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Effect of kalogira (*Nigella sativa*) and BHA (beta hydroxyl anisole) on quality control and shelf- life of beef meatballs

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ABSTRACT

The experiment was conducted to find out the effect of different levels of kalogira extract and synthetic antioxidant on fresh and preserved beef meatballs as source of value added meat products. Five treatment groups as control group (T_1), BHA (T_2- 0.1%), 0.1%, 0.2% and 0.3% kalogira extract treated as (T_3), (T_4), and (T_5). respectively. Day's intervals of experiment were on 0, 15th, 30th, and 60th. DM content increased significantly ($p<0.05$) with advancement of different days intervals. CP, EE and Ash content at different treatment levels differ significantly ($p<0.05$). FFA values, TBARs, PV were decreased significantly ($p<0.05$) at different treatment levels. Color, flavor, tenderness, juiciness, overall acceptability, raw p^H, cooked p^H were increased at different treatment levels significantly ($p<0.05$). Cooking loss (%) with advancement of different days intervals were differ significantly ($p<0.05$). In microbiological assessment, TCC (*log* CFU/g) and TYMC (*log* CFU/g) was decreased significantly ($p<0.05$) at different treatment comparison to control, Addition of BHA and Kalogira extract significantly reduced aerobic plate count and storage time had negative effect on total bacterial counts and coli form group for all samples. But TVC (*log* CFU/g) increased significantly with increasing storage period. Hence, it may be concluded that kalogira extract had potential source of antioxidants and antimicrobial activities as similar characteristics of synthetic antioxidant BHA.

INTRODUCTION

Value added of meat is defined as the addition of time, place, and/or form utility to meet the preferences of consumers. In other words, value added meat is figuring out what consumers want. In recent years, natural antioxidants have increasingly replaced artificial preservatives in food products. Natural antioxidants like kalogira extracts have many of same functions as artificial antioxidants like butyrate hydroxyl anisole; can increase shelf-life of stored products without affecting qualities. This research explores the effects of natural antioxidants on quality changes of beef meatball during refrigeration storage. Kalogira is the potential sources of natural antioxidants because they produce various ant oxidative compounds to counteract reactive oxygen species (ROS). In food, ROS can cause lipid per oxidation, which leads to the deterioration of food (Miller *et al.*, 1997). The oxidative deterioration of lipid-containing food is responsible for rancid odors and flavors that can develop during processing and storage; this deterioration decreases nutritional quality and safety of food due to formation of secondary and potentially toxic compounds. The addition of kalogira extracts as natural antioxidant can help to prevent this process and increase shelf- life of foods. Lipid oxidation promotes production of rancid flavors and odors while also reducing shelf-life, nutritional quality, and safety of food products. To prevent autoxidation process, antioxidants have been utilized for many years (Lahucky *et al.*,2010). Antioxidants have an ability to prevent oxidative damage of a tissue indirectly by enhancing natural defenses of cell and directly by scavenging free radical species. Fatty acids contribute to a wide range of quality attributes for meat such as color stability, drip loss and the development of oxidative rancidity (Kouba *et al.*, 2003).

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Over the years, synthetic antioxidants like butyrate hydroxyl anisole, butyrate hydroxyl toluene and tertiary butyl hydroquinone have been widely used to preserve meatballs (Fasseas *et al.*, 2007). The use of these antioxidants has been questionable since they have been discovered to possess toxic, pathogenic and carcinogenic effects to humans and animals (Hayes *et al.*, 2010). In addition, consumers have shifted their interest to natural antioxidants since they are considered safer than synthetic antioxidants (Jung *et al.*, 2010). It has also been reported that these natural antioxidants, especially of plant source, have greater application potential for consumer's acceptability, palatability, stability and shelf- life of meat products one such plant with a potential to be used as an antioxidant is kalogira extracts. There was not more research so far conducted before my experiment on beef meatballs with kalogira extracts in Bangladesh. When meat products are enriched with natural antioxidant we can recommend this as natural antioxidant. The aim of preservation is not only to retard food spoilage but also to control undesirable changes of wholesomeness, nutritive value and growth of microorganisms. Yet no investigation on different levels of kalogira has been carried out. Hence, the study was to examine the addition of kalogira extract on sensory, proximate, physicochemical, biochemical, microbiological analysis and recommend value added beef meatball enriched with kalogira extract and compare its effectiveness as natural antioxidant and with a synthetic antioxidant (BHA) in delaying lipid oxidation to extend the shelf-life of meatball during refrigeration.

MATERIALS AND METHODS

Materials collection

Slaughtered boneless beef of 3 kg was collected from "Sheep, Goat & Horse Farm", Bangladesh Agricultural University; Mymensingh The meat sample was immediately transferred to the "Animal Science Laboratory" at Bangladesh Agricultural University, Mymensingh.

Sample preparation

About 3 kg of fresh beef sample was taken for the preparation of beef meatball. First beef was properly cleaned with fresh water and fat was trimmed with sharp knife. Then beef was grinded properly and spices, garlic paste, onion paste , ginger paste, BHA, kalogira extract, eggs, salt, Ice flakes, refined vegetable oil, refined wheat flower, sauce was mixed with grinded beef properly as per experimental design. Five treatment groups as control (T_1), BHA (T_2- 0.1%), 0.1%, 0.2% and 0.3% kalogira extract treated as (T_3), (T_4), and (T_5). Then beef meatball of proper shape was prepared separately. It was then boiled in hot water for 2-3 minute. Then water was removed from meatball properly and was fried in hot oil until reddish brown color was obtained.

Packaging of the sample

After frying the meatballs, they were packaged in polyethylene bags separately. Then, it was kept into the freeze for the research at different days intervals.

Sensory evaluation

Different sensory attributes were examined. Each meatball sample was evaluated by a trained 8-member panel. The sensory questionnaires measured intensity on a 5-point balanced semantic scale for the attributes color, smell, tenderness, juiciness, and overall acceptability. Eight training sessions were held to familiarize the judges with the attributes to be evaluated and the scale to be used (Rubio *et al.*, 2007). Prior to sample evaluation, all panelists participated in orientation sessions to familiarize with the scale attributes of beef meatball using intensity scale. All samples were served in the Petri dishes. Sensory evaluation was accomplished at 0 day and repeated at 15, 30 and 60 day.

Proximate components

Proximate composition on Dry Matter, Ether Extract, Crude Protein and Ash were carried out according to methods (AOAC, 2016).

Raw and cooked p^H measurement

pH value of raw, cooked and cooking loss meatball was measured using pH meter from raw

meatball homogenate. It was prepared by blending 5 g of meat with 10 ml distilled water.

Biochemical analysis

There were three types of biochemical analysis viz. FFA, Peroxide Value, Thiobarbituric Acid value. FFA value was determined according to Rukunudin *et al.* (1998). Peroxide value was determined according to Sallam *et al.* (2004). Lipid oxidation was assessed in triplicate using the 2-thiobarbituric acid method described by Schmedes and Holmer (1989).

Microbial assessment

For microbial assessment total viable, coli form and yeast-mould count was undertaken. Ten g beef meatball sample was aseptically excised from stored stock sample. Each stored beef meatball samples was thoroughly and uniformly macerated in a mechanical blender using sterile diluents (0.1% peptone water) as per recommendation of International Organization for Standardization (ISO, 1995). A quantity of ten gram of minced meatball sample was taken aseptically transferred into a sterile container containing 90 ml of 0.1% peptone water. A homogenized suspension was made in a sterile blender. Thus 1:10 dilution of the samples was obtained. Later on using whirly mixture machine different serial dilutions ranging from 10^{-2} to 10^{-6} were prepared. Microbiological analyses were determined by Ikhlas *et al.* (2011).

Statistical analysis

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

RESULTS AND DISCUSSION

Sensory evaluation

It was found that sensory quality after fortification with kalogira extracts was deteriorated with increased storage period. The range of overall observed color score at different treatment was 3.55 to 4.08, flavor score was 3.55 to 3.92, tenderness score was 3.50 to 4.17, juiciness score was 3.50 to 4.08 and overall acceptability score was 3.55 to 4.17. The range of different day's intervals of overall observation of overall acceptability score was 4.57 to 2.80. Among five treatments most preferable color, flavor, tenderness and juiciness was observed from o.2% kalogira extract and BHA group (Table 1). The present findings is in agreement with Gonzalez *et al.* (2008) where he stated that dried plum ingredients in raw and precooked pork sausage negatively affect sensory attributes viz. color, texture, odor, and flavor as well as the nutritional quality of the product.

Proximate analysis

The DM content was increased with increased storage period because moisture loss was decreased with storage period. The range of overall observed DM content at different treatments was 58.13 to 48.07. The range of overall observation of different days of interval DM content was 53.85 to 50.35. Among five treatments most preferable DM content was observed at 0.1% Kalogira seed extract group (Table 2) .The highest amount of DM content indicates this product is less preferable. The most preferable DM content was observed from 0 day and less preferable DM content from 60^{th} day. Similar results were reported for Indonesian traditional meatballs with moisture content ranged from 69.52 to 71.17% (Purnomo and Rahardiyan 2008). Devatkal *et al.*,(2010) reported that incorporation pomegranate rind and seed powder extracts did not affect DM content of goat meat patties. The range of overall observed moisture at different treatments was 41.87 to 51.93. The CP content was decreased with increased storage period. The range of overall observed CP content at different treatments was 21.89 to 23.27%. Among three treatments most preferable CP content was observed from 1% kalogira extract group. The range of overall observed of different days of intervals of CP content was 23.32 to 22.24%. The range of overall observed EE content at different treatments was 11.38 to 12.34%. The range of overall observed of different days of intervals of EE content was 11.37 to 12.38%. The EE content was no changed with increased storage period. Among three treatments of kalogira extract, the most preferable EE content was

observed at 0.3% Kalogira extract group. The most preferable EE content was observed at 0 day and less preferable EE content at 60th day. The data show that the highest amount of EE content was increased to 11.37 % in all treatments after 60 days of storage. Kalogira extract group contains higher amount of EE than control group. The Malaysian Food Regulation of 1985 stated that manufactured meat should not contain more than 30% fat. Malaysian beef meatballs can be classified as low-fat meatballs since fat content ranges from 1.69 to 11.09. The range of overall observed ash content at different treatments was 3.43 to 3.79%. The range of overall observed of different day's intervals of ash content was .48 to 3.64%. Among these three treatments, the most preferable Ash content was observed from 0.1% Kalogira seed extract group. The lowest amount of ash content indicates this product is most preferable for consumers' health. The most preferable Ash content was observed at 0 day and less preferable ash content at 60th day. The ash content was significantly changed with increased storage period. The same trend was also observed by Konieczny *et al.* (2007) and they reported that ash content increased during frozen storage which is related to our findings.

Table 1
Effect of kalogira extract and BHA on sensory parameters in beef meatballs.

Parameters	DI	Treatments					Mean	Level of significance		
		T_1	T_2	T_3	T_4	T_5		Treat.	DI	T*DI
Color	0	4.50±0.50	4.67±0.33	4.67±0.33	4.67±0.33	5.00±0.00	4.71^{a}±0.30	.247	<.0001	.968
	15	4.33±0.33	4.67±0.33	4.67±0.33	4.67±0.33	4.33±0.33	4.53^{a}±0.33			
	30	3.33±0.33	3.67±0.33	3.67±0.33	3.67±0.33	3.33±0.67	3.53^{b}±0.33			
	60	2.33±0.33	3.00±0.00	2.67±0.33	3.33±0.33	3.00±0.00	2.87^{c}±0.20			
	Mean	3.55^{b}±0.37	4.00^{ab}±0.25	3.92^{ab}±0.33	4.08^{a}±0.33	3.92^{ab}±0.25				
Flavor	0	4.50±0.50	4.67±0.33	4.67±0.33	4.67±0.33	4.67±0.33	4.64^{a}±0.36	.590	<.0001	.976
	15	4.33±0.33	4.67±0.33	4.00±0.56	4.00±0.00	4.33±0.33	4.27^{a}±0.31			
	30	3.33±0.33	3.67±0.33	3.33±0.33	3.67±0.33	3.33±0.33	4.47^{b}±0.33			
	60	2.33±0.33	2.67±0.33	2.67±0.33	3.00±0.00	2.67±0.33	2.67^{c}±0.26			
	Mean	3.55^{a}±0.37	3.92^{a}±0.33	3.67^{a}±0.39	3.84^{a}±0.17	3.75^{a}±0.33				
Tenderness	0	4.50±0.50	4.67±0.33	4.33±0.33	4.33±0.33	4.33±0.67	4.43^{a}±0.50	.110	<.0001	.948
	15	4.67±0.33	4.67±0.33	3.67±0.33	4.00±0.00	4.00±0.58	4.20^{a}±0.31			
	30	3.33±0.33	4.00±0.00	3.00±0.58	3.00±0.00	3.33±0.33	3.33^{b}±0.25			
	60	2.67±0.33	3.33±0.33	3.00±0.00	3.00±0.58	2.67±0.33	2.93^{b}±0.31			
	Mean	3.73^{ab}±0.37	4.17^{a}±0.42	3.50^{b}±0.31	3.58^{ab}±0.23	3.58^{ab}±0.48				
Juiciness	0	4.50±0.50	4.67±0.33	4.33±0.33	4.67±0.33	4.33±0.33	4.50^{a}±0.36	.154	<.0001	.954
	15	4.67±0.33	4.67±0.33	4.00±0.00	4.00±0.58	3.67±0.33	4.20^{a}±0.31			
	30	3.33±0.33	3.67±0.33	3.33±0.33	3.33±0.33	3.33±0.33	3.40^{b}±0.33			
	60	2.67±0.33	3.33±0.33	2.67±0.33	3.00±0.00	2.67±0.33	2.87^{c}±0.26			
	Mean	3.73^{ab}±0.37	4.08^{a}±0.33	3.58^{ab}±0.25	3.75^{ab}±0.31	3.50^{b}±0.33				
Overall acceptability	0	4.50±0.50	4.67±0.33	4.33±0.33	4.67±0.33	4.67±0.33	4.57^{a}±0.36	.074	<.0001	.927
	15	4.33±0.33	4.67±0.33	4.33±0.33	4.67±0.33	4.00±0.00	4.40^{a}±0.26			
	30	3.33±0.33	3.67±0.33	3.67±0.33	4.00±0.00	3.33±0.33	3.60^{b}±0.26			
	60	2.33±0.33	2.67±0.33	3.00±0.00	3.33±0.33	2.67±0.33	2.80^{c}±0.26			
	Mean	3.55^{b}±0.37	3.92^{ab}±0.33	3.83^{ab}±0.25	4.17^{a}±0.25	3.67^{b}±0.25				

Sensory scores were 5 for excellent, 4 for very good, 3 for good, 2 for fair, and 1 for poor. Mean in each row having different superscript varies significantly at values *P < 0.05. Again, mean values having same superscript in each row did not differ significantly at P > 0.05. T_1=Controlled group, T_2= Beta Hydroxyl Anisole (BHA) group, T_3=0.1% Kalogira seed extract, T_4=0.2% Kalogira seed extract, T_5=0.3% Kalogira seed extract, DI=Days of Intervals, Treat= Treatment, T*DI=Interaction of Treatment and Days of Intervals.

Table 2
Effects of Kalogira and BHA on proximate components in beef meatballs.

Parameters	DI	Treatments					Mean	Level of significance		
		T_1	T_2	T_3	T_4	T_5		Treat.	DI	T*DI
DM	0	55.51±0.03	54.97±0.02	47.25±0.14	47.40±0.33	48.33±0.23	50.35^{d}±0.15	<.0001	<.0001	<.0001
	15	57.04±0.04	55.34±0.02	48.17±0.03	47.68±0.04	48.40±0.23	51.33^{c}±0.05			
	30	58.03±0.04	56.06±0.05	47.91±0.04	48.12±0.06	48.68±0.06	51.76^{b}±0.05			
	60	61.07±0.09	58.01±0.01	48.92±0.07	50.01±0.06	51.23±0.18	53.85^{a}±0.08			
	Mean	58.13^{a}±0.05	56.09^{b}±0.03	48.07^{e}±0.07	48.31^{d}±0.12	49.16^{c}±0.18				
CP	0	22.40±0.19	23.12±0.02	23.82±0.04	23.51±0.01	23.45±0.01	23.32^{a}±0.05	<.0001	<.0001	<.0001
	15	22.07±0.10	23.00±0.01	23.66±0.01	23.39±0.01	23.32±0.02	23.09^{b}±0.03			
	30	21.99±0.03	22.88±0.04	23.05±0.01	23.20±0.02	23.17±0.03	22.86^{c}±0.03			
	60	21.28±0.16	22.19±0.04	22.54±0.01	22.61±0.01	22.61±0.01	22.24^{d}±0.05			
	Mean	21.89^{d}±0.12	22.80^{c}±0.03	23.27^{a}±0.02	23.18^{b}±0.01	23.14^{b}±0.02				
EE	0	12.23±0.19	11.56±0.07	12.65±0.01	12.53±0.01	12.90±0.01	12.38^{a}±0.06	<.0001	<.0001	<.0001
	15	12.01±0.01	11.48±0.01	12.10±0.01	12.37±0.01	12.72±0.02	12.15^{b}±0.01			
	30	11.90±0.02	11.33±0.01	11.94±0.01	12.22±0.03	12.26±0.01	11.93^{c}±0.02			
	60	11.38±0.23	11.16±0.02	11.42±0.02	11.43±0.02	11.47±0.01	11.37^{d}±0.06			
	Mean	11.85^{d}±0.11	11.38^{e}±0.03	12.03^{c}±0.01	12.14^{b}±0.02	12.34^{a}±0.01				
Ash	0	3.46±0.14	3.48±0.07	3.29±0.01	3.27±0.01	3.89±0.01	3.48^{c}±0.06	<.0001	.0005	<.0001
	15	3.66±0.08	3.35±0.02	3.39±0.01	3.27±0.01	3.93±0.02	3.52^{bc}±0.03			
	30	3.62±0.16	3.41±0.01	3.48±0.02	3.83±0.01	3.87±0.01	3.64^{a}±0.04			
	60	3.74±0.10	3.47±0.02	3.60±0.02	3.52±0.03	3.49±0.01	3.56^{b}±0.04			
	Mean	3.63^{b}±0.12	3.43^{c}±0.03	3.44^{c}±0.02	3.47^{c}±0.01	3.79^{a}±0.01				

Mean in each row having different superscript varies significantly at values $P < 0.05$. Again, mean values having same superscript in each row did not differ significantly at $P > 0.05$.T_1=Controlled group, T_2= Beta Hydroxyl Anisole (BHA) group, T_3= 0.1% Kalogira seed extract, T_4=0.2% Kalogira seed extract, T_5=0.3% Kalogira seed extract, DI=Days of Intervals, Treat= Treatment and T*DI=Interaction of Treatment and Days of Intervals.

Physicochemical properties

The range of overall observed raw p^H at different treatments was 5.68 to 5.78. Among five treatments most preferable raw p^H was observed from 0.2% kalogira extract group. The highest amount of raw p^H indicates this product is most preferable for consumers' health (Table 3). The raw p^H among these treatments was decreased with increased storage period. Choi *et al.* (2009) reported that meat batter containing dietary fiber from rice bran have higher pH values. The decrease in raw p^H values was lower in untreated samples than treated ones due to the effect of natural antioxidants which were retarded the formation of free fatty acids. It is also obvious that the values of raw p^H for the product were higher than that of raw p^H values of meat and this could be due to interaction effect of other ingredients which were added during processing of meat products. The highest amount of raw p^H indicates this product is most preferable for consumers' health than other treatment groups. The range of overall observed of different days of intervals of raw p^H 5.65 to 5.85. The most preferable raw p^H was observed at 0 day and less preferable raw p^H was observed at 60^{th} day observation. The raw p^H was decreased with increased storage period. The data showed a slight decrease in the raw p^H values and an increased in the acidity values for all samples along with storage time during the 60 days of storage increasing of free fatty acids due to

rancidity. Similar results have also been found in the study of antioxidant treatments during storage time using a mixture of BHA and BHT in precooked pork patties (Biswas *et al.* 2004). (Aksu and Kaya 2005) reported a study related to Kavurma, a cooked Turkey meat product that is usually sliced and consumed. They found that the pH of Kavurma slightly increased after 30 days of storage time the data showed a slight increase in cooked pH values and a decrease in acidity values for all samples along with natural antioxidants. The range of overall observed cooked p^H at different treatments was 5.87 to 5.99. The highest amount of cooked p^H indicates this product is most preferable for consumers' health. The lowest amount of cooking loss indicates this product is most preferable for consumers' choices. The range of overall observed of different days of intervals of cooking loss was 5.80 to 6.09. The cooking loss was decreased with increased storage period. The range of overall observed cooking loss at different treatments was 24.21 to 23.92%. The different superscript was observed at controlled group than other four treatment groups indicates there were significant ($p<0.05$) differences of cooking loss of control group. The increased loss of such nutrients deteriorates meat nutritional quality and lowers its purchase (Jama *et al.*,2008) evenly similar trend with this experiment. Turhan *et al.* (2005) reported that addition of hazelnut pellicle fiber was found to be effective in improving cooking yield, dimensional changes and thickness of beef burgers. Cooking yield was an important data that could be used by meat industry to predict the behavior of their products during processing (Ulu, 2006). The values of cooking yield were similar to the results in high-fat Kung-wan meatballs reported by Huang *et al.* (2005) or in low-fat Kung-wan meatballs reported by Hsu and Sun (2006). The cooking yield of the Kung-Wan significantly decreased with higher natural antioxidant extract levels which is nearly with this experiment.

Table 3
Physicochemical Parameters of Beef Meatballs Using Turmeric Powder and BHA.

Parameters	DI	Treatments					Mean	Level of significance		
		T_1	T_2	T_3	T_4	T_5		Treat.	DI	T*DI
Raw P^H	0	5.76±0.07	5.87±0.01	5.88±0.01	5.86±0.003	5.85±0.01	5.85^{a}±0.02	<.0001	<.0001	0.010
	15	5.72±0.02	5.82±0.01	5.82±0.01	5.82±0.01	5.83±0.01	5.80^{b}±0.01			
	30	5.65±0.02	5.68±0.02	5.75±0.01	5.77±0.01	5.77±0.01	5.73^{c}±0.01			
	60	5.60±0.01	5.66±0.01	5.65±0.01	5.67±0.01	5.66±0.01	5.65^{d}±0.01			
	Mean	5.68^{b}±0.03	5.76^{a}±0.01	5.78^{a}±0.01	5.78^{a}±0.01	5.78^{a}±0.01				
Cooked P^H	0	6.05±0.01	6.06±0.01	6.10±0.01	6.11±0.01	6.11±0.01	6.09^{a}±0.01	<.0001	<.0001	<.0001
	15	5.99±0.01	6.05±0.01	6.03±0.003	6.02±0.01	6.03±0.003	6.02^{b}±0.01			
	30	5.77±0.01	6.01±0.003	5.97±0.01	5.96±0.01	5.97±0.01	5.94^{c}±0.01			
	60	5.72±0.01	5.84±0.02	5.81±0.01	5.83±0.01	5.81±0.01	5.80^{d}±0.01			
	Mean	5.87^{b}±0.01	5.99^{a}±0.01	5.98^{a}±0.01	5.98^{a}±0.01	5.98^{a}±0.01				
Cooking loss	0	27.38±0.13	26.10±0.06	26.62±0.03	26.53±0.03	27.06±0.14	26.69^{a}±0.08	0.021	<.0001	<.0001
	15	25.67±0.24	24.92±0.07	25.75±0.06	25.78±0.10	25.43±0.05	25.51^{b}±0.10			
	30	23.84±0.23	22.59±0.21	22.45±0.05	22.59±0.08	22.18±0.01	22.73^{c}±0.12			
	60	21.00±0.44	22.08±0.04	22.09±0.01	22.02±0.04	21.66±0.04	21.77^{d}±0.11			
	Mean	24.21^{a}±0.26	23.92^{b}±0.10	24.23^{a}±0.04	24.23^{a}±0.06	24.09^{ab}±0.06				

Mean in each row having different superscript varies significantly at values $P < 0.05$. Again, mean values having same superscript in each row did not differ significantly at $P > 0.05$. T_1=Controlled group, T_2= Beta Hydroxy Anisole (BHA) group, T_3=0.1% Kalogira seed extract, T_4=0.2% Kalogira seed extract, T_5=0.3% Kalogira seed extract, DI=Days of Intervals, Treat=Treatment and T*DI=Interaction of Treatment and Days of Intervals.

Biochemical properties

The range of overall observed peroxide value at different treatments was 4.54 to 4.17. The range of overall observed FFA value at different treatments was 0.45 to 0.36. The range of overall observed of different days of intervals of FFA was 0.32 to 0.44. The FFA value was increased with storage period. The most preferable FFA value was observed at 0 day and less preferable FFA was observed at 60th day of observation. The range of overall observed of different days of intervals of peroxide value was 3.96 to 4.59 (Table 4). Among five treatments most preferable peroxide value was observed from 0.3% Kalogira extract group. The lowest amount peroxide value indicates this product is most preferable for consumes health. During storage, peroxide value increased in all treatments. Lund *et al.*,(2007) reported on peroxide values in sausage with three treatments (rosemary extract, collagen fiber preparation impregnated with rosemary extract and collagen hydrolyses impregnated with rosemary extract); samples with three treatments showed lower values than control group.

The peroxide value of plain meat loaf was 0.38, 1.33 and 2.40 at 0, 3 and 6 days, respectively, in aerobic storage at 4ºC. The range of overall observed of different days of intervals of TBARS value was 0.39 to 0.54. The control sample, without any added antioxidants, this may be showed a higher level of TBARS than samples treated with 0.3%, 0.2%, and 0.1% Kalogira extract or BHA. The TBARS level of samples treated with 0.3%, 0.2%, 0.1% kalogira seed extract was also lower than those treated with BHA; this difference was especially significant ($P < 0.05$) after 60 days of storage time. The TBA value increased until storage day 60. The TBARS value on day 60 was 0.58 for control samples, 0.49, 0.47, 0.47, 0.46 for those treated with BHA, 0.3%, 0.2%, 0.1% Kalogira extract. Among five treatments most preferable TBARS value was observed from 0.3% Kalogira extract group.. The lowest amount of TBARS value indicates this product is most preferable for consumes health. The results of this study confirm that BHA, 0.3%, 0.2%, 0.1% kaligitra seed extract can delay lipid oxidation significantly, reducing the potential risk induced by lipid oxidation. They also tend to be better than synthetic antioxidant BHA, which has been commercially used in food industry. Lipid oxidation developed in all five types of meatballs (control, dittany 0.05%, dittany 0.10%, rosemary 0.05% and rosemary 0.10%) as evidenced by an increasing TBARS value. They also found that dittany and rosemary at a concentration 0.10% protected the product significantly. The levels were 0.73, 3.90 and 4.44/kg meat, respectively, in samples treated with ethanolic extract (0.15%) and 0.59, 1.24 and 1.77/kg meat, respectively, in samples treated with dried powder (0.51%). The TBARS extracts in both sausage control group (CG) and the experimental group (EG) increased over almost the entire study period. Nassu *et al.* (2003) examined lipid oxidation and reported that TBARS values of fermented goat meat sausage were different at beginning of storage depending on treatment.

Meat with higher lipid oxidation values also showed higher protein oxidation and greater metmyoglobin formation. The peroxide value of plain meat loaf was 0.38-1.33 and 2.40 at 0, 3 and 60 days, respectively, in aerobic storage at 4ºC

Natural antioxidants, in particular polyphones, are the major plant compounds which have the ability to attenuate the oxidative damage of a tissue indirectly by enhancing natural defenses of cell and/or directly by scavenging the free radical species combat pathological disorders generated by physicochemical Reactive Oxygen Species (ROS) (Du *et al.*,2010).To prevent the autoxidation process antioxidants have been utilized for many years (Lahucky *et al.*, 2010). Antioxidants have an ability to prevent the oxidative damage of tissue indirectly by enhancing natural defenses of cell and directly by scavenging the free radical species (Verma *et al.*, 2009). It has also been reported that these natural antioxidants, especially of plant source, have greater application potential for consumer's acceptability, palatability, stability and shelf-life of meat products (Jung *et al.*, 2010). One such plant with a potential to be used as an antioxidant is kaligira extract. It has various functions, including pharmacological activities and antioxidant properties.

Table 4
Effect of Kalogira seed extract and BHA on bio-chemical parameters in beef meatballs.

Parameters	DI	Treatments					Mean	Level of significance		
		T_1	T_2	T_3	T_4	T_5		Treat.	DI	T*DI
FFA (%)	0	0.38±0.001	0.34±0.001	0.31±0.001	0.31±0.002	0.29±0.001	0.32^{d}±0.001	<.0001	<.0001	<.0001
	15	0.40±0.01	0.38±0.001	0.37±0.001	0.36±0.001	0.34±0.001	0.37^{c}±0.001			
	30	0.47±0.01	0.41±0.001	0.42±0.002	0.41±0.001	0.40±0.001	0.42^{b}±0.001			
	60	0.53±0.03	0.43±0.002	0.43±0.002	0.42±0.001	0.40±0.005	0.44^{a}±0.008			
	Mean	0.45^{a}±0.01	0.39^{b}±0.001	0.38^{bc}±0.002	0.37^{c}±0.001	0.36^{d}±0.002				
PV (meq/kg)	0	4.10±0.07	3.97±0.01	4.02±0.003	4.08±0.01	3.96±0.01	4.02^{d}±0.02	<.0001	<.0001	<.0001
	15	4.33±0.03	4.04±0.01	4.23±0.01	4.09±0.01	4.02±0.01	4.14^{c}±0.01			
	30	4.74±0.03	4.53±0.01	4.45±0.01	4.18±0.03	4.09±0.01	4.40^{b}±0.02			
	60	4.85±0.01	4.63±0.01	4.53±0.01	4.47±0.01	4.45±0.02	4.58^{a}±0.01			
	Mean	4.54^{a}±0.03	4.29^{b}±0.01	4.31^{b}±0.01	4.20^{c}±0.02	4.13^{d}±0.01				
TBARS (mg-MA/kg)	0	0.41±0.004	0.40±0.001	0.40±0.002	0.40±0.001	0.40±0.001	0.40^{d}±0.002	<.0001	<.0001	<.0001
	15	0.48±0.002	0.44±0.003	0.42±0.001	0.41±0.003	0.40±0.001	0.43^{c}±0.002			
	30	0.55±0.001	0.50±0.001	0.55±0.003	0.55±0.003	0.54±0.001	0.54^{b}±0.002			
	60	0.82±0.003	0.62±0.001	0.62±0.002	0.62±0.001	0.62±0.002	0.66^{a}±0.002			
	Mean	0.58^{a}±0.003	0.49^{d}±0.02	0.50^{b}±0.002	0.49^{c}±0.002	0.49^{d}±0.001				

Mean in each row having different superscript varies significantly at values $P < 0.05$. Again, mean values having same superscript in each row did not differ significantly at $P > 0.05$. T_1=Controlled group, T_2= Beta Hydroxyl Anisole (BHA) group, T_3=0.1% Kalogira seed extract, T_4=0.2% Kalogira seed extract, T_5=0.3% Kalogira seed extract, DI=Days of Intervals, Treat=Treatment and T*DI=Interaction of Treatment and Days of Intervals.

Table 5
Effect of Kalogira seed extract and BHA on different microbe's population in beef meatballs.

Parameters	DI	Treatments					Mean	Level of significance		
		T_1	T_2	T_3	T_4	T_5		Treat.	DI	T*DI
TVC (*log*CFU/g)	0	4.65±0.04	4.57±0.04	4.59±0.01	4.54±0.02	4.51±0.01	4.57^{c}±0.02	<.0001	<.0001	0.006
	30	4.95±0.02	4.76±0.02	4.75±0.01	4.73±0.01	4.71±0.01	4.78^{b}±0.01			
	60	5.45±0.04	5.16±0.03	5.19±0.01	5.15±0.01	5.12±0.01	5.22^{a}±0.02			
	Mean	5.06^{a}±0.04	4.83^{bc}±0.03	4.85^{b}±0.01	4.81^{cd}±0.01	4.78^{d+}±0.01				
TCC (*log*CFU/g)	0	1.22±0.04	1.15±0.02	1.20±0.01	1.16±0.01	1.13±0.01	1.17^{a}±0.02	<.0001	<.0001	.574
	30	1.10±0.01	1.04±0.02	1.05±0.01	1.02±0.01	1.01±0.01	1.04^{b}±0.01			
	60	1.02±0.01	0.95±0.03	0.94±0.01	0.92±0.01	0.91±0.01	0.95^{c}±0.01			
	Mean	1.10^{a}±0.02	1.05^{bc}±0.02	1.06^{b}±0.01	1.03^{cd}±0.01	1.01^{d}±0.01				
TYMC (*log*CFU/g)	0	1.98±0.02	1.85±0.02	1.87±0.01	1.85±0.01	1.81±0. 01	1.86^{a}±0.01	0.021	<.0001	.001
	30	1.55±0.02	1.38±0.01	1.41±0.01	1.38±0.01	1.32±0.01	1.41^{b}±0.01			
	60	1.14±0.01	1.08±0.01	1.11±0.01	1.07±0.01	1.02±0.01	1.08^{c}±0.01			
	Mean	1.50^{a}±0.02	1.43^{c}±0.01	1.46^{b}±0.01	1.43^{c}±0.01	1.38^{d}±0.01				

Mean in each row having different superscript varies significantly at values $P < 0.05$. Again, mean values having same superscript in each row did not differ significantly at $P > 0.05$. T_1=Controlled group, T_2= Beta Hydroxy Anisole (BHA) group, T_3=0.1% Kalogira seed extract mixed group, T_4=0.2% Kalogira seed extract mixed group, T_5=0.3% Kalogira seed extract, DI=Days of Intervals, Treat= Treatment and T*DI=Interaction of Treatment and Days of Intervals.

Microbiological assessment

The range of overall observed aerobic plate count from the beef meatballs was 5.06–4.36 (log CFU/g) at different treatments. Among five treatments, the plate count in the control sample (5.06 log CFU/g) was significantly higher than in the samples treated with BHA, 0.1%, 0.2%, and 0.3% of Kalogira seed extracts. The less amount of TVC value indicates this product is most preferable for consumers' health (Table 5). The initial value of TVC for fresh beef (beef not frozen and thawed) was 5.12 logs CFU/g beefs, indicating good quality beef. The less amount of TVC value indicates this product is most preferable for consumers' health. The range of overall observed of different days of intervals of TVC value 4.45 to 4.86. During storage TVC value was increased. The initial TCC of fresh beef (beef not frozen and thawed) was 1.25 logs CFU/g beef The range of overall observed total coli form count from beef meatballs was 1.10–0.91 (logs CFU/g), at different treatments. During storage TCC value and TYMC value was decreased. The range of overall observed total yeast-mold count from the beef meatballs was 1.50 to1.25 (log CFU/g) at different treatment. The range of overall observed of different days of intervals of TYMC values was 1.79 to 1.04. The less amount of TYMC value indicates this product is most preferable for consumers' health. Fernández-López *et al.* (2003) reported on the results of a research study related to antimicrobials in beef meatballs. Some bacteria may be present in the product, but their growth is controlled under storage conditions (Fernandez Lopez *et al.*, 2005).

CONCLUSIONS

Present study may be concluded that 0.2% concentration of kalogira extract as natural antioxidant will be used in future manufacturing meatballs with providing herbal remedy against a variety of ailments, antioxidant and antimicrobial agents as value addition through inhibiting lipid oxidation, adding flavor & prolonged shelf- life of stored meat and meat products instead of synthetic antioxidant (BHA). So, we can recommend that beef meatball with 0.2% kalogira extract level and BHA treated beef meatballs are more acceptable to consumers than others treated beef meatballs.

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