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*Original Article*

# Effect of tulsi (*ocimum sanctum*) leaves extract on mutton meatball as a source of natural antioxidant stored at refrigerated temperature

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**A B S T R A C T**

This study was conducted to assess the qualities of fresh and preserved mutton meatballs after inoculating tulsi (*Ocimum sanctum*) leaves extract at different levels as a source of natural antioxidant. For this purpose, incorporation of 0, 0.1, 0.2 and 0.3% tulsi leaves extract on mutton meatball were grouped as $T_0$, $T_1$, $T_2$ and $T_3$, respectively. Days of intervals for experiment were 0, 5, and 10 days. Samples were stored at 4˚C to study the quality and shelf life. To evaluate the qualities of the samples sensory, proximate, and physicochemical properties were conducted. Besides, microbial analyses were also performed to ensure its safety for consumers. For different treatments level and at different storage period, significant ($p<0.05$) variation among the qualities of mutton meatballs were obtained. On the basis of biochemical, microbial and nutrient quality, 0.3% tulsi leaves extract was found suitable for formulation of value added mutton meatball at refrigerated preservation.



## Introduction

There are a growing interest for natural antioxidants to preserve meat and related products. Inoculation of different natural components in meat has been practiced to get expected advantages of natural antioxidant over synthetic preservatives for human health as well as for food safety and consumer demand (Mohamed and Mansour, 2012; Nurul *et al*., 2010). Due to the presence of phenolic contents, some plants compounds show antioxidant properties and very little difference was found compared to synthetic antioxidant (Mohamed and Mansour, 2012; Sellami *et al*., 2009; Fecka and Turek, 2008; Durling *et al*., 2007). Lipid per oxidation is a complex process occurring in aerobic cells and reflects the interaction between molecular oxygen and polyunsaturated fatty acids (Verma *et al*., 2009). Rancidity, odd flavor and bad odor develop in different food products for the reason of lipid oxidation which as well deteriorates the quality, food safety and minimizes the storage period. To prevent or delay the auto-oxidation process, antioxidants have been utilized for many years in meat and meat products (Lahucky *et al*., 2010). The synthetic antioxidants like butylated hydroxy anisole, butylated hydroxytoluene etc. currently being used in meat products have been found to exhibit various health affects especially mutagenic and carcinogenic (Shahidi *et al*., 1992).

BHA (butylated hydroxyanisole) is an artificial antioxidant and the antioxidant action of tulsi (*Ocimum sanctum*) leaf is almost similar with BHA. Thus tulsi leaf extract helps to extend the storage period of different food products supposed to oxidation. Plant secondary metabolites like flavonoids,

phenolic compounds were found at high level in the leaf of tulsi (*Ocimum sanctum*). 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; Umadevi, 2001). 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). The leaves of the plant have been shown to possess good antioxidant potentials in experimental animals (Sethi *et al*., 2003).

Salt is considered as low cost hygroscopic compound which contributes in the extension of shelf-life and in antimicrobial activity for different food items by reducing the water activity. So, wide uses of salt as food additives has been found at industrial level due to its low expense. Moreover, the biochemical mechanisms of salt like increment or downfall of enzymatic activity attributed it as flavor producer for different food and food products.

Different experiments were conducted by adding *Zizyphus jujube*, olive leaf and blueberry extract in meat products to observe the overall quality and storage period. Positive response was documented for all these naturally occurring compounds. The antimicrobial property of *Zizyphus jujuba* extracts, antioxidant property of olive leaf extracts and the improvement of sensory quality of meat was found in blueberry extract. The addition of olive leaf extract, blueberry extract and *Zizyphus jujuba* extract reduced TBARS in meatball. The addition of these extracts was as effective as antioxidant and antimicrobial agents for improving the quality of meatball (Veli and Yasemin, 2012).

*Ocimum sanctum*, also known as *Ocimum tenuiflorum*, is revered as "Queen of herbs" 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).

No studies yet been done on the effect of *tulsi* leaf extract on mutton as source of antioxidant for preservation in Bangladesh. Having the above views in mind this experiment was undertaken to study the incorporation of different levels of *tulsi* leaf extract as natural antioxidant in mutton meatballs.

## 2. Materials and methods

### 2.1 Sample Preparation for different treatments

About 1.5 kg of fresh mutton sample was taken for the preparation of mutton meatball. At-first only water was used to clean and then trimming was done to remove the external fat of mutton. After grinding the mutton sample, different native spices, oil, salt, ice, 5% corn flour was mixed with the grinded sample properly as per experimental design. There were four treatment groups. These were treated as 0% ($T_0$), 0.1% ($T_1$), 0.2% ($T_2$), and 0.3% ($T_3$) tulsi leaves extract. Then mutton meatball of proper shape was prepared separately. Then these meatballs was boiled at 100 $^{o}$c for 3-4 minutes and roasted by oil until reddish color observed. Four types of meatballs were prepared properly about 20-25 g size. These samples of different treatments were used for sensory, physicochemical, biochemical and microbiological analyses.

### 2.2 Experimental procedure

To characterize the quality of different treatment groups at different storage period, sensory, physicochemical, biochemical and microbiological analyses were conducted for each treatment group at 0, 5$^{th}$, 10$^{th}$ days interval. Each treatment had three replications. The meat ball preparation and analyses were performed at Department of Animal Science, Bangladesh Agricultural University, Mymensingh.

### 2.3 Sensory evaluation

Six member panel was prepared to evaluate the sensory properties of meatball samples at 0 day and repeated at 5$^{th}$ days, 10$^{th}$ days of storage period. A 5 point semantic scale (weak to strong) was used to mark the sensory qualities of meatball for several characters like flavor, color, tenderness, juiciness, and overall acceptability. The judges evaluated the samples based on the above criterions. Panelists were selected among department staff and students and trained according to the American Meat Science Association guidelines (AMSA, 1995).

At the same atmospheric condition in separate chamber, the sensory properties were evaluated. For this purposes, panelists were attended in the orientation program to make them known with the scale for judging sensory quality (color, flavor, juiciness, tenderness, overall acceptability) of mutton meatball. Sensory scores were 5 for excellent, 4 for very good, 3 for good, 2 for fair and 1 for poor (Rahman, 2012). All samples were served in the Petri dishes.

### 2.4 Physicochemical analysis

#### 2.4.1 Proximate Composition

Only Crude Protein (CP) of the meatballs was measured according to the micro kjeldahl method. Each value was taken for three times and the mean value was reported.

#### 2.4.2 pH measurement

The pH value of cooked meatballs were measured using digital pH meter (CON60; Trans- Wiggens) from homogenate. 5 g blended meatball sample and 10 ml distilled water were used to prepare the homogenate.

### 2.5 Biochemical analysis

Three types of biochemical analysis were done. These are Free Fatty Acid (FFA), Peroxide Value (POV), Thiobarbituric Acid value (TBARS).

Following the method of Rukunudin *et al*. (1998), FFA value of the meatball was measured.

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

Thiobarbituric Acid value (TBARS) was assessed by the method described by Schmedes and Holmer (1989).

### 2.6 Microbial assessment

A quantity of 10 g of mutton meatball sample were aseptically excised from stored stock sample. Each of the stored mutton meatball samples was thoroughly and uniformly macerated in a mechanical blender using a sterile diluent (0.1% peptone water) as per recommendation of International Organization for Standardization (ISO, 1995). For microbial assessment total viable count, total coliform count and total yeast-mould count were measured. Under aseptic condition, 10g minced sample were taken into a sterilized container having 90ml of .1% peptone water. A homogenized suspension was made in a sterile blender. Thus 1:10 dilution of the samples was obtained. Later on using whirly mixture machine different serial dilutions ranging from $10^{-2}$ to $10^{-6}$ were prepared according to the instruction of the standard method (ISO, 1995). The media employed for

these bacteriological analysis included plate count agar (PCA) for total viable count, Macconkey agar (MA) for total coliform count and potato dextrose agar (PDA) for total yeast mold count. The media were prepared according to the guideline of the manufacturer. After inoculation, the PCA and MA media were incubated at 35°C for 24-48 hours and the PDA agar at 25°C for 48-72 hours. The microbiological count was expressed as $\log_{10}$ CFU/g.

### 2.7 Statistical model and analysis

The model was prepared as factorial experiment having two factors A (Treatments) and B (Days of Intervals):

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

Where:

*yijk* = *k* th observation in *i* th level for factor *A* and *j* th level for factor *B*

$\mu$ = the overall mean

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

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

SAS Statistical Discovery software, NC, USA. DMRT test was used for Data analyses.

## 3. Results and discussion

### 3.1 Sensory Evaluation

The color, flavor, juiciness and overall acceptability score of different treatments with days intervals were shown in Table 1. Among four treatments most preferable color was observed from $T_3$ group and unwanted color was found in control group. The most preferable color was observed from 0 day and less preferable color at 10th day. Singh *et al.* (2011), Kandeepan *et al.* (2010), Chidanandaiah & Sanyal (2009) and Kilinc (2009) found that with the advancement of storage period, the color and overall outlook was decreased in scores. The most preferable flavor was observed in $T^3$ group and the lowest flavor from $T_0$ group. With the increasing of storage period the flavor decreased for different treatments. Decline in flavor scores of meat products during storage was reported by Zargar *et al.* (2014) and Thomas *et al.* (2006) in different meat products. The most preferable juiciness score was observed at $T_3$ group. The most preferable tenderness was observed from 0 day and less preferable tenderness from 10th day. Thomas *et al.* (2006) found the reduced scores for juiciness of the products of meat at different storage temperature. Among these four treatments most preferable overall acceptability was observed at $T_3$ group and less preferable overall acceptability was observed at control and $T_0$ group. The different superscripts were observed from 0, 5th and 10th days observation indicates there were significant differences ($p<0.05$) between those days observation. The data show that the quality of meatball deteriorated with the increasing duration. Decrease in overall acceptability scores of meatball might be due to decrease in the values of other sensory attributes. Comparable results were reported by Chacko and Patterson (2011) in the qualities of octopus meatballs and by Agnihotri *et al*. (2006) in goat meatballs.

### 3.2 Change in physiochemical properties

#### 3.2.1 Crude Protein (CP)

The CP content of different treatments with days intervals are shown in Table 2. CP percentage of different treatment groups ranged from 23.32 to 24.08. The values with dissimilar superscripts differ significantly ($p<0.05$) for CP content of different treatments. The different superscript observed at 0, 5th, and 10th days indicates there were significant differences ($p<0.05$) of CP content among these days. Most preferable CP content was observed at 0 days. Less preferable CP content was observed at 10 days. The protein result was higher compared to the protein content of Indonesian Mutton meatballs where the range of CP was 13.38 to 14.44% (Purnomo and Rahardiyan, 2008). Traditional koefte meatballs showed higher protein content (25.51%) reported by Ulu (2004). Koefte meatballs prepared with different levels of fat and flour also showed a higher protein content, ranged from 16.1 to 19.58% (Serdaroglu *et al*., 2005). Pork meatballs were also reported to have higher protein content, ranged from 17.30 to 19.26 % mentioned by Huang *et al*. (2005) and 25.51 to 29.85% by Ulu (2004).

**Table 1. Effect of Tulsi on sensory quality in mutton meatballs.**

| Parameters | DI | Treatments | | | | Mean | Level of significance | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $T_0$ | $T_1$ | $T_2$ | $T_3$ | | Treat. | DI | T × DI |
| Color | 0 | 4.96±0.0088 | 4.90±0.0057 | 4.91±0.0057 | 4.92±0.0033 | $4.91^{a}$±0.01 | <0.05 | <0.05 | <0.05 |
| | 5 | 4.31±0.0088 | 4.87±0.003 | 4.88±0.0057 | 4.90±0.0057 | $4.74^{b}$±0.31 | | | |
| | 10 | 3.31±0.0115 | 4.84±0.0033 | 4.84±0.0057 | 4.87±0.0057 | $4.46^{c}$±0.80 | | | |
| | Mean | $4.19^{c}$±0.83 | $4.87^{b}$±0.03 | $4.87^{b}$±0.02 | $4.89^{a}$±0.02 | | | | |
| Odor | 0 | 4.50±0.0057 | 4.47±0.0057 | 4.42±0.0033 | 4.44±0.0057 | $4.45^{a}$±0.03 | <0.05 | <0.05 | <0.05 |
| | 5 | 4.33±0.0000 | 4.35±0.0057 | 4.38±0.0057 | 4.35±0.0057 | $4.35^{b}$±0.03 | | | |
| | 10 | 3.50±0.0057 | 4.09±0.0057 | 4.06±0.0033 | 4.03±0.0057 | $3.92^{c}$±0.27 | | | |
| | Mean | $4.11^{d}$±0.54 | $4.30^{c}$±0.22 | $4.28^{b}$±0.22 | $4.27^{a}$±0.22 | | | | |
| Juiciness | 0 | 4.57±0.0057 | 4.50±0.0057 | 4.50±0.0033 | 4.52±0.0057 | $4.52^{a}$±0.01 | <0.05 | <0.05 | <0.05 |
| | 5 | 4.51±0.0057 | 4.39±0.0057 | 4.30±0.0057 | 4.41±0.0057 | $4.40^{b}$±0.07 | | | |
| | 10 | 3.71±0.0057 | 4.29±0.0057 | 4.27±0.0057 | 4.30±0.0057 | $4.14^{c}$±0.29 | | | |
| | Mean | $4.26^{d}$±0.48 | $4.39^{b}$±0.12 | $4.35^{c}$±0.14 | $4.41^{a}$±0.12 | | | | |
| Overall Acceptability | 0 | 4.67±0.0057 | 4.45±0.0057 | 4.45±0.0033 | 4.47±0.0057 | $4.51^{a}$±0.09 | <0.05 | <0.05 | <0.05 |
| | 5 | 4.50±0.0057 | 4.30±0.0057 | 4.30±0.0057 | 4.35±0.0057 | $4.36^{b}$±0.07 | | | |
| | 10 | 3.33±0.0057 | 4.25±0.0057 | 4.27±0.0057 | 4.29±0.0057 | $4.03^{c}$±0.49 | | | |
| | Mean | $4.17^{c}$±0.73 | $4.33^{b}$±0.11 | $4.34^{b}$±0.10 | $4.40^{a}$±0.08 | | | | |

Sensory scores were 5 for excellent, 4 for very good, 3 for good, 2 for fair, and 1 for poor. Mean in each row and column having different superscripts vary significantly at values $*P < 0.05$. $T_0$ = 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 extract on bio-chemical (CP%) parameters in mutton meatballs.**

| Parameters | DI | Treatments | | | | Mean | Level of significance | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $T_0$ | $T_1$ | $T_2$ | $T_3$ | | Treat. | DI | T×DI |
| Crude Protein (CP) % | 0 | 24.39±0.06 | 23.48±0.06 | 23.86±0.06 | 24.28±0.06 | $24.02^{a}$±0.06 | <0.05 | <0.05 | <0.05 |
| | 5 | 23.01±0.03 | 22.90±0.06 | 23.34±0.06 | 24.11±0.06 | $23.34^{b}$±0.05 | | | |
| | 10 | 22.57±0.06 | 22.21±0.05 | 22.84±0.06 | 23.85±0.05 | $22.87^{c}$±0.06 | | | |
| | Mean | $23.32^{c}$±0.95 | $22.86^{d}$±0.50 | $23.35^{b}$±0.51 | $24.08^{a}$±0.22 | | | | |

Mean in each row and column having different superscripts vary significantly at values $P < 0.05$. $T_0$ = 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 = Day Intervals, Treat = Treatment, T × DI = Interaction of Treatment and Day Intervals, CP (%) = Crude Protein

### 3.2.2 pH of cooked meat

The cooked pH of different treatments with day's intervals are shown in Table 3. The pH of different treatments for meatballs ranged 6.43 to 6.49. Values with dissimilar superscripts differ significantly (p<0.05). For different treatment groups, pH value increased slightly and acidity value decreased. The range of overall observed cooked pH for different days intervals was 6.33 to 6.55. The different superscript observed at 0, 5$^{th}$ and 10$^{th}$ days of storage period which indicates significant (p<0.05) differences among these observations. The most preferable pH value was found at 0 day and less preferable pH was found at 10$^{th}$ day of storage period. These results are similar to the findings of Sallam *et al.* (2004). They found that pH value changed significantly with the storage period which supposed to increase with time. Biswas *et al.* (2004) also reported similar type of result in an experiment where BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) were incorporated with the pork at different storage period. The report of Verma *et al.* (2013) showed the reduction of pH in the nugget of sheep meat which was previously mixed with guava powder. Banerjee *et al.* (2012) also found the decrease in pH value for the nugget of goat meat in which broccoli powder was incorporated.

## 3.3 Biochemical properties

### 3.3.1 Free Fatty Acid value (FFA %)

Free Fatty Acid value (FFA %) of different treatment levels with day intervals shown in Table 4. The different superscript was observed from the entire treatment group which exhibits the significant variation (p<0.05) among these observation. The most preferable value was observed from $T_3$ due to higher level of tulsi leaf extract. The range of FFA at different days of intervals was 0.34% to 0.38%. The different superscript observed at 0, 5$^{th}$ and 10$^{th}$ days of observation which also indicate the significant variation (p<0.05) among these observations. Increase of FFA value was found with the increasing of storage time. The most preferable FFA was observed from 0 day and less preferable FFA was observed from 10th day of observation. The significant (p<0.05) increase in FFA content of the products during storage might be due to growth of lipolytic microorganisms (Das *et al.*, 2008). Lipid degradation by microbes of enzymatic process produces free fatty acid which was reported by Das *et al.* (2012).

**Table 3. Effect of Tulsi extract on bio-chemical (pH) parameters in mutton meatballs.**

| Parameters | DI | Treatments | | | | Mean | Level of significance | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $T_0$ | $T_1$ | $T_2$ | $T_3$ | | Treat. | DI | T×DI |
| pH | 0 | 6.48±0.01 | 6.46±0.02 | 6.37±0.02 | 6.32±0.02 | 6.55a±0.04 | <0.05 | <0.05 | <0.05 |
| | 5 | 6.44±0.01 | 6.41±0.01 | 6.36±0.01 | 6.30±0.01 | $6.38^{b}$±0.07 | | | |
| | 10 | 6.36±0.01 | 6.35±0.01 | 6.35±0.01 | 6.27±0.01 | $6.33^{c}$±0.05 | | | |
| | Mean | $6.43^{a}$±0.06 | $6.41^{a}$±0.06 | $6.36^{b}$±0.01 | 6.49c±0.03 | | | | |

Mean in each row and column having different superscripts vary significantly at values $P < 0.05$. $T_0$ = 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 = Day Intervals, Treat = Treatment, T × DI = Interaction of Treatment and Day Intervals

### 3.3.2 Peroxide Value (POV-meq/kg)

Table 4 shows the effects of natural antioxidant treatments with controlled group ($T_0$) on POV. For different treatment group, the POV ranged from 4.31 to 3.95. During the storage period, the POV was found higher only in the control group. As shown in Table 4, the higher anti-oxidative effect on POV came from $T_3$ group. The different superscript observed from the treatment groups indicates there were significantly differences (p<0.05) of peroxide value among these treatments. Among these four treatment group, the most preferable POV was observed from $T_3$ group. The low POV in meat and meat products is expected because of its food safety issue for human health. The range of POV at different days of intervals was 3.96 to 4.20. The different superscript was observed at 0, 5$^{th}$ and 10$^{th}$ days of observation indicate there were significant differences (p<0.05) among these observations. During storage, all treatment had increased POV. The report from another experiment showed the higher POV at prolonged storage period with the absence or presence of antioxidant. But generally antioxidant can reduce the POV of food products when compared with control group. In a meat product (Salame), with the extended storage period high POV was reported by Novelli *et al.*, (1998) where at 0, 1 and 3 months of storage period, POV was 1.67, 4.02 and 4.20 meq O2/kg fat respectively. At 0, 30, 60, 90, 120, 150 and 180 days of storage for rosemary treated sheep meat burger, the POV was found 0.24, 0.45, 0.66, 1.05, 1.27, 1.46 and 1.59 meq peroxides/kg fat respectively (Georgantelis *et al.*, 2007).

**Table 4. Effect of Tulsi extract on bio-chemical parameters in mutton meatballs.**

| Parameters | DI | Treatments | | | | Mean | Level of significance | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $T_0$ | $T_1$ | $T_2$ | $T_3$ | | Treat. | DI | T × DI |
| TBARS (mg-MA/kg) | 0 | 0.44±0.03 | 0.38±0.03 | 0.36±0.03 | 0.35±0.03 | $0.38^{c}$±0.03 | <0.05 | <0.05 | <0.05 |
| | 5 | 0.47±0.07 | 0.40±0.06 | 0.38±0.02 | 0.37±0.04 | $0.41^{b}$±0.03 | | | |
| | 10 | 0.58±0.04 | 0.49±0.01 | 0.46±0.05 | 0.44±0.02 | $0.49^{a}$±0.05 | | | |
| | Mean | $0.49^{a}$±0.07 | $0.42^{b}$±0.06 | $0.40^{c}$±0.05 | $0.39^{c}$±0.04 | | | | |
| POV (meq/kg) | 0 | 4.03±0.04 | 3.96±0.02 | 3.95±0.04 | 3.89±0.03 | $3.96^{c}$±0.05 | <0.05 | <0.05 | <0.05 |
| | 5 | 4.32±0.05 | 4.02±0.04 | 3.99±0.09 | 3.95±0.08 | $4.07^{b}$±0.16 | | | |
| | 10 | 4.57±0.02 | 4.15±0.01 | 4.07±0.03 | 4.00±0.03 | $4.20^{a}$±0.25 | | | |
| | Mean | $4.31^{a}$±0.27 | $4.04^{b}$±0.09 | $4.00^{c}$±0.06 | $3.95^{d}$±0.05 | | | | |
| FFA (%) | 0 | 0.38±0.01 | 0.34±0.01 | 0.32±0.01 | 0.31±0.01 | $0.34^{c}$±0.02 | <0.05 | <0.05 | <0.05 |
| | 5 | 0.41±0.02 | 0.36±0.01 | 0.35±0.01 | 0.34±0.01 | $0.37^{b}$±0.02 | | | |
| | 10 | 0.43±0.05 | 0.37±0.03 | 0.37±0.02 | 0.36±0.03 | $0.38^{a}$±0.02 | | | |
| | Mean | $0.41^{a}$±0.03 | $0.36^{b}$±0.02 | $0.35^{c}$±0.03 | $0.34^{c}$±0.02 | | | | |

Mean in each row and column having different superscript varies significantly at values $P < 0.05$. $T_0$ = 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 = 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.

### 3.3.3 Thiobarbituric Acid Value (TBARS)

The TBARS values of treatments has been presented in table 4. The TBARS for different treatment group ranged from 0.49 to 0.39. The unlike superscript with the value of TBARS in treatment showed the significant variation among the treatment group. Most preferable TBARS was found in $T_3$ group. For human health safety, meat products are always desired to have less TBARS content. At the different days of storage period, TBARS ranged from 0.38 to 0.49. The different superscript observed from 0, 5$^{th}$ and 10$^{th}$ days of observation indicates there were significant differences ($p<0.05$) among these fourth days observation. The TBARS values increased significantly ($p<0.05$) during storage in all treatments. Similar findings were reported by Chidanandaiah & Sanyal (2009) in meat patties and Modi *et al*. (2003) in buffalo meat burger during refrigerated storage. Similar findings were reported by Nassu *et al*. (2003) in goat meat sausage during refrigerated storage.

## 3.4 Microbiological assessment

### 3.4.1 Total viable count (TVC)

The TVC value of different treatment levels with different days of intervals shown in Table 5. For different treatment, the TVC of mutton meatballs ranged from 4.41 to 5.02 (log CFU/g). The same superscript was observed from all treatments indicate there were no significant differences ($p>0.05$) of TVC values among these four treatment groups. The plate count in the $T_0$ group (5.02 log CFU/g) was significantly higher than the treated samples ($T_3$). Considering food safety issue, less TVC value is always expected. At different days of storage period the TVC ranged from 4.53 to 4.67 log CFU/g. The different superscript was observed from 0, 5$^{th}$, and 10$^{th}$ days intervals indicate there were significant differences ($p<0.05$) of TVC values. The amount of TVC was increased with the storage periods. The multiplication of aerobic plate microbes can be retarded significantly by using Diallyl sulfide and diallyl disulfide for long term storage period. Fernández- López *et al*. (2005) reported that antimicrobial compounds help to retard the microbial growth of food by physical interaction.

### 3.4.2 Total coliform count (TCC)

The TCC value of different treatment levels with different days of intervals shown in Table 5. The total coliform count of the mutton meatballs for different treatments ranged from 1.07 to 1.28 (log CFU/g). The TCC value with dissimilar superscripts for treatment groups differ significantly ($p<0.05$). The TCC was found significantly higher in control group (1.28 log CFU/g) than other group. Most preferable TCC content was observed at $T_3$ group. Generally less TCC is expected in food products for human health safety. TCC value for different storage period ranged from 1.13 to 1.10. During storage, TCC value was decreased. The different superscript observed from 0, 5$^{th}$ and 10$^{th}$ days of observation indicate that there were significant differences ($p<0.05$) among these fourth days of observation. Antioxidant mainly acts on fat where it slows the auto-oxidation rate and as well hinder the microbial metabolism on fat. Therefore, reduced TCC was found in antioxidant treated group. In a study, Camo *et al*. (2008) used rosemary active film, rosemary extract before packaging, oregano active film for sheep meat storage under illumination for 0, 5, 8, 11 and 13 days. They reported that coliform counts in all samples gradually decreased with storage time.

**Table 5. Effect of Tulsi extract on microbial quality in mutton meatballs.**

| Parameters | DI | Treatments | | | | Mean | Level of significance | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $T_0$ | $T_1$ | $T_2$ | $T_3$ | | Treat. | DI | T× DI |
| TCC (log CFU/g) | 0 | 1.22±0.01 | 1.15±0.02 | 1.13±0.04 | 1.12±0.03 | $1.13^{a}$±0.03 | <0.05 | <0.05 | <0.05 |
| | 5 | 1.29±0.02 | 1.13±0.05 | 1.08±0.04 | 1.07±0.01 | $1.12^{b}$±0.09 | | | |
| | 10 | 1.32±0.01 | 1.09±0.03 | 1.06±0.03 | 1.03±0.01 | $1.10^{c}$±0.11 | | | |
| | Mean | $1.28^{a}$±0.05 | $1.12^{b}$±0.03 | $1.09^{c}$±0.03 | $1.07^{d}$±0.04 | | | | |
| TYMC (log CFU/g) | 0 | 1.98±0.02 | 1.83±0.02 | 1.78±0.07 | 1.78±0.04 | $1.84^{a}$±0.07 | <0.05 | <0.05 | <0.05 |
| | 5 | 2.03±0.01 | 1.74±0.05 | 1.75±0.03 | 1.74±0.05 | $1.83^{b}$±0.11 | | | |
| | 10 | 2.14±0.03 | 1.75±0.03 | 1.73±0.01 | 1.72±0.01 | $1.81^{c}$±0.18 | | | |
| | Mean | $2.05^{a}$±0.08 | $1.77^{b}$±0.04 | $1.75^{c}$±0.03 | $1.74^{c}$±0.03 | | | | |
| TVC (log CFU/g) | 0 | 4.65±0.04 | 4.52±0.01 | 4.49±0.01 | 4.46±0.09 | $4.53^{a}$±0.06 | <0.05 | <0.05 | <0.05 |
| | 5 | 4.95±0.01 | 4.46±0.05 | 4.44±0.04 | 4.42±0.05 | $4.56^{b}$±0.23 | | | |
| | 10 | 5.45±0.01 | 4.45±0.02 | 4.41±0.04 | 4.37±0.07 | $4.67^{c}$±0.49 | | | |
| | Mean | $5.02^{a}$±0.40 | $4.47^{b}$±0.04 | $4.44^{c}$±0.05 | $4.41^{d}$±0.05 | | | | |

Mean in each row and column having different superscripts vary significantly at values $P < 0.05$. $T_0$ = 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 = Day Intervals, Treat = Treatment, T x DI = Interaction of Treatment and Day Intervals, TVC = Total Viable Count, TCC = Total Coliform Count, TYMC = Total Yeast-Mold Count.

### 3.4.3 Total yeast-mold count (TYMC)

For different treatment groups as well as for different storage period, the TYMC value has been shown in table 5. The total yeast-mold count ranged from 1.74 to 2.05 (log CFU/g) for different treatment levels. The unlike superscripts of TYMC for different treatment groups differ significantly ($p<0.05$). In control group, the higher level of TYMC (2.05 log CFU/g) was found than other treatment groups. In food products, less TYMC is considered good for human health. The range of overall observed of different days of intervals of TYMC value was 1.81 to 1.84. During storage TYMC value was decreased. The different superscript at 0, $5^{th}$ and $10^{th}$ days of observation indicated that there were significant differences ($p<0.05$) among these four days of observation. A study was conducted by Fernández-López *et al.*, (2005) on antimicrobial components in mutton meatball. They reported the absence of yeast and mold in all cooked meatball samples. In thin layer chromatograph, the removal of pathogenic mold- *Cladosporium cucumerinum* and pathogenic yeast- *Candida albicans* was found by dichloromethane root extract of *Cosmos caudatus*. In some studies, it had been reported that the essential oil from plant sources had antimicrobial activity on meat and meat products against spoilage and pathogenic microorganisms (Busatta *et al*., 2008; Carraminana *et al*., 2008; Gutierrez *et al*., 2009).

## 4. Conclusion

The results showed that the qualities of mutton meatball could be preserved for extended days by the use of tulsi leaf extract as a source of natural antioxidant. The freshness, sensory, physicochemical, biochemical and microbial properties of these meat balls are highly linked with the anti-oxidant properties of tulsi leaf.

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