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Effect of Different Levels of Orange Peel Extract on the Quality and Shelf Life of Beef Muscle during Frozen Storage

F. Haque [a, 1], M. H. Rahman[b,*,#], M. Habib[c], M. S. Alam[d], M. M. Monir[d], M. M. Hossain[a]

[a] *Department of Animal Science, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh*
[b] *Department of Animal Products and By-products Technology, Patuakhali Science and Technology University, Barishal-8210, Bangladesh*
[c] *Department of Agricultural Extension, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh*
[d] *Department of General Animal Science and Animal Nutrition, Patuakhali Science and Technology University, Barishal-8210, Bangladesh*
[*] *Corresponding author: M. H. Rahman*

Abstract*: The aim of this research work was to explore the effect of different levels of orange peel extract as a natural antioxidant and antimicrobial agent on fresh and preserved beef. The beef were subjected to four treatment with different level of orange peel extract (T_0 = fresh raw beef; T_1 = 0.2% orange peel extract, T_2 = 0.3% orange peel extract and T_3 = 0.4% orange peel extract) and carried out to evaluate sensory, proximate components, physicochemical quality, biochemical quality and microbiological assessment as natural antioxidant and antimicrobial agent for maintaining beef qualities and the shelf-life of beef for 0, 15th, 30th and 60th day of storage period. Color, flavor, tenderness, juiciness, overall acceptability at different levels of orange peel extract were almost similar to control but decreased significantly ($p<0.05$) with increased storage periods (different days of intervals). A significant ($p<0.05$) change were observed in raw pH and cooking loss at different treatment levels with increased storage periods. Among the proximate components DM, EE and Ash content of beef increased significantly ($p<0.05$) in all treatment and with increased storage periods. But CP content of beef samples were decreased significantly ($p<0.05$) in different treatment groups with prolonged storage. FFA, POV, TBARS differ significantly ($p<0.05$) at different treatment levels with prolonged preservation periods. Total viable bacteria, total coliform and total yeast-mould count of beef decreased significantly ($p<0.05$) at different treatment levels and storage periods in comparison to control beef. The beef muscle remained stable with minor changes in sensory, physic-chemical and microbiological quality during frozen storage (-20°C) for 60 days. The best results were obtained at 0.4% orange peel extract among the treatments. Therefore, it may be concluded that 0.4% orange peel extract has the best potential as natural antioxidant and antimicrobial property in terms of beef color for commercial value, nutrient quality, physicochemical, biochemical and microbial assessment for beef quality.*

Key words*: Orange Peel, Quality, Shelf Life, Beef, Antioxidant and TBARS.*

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I. Introduction

Meat typically spoils due to one of the two major causes- microbial growth or chemical deterioration. In chemical deterioration, lipid oxidation is important in the meat industry (Raghavan and Richards, 2007). Because lipid oxidation imparts adverse effects not only on sensory attributes such as color, texture, odor, and flavor but also on nutritional quality of meat (Nunez de Gonzalez *et al*., 2008). Before meat is cooked, lipids in the meat undergo autoxidation (Angelo *et al*., 1990), which requires oxygen as an oxidizing agent (Rojas and Brewer, 2008).

Frozen processed meat and meat products are available in the home consumption and markets. Nowadays, frozen meat has a huge demand with high commercial value. The most important quality during long-term storage of frozen meat includes loss of color, fat oxidation, protein denaturation, and formation of ice crystals (Erickson, 1997, Gonçalves and Junior, 2009).

The common strategy adopted for preventing lipid oxidation is the use of antioxidants (Tang *et al.*, 2001). The inherent antioxidant systems present in meat are known as endogenous antioxidants whereas exogenous antioxidant systems include both synthetic and natural antioxidants that are added during processing of meat products (Decker and Mei, 1996). Well-known endogenous systems in fresh meats include tocopherols, carnosine, lipoic acid, and various enzymatic systems (Decker and Mei., 1996).

For several years, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) have been used as antioxidants in meat and poultry products (Formanek *et al*., 2001; Jayathilakan *et al*., 2007; Biswas *et al*., 2004). The use of these synthetic antioxidants has been scrutinized in these days due to their potential toxicological effects (Raghavan and Richards, 2007; Naveena *et al*., 2008; Nunez de Gonzalez *et al*., 2008) and at the same time, increasing trend of "green" consumerism (DeSilva, 1996; Smid and Gorris, 1999). The use of natural antioxidants became a new concept of making food safe which has a natural or "green" image. This has led the researchers and food processors to look for the natural antioxidants with broad spectrum antimicrobial and anti-oxidant activity (Ahn *et al.,* 2002; Baratta *et al.,* 1998; Tomaino *et al.,* 2005).

Natural antioxidants mainly come from plants, fruits and vegetables which are rich source of phytochemicals such as ascorbic acid, carotenoides, flavonoides and other phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols). Citrus fruits and waste are of great value since it contains large amount of various carotenoids, flavonoids, dietary fiber, sugars, polyphenols, essential oils, ascorbic acid, as well as some trace elements (Sharma *et al*., 2017). Orange peels are considered the primary citrus by-products and discarded as wastes. Citrus fruit juices and orange peels are known as antimicrobial agents against the bacteria and the fungus (Pandey *et al*., 2011; Al-Ani *et al.,* 2010).

Because of the preferred flavor, delightful taste, affordable economic reach and consumer awareness of the increasingly recognized potential health properties, citrus fruits and their by-products are very prevalent in both developed and developing countries (Ting, 1980). Orange (*Citrus sinensis* L.), a hesperidium belonging to the Rutaceae family, is the most widely grown and commercialized citrus species. Besides sugars, acids, and polysaccharides, oranges are an important source of phytochemicals such as phenolics, ascorbic acid and carotenoids. These compounds, also known as nutraceuticals, provide health benefits due to a reduction of chronic illness such as cancer and cardiovascular diseases (Diplock, 1994; Faulks & Southon, 2001).

The search for alternative methods to retard oxidative processes in meat and meat products has led researchers to investigate natural antioxidants. Addition of antioxidants to meat products is known to be effective in metmyoglobin formation and lipid oxidation. The application of vitamin E (McCarthy *et al.,* 2001), ascorbic acid (Sánchez-Escalante *et al.,* 2003), rosemary (Sebranek *et al.,* 2005) to meat products is well documented. In order to keep pace with the recent trends and the present demands of meat industry, this study was undertaken to determine the effect of different levels of orange peel extract on the sensory characteristics, proximate, physicochemical, biochemical, microbial quality and stability of beef muscle under frozen storage to compare with fresh beef muscle.

II. Materials and methods

2.1 Sample collection and preparation

The meat samples were obtained from a 2-year old bull weighing 250 kg. The required quantity of beef muscle was purchased within 2 hours of slaughter. After removing the fat, ligaments, bone and tendons from the muscles, they were randomly divided into thirty six samples for Completely Randomized Design (CRD) model. The orange peel extract was mixed with beef sample at the rate of 0.2, 0.3 and 0.4% for T_1, T_2 and T_3 treatment groups, respectively. Then the beef samples were frozen in a blast freezer set at -20ºC with a wind speed of 2.6 m/s till further use. The fresh beef was used as control (T_0) i.e. not frozen or thawed. The temperature of the freeze was checked regularly. The samples were stored for 60 d. The samples were analyzed at 0, 15^{th}, 30^{th} and 60^{th} day for different parameters.

2.2 Sensory evaluation

Sensory evaluation raw beef samples were analyzed for their freshness, texture, odor, spoilage/decay and overall acceptability by 10 trained and untrained panelists familiar with beef evaluation after 0, 15^{th}, 30^{th} and 60^{th} day of preservation. Panelists were selected among department staff and students and trained according to the American Meat Science Association guidelines (AMSA, 2015). Sensory evaluation was carried out in individual booths under controlled conditions of light, temperature and humidity. Prior to sample evaluation, all panelists participated in orientation sessions to familiarize with the scale attributes (off-odor, freshness, overall and so on) of raw beef using an intensity scale. Sensory qualities of the samples were evaluated after refrigerator thawing using a 5-point scoring method. Sensory scores were 5 for excellent, 4 for very good, 3 for good, 2 for fair and 1 for poor (Rahman *et al.,* 2014). In particular, for spoilage of samples, panelists observed the degree of spoilage by appearance (discoloration and slime formation). All samples were served in the petri-dishes.

2.3 Proximate composition

Proximate analysis such as moisture, crude protein, ether extract and ash value were determined by following the AOAC (2011) methods. All determination was done in triplicate.

2.4 Physicochemical properties of beef

The physicochemical properties like pH, cooking loss, and lipid oxidation as free fatty acids (FFA) value, peroxide value and thiobarbituric acid reacting substances (TBARS) value were analyzed at 0 d and repeated in 15th, 30th and 60th day of preservation.

2.4.1 pH

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 × *g*, and the pH was measured using a pH meter (Seven Easy pH, Mettler-Toledo GmbH, Switzerland).

2.4.2 Cooking loss

To determine cooking loss, weighed 5±1 g samples and wrapped in a heat-stable foil paper and kept in water bath at 80°C for 30 min. The internal temperature was not measured, but from a study (Sultana *et al.*, 2009) it was estimated that the optimum internal meat temperature (75-80°C) would be gained by 30 min. Samples are dried and weighed out. After draining the drip cooked loss was calculated as follows:

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

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

2.4.3 Free fatty acid value (FFA)

FFA value was determined according to Rukunudin *et al.* (1998). Beef samples (5 g) were dissolved with 30 ml chloroform using a homogenizer (IKA T25digital Ultra-Turrax, Germany) at 10,000 rpm for 1 min. The sample was filtered under vacuum through Whatman filter paper number 1 to remove meat particles. After five drops of 1% ethanolic phenolphthalein were added as indicator to filtrate, the solution was titrated with 0.01 N ethanolic potassium hydroxide. The formula is mentioned below: FFA (%) =

$$\frac{\text{ml titration} \times \text{normality of KOH} \times 28.2}{\text{g of sample}}$$

2.4.4 Peroxide value (POV)

The peroxide value (POV) was determined according to the method of Sallam *et al.* (2004). The samples (3 g) were weighed in a 250-ml glass stopper Erlenmeyer flask. Then it was heated for 3 min at 60°C in a water bath to melt the fat. After that the flask thoroughly agitated for 3 min with 30 ml acetic acid-chloroform solution (3:2 v/v) to dissolve the fat. Whatman filter paper number 1 was used in filtration process to remove beef particles from the filtrate. After adding saturated potassium iodide solution (0.5 ml) to filtrate and continued with addition of starch solution as indicator. The titration was continued against standard solution of sodium thiosulfate. POV was calculated by following equation and expressed as milli 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).

2.4.5 Thiobarbituric acid reactive substance (TBARS)

Lipid oxidation was assessed in triplicate using the 2-thiobarbituric acid (TBA) method described by Schmedes and Holmer (1989). Beef samples (5 g) were blended with 25 ml of 20% trichloroacetic acid solution (200 g/l of tricholoroacetic acid in 135 ml/l phosphoric acid solution) in a homogenizer (IKA) for 30s. The homogenized sample was filtered with Whatman filter paper number 4, and 2 ml of the filtrate was added to 2 ml of 0.02 M aqueous TBA solution (3 g/l) in a test tube. The test tubes were incubated at 100°C for 30 min and cooled with running tap water. The absorbance was measured at 532 nm using a UV-VIS spectrophotometer (UV-1200, Shimadzu, Japan). The TBA value was expressed as mg malonaldehyde per kg of beef sample.

2.5 Microbial assessment

Beef samples (25 g) were aseptically homogenized with 225 ml of sterile peptone water (EMD Buffered peptone water granulated, EMD Chemicals Inc., USA) (1 g/l) in a stomacher bag with stomacher blender (Stomacher® 400 Circulator, Seward Ltd., U.K.) for 5 min. Serial dilutions were prepared. Total viable counts (TVC) were measured by pouring 0.1 ml of each dilution on duplicate plates and then were poured by plate count agar (EMD dehydrated plate count agar granulated, EMD Chemicals Inc., USA). After 48 h of incubation at 37°C, built up colonies were counted according to ISO (1995) and results were expressed as Log CFU/g beef sample. Total coliform was measured by spreading 0.1 ml of each dilution with a bent sterile polypropylene rod on duplicate plates of prepoured and dried MacConkey agar (EMD dehydrated MacConkey

agar granulated, EMD Chemicals Inc., USA). After 48 h of incubation at 37°C, built up colonies were counted according to ISO (1995) and results were expressed as Log CFU/g sample. Total yeast and mould was measured by spreading 0.1 ml of each dilution with a bent sterile polypropylene rod on duplicate plates of prepoured and dried standard potato dextrose agar (EMD Dehydrated potato dextrose agar granulated). After 72 h of incubation at 25°C, built up colonies were counted according to ISO (1995) and results were expressed as Log CFU/g sample.

2.6 Statistical model and analysis

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

$y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \varepsilon_{ijk}$; $i = 1,\ldots,a$; $j = 1,\ldots,b$; $k = 1,\ldots,n$

Where,

y_{ijk} = observation k in level i of factor A and level j of factor B

μ = the overall mean

A_i = the effect of level i of factor A

B_j = the effect of level j of factor B

The three treatments (T_1 = 0.2% orange peel extract, T_2 = 0.3% orange peel extract, T_3 = 0.4% orange peel extract) were resulted from four repeated intervals (days). Different tests were repeated thrice for each interval. Data were statistically analyzed using Completely Randomized Design (CRD) model procedure by JMP, SAS Statistical Discovery software, NC, USA. The 3×4 factorial design was used for cycle-treatment interaction analysis. Tukey HSD test was used to determine the significance of differences among treatments means.

III. Results

3.1 Sensory evaluation

3.1.1 Color

The color of beef samples was observed at different concentration of orange peel extract during refrigerated storage (Table 1). The color was almost similar to control but slightly varied by length of preservation period. There was no significant (P>0.05) difference among the treatments but color was significantly (P>0.05) differ among the preservation periods. Beef with 0.3% orange peel extract showed the highest color score at 15th days of preservation and 0.4% orange peel extract showed lowest color score at 60th day, respectively.

Table 1: Effect of different levels of Orange peel extract and storage periods on sensory quality of beef (mean±SE)

Parameters	DI	Treatments				Mean	Level of significance		
		T_0	T_1	T_2	T_3		Treat.	DI	T*D1
Color	0	4.66±0.33	5.00±0.0	4.66±0.33	4.66±0.33	4.75^{a}±0.25	NS	**	NS
	15	4.66±0.33	4.66±0.33	5.00±0.0	4.00±0.57	4.58^{a}±0.31			
	30	3.33±0.33	3.66±0.33	4.00±0.57	3.33±0.33	3.58^{b}±0.39			
	60	2.33±0.33	3.66±0.33	3.66±0.33	3.33±0.33	3.25^{b}±0.33			
	Mean	3.75^{a}±0.33	4.25^{a}±0.25	4.33^{a}±0.31	3.83^{a}±0.39				
Flavor	0	4.33±0.33	4.33±0.33	4.66±0.33	4.66±0.33	4.50^{a}±0.33	NS	**	NS
	15	4.33±0.33	4.66±0.33	4.33±0.33	4.33±0.33	4.41^{a}±0.33			
	30	3.66±0.33	4.00±0.57	4.33±0.33	4.33±0.33	4.08^{a}±0.39			
	60	2.66±0.33	3.33±0.33	3.66±0.33	3.33±0.33	4.25^{b}±0.33			
	Mean	3.75^{a}±0.33	4.08^{a}±0.39	4.25^{a}±0.33	4.16^{a}±0.33				
Tenderness	0	4.33±0.33	4.66±0.33	4.66±0.33	4.33±0.33	4.5^{a}±0.33	NS	**	NS
	15	4.00±0.00	4.66±0.33	4.66±0.33	3.66±0.33	4.25^{a}±0.25			
	30	3.66±0.33	3.66±0.33	3.66±0.33	3.33±0.33	3.58^{b}±0.33			
	60	2.66±0.33	3.33±0.33	3.66±0.33	3.66±0.33	3.33^{b}±0.33			
	Mean	3.66^{a}±0.25	4.08^{a}±0.33	4.16^{a}±0.33	3.75^{a}±0.33				
Juiciness	0	4.66±0.33	4.66±0.33	4.66±0.33	4.33±0.33	4.58^{a}±0.33	NS	**	NS
	15	4.66±0.33	4.66±0.33	4.66±0.33	4.33±0.33	4.58^{a}±0.33			
	30	3.33±0.33	3.66±0.33	3.66±0.33	3.66±0.33	3.58^{b}±0.33			
	60	2.33±0.33	3.00±0.57	3.33±0.33	3.33±0.33	3.00^{c}±0.39			
	Mean	3.75^{a}±0.33	3.99^{a}±0.39	4.08^{a}±0.33	3.91^{a}±0.33				
Overall acceptability	0	4.33±0.33	4.33±0.33	4.66±0.33	4.66±0.33	4.5^{a}±0.33	NS	**	NS
	15	4.33±0.33	4.66±0.33	4.66±0.33	4.66±0.33	4.58^{a}±0.33			

	30	3.66±0.33	3.66±0.33	3.66±0.33	3.33±0.33	3.58^{a}±0.33			
	60	3.00±0.57	3.33±0.33	3.33±0.33	2.66±0.33	3.08^{b}±0.39			
	Mean	3.83^{a}±0.39	4.0^{a}±0.33	4.08^{a}±0.33	3.83^{a}±0.33				

Means with different superscripts in each column and row are significantly different (*p<0.05, **p<0.01). NS was not significantly different. Sensory scores were based on 5 point descriptive scale, where 5=Excellent, 4=Very good, 3=Good, 2=Fair, 1=Poor. T_0 = control, T_1 = 0.2% orange peel extract, T_2 = 0.3% orange peel extract, T_3 = 0.4% orange peel extract, DI = Days of Intervals, Treat = Treatment, T*DI = Interaction of Treatment and Day Intervals.

3.1.2 Flavor

The flavor was almost similar to control but differed with concentration of orange peel extract and days of preservation periods (Table 1). No significant change of flavor of beef samples among treatment groups but significant (p<0.05) difference among days of intervals. The most preferable good flavor was observed at 0.3% orange peel extract beef samples of 15 days of preservation and 0.4% orange peel extract beef samples showed the lowest flavor at 60 days of intervals.

3.1.3 Tenderness

The tenderness score of different treatments with days of intervals was shown in Table 1. There was no significant difference of tenderness score among treatment groups and most preferable tenderness was observed at 0.3% orange peel extract beef samples and less preferable tenderness was observed in control group. There was also significant (p<0.05) difference among days of observation and the most preferable tenderness was observed at 15^{th} days and less preferable tenderness at 60 day.

3.1.4 Juiciness

Beef samples with different concentration of orange peel extract did not affect (p>0.05) juiciness among the treatment groups but within days of preservation intervals significant (p<0.05) change was found (Table 1). Among the treatments most preferable juiciness score was observed at 0.3% orange peel extract beef samples of 15 days of preservation and less preferable juiciness was observed at control group of 60 days of preservation.

3.1.5 Overall acceptability

There was no significant difference (p>0.05) of overall acceptability among treatment groups but slightly change within days of intervals of observation. The overall acceptability score of different treatments with day intervals shown in Table 1. The most preferable overall acceptability was observed in 0.3% orange peel extract beef samples at 15 days of preservation and less preferable overall acceptability was observed in control group after 60 days of storage.

3.2 Proximate analysis

3.2.1 Dry matter (DM)

The results showed significant (p<0.05) change of dry matter content of beef samples treated not only with different level of orange peel extract but also with different days of preservation (Table 2). The mean value of DM content at different treatments was observed 25.35 to 27.40%. The highest DM content was found in fresh beef samples at 60 days of preservation and the lowest in beef sample with 0.4% orange peel extract at 0 day of preservation.

3.2.2 Crude protein (CP)

The slight decrease of crude protein content was observed in beef samples treated with different level of orange peel extract with days of interval of preservation period. The range of CP content observed at different treatment groups was 22.65 to 22.96% (Table 2). There was no significant change (p>0.05) in CP content among the treatment groups but significant change was observed (p<0.05) in days of intervals of preservation period.

3.2.3 Ether extracts (EE)

Ether Extract content of the beef samples treated with orange peel extract was shown in Table 2. The mean ether extract content of treated samples was decreased (p<0.05) with days of intervals of preservation. The highest EE content was observed in fresh sample with 0 day of preservation and lowest at 0.4% orange peel extract with 60 days of preservation.

Table 2: Effect of different levels of Orange peel extract and storage periods on proximate components of beef (mean±SE)

Parameters	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat.	DI	T*DI
DM (%)	0	25.40±0.03	25.02±0.03	24.68±0.02	24.18±0.03	24.81^{d}±0.03	**	**	**
	15	26.36±0.31	26.84±0.03	25.08±0.05	24.73±0.05	25.75^{c}±0.011			
	30	27.74±0.05	28.20±0.04	26.74±0.04	25.32±0.03	27.03^{b}±0.04			
	60	30.13±0.03	29.30±0.02	28.38±0.05	27.19±0.03	28.75^{a}±0.03			
	Mean	27.40^{a}±0.11	27.34^{a}±0.03	26.21^{b}±0.04	25.35^{c}±0.03				
CP (%)	0	23.23±0.03	23.32±0.04	23.36±0.02	23.47±0.03	23.35^{a}±0.03	**	**	**
	15	22.86±0.03	23.12±0.03	23.02±0.03	23.12±0.02	23.03^{b}±0.03			
	30	22.41±0.02	22.85±0.05	22.25±0.03	22.69±0.03	22.55^{c}±0.03			
	60	22.09±0.02	22.43±0.04	21.88±0.04	22.20±0.02	22.15^{d}±0.03			
	Mean	22.65^{c}±0.03	22.93^{a}±0.04	22.94^{c}±0.03	22.96^{b}±0.03				
EE (%)	0	3.35±0.01	3.31±0.01	3.19±0.02	3.08±0.02	3.23^{d}±0.02	**	**	*
	15	3.54±0.01	3.43±0.008	3.31±0.01	3.23±0.01	3.38^{c}±0.01			
	30	3.67±0.01	3.55±0.01	3.45±0.02	3.35±0.01	3.51^{b}±0.01			
	60	3.82±0.01	3.74±0.01	3.55±0.01	3.45±0.01	3.64^{a}±0.01			
	Mean	3.60^{a}±0.01	3.51^{b}±0.01	3.38^{c}±0.02	3.28^{d}±0.01				
Ash (%)	0	0.44±0.04	0.55±0.02	0.61±0.002	0.68±0.01	0.57^{d}±0.02	**	**	**
	15	1.01±0.03	0.83±0.01	0.95±0.003	0.95±0.01	0.94^{c}±0.02			
	30	1.03±0.02	1.09±0.01	1.24±0.02	1.28±0.01	1.16^{b}±0.02			
	60	1.45±0.01	1.34±0.02	1.47±0.02	1.51±0.01	1.44^{a}±0.02			
	Mean	0.98^{c}±0.03	0.95^{c}±0.02	1.07^{b}±0.02	1.11^{a}±0.01				

Means with different superscripts in each column and row are significantly different (*p<0.05, **p<0.01). NS was not significantly different. Where, T_0 = control, T_1 = 0.2% orange peel extract, T_2 = 0.3% orange peel extract, T_3 = 0.4% orange peel extract, DM= Dry matter, CP= Crude protein, EE= Ether extracts, DI = Days of Intervals, Treat = Treatment, T*DI = Interaction of Treatment and Day Intervals.

3.2.4 Ash

The ash content of beef samples of different treatment groups with days of intervals was also shown in Table 2. Ash content of beef samples was increased significantly (p<0.05) among the treatment groups with days of intervals of preservation. The highest ash content was observed at 0.4% orange peel extract with 60 days of preservation and the lowest in fresh sample with 0 day of preservation.

3.3 Physicochemical properties

3.3.1 pH of raw meat

The initial pH of fresh beef sample was 5.76. As the increased of preservation period pH was also decreased slightly. Beef samples with different treatments showed the highest pH at 0 day of preservation whereas 60 day of preservation showed the lowest pH (Table 3). Among the treatment groups significant (p<0.05) change of pH value was observed. Moreover significant (p<0.05) interactive effects in treatment groups and days of intervals of preservation was also observed. In this study, beef treated with 0.4% orange peel extract had the highest pH value at 0 day of preservation and fresh sample had the lowest pH value at 60 day of preservation (Table 3).

Table 3: Effect of different levels of Orange peel extract and storage periods on physicochemical quality of beef (mean±SE)

Parameters	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat.	DI	T*DI
Raw p^H	0	5.76±0.008	5.81±0.01	5.88±0.02	5.96±0.008	5.85^{a}±0.01	**	**	**
	15	5.67±0.01	5.77±0.03	5.40±0.01	5.83±0.01	5.67^{b}±0.02			
	30	5.47±0.01	5.61±0.01	5.65±0.01	5.72±0.01	5.61^{c}±0.01			
	60	5.45±0.01	5.50±0.01	5.60±0.01	5.70±0.01	5.56^{d}±0.01			
	Mean	5.59^{d}±0.01	5.67^{b}±0.02	5.63^{c}±0.01	5.80^{a}±0.01				
Cooking loss (%)	0	39.28±0.15	37.89±0.04	36.63±0.06	34.37±0.03	30.04^{a}±0.07	**	**	**
	15	37.65±0.24	35.86±0.06	35.45±0.07	32.35±0.08	35.33^{b}±0.11			
	30	33.56±0.15	32.52±0.03	32.42±0.04	30.91±0.003	32.35^{c}±0.06			
	60	30.13±0.07	29.92±0.04	29.52±0.02	29.11±0.04	29.67^{d}±0.04			
	Mean	35.16^{a}±0.15	30.05^{b}±0.04	33.50^{c}±0.05	31.69^{d}±0.05				

Means with different superscripts in each column and row are significantly different (*p<0.05, **p<0.01). NS was not significantly different. Where, T_0 = control, T_1 = 0.2% orange peel extract, T_2 = 0.3% orange peel extract, T_3 = 0.4% orange peel extract, DI = Days of Intervals, Treat = Treatment, T*DI = Interaction of Treatment and Day Intervals.

3.3.2 Cooking loss (CL)

A significant (P<0.05) difference was noted for cooking loss among treated beef samples and days of preservation periods. The initial cooking loss was 39.28% (Table 3). As the increased of preservation period cooking loss was decreased slightly. Table 3 represents that there were significant interactive effects among treatment groups and days of interval. Beef samples with different treatments showed the highest cooking loss at 0 day of preservation whereas 60 day of preservation showed the lowest cooking loss (Table 3).

3.4 Biochemical properties

3.4.1 Free fatty acid value (FFA)

At the beginning of storage (0 day) control, 0.2%, 0.3% and 0.4% orange peel extract treated beef samples exhibited FFA values were 0.78, 0.67, 0.61 and 0.59% respectively. Also, FFA values of all samples treated with orange peel extract and control group significantly (p<0.01) increased with different rates depending on amount of the addition and time of storage (Table 4). Control fresh beef samples exhibited significantly (p<0.01) higher FFA values at any given time of storage as compared to 0.2, 0.3 and 0.4% orange peel extract treated beef samples (Table 4). Fresh beef samples had the highest FFA value at 60 day of storage and 0.4% orange peel extract treated beef samples had the lowest POV value at 0 day of storage.

3.4.2 Peroxide value (POV)

The peroxide values had an increasing trend but were lower in treated samples than the control cases. Control fresh beef samples exhibited significantly (p<0.01) higher POV values at any given time of frozen as compared to 0.2, 0.3 and 0.4% orange peel extract treated beef samples (Table 4). The lowest amounts of peroxide on the first day for the treated samples were respectively 1.66, 1.55, 1.53 (Meq/kg) while the highest amounts on the 60th day for the control samples were 3.18 (Meq/ kg). Significant interactive effects (p<0.01) were found on POV value in different treatment groups and preservation periods. Fresh beef samples had the highest POV value at 60 day of storage and 0.4% orange peel extract treated beef samples had the lowest POV value at 0 day of storage.

Table 4: Effect of different levels of Orange peel extract and storage periods on biochemical quality of beef (mean±SE)

Parameters	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat.	DI	T*DI
FFA (%)	0	0.78±0.001	0.67±0.001	0.61±0.001	0.59±0.001	0.66^{d}±0.001	**	**	**
	15	0.85±0.002	0.71±0.002	0.63±0.001	0.61±0.001	0.70^{c}±0.002			
	30	0.91±0.001	0.74±0.001	0.67±0.001	0.63±0.008	0.74^{b}±0.003			
	60	0.99±001	0.82±0.001	0.70±0.002	0.68±0.001	0.80^{a}±0.001			
	Mean	0.88^{a}±0.001	0.74^{b}±0.001	0.65^{c}±0.001	0.63^{d}±0.003				
POV (meq/kg)	0	1.96±0.01	1.66±0.01	1.55±0.02	1.53±0.01	1.68^{d}±0.01	**	**	**
	15	2.34±0.02	1.75±0.01	1.65±0.02	1.58±0.01	1.83^{c}±0.02			
	30	2.65±0.02	1.85±0.02	1.78±0.02	1.65±0.01	1.98^{b}±0.02			
	60	3.18±0.02	1.97±0.01	1.93±0.01	1.84±0.02	2.23^{a}±0.02			
	Mean	2.53^{a}±0.02	1.81^{b}±0.01	1.73^{c}±0.02	1.65^{d}±0.01				
TBARS (mg-MA/kg)	0	0.47±0.001	0.44±0.008	0.41±0.001	0.40±0.0008	0.45^{d}±0.005	**	**	**
	15	0.51±0.001	0.48±0.001	0.47±0.001	0.43±0.001	0.47^{c}±0.001			
	30	0.61±0.001	0.57±0.001	0.53±0.001	0.43±0.001	0.54^{b}±0.001			
	60	0.79±0.001	0.70±0.008	0.69±0.005	0.59±0.0008	0.69^{a}±0.005			
	Mean	0.60^{a}±0.001	0.55^{b}±0.005	0.53^{c}±0.002	0.46^{d}±0.005				

Means with different superscripts in each column and row are significantly different (*p<0.05, **p<0.01). NS was not significantly different. Where, T_0 = control, T_1 = 0.2% orange peel extract, T_2 = 0.3% orange peel extract, T_3 = 0.4% orange peel extract, DI = Days of Intervals, Treat = Treatment, T*DI = Interaction of Treatment and Day Intervals.

3.4.3 Thiobarbituric acid reactive substances (TBARS)

Data in Table 4 showed the changes in TBARS values of the orange peel extract treated beef samples during frozen storage for 60 days at -20°C. Results of the current study indicated that TBARS values increased over the storage time for all treated beef samples. Control fresh beef samples exhibited significantly (p<0.01) higher TBARS values at any given time of frozen as compared to 0.2, 0.3 and 0.4% orange peel extract treated beef samples. Significant interactive effects (p<0.01) were found on TBARS value in different treatment groups and preservation periods. Fresh beef samples had the highest TBARS value at 60 day of storage and 0.4% orange peel extract treated beef samples had the lowest TBARS value at 0 day of storage.

3.5 Microbial property assessment

3.5.1 Total viable count (TVC)

Total plate counts (TVC) of beef samples were evaluated and the counts (as log10 CFU/gm) are presented in Table 5. The initial value of TVC for control was 4.64 Log CFU/gm, indicating good quality beef. TVC values of all samples treated with orange peel extract and control group were affected significantly (p<0.01) depending on amount of the addition and time of storage. 0.4% orange peel extract treated beef sample had the lowest TVC value and control group had the highest TVC value was observed in this study (Table 5).

Table 5: Effect of different levels of Orange peel extract and storage periods on microbial population of beef (mean±SE)

Parameter	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat.	DI	T*DI
TVC (logCFU/g)	0	4.64±0.2	4.56±0.02	4.42±0.005	4.36±0.02	4.5^{c}±0.02	NS	**	NS
	15	4.87±0.009	4.68±0.01	4.70±0.02	4.71±0.02	4.74^{b}±0.01			
	30	4.85±0.08	4.78±0.009	4.76±0.01	4.72±0.005	4.78^{b}±0.03			
	60	5.06±0.31	5.13±0.02	5.19±0.004	5.14±0.005	5.13^{a}±0.08			
	Mean	4.86^{a}±0.11	4.79^{a}±0.02	4.77^{a}±0.01	4.73^{a}±0.01				
TCC (logCFU/g)	0	1.19±0.007	1.17±0.01	1.20±0.009	1.16±0.009	1.18^{a}±0.01	*	**	NS
	15	1.15±0.03	1.15±0.03	1.15±0.004	1.11±0.002	1.14^{b}±0.02			
	30	1.13±0.01	1.09±0.02	1.04±0.01	1.06±0.002	1.08^{c}±0.02			
	60	1.04±0.01	1.05±0.02	0.97±0.03	0.95±0.03	1.00^{d}±0.02			
	Mean	1.13±0.01	1.11±0.02	1.09±0.01	1.07±0.02				
TYMC (logCFU/g)	0	1.95±0.01	1.87±0.002	1.86±0.003	1.87±0.0003	1.89^{a}±0.01	**	**	**
	15	1.75±0.01	1.61±0.002	1.56±0.01	1.56±0.01	1.62^{b}±0.01			
	30	1.55±0.01	1.38±0.02	1.42±0.0.02	1.38±0.01	1.43^{c}±0.01			
	60	1.12±0.01	1.10±0.01	1.11±0.005	1.10±0.005	1.1^{d}±0.01			
	Mean	1.60^{a}±0.01	1.48^{b}±0.01	1.5^{b}±0.01	1.48^{b}±0.01				

Means with different superscripts in each column and row are significantly different (*p<0.05, **p<0.01). NS was not significantly different. Where, T_0 = control, T_1 = 0.2% orange peel extract, T_2 = 0.3% orange peel extract, T_3 = 0.4% orange peel extract, DI = Days of Intervals, Treat = Treatment, T*DI = Interaction of Treatment and Day Intervals.

3.5.2 Total coliform count (TCC)

The initial TCC of fresh beef (beef not frozen and thawed) was 1.19 log CFU/g beef. TCC was satisfactory in control beef samples. The TCC value of different treatment groups with different days of intervals shown in Table 5. There was no significant difference in addition of orange peel extract on TCC value of beef samples but significant (p<0.01) effect on time of storage. In this study, 0.4% orange peel extract treated beef sample had the lowest TCC content and control group had the highest value of TCC.

3.5.3 Total yeast-mould count (TYMC)

The TYMC value of different treatment levels with different days of storage shown in Table 5. TYMC values of all samples treated with orange peel extract and control group significantly (p<0.01) decreased with different rates depending on amount of the addition and time of storage (Table 5). Control fresh beef samples exhibited significantly (p<0.01) higher TYMC values than 0.2, 0.3 and 0.4% orange peel extract treated beef samples (Table 5). Fresh beef samples had the highest TYMC value at 0 day of storage and 0.4% orange peel extract treated beef samples had the lowest TYMC value at 60 day of storage.

IV. Discussion

4.1 Sensory evaluation

During the experimental analysis, it was observed that all the sensory attributes i.e. color, flavor, tenderness, juiciness and overall acceptability of beef samples treated with different level of orange peel extract showed non-significant differences (P<0.05) with the control. It might be due to phenolic compounds content of orange peel extract, which act as antioxidant. These observations were similar to those recorded by Narkhede (2012), who reported non-significant (P<0.05) variations in sensory scores of chicken meat containing natural antioxidants as compared to control. Meanwhile, these sensory attributes were slightly decreased by increasing the storage time due to slight progress of fat oxidation and red oxymyoglobin to metmyoglobin. A decrease in appearance and color scores of meat and meat products with increase in storage period was also reported by Nerín *et al*. (2006), Kilinc (2009), Chidanandaiah and Sanyal (2009), Kandeepan *et al.* (2010), Mathur *et al.* (2011) and Ashour *et al.* (2014) which were similar to present study. The progressive decrease in flavor could be correlated to increase in TBARS values of meat stored under aerobic conditions. Decline in flavor scores of meat products during storage was also reported by Thomas *et al.* (2006), Zargar *et al.* (2014) and Malav *et al.* (2015) in different meat products also agreed to present study's results. Tenderness is interrelated to DM content

of the beef. With the increasing of storage period, DM was increased consequently tenderness was decreased with storage period (Rahman *et al.*, 2014). The result of this experiment is also related to Chidanandaiah *et al.* (2009), Lui *et al.* (2010) and Raja *et al.* (2014) findings. The findings were a decline in the juiciness scores of different meat products during freezing storage because beef cells were ruptured with ice crystals. The overall acceptability also decreased during storage because of the decline in the sensory scores of all parameters. This decrease in overall acceptability was confirmed by the results of Malav *et al.* (2015) who reported that the overall acceptability of mutton decreased during storage.

4.2 Proximate analysis

4.2.1 Dry matter (DM)

In this study, there was an increasing trend of DM content of beef samples treated with different level of orange peel extract with the increasing of storage period. The primary reason would be an evaporative loss from the hot carcass as it is transferred to the refrigerator and prolonged preservation period. Similar results also found by Modi *et al.* (2008) and Al-Bachir and Zeinou (2014). The same trend was also observed by Konieczny *et al.* (2007) and they reported that DM content increased during frozen storage. Naveena *et al. (*2008) have reported an increase in storage period with an increase in the DM content of pomegranate peel extract and pomegranate rind powder extract, respectively.

4.2.2 Crude protein (CP)

The range of CP content observed at different treatment groups was 22.65 to 22.96%. So, there was slight decreasing trend with storage period due to slight protein break down. The same trend was also observed by Konieczny *et al.* (2007) and reported that CP content decreased during frozen storage. Similar trend was also observed by Shewalkar (2011) and Narkhede (2012) in chicken nuggets. Anti-oxidant property of orange peel solution may have the effect on inhibiting the oxidation of beef which may uphold the CP level in treated samples.

4.2.3 Ether extracts (EE)

The EE content of this study was increased with the increased storage period due to phenolic compounds present in orange peel extract. This observation was similar to those recorded by Verma *et al.* (2012a, b), Suradkar *et al.* (2013) and Verma *et al.* (2013), who reported an increase trend in the fat content of sheep meat on incorporation of guava powder. Similar trend was also observed by Shewalkar (2011) and Narkhede (2012) in chicken nuggets.

4.2.4 Ash

Ash content of the beef samples in this study was observed an increasing trend with the storage period due to the increase of DM content of beef treated with orange peel extract. Similar results were also reported by Serdaroglu *et al.* (2005) on the ash content of koefte beef meatballs. The same trend was also observed by Konieczny *et al.* (2007) and they reported that ash content increased of beef on incorporation of natural antioxidant during frozen storage.

4.3 Physicochemical properties

4.3.1 pH of raw meat

Meat pH is considered as one of the most important technological properties as it alters pigment and lipid stability. The pH of the samples treated with orange peel extract remained almost similar up to 60 days of frozen storage (−20±1°C). This might be due to inhibition of microbial growth at frozen storage and antioxidant activity of orange peel extract. The decrease in the raw pH values with storage time in the treated samples due to the loss of minerals and small protein compounds as exudates, thereby changing the ionic balance in the beef which resulted in a decreased pH (Vieira *et al*., 2009). This decrease could be due to the presence of some organic acids in orange peel extract which control sample to be slightly acidic. Braddock, (1995) reported that the pH values of samples effect of natural antioxidants which retarded the formation of free fatty acids. The present study's foundlings supported by this statement.

4.3.2 Cooking loss (CL)

Cooking loss refers to the reduction in weight of meat during the cooking process (Jama *et al.*, 2008). Cooking loss is an important data that are used by the meat industry to predict the behavior of their products during processing (Ulu, 2006). Major components of cooking losses are thawing, dripping and evaporation. Control sample recorded the significant highest cooking loss in this study. From the obtained results of this study, it was noticed that % cooking loss decreased in beef samples incorporated with orange peels extract. Decrease in cooking loss could be attributed to the increase in emulsion stability and due to the high ability of

orange peel extract to retain moisture and fat in the matrix. This finding is supported by Aleson-Carbonell *et al.* (2005), on the incorporation of lemon albedo fibers in beef patties formulation which shows that dietary fibers increased cooking yield, because of their ability to keep moisture and fat in the formulation. The meat also tended to shrink during the cooking process due to the denaturation of meat protein; the loss of water and fat also contributed to the shrinking process (Serdaroglu *et al.,* 2005). The observations of the present study were similar to those recorded by RochaGarza and Zayas (1996), who reported that, in meat products, quality attributes such as texture, structural binding and yield are determined by the ability of the protein matrix to retain water and bind fat. There were same relationship between moisture and fat retention and cooking yield with the addition levels of powder citrus peel to the beef products formulations.

4.4 Biochemical properties

4.4.1 Peroxide value (POV)

Fresh beef undergoes many undesirable changes during preservation incorporation with natural antioxidants (Hayam *et al.,* 2018) and freezing temperatures (Rahman *et al.,* 2015). Peroxide, TBARS and acid value were chosen as representative for primary, secondary lipid oxidation and lipid hydrolysis, respectively, in this study. The peroxide value (POV) generally serves as a useful indicator of the extent of oxidation of lipids, fats and oils. POV directly measures the lipid peroxides, which are primary lipid oxidation products. In present study, POV of the treated beef samples during freezing storage showed significant ($P<0.05$) rise when compared with control at zero day. The increase of POV in the beef during subsequent storage might be the result of catalysis of intracellular compounds due to the destruction of the cell structure as reported by Narkhede (2012). Significant ($P<0.05$) variations in POVs were observed irrespective of the addition of antioxidants as orange peel extract in beef. It was also evident that the products with natural antioxidants revealed significantly ($P<0.05$) low POV as compared to control. This might be due to high total phenolic compound present in orange peel extract. Sallam *et al.* (2004) reported the similar trend of increased peroxide value over storage time in products with or without antioxidants.

4.4.2 Free fatty acid value (FFA)

Free fatty acids are not only the products of enzymatic degradation but also microbial degradation of lipids. FFA gives an idea about stability of lipid during preservation (Rahman *et al.,* 2015). The significant increase in FFA levels of beef muscle treated with orange peel extract during 60 days of frozen storage might be due to growth inhibition of lipolytic microbes, total myofibrillar protein solubility, and intramuscular free fatty acids concentration decreased ($p<0.05$) in frozen storage which is in agreement with Qi *et al.* (2012), Rahman *et al.* (2014) and Hayam *et al.* (2018). Antioxidants have an ability to prevent or reduce the oxidative damage of muscle tissue indirectly by enhancing natural defenses of cell and/or directly by scavenging the free radical species (Verma *et al.,* 2009). In addition, orange peel extract may be believed to intercept the free radical chain of oxidation and to give hydrogen from the phenolic hydroxyl groups, there, by forming a stable end product that does not initiate or propagate further oxidation of lipids. Similar observations were reported by Sherwin (1998) and Morcuende *et al.* (2003).

4.4.3 Thiobarbituric acid reactive substances (TBARS)

TBARS is a secondary oxidation product commonly used as a measurement of lipid oxidation (Rahman *et al.,* 2015). The secondary by-products of lipid oxidation like aldehydes, possessing cytotoxic and genotoxic properties due to their high reactivity. TBA and peroxide values correlated positively with each other and increased significantly during storage time. Lipid oxidation is an important quality deteriorating determinant for meat and meat products, as it may lead to rancidity of lipid (Jin *et al.,* 2009; Nolsøe and Undeland 2009). Natural preservatives can protect the human body from these toxic compounds and free radicals and can retard the progress of many chronic diseases as well lipid oxidation and microbial growth in foods due to their phenolic compounds (Camo *et al.,* 2008; APHA, 2001). In the current study, TBARS values increased slightly over the storage time for all treated beef samples compared to control fresh beef samples, which exhibited significantly ($p<0.01$) higher TBARS values at any given time of storage. However, the values were within the spoilage limit reported by Kowale *et al.* (2008). Amongst the treatments, TBARS value was observed to be significantly ($P<0.05$) lower in 0.4% orange peel extract treated beef, irrespective of type used, as compared to control while among treatment groups. It might be due to the high phenolics content of orange peel extract. This fact has been supported by the finding of Abu-Amsha *et al.* (1996) who observed that total phenolics content and antioxidant activity were directly correlated with each other. Similar results were recorded by Abd El-Khalek and Zahran (2013). Kim *et al.* (2013) reported that the addition of edible plant extracts significantly lowered TBARS values in fresh ground beef compared with non-treated samples. The finding of the current study was also in agreement with Reddy *et al.* (2013) and Hadi (2017), reported that the restructured mutton

slices treated with grape seed extract had significantly (P<0.05) lower TBARS values and free fatty acids (FFA %) compared to control.

4.5.2 Microbial property assessment

4.5.1 Total viable count (TVC)

In the present study the range of TVC value was 4.5-5.13 log CFU/g beef, indicating good quality beef (Dempster, 1986 and Joy, 1986). The shelf-life of control meat is usually limited by microbial spoilage. All the treatments showed significantly (P<0.05) increased microbial count with the storage period but the increment in all the stored products was within the limit of acceptability. Similar results were also found by Bhat *et al.* (2011). Phenolic acids are natural constituents of orange peel extract which exhibit antibacterial activity. The antimicrobial activity of phenolic acids is attributed to depression of internal pH of microbial cells by ionization of acid molecules and disruption of substrate transport by altering cell membrane permeability. Present findings were in accordance with those recorded earlier by Narkhede (2012). The antioxidant compounds blocked the deteriorating of fat and helped prevent the metabolism of fat by bacteria. As a result, bacterial growth was lower in beef treated with orange peel extract. In this study 0.4% orange peel extract treated beef sample had the lowest TVC value compared with control group had the highest TVC value. Similar results were achieved by Alahakoon *et al.* (2013), who found that significant effects of citrus peel extract and onion peel extract added to chicken breast meat sample on microbial growth inhibition during storage at different temperatures. Klangpetch *et al.* (2016) reported also that total viable count (TVC) of all samples increased during storage. Hanan *et al.* (2013) reported that, the use of fruit by-products were significantly (p<0.05) reduced total bacterial, lactic acid bacteria and total mold and yeast counts and extended the shelf-life of ground meat compared with the control. The founding of the present study was disagreed with this due to cross-contamination from the environment (i.e., the air or beef handlers) or from the survival of spores or resistant cells was possible in this study.

4.5.2 Total coliform count (TCC)

In the current study TCC had a decreasing trend either with or without orange peel extract treated beef samples at the end of preservation. Similar observations were recorded by Stika *et al.* (2007), Camo *et al.* (2008) and Zehra *et al.* (2014), who found that coliform counts gradually decreased or absence in frozen storage and antioxidant-treated raw restructured beef steaks made from mature cows. Shewalkar (2011) and Narkhede (2012) reported that TCC organisms were not detected in the chicken nuggets either with or without antioxidants at the end of storage study. These bacteria are indicator of fecal contamination. Therefore, presence of these microorganisms in the current study indicated cross contamination during post processing handling of beef.

4.5.3 Total Yeast-Mould Count (TYMC)

It was observed that all the treatments had significantly (P<0.01) differ from each other in respect of TYMC. Similar findings were also documented by Gutierrez *et al*. (2009) and Narkhede (2012) in which he had documented significantly (P<0.05) lower TYMC in the beef and chicken nuggets treated with natural antioxidants.

V. Conclusion

Results of the current study represented that orange peel extract improves the sensory characteristics and chemical quality of beef muscle at frozen storage by increasing cooking yield, lowering the pH, peroxide value, free fatty acid value and TBARS value. Due to its antibacterial and antioxidant activity, orange peel extract can be used as a natural preservative and alternative to chemical compounds to increase beef's shelf life and prevent microbial spoilage. It can be concluded that orange peel extract added @ 0.3% and 0.4% as natural food additives which have potential to serve as an effective alternative to synthetic antioxidants such as BHA, BHT etc. for improving quality and safety of meat and meat products.

References

[1]. Abd El-Khalek, HH, Zahran DA. Utilization of Fruit by-Product in Ground Meat Preservation. Food Science and Quality Management. 2013; 11: 49-60.
[2]. Abu-Amsha R, Croft KD, Puddey IB, Proudfoot JM, Beilin LJ. Phenolic content of various beverages determines the extent of inhibition of human serum and low-density lipoprotein oxidation in vitro: identification and mechanism of action of some cinnamic acid derivatives from red wine. Clinical Science. 1996; 91: 449-458.
[3]. Ahn JI, Grun U, Fernando LN. Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooled beef. Journal of Food Science. 2002; 67: 1364-1369.
[4]. Alahakoon AU, SikBae Y, Kim HJ, Jung S, Jayasena DD, Yong HI, Kim, SH, Jo C. The effect of citrus and onion peel extracts, calcium lactate, and phosvitin on microbial quality of seasoned chicken breast meat. CNU Journal of Agricultural Science. 2013; 40(2): 131-137.
[5]. Al-Ani WN, Al-Haliem SM, Tawfik NO. Evaluation of antibacterial activity of citrus juice. An in-vitro study. Al-Rafidain Dental. 2010; 10(2): 376-382.

[6]. Al-Bachir M, Zeinou R. Effect of gamma irradiation on the microbial load, chemical and sensory properties of goat meat. Acta Alimentaria. 2014; 43(2): 264-272.
[7]. Aleson-Carbonell L, Fernandez-Lopez J, Perez-Alvarez JA, Kuri V. Functional and sensory effects of fibre-rich ingredients on breakfast fresh sausages. Food Science and Technology International. 2005; 11(2): 89-97.
[8]. AMSA. Research guidelines for cookery sensory evaluation and instrumental tenderness measurements of fresh meat. March, 2015: Second edition (version 1.0).
[9]. Angelo AJ, Crippen KL, Dupuy HP, James JrC. Chemical and sensory studies of antioxidant treated beef. Journal of Food Science. 1990; 55(6): 1501-1505.
[10]. AOAC. Official method of analysis of the Association of Official Analytical Chemists. 18th ed. 2011; Washington, D.C.
[11]. APHA. American Public Health Association. Compendium of methods for the microbiological examination of foods, 4^{th} Edt. Downes FP, Ito K, Eds. American Public Health Association: 2001; Washington, DC, USA.
[12]. Ashok KK, Narayani, Subanthini, Jayakumar. Antimicrobial Activity and Phytochemical Analysis of Citrus Fruit Peels-Utilization of Fruit Waste. International Journal of Engineering Science and Technology. 2011; 3(6): 5414-5421.
[13]. Ashour MMS, Moawad RK, Bareh GF. Quality Enhancement and Shelf-Life Extension of Raw Beef Patties Formulated with Lactate/Thyme Essential Oil during Refrigerated Storage. Journal of Applied Sciences Research. 2014; 9(13): 6699-6709.
[14]. Baratta MT, Dorman HJD, Deans SG. Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oil. Journal of Essential oil Research. 1998; 10: 618-627.
[15]. Bekhit AED, Geesink GH, Ilian MA, Morton JD, Bickerstaffe R. The effects of natural antioxidants on oxidative processes and metmyoglobin reducing activity in beef patties. Journal of Food Chemistry. 2003; 81: 175-187.
[16]. Bhat ZF, Pathak V, Bukhari SAA, Anmad SR, Bhat H. Quality changes in chevonherrisa (meat based product) during refrigerated storage. International Journal of Meat Science. 2011; 1(1): 52-61.
[17]. Biswas AK, Keshri RC, Bisht GS. Effect of enrobing and antioxidants on quality characteristics of precooked pork patties under chilled and frozen storage conditions. Meat Science. 2004; 66: 733-741.
[18]. Braddock RJ. By-products of citrus fruits. Food Technology. 1995; 49: 74-77.
[19]. Camo J, Beltrán JA, Roncalés P. Extension of the display life of lamb with an antioxidant active packaging. Meat Science. 2008; 80: 1086-1091.
[20]. Camo J, Beltrán JA, Roncalés P. Extension of the display life of lamb with an antioxidant active packaging. Journal of Meat Science. 2008; 80(4): 1086-1091.
[21]. Chidanandaiah KRC, Sanyal MK. Effect of sodium alginate coating with preservatives on the quality of meat patties during refrigerated storage. Journal of Muscle Foods. 2009; 20(3): 275-292.
[22]. Chidanandaiah KRC, Sanyal MK. Effect of sodium alginate coating with preservatives on the quality of meat patties during refrigerated storage. Journal of Muscle Foods. 2009; 20(3): 275-292.
[23]. Decker EA, Mei L. Antioxidant mechanisms and applications in muscle foods. In Proceedings of the 49th Reciprocal Meat Conference. 1996; 64-72.
[24]. Dempster JF. Bacteriological status of minced beef. Irish Journal of Food Science and Technology. 1986; 2: 1-11.
[25]. DeSilva KT. A manual on the essential oil industry (Ed.). United Nations Industrial development organization, Vienna. 1996.
[26]. Diplock AT. Antioxidants and Disease Prevention. Molecular Aspects of Medicine. 1994; 15: 293-376.
[27]. Erickson MC. Lipid oxidation: Flavor and nutritional quality deterioration in frozen foods. In Quality in frozen food. 1997; 141-173.
[28]. Faulks M, Southo S. Carotenoids, Metabolism and Disease. In: Wildman R.E.C. (Ed.). Handbook of Nutraceuticals and Functional Foods. CRC Press, Florida, USA. 2001.
[29]. Formanek Z, Kerry JP, Higgins FM, Buckley DJ, Morrissey PA, Farkas J. Addition of synthetic and natural antioxidants to alpha-tocopheryl acetate supplemented beef patties: effects of antioxidants and packaging on lipid oxidation. Meat Science. 2001; 58(4): 337-341.
[30]. Gonçalves AA, Junior CSGG.The effect of glaze uptake on storage quality of frozen shrimp. Journal of Food Engineering. 2009; 90 (2): 285-290.
[31]. Gutierrez J, Barry-Ryan C, Bourke P. Antimicrobial activity of plant essential oils using food model media: Efficacy, synergistic potential and interactions with food components. Journal of Food Microbiology. 2009; 26: 142-150.
[32]. Hadi HG. Lipid Oxidation, Color Changes, and Microbiological Quality of Frozen Beef Burgers Incorporated with Shirazi Thyme, Cinnamon, and Rosemary Extracts. Journal of Food Quality. 2017; 1-9.
[33]. Hanan HA, Dalia AZ. Utilization of Fruit by-Product in Ground Meat Preservation. Food Science and Quality Management. 2013; 11:49-60.
[34]. Hayam M Ibrahim, Ibrahim M Hassan, Ahmed AM Hamed. Application of Lemon and Orange Peels in Meat Products: Quality and Safety. International Journal of Current Microbiology and Applied Science. 2018; 7(4): 2703-2723.
[35]. ISO. Recommendation of the meeting of the subcommittee, International Organization for Standardization, on meat and meat products. ISO/TC- 36/SC-6. 1995; 10-18.
[36]. Jama N, Muchenje V, Chimonyo M, Strydom PE, Dzama K, Raats JG. Cooking loss components of beef from Nguni, Bonsmara and Angus steers. African Journal of Agricultural Research. 2008; 3(6): 416-420.
[37]. Jay JM. Modern food Microbiology. 4th Edn., CBS Publishers and Distributors, New Delhi. 1986.
[38]. Jayathilakan K, Sharma GK, Radhakrishna K, Bawa AS. Antioxidant potential of synthetic and natural antioxidants and its effect on warmed-over-flavour in different species of meat. Food Chemistry. 2007; 105(3): 908-916.
[39]. Jin SK, Kim IS, Choi YJ, Kim BG, Hur SJ. The development of imitation crab sticks containing chicken breast surimi. LWT-Food Science and Technology. 2009; 42:150-156.
[40]. Kandeepan G, Anjaneyulu ASR, Kondaiah N, Mendiratta SK. Quality of buffalo meat keema at different storage temperature. African Journal of Food Science. 2010;4(1): 410-417.
[41]. Kilinc B. Microbiological, sensory and color changes of anchovy (*Engraulis encrasicholus*) patties during refrigerated storage. Journal Muscle Foods. 2009; 20**:** 129-137.
[42]. Kim SJ, Min SC, Shin HJ, Lee YJ, Cho AR, Kim SY, Han. Evaluation of the antioxidant activities and nutritional properties of ten edible plant extracts and their application to fresh ground beef. Journal of Meat Science. 2013; 93: 715-722.
[43]. Klangpetch W, Phromsurin K, Hannarong K, Wichaphon J, Rungchang S. Antibacterial and antioxidant effects of tropical citrus peel extracts to improve the shelf life of raw chicken drumettes. International Food Research Journal. 2016; 23(2): 700-707.
[44]. Konieczny P, Stangierski J, Kijowski J. Physical and chemical characteristics and acceptability of home style beef jerky. Journal of Meat Science. 2007; 76: 253-257.

[45]. Kowale BN, Kulkarni VV, Rao VK. Tests for determination of keeping quality of meat. In Methods in Meat Science. Jaypee Brothers Medical Publishers (P) Ltd. 2008; 140-141.
[46]. Lui Z, Xiong Y, Chen J. Protein oxidation enhances hydration but suppresses water-holding capacity in *Porcine Longissimus* muscle. Journal of Agricultural and Food Chemistry. 2010; 58: 10697-10704.
[47]. Malav OP, Sharma BD, Kumar RR, Talukder S, Ahmed SR. Antioxidant potential and quality characteristics of functional mutton patties incorporated with cabbage powder. Journal of Nutritional Food Science. 2015; 45(4): 542-63.
[48]. Manthey A, Grohmann K. Phenols in citrus peel byproducts: concentrations of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses. Journal Agricultural Food Chemistry. 2001; 49: 3268-3273.
[49]. Mathur A, Satish K, Verma, Purohit R, Gupta V, Dua VK, Prasad GBKS, Mathu D, Santosh K, Singh S. Evaluation of in vitro antimicrobial and antioxidant activities of peel and pulp of some citrus fruits. IJPI's Journal of Biotechnology and Bio-therapeutics. 2011; 1(2): 1-17.
[50]. Mc Carthy TL, Kerry JP, Kerry JF, Lynch PB, Buckley DJ. Evaluation of the antioxidant potential of natural food/plant extracts as compared with synthetic antioxidants and vitamin E in raw and cooked pork patties. Journal of Meat Science. 2001; 57: 45-52.
[51]. Modi VK, Mahendrakar NS, Narasimha DR, Sachindra NM. Quality of buffalo meat burger containing legume flours as binders. Journal of Meat Science. 2008; 66(1):143-149.
[52]. Morcuende D, EstevesM, Riuz J, Cava R. Oxidative and lipolitic deterioration of different muscle from free-range reared Iberian pigs under refrigerated storage. Journal of Meat Science. 2003; 65: 1157-1164.
[53]. Narkhede HP. Incorporation of grape seed extract and dried holy basil powder as natural antioxidants to develop functional chicken nuggets. M.V.Sc. Thesis. Maharashtra Animal and Fishery Sciences University, Nagpur. 2012.
[54]. Naveena BM, Sen AR, Vaithiyanathan S, Babji Y, Kondaiah N. Comparative efficacy of pomegranate juice, pomegranate rind powder extract and BHT as antioxidants in cooked chicken patties. Meat Science. 2008; 80: 1304-1308.
[55]. Naveena BM, Sen AR, Vaithiyanathan S, Babji Y, Kondaiah N. Comparative efficacy of pomegranate juice, pomegranate rind powder extract and BHT as antioxidants in cooked chicken patties. Meat Science. 2008; 80: 1304-1308.
[56]. Nolsøe H, Undeland I. The acid and alkaline solubilization process for the isolation of muscle proteins: State of the art. Food and Bioprocess Technology. 2009; 2: 1-27.
[57]. Nunez de Gonzalez MTN, Hafley BS, Boleman RM, Miller RK, Rhee KS, Keeton JT. Antioxidant properties of plum concentrates and powder in precooked roast beef to reduce lipid oxidation. Meat Science. 2008; 80(1): 997-1004.
[58]. Pandey A, Kaushik A, Tiwari K. Evaluation of antimicrobial activity and phytochemical analysis of Citrus lemon. Journal of Pharma Bio-medicinal Science. 2011; 13(17) 1-5.
[59]. Qi J, Li C, Chen Y, Gao F, Xu X, Zhou GH. Changes in meat quality of ovine longissimusdorsi muscle in response to repeated freeze and thaw. Meat Science. 2012; 92: 619-626.
[60]. Raghavan S, Richards MP. Comparison of solvent and microwave extracts of cranberry press cake on the inhibition of lipid oxidation in mechanically separated turkey. Food Chemistry. 2007; 102(3): 818-826.
[61]. Rahman MH, Hossain MM, Rahman SM, Amin MR, Oh DH. Evaluation of Physicochemical Deterioration and Lipid Oxidation of Beef Muscle Affected by Freeze-thaw Cycles. Korean Journal for Food Science of Animal Resources. 2015; 35(6): 772-782.
[62]. Rahman MH, Hossain MM, Rahman SM, Hashem MA, Oh DH.. Effect of Repeated Freeze-Thaw Cycles on Beef Quality and Safety. Korean Journal for Food Science and Animal Resources. 2014; 34(4): 482-495.
[63]. Raja WH, Kumar S, Bhat ZF, Kumar P. Effect of ambient storage on the quality characteristics of aerobically packaged fish curls incorporated with different flours. Springer Plus. 2014; 3(1): 106.
[64]. Reddy GVB, Sen AR, Nair PN, Reddy KS, Reddy KK, Kondaiah N. Effects of grape seed extract on the oxidative and microbial stability of restructured mutton slices. Journal of Meat Science. 2013; 95(2): 288-294.
[65]. Rocha-Garza AE, Zayas JF. Quality of broiled beef patties supplemented with wheat germ protein flour. Journal of Food Science. 1996; 61: 418- 421.
[66]. Rojas MC, Brewer MS. Effect of natural antioxidants on oxidative stability of frozen, vacuum-packaged beef and pork. Journal of Food Quality. 2008; 31(2):173-188.
[67]. Rukunudin IH, White PJ, Bern CJ, Bailey TB. A modified method for determining free fatty acids from small soybean sample sizes. Journal of American Oil Chemical Society. 1998; 75: 563-568.
[68]. Sallam KI, Ishioroshi M, Samejima K. Antioxidant and antimicrobial effect of garlic in chicken sausage. Journal of Lebensmittel-Wissenschaft & Technologie. 2004; 37: 849-855.
[69]. Sallam KI, Ishioroshi M, Samejima K. Antioxidants and antimicrobial effects of garlic in chicken sausage. Journal of Lebensmittel-Wissenschaft & Technologie. 2004; 37: 849-855.
[70]. Sánchez-Escalante A, Djenane D, Torrescano G, Beltrán JA, Roncalés P. Antioxidant action of borage, rosemary, oregano and ascorbic acid in beef patties packaged in modified atmosphere. Journal of Food Science. 2003; 68: 339-344.
[71]. Schmedes A, Holmer G. A new thiobarbituric acid (TBA) method for determining free malondialdehyde (MDA) and hydroperoxides selectively as a measure of lipid peroxidation. Journal of American Oil Chemists' Society. 1989; 66: 813-817.
[72]. Sebranek JG, Sewalt VJH, Robbins KL, Houser TA. Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. Meat Science. 2005; 69: 289-296.
[73]. Serdaroglu M, YldzTurp G, Abrodimov K. Quality of low-fat meatballs containing Legume flours as extenders. Journal of Meat Science. 2005; 70(1): 99-105.
[74]. Serdaroglu M, YldzTurp G, Abrodimov K. Quality of low-fat meatballs containing Legume flours as extenders. Journal of Meat Science. 2005; 70(1): 99-105.
[75]. Sharma K, Mahato N, Cho MH, Lee YR. Converting citrus wastes into value-added products: Economic and environmentally friendly approaches. Nutrition. 2017; 34: 29-46.
[76]. Sherwin ER. Oxidation and antioxidants in fat and oil processing. Journal of the American Oil Chemists' Society. 1998; 55: 809-814.
[77]. Shewalkar AA. Efficacy of natural antioxidants as a preservative in chicken nuggets. M.V.Sc. Thesis. Maharashtra Animal and Fishery Sciences University, Nagpur. 2011.
[78]. Smid EJ, Gorris LGM. Natural antimicrobials for food preservation. In Rahman, M.S. (Ed.), Handbook of food preservation. Marcel Dekker, New York. 1999; 285-308.
[79]. Stika JF, Xiong JF, Suman YL, Blanchard SP, Moody SP: Frozen storage stability of antioxidant treated raw restructured beef steaks made from mature cows. Journal of Meat Science. 2007; 77 (4): 562-569.
[80]. Sultana A, Huque KS, Amanullah SM. Development of tasty marinating kit for tenderization and preservation of beef chuck. The Bangladesh Veterinarian. 2009; 26:23-30.

[81]. Suradkar US, Bumla NA, Maria A, Zanjad PN, Sofi AH. Effect of incorporation of bread crumbs on the physicochemical and sensory quality of chicken nuggets. International Journal of Food Nutrition and Safety. 2013; 3: 1-6.
[82]. Tang S, Kerry JP, Sheehan D, Buckley DJ, Morrisey PA. Antioxidative effect of added tea catechins on susceptibility of cooked red meat, poultry and fish patties to lipid oxidation. Food Research International. 2001; 34: 651-657.
[83]. Thomas R, Anjaneyulu ASR, Kondaiah N. Quality and shelf life evaluation of emulsion and restructured buffalo meat nuggets at cold storage (4±1°C). Journal of Meat Science. 2006; 72**:** 373-379.
[84]. Ting SV. Nutrients and nutrition of citrus fruits. In: Nagy S, Attaway JA, editors. Citrus Nutrition and Quality. Washington, D.C. American Chemical Society. 1980; 3-24.
[85]. Tomaino A, Cimino F, Zimbalatti V, Venuti V, Sulfaro V, De Pasquale A, Saija A. Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. Journal of Meat Food Chemistry. 2005; 89: 549-554.
[86]. Ulu H. Effects of carrageenam and guar gum on the cooking and textural properties of low fat meatballs. Journal of Meat Food Chemistry. 2006; 95: 600-605.
[87]. Verma AK, Banerjee R & Sharma BD. Quality of low fat chicken nuggets: Effect of sodium chloride replacement and added chickpea (*Cicer arietinum* L.) hull flour. Asian Australas Journal of Animal Science. 2012a; 25: 291-298.
[88]. Verma AK, Rajkumar V, Banerjee R, Biswas S, Das AK. Guava (*Psidium guajava* L.) Powder as an antioxidant dietary fibre in sheep meat nuggets. Asian Australasian Journal of Animal Science. 2013; 26: 886-895.
[89]. Verma AK, Sharma BD, Banerjee R. Quality characteristics of low-fat chicken nuggets: effect of common salt replacement and added bottle gourd. (*Lagenaria siceraria* L.). Journal of Food Science and Agriculture. 2012b; 92: 1848-1854.
[90]. Verma AR, Vijayakumar M, Mathela CS, Rao CV. In vitro and in vivo antioxidant properties of different fractions of Moringa oleifera leaves. Journal of Meat Food and Chemical Toxicology. 2009; 47: 2196-2201.
[91]. Vieira C, Diaz MY, Martínez B, García-Cachán MD. Effect of frozen storage conditions (temperature and length of storage) on microbial and sensory quality of rustic crossbred beef at different stages of aging. Meat Science. 2009; 83:398-404.
[92]. Zargar FA, Kumar S, Bhat ZF, Kumar P. Effect of pumpkin on the quality characteristics and storage quality of aerobically packaged chicken sausages. Springer Plus. 2014; 3(1): 39.
[93]. Zehra KE, Kezban C. Antioxidant Active Packaging with Soy Edible Films and Oregano or Thyme Essential Oils for Oxidative Stability of Ground Beef Patties. Journal of Food Quality. 2014; 37(3):203-212.

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