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Formulation of value added beef meatball using *tulsi* (*Ocimum sanctum*) leaf extract as a source of natural antioxidant

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Abstract

The present study was undertaken to evaluate the effect of different levels of *tulsi* leaf extract on fresh and preserved beef meatballs. Four types of beef meatballs were formulated for this purpose. Meatballs were made with 0 (control), 0.1, 0.2 and 0.3% *tulsi* leaf extract, respectively and preserved at –20˚C. Quality and safety evaluation of meatballs were determined by sensory, physicochemical, biochemical and microbiological tests. The analyses were conducted at 0, 15th, 30th and 60th days of interval. Considering CP, tenderness, juiciness, overall acceptability, cooking loss, Free Fatty Acid (FFA), Per oxide Value (POV) and Thiobarbituric Acid Reactive Substances (TBARS) value, it can be concluded that *tulsi* leaf extract @ 0.1, 0.2 and 0.3% can be used in the formulation of beef meatball. In case of sensory evaluation 0.2% tulsi leaf extract is appreciated but on the basis of nutrient quality, physicochemical properties, biochemical analysis and microbial analysis 0.3% tulsi leaf extract is more satisfactory as a source of natural antioxidant than that of other treatment groups. Therefore, it may be concluded that 0.3% *tulsi* leaf extract can be added as a functional ingredients in beef meatball.

Introduction

Lipid peroxidation causes meat spoilage. It occurs during processing and storage when meatballs are exposed to oxygen, heat, and light (Fasseas *et al*., 2007). Antioxidant have an ability to prevent or reduce the oxidative damage of a tissue indirectly by enhancing natural defense of cell and/or directly by scavenging the free radical species (Verma *et al*., 2009). Over the years, synthetic antioxidants such as beta hydroxyl anisole, butyrate hydroxyl toluene and tertiary butyl hydroquinone etc. have been widely used to preserve meat and meat products (Fasseas *et al*., 2007). The use of these antioxidants is questionable since they have been discovered as toxic, mutagenic and carcinogenic effect to human and animal (Hayes *et al*., 2010). Hence there has been a growing interest in the use of natural antioxidants as alternative remedy for synthetic antioxidants. In addition, consumers have shifted their interest to natural antioxidants since they are considered safer than the 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 (Jung *et al*., 2010). Tulsi is grown in tropical and sub tropical including Indian Regions (Banerjee *et al*., 1996). This plant has been evaluated pharmacologically for antimicrobial, immunomodulatory, anti-stress, anti-inflammatory, antipyretic, anti-asthmatic, hypoglycemic, hypotensive and analgesic activities. tulsi has been found to be utmost effective in various types of animal models (Chiang et al., 2005). Phenolics and flavonoids are the authentic antioxidants found in tulsi leaf that have been reported to be safe and bioactive (Sreelatha and Padma, 2009). *Tulsi* is a naturally occurring antioxidants that have many of the same functions as artificial antioxidants like butyrate hydroxyl anisole. It can increase shelf life of stored products without affecting qualities. It contains several compounds having multiple phenolic hydroxyl groups, such as apigenin, luteolin, vitexin, isovitexin, orientin, aesculetin, aesculin, chlorogenic acid and caffeic acid (Koushik and Gopal, 2013). The phenolic compounds, namely, cirsilineol, cirsimaritin, isothymusin, apigenin, rosmarinic acid and eugenol possess good antioxidant activity and have significant ability to scavenge highly reactive free radicals (Pandey and Madhuri, 2010). *Tulsi*, also known as *Ocimum tenuiflorum*, is revered as "Queen of herbs" due to its greater medicinal values and often consumed as herbal tea. Marked by its strong aroma and astringent taste, it is regarded as a kind of "elixir of life", as it is believed to promote longevity (Puri and Singh, 2002). *Tulsi* is known as "Queen of plant", "The mother medicine of nature" (Singh et al., 2010). No investigation on different levels of *tulsi* leaf extract on beef meatball has been carried out yet. With these view, the present study was undertaken to investigate the possibility of using different levels of *tulsi* leaf extract in beef meatballs to evaluate shelf-life, nutritional and microbiological status of beef meatball under different storage conditions.

Materials and Methods

Collection of meat sample

Boneless beef of 2.5kg from freshly slaughtered cattle was collected from "Local market", Bangladesh Agricultural University, Mymensingh at 10.00 a.m. The

meat sample was immediately transferred to the "Animal Science Laboratory" and stored at room temperature for 1 hour.

Preparation of jar and other instruments

All necessary instruments and jars or containers were cleaned with hot water and detergent powder and then autoclaved for sterilization and dried before starting the experimental activities.

Sample preparation

About 2.5 kg of fresh beef sample was taken for the preparation of beef meatball. First, the beef was cleaned with fresh water and the fat was trimmed with sharp knife. The beef sample was grinded properly and the spices, garam masala, salt, ice flakes, refined vegetable oil, refined wheat flower, sauce were mixed with grinded beef properly as per experimental design. There were four treatment groups as 0% (T_0), 0.1% (T_1), 0.2% (T_2), and 0.3% (T_3) *tulsi* leaf extract. The beef meatball of proper shape was prepared separately. It was then boiled in hot water for 2–3 minutes, water was removed from the meatball and fried in hot oil until reddish brown color was obtained. After frying the meatballs, they were packaged in polyethylene bags separately and kept into the freezer for further research at different day's of intervals.

Sensory evaluation

Different sensory attributes were examined. The meatball samples were evaluated by a trained 8-member panel. The sensory questionnaires measured intensity on a 5-point balanced semantic scale for the attributes of 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 the orientation sessions to familiarize with the scale attributes (color, smell, juiciness, tenderness, and overall acceptability) 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 days.

Proximate composition

Proximate composition such as Dry Matter (DM), Ether Extract (EE), Crude Protein (CP) and Ash were carried out according to the methods of AOAC (1995).

Physicochemical properties measurement

All pH value of raw, cooked and cooking loss meatball was measured using pH meter (Hanna HI99163) from raw meatball homogenate. It was prepared by blending 5 g of meat with 10 ml distilled water.

Biochemical analysis and microbial assessment

Free Fatty Acid (FFA), Per Oxide Value (POV) and Thiobarbituric Acid Reactive Substances (TBARS) value were determined according to Sharma *et al,* (2012). Total Viable Count (TVC), Total Coliform Count (TCC) and Total Yeast Mold Count (TYMC) were determined according to Ikhlas *et al*., (2011). All determination was done in triplicate and mean value was reported.

Statistical analysis

Data were analyzed using 4x4 factorial experiment in completely randomized design replicated three times per cell using SAS 9.1.3 version Statistical Discovery software, NC, USA. Duncan's Multiple Range Test (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 *tulsi* leaf extracts was deteriorated with increased storage period. The range of overall observed color score at different treatment was 3.75 to 4.33, flavor score was 3.75 to 4.17, tenderness score was 3.58 to 4.17, juiciness score was 3.83 to 4.00 and overall acceptability score was 3.83 to 4.08 (Table 1). The range of different day's intervals of overall observation of overall acceptability score was 3.08 to 4.58. Among four treatments most preferable color and juiciness was observed from 0.2 % (T_2), tenderness and overall acceptability was observed from 0.1% (T_1) and flavor was observed from 0.3% (T_3) *tulsi* leaf extract. Texture attributes viz, hardness, springiness, color, odor, gumminess and flavor as well as the nutritional quality of the product were significantly higher for clove and cardamom burfi. Sensory evaluation revealed that among different herbs, cardamon is highly preferred in burfi followed by ginger, turmeric , clove, curry leaves and *tulsi* (Prasad et al., 2017). It is in agreement with the present findings where *tulsi* leaf extract significantly affect different sensory attributes.

Proximate components

Overall DM content at different treatment was 53.99 to 59.29%. The highest value was observed in 0 day and decreased gradually up to 60th day (Table 2). The highest amount of DM content indicates this product is more preferable. DM content was increased with increased storage period because moisture loss was decreased with storage period. Similar results were reported for Indonesian traditional meatballs with a moisture content ranged from 69.52 to 71.17% (Purnomo and Rahardiyan 2008). The CP content at different treatments was 41.87 to 51.93. Synthetic antioxidant group contain more amount of CP than control group. The most preferable CP content was observed at 0.3 % (T_4) *tulsi* leaf extracts group. The CP content at different days of interval was 23.32 to 22.24%. The most preferable CP content was observed at 0 day and less preferable at 60th day. The EE content at different treatments was 11.38 to 12.06 %. The most preferable EE content was observed at 0.2 (T_2)

and 0.3% (T_3) *tulsi* leaf extracts. The range of EE content at different days of interval was 11.82 to 11.89%. The most preferable EE content was observed at 0 day and less preferable EE content was at 60th day (Table 2). Serdaroglu *et al*. (2005) reported a similar fat content ranged from 7.9 to 8.8% in low-fat traditional Turkey koefte beef meatballs. Overall ash content at different treatments was 3.49 to 3.67%. Control group contain higher amount of ash than treated with *tulsi* leaf extracts group except T_3.

Physicochemical properties

The range of overall observed raw pH at different treatments was 5.69 to 5.81%. Among four treatments most preferable raw pH was observed at 0.3% (T_3) ***t****ulsi* leaf extrac**t** (Table 3). The highest amount of raw pH indicated the higher preferability of the products for consumers' health. The raw pH among the treatments was decreased with increased storage period. The raw p^H at different days of interval was 5.70 to 5.79%. The most preferable raw p^H was observed at 15th day and less preferable was observed at 60th day. Overall cooked p^H at different treatments was 5.93 to 6.10%. The most preferable cooked p^H was observed at 0.3% (T_3) *tulsi* leaf extrac**t**. The most preferable cooked p^H was observed at 30th day and less preferable cooked p^H at 60th day. These results are similar to those of Sallam *et al*. (2004), who reported that storage time had a significant ($p< 0.05$) effect on pH values, which tended to increase with storage time (up to 15 days with present finding). The overall cooking loss at different treatments was 23.97 to 24.95%. The highest cooking loss was observed at 0.3% (T_3) *tulsi* leaf extrac**t**. The overall cooking loss at different days of interval was 22.35 to 26.32%. The lowest cooking loss was observed at 60th day and the highest cooking loss was observed at 0 day of observation. Cooking loss refers to the reduction of weight of meatballs during cooking process (Jama *et al.,* 2008).

Biochemical properties

The biochemical effects of *tulsi* leaf extract on beef meatball is shown in Table 4. The overall FFA, POV and TBARS value at different treatment was 0.36 to 0.44%., 4.47 to 4.20 and 0.49 to 0.59, respectively. The overall FFA, POV and TBARS at different days of interval were 0.35 to 0.44%., 4.03 to 4.59% and 0.40 to 0.69%, respectively. The most preferable FFA value was observed at 0 day and less preferable FFA value was observed at 60th day. The most preferable value was observed from 0.3% (T_3) *tulsi* leaf extrac**t.** The FFA value (0.44) in the control group was significantly ($P <$ 0.01) higher than the values of the samples treated with 0.1, 0.2, and 0.3% *tulsi* leaf extracts. Lee and Kunz (2005) found that fermented sausages showed an increasing FFA content over time. It has 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). Throughout the storage time, POV were generally higher in control group compared to treatment groups (Table 4). The most preferable POV was observed at 0.3% (T_3) *tulsi* leaf extracts. The lowest amount of POV indicates that this product is most preferable for consumers health. The control sample showed a higher level of TBARS than samples treated with 0.1, and 0.3% except 0.2% *tulsi* leaf extracts. This difference was especially significant ($p < 0.01$) after 60th days of storage time. 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). 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). *Tulsi* leaf extracts can be a potential source of natural antioxidant which can be used in meat products.

Microbiological assessment

Effect of *tulsi* leaf extracts on beef meatball on microbial population is shown in Table 5. TVC value of fresh beef was 5.12 logs CFU/g beef, indicated good quality beef. The overall total viable count, TCC value and TYMC of beef meatball was 4.74 to 4.86, 1.07 to 1.13, and 1.47 to 1.69 (log CFU/g), respectively at different treatment levels. The range of TVC value, TCC value and TYMC values at different days of interval was 4.50 to 5.13, 1.01 to 1.18 and1.11 to 1.89, respectively. The TCC value of fresh beef was 1.13 logs CFU/g beef. Among these four treatments, the TCC in the control sample (1.13 logs CFU/g) was significantly higher than in the samples treated with 0.1, 0.2, and 0.3% of ***t****ulsi* leaf extracts. The different superscript was observed from different treatment indicated that there were significant differences of TYMC values among these four treatment groups. Among four treatments, the total yeast-mold count in the control sample (1.69 log CFU/g) were significantly ($p<0.01$) higher than in the samples treated with, 0.1, 0.2, and 0.3% of ***t****ulsi* leaf extracts. Some bacteria may be present in the product, but their growth is controlled under storage conditions (Fernandez-Lopez *et al.,* 2005).

Table 1. Effect of *tulsi* leaf extract on sensory parameters in beef meatballs

Parameters	DI	Treatments				Mean	Level of significance		
		T_0	T_1	T_2	T_3		Treat.	DI	T*DI
Color	0	4.67±0.33	5.00±0.00	4.67±0.33	4.67±0.33	4.75^{a}±0.25	0.0906	<0.0001	0.2244
	15	4.67±0.33	4.00±0.00	5.00±0.00	4.00±0.58	4.42^{a}± 0.23			
	30	3.33±0.33	3.67±0.33	4.00±0.58	3.33±0.33	3.58^{b}±0.45			
	60	2.33±0.33	3.67±0.33	3.67±0.33	3.33±0.33	3.25^{b}±0.33			
	Mean	3.75^{a}±0.33	4.08^{ab}±0.16	4.33^{a}±0.31	3.83^{ab}±0.39				
Flavor	0	4.33±0.33	4.33±0.33	4.67±0.33	4.67±0.33	4.50^{a}±0.33	0.3414	<0.0001	0.9622
	15	4.33±0.33	4.67±0.33	4.33±0.33	4.33±0.33	4.42^{a}±0.33			
	30	3.670.33	4.00±0.58	4.00±0.00	4.33±0.33	4.00^{a}±0.31			
	60	2.67±0.33	3.33±0.33	3.33±0.33	3.33±0.33	3.17^{b}±0.33			
	Mean	3.75^{a}±0.33	4.08^{a}±0.39	4.08^{a}±0.25	4.17^{a}±0.33				
Tenderness	0	4.00±0.00	4.67±0.33	4.67±0.33	4.33±0.33	4.42^{a}±0.25	0.0396	<0.0001	0.4427
	15	4.00±0.00	4.67±0.33	4.67±0.33	3.67±0.33	4.25^{a}±0.25			
	30	3.67±0.33	3.67±0.33	3.67±0.33	3.33±0.33	3.58^{b}±0.33			
	60	2.67±-0.33	3.67±0.33	3.33±0.33	3.67±0.33	3.33^{b}±0.33			
	Mean	3.58^{b}±0.16	4.17^{a}±0.33	4.08^{a}±0.33	3.75^{ab}±0.33				
Juiciness	0	4.67±0.33	4.33±0.33	4.67±0.33	4.33±0.33	4.50^{a}±0.33	0.8848	<0.0001	0.4226
	15	5.00±0.00	4.33±0.33	4.67±0.33	4.33±0.33	4.58^{a}±0.25			
	30	3.33±0.33	3.33±0.33	3.67±0.33	3.67±0.33	5.50^{b}±0.33			
	60	2.33±0.33	3.33±0.33	3.00±0.33	3.00±0.33	3.00^{c}±0.39			
	Mean	3.83^{a}±0.25	3.83^{a}±0.33	4.00^{a}±0.39	3.92^{a}±0.33				
Overall acceptability	0	4.33±0.33	4.67±0.33	4.33±0.33	4.67±0.33	4.50^{a}±0.33	0.6849	<0.0001	0.9493
	15	4.33±0.33	4.67±0.33	4.67±0.33	4.67±0.33	4.58^{a}±0.33			
	30	3.67±0.33	3.67±0.33	3.67±0.33	3.33±0.33	3.58^{b}±0.33			
	60	3.00±0.58	3.33±0.33	3.33±0.33	2.67±0.33	3.08^{b}±0.39			
	Mean	3.83^{a}±0.39	4.08^{a}±0.33	4.00^{a}±0.33	3.83^{a}±0.33				

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_0 = 0% *tulsi* leaves extract, T_1 = 0.1% *tulsi* leaves extract, T_2 = 0.2% *tulsi* leaves extract, T_3 = 0.3% *tulsi* leaves extract, DI=Days of Intervals, Treat= Treatment, T*DI=Interaction of Treatment and Day Intervals.

Table 2. Effect of *tulsi* leaf extract on proximate components in beef meatballs

Parameters	DI	Treatments				Mean	Level of significance		
		T_0	T_1	T_2	T_3		Treat.	DI	T*DI
DM%	0	56.3±0.16	55.49±0.15	54.39±0.17	52.44±0.13	54.66^{d}±0.15	<0.0001	<0.0001	<0.0001
	15	58.87±0.05	56.57±0.06	54.67±0.07	53.54±0.26	55.91^{c}±0.11			
	30	59.48±0.19	58.58±0.19	55.41±0.10	54.33±0.13	56.95^{b}±0.15			
	60	61.53±0.16	60.06±0.12	57.66±0.07	55.65±0.18	58.73^{a}±0.13			
	Mean	59.29^{a}±0.14	57.68^{b}±0.13	55.53^{c}±0.10	53.99^{d}±0.17				
CP%	0	22.33±0.11	23.09±0.07	23.35±0.03	23.53±0.06	23.07^{a}±0.07	<0.0001	<0.0001	<0.0001
	15	22.19±0.03	22.91±0.04	23.16±0.02	23.14±0.02	22.85^{b}±0.03			
	30	22.04±0.05	22.86±0.32	22.92±0.03	22.94±0.04	22.69^{c}±0.11			
	60	21.69±0.04	22.33±0.02	22.27±0.01	22.23±0.02	22.13^{d}±0.07			
	Mean	22.06^{c}±0.06	22.79^{b}±0.11	22.92^{a}±0.02	22.96^{a}±0.03				
EE%	0	12.20±0.08	11.52±0.06	11.84±0.03	11.83±0.03	11.85^{a}±0.05	<0.0001	0.6444	<0.0001
	15	12.06±0.04	11.49±0.02	11.94±0.03	11.91±0.03	11.85^{a}±0.03			
	30	11.83±0.09	11.31±0.05	12.09±0.00	12.04±0.04	11.82^{a}±0.04			
	60	11.41±0.20	11.20±0.02	12.36±0.04	12.22±0.03	11.89^{a}±0.07			
	Mean	11.87^{b}±0.10	11.38^{c}±0.04	12.06^{a}±0.02	12.06^{a}±0.03				
ASH%	0	3.48±0.02	3.47±0.04	3.48±0.03	3.53±0.01	3.49^{b}±0.02	0.0005	<0.0001	0.3534
	15	3.64±0.04	3.37±0.03	3.56±0.02	3.65±0.04	3.55^{b}±0.03			
	30	3.51±0.11	3.45±0.04	3.51±0.01	3.67±0.01	3.54^{b}±0.04			
	60	3.78±0.09	3.66±0.13	3.65±0.03	3.83±0.00	3.73^{a}±0.06			
	Mean	3.60^{ab}±0.06	3.49^{c}±0.06	3.55^{bc}±0.22	3.67^{a}±0.03				

Mean in each row having different superscript varies significantly at values P < 0.05., T_0 = 0% *tulsi* leaf extract, T_1 = 0.1% *tulsi* leaves extract, T_2 = 0.2% *tulsi* leaves extract, T_3 = 0.3% *tulsi* leaf extract, DI=Day Intervals, Treat= Treatment, T*DI=Interaction of Treatment and Day Intervals.

Table 3. Effect of *tulsi* leaf extract on physicochemical parameters in beef meatballs

Parameters	DI	Treatments				Mean	Level of significance		
		T_0	T_1	T_2	T_3		Treat.	DI	T*DI
Raw pH	0	5.68±0.01	5.69±0.03	5.72±0.01	5.82±0.02	5.73^{b}±0.02	<0.0001	<0.0001	0.2146
	15	5.72±0.02	5.81±0.01	5.80±0.02	5.82±0.04	5.79^{a}±0.02			
	30	5.68±0.00	5.73±0.02	5.72±0.02	5.81±0.02	5.74^{b}±0.01			
	60	5.67±0.03	5.66±0.03	5.67±0.01	5.80±0.04	5.70^{b}±0.03			
	Mean	5.69^{c}±0.01	5.72^{b}±0.02	5.73^{b}±0.01	5.81^{a}±0.03				
Cooked pH	0	5.99±0.01	5.99±0.01	6.10±0.01	6.24±0.03	6.08^{b}±0.01	<0.0001	<0.0001	0.0002
	15	6.09±0.01	6.19±0.02	6.15±0.01	6.14±0.00	6.12^{a}±0.01			
	30	5.93±0.00	5.96±0.01	6.07±0.04	6.06±0.04	6.00^{c}±0.02			
	60	5.73±0.02	5.82±0.01	5.91±0.01	5.93±0.04	5.85^{d}±0.02			
	Mean	5.93^{d}±0.01	5.97^{c}±0.01	6.06^{b}±0.02	6.10^{a}± 0.03				
Cooking loss (%)	0	27.21±0.17	26.12±0.06	26.01±0.01	25.94±0.02	26.32^{a}±0.06	0.0071	<0.0001	0.0589
	15	25.71±0.23	24.90±0.07	25.93±0.01	25.87±0.01	25.60^{b}± 0.08			
	30	23.80±0.46	23.94±0.29	23.27±0.72	22.11±0.07	23.28^{c}±0.38			
	60	23.08±0.75	22.39±0.78	21.96±0.18	21.96±0.38	22.35^{d}± 0.52			
	Mean	24.95^{a}±0.40	24.34^{b}±0.30	24.29^{b}±0.23	23.97^{b}±0.12				

Mean in each row having different superscript varies significantly at values p<0.05. T_0 = 0% *tulsi* leaves extract, T_1 = 0.1% *tulsi* leaf extract, T_2 = 0.2% *tulsi* leaf extract, T_3 = 0.3% *tulsi* leaf extract, DI=Day Intervals, Treat= Treatment, T*DI= Interaction of Treatment and Day Intervals.

Table 4. Effect of *tulsi* leaf extract on biochemical parameters in beef meatballs

Parameters	DI	Treatments				Mean	Level of significance		
		T_0	T_1	T_2	T_3		Treat.	DI	T*DI
FFA (%)	0	0.38±0.00	0.34±0.00	0.34±0.00	0.33±0.00	0.35^{d}±0.00	<0.0001	<0.0001	<0.0001
	15	0.40±0.01	0.38±0.00	0.34±0.00	0.33±0.00	0.37^{c}±0.002			
	30	0.46±0.01	0.41±0.00	0.36±0.00	0.35±0.00	0.39^{b}±0.002			
	60	0.52±0.01	0.43±0.00	0.42±0.00	0.41±0.00	0.44^{a}±0.002			
	Mean	0.44^{a}±0.01	0.39^{b}±0.00	0.37^{c}±0.00	0.36^{d}±0.00				
POV (meq/kg)	0	4.06±0.06	3.97±0.01	4.03±0.01	4.03±0.01	4.03^{d}±0.02	<0.0001	<0.0001	<0.0001
	15	4.28±0.06	4.13±0.02	4.24±0.02	4.19± 0.02	4.19^{c}±0.03			
	30	4.71±0.01	4.51±0.01	4.41±0.02	4.19±0.04	4.45^{b}±0.02			
	60	4.85±0.01	4.56±0.04	4.47±0.03	4.48±0.09	4.59^{a}±0.04			
	Mean	4.47^{a}±0.03	4.29^{b}±0.02	4.29^{b}±0.02	4.20^{c}±0.04				
TBARS (mg-MA/kg	0	0.41±0.00	0.40±0.00	0.49±0.00	0.49±0.00	0.40^{d}±0.00	<0.0001	<0.0001	<0.0001
	15	0.48±0.00	0.43±0.00	0.42±0.00	0.42±0.00	0.44^{c}±0.00			
	30	0.61±0.00	0.58±0.02	0.54±0.00	0.51±0.01	0.56^{b}±0.01			
	60	0.80±0.00	0.70±0.01	0.63±0.00	0.62±0.00	0.69^{a}±0.00			
	Mean	0.58^{a}±0.00	0.53^{b}±0.01	0.59^{c}±0.00	0.49^{d}±0.00				

Mean in each row having different superscript varies significantly at values P < 0.05. T_0 = 0% tulsi leaves extract, T_1 = 0.1% *t*ulsi leaf extract, T_2 = 0.2% tulsi leaf extract, T_3 = 0.3% tulsi leaf extract, DI = Day Intervals, Treat = Treatment, T*DI = Interaction of Treatment and Day Intervals FFA = Free Fatty Acid, POV = Per Oxide Value, TBARS = Thiobarbituric Acid Reactive Substances.

Table 5. Effect of *tulsi* leaf extract on microbial population in beef meatballs

Parameters	DI	Treatments				Mean	Level of significance		
		T_0	T_1	T_2	T_3		Treat.	DI	T*DI
TVC (*log* CFU/g)	0	4.65±0.03	4.57±0.04	4.43±0.00	4.37±0.02	4.50^{c}±0.02	0.2396	<0.0001	0.5151
	15	4.87±0.01	4.68±0.01	4.71±0.02	4.72±0.03	4.74^{b}±0.02			
	30	4.86±0.08	4.78±0.01	4.76±0.01	4.72±0.00	4.78^{b}±0.02			
	60	5.06±0.31	4.43±0.01	5.19±0.00	5.14±0.00	5.13^{a}±0.08			
	Mean	4.86^{a}±0.17	4.79^{a}±0.02	4.77^{a}±0.01	4.74^{a}±0.01				
TCC (*log* CFU/g)	0	1.19±0.01	1.17±0.01	1.20±0.01	1.16±0.01	1.18^{a}±0.01	0.0026	<0.0001	0.0795
	15	1.17±0.03	1.12±0.00	1.15±0.00	1.12±0.00	1.14^{b}±0.01			
	30	1.11±0.00	1.19±0.02	1.05±0.01	1.06±0.03	1.08^{c}±0.26			
	60	1.04±0.01	1.06±0.03	0.98±0.03	0.96±0.03	1.01^{d}±0.02			
	Mean	1.13^{a}±0.01	1.11^{ab}±0.01	1.09^{bc}±0.01	1.07^{c}±0.02				
TYMC (*log* CFU/g)	0	1.96±0.02	1.87±0.02	1.86±0.00	1.87±0.00	1.89^{a}±0.01	<0.0001	<0.0001	<0.0001
	15	1.76±0.01	1.56±0.01	1.62±0.00	1.56±0.02	1.63^{b}±0.01			
	30	1.55±0.02	1.38±0.02	1.42±0.02	1.39±0.02	1.43^{c}±0.02			
	60	1.12±0.02	1.08±0.01	1.12±0.00	1.11±0.01	1.11^{d}±0.01			
	Mean	1.69^{a}±0.02	1.47^{c}±0.01	1.50^{b}±0.00	1.48^{c}±0.01				

Mean in each row having different superscript varies significantly at values P < 0.05. T_0 = 0% tul*si* leaves extract, T_1 = 0.1% *tulsi* leaves extract, T_2 = 0.2% tulsi leaves extract, T3 = 0.3% *tulsi* leaves extract, DI = Day Intervals, Treat = Treatment, T*DI = Interaction of Treatment and Day Intervals, TVC = Total Viable Count, TCC = Total Coliform Count, TYMC = Total Yeast-Mold Count.

Conclusion

From the study it may be concluded that 0.3% of *tulsi* leaf extracts as natural antioxidant may be used in beef meatballs preparation. On the basis of sensory evaluation, physicochemical properties, biochemical analysis and microbial assessment indicated that 0.3% *tulsi* leaf extracts showed better results in the preparation of beef meatball compare to control and other two treatments.

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