peroxide Value (POV)

During storage, the peroxide value significantly (P<0.05) increased in all treatments. The initial POV value of the control sample was 1.86 meq./ kg lipid and increased to 2.31 meq./kg lipid after 12 days storage, significantly (P<0.05) higher than other treatments (Table 1). The different superscript was observed from 0, 4^{th}, 8^{th} and 12^{th} days of observation indicates there were significant difference among these fourth days observation. Peroxide value of control and treatments showed a highly significant difference (P<0.05) in between the treatments and in between the storage period. Similar, results were reported by Gheisari, (2011), who found a significant increase in peroxide value with the storage period in chicken meat stored under refrigeration temperature. Georgantelis *et al.,* (2007a) observed that 1 % chitosan, individually or in combination with other natural antioxidants, was more effective in decreasing lipid oxidation in frozen beef patties.

Crude Protein (CP)

The different superscripts were observed in all treatments groups CP content significantly (P<0.05) decrease compared to control. The different superscripts were observed at 0, 4^{th}, 8^{th} and 12^{th} days of observation which indicate that there were significant (P<0.05) differences among these four days of observation. The CP content was decreased with the increased storage period. The most preferable CP content was observed at 0 day and less preferable CP content was observed at 12 day. The same trend was also observed by (Konieczny *et al.,* 2007) and they reported that CP content decreased during frozen storage.

Cooking Loss (CL)

The different superscripts were observed in all treatments groups CL content significantly (P<0.05) decrease compared to control. Among these three treatments, most preferable cooking loss was observed at 2% chitosan than other groups. The different superscripts were observed at 0, 4^{th}, 8^{th} and 12^{th} days of observation which indicate that there were significant (P<0.05) differences among these four days of observation. Among this treatment groups and days of interval Duncan grouping letters a, b, c and d are indicate that there were significant difference. The cooking loss was decreased with the increased storage period. The less preferable cooking loss was observed at 12^{th} day and most preferable cooking loss was observed at 0 day observation. Cooking loss refers to the reduction in weight of meat during the cooking process (Jama *et al.,* 2008). Major components of cooking losses are thawing, dripping and evaporation.

Microbiological Assessments

Total Viable Count (TVC)

Total viable counts were affected significantly (P<0.05) by dipping the samples in 1%, 1.5% and 2% chitosan solution compared with samples dipped in distilled water (Control group). The increase in the number of microorganisms in the treated samples with chitosan was significantly (P<0.05) less than those treated with only water. The initial value of TVC for fresh beef (beef not frozen and thawed) was 5.67 $\log_{10}$ CFU/g beef, indicating good quality beef. The range of overall

observed of different days of intervals of TVC value was 6.69 to 5.60. The different superscript was observed from different treatments indicate there were significant differences of TVC values among these four treatment groups. Among four treatments, the plate count in the control sample (6.03$\log_{10}$ CFU/g) was significantly higher than in the samples treated with chitosan solution 1%, 1.5%, 2% respectively. The results of the study revealed a significant (P<0.05) difference in standard plate count among storage period and among treatments and standard plate count increased significantly (P<0.05) with storage period. Results also showed that 2% chitosan solution had better antimicrobial capacity than control sample, and chitosan solution (1%, 1.5%) had possible synergistic effect on microbial inhibition. Georgantelis *et al.,* (2007b) also reported that, in pork sausages, the lowest microbial counts were obtained in samples containing chitosan and rosemary, indicating a possible synergistic effect. The antimicrobial activity of chitosan is well documented against a number of food spoilage and pathogenic microorganisms with MIC varying from 0.01% to 1% (Sagoo *et al.,* 2002).

Total Coliform Count (TCC)

The results of total coliform count of beef dipped in chitosan with different treatments (1% chitosan solution, 1.5% chitosan solution, 2% chitosan solution and control group) during 12 days of refrigerated storage are represented table 2. Total coliform counts were affected significantly (P<0.05) by dipping the samples in 1%, 1.5% and 2% chitosan solution compared with samples dipped in distilled water (Control group). The increase in the number of coliform in the treated samples with chitosan was significantly (P<0.05) less than those treated with only water. The different superscript was observed from different treatments indicate there were significant differences of TCC values among these four treatment groups. Among four treatments, the coliform count in the control sample (2.06 $\log_{10}$ CFU/g) was significantly higher than in the samples treated with chitosan solution 1%, 1.5%, 2% respectively. During storage TCC was increased gradually in different treatments at increasing storage days. Chitosan treated samples reached the acceptable limit on day 12, indicating a significantly delayed microbial spoilage

In 2% chitosan treated sample was highly preferable than other treatment groups. Generally, the addition of chitosan affected color (P<0.05) and was dependent on the concentration (Barbera *et al.*, 2011). Also, in fresh ground beef patties, chitosan in combination with rosemary extract showed synergistic effect resulting in the most intense red color stabilization and anti-oxidative protection (Mokhtar*et al.,* 2014).

(P<0.05) than control treatment. Results also showed that 2% chitosan solution had better antimicrobial capacity than control sample, and chitosan solution (1%, 1.5%) had possible synergistic effect on microbial inhibition (No *et al.*, 2002).

Total Yeast-Mold Count (TYMC)

Yeast and mold counts were affected significantly (P<0.05) by dipping the samples in 1%, 1.5% and 2% chitosan solution compared with samples dipped in distilled water (Control group). The increase in the number of yeast and mold in the treated samples with chitosan was significantly (P<0.05) less than those treated with only water. The initial value of yeast and mold counts for fresh beef (beef not frozen and thawed) was 1.44 $\log_{10}$ CFU/g beef, indicating good quality beef. The results of the study revealed a significant (P<0.05) difference in yeast and mold counts among storage period and among treatments and Yeast and mold count increased significantly (P<0.05) with storage period. Results also showed that 2% chitosan solution had better antimicrobial capacity than control sample, and chitosan solution (1%, 1.5%) had possible synergistic effect on microbial inhibition. Microbial colonization decreases with increasing concentration of chitosan, which was also confirmed by Ulbin-Figlewicz*et al.,* (2014). Similar results were reported by Roller *et al.,* (2002) chitosan (0.6%) incorparated into the sausages did not reduce significantly the yeast and molds counts during storage at 4°C.

Sensory Evaluation

Color

The color was affected significantly (P<0.05) by dipping the samples in 1%, 1.5% and 2% chitosan solution compared with samples dipped in distilled water (control group). The different superscripts were observed in all treatments groups which indicate there were significant (P<0.05) difference of color content. In different treatment groups color content significantly (P<0.05) decreased but in the control group color content decreased rapidly.

Odor

The same superscript was observed from all treatments which indicate that there were significant ($P<0.05$) differences of odor of all treatment. The most preferable good odor was observed from 2% chitosan solution treatment and the lowest odor from control group. The range of odor among different days of intervals was 4.46 to 3.45. The odor of different treatments was decreased with increased storage period. The different superscripts were observed at 0 to 12th days observation indicates that there were little changes of odor values. It showed that the quality was deteriorated with increased storage period. Roller *et al.,* (2002) also reported that the addition of chitosan to sausages would not lead to off-odors and that the appearance would not be rendered objectionable, either of which could potentially lead to rejection by the consumer. Sagoo *et al.,* (2002) determined that shelflife of the sausage was extended from seven days to fifteen days with chitosan. Kanatt *et al.,* (2008) reported that mixture of chitosan and mint extract enhanced the shelflife of pork cocktail salami stored at 0-3°C.

Overall Acceptance

The overall acceptance of food by consumers determines the future of that food in the market. Therefore, increasing the consumer acceptance of food processed with new technologies will accelerate their market share in food industry. The overall acceptance score of different treatments with days of intervals is shown in Table 3. Different treatments (chitosan) resulted in significantly ($P<0.05$) higher overall acceptance scores than the control at the end of storage. Beef samples treated with chitosan solutions (1%, 1.5%, 2%) had slightly significant difference ($P<0.05$) on the changes of overall acceptance scores and high concentration chitosan (2%) treated samples revealed slightly change of flavor with storage time extending. Chitosan 2% solution treated samples were mostly accepted by panelists might be attributed to oxidative and microbial stability with possible synergistic effect to chitosan.

Conclusion

Beef can be preserved for 12 days in different techniques with more or less difference in the quality. The results of the present study it may also be concluded that 2% concentration of chitosan (CHI) will be used in future for manufacturing beef with providing antioxidant and antimicrobial agents as value addition through inhibiting lipid oxidation & prolonged the shelf- life of stored meat and meat products instead of synthetic antioxidant.

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