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Research Article

Addition of wheat flour in chicken meatball increases the food value through prompting sensory, physicochemical, biochemical and microbial properties

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Abstract

The present study was undertaken to evaluate the effect of different levels (0%, 20%, 25%, and 30%) of wheat flour on the quality characteristics of chicken meatball. After formulation, samples were stored at -20˚C temperature for 60 days and were analyzed on 15th day, 30th day, 45th day and 60th day. The products were analyzed for various sensory (color, flavor, texture, Juiciness, tenderness, overall acceptability), physicochemical (proximate analysis, p^H, cooking loss), biochemical (TBARs, POV, FFA) and microbiological (TVC, TCC, TYMC) characteristics. Data were analyzed in a 4x3 factorial experiment in CRD which is replicated three times per cell. Most preferable sensory evaluation (tenderness, overall acceptability and juiciness), preferable physicochemical properties (raw p^H, cooked p^H), most acceptable proximate analysis (DM and ash), biochemical properties (PV and TBA values), and microbiological properties (TVC and TCC value) showed significant ($p<0.05$) result in 25% wheat flour group among the four treatments. Most found in 25% wheat flour group. Among four treatments, the total yeast-mold count in the control sample (1.70 log CFU/g) was significantly ($p<0.05$) higher than in the samples treated with 20%, 25% and 30% wheat flour group. The less amount of TYMC value indicates this product is most preferable for consumers' health. 30% and 25% wheat flour were the most preferable for TYMC. Summary of the present study reveals that meatballs made with 25% wheat flour had the highest tenderness, overall acceptability, raw p^H, cooked p^H, DM, ash, PV and TBA. Prebiotic is a non-digested food ingredient that promotes the growth of beneficial microorganism in the intestine.

Introduction

Modern-day consumers are concerned of the quality and safety of processed meat products. However, the high saturated fat content of such products results in a restriction of consumption for those who are prone to cardiovascular diseases and/or suffer from overweight (Weiss et al., 2010). Yet, fat is an important constituent of human nutrition and contribute to the flavor, tenderness, juiciness, appearance, texture and shelf life of meat products. Thus, the challenge for meat industry is to develop low-fat meat products without compromising sensory and texture characteristics (Das et al., 2022; Mun et al., 2009). Meat processors have therefore had to develop alternative, natural, functional and cost-effective ingredients that can successfully replace the less desirable fat component, while maintaining product quality. Interestingly, in recent years also in the upmarket sector some new developments regarding increased utilization of non-meat additives can be noted. The consumption of poultry meat and poultry meat products is growing all over the world (Ali et al., 2022; Bithi et al., 2020; Boby et al., 2021; Disha et al., 2020; Hossain et al., 2021; Islam et al., 2018; Jahan et al., 2018; Khatun et al., 2022; Siddiqua et al., 2018; Mielniket al., 2002). In recent years, poultry meat has gained much popularity among consumers. Their distribution in the wild spreads over large areas of Asia, Europe and Africa.

Minced meat is used for the preparation of a variety of products such as patties, sausages and meat balls. The liquid loss that occurs during cooking of processed meats may be reduced by the use of appropriate additives like carbohydrates, proteins, salt and phosphates (Hsu et al, 1999). Grinding of meat disrupts the integrity of muscle membranes and exposes lipid membranes to metal ions and facilitates the inter action of pro-oxidants with unsaturated fatty acids resulting in generation of free radicals and propagation of oxidative reaction (Asghar et al, 1988). Among the different meat products meatball is one of the tasty and popular foods. Therefore, numbers of studies about the nutrition and quality of meatballs already have been performed in different parts of the world. The studies on the physicochemical and sensory characteristics of low-fat meatballs with added wheat bran (Yilmaz, 2005), the improvement of low-fat meatball characteristics by adding whey powder (Serdaroglu, 2006), the effect of rice bran on the sensory and physicochemical properties of emulsified pork meatballs (Huang et al., 2005), the quality of low-fat meatballs containing legume flour as an extender (Serdarogluet al., 2005) and the effect of ingredients on the characteristics and quality of meatballs (Hsu and Yu, 1999; Serdaroglu, 2006; Hsu and Lung-Yueh Sun, 2006). In particular, non-meat proteins and carbohydrates are often used to enhance the texture meat products (Hongsprabhas and Barbut, 1999). Carbohydrate-based ingredients are known to be good water binders and numerous studies have focused on the effects of these nonmeat ingredients on sensory, cooking and water-binding properties in ground beef (Desmond et al., 1998). In the past, starch was added as a source of carbohydrates and to thicken the texture of meatballs (Huda et al., 2009). Today, starch is extensively used not only as a stabilizer, texturizer, water or fat binder and emulsifier but also increase the gel strength and freeze-thaw stability of meatballs (Serdarogluet al., 2005). Preservation not only retard the food spoilage but also control undesirable changes of wholesomeness, nutritive value and growth of microorganisms. Freezing is the only known method by which meatball can be preserved in a condition similar to their normal state.

Considering above all aspects present study was performed to investigate the suitable level of wheat flour adding and its effects on the proximate composition, physicochemical properties and sensory qualities of chicken meatballs.

Materials and Methods

Meat Sample Collection

Boneless poultry meat (broiler) of 2.5 kg from freshly slaughtered chicken was collected from "Poultry Farm", Bangladesh Agricultural University, Mymensingh. The meat sample was immediately transferred to the "Animal Science Laboratory".

Preparation of Jar and Other Instruments

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

Sample Preparation:

Collected meat sample was taken for the preparation of chicken meatball. First the broiler meat was properly cleaned and trimmed. Then it was grinded properly and mixed with the spices, garam masala, salt, Ice flakes, refined vegetable oil, refined wheat flower, sauce properly as per experimental design. There were four treatment groups T_1 -0%, T_2 - 20% flour, T_3-25% flour, T_4-30% wheat flour. Then meatball of proper shape was prepared separately. It was then boiled in hot water for 2-3 minutes. Then the water was removed from the meatball properly and was fried in hot oil until reddish brown color was obtained.

Sensory evaluation

Each meatball sample was evaluated by a trained 6-member panel with questionnaires measured intensity on a 5-point balanced semantic scale (weak to strong) for the following attributes color, smell, tenderness, juiciness, and overall acceptability. Panelists were selected according to the American Meat Science Association guidelines (AMSA, 1995). Sensory evaluation was carried out in individual booths under controlled conditions of light, temperature and humidity after thawing of before cook and after cook using a 5-point scoring method. Sensory scores were 5 for excellent, 4 for very good, 3 for good, 2 for fair and 1 for poor (Rahman et al., 2012). Sensory evaluation was accomplished at 0 day and repeated at 15 day, 30 day and 60 day; up to the end of refrigerated storage at -20 ± 1ºC.

Proximate Composition

Crude Protein

Crude protein was determined by micro kjeldahl method. The nitrogen values thus obtained were converted to total crude protein by multiply with a factor of 6.25. The calculation is as follows:

$$\frac{\text{Titrate required (ml)} \times .014 \text{ (milliequivalent of } N_2) \times \text{Strength of HCl} \times 100}{\text{Weight of sample}}$$

$$\text{\% of CP} = \text{\% of nitrogen} \times \text{conversion factor (6.25)}$$

Ether extract

Ether extract content was determined by Soxhlet apparatus using diethyl ether. The calculated value for ether extract content was obtained as percent of the sample.

The formula is mentioned below:

$$\text{\% of ether extract} = \frac{\text{Weight of the ether extract}}{\text{Weight of the sample}} \times 100$$

Ash

The ash was detected using the formula is mentioned below:

$$\text{\% of ash content} = \frac{E}{C} \times 100$$

Where, E = Weight of ash and C = Weight of sample

Biochemical analysis

Free fatty acid (%) analysis

Free fatty acid value was determined according to Rukunudinet al. (1998) using the following formula:

FFA (%) = ml titration × Normality of KOH × 28.2/g of sample

Peroxide Value (POV) analysis (meq/kg)

Peroxide value (POV) was determined according to Sallamet al. (2004) using the following formula:

POV was calculated and expressed as milliequivalent peroxide per kilogram of sample:

$$POV(meq/kg) = \frac{S \times N}{W} \times 100$$

Where S is the volume of titration (mL), N the normality of sodium thiosulfate solution (n = 0.01) and W the sample weight (g).

Thiobarbituric Acid Values (TBARS)

Lipid oxidation was assessed in triplicate using the 2-thiobarbituric acid (TBA) method described by Schmedes and Holmer (1989).

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

pH 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.

Cooking Loss

Cooking loss was practiced at 0 day, 30^{th} day and 60^{th} day using the following formula:

Cook loss (%) = $[(w_2\text{-}w_3) \div w_2]$ x 100; where, w_2 = meat weight before cooking and w_3 = meat weight after cooking.

Microbial assessment

Preparation of samples for TVC, TCC and Yeast-Mould count

A quantity of 10 g of meatball sample were aseptically excised from stored stock sample. Each of the stored meatball samples were 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).A quantity of ten (10) gram of the 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 according to the instruction of the standard method (ISO, 1995).

Enumeration of total viable count (TVC), total coliform count (TCC) and total Yeast-Mould count (TYMC)

The commercial media were prepared according to the manufacturer's instructions. For the determination of TVC, TCC and TYMC, 0.1 ml of each ten-fold dilution was transferred and spread on triplicate plate count agar (PCA) for TVC,MacConkey agar (MA) for TCC and potato dextrose agar (PDA) for TYMC using a sterile pipette for each dilution. The diluted samples were spread as quickly as possible on the surface of the plate with a sterile glass spreader. One sterile spreader was used for each plate. The plates were then kept in an incubator at 35°C for 24-48 hours for TVC and TCC, side by side 25°C for 48-72 hours for TYMC. Following incubation, plates exhibiting 30-300 colonies were counted. Colonies were counted with the aid of a colony counter. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the total viable count. The total viable count was calculated according to ISO (1995). The results of the total bacterial count were expressed as the number of organism of colony forming units per gram (CFU/g) of chicken meatball samples.

Statistical model and analysis

The proposed model for the planned experiment was factorial experiment with two factors A (Treatments) and B(Days of Intervals) is:

yijk= μ + Ai + Bj+(AB)ij+ εijki= 1,…,a; j = 1,…,b; k = 1,…,n

where:

yijk= observation k in level iof factor A and level j of factor B

μ = the overall mean

Ai = the effect of level iof factor A

Bj= the effect of level j of factor B

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

The observation of color, flavor, tenderness, juiciness and overall acceptability of different treatments and days of intervals is shown in Table 1. The totally different superscript was observed from four treatment groups indicates there were significantly differences ($p<0.05$). The same superscript was observed from four treatment groups indicates there were no significantly differences ($p>0.05$).

Table 1. Effect of Wheat flour on sensory parameters in chicken meatballs

Parameters	DI	Treatments				Mean ± SEM	Level of significance		
		T_1	T_2	T_3	T_4		Treat.	DI	T*DI
Color	0	4.33±0.33	4.67±0.00	4.67±0.33	4.67±0.33	4.58^{a}±0.33	0.4014	0.0331	0.9524
	30	3.33±0.33	3.67±0.33	4.00±0.58	4.33±0.33	3.83^{b}±0.39			
	60	4.33±0.33	4.33±0.67	4.33±0.33	4.67±0.33	4.42^{a}±0.41			
	Mean ± SEM	4.00^{a}±0.33	4.22^{a}±0.44	4.33^{a}±0.41	4.56^{a}±0.33				
Flavor	0	4.33±0.33	4.67±0.33	4.67±0.33	5.00±0.00	4.67^{a}±0.25	0.8013	0.0036	0.1102
	30	3.33±0.33	3.67±0.33	4.00±0.58	4.33±0.33	3.83^{b}±0.39			
	60	5.00±0.00	4.33±0.33	4.33±0.33	4.00±0.00	4.42^{a}±0.16			
	Mean ± SEM	4.22^{a}±0.22	4.22^{a}±0.33	4.33^{a}±0.41	4.44^{a}±0.11				
Tenderness	0	4.67±0.33	4.67±0.33	5.00±0.00	4.67±0.33	4.75^{a}±0.25	0.1764	0.0516	0.4128
	30	3.33±0.33	4.33±0.33	4.33±0.33	4.67±0.33	4.17^{b}±0.33			
	60	4.33±0.33	4.67±0.33	4.67±0.33	4.33±0.33	4.50^{ab}±0.33			
	Mean ± SEM	4.11^{a}±0.33	4.56^{a}±0.33	4.67^{a}±0.22	4.56^{a}±0.33				
Juiceness	0	4.67±0.33	4.67±0.33	4.33±0.33	5.00±0.00	4.67^{a}±0.25	0.1764	0.0019	0.3202
	30	3.33±0.33	3.67±0.33	4.00±0.58	4.33±0.33	3.83^{b}±0.39			
	60	4.67±0.33	4.00±0.00	5.00±0.00	4.67±0.33	4.58^{a}±0.16			
	Mean ± SEM	4.22^{a}±0.33	4.11^{a}±0.22	4.44^{a}±0.30	4.67^{a}±0.22				
Over all Acceptability	0	4.33±0.33	4.67±0.33	4.67±0.33	4.67±0.33	4.58^{a}±0.33	0.6393	0.0089	0.6951
	30	3.33±0.33	3.67±0.33	4.00±0.58	4.33±0.33	3.83^{b}±0.39			
	60	4.67±0.33	4.67±0.33	4.67±0.33	4.33±0.33	4.58^{a}±0.33			
	Mean ± SEM	4.11^{a}±0.33	4.33^{a}±0.33	4.44^{a}±0.41	4.44^{a}±0.33				

In each Table column mean values having different superscript varies significantly at $p < 0.05$. Again, mean values having same superscript in each row did not differ significantly at $p > 0.05$. T1=Control group, T2= 20% wheat flour group, T3=, 25% wheat flour group, T4=, 30% wheat flour group, DI=Day Intervals, Treat= Treatment, T*DI=Interaction of Treatment and Days of Interval, SEM= Standard error mean.

Color

The range of overall observed color score at different treatment was 4.00 to 4.56. Among four treatments most preferable color was observed from 30% wheat flour group (p<0.05) and less preferable color was observed from control group. The range of different days of interval of overall observation of color score was 3.83 to 4.58. The most preferable color was observed from 0 day and less preferable color from 30th day. The data show that the lowest test score was reduced to 3.83 in all treatments after 30 days of storage. The decreased color test scores during storage resulted from the denaturation of proteins, particularly the myofibrillar protein that affects gel formation (Aksu et al., 2005**)**. Among four treatments significantly higher color score was observed in 30% wheat flour group than other treatments which was similar to the findings of a reasearch (Naveena et al., 2008). Meatball redness decreased with more wheat flour addition reported by **(**Yilmaz 2004) which is similar to our study.

Flavor

The range of odor score among four treatments was 4.22 to 4.44. The most preferable good odor was observed in 30% wheat flour group and the lowest odor from control group and the 20% wheat flour group. The range of odor among different day intervals was 3.83 to 4.67. The odor of different treatments was decreased in 0 to 30th days and increase in 30th to 60th day's storage. There were significant (p<0.05) difference among 0 and 30th days observation and also between 30th and 60th days observation. Present study suggesting that the quality was deteriorated with increased storage period. Odor is one of the major causes of quality deterioration reported by a study (Raghavan & Richards, 2007).These issues leave the meat and poultry industry in need of economical and effective natural antioxidants that can replace synthetic antioxidants without negatively affecting the quality of finished products and consumer perceptions.

Tenderness

The range of overall observed tenderness score at different treatments was 4.11 to 4.67. Among these four treatments most preferable tenderness was observed from 25% wheat flour group and less preferable tenderness was observed from control group. The range of different day intervals of overall observation of tenderness score was 4.17 to 4.75. There were significant differences (p<0.05) among these 0, 30th and 60th days observation. The most preferable tenderness was observed from 0 day and less preferable tenderness from 30th day. Tenderness is interrelated dry matter content of the meatballs. With the increasing of storage period dry matter was increased consequently tenderness was decreased with day intervals. The result of this experiment is also related to another findings (Lui et al., 2010). Consumers use tenderness as an indicator of meatballs softness or even eating quality were reported by two studies (Ngapo et al., 2005; Mancini and Hunt 2008) which is similar to our present study. Several researchers have associated tenderness of meat with the breakdown of myofibrillar proteins affected by the presence of calcium-dependent proteases or calpains (Muchenje et al., 2009a).

Juiciness

The range of overall observed juiciness score at different treatments was 4.11 to 4.67. Among these four treatments most preferable juiciness score was observed from 30% wheat flour group and less preferable juiciness was observed from 20% wheat flour group. The range of different day intervals of overall observation of juiciness score was 3.83 to 4.67. There were significant differences (p<0.05) among these 0 and 30th days observation and also in 30th and 60th days observation. The most preferable tenderness was observed from 0 day and less preferable tenderness from 30th day. The data show that the lowest test score was reduced to 3.83 in all treatments after 30th days of storage. That's why meatballs leak juices when they were stored. If meatballs refrozen accelerating further moisture loss, and when this meatball eventually cooked, any one may find it dense and dry in texture is also related to another findings (Lui et al.,2010). Consumers use juiciness as an indicator of meatballs freshness or even eating quality (Ngapoet al., 2005; Mancini and Hunt, 2008).

Overall acceptability

The range of overall observed overall acceptability score at different treatments was 4.11 to 4.44. Most preferable overall acceptability was observed from 25% and 30% wheat flour group and less preferable overall acceptability was observed from control group. The range of different day intervals of overall observation of overall acceptability score was 3.83 to 4.58. There were significant ($p<0.05$) differences between 0 and 30th days observation and also between 30th and 60th days observation. The most preferable overall acceptability was observed from 0 day and 60thday and less preferable overall acceptability from 30th day. Our data showing that the lowest test score was reduced to 3.83 in all treatments after 30 days of storage. During the processing of meat and meat products, many functional compounds can be generated: many peptides produced from fermentation and enzyme-induced hydrolysis showed physiological benefits to human (Saiga et al., 2003) which is also related to our result.

Proximate Analysis

The DM, CP, EE, Ash content of different treatments with day intervals shown in Table 2.

Table 2. Effects of Wheat flour on proximate components in chicken meatballs

Parameters	DI	Treatments				Mean ± SEM	Level of significance		
		T_1	T_2	T_3	T_4		Treat.	DI	T*DI
DM%	0	53.73±0.09	52.35±0.11	49.40±0.14	48.54±0.06	51.00^{b}±0.1			
	30	56.36±0.59	55.16±0.11	56.95±2.03	60.06±1.03	57.12^{a}±0.94			
	60	58.16±1.90	58.13±2.29	57.13±2.09	60.07±0.99	58.37^{a} ±1.32			
	Mean ± SEM	56.08^{a}±0.86	55.21^{a} ±0.84	54.49^{a} ±1.42	56.22^{a} ±0.69		0.3374	<0.0001	0.0248
CP%	0	21.42±0.29	20.69±0.17	20.14±0.16	20.03±0.13	20.57^{b}±0.19			
	30	21.42±0.29	20.39±0.05	20.75±0.59	21.69±0.33	21.06^{a}±0.32			
	60	21.68±0.18	21.77±0.14	20.84±0.39	20.19±0.35	20.19^{a}±0.35			
	Mean ± SEM	21.51^{a}±0.25	20.95^{b}±0.12	20.58^{b}±0.38	20.64^{b}±0.27		0.0027	0.0271	0.0035
EE%	0	7.88±0.27	8.24±0.17	9.19±0.13	8.74±0.13	8.51^{a}±0.18			
	30	8.19±0.23	8.88±0.56	8.39±0.31	8.80±0.11	8.57^{a}±0.30			
	60	8.21±0.27	8.53±0.37	8.33±0.	8.62±0.08	8.42^{a}±0.27			
	Mean ± SEM	8.09^{b}±0.26	8.55^{ab}±0.37	8.63^{a}±0.26	8.72^{a}±0.11		0.0509	0.7707	0.2201
ASH%	0	1.16±0.02	1.75±0.16	1.07±0.03	1.40±0.06	1.35^{ab}±0.07			
	30	1.20±0.02	1.41±0.19	1.15±0.01	1.30±0.06	1.27^{b}±0.07			
	60	1.53±0.05	1.37±0.03	1.33±0.03	1.40±0.02	1.41^{a}±0.03			
	Mean ± SEM	1.30^{bc}±0.03	1.51^{a}±0.13	1.19^{c}±0.02	1.37^{b}±0.05		<0.0001	0.0215	0.0003

In each table column mean values having different superscript varies significantly at $p < 0.05$. Again, mean values having same superscript in each row did not differ significantly at $p > 0.05$. T1=Control group, T2= 20% wheat flour group, T3=, 25% wheat flour group, T4=, 30% wheat flour group, DI=Day Intervals, Treat= Treatment, T*DI=Interaction of Treatment and Days of Interval, SEM= Standard error mean.

Dry Matter (DM)

The range of overall observed Dry Matter content at different treatments was 54.49 to 56.22 %. Most preferable dry matter content was observed from 30% wheat flour group. The lowest amount dry matter content indicates this product is most preferable. Less preferable dry matter content was observed from 25% wheat flour group. The highest amount of dry matter content indicates this product is less preferable. The range of overall observation of different days of intervals of dry matter content was 51.00 to 58.37%. The different superscript was observed from 0 and 30thdays of observation indicates there were significant ($p<0.05$) differences among this observation. The dry matter content was increased with the increasing of storage period because moisture loss was decreased with the storage period. The most preferable dry matter content was observed from 0 day and less preferable dry matter content from 60th day. Present data shows that the highest amount dry matter content was increased to 58.37 in all treatments after 60 days of storage. Similar results were reported for Indonesian traditional meatballs with a dry matter content ranged from 56.17 to 60.32% (Purnomo &Rahardiyan, 2008). A study reported that incorporation pomegranate rind and seed powder extracts did not affect the dry matter content of goat meat patties(Devatkalet al., 2010). Another study reported that an increase in storage period with an increase in the dry matter content of pomegranate peel extract and pomegranate rind powder extract, respectively (Naveenaet al., 2008).

Crude Protein (CP)

The range of overall observed crude protein content at different treatments was 20.58 to 21.51%. There were no significant difference of crude protein among this treatment of 20%, 25% and 30% wheat flour. Control group contain more amount of CP than other groups. The highest amount of CP content indicates this product is most preferable for consumers' health. Less preferable CP content was observed from 25% wheat flour group. The range of overall observed of different days of intervals of CP content was 20.06 to 21.12 %. There were significant ($p<0.05$) differencesbetween 0, 30thbut no significant ($p<0.05$) differencesbetween 30th to 60th days of observation. The most preferable CP content was observed from 60th day and less preferable CP content was observed from 0 day. The protein result was lower compared to the protein content of Indonesian Beef Meatballs which ranged from 13.38 to 14.44%(Purnomo and Rahardiyanet al., 2008), similar results were reported to our findings. Traditional Taiwan meatballs, called kung-wang showed broad range of protein content, ranging from 12 to 22% (Hsu and Yu, 1999), also similar to our findings.

Ether Extract (EE)

The range of overall observed EE content at different treatments was 8.09 to 8.72%. Less preferable EE content was observed from 30% wheat flour group. The range of overall observed of different days of intervals of EE content was 8.42 to 8.57%. There were no significant differences among these (0, 30th and 60th) days of observation ($p>0.05$). The EE content was no changed with the increased storage period. The most preferable EE content was observed from 60 day and less preferable EE content from 30th day. 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%. Low-fat traditional Turkey koefte beef meatballs reported a similar fat content ranged from 7.9 to 8.8% (Serdarogluet al., 2005), similar to our findings which was lower than the normal traditional koefte with a fat content of 14.70%.The lower fat content of commercial beef meatballs illustrates the trend in the perception of Malaysian consumers on the negative effects of high fat content and high cholesterol on health. A study on chickpea hull flour reported significant decrease in low fat chicken nuggets incorporated with chickpea hull flour (Verma et al., 2012)similar to our findings.

Ash

The range of overall observed Ash content at different treatments was 1.19 to 1.51%. 25% wheat flour group contain lower amount of Ash than control group. The lowest amount of Ash content indicates this product is most preferable for consumers' health. The range of overall observed of different days of intervals of Ash content was 1.27to 1.41%. There was no significant difference between 0, 30th days of observation but from30th and 60th days of observation indicates there were significant ($p<0.05$) differences. The Ash content was significantly changed with the increased storage period. The most preferable Ash content was observed from 30th day and less preferable Ash content from 60th day. Present data indicating that the highest amount of Ash content was increased to 1.41% in all treatments after 60 days of storage. The ash content of Malaysian commercial beef meatballs ranged from 1.76 to 3.40% similar to our findings. Similar results were also reported by on the ash content of koefte beef meatballs study (Serdarogluet al., 2005), which ranged from 2.6 to 2.8%. The ash content of low fat chicken meatballs ranged from 2.34 to 3.34% reported by (Yilmaz, 2004).

Physicochemical properties

The raw P^H, cooked P^H and cooking loss of different treatments with day intervals shown in Table 3.

Table 3. Effect of Wheat flour on physicochemical in chicken meatballs

Parameters	DI	Treatments				Mean ± SEM	Level of significance		
		T_1	T_2	T_3	T_4		Treat.	DI	T*DI
Raw pH	0	5.95±0.04	5.81±0.07	6.07±0.07	5.76±0.07	5.90^a±0.06			
	30	6.11±0.01	6.17±0.02	6.02±0.15	5.75±0.04	6.02^a±0.05			
	60	5.56±0.01	5.36±0.08	5.70±0.11	5.73±0.14	5.58^b±0.08			
	Mean ± SEM	5.87^{ab}±0.02	5.78^b±0.06	5.93^a±0.11	5.74^b±0.08		0.0393	<0.0001	0.0031
Cooked pH	0	6.05±0.01	6.06±0.03	6.04±0.03	6.08±0.01	6.06^a±0.02			
	30	6.16±0.02	6.21±0.00	6.17±0.08	6.00±0.02	6.14^a±0.03			
	60	5.72±0.07	5.51±0.09	5.82±0.09	5.92±0.11	5.74^b±0.09			
	Mean ± SEM	5.98^a±0.03	5.93^a±0.04	6.01^a±0.06	6.00^a±0.05		0.3953	<0.0001	0.0022
Cooking Loss%	0	27.24±0.15	26.34±0.11	28.04±0.20	27.80±0.15	27.36^a±0.15			
	30	26.69±0.29	27.35±0.50	27.46±0.45	27.01±0.41	27.13^a±0.41			
	60	26.93±0.48	27.48±0.22	26.83±0.27	27.45±0.43	27.17^a±0.35			
	Mean ± SEM	26.95^a±0.31	27.02^a±0.28	27.44^a±0.31	27.42^a±0.33		0.2003	0.5988	0.0338

In each table column mean values having different superscript varies significantly at $p < 0.05$. Again, mean values having same superscript in each row did not differ significantly at $p > 0.05$. T1=Control group, T2= 20% wheat flour group, T3=, 25% wheat flour group, T4=, 30% wheat flour group, DI=Day Intervals, Treat= Treatment, T*DI=Interaction of Treatment and Days of Interval, SEM= Standard error mean.

Raw pH

The range of overall observed raw pHat different treatments was 5.74 to 5.93%. Most preferable raw pHwas observed from 25% wheat flour group. 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 was 5.58% to 6.02. There were no significant differences ($p>0.05$) between two (0, 30th days) observations rather 30th to 60th days of observation indicates there were significant differences. The raw $P^H$was decreased with the increased storage period. The most preferable raw pHobserved in 30 day and minimum pH observed in 0 day.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). The pH of Kavurma slightly increased after 300 days of storage time (Aksu et al., 2005). A study reported that there is no difference in the P^H of control and test antioxidants like grape seed, bearberry and rosemary extracts incorporated raw and cooked pork meat products(Carpenter et al., 2007)which is similar to our present findings**.**

Cooked pH

The most preferable cooked pHobserved in 30 day and minimum P^H observed in 0 day. The decrease in the cooked P^H values was lower in the untreated samples than the 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 Cooked P^H for the product were higher than that of the Cooked P^H values of meatballs and this could be due to the interaction effect of the other ingredients which were added during the processing of meat products. Among these four treatments most preferable cooked $P^H$was observed from 25% wheat flour group. The highest amount of cooked 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 cooked pH was 5.74 to 6.14. There were no significant differences between 0, 30th days of observation these two observationsrather 30th to 60th days of observation indicates there were significant differences ($p<0.05$) among these two days of observation. The Cooked P^H was decreased with the increased storage period.

The most preferable cooked P^Hcontent was observed from 0 day and less preferable cooked pH was observed from 60th day observation. The highest P^H values (6.11) were obtained from the 25% wheat bran added meatball sample. Which is similar to another study (Yılmaz and Daglıoglu,2003) for meatball. The P^H values of all patties increased ($P<0.05$) during storage which is similar to our result.

Cooking loss

The range of overall observed cooking loss at different treatments was 26.95 to 27.44%. Most preferable cooking loss was observed from control group. The lowest amount of cooking loss indicates this product is most preferable for consumers' choices than other treatment groups. The range of overall observed of different days of intervals of cooking loss was 27.13 to 27.36%. There were no significant differences (p>0.05) among 0, 30th and 60th days of observation. 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 30th day observation. The differences among the weight losses of the meatballs were significant ($P < 0.05$). High fat meatballs had the highest weight losses obtained(Yilmaz,2004). Major components of cooking losses are thawing, dripping and evaporation. Thawing loss refers to the loss of fluid in meatballs resulting from the formation of exudates following freezing and thawing by (Jama et al., 2008). 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 cooking yield of the Kung-Wan significantly decreased with higher natural antioxidant extract levels (Hsu and Sun, 2006) which is similar to our findings.

Biochemical properties

The FFA, PV, TBARS, of different treatments with day intervals shown in Table 4.

Table 4. Effect of Wheat flour on bio-chemical parameters in chicken meatballs

Parameters	DI	Treatments				Mean ± SEM	Level of significance		
		T_1	T_2	T_3	T_4		Treat.	DI	T*DI
FFA%	0	0.33±0.01	0.30±0.01	0.35±0.03	0.37±0.01	0.34^{b}±0.02	0.2753	0.0834	0.3099
	30	0.37±0.001	0.36±0.02	0.37±0.01	0.34±0.02	0.36^{a}±0.02			
	60	0.37±0.00	0.34±0.01	0.36±0.01	0.35±0.02	0.36^{ab}±0.01			
	Mean ± SEM	0.36^{a}±0.01	0.34^{a}±0.01	0.36^{a}±0.02	0.36^{a}±0.02				
PV%	0	3.86±0.10	3.83±0.08	2.87±0.09	3.60±0.07	3.54^{a}±0.08	0.0631	0.9951	<0.0001
	30	3.53±0.06	3.39±0.12	3.70±0.06	3.50±0.13	3.53^{a}±0.09			
	60	3.49±0.18	3.52±0.08	3.65±0.06	3.47±0.06	3.53^{a}±0.09			
	Mean ± SEM	3.63^{a}±0.11	3.58^{ab}±0.09	3.41^{b}±0.07	3.52^{ab}±0.09				
TBARS%	0	0.09±0.01	0.11±0.00	0.11±0.00	0.11±0.00	0.11^{b}±0.00	0.1527	0.0185	0.1170
	30	0.11±0.00	0.11±0.00	0.12±0.00	0.11±0.00	0.11^{ab}±0.00			
	60	0.12±0.01	0.12±0.00	0.11±0.00	0.12±0.01	0.12^{a}±0.01			
	Mean ± SEM	0.11^{b}±0.01	0.11^{ab}±0.00	0.11^{a}±0.00	0.11^{ab}±0.00				

In each table column mean values having different superscript varies significantly at $p < 0.05$. Again, mean values having same superscript in each row did not differ significantly at $p > 0.05$. T1=Control group, T2= 20% wheat flour group, T3=, 25% wheat flour group, T4=, 30% wheat flour group, DI=Day Intervals, Treat= Treatment, T*DI=Interaction of Treatment and Days of Interval, SEM= Standard error mean.

Free Fatty Acid values (FFA%)

FFA results appeared to be consistent with those of TBA and POV. Table 4 shows that the number of FFA increased with storage time. The range of overall observed of different days of intervals of FFA was 0.34 to 0.36. There were significant differences among 0, 30th and 60th days of observation. The FFA value was increased with storage period. The most preferable FFA was observed from 0 day and less preferable FFA was observed from 30th day of observation. The range of overall observed FFA value at different treatments was 0.34 to 0.36. Generally, the control samples treatments were slightly different. At the end of the storage time (day 60), the FFA value in the 30% wheat flour sample (0.36) was significantly ($P < 0.05$) higher than the other values. There had been significant difference ($P < 0.05$) among them throughout most of the storage process. Meat is biologically complex and model systems have been used in an attempt to gain a fundamental understanding of the lipid and protein oxidation processes (Lee et al., 2003) which is similar to our work. The fatty acid profiles reported in this study are within the ranges reported by another study (Muchenjeet al., 2009**).** Lipid oxidation promotes production of rancid flavors and odors while also reducing the shelf-life, nutritional quality, and safety of food products (Junet al., 2012). To prevent or delay the autoxidation process antioxidants have been utilized for many years. Antioxidants have an ability to prevent or reduce the oxidative damage of a tissue indirectly by enhancing nature of cell and/or directly by scavenging the free radical species (Verma et al., 2009).

Peroxide Value (PV-meq/kg)

The range of overall observed peroxide value at different treatment levels was 3.41 to 3.63. Throughout the storage time, peroxide values were generally higher in control samples than in others. As shown in Table 4, the higher peroxide value came from 20%, 30% and 25%wheat flour. During storage, the peroxide value decreased in all treatments. Most preferable peroxide value was observed from 25% wheat flour group. The lowest amount peroxide value indicates this product is most preferable for consumes health. Less preferable peroxide value was observed from control group. The highest amount of peroxide value indicates this product is less preferable. The range of overall observed of different days of intervals of peroxide value was 3.53 to 3.54. There were no significant differences (p>0.05) among these day0, 30th and 60th days of observation. The most preferable peroxide value was observed in 30 and 60 days and lowest amount in 0 day. During storage, the peroxide value increased in all treatments. Study onincreasing peroxide values with longer storage time in a sausage product (Salame) noted that peroxide values of 1.67, 4.02 and 4.20 meq O2/kg fat were found at 0, 1 and 3 months of frozen storage with no antioxidant treatments(Novelliet al., 1998) which is similar to our findings. Meat with higher lipid oxidation values also showed higher protein oxidation and greater met myoglobin formation (Ismailet al., 2011).

Thiobarbituric Acid Value (TBARS)

Generally, TBA levels significantly ($P < 0.05$) increased with storage time, showing decreasing shelf life. There were no significant differences among these 0, 30th and 60thday observation. The control sample, without any added antioxidants, showed a higher level of TBA than samples treated with 20%, 25%, 30%wheat flour. The TBA value on day 60 was 0.11 for the control samples, 0.11, 0.11 for those treated with 20%, 25%, and 30% wheat flour. Most preferable TBA value was observed from 20%,25% and 30% wheat group. Less preferable TBA value was observed from controlled group. The highest amount of peroxide value indicates this product is less preferable. Some studies of natural antioxidants also showed that they have the potential to replace synthetic antioxidants in food (Racanicciet al., 2004). 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. At the end of the storage period (10 days), increasing TBARS levels were observed (Vosenet al.,2004) which is similar to our findings.

Microbiological assessment

The TVC, TCC, TYMC of different treatments with day intervals shown in Table 5.

Table 5. Effect of Wheat flour on different microbe's population in chicken meatballs

Parameters	DI	Treatments				Mean ± SEM	Level of significance		
		T_1	T_2	T_3	T_4		Treat.	DI	T*DI
TVC	0	6.37±0.10	7.03±0.04	7.04±0.13	7.18±0.04	6.91^{a}±0.08	0.0043	0.0009	0.0004
	30	6.78±0.11	6.79±0.11	6.75±0.05	7.04±0.14	6.84^{a}±0.10			
	60	6.51±0.18	6.42±0.20	7.04±0.09	6.27±0.10	6.56^{b}±0.14			
	Mean ± SEM	6.55^{b}±0.13	6.75^{ab}±0.12	6.94^{a}±0.09	6.83^{a}±0.09				
TCC	0	1.17±0.02	1.27±0.12	1.62±0.09	1.54±0.06	1.40^{a}±0.07	0.0408	0.0081	0.0147
	30	1.13±0.04	1.10±0.04	1.30±0.02	1.28±0.09	1.21^{b}±0.05			
	60	1.43±0.06	1.30±0.12	1.21±0.09	1.33±0.09	1.32^{ab}±0.09			
	Mean ± SEM	1.25^{ab}±0.04	1.23^{b}±0.09	1.37^{a}±0.07	1.38^{a}±0.08				
TYMC	0	1.96±0.02	1.90±0.01	1.90±0.01	1.94±0.02	1.92^{a}±o.01	0.6972	<0.0001	0.0403
	30	1.78±0.06	1.54±0.12	1.48±0.09	1.46±0.00	1.57^{b}±0.07			
	60	1.36±0.13	1.55±0.06	1.61±0.10	1.48±0.08	1.50^{b}±0.09			
	Mean ± SEM	1.70^{a}±0.07	1.67^{a}±0.06	1.66^{a}±0.07	1.63^{a}±0.03				

In each table column mean values having different superscript varies significantly at $p < 0.05$. Again, mean values having same superscript in each row did not differ significantly at $p > 0.05$. T1=Control group, T2= 20% wheat flour group, T3=, 25% wheat flour group, T4=, 30% wheat flour group, DI=Day Intervals, Treat= Treatment, T*DI=Interaction of Treatment and Days of Interval, SEM= Standard error mean.

Total viable count (TVC)

The range of overall observed aerobic plate count from the chicken meatballs was 6.55 to 6.94 at different treatment levels. Among four treatments, the plate count in the 25% wheat flour group was significantly ($p<0.05$) higher than all treatments. The less amount of TVC value indicates this product the control group is most preferable for consumers' health. The range of overall observed of different days of intervals of TVC value was 6.56 to 6.91. During storage TVC value was increased. There is no significant difference between0, 30th days' observations. There were significant differences among30th and 60thdays of observation. The most preferable aerobic plate count was observed in 60 days and minimum in 0 day. The antioxidant compounds blocked the deteriorating of fat and helped prevent the metabolism of fat by bacteria. As a result, bacterial growth was lower in chicken meatballs treated with antioxidants. However, a number of studies have demonstrated that compounds existing in many spices also possess antimicrobial activity (Zhang et al., 2010). The frozen storage stability study of antioxidant-treated raw restructured beef steaks made from mature cows and resulted that initial level of contamination (aerobic count) was relatively low (3.18 log10 CFU/g) (Stikaet al., 2007). The effects of antioxidant-active packaging study on the display life of lamb meat and found that microbial counts in all samples gradually increased with storage time, reaching final values of 7–8 log10 CFU/cm2(Camo et al., 2008). Significant difference ($P < 0.05$) produced from day 8 and onwards.

Total coliform count (TCC)

The range of overall observed total coliform count from the chicken meatballs was 1.23–1.38 (log CFU/g), at different treatment levels. The total coliform count in the 30% wheat flour sample (1.38 log CFU/g) was significantly ($p<0.05$) higher than control, 20%, 25% wheat flour sample. The less amount of TCC value indicates this product is most preferable for consumers' health. The range of overall observed of different days of intervals of TCC value was 1.21 to 1.40. During storage TCC value was decreased. There were significant differences among 0, 30th and 60th days of observation. The most preferable TCC was observed in 30 days. Antioxidant-active packaging on the display life of lamb meat study found that coliform counts in all samples gradually decreased with storage time(Camo et al., 2008). The frozen storage stability of antioxidant-treated raw restructured beef steaks study revealed that aerobic coliform counts on agar incubated at 26ºC for 72 h to detect total coliforms was relatively low (1.18 logCFU/g) (Stikaet al., 2007) which is similar our study.

Total yeast-mould count (TYMC)

The range of overall observed total yeast-mold count from the chicken meatballs was 1.63 to1.70 (log CFU/g), at different treatment levels. Among four treatments, the total yeast-mold count in the control sample (1.70 log CFU/g) was significantly ($p<0.05$) higher than in the samples treated with 20%, 25% and 30% wheat flour group. 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 1.92 to 1.50. During storage TYMC value was decreased. There were significant ($p<0.05$) differences among 0, 30th and 60thdays of observation. The most preferable TYMC in 0 day and minimum in 60 days. Aresearch study related to antimicrobials in beef meatballsreported that the presence of mold and yeasts was not detected in any cooked meatball samples (Fernandez-Lopez et al., 2005) which is similar our work.

Conclusions

In conclusion, meatballs made with the addition of 25% wheat flour had the highest tenderness, overall acceptability, raw p^H, cooked p^H, DM, ash, PV and TBA. It is recommended that further studies of the wheat flour inclusion in meatballs production be carried out to ensure the availability of cheaper, nutritious and acceptable convenience food in the Bangladeshi market.

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