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Effect of *Moringa oleifera* leaf extract and synthetic antioxidant on quality and shelf-life of goat meat nuggets at frozen storage

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M. H. Rahman[1]*, M. S. Alam[2], M. M. Monir[2] and S. M. E. Rahman[3]

[1]Department of Animal Products and By-products Technology, Patuakhali Science and Technology University, Barishal-8210, Bangladesh.
[2]Department of General Animal Science and Animal Nutrition, Patuakhali Science and Technology University, Barishal-8210, Bangladesh.
[3]Department of Animal Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

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ABSTRACT

The experiment was conducted to evaluate the antioxidative activity of *Moringa oleifera* leaf extract against 0.1% beta hydroxyl anisole (BHA) added to goat meat nuggets. Goat meat nuggets were formulated having five treatment groups namely control, 0.1% BHA, 0.1%, 0.2% and 0.3% *M. oleifera* leaf extract, respectively. Sensory tests were performed where color, flavor, tenderness, juiciness, and overall acceptability increased significantly ($p<0.05$) in treated groups (especially in 0.3% *M. oleifera* leaf extract) in comparison to the control and BHA. pH, cooking loss, free fatty acids (FFA), thiobarbituric acid values (TBARS), peroxide value (POV) and microbiological examination were also determined for treated nuggets. pH and cooking loss of ready to eat nuggets decreased significantly ($p<0.05$) in comparison to control and BHA with prolonged storage period. However, FFA, TBARS, POV, total viable count (TVC) (log CFU/g), total coliform count (TCC) (log CFU/g) and total yeast and mould count (TYMC) (log CFU/g) were also decreased significantly ($p<0.05$) among treated groups in comparison to the control and BHA with increased storage period. So, *M. oleifera* leaf extract treated nugget's quality remain stable with minor changed in sensory, physicochemical and microbiological quality during frozen storage (-18±1°C) for 45 days. Therefore it can be concluded from this study that synthetic antioxidants could be successfully replaced by *M. oleifera* leaf extract in producing commercially available quality comminuted meat products.

INTRODUCTION

The consumption trend of value added or processed comminuted meat products (ready-to-eat/ready-to-cook) from wholesome meat have been increased recently in Bangladesh. Goat meat nugget is one of the most popular ready-to-eat fast food among both Muslim and Hindu consumers due to no religious restriction.

Moreover, value added meat products as nugget contains lipids derived either from muscle itself or from vegetable oil during processing which are perishable by nature. Lipid oxidation and microbial growth are major causes of quality deterioration which reduced shelf-life of comminuted meat products. Lipid oxidation also limiting the quality and acceptability of meat products by degradation of quality parameters such as color, flavor, odor, texture and even physicochemical quality (Contini et al.,

*Corresponding author. E-mail: hafiz_08@pstu.ac.bd, hafiz.bau08@yahoo.com.

2014; Rahman et al., 2014), while microbial contamination can cause public health hazards and economic loss in terms of food poisoning and meat products spoilage. Another serious impact of oxidative rancidity is that it can cause health risks due to the formation of oxidation products that are suspected to be toxic. These oxidation products can cause oxidation damage in living organisms as well as mutagenesis and carcinogenesis [for example, lipid peroxide, malondialdehyde (MDA). Lipid oxidation also reduces the nutritional value of the food. Previous research has confirmed that lipid oxidation and microbial growth in meat products can be controlled or minimized by using antioxidants (Mielnik et al., 2003; Lahucky et al., 2010). There are two types of antioxidants are used in food industry, one is natural and another is synthetic (Matthews and Strong, 2005). Spices and herbs have a long history of safe usage as natural antioxidant whereas butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butyl hydroquinone (TBHQ) have been widely used as synthetic one with side effects (Fasseas et al., 2007). The use of these antioxidants is questionable since they have been discovered as toxic, pathogenic and carcinogenic to human health (Hayes et al., 2010).

So, the consumer preference for natural antioxidants has been increased and to meet up this demand not only the meat scientists but also the meat processors are giving emphasis on natural antioxidants as an alternative of synthetic antioxidant (Karre et al., 2013). It has been reported that, the use of natural antioxidants, especially from plant origins, have greater application potential for consumer's acceptability, palatability, stability and shelf-life of meat products (Jung et al., 2010) because they containing a number of valuable antioxidant and antimicrobial properties that can not only prevent or reduce oxidation of lipids, fats and oils but also increase shelf life of the products (Shahidi and Zhong, 2010).

One such a potential source of natural antioxidant is Moringa (*Moringa oleifera*) leaf extract. Moringa leaves have been reported to be a rich source of ascorbic acid, flavonoids, phenolics, carotenoids, proteins, calcium, potassium etc. and they act as good natural antioxidants (Sreelatha and Padma, 2009; Das et al., 2012; Dubey et al., 2013). *M. oleifera* leaf extracts have been confirmed as having high antioxidant and antimicrobial activity and had no adverse effects on human health (Das et al., 2012; Stohs and Hartman, 2015). This tree is native to sub-Himalayan tracts of India, Pakistan, Bangladesh, Thailand and Philippines (Lin et al., 2018). To the best of our knowledge, this is the first study which investigated the potential antioxidant activity of *M. oleifera* leaf extracts in comminuted value added meat products in Bangladesh. The purpose of this study was to know the comparative effectiveness of natural antioxidant extracted from *M. oleifera* leaf against an artificial antioxidant (BHA) in delaying lipid oxidation and change in sensory, physicochemical and microbiological quality in goat meat nuggets at frozen storage.

MATERIALS AND METHODS

Goat meat

About 10 kg goat meat was purchased from local market within 1 h of slaughtered and brought to the laboratory within 10 min. Then meat was deboned, trimmed of separable fat and connective tissue and chilled in a refrigerator at 4±1°C for about 6 h and frozen at -18°C for further use. The meat was cut into small pieces after partial thawing for 6 h at 4°C. Goat meat was doubled minced (10 mm plate followed by 8 mm plate) with a meat mincer (Tallers Ramon Model P-22, Barcelona). This ground goat meat was used in nuggets formulations.

Natural extract

For preparation of *M. oleifera* leave extract, fresh mature leaves were collected from the Regional Agricultural Research Station (Barishal) of Bangladesh Agricultural Research Institute. The leaves were properly cleaned, washed, chopped and air-dried using a fan, powdered, passed through sieve No. 20 and extracted (100 g) successively with 600 ml of water in a Soxhlet extractor for 18-20 h. The extract was concentrated to dryness under reduced pressure and controlled temperature (40-50°C). The yield (w/w) of the extract from fresh leaves was about 8-9%. Food grade BHA was used as synthetic antioxidant and compared against the natural antioxidant extracts. The five treatment groups were control (T_0), 0.1% BHA (T_1), 0.1% (T_2), 0.2% (T_3) and 0.3% (T_4) *M. oleifera* leaf extract added goat meat nuggets, respectively.

Nuggets preparation

Goat meat nuggets (control) were formulated using 70% goat meat, 10% refined vegetable oil (soybean oil), 3% refined wheat flour, 10% ice flakes, 3% condiment mix (4 parts onion and 1 parts garlic), 1.6% refined common salt, 1.8% dry spice mix, 0.3% sugar and 0.3% tripolyphosphate. 150 ppm sodium nitrite was also added to above formulations. 2 kg nuggets formulation was made for each treatment. In treated formulation 0.1% BHA, 0.1%, 0.2% and 0.3% *M. oleifera* leaf extract were added respectively. Minced goat meat and all the ingredients were thoroughly mixed in a bowl chopper (Seydelmann K20 Ras, Germany) to prepare the emulsion. Goat meat emulsion (~750 g) was placed into

stainless steel block (18×12×4 cm); packed compactly and covered. The goat meat block from all the treatments were clipped and cooked in a steam oven at atmospheric pressure for 35 min. The temperature of the steam oven during cooking was over 100°C and the internal temperature of the cooked meat blocks was 85 ± 1°C recorded by a probe type thermometer (Oakton, China). After cooling to room temperature, meat block chilled overnight at 4 ± 1°C and cut into slices of 15 mm thickness using a meat slicer (Electrolux, Model H 300, Italy). The slices were manually cut into nuggets. About 200 g nuggets were packed in zippered bag; kept at -18 ± 1°C and analyzed at 0, 15th, 30th, and 45th day after thawing in a refrigerator (4 ± 1°C) for 7-8 h. The experiment was replicated thrice.

Sensory attributes

Different sensory attributes of nuggets were evaluated by a trained 6-member panel selected according to the American Meat Science Association guidelines (AMSA, 2015). Evaluation of sensory attributes of goat meat nuggets (color, flavor, tenderness, juiciness, and overall acceptability) were carried out in individual booths under controlled conditions of light, temperature and humidity 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). All samples were served in the petri-dishes. Sensory evaluation was accomplished at 0 day and repeated at 15th, 30th and 45th day.

pH values

The pH value of nuggets (before cooking) was measured with a pH meter (Metter Toledo Delta 320, Switzerland). For this, 5 g homogenate samples were dissolved in 45 ml aquadest in a glass beaker with a homogenizer. Then pH value was determined from the reading of pH meter.

Cooking loss

Cooking loss was determined according to Serdaroglu (2006) by measuring the difference in the sample weight before and after cooking.

Cook loss (%) = $[(w_1-w_2) \div w_1] \times 100$

Where, w_1, Nuggets weight (g) before cooking; w_2, Nuggets weight (g) after cooking.

Peroxide values

The 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 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 meat 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 of nuggets was calculated by the 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); W, is the sample weight (g).

Free fatty acid values

FFA value was determined according to the method of Rukunudin et al. (1998). The samples (5 g) were dissolved with 30 ml chloroform using a homogenizer at 10,000 rpm for 1 min. Whatman filter paper number 1 was used in filtration process to remove meat particles from the filtrate. After addition of five drops 1% ethanolic phenolphthalein as indicator to filtrate, the titration was continued against 0.01 N ethanolic potassium hydroxide solutions and FFA value was calculated. The formula is mentioned below:

$$\text{FFA (\%)} = \frac{\text{ml titration} \times \text{normality of KOH} \times 28.2}{\text{g of sample}}$$

Thiobarbituric acid values

The 2-thiobarbituric acid (TBA) values were determined by the method described by Schmedes and Holmer (1989). The samples (5 g) were blended with 25 ml of 20% trichloroacetic acid solution (200 g/l of trichloroacetic acid in 135 ml/l phosphoricacid solution) in a homogenizer for 30 s. The homogenized samples were filtered through Whatman filter paper number 4 to remove meat particles from the filtrate. Then 2 ml of 0.02 M aqueous TBA solution (3 g/l) was added to 2 ml of filtrate in a test tube. After that test tubes were incubated at 100°C for 30 min and cooled under running tap water. The absorbance of supernatant solutions was measured at 532 nm using a UV-VIS spectrophotometer (UV-1200, Shimadzu, Japan). The TBA values were calculated from a standard curve and expressed as mg malonaldehyde per kilogram (MA/kg) of nuggets sample.

Microbiological analysis

TVC, TCC and TYMC were performed according to the guidelines described by ISO (1995). A composite nuggets sample (25 g) was aseptically homogenized with 225 ml of sterile peptone water (EMD Buffered peptone water granulated, EMD Chemicals Inc., Gibbstown, NJ, USA) (1 g/l) in a stomacher bag with stomacher blender (Stomacher® 400 Circulator, Seward Ltd., West Sussex, U.K.) for 5 min. Serial dilutions were prepared. TV was 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 incubation at 37°C, built up colonies were counted according to ISO (1995) and results were expressed as log CFU/g nuggets sample. TCC was measured by spreading 0.1 ml of each dilution with a bent sterile polypropylene rod on duplicate plates of pre-poured and dried MacConkey agar (EMD Dehydrated MacConkey agar granulated, EMD Chemicals Inc., USA). After 48 h incubation at 37°C, built up colonies were counted according to ISO (1995) and results were expressed as log CFU/g nuggets sample. TYMC was measured by spreading 0.1 ml of each dilution with a bent sterile polypropylene rod on duplicate plates of pre-poured and dried standard potato dextrose agar (EMD Dehydrated potato dextrose agar granulated, EMD chemicals Inc., USA). After 72 h incubation at 25°C, built up colonies were counted according to ISO (1995) and results were expressed as log CFU/g nuggets sample.

Statistical analysis

All analyses were run in triplicate. Data were analyzed using analysis of variance ($p<0.05$) and the means separated by Tukey HSD test using SAS. The proposed model for the planned 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.

RESULTS AND DISCUSSION

Sensory attributes

Color

The color score in different treatments ranged 3.33 to 5.00. There were significant differences ($p<0.05$) of nugget's color in treated groups with increased storage period. Table 1 representing the color of nuggets which was almost similar among the treatments except control and slightly increased in *M. oleifera* leaf extract treated groups (specially 0.3%) in comparison to control and BHA which may be attributed by β-carotene, proteins, calcium, potassium etc. present in Moringa leaves and they act as good antioxidants. Color stability is related to antioxidant levels in meat samples and effect on metmyoglobin formation and as a result on color protection (Insani et al., 2008). The scores for appearance and color were significantly ($p<0.05$) higher for 0.3% Moringa leaf extract treated nuggets in comparison to control and other treatments throughout the storage period which agreed to Das et al. (2012) findings in goat meat patties treated with Moringa leaf extract. This might be attributed to high antioxidant content of 0.3% Moringa leaf extract which prevent oxidation of myoglobin content in goat meat nuggets. After 0^{th} day observation, the color scores showed a decreasing trend with advanced storage period which might be due to pigment discoloration, denaturation of proteins, particularly the myofibrillar protein (actin and myosin) and lipid oxidation and non-enzymatic browning resulting from reaction between lipid oxidation products and amino acids which was also reported by Bhat et al. (2013a, b).

Flavor

The scores for flavor were significantly ($p<0.05$) higher for nuggets incorporated with 0.3% *M. oleifera* leaf extract in comparison to control on 0^{th}, 15^{th}, 30^{th} and 45^{th} day of storage (Table 1). The flavor score in different treatments ranged 3.0 to 5.00 and was almost similar among the treatments except control and slightly decreased with prolonged storage period. The most preferable flavor was observed in 0.3% Moringa leaf extract (T_4) over the storage period and less preferable flavor was observed in control (T_0) (Table 1). *M. oleifera* leaves contain flavonoids, polyphenols, carotenoids and other bioactive compounds which may be attributed the flavor score of nuggets. The progressive decrease in flavor could be correlated to the increased lipid oxidation, liberation of fatty acids, increased microbial load and loss of volatile flavor components from spices and condiments with prolonged storage under aerobic conditions (Zargar et al., 2014). Similar decline trend in flavor scores during storage was also reported by Singh et al. (2015) in chevon cutlets treated with clove oil and Zargar et al. (2014) in chicken sausages treated with pumpkin.

Tenderness

The scores for tenderness of goat meat nuggets showed

Table 1. Effect of BHA and different levels of *M. oleifera* leaf extract on sensory attributes of goat meat nuggets at different storage time (mean±SE).

Sensory attributes	SP	Treatments					Mean	Level of significance		
		T_0	T_1	T_2	T_3	T_4		Treat.	SP	T*SP
Color	0	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00^{a}±0.00	*	*	*
	15	3.75±0.33	4.50±0.21	4.58±0.19	4.67±0.33	5.00±0.00	4.50^{b}±0.15			
	30	3.55±0.33	3.92±0.21	4.00±0.19	4.50±0.15	4.67±0.33	4.13^{c}±0.19			
	45	3.33±0.33	3.67±0.21	3.58±0.19	3.92±0.15	4.10±0.13	3.72^{d}±0.21			
	Mean	3.91^{d}±0.33	4.27^{c}±0.21	4.29^{c}±0.19	4.52^{b}±0.15	4.69^{a}±0.13				
Flavor	0	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00^{a}±0.00	*	*	*
	15	4.50±0.33	4.67±0.33	4.67±0.33	4.67±0.33	5.00±0.00	4.70^{b}±0.13			
	30	3.33±0.33	4.50±0.21	4.10±0.19	4.50±0.15	4.67±0.33	4.22^{c}±0.19			
	45	3.00±0.57	3.33±0.33	3.33±0.33	3.67±0.33	4.10±0.13	3.49^{d}±0.33			
	Mean	3.96^{e}±0.39	4.38^{c}±0.33	4.28^{d}±0.33	4.46^{b}±0.33	4.69^{a}±0.13				
Tenderness	0	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00^{a}±0.00	*	*	*
	15	4.75±0.33	4.67±0.33	4.67±0.33	5.00±0.00	5.00±0.00	4.82^{b}±0.13			
	30	4.67±0.33	4.10±0.21	4.33±0.19	4.67±0.33	4.67±0.33	4.49^{c}±0.15			
	45	4.10±0.33	3.58±0.21	3.67±0.33	4.00±0.15	4.33±0.13	3.94^{d}±0.19			
	Mean	4.63^{b}±0.33	4.34^{d}±0.21	4.42^{c}±0.33	4.67^{b}±0.21	4.75^{a}±0.13				
Juiciness	0	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00^{a}±0.00	*	*	*
	15	4.67±0.33	4.67±0.33	4.67±0.33	5.00±0.00	5.00±0.00	4.80^{b}±0.13			
	30	3.33±0.33	3.67±0.33	4.00±0.19	4.50±0.15	4.67±0.33	4.03^{c}±0.21			
	45	2.92±0.57	3.33±0.33	3.67±0.33	4.00±0.57	4.50 ±0.13	3.68^{d}±0.33			
	Mean	3.98^{e}±0.33	4.17^{d}±0.33	4.34^{c}±0.33	4.63^{b}±0.21	4.79^{a}±0.18				
Overall acceptability	0	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00^{a}±0.00	*	*	*
	15	4.10±0.33	4.75±0.21	4.67±0.33	5.00±0.00	5.00±0.00	4.70^{b}±0.13			
	30	3.33±0.33	4.10±0.21	4.25±0.19	4.50±0.15	4.67±0.33	4.17^{c}±0.19			
	45	2.67±0.57	3.00±0.57	3.33±0.33	3.58±0.15	3.67±0.33	3.25^{d}±0.33			
	Mean	3.78^{e}±0.33	4.21^{d}±0.21	4.31^{c}±0.33	4.52^{b}±0.15	4.59^{a}±0.33				

Means with different superscripts in each column and row are significantly different (*p<0.05). 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,** 0.1% BHA; **T_2,** 0.1% Moringa leaf extract; **T_3,** 0.2% Moringa leaf extract; **T_4,** 0.3% Moringa leaf extract; **SP,** storage period; **Treat.,** treatment; **T*SP,** interaction between treatment and storage period.

a declining trend with advancement of storage period. Table 1 also described that tenderness was almost similar to control but slightly decreased by treatments. The tenderness score in different treatments ranged 3.58 to 5.00 during storage. The tenderness of nuggets were significantly ($p<0.05$) differed among treatments. Nuggets treated with 0.3% Moringa leaf extract (T_4) showed most preferable tenderness among treatments and BHA treated nuggets (T_1) showed less preferable tenderness with increased storage period (Table 1). The probable reasons may be due to increased loss of moisture from products which lead to hardening of the texture, breakdown of fat, and degradation of muscle fibre proteins by bacterial action resulting into decreased water binding. Similar results were presented by Zargar et al. (2014) in chicken sausages and Bhat et al. (2013a) in chicken *seekh kebabs*. Tenderness is interrelated with DM content of the nuggets. With the increasing of storage period DM was increased, which accelerated tenderness of nuggets. The result of this experiment is also related to Das et al. (2012) findings in goat meat patties treated with Moninga leaf extract. The most preferable tenderness was observed at 0.3% Moringa leaf extract treated nuggets. This might be due to protect rancidity of fat and prolongs shelf-life of nuggets through its anti-oxidative properties which decreased shear force measurements thus improving tenderness.

Juiciness

The juiciness score in different treatments ranged 2.92 to

Table 2. Effect of BHA and different levels of *M. oleifera* leaf extract on pH value and cooking loss of goat meat nuggets at different storage time (mean±SE).

Sensory attributes	SP	Treatments						Level of significance		
		T_0	T_1	T_2	T_3	T_4	Mean	Treat.	SP	T*SP
pH	0	6.01±0.03	5.87±0.01	5.94±0.02	5.96±0.01	6.05±0.03	5.97^{a}±0.02	*	*	*
	15	5.79±0.01	5.84±0.01	5.86±0.01	$5.90^{\pm}$0.01	6.01±0.01	$5.88^{b\pm}$0.02			
	30	5.64±0.01	5.80±0.01	5.81±0.01	$5.82^{\pm}$0.01	5.94±0.01	5.80^{c}±0.01			
	45	5.48±0.01	5.67±0.01	5.72±0.01	$5.77^{\pm}$0.01	5.82±0.01	5.70^{d}±0.04			
	Mean	5.73^{e}±0.02	5.80^{d}±0.03	$5.83^{c\pm}$0.02	$5.86^{b\pm}$0.02	5.96^{a}±0.02				
Cooking loss	0	26.10±0.23	25.50±0.21	24.08±0.02	23.94±0.01	23.90±0.01	24.70^{a}±0.04	*	*	*
	15	24.67±0.24	23.72±0.18	22.91±0.01	22.80±0.01	22.77±0.01	23.37^{b}±0.10			
	30	22.91±0.23	21.78±0.21	21.25±0.01	21.96±0.06	21.85±0.07	21.95^{c}±0.19			
	45	21.78±0.26	21.07±0.21	20.03±0.01	20.45±0.01	20.33±0.01	20.73^{d}±0.13			
	Mean	$23.87^{a\pm}$0.25	23.02^{b}±0.20	22.07^{e}±0.02	22.29^{c}±0.04	22.21^{d}±0.10				

Means with different superscripts in each column and row are significantly different (*p<0.05). **T_0,** Control; **T_1,** 0.1% BHA; **T_2,** 0.1% Moringa leaf extract; **T_3,** 0.2% Moringa leaf extract; **T_4,** 0.3% Moringa leaf extract; **SP,** storage period, **Treat.,** treatment; **T*SP,** interaction between treatment and storage period.

5.00 over the storage period. The juiciness scores decreased as the days of storage progressed for all the treated goat meat nuggets that may be due to the gradual loss of moisture from the products. Juiciness of nuggets was significantly ($p<0.05$) decreased with increased storage period. Preferable juiciness was observed at 0.2% and 0.3% Moringa leaf extract (T_3 & T_4) treated nuggets over the storage period and less preferable juiciness was observed in control (Table 1). The results were in accordance with findings of Lui et al. (2010), Bhat et al. (2013a, b) and Singh et al. (2015) who also reported a decline in the juiciness scores of different meat products during refrigerated storage. The most preferable juiciness score was observed at 0.3% Moringa leaf extract treated nuggets that might be attributed to the gradual loss of moisture from the products, which agreed with Das et al. (2012) findings in goat meat patties treated with Moninga leaf extract.

Overall acceptability

The overall acceptability of nuggets was also observed in different treatments during storage period. The overall acceptability score of nuggets in different treatments ranged 2.67 to 5.00 over the storage period. Table 1 indicating that overall acceptability of nuggets was almost similar except control. There were significant ($p<0.05$) differences in overall acceptability of nuggets among the treatments. The most preferable overall acceptability was observed in 0.3% Moringa leaf extract (T_4) over the storage period in comparison to control and BHA (T_0 & T_1) (Table 1). Continuous decrease in overall acceptability scores might be reflective of the decline in scores of appearance and color, flavor, tenderness and juiciness. Similar observation was also reported by Bhat et al. (2013a) in chicken *seekh kebabs,* Zargar et al. (2014) in chicken sausages, and Singh et al. (2015) in chevon cutlets. The most preferable overall acceptability was observed at 0.3% Moringa leaf extract treated nuggets that might be due to less declining in scores of color, flavor, tenderness and juiciness due to high antioxidant properties of Moringa leaf extract. Similar observation was also reported by Das et al. (2012) in goat meat patties treated with Moninga leaf extract.

pH values

The initial pH of ready to eat raw nuggets was 6.01 and decreased to 5.48 after storage period. The pH of raw nuggets was significantly ($p<0.05$) decreased by treatments and storage period. The most preferable pH was observed in 0.3% Moringa leaf extract (T_4) over the storage period in comparison to control and BHA treated nuggets. As the storage period increased pH value of nuggets in all treatments decreased gradually (Table 2). The decreasing trend of pH value was probably due to the accumulation of lactic acids from the secretions of microorganisms and thaw loss of goat meat nuggets. Bacteria and mold have a tendency to decrease with increasing storage time, and they secrete components that decreasing the pH. Bhat et al. (2015) who observed a similar decline in pH of chicken nuggets incorporated with *Aloe vera*. The current result is also in agreement with the findings of Verma et al. (2013) who also observed a similar decrease in the pH of sheep meat nuggets incorporated with guava powder. Banerjee et al.

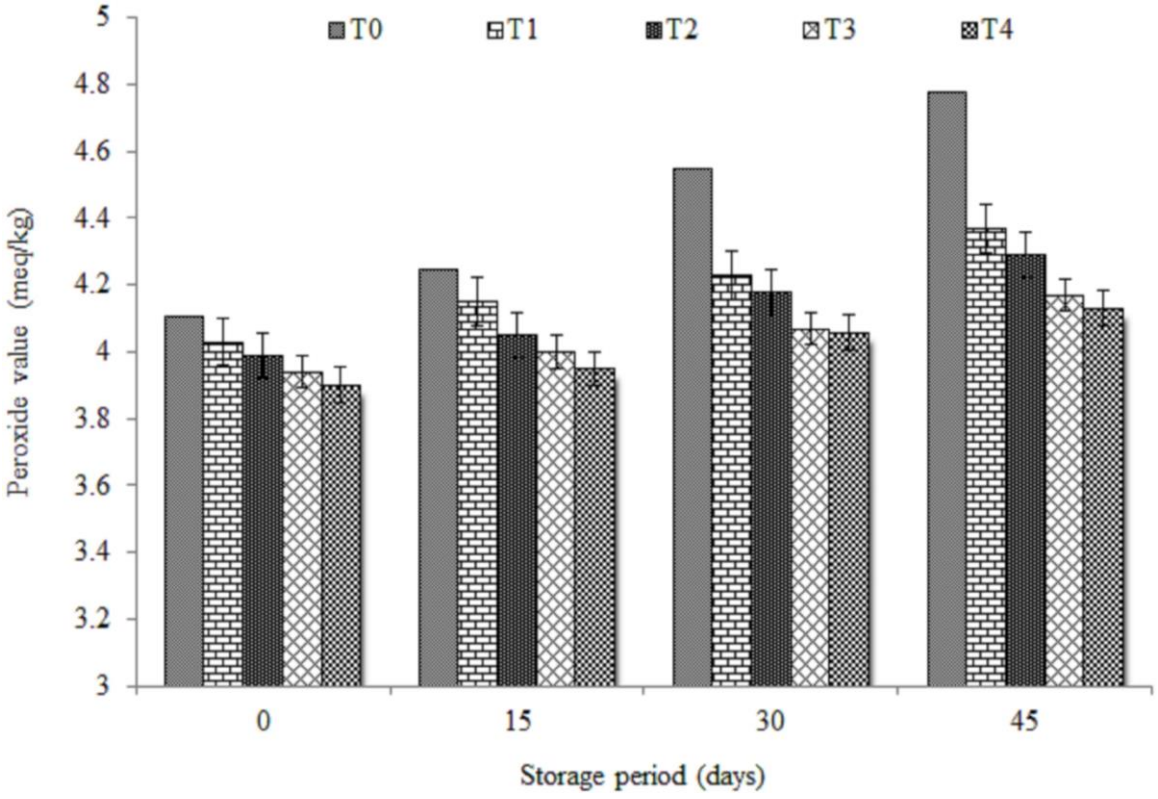

Figure 1. Effect of BHA and different levels of *M. oleifera* leaf extract on peroxide value of goat meat nuggets at different storage time.

(2012) also reported similar findings in goat meat nuggets with the incorporation of broccoli powder extract. Similar findings also reported by Sallam et al. (2004) in chicken sausage incorporated with garlic and Das et al. (2012) in goat meat patties treated with Moninga leaf extract. Therefore, low pH value is a positive character in nugget preparation because microorganism growth is reduced in low pH conditions which will increase shelf life of the product.

Cooking loss

Cooking loss refers to the reduction in weight of products during the cooking process. Cooking loss of goat meat nuggets is an important parameter which is used to predict the behavior of the products during processing. There were significant ($p<0.05$) differences in cooking loss of nuggets samples in different treatments during storage. Cooking loss in different treatments ranged 26.38 to 19.83% and almost similar to control (Table 2). As the storage period increased cooking loss of nuggets in different treatments also decreased. The most preferable cooking loss was observed in 0.3% Moringa leaf extract (T_4) in comparison to control and BHA treated nuggets. Decreasing trend of cooking loss may be due to the denatured meat protein and less loss of water and fat from nuggets. The results of cooking loss of the current study were in accordance with Das et al. (2012) findings in goat meat patties treated with Moninga leaf extract.

Peroxide values

Determination of peroxide value is a simple way to know the degree of primary lipid oxidation. Peroxide value used as primary oxidation measurement of lipid oxidation as indicator of meat and meat products quality. Figure 1 showed the effects of different antioxidant treatments on peroxide test. Early in the storage period (0 days), antioxidants had no effect on the results of peroxide test. Although there were significant differences ($p<0.05$) based on antioxidant treatment, longer storage time was associated with increased peroxide value. The data showed that the peroxide value was increased slowly over the observation period in Moringa leaf extract treated nuggets than BHA treated nuggets. 0.3% Moringa leaf extract (T_4) treated nuggets having more antioxidant functions than other treatments.

Throughout the storage time, peroxide values were

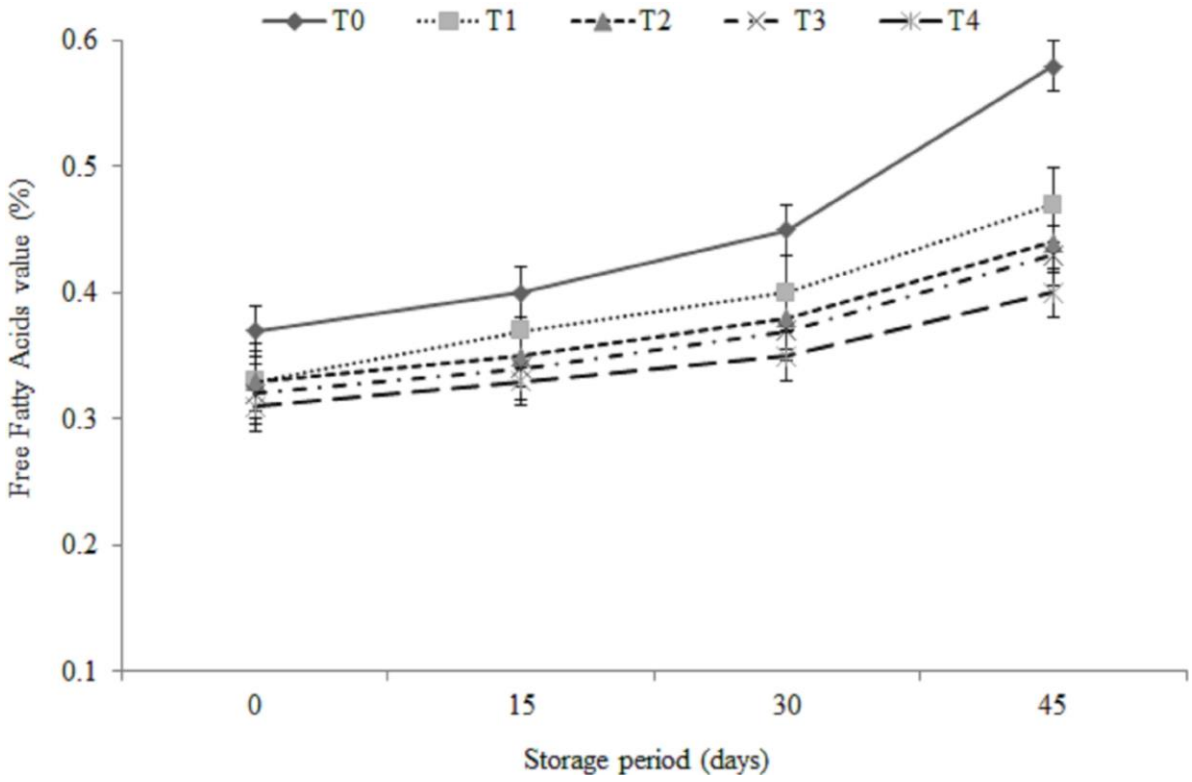

Figure 2. Effect of BHA and different levels of *M. oleifera* leaf extract on free fatty acids value of goat meat nuggets at different storage time.

generally higher in control samples than in others. Higher antioxidative effect on peroxide value was observed in *M. oleiferia* leaf extract treated nuggets than control and BHA treated samples. This might be due to high total phenolic compound present in *M. oleiferia* leaf extract reported by Das et al. (2012). During storage period, the peroxide value increased very slightly in all treatments except control which was also agreed with Georgantelis et al. (2007) findings on beef burger treated with rosemary extract.

Free fatty acid values

Determination of free fatty acid value is a simple way to know the degree of lipid hydrolysis. FFA gives an idea about stability of lipid and fat during preservation. Figure 2 exhibiting the effects of different antioxidant treatments on free fatty acid value. At the beginning of storage (day 0), the FFA value ranged from 0.31 to 0.37 % in different treatments. Throughout the storage time, FFA values were generally higher in control samples than in others. During storage period, FFA values was significantly ($p<0.05$) increased in all treatments but the increasing rate was slower in Moringa leaf extract treated nuggets than control and BHA treated nuggets. Generally, FFA values always appeared to be consistent with the values of TBA and POV. Moreover, free fatty acids are not only the products of enzymatic degradation but also microbial degradation of lipids and fats. The significant increase in FFA levels of goat meat nuggets treated with Moringa leaves extract during 45 days of frozen storage might be due to growth inhibition of lipolytic microbes, total myofibrillar protein solubility, and intramuscular free fatty acids concentration decreased in frozen storage which is in agreement with Qi et al. (2012), Rahman et al. (2014) and Ibrahim et al. (2018) findings.

Thiobarbituric acid values

Determination of TBARS value is a simple way to know the degree of secondary lipid oxidation. Lipid oxidation is an important quality parameter for meat and meat products, as it may lead to rancidity by lipolysis (Jin et al., 2009; Nolsøe and Undeland, 2009). TBARS values were significantly ($p<0.05$) low in Moringa leaf extract incorporated nugget samples compared to the control and BHA containing nuggets throughout the storage period. This lower TBARS values may be due to inhibition

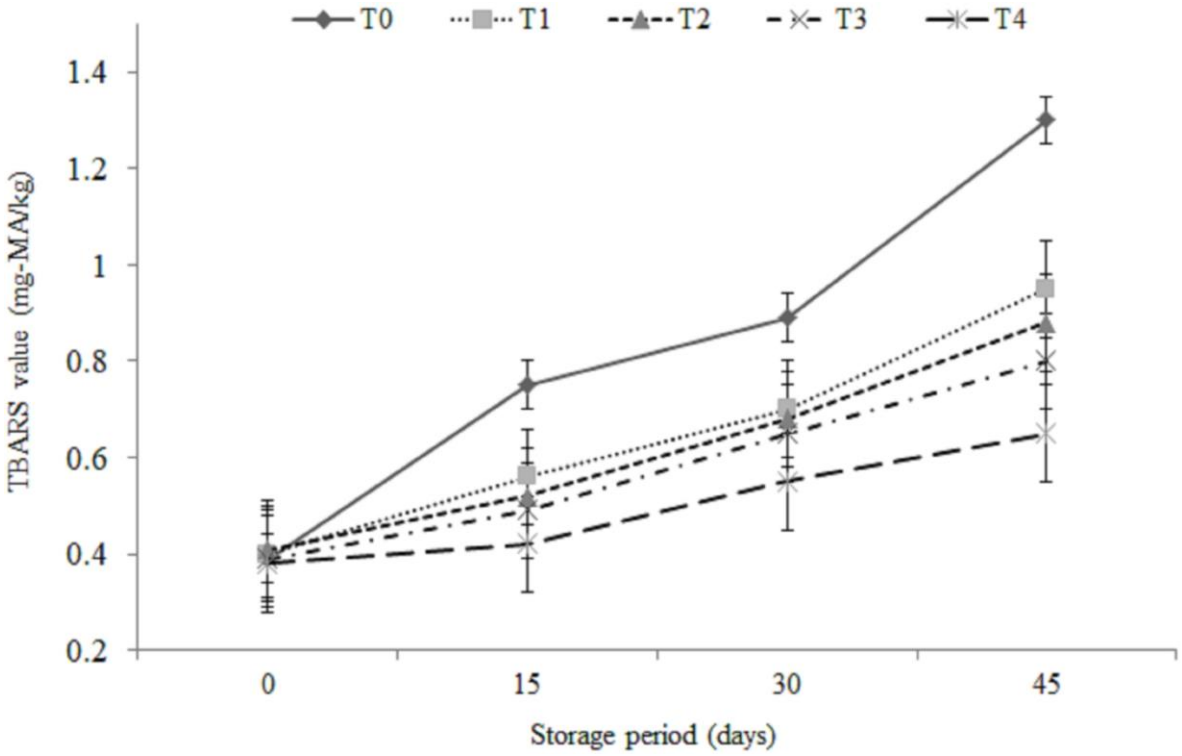

Figure 3. Effect of BHA and different levels of *M. oleifera* leaf extract on TBARS of goat meat nuggets value at different storage time.

of lipid peroxidation by *M. oleifera* leaves extract which containing β-carotene, polyphenols and bioactive compounds that have antioxidant effects due to their redox properties. Figure 3 presenting the significant ($p<0.05$) increased of TBARS values in different treatments with advanced storage period. But the increasing rate of TBARS values was slow in Moringa leaf extract treated nuggets in comparison with control and BHA. So, TBARS values may not able to affect the shelf life of the product. This increasing trend of TBARS values may be due to lipolysis of fat because lipolysis has been considered to be a promoter of lipid oxidation due to an accumulation of FFAs that are prone to lipid peroxidation, particularly long chain unsaturated FFAs (Das et al., 2008, 2012). Similar increase in malondialdehyde content was also reported by Bhat et al. (2015) in chicken nuggets treated with clove oil; Zargar et al. (2014) in chicken sausages treated with pumpkin and Bhat et al. (2013b) in chicken meatballs during refrigerated storage. The results of current study was also accordance with the findings of Ibrahim et al. (2018) who also observed significantly ($p<0.05$) lower TBARS values for the products treated with orange peel extract and Singh et al. (2015) who also observed significantly ($p<0.05$) lower TBARS values for the chevon cutlets incorporated with clove oil. Comparatively lower malondialdehyde contents of products were also reported by Banerjee et al. (2012) in goat meat nuggets containing broccoli powder extract, Das et al. (2013) in chicken nuggets containing fermented bamboo shoot, Das et al. (2012) in goat meat patties treated with Moringa leaves extract. Therefore, low TBARS value is a good sign in nugget preparation because of secondary lipid oxidation is somewhat arrested by antioxidative properties of Moringa leaf and able to increase shelf life of the product.

Microbiological analysis

Total viable count

The initial (0 day) TVC of goat meat nuggets samples was 4.8 to 5.12 log CFU/g in different treatments representing in Table 3. TVC was satisfactory in treated nugget samples. A significant ($p<0.05$) decreased of TVC was seen as the storage period increased. TVC was gradually decreased in all treatments but more preferable TVC was found in 0.3% Moringa leaf extract (T_4) in comparison to control and BHA treated samples. Bacterial growth may be controlled in current study by blocking the deterioration of fat by antioxidant compounds present in treatments and helped to prevent

Table 3. Effect of BHA and different levels of *M. oleifera* leaf extract on microbilogical analysis of goat meat nuggets at different storage time (mean±SE).

Microbiological analysis	SP	Treatments						Level of significance		
		T_0	T_1	T_2	T_3	T_4	Mean	Treat.	SP	T*SP
Total viable count (TVC)	0	5.12±0.11	4.95±0.11	4.88±0.11	4.83±0.11	4.80±0.11	4.92^{a}±0.10			
	15	4.92±0.13	4.84±0.13	4.70±0.13	4.62±0.13	4.56±0.13	4.73^{b}±0.10			
	30	4.75±0.14	4.62±0.14	4.50±0.14	4.43±0.14	4.35±0.14	4.53^{c}±0.10	*	*	*
	45	4.38±0.10	4.26±0.10	4.23±0.10	4.18±0.10	4.10±0.10	4.23^{d}±0.10			
	Mean	4.79^{a}±0.11	4.67^{b}±0.11	4.58^{c}±0.11	4.52^{d}±0.10	4.45^{e}±0.10				
Total coliform count (TCC)	0	1.25±0.01	1.22±0.01	1.15±0.01	1.11±0.01	1.09±0.01	1.16^{a}±0.01			
	15	1.11±0.01	1.08±0.01	1.05±0.01	1.03±0.01	1.00±0.01	1.05^{b}±0.01			
	30	1.06±0.01	1.00±0.01	<1±0.00	<1±0.00	<1±0.00	0.41^{c}±0.01	*	*	*
	45	<1±0.00	<1±0.00	<1±0.00	<1±0.00	<1±0.00	$<1^{c}$±0.00			
	Mean	0.86^{a}±0.02	0.83^{a}±0.02	0.55^{b}±0.02	0.54^{b}±0.02	0.52^{b}±0.02				
Total yeast-mould count (TYMC)	0	1.85±0.51	1.8±0.51	1.79±0.51	1.72±0.51	1.67±0.51	1.77^{a}±0.51			
	15	1.22±0.02	1.15±0.02	1.06±0.02	1.00±0.02	1.00±0.02	1.09^{b}±0.02			
	30	1.00±0.02	<1±0.00	<1±0.00	<1±0.00	<1±0.00	0.2^{c}±0.21	*	*	*
	45	<1 ±0.00	<1±0.00	<1±0.00	<1±0.00	<1±0.00	$<1^{c}$±0.00			
	Mean	1.02^{a}±0.21	0.74^{b}±0.21	0.71^{b}±0.21	0.68^{c}±0.21	0.67^{c}±0.21				

Means with different superscripts in each column and row are significantly different (*p<0.05). **T_0,** Control; **T_1,** 0.1% BHA; **T_2,** 0.1% Moringa leaf extract; **T_3,** 0.2% Moringa leaf extract; **T_4,** 0.3% Moringa leaf extract; **SP,** storage period; **Treat.,** treatment; **T*SP,** interaction between treatment and storage period.

the metabolism of fat by bacteria. As a result, bacterial growth was lower in goat meat nuggets treated with antioxidants both natural and synthetic. Bacterial growth may also be lowered in goat meat nuggets due to use of spices in nuggets preparation, which also possess antimicrobial activity. TVC showed a decreasing trend from day 0 to 45 day of observation but the decreasing rate was more in Moringa leaf extract treated nuggets than control and BHA containing nuggets. The lower amount of TVC value indicated that the shelf life of the product may be prolonged. The gradual decrease in TVC in treated nuggets might be attributed to the high bioactive compounds present in Moringa leaf extract responsible for antimicrobial properties. Similar findings were reported by Kim et al. (2013) in meat patties; Singh et al. (2015) in chevon cutlets and Bhat et al. (2015) in chicken nuggets who also observed a similar decreasing trend in the TVC of the products treated with tomato powder, *A. vera* and clove oil, respectively. The result of this experiment was also accordance with the findings of Zargar et al. (2014) in chicken sausages; Bhat et al. (2013a) in chicken *seekh kababs* and Bhat et al. (2013b) in chicken meatballs during refrigerated storage.

Total coliform count

Table 3 representing TCC and as the storage period increased TCC was decreasing significantly ($p<0.5$) and after 30th day of observation TCC was degenerating in Moringa leaf extract treated nuggets by counting less than 1 log CFU/g. The initial (0 day) TCC of goat meat nuggets samples was 1.25 to 1.09 log CFU/g indicating better sanitary measures adopted during processing. This result may also indicate the good shelf life of the product. The degenerating trend of TCC bacteria may be due to presence of pterygospermin in Moringa leaf which acts as a preservative that inhibits the growth of the *Escherichia coli, Enterobacter aerogenes* etc. and the destruction of these bacteria during cooking above their thermal death point of 57°C. Further, hygienic practices followed during the formulation and packaging of nuggets might also be one of the reasons for the absence of coliforms. Similar results were reported by Bhat et al. (2013a, b), Bhat et al. (2015) and Singh et al. (2015) who also reported zero count of coliforms for the meat products heated to such a high temperature.

Total yeast-mould count

TYMC was satisfactory in treated nugget samples and a significant ($p<0.5$) decrease of TYMC was also observed in different treatments over the storage period. During 0 day of storage, TYMC in the control goat meat nuggets was 1.85 log CFU/g indicating the highest than other treatments. As the storage period increased TYMC was also decreased and after 15th day of observation TYMC

was degenerating in BHA and Moringa leaf extract treated nuggets by counting less than 1 log CFU/g. This result may also indicate the good shelf life of the product. The lower TYMC of the treated nuggets may be attributed by the antifungal properties of Moringa leaf extract and high cooking temperature of nuggets. Moringa leaf extract contains several low weight proteins and peptides which are responsible for the antibacterial and antifungal activity. This antimicrobial effect agrees with Tesfay et al. (2017), who demonstrated that *M. oleifera* leaves extracts had antifungal properties against *Colletotrichum, Alternaria* and *Lasiodiplodia* strains. Similar findings were also observed by Das et al. (2013) in chicken nuggets treated with fermented bamboo shoots, Singh et al. (2015) in chevon cutlets treated with clove oil and Bhat et al. (2015) in chicken nuggets treated with *A. vera*, respectively.

Conclusion

The current study concludes that *M. oleifera* leaf extract is significantly more effective than BHA by providing antioxidant and antimicrobial benefits to goat meat nuggets in maintaining sensory quality, microbial quality and low POV, FFA, TABRS values during frozen storage. Addition of Moringa leaf extracts at 0.3% significantly arrests lipid oxidation as well as reduces microbial load without altering the sensory attributes of goat meat nuggets which are very important for the food industry because consumer acceptance depends largely on these attributes. From the findings of this study, it may be suggested that 0.3% Moringa leaf extracts may be able to replace synthetic antioxidants as a potential source of natural antioxidant in processed comminuted meat products to retain quality and to extend the shelf-life of the products, which are seen as health beneficial than those of synthetic origin by consumers and meat processors alike.

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