International Journal of Applied Research
Journal HP: www.intjar.com, ISSN: 2411-6610

Formulation of value added beef meatballs using bottle gourd (*Lagenaria siceraria*) leaf extracts and wheat flour

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ARTICLE INFO

Article history

Accepted 9 Nov 2016
Online release 15 Nov 2016

Keyword

Value added
Meatballs
Bottle gourd leaf extract
Wheat flour

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ABSTRACT

The study was carried out to determine appropriate level of wheat flour with bottle gourd leaves extract which can be used in meatball production. Four treatment groups treated as control group (T_1), (T_2- 5% bottle gourd leaves extract + 5% wheat flour), T_3 (5% bottle gourd leaves extract+ 8% wheat flour) and T_4 (5% bottle gourd leaves extract +10% wheat flour) respectively. Meatballs were refrigerated under an aerobic condition. Different analysis like proximate, sensory, physicochemical, biochemical and microbiological analyses were used for quality assessment. DM matter content was increased with storage period. Color, cooking loss, flavor, tenderness and overall acceptability were differed significantly at different treatment levels ($p<0.05$). Crude protein, ether extract, raw and cooked pH were decreased significantly ($p<0.05$) with storage period. TBRAS value, total viable count, total coli form count and yeast-mould count of meatballs were differed significantly ($p< 0.05$) at different levels. T_2 was more acceptable than other treatment groups contain 5% bottle gourd leaves extract and 5% wheat flour. It may be concluded that wheat flour as prebiotics and bottle gourd leaves extract as antioxidant and antimicrobial can be used in manufacture of meatball successfully.

Introduction

Beef is the largest source of meat in Bangladesh. Meatball is processed comminuted meat and is very popular among some countries within the Asian region. Meatball is ground or minced meat rolled into a small ball, sometimes along with other ingredients, such as bread/biscuit crumbs, minced onion, eggs, butter and seasonings. Many non-meat ingredients are also added to meatball to increase their nutritional and functional value. Oxidation of lipids can have a marked negative effect on nutritional value, and could be responsible for production of toxic compounds (McCarthy et al, 2001). Lipid oxidation and bacterial contamination are the main factors that determine food quality loss and shelf-life reduction. To prevent the 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/or directly by scavenging the free radical species (Verma *et al*. 2009).

Bottle gourd leaf extracts have many benefits for human body which aids in digestion, weight loss, reduce stress, reducing high blood pressure and keeping heart health, reducing burning sensation from high acidic urination. People have become more conscious about reduced fat in diet, balanced protein sources and more importantly the inclusion of recommended levels of dietary fiber sources in daily diet.

In view of fact that natural spices are widely used in a variety of food products, it is important to know the effects they have on keeping qualities of products. It has also been reported that 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 bottle gourd leaf extracts. The plant bottle gourd is a common vegetable used throughout Bangladesh. Since time immemorial the fruit has been used as immune suppressant (Sankari *et al.* 2010). Therefore, there has been a growing interest in natural ingredients to be used in food and food products as preservatives instead of synthetic chemicals. Consequently, search for natural additives, especially of plant origin, has notably increased in recent years (Naveena *et al* 2008). No study yet been conducted on the extract of bottle gourd leaf in meat products. Bottle gourd leaves extract along with wheat flour can be very beneficial for producing products. They are also available throughout the country. If we can utilize them properly, they can be a great source of nutrition in this study, the effect of different level of wheat flour like 5%, 8% and 10 % with 5% bottle gourd leaves extract on beef meatballs are discussed properly.

The study is to investigate suitable amount of bottle gourd leaf extract and wheat flour for beef meatball formulation can be act as prebiotics and natural antioxidant. Hence, the study was to examine the addition of bottle gourd leaf extracts on sensory, proximate, physicochemical, biochemical, microbiological analysis, to find out exact level of bottle gourd leaves and wheat flour and recommend value added beef meatball enriched with bottle gourd leaf extracts and wheat flour.

Materials and methods

Materials collection

Boneless beef 2.5 kg from freshly slaughtered cattle was collected from Local market (Seshmoor). The meat sample was immediately transferred to the "Animal Science Laboratory" in Bangladesh Agricultural University Mymensingh.

Sample preparation

About 2.5 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 of with sharp knife. Then beef was grinded properly and spices, salt, ice flakes, refined vegetable oil, refined bottle gourd leaf extracts, wheat flour, sauce was mixed with grinded beef properly as per experimental design. There were four treatment groups treated as control group (T_1), 5% bottle gourd leaf extract +5% wheat flour (T_2), 5% bottle gourd leaf extract +8% wheat flour (T_3), and 5% bottle gourd leaf extract +10% wheat flour (T_4). Then beef meatball of proper shape was prepared separately. It was then boiled in hot water for 2-3 minutes. Then water was removed from meatball properly and was fried in hot oil until reddish brown color was obtained.

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 (weak to strong) for the following attributes color, smell, tenderness, juiciness, and overall acceptability. Eight training sessions were held to familiarize the judges with 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 (color, smell/flavor, juiciness, tenderness, 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 45 day.

Proximate composition

Proximate composition like Dry Matter (DM), Ether Extract (EE), Crude Protein (CP) and Ash were carried out according to the methods (AOAC, 2016). All determination was done in triplicate and mean value was reported.

Physicochemical properties measurement

Raw P^H measurement

P^H value of raw meatball was measured using pH meter from raw meatball homogenate. The homogenate was prepared by blending 5 g of meat with 10 ml distilled water.

Cooked P^H measurement

P^H value of cooked meatballs was measured using pH meter from cooked meatball homogenate. The homogenate was prepared by blending 5 g of meat with 10 ml distilled water.

Biochemical analysis

There were three types of biochemical analysis. These are Free Fatty Acid (FFA), Peroxide Value (POV), Thiobarbituric Acid value (TBARS). Free fatty acid value was determined according to Rukunudin *et al*. (1998).Peroxide value (POV) was determined according to Sallam *et al*. (2004). Lipid oxidation was assessed in triplicate using the2-thiobarbituric acid (TBA) method described by Schmedesand Holmer (1989). P^H value of raw and cooked meatball was measured using pH meter from raw meatball homogenate. The homogenate was prepared by blending 5 g of meat with 10 ml distilled water.

Microbial assessment

For microbial assessment total viable count, total coli form count and total yeast-mould count was undertaken. To determine these parameters the procedures which were followed are described below:

Quantity of 10 g beef meatball sample was aseptically excised from stored stock sample. Each of the stored beef meatball samples was thoroughly and uniformly macerated in a mechanical blender using a sterile diluents (0.1% peptone water) as per recommendation of International Organization for Standardization (ISO, 1995).A quantity of ten (10) gram of the minced meat 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 statistically 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

Value added meatball was formulated through fortification with different level of bottle gourd leaf extracts as antioxidants and antimicrobial agents. From the study it was found that the sensory quality after fortification with bottle gourd leaf extracts was deteriorated with increased storage period (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 such as color, texture, odor, and flavor as well as the nutritional quality of product. Some authors also

reported that inclusion of fibers in various meat products increased hardness (Fernandez-Gines et al., 2004).That's why meatballs leak juices when they were stored. If meatballs refrozen accelerating further moisture loss, and when this meatballs eventually cook, any one may find it dense and dry in texture. The range of overall observed color score at different treatment was 3.50 to 4.41. The range of flavor score among four treatments was 3.66 to 4.5. The range of overall observed tenderness score at different treatments was 3.33 to 4.16. The range of overall observed juiciness score at different treatments was 3.33 to 4.16. The range of overall observed of overall acceptability score at different treatments was 3.5 to 4.16. The range of different day's intervals of overall observation of overall acceptability score was 4.58 to 2.83. Most preferable color was observed from T_2 among four treatments group and less preferable color was observed from control group. T_2 and T_3 and the lowest flavor from controlled group. Among these four treatments most preferable tenderness was observed from T_2 bottle gourd leaf extract group. Among four treatments most preferable juiciness score was observed from T_2 bottle gourd leaf extract group.

Table 1: Effects of bottle gourd leaf extract on sensory parameters in beef meatballs.

Parameters	DI	Treatments				Mean	Level of significance		
		T_1	T_2	T_3	T_4		Treat.	DI	T*DI
Color	0	4.00±0	5±0	4.33±0.57	4.33±0.33	4.41±0.28^{a}	.0016	<.0001	.8723
	15	4.33±0.57	4.66±0.57	4.66±0.57	4.33±0.33	4.5±0.57^{a}			
	30	3.33±0.57	4.33±0.57	3.66±0.57	3.66±0.58	3.75±0.57^{b}			
	60	2.33±0.57	3.66±0.57	3.33±0.57	3±0	3.08±0.42^{c}			
	Mean	3.5^{c}±0.42	4.41^{a}±.42	4.00^{ab}±0.57	3.83^{bc}±0.42				
Flavor	0	4.33±0.57	5±0	4.33±0.57	4.00±0	4.41±0.28^{a}	0.0017	<.0001	.7178
	15	4.33±0.57	4.66±0.57	4.33±0.57	4.33±0.57	4.41±0.57^{a}			
	30	3.66±0.57	4.66±0.57	4.33±0.57	4±0	4.16±0.42^{a}			
	60	2.33±0.57	3.66±0.57	3.33±0.57	2.66±0.57	3.00±0.57^{b}			
	Mean	3.66^{b}±0.57	4.5^{b}±0.42	4.08^{ab}±0.57	3.75±0.28				
Tenderness	0	4.33±0.57	5±0	4.66±0.57	4±0.00	4.5±0.28^{a}	0.0036	<.0001	0.9986
	15	4±0	4.33±0.57	4.33±0.57	3.66±0.57	4.08±0.42^{a}			
	30	3.33±0.57	4.00±0.00	3.66±0.57	3.33±0.57	3.41±0.42^{a}			
	60	2.66±0.57	3.33±0.57	3.00±1	2.33±0.57	2.58±0.67^{c}			
	Mean	3.58 ^{bc}±.42	4.16^{a}±0.28	3.91^{ab}±0.67	3.33^{c}±0.42				
Juiciness	0	4.33±0.57	5±0	4.66±0.57	4.00±0.00	4.5±0.28^{a}	.0009	<.0001	.8224
	15	3.66±0.57	4.66±0.57	4.33±0.57	4.00±0.00	4.08±0.42^{a}			
	30	3.33±0.57	3.66±0.57	3.33±0.57	3.66±0.57	3.41±0.57^{b}			
	60	2.00±0	3.33±0.57	2.66±0.57	2.33±0.57	2.58±0.42^{c}			
	Mean	3.33^{c}±0.42	4.16^{a}±0.42	3.75^{ab}±0.57	3.33^{b}±0.28				
Overall acceptability	0	4.33±0.57	4.66±0.57	4.66±0.57	5.00±0	4.41±0.42^{a}	.0146	<.0001	.7859
	15	4.33±0.57	4.66±0.57	4.66±0.57	4.66±0.57	4.58±0.57^{a}			
	30	3.66±0.57	4.00±0	3.66±0.57	3±0	4.58±0.28^{b}			
	60	2.66±0.57	3.33±0.57	3.00±0.00	2.33±0.57	2.83±0.42^{c}			
	Mean	3.75^{ab}±0.57	4.16^{a}±0.42	4.00^{a}±0.42	3.5^{b}±0.28				

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= Control group, T_2= 5% bottle gourd leaves extract + 5% Wheat flour, T3= 5% bottle gourd leaves extract + 8% Wheat flour, T4= 5% bottle gourd leaves extract + 10% Wheat flour, DI=Day Intervals, Treat= Treatment, T*DI=Interaction of Treatment and Day Intervals.

Proximate analysis

The DM content was increased with the increased storage period because moisture loss was decreased with storage period. The range of overall observed DM content at different treatments was 51.53 to 41.16%. The range of overall observation of different days of interval DM content was 48.12% to 43.64%. Among four treatments most preferable DM content was observed from T_2 (5%) bottle gourd leaf extract). DM increased for the moisture loss of beef meatball samples with advance storage time during freezing. The different days intervals indicates there were significant differences ($p<0.05$) among four days observation. The most preferable DM content was observed from 0 day and less preferable DM content from 45th day (Table 2). The same trend was also observed by (Konieczny *et al.*, 2007) and they reported that dry matter content increased during frozen storage The data show that the highest amount DM content was increased to 48.09% in all treatments after 45days of storage. Similar results were reported for Indonesian traditional meatballs with a moisture content ranged from 69.52 to 71.17% (Purnomo and Rahardiyan 2008). Devatkal et al., (2010) also reported that incorporation pomegranate rind and seed powder extracts did not affect the DM content of goat meat patties. The range of overall observed moisture at different treatments was 43.61 to 49.18%. CP content was decreased with increased storage period. The range of overall observed CP content at

different treatments was 21.86 to 21.27%. Among these three treatments most preferable CP content was observed from T_2. The range of overall observed of different days intervals of CP content was 22.57 to 20.75%. The range of overall observed EE content at different treatments was 12.22 to 11.50%. The range of overall observed of different days of intervals of EE content was 12.27 to 11.35%. Bottle gourd leaf extracts 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 the fat content ranges from 1.69 to 11.09. Verma et al. (2012) reported significant decrease in low fat chicken nuggets incorporated with chickpea hull flour. The range of overall observed ash content at different treatments was 2.46 to 1.19%. The range of overall observed of different day's intervals of ash content was 1.55 to 1.78%. 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 2: Effects of bottle gourd leaf extract on proximate components in beef meatballs.

Parameters	DI	Treatments				Mean	Level of significance		
		T_1	T_2	T_3	T_4		Treat.	DI	T*DI
DM (%)	0	48.68±0.03	39.74±0.05	41.24±0.01	44.89±0.02	43.64±0.02^{d}	<.0001	<.0001	<.0001
	15	50.71±0.04	40.85±0.01	42.12±0.01	46.23±0.02	44.97±0.02^{c}			
	30	52.40±0.02	41.62±0.01	43.22±0.01	48.46±0.01	46.42±0.01^{b}			
	45	54.32±0.01	42.42±0.01	45.64±0.01	50.11±0.01	48.12±0.01^{a}			
	Mean	51.53^{a}±0.02	41.16^{d}±0.02	43.05^{c}±0.01	47.42^{b}±0.02				
CP (%)	0	24.47±0.3	22.68±0.01	21.85±0.01	21.24±0.01	22.57±0.01^{a}	<.0001	<.0001	<.0001
	15	22.15±0.45	22.73±0.02	21.64±0.02	21.62±0.03	22.02±0.13^{b}			
	30	20.54±0.03	21.61±0.01	21.44±0.02	21.10±0.02	21.17±0.02^{c}			
	45	21.21±0.01	20.44±0.005	21.21±0.01	21.14±0.02	20.75±0.01^{d}			
	Mean	21.84^{b}±0.13	21.86^{a}±0.02	21.54^{c}±0.01	21.27^{d}±0.02				
EE (%)	0	12.12±0.18	11.79±0.11	12.57±0.42	12.42±0.05	12.27±0.19^{a}	<.0001	<.0001	<.0059
	15	12.20±0.18	11.50±0.06	12.44±0.28	12.62±0.19	12.14±0.17^{a}			
	30	11.64±0.46	11.56±0.27	11.77±0.20	12.40±0.16	11.84±0.27^{b}			
	45	11.54±.03	11.16±0.06	11.27±0.07	11.43±0.05	11.34±0.05^{c}			
	Mean	11.87^{b}±0.21	11.50^{c}±0.2	12.01^{b}±0.2	12.22^{ab}±0.11				
Ash (%)	0	1.12±0.03	1.11±0.01	1.34±0.01	2.63±0.03	1.55±0.02^{c}	<.0001	<.0001	<.0001
	15	1.34±0.03	1.13±0.02	1.41±0.01	2.51±0.02	1.6±0.02^{b}			
	30	1.91±0.03	1.21±0.02	1.27±0.03	2.07±0.04	1.61±0.03^{b}			
	45	1.94±0.03	1.32±0.02	1.44±0.03	2.44±0.02	1.78±0.02^{a}			
	Mean	1.58^{b}±0.03	1.19^{d}±0.01	1.36^{c}±0.02	2.46^{a}±0.02				

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= Control group, T_2= 5% bottle gourd leaves extract + 5% Wheat flour, T3= 5% bottle gourd leaves extract + 8% Wheat flour, T4= 5% bottle gourd leaves extract + 10% Wheat flour, DI=Day Intervals, Treat= Treatment, T*DI=Interaction of Treatment and Day Intervals.

Physicochemical properties

The range of overall observed raw pH at different treatments was 5.67 to 5.80 %. Among four treatments most preferable raw PH was observed from T_2 group. The highest amount of raw pH indicates this product is most preferable for consumers' health than other treatment groups. The decrease in the raw PH values was lower in the untreated samples than treated ones due to the effect of natural antioxidants which retarded the formation of free fatty acids. It is also obvious that the values of raw PH for the product were higher than that of the raw pH values of meat. The raw PH was decreased with increased storage period (Table 3). Bacteria and mold have a tendency to increase with increasing storage time. Choi et al. (2009) reported that meat batter containing dietary fiber from rice bran have higher pH values. . The data showed a slight decrease in the raw pH values and an increase in the acidity values for all samples along with storage time during the 45 days of storage as a result of the increase of free fatty acids due to rancidity. The data showed a slight increase in cooked pH values and a decrease in the acidity values for all samples along with addition of synthetic antioxidant and natural antioxidants as a result of decrease of free fatty acids due to lower rancidity. The range of overall observed of different days of intervals of raw pH was treatment groups. The range of overall observed of different days of intervals of raw pH was 5.71 to 5.78%. The range of overall observed cooked pH at different treatments was 5.99 to 6.07. The highest amount of cooked pH indicates this product is most preferable for consumers' health than other treatment groups. The lowest amount of cooking loss indicates this product is most preferable for consumers' choices than other treatment groups. The range of overall observed cooking loss at different treatments was 24.96 to 23.35%. The range of overall observed of different days of intervals of cooking loss was 5.87 to 6.17. The cooking loss was decreased with the increased storage period. The less preferable cooking loss was observed from 0 day and most preferable cooking loss was observed from 45th day observation. Cooking loss refers to the reduction in weight of meatballs during the cooking

process (Jama et al. 2008). Cooking loss in meat cuts is important for maintaining an attractive retail display of meat. 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 is an important data that are used by the 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). The cooking yield of the Kung-Wan significantly decreased with higher natural antioxidant extract levels (Hsu and Sun, 2006). The cooked pH was decreased with the increased storage period.

Table 3: Effects of bottle gourd leaf extract on physicochemical parameters in beef meatballs.

Parameters	DI	Treatments				Mean	Level of significance		
		T_1	T_2	T_3	T_4		Treat.	DI	T*DI
Raw pH	0	5.67±0.01	5.79±0.01	5.72±0.02	5.69±0.01	5.72±0.01^{b}	<.0001	<.0001	<.0001
	15	5.71±0.03	5.80±0.02	5.80±0.01	5.82±0.03	5.78±0.02^{a}			
	30	5.68±0.02	5.78±0.03	5.75±0.04	5.72±0.02	5.73±0.02^{b}			
	45	5.64±0.04	5.81±0.04	5.68±0.03	5.70±0.02	5.71±0.03^{b}			
	Mean	5.68^{c}±0.02	5.80^{a}±0.02	5.74^{b}±0.02	5.73^{b}±0.02				
Cooked pH	0	6.17±0.01	6.16±0.01	6.12±0.01	6.25±0.03	6.17±0.01^{a}	<.0001	<.0001	<.0001
	15	6.08±0.05	6.10±0.01	6.14±0.02	6.14±0.02	6.11±0.02^{b}			
	30	5.92±0.01	5.96±0.01	6.10±0.01	6.01±0.03	5.99±0.01^{c}			
	45	5.80±0.01	5.85±0.01	5.94±0.02	5.89±0.01	5.87±0.01^{d}			
	Mean	5.99^{c}±0.02	6.01^{b}±0.01	6.07^{a}±0.01	6.07^{a}±0.02				
Cooking loss (%)	0	27.17±0.32	25.16±2.21	25.14±0.88	25.54±1.28	25.75±1.17^{a}	0.1024	<.0001	<.0001
	15	26.08±0.81	24.31±1.65	24.69±1.42	24.67±0.50	24.94±1.05^{a}			
	30	23.40±0.46	21.97±0.82	23.20±1.37	23.92±0.56	23.12±1.05^{c}			
	45	23.12±1.26	21.95±0.68	21.99±0.33	22.39±1.36	22.36±0.90^{b}			
	Mean	24.96^{a}±0.71	23.35^{b}±1.34	23.75^{b}±1	24.13^{ab}±0.92				

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= Control group, T_2= 5% bottle gourd leaves extract + 5% Wheat flour, T_3= 5% bottle gourd leaves extract + 8% Wheat flour, T_4= 5% bottle gourd leaves extract + 10% Wheat flour, DI=Day Intervals, Treat= Treatment, T*DI=Interaction of Treatment and Day Intervals.

Biochemical analysis

Lipid oxidation promotes production of rancid flavors and odors while also reducing the shelf-life, nutritional quality, and safety of food products. 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). During storage, the peroxide value increased in all treatments. The range of overall observed peroxide value at different treatment levels was 4.08 to 4.26. The range of overall observed of different days of intervals of peroxide value was 4.05 to 4.35. Throughout the storage time, peroxide values were generally higher in control samples than in others. The range of overall observed of different days of intervals of FFA was 0.29 to 0.36. The range of overall observed FFA value at different treatments was 0.28 to 0.37 (Table 4). Meat with higher lipid oxidation values also showed higher protein oxidation greater metmyoglobin formation. Lund et *al*. (2007) reported on the 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 these three treatments showed lower values than the control. 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. Natural antioxidants, in particular polyphenols, 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 psysicochemical's Reactive Oxygen Species (ROS) (Du *et al*. 2010). Rhee and Myers (2003) examined peroxide values in plain meat loaf made from ground goat meat and reported a similar trend in peroxide value during storage. Generally, TBA levels significantly ($p < 0.05$) increased with storage time, showing decreasing shelf life. The range of overall observed of different days of intervals of TBA value was 0.40 to 0.62. Among these four treatments, most preferable TBA value was observed from T_4. The lowest amount of TBA value indicates this product is most preferable for consumer's health. For all treatments, TBARS values increased at the beginning to end of storage time.

The results of this study confirm that BHA, 3%, 2%, 1% bottle gourd leaf extract can delay lipid oxidation significantly, reducing the potential risk induced by lipid oxidation. The results of this study confirm that 1%, 2%, 3% bottle gourd leaf extracts can delay lipid oxidation significantly, reducing the potential risk induced by lipid oxidation. Higher values were observed in the control treatment, while values of 0.025 and 0.05% were observed in the sample treated with rosemary extract. The control sample, without any added antioxidants, showed a higher level of TBA than samples treated with 3%, 2%, 1% bottle gourd leaf extract or BHA. The TBA level of

samples treated with 3%, 2%, 1% bottle gourd leaf extract was also lower than those treated with BHA; this difference was especially significant ($p < 0.05$) after 45 days of storage time. The TBA value increased until storage day 45. The TBA value on day 45 was 0.63% for the control samples. The highest amount of peroxide value indicates this product is less preferable.

Table 4: Effect of Bottle gourd leaves extract and wheat flour on bio-chemical parameters in beef meatballs.

Parameters	DI	Treatments				Mean	Level of significance		
		T_1	T_2	T_3	T_4		Treat.	DI	T*DI
FFA (%)	0	0.28±0.01	0.24±0.01	0.29±0.008	0.34±0.02	0.29±0.01^{d}	.0001	.0001	.5904
	15	0.30±0.004	0.27±0.01	0.31±0.006	0.35±0.01	0.31±0.007^{c}			
	30	0.33±0.01	0.29±0.002	0.34±0.01	0.39±0.01	0.33±0.008^{b}			
	45	0.33±0.01	0.32±0.02	0.37±0.06	0.41±0.007	0.36±0.02^{a}			
	Mean	0.31^{c}±0.01	0.28^{d}±0.01	0.33^{b}±0.02	0.37^{a}±0.04				
PV(meq/kg)	0	4.12±0.03	4.06±0.04	3.97±0.05	4.04±0.08	4.05±0.05^{d}	.0001	.0001	.595
	15	4.16±0.015	4.08±0.03	4.05±0.06	4.12±0.04	4.10±0.03^{c}			
	30	4.30±0.01	4.26±0.02	4.11±0.05	4.25±0.03	4.23±0.02^{b}			
	45	4.47±0.03	4.36±0.02	4.20±0.01	4.36±0.04	4.35±0.02^{a}			
	Mean	4.26^{a}±0.02	4.19^{b}±0.02	4.08^{c}±0.04	4.19^{b}±0.04				
TBARS (mg-MA/kg)	0	0.41±0.003	0.04±0.003	0.39±0.001	0.38±0.007	0.40±0.003^{d}	.0001	.0001	.0001
	15	0.48±0.001	0.43±0.004	0.41±0.004	0.40±0.003	0.43±0.003^{c}			
	30	0.55±0.003	0.50±0.006	0.49±0.004	0.49±0.004	0.51±0.004^{b}			
	45	0.75±0.004	0.58±0.007	0.58±0.004	0.58±0.02	0.62±0.008^{a}			
	Mean	0.55^{a}±0.002	0.48^{b}±0.005	0.47^{c}±0.003	0.46^{d}±0.008				

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= Control group, T_2= 5% bottle gourd leaves extract + 5% Wheat flour, T_3= 5% bottle gourd leaves extract + 8% Wheat flour, T4= 5% bottle gourd leaves extract + 10% Wheat flour, DI=Day Intervals, Treat= Treatment, T*DI=Interaction of Treatment and Day Intervals.

Table5: Effect of Bottle gourd leaves extract and wheat flour on different microbe's population in beef meatballs.

Parameters	DI	Treatments				Mean	Level of significance		
		T_1	T_2	T_3	T_4		Treat.	DI	T*DI
TVC (*log*CFU/g)	0	4.66±0.03	4.55±0.02	4.43±0.01	4.37±0.01	4.5±0.01^{c}	<.0800	<.0001	.3754
	15	4.87±0.01	4.66±0.04	4.70±0.03	4.72±0.04	4.74±0.03^{b}			
	30	4.94±0.04	4.78±0.01	4.76±0.02	4.72±0.01	4.8±0.002^{b}			
	45	5.06±0.54	5. 13 ±0.03	5.19±0.006	5.14±0.009	5.3±0.14^{a}			
	Mean	4.88^{a}±0.15	4.78^{b}±0.02	4.77^{ab}±0.01	4.74^{b}±0.01				
TCC (*log*CFU/g)	0	1.19±0.02	1.16±0.01	1.20±0.01	1.17±0.02	1.18±0.01^{a}	.0002	<.0001	.0338
	15	1.19±0.06	1.11±0.02	1.15±0.005	1.11±0.002	1.14±0.01^{b}			
	30	1.11±0.008	1.05±0.04	1.04±0.01^{b}	1.08±0.04	1.07±0.02^{c}			
	45	1.04±0.01	0.966±0.01	0.98±0.002	1.05±0.04	1.01±0.01^{d}			
	Mean	1.13^{a}±0.02	1.07^{c}±0.01	1.09^{bc}±0.002	1.1^{b}±0.02				
TYMC (*log*CFU/g)	0	1.96±0.02	1.87±0.01	1.86±0.009	1.86±0.005	1.89±0.03^{a}	<.0001	<.0001	<.0001
	15	1.75±0.02	1.56±0.01	1.61±0.004	1.56±0.03	1.62±0.01^{b}			
	30	1.54±0.03	1.37±0.02	1.42±0.03	1.38±0.01	1.43±0.02			
	45	1.12±0.008	1.07±0.01	1.12±0.009	1.10±0.01	1.1±0.009^{d}			
	Mean	1.6^{a}±0.01	1.47^{c}±0.01	1.5^{b}±0.01	1.47^{c}±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= Control group, T_2= 5% bottle gourd leaves extract + 5% Wheat flour, T3= 5% bottle gourd leaves extract + 8% Wheat flour, T4= 5% bottle gourd leaves extract + 10% Wheat flour, DI=Day Intervals, Treat= Treatment, T*DI=Interaction of Treatment and Day Intervals.

Microbiological assessment

The range of overall observed aerobic plate count from the beef meatballs was 4.88–4.74 (log10 CFU/g), at different treatment levels. Among four treatments, the plate count in the control sample (4.88 logs CFU/g) was significantly higher than in the samples treated with 1%, 2%, and 3% of bottle gourd leaf extracts. 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 was 4.5 to 5.3. During storage TVC value was increased. Plant-derived spices are generally used in foods for flavorings and medicinal purposes (Table 5). However, a number of studies have demonstrated that compounds existing in many spices also possess antimicrobial activity (Zhang et al. 2009). The initial TCC of fresh beef was 1.25 logs CFU/g beef. The range of overall observed total coli form count from the beef meatballs was 1.10 to 1.06 (log CFU/g), at different treatment levels. During storage TCC value was decreased. During storage TYMC value was decreased. The range of overall observed total yeast-mold count from the beef meatballs was 1.47 to 1.6 (logCFU/g), at different treatment levels. Among four treatments, the total yeast-mold count in the control sample (1.60 logs CFU/g) was significantly higher than others and lowest total yeast mould count was T2 and T4. The less amount of TYMC value indicates this product is most preferable for consumers' health. The range

of overall observed of different days of intervals of TYMC value was 1.1 to 1.89. During storage TYMC value was decreased. Fernández-López et al. (2003) reported on the results of a research study related to antimicrobials in beef meatballs. They noted that the presence of mold and yeasts were not detected in any cooked meatball samples. The initial value of TVC for fresh beef (beef not frozen and thawed) was 5.12 log CFU/g beef, indicating good quality beef

Acknowledgement

The financial support of the Bangladesh Agricultural University Research System (BAURES), Mymensingh is greatly appreciated.

Conclusions

From the findings sensory, proximate, biochemical and microbiological studies 5% bottle gourd leaf extract +5% wheat flour (T_2) shown better performance than other treatments in meat preservation and most preferable color, odor tenderness, juiciness, overall acceptability, raw pH, cooked p^H, DM, Ash, POV and TBARs was found at this treatment. So, it may be concluded that 5% bottle gourd leaf extract +5% wheat flour (T_2)) can be used in beef meatball as prebiotics, natural antioxidant and antimicrobial agent to increase shelf-life of meatball.

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