

Development of restructured chicken block utilizing gizzard and its refrigerated storage stability

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Revised: 3 July 2010 / Accepted: 29 September 2010 / Published online: 28 October 2010
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Abstract A study was planned to develop restructured chicken product by incorporating optimum level of gizzard and fat and evaluate their quality attributes under refrigerated storage condition (4 ± 10 C). Incorporation of gizzard did not show any change in pH, but cooking yield reduced significantly ($P<0.05$) at 40% level. There was a significant ($P<0.05$) decrease in fat content and increase in protein and ash content with increase in the level of gizzard in the formulation. Significant ($P<0.05$) improvement in the sensory attributes of chicken blocks was noticed by addition of gizzard. Incorporation of fat resulted in no change in pH, significant ($P<0.05$) reduction in cooking yield, moisture and protein content and increase in fat and total ash content. Products incorporated with fat rated better than control for various sensory attributes. Significant ($P<0.05$) increase in thiobarbituric acid reactive substance (TBARS), tyrosine value, stand plate count and psychrophilic count was observed during refrigerated storage (4 ± 10 C) in both control (no gizzard and fat) and test chicken blocks incorporated with gizzard and fat. The restructured chicken products were found acceptable up to 10 days under refrigerated storage condition (4 ± 10 C).

Keywords Chicken gizzard · Restructured · Chicken block · Low fat · Quality · Refrigerated storage

Introduction

Broiler production in India touched about one billion in the year 2006 and India has emerged as the 5th largest poultry meat producer in the world producing 1.92 million tones of meat in 2006 (Ministry of Agriculture 2008). With the present growth rate of over 15%, broiler production is expected to reach 4 billion by the year 2020 (Fakhruddin et al. 2001). Presently, chicken meat has become the highest contributor (37%) to total meat production in India (FAO 2008). The CII-McKinsey report (1997) on “Food and Agriculture Integrated Development Action (FAIDA)” has identified poultry products as having the third highest growth potential after wheat and milk based products.

With the growing poultry production and processing activities, there would be an increased availability of the edible byproducts. Gizzard is one of the principal edible byproducts of poultry processing which is being marketed as variety meats along with dressed chicken. It forms nearly 3% of dressed chicken (Charonpong and Chen 1980) and as such it is less preferred by the consumer due to its peculiar flavour and texture. Gizzard contains approximately 20% proteins (Kondaiah and Panda 1987; Rao et al. 1994) and has potential for using in cost effective, convenient ready to eat chicken products. Studies on development of fried chicken gizzard and its storage stability has been reported (Pangas et al. 1998). Further, utilization of this byproduct would increase the profitability of broiler industry.

In its broadest sense, any meat product that is partially or completely disassembled and then reformed into the same or a different form is called restructured product. Production of improved quality processed product such as restructured product has been indicated as a good end product from low value meats. Restructured products have characteristics somewhere between ground meats and intact

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muscle steaks (Mandigo 1986). By restructuring, product characteristics such as shape, colour, texture, juiciness and flavour can be improved. Cured products are very popular throughout the world. Cured meat products have the advantages of higher cooking yield, uniform colour, easy slicing, better fat control and salability (Pearson and Gillett 1996).

The demand for processed poultry meat products is rapidly increasing in India due to low cost, highly desirable flavor and taste and also due to relatively high unsaturated fat content. The ground meat products continue to command a major share of meat consumption. In light of the above facts this study was planned to develop restructured chicken block incorporating ideal levels of gizzard and fat and to study the quality and shelf life under refrigeration storage condition.

Materials and methods

Chicken meat, gizzard and fat Broiler chickens of about 16 weeks of age were obtained from the Instructional Farm of Rajiv Gandhi College of Veterinary and Animal Sciences (RAGACOVAS), Puducherry and slaughtered under hygienic conditions using semi automatic poultry dressing unit (RND Practical Engineers, Pune) in the Department of Livestock Products Technology (LPT) adopting standard procedures. The dressed carcasses were chilled and deboned manually and the meat obtained was packed in low-density polyethylene (LDPE) bags (200 gauge) and stored in the freezer (-18 ± 1 °C) till further use. Gizzard and fat obtained during slaughter of broiler chicken were collected hygienically, cleaned and packed separately in LDPE bags (200 gauge) and stored in frozen condition (-18 ± 1 °C). The frozen meat, gizzard were thawed overnight in the refrigerator and minced in a meat mincer (Mado Shop Mincer Junior, Germany) using 8 mm plate before use.

Product preparation Several preliminary trials were conducted to standardize the procedure to develop restructured chicken block following the formulation of Mandal et al. (2002). The standardized recipe for the product is deboned meat 100% with salt 2%, sugar 1%, phosphates 0.4%, nitrite 150 ppm and water 10%. All the curing ingredients were kept within permissible limits.

The frozen deboned meat was thawed overnight in the refrigerator (4 ± 1 °C). The thawed meat was minced using 8 mm plate in a meat mincer (Mado Shop Mincer Junior, Germany). The minced meat was mixed manually with curing ingredients and water, then further mixed uniformly using home mixer grinder for 30 s (Sumeet Machines Ltd, Mumbai) for better extraction of proteins. Thus obtained meat mix (approximately 600 g) was filled in clean

stainless steel mould lined with food grade aluminum foil. Finally the moulds were covered with lid and kept overnight in refrigerator (4 ± 1 °C) for curing. Next day the moulds were cooked in water at 90 °C for 45 min followed by cooling under potable tap water and chilled overnight in refrigerator (4 ± 1 °C) for setting. The resultant product is utilized as control.

Incorporation of different levels of gizzard Restructured chicken blocks were prepared incorporating minced gizzard into the control recipe at 20%, 30% and 40% replacing lean meat by following the procedure mentioned above. The finished products incorporated with different levels of gizzards were evaluated for different physicochemical properties such as pH, cooking yield and chemical composition viz., moisture, protein, crude fat and total ash and the sensory quality. Based on the physicochemical and sensory qualities, the product with the better merit was selected for further studies.

Incorporation of different levels of fat Chicken blocks were made by incorporating fat at 3%, 5% and 7% level in the formulation with selected level of gizzard. The required amount of fat was mixed at the second phase using mixer grinder. The finished products incorporated with different levels of fat were compared on different physicochemical properties and sensory quality as mentioned earlier. The product with better merit was selected for storage studies.

Storage studies under refrigeration The control (no gizzard and fat) and test (with selected level of gizzard and fat) products were aerobically packed in LDPE bags and evaluated for various physico-chemical (pH, thiobarbituric acid reactive substances and tyrosine value), microbiological (standard plate count, psychrophilic count, coliform count and yeast and mould count) and sensory attributes under refrigerated storage condition for a period of 15 days (0, 5, 10 and 15 days).

pH The pH of chicken meat was determined by adopting the method of AOAC (1995). Five gm of the product was homogenized with 45 ml of distilled water using waring blender at 3000 rpm for 15 s then pH of the homogenate was recorded by immersing combined glass electrode of the pH meter (ELICO Model LI-120, Hyderabad).

Cooking yield The weights of meat blocks were recorded before and after cooking. The cooking yield was calculated and expressed in percentage as follows.

$$\text{Cooking yield(\%)} = \frac{\text{Weight of cooked chicken meat blocks}}{\text{Weight of raw chicken meat blocks}} \times 100$$

Chemical composition The moisture, protein, fat and total ash contents of restructured chicken block were determined by standard methods of analysis in accordance with AOAC (1995).

TBARS value The distillation method described by Talradgis et al. (1960) was followed for the determination of TBARS value expressing as mg malonaldehyde/kg of sample.

Tyrosine value Tyrosine value of restructured chicken block was estimated adopting the procedure of Strange et al. (1977).

Microbiological analysis All the microbiological parameters viz, standard plate count, psychrophilic count, coliform and yeast & mould count were determined following procedures recommended by APHA (1984). Readymade media (Hi-Media, Mumbai) were used for the analysis.

Sensory analysis The products were evaluated organoleptically using semi-trained panelists consisting of faculty and post-graduate students using eight-point hedonic scale (Keeton 1983). The panelists were explained about the nature of experiment without disclosing the identity of the samples. They were requested to record their preferences on an 8 point hedonic scale (8=like extremely, 1=dislike extremely) for appearance, colour, flavour, juiciness, texture and overall acceptability as given in the score sheet. Taste panel was conducted around 3–4 pm every time. Plain water was provided to each panelist to rinse the mouth in between the samples.

Statistical analysis Three trials were conducted for each experiment and samples were analyzed in duplicate. The data recorded were analyzed using SPSS version 10.0 of

windows (SPSS, Chicago, USA). One way analysis of variance (ANOVA) was applied on all parameters analyzed for restructured chicken block incorporated with different levels of gizzards and fat. The data on all parameters obtained from on refrigerated (4 ± 1 °C) storage were analyzed using 2-way ANOVA. The data were tabulated and significant effects were tested using the least significant difference (LSD) test (Snedecor and Cochran 1986).

Results and discussion

Incorporation of different levels of gizzard The data (Table 1) on the incorporation of different levels of gizzard on physicochemical properties of restructured chicken block revealed that cooking yield decreased significantly ($P<0.05$) on incorporation of gizzards at 40% level (97.8%). Similar to the findings of the present study Kondaiah et al. (1993) reported the yield of 96 to 97.6% in mutton nuggets incorporated with chicken skin, heart and gizzard at 15% and 25% levels. Whereas, Reddy and Vijayalakshmi (1998) reported much lower cooking yield of chicken sausages (75.13–79.39%) containing skin, gizzard, heart and yolk at levels of 15 and 18%. The higher cooking yield of the product in the present study compared to the above reports might be due to high quality of raw materials used and use of sodium pyrophosphate.

In the present study pH (6.43–6.55) did not change significantly as the level of incorporation of gizzards increased in the product. Reddy and Vijayalakshmi (1998) reported a pH of 5.64 to 5.8 in chicken sausages incorporated with chicken skin, gizzard, heart and yolk.

The moisture, protein, fat and ash content of chicken block incorporated with different levels of gizzards were

Table 1 Effect of incorporation of different levels of gizzards on physicochemical and sensory properties of restructured chicken block (Mean \pm SE)

	Levels of gizzard (%)				
	Parameter	0	20	30	40
<i>Physico-chemical</i>					
	pH	6.4 \pm 0.01	6.4 \pm 0.01	6.5 \pm 0.01	6.5 \pm 0.01
	Cooking yield (%)	98.4 \pm 0.24 ^a	98.5 \pm 0.04 ^a	98.4 \pm 0.03 ^a	97.8 \pm 0.20 ^b
	Moisture (%)	71.3 \pm 0.13	71.2 \pm 0.11	71.3 \pm 0.05	71.4 \pm 0.04
	Protein (%)	18.6 \pm 0.06 ^a	19.2 \pm 0.04 ^b	19.3 \pm 0.05 ^b	19.4 \pm 0.02 ^b
	Crude fat (%)	5.4 \pm 0.07 ^a	4.8 \pm 0.09 ^b	4.4 \pm 0.10 ^c	4.1 \pm 0.06 ^d
	Total ash (%)	2.1 \pm 0.03 ^a	2.1 \pm 0.01 ^a	2.2 \pm 0.02 ^b	2.3 \pm 0.04 ^c
<i>Sensory Quality</i>					
	Appearance & colour	5.7 \pm 0.17 ^a	6.6 \pm 0.12 ^b	6.5 \pm 0.13 ^b	6.8 \pm 0.13 ^b
	Flavour	6.3 \pm 0.17 ^a	6.8 \pm 0.14 ^b	6.6 \pm 0.13 ^a	6.6 \pm 0.11 ^a
	Juiciness	6.1 \pm 0.16 ^a	6.6 \pm 0.12 ^b	6.6 \pm 0.10 ^b	6.5 \pm 0.08 ^b
	Texture	6.1 \pm 0.18 ^a	6.7 \pm 0.13 ^b	6.6 \pm 0.11 ^b	6.6 \pm 0.14 ^b
	Overall acceptability	6.3 \pm 0.16 ^a	7.0 \pm 0.12 ^b	6.7 \pm 0.14 ^b	6.8 \pm 0.13 ^b

N=6, for Physico-chemical parameters, *N*=18, for sensory attributes

Sensory scores were assessed on 8 point hedonic scale where 8=like extremely, 1=dislike extremely

*Means with different superscripts in the same row differ significantly ($P<0.05$)

between 71.22–71.46%, 19.26–19.44%, 4.10–4.80% and 2.15–2.38%, respectively. The protein and total ash contents increased significantly ($p < 0.05$), whereas, the fat content decreased significantly ($p < 0.05$) as the level of incorporation of gizzards increased due to higher protein (19.5%) and lower fat content (1.95%) in raw gizzards compared to chicken meat (protein-18.3% and fat-5.12%). In contrast to our results, Kondaiah et al. (1993) and Reddy and Vijayalakshmi (1998) reported much lower moisture content and much higher fat content in mutton nuggets and chicken sausages incorporated with skin, heart, gizzard and yolk. However, the protein content recorded in the present study was in close agreement with the values reported by them.

In the present study the sensory scores (Table 1) of the product increased significantly ($p < 0.05$) for all the parameters up to 40% level of gizzard incorporation. Similar to our findings Malik and Panda (1994) and Reddy and Vijayalakshmi (1998) reported higher acceptability scores of mutton blocks incorporated with 25% gizzard and 5% heart and chicken sausages incorporated with skin, heart, gizzard and yolk at levels of 15 and 18%, respectively.

Since there was no difference in the sensory attributes of chicken blocks with different levels of gizzards and all were significantly ($p < 0.05$) better than control, chicken blocks with 40% gizzard was selected for further studies, as it works out to be cheaper when lean is replaced with low priced gizzards.

Incorporation of different levels of fat The effects of different levels of fat on physicochemical properties of chicken block with 40% gizzard were studied (Table 2). The results showed significant decrease ($P < 0.05$) in the cooking yield in all three levels of fat incorporation (96.69, 95.52 and 95.74%) compared to that of control (97.8%).

The lower cooking yields might be due to improper extraction of meat proteins and emulsion formation during mixing leading to lower retention of the fat during cooking. Similar to our findings Nath et al. (1995) reported lower cooking yields of chicken patties incorporated with different levels of fat (12–18%).

In the present study pH did not change significantly as the level of incorporation of fat increased in the product which were in close agreement with the observations of Nath et al. (1995) and Pandey et al. (1998) in chicken patties and egg patties.

The moisture, protein, fat and ash content (Table 2) of restructured chicken block incorporated with different levels of fat ranged between 66.40–69.41%, 17.83–19.01%, 6.80–10.81% and 2.16–2.31%, respectively. Significant ($P < 0.05$) decrease in protein and moisture content and increase in fat and ash content was observed with increase in the level of incorporation of fat. Similarly, Nath et al. (1995) reported an increase in fat and decrease in protein and moisture content of chicken patties incorporated with different levels of fat.

Incorporation of fat at different levels significantly ($P < 0.05$) improved the sensory quality (Table 2) of the product compared to the control. However, there were no significant differences in the sensory scores of chicken blocks among different levels of fat incorporation. Similar to our findings Nath et al. (1995) noticed improvement in the acceptability scores as the level of fat incorporation increased in chicken patties up to 18%.

Since, there were no significant differences in the sensory scores of chicken blocks with different levels of fat, the 3% fat incorporation (6.80% fat in the final product) in chicken block was selected, considering the cost and health benefit and to categorize it as low fat meat product.

Table 2 Effect of incorporation of different levels of fat on physicochemical and sensory properties of restructured chicken block with 40% gizzard (Mean±SE)

$N=6$, for Physico-chemical parameters, $N=18$, for sensory attributes
Sensory scores were assessed on 8 point hedonic scale where 8=like extremely, 1=dislike extremely
*Means with different superscripts in the same row differ significantly ($P < 0.05$)

Parameter	Levels of fat (%)			
	0	3	5	7
<i>Physico-chemical</i>				
pH	6.5±0.01	6.5±0.01	6.5±0.01	6.5±0.01
Cooking yield	97.8±0.23 ^a	96.6±0.74 ^b	95.5±0.75 ^c	95.7±0.37 ^c
Moisture	71.4±0.14 ^a	69.4±0.14 ^b	68.2±0.31 ^c	66.4±0.12 ^d
Protein	19.4±0.10 ^a	19.0±0.10 ^a	18.9±0.14 ^a	17.8±0.12 ^b
Crude fat	4.1±0.06 ^a	6.8±0.31 ^b	8.6±0.12 ^c	10.8±0.28 ^d
Total ash	2.3±0.04 ^a	2.1±0.02 ^b	2.2±0.01 ^b	2.3±0.02 ^c
<i>Sensory quality</i>				
Appearance & colour	5.7±0.14 ^a	6.8±0.10 ^b	6.7±0.10 ^b	6.6±0.11 ^b
Flavour	6.3±0.13 ^a	6.8±0.12 ^b	6.6±0.09 ^b	6.8±0.11 ^b
Juiciness	6.1±0.13 ^a	6.6±0.12 ^b	6.6±0.11 ^b	6.6±0.13 ^b
Texture	6.2±0.15 ^a	6.7±0.11 ^b	6.6±0.11 ^b	6.7±0.13 ^b
Overall acceptability	6.3±0.13 ^a	6.9±0.10 ^b	6.8±0.11 ^b	6.8±0.11 ^b

Table 3 Effect of refrigeration (4 ± 1 °C) storage on physico-chemical and microbial quality of low fat restructured chicken block with 40% gizzard and 3% fat (Mean \pm SE)

Treatment/ parameter	Storage period (days)			
	0	5	10	15
<i>pH</i>				
Control	6.4 \pm 0.04 ^A	6.4 \pm 0.03 ^A	6.4 \pm 0.03 ^A	6.3 \pm 0.03 ^A
Test product	6.5 \pm 0.02 ^B	6.5 \pm 0.02 ^B	6.5 \pm 0.03 ^B	6.4 \pm 0.04 ^B
<i>TBARS (mg malonaldehyde/kg sample)</i>				
Control	0.20 \pm 0.02 ^{Aa}	0.34 \pm 0.01 ^b	0.50 \pm 0.05 ^{Ac}	0.77 \pm 0.01 ^{Ad}
Test product	0.29 \pm 0.01 ^{Ba}	0.37 \pm 0.01 ^b	0.63 \pm 0.02 ^{Bc}	0.96 \pm 0.01 ^{Bd}
<i>Tyrosine value (mg/100 g)</i>				
Control	19.9 \pm 0.18 ^{Aa}	22.3 \pm 0.12 ^b	24.5 \pm 0.41 ^c	26.1 \pm 0.24 ^{Ad}
Test product	20.5 \pm 0.10 ^{Ba}	22.3 \pm 0.12 ^b	24.3 \pm 0.28 ^c	26.4 \pm 0.21 ^{Bd}
<i>SPC (log₁₀ CFU/g)</i>				
Control	2.4 \pm 0.04 ^a	3.3 \pm 0.09 ^b	3.5 \pm 0.03 ^c	4.3 \pm 0.09 ^d
Test product	2.4 \pm 0.03 ^a	3.2 \pm 0.09 ^b	3.6 \pm 0.04 ^c	4.4 \pm 0.05 ^d
<i>Psychrophiles (log₁₀ CFU/g)</i>				
Control	1.3 \pm 0.03 ^{Aa}	2.4 \pm 0.10 ^{Ab}	3.1 \pm 0.02 ^{Ac}	3.9 \pm 0.09 ^{Ad}
Test product	1.4 \pm 0.03 ^{Ba}	2.8 \pm 0.04 ^{Bb}	3.2 \pm 0.02 ^{Bc}	3.7 \pm 0.07 ^{Bd}

N=6

*Means with different superscripts (capital letters in same column and small letters in the same row) differ significantly ($P<0.01$)

Refrigerated storage study The control product and the chicken block with 40% gizzard and 3% fat (test product) were prepared freshly and used for refrigeration storage studies. The physicochemical and microbiological quality of control and test products during the refrigerated storage period were presented in Table 3.

The pH of the test product was significantly higher in comparison to control throughout the storage, however, pH of both the products were not affected due to the storage. The results of the present study were in agreement with the results reported by Nath et al. (1995) in chicken patties and Mandal et al. (2002) in restructured cured chicken during refrigeration storage.

In the present study the TBARS values (Table 3) of test product as well as control increased significantly ($P<0.05$) throughout the storage period indicating an increase in the lipid oxidation. Increase in lipid oxidation by its reaction with TBARS reagent in muscle foods with storage time has been reported (Rao and Kowale 1988). However, the values at 15th day were close to the threshold values of 1–2 mg/kg for spoilage as reported by Watts (1962). The values in the present study were in conformity with the earlier findings on different chicken products (Nath et al. 1995; Bhojar et al. 1997; Mandal et al. 2002).

The tyrosine value (Table 3) of the test product and control increased significantly ($P<0.05$) throughout the storage period which might be attributed to increased microbial proteolytic activity as evident from increased microbial counts during storage. The tyrosine value indicates presence of free amino acids which generally increases due to microbial protease activity. Similar to the present findings Mandal et al. (2002) reported significant

increase in tyrosine values of restructured chicken block during refrigerated storage.

The microbial counts (Table 3) of the test product and control increased significantly ($P<0.05$) during refrigerated

Table 4 Effect of refrigeration (4 ± 1 °C) storage on the sensory quality of low fat restructured chicken block with 40% gizzard and 3% fat (Mean \pm SE)

Parameters	Storage period (days)		
	0	5	10
<i>Appearance & Colour</i>			
Control	6.0 \pm 0.13 ^{Aa}	5.6 \pm 0.12 ^{Ab}	5.3 \pm 0.12 ^{Ac}
Test product	7.1 \pm 0.10 ^{Ba}	6.7 \pm 0.09 ^{Bb}	6.3 \pm 0.08 ^{Bc}
<i>Flavour</i>			
Control	5.9 \pm 0.14 ^{Aa}	6.0 \pm 0.13 ^{Aa}	4.8 \pm 0.09 ^{Ab}
Test product	7.0 \pm 0.11 ^{Ba}	6.9 \pm 0.11 ^{Ba}	6.1 \pm 0.11 ^{Bb}
<i>Juiciness</i>			
Control	5.7 \pm 0.13 ^A	5.8 \pm 0.12 ^A	5.9 \pm 0.10 ^A
Test product	6.8 \pm 0.09 ^B	7.0 \pm 0.08 ^B	6.9 \pm 0.10 ^B
<i>Texture</i>			
Control	6.2 \pm 0.15 ^{Aa}	6.1 \pm 0.13 ^{Aa}	5.8 \pm 0.13 ^{Ab}
Test product	7.0 \pm 0.11 ^{Ba}	6.9 \pm 0.11 ^{Ba}	6.7 \pm 0.13 ^{Bb}
<i>Overall Acceptability</i>			
Control	6.2 \pm 0.13 ^{Aa}	5.9 \pm 0.11 ^{Ab}	5.2 \pm 0.12 ^{Ac}
Test product	7.2 \pm 0.11 ^{Ba}	7.0 \pm 0.10 ^{Ba}	6.2 \pm 0.11 ^{Bb}

N=18

Sensory scores were assessed on 8 point hedonic scale where 8=like extremely, 1=dislike extremely

*Means with different superscripts (capital letters in same column and small letters in the same row) differ significantly ($P<0.05$)

storage, but were found to be within the safer limits. The microbial counts recorded in the present study were lower than those reported by Bhoyar et al. (1997) and Mandal et al. (2002). The reasons for the low counts in the product could be due to hygienic measures practiced during processing and the effect of curing. Similar to our finding a gradual increase in the aerobic and psychrophilic counts throughout the storage period of 15 days were reported by Bhoyar et al. (1997) and Mandal et al. (2002). However, Cremer and Chipley (1977) suggested that an aerobic bacterial count of log 5.33 and log 4.6 for psychrophiles is considered to be indicative of unacceptability of cooked meat products. Coliforms and yeast & moulds were not detected in the cooked products throughout the storage period of 15 days reflecting the good hygienic practices during the processing of product and also due to the cooking of product to an internal temperature of 80 °C, which might have been lethal to the coliforms. Similarly, Pal et al. (2003) and Sachdev and Gopal (2000) observed no coliforms during refrigeration storage of chicken sausages and cooked chicken rolls, respectively.

Sensory scores (Table 4) of the test product and control in respect of appearance, flavor, texture and acceptability decreased significantly ($P < 0.05$) on the 10th day of storage, however, there was no significant difference between 0 day and 5 day scores. The sensory scores for appearance, flavor, texture and acceptability were significantly ($P < 0.05$) better in test product than control on 0, 5 and 10 day of storage. The product was not subjected to sensory evaluation on 15th day due to oxidative changes. Similar to the findings of the present study, a decrease in the sensory scores of various meat products during refrigerated storage has been reported (Nath et al. 1996; Bhoyar et al. 1997; Pandey et al. 1998; Mandal et al. 2002).

Conclusion

Highly acceptable restructured chicken block was developed with incorporation of gizzard and fat and the prepared product showed a shelf life of 10 days under refrigerated storage condition.

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