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Storage Quality and Oxidative Stability Attributes of Almond (*Prunus dulcis*) Fortified Chevon Nuggets

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ABSTRACT

The present study was conducted to evaluate the possibility of utilization of almonds in the development of value added healthier chevon nuggets and to study their physico-chemical, sensory and storage properties. Four levels of almonds (0, 5, 10 and 15%) were incorporated replacing lean meat in the formulation. The products developed were assessed for various physico-chemical and sensory parameters. Based on these parameters, chevon nuggets containing (5%) levels of almond were selected and further studied for its storage quality. The optimized nuggets were aerobically packaged in low density polyethylene pouches along with control and evaluated for storage quality (Oxidative stability, microbiological and sensory parameters) for 21 days under refrigerated conditions (4±1ºC). Based on various parameters, a 5% level of almonds was adjudged as optimum for the preparation of almond enriched chevon nuggets. The storage quality parameters like Free fatty acid value, Thiobarbituric acid reactive substances value, Total plate count and Psychrophillic count increased significantly ($P<0.05$) whereas all the sensory parameters decreased significantly ($P<0.05$) with increasing days of storage. The product can be successfully stored for 14 days under refrigerated conditions (4±1ºC) without marked loss in quality.

Keywords: Almonds (*Prunus dulcis*), Chevon, Nuggets, Oxidative stability

Over the last fifty years, meat products have come under increasing perusal by medical, nutritional and consumer groups because of the direct association between their consumption and the risk of some of the major degenerative and chronic diseases like ischemic heart disease, cancer, hypertension and obesity. Hence, functional meat products are seen as a replacement to satisfy consumer demands, and also to achieve the nutritional and dietary goals (Jimenez-Colmenero, 2007). Diet and nutrition play important role in the promotion and maintenance of health throughout life. According to a report by World Health Organization (WHO), the faulty dietary lifestyle acts as a major risk factor for the development of non-communicable diseases like coronary heart disease (CHD), cancer, type 2 diabetes, obesity, osteoporosis and periodontal disease (WHO, 2003). Chronic diseases alone contribute to approximately 60% of deaths, almost half of which are cardiovascular (WHO, 2003). However, luckily the deaths and disabilities due to CHD and strokes can be reduced by more than 50% by certain dietary innovations to reduce major risk factors such as high blood pressure, high cholesterol, obesity and smoking (WHO, 2007). Faulty dietary practices include consumption of diets containing higher levels of saturated fats, salt and lower levels of fruits and vegetables (WHO, 2009), on the other hand the recommendations for reducing CVD in industrialized countries commonly specify less fat (WCRF/AICR, 1997; WHO, 2003). Among the different plant foods, it is widely recognized that the components of almond can deliver health benefits beyond their nutritional value. Nuts, especially almonds are rich in monounsaturated (oleic) fatty acids and also have a higher polyunsaturated (linoleic and α-linolenic) fatty acid content than typical vegetable oil (Mostafa *et al*., 2013).

The ameliorative effect of almonds is attributed to the antioxidant action of the vitamin-E found in the almonds, as well as to the LDL-lowering effect of almonds mono-unsaturated fats. If almonds are substituted for common fats in human feeding-trials, LDL cholesterol value has been reported to reduce from 8 to 12% (Sang *et al.*, 2002; Frison-Noorie *et al.*, 2002). Because of the evident health benefit, there is a need to stress the importance of including a certain amount of almond in human diets. In light of these considerations and the nutritional profile defined, the present study was undertaken to analyse the quality attributes of almond enriched chevon nuggets and to study the effect of refrigerated storage on the storage quality of developed nuggets.

MATERIALS AND METHODS

Procurement of materials for the preparation of chevon nuggets

Leg cut of adult Bhakarwal goat carcass slaughtered by ritual method was purchased from the local market of Jammu. The lean meat was obtained after deboning and trimming the fat manually. The lean meat so obtained was packed in polythene bags and stored as frozen (-18 ± 2˚C) until it was used.. Condiments paste used contained fresh onion, garlic and ginger in the ratio of 3:2:1. The spice mix formula was standardized in the laboratory. The dried almonds were obtained from local market of Srinagar. The shells of these nuts were removed manually and the kernels were ground in powder form and packed in polythene bags and refrigerated at 4 ± 1˚C.

Preparation of chevon nuggets

Lean meat was cut into smaller pieces and minced twice in a mincer (Marsango, Italy). The formulation for the preparation of chevon nuggets was earlier standardized through preliminary trials and contained lean meat 68%, added water 9%, vegetable oil 8%, condiment mixture 3%, refined wheat flour 4%, whole egg liquid 2.5%, spice mixture 2.5%, common salt 2%, sugar 0.5%, monosodium glutamate 0.3%, sodium hexametaphosphate 0.2% and sodium nitrite 120ppm. Meat emulsion was prepared by using Bowl chopper (Marsango, Italy). The batter so obtained was moulded in oil coated rectangular stainless steel boxes and steam cooked for 30±2 minutes. The boxes were allowed to cool at room temperature after removal from pressure cooker. The chevon block so obtained was cut into nuggets.

Physico-chemical properties

The pH of chevon nuggets was determined as per the procedure of Keller *et al.* (1974) by using a digital pH meter (Systronics Digital pH Meter 803). The proximate components viz., moisture, crude protein, crude fat and ash contents in both treatment samples and control were determined by using standard procedures prescribed by AOAC (2000). Emulsion stability of meat emulsion was determined as per Townsend *et al.* (1968). The weight of product was recorded before and after cooking and cooking yield was calculated as a percentage.

Storage studies

Free fatty acid value was determined by method of US Army laboratories (Natick) described by Koniecko (1979). Thiobarbituric acid reactive substances (TBARS) value of cooked products during storage was determined as per Witte *et al.* (1970). The microbiological characteristics viz., total plate count, psychrophillic count, coliform count and yeast and mold count were determined by methods of APHA (1984).

Sensory Evaluation

The sensory evaluation of all sample products was performed by a panel of seven trained members based on 8-point hedonic scale, wherein 8 denoted "extremely desirable" and 1 denoted "extremely undesirable" (Keeton *et al.*, 1983). The attributes of sensory evaluation included colour and appearance, flavour, juiciness, texture and overall acceptability. Coded samples were presented to the panelist by cutting nuggets into 1cm×1cm cubes. Refrigerated storage samples were warmed in microwave oven at 90°C for 5 mins and then presented to panelists. Water was provided for oral rinsing between two sample testing.

Statistical Analysis

The statistical analysis of data was carried out by analysis of variance and least significant difference tests (Snedecor and Cochran, 1980). In significant effects, least significant differences were calculated at appropriate level of significance for a pair wise comparison of treatment means. The results were analyzed at 95%confidence level and the level of significance was (0.05).The value for n=6 for proximate and physiochemical paprameters while n=21 for sensory evaluation.

RESULTS AND DISCUSSION

Physicochemical characteristics

The mean values of various physicochemical parameters namely pH, proximate composition, emulsion stability and cooking yield of chevon nuggets incorporated with 0, 5, 10 and 15 percent levels of almond are presented in Table 1.

pH and proximate composition

A non-significant (P>0.05) effect on pH value was observed in chevon nuggets after the incorporation of 5, 10 and 15% levels of almond though there was a gradual increase in pH with increased levels of almond incorporation. This may be due to phenolic content of almond. These results are in contrast to the findings of Rajkumar *et al*. (2014) who reported significant (P<0.05) increase in pH of almond incorporated goat meat nuggets.

The moisture value followed a gradual decreasing trend with increase in the level of almond incorporation. Moisture was recorded lowest at 15% level and was comparable to the moisture value at 10% level, while as both 10% and 15% levels differed significantly (P<0.05) from 5% and control treatments. Moisture value in control and 5% treatment level was comparable to each other. Mostafa *et al*. (2013) reported 1.6% moisture content in almonds on dry matter basis, which might be the possible reason for decreasing trend in moisture values of chevon nuggets incorporated with almond. The decrease in moisture content of almond incorporated meat was also reported earlier (Rajkumar *et al*., 2014).

Incorporation of almond had a profound effect on protein content of chevon nuggets, with 10% and 15% levels recording significantly (P<0.05) higher values as compared to control. However, there was no significant (P>0.05) difference observed within the treatments of almond incorporation. Protein value at 5% level of almond incorporation was comparable to control. Mostafa *et al*. (2013) reported 23.6% protein content in almonds on dry matter basis which is somewhat higher than meat protein value thus a possible reason for higher protein content in almond incorporated chevon nuggets. Similar effect on protein value due to incorporation of almond in meat has been reported previously (Rajkumar *et al*., 2014).

There was no significant (P>0.05) increase in fat content up to 5% level of almond incorporation although fat content of 10% and 15% levels were recorded significantly (P<0.05) higher than control at the same time both levels being comparable to each other. The higher fat content in almond incorporated chevon nuggets is due to higher lipid content (56.8%) in almonds (Mostafa *et al*., 2013). These results are in consonance with the findings of Rajkumar *et al*. (2014) in goat meat nuggets.

Table 1: Effect of different levels of almond on physico-chemical properties of cooked chevon nuggets (Mean± SE)*

PARAMETERS	Levels of almond (%)			
	0	5	10	15
pH	6.33 ± 0.13	6.5 ± 0.14	6.66 ± 0.07	6.67 ± 0.08
Moisture (%)	$58.75±0.43^{a}$	$57.64±0.49^{a}$	$55.51±0.6^{b}$	$55.47±0.50^{b}$
Protein (%)	$17.92±0.43^{a}$	$18.89±0.28^{ab}$	$19.48±0.37^{b}$	$19.77±0.37^{b}$
Fat (%)	$11.11±0.35^{a}$	$11.90±0.40^{ab}$	$13.14±0.47^{bc}$	$14.03±0.51^{c}$
Ash (%)	$1.73 ± 0.09^{a}$	$1.90 ± 0.09^{ab}$	$2.1 ± 0.12^{ab}$	$2.25 ± 0.07^{b}$
Emulsion stability (%)	$80.29± 1.17^{a}$	$83.70± 0.85^{b}$	$83.91 ± 1.044^{b}$	$84.07± 1.17^{b}$
Cooking yield (%)	$81.02±1.12^{a}$	$84.56±1.03^{b}$	$84.70±1.05^{b}$	$84.35±0.90^{b}$

*****Mean ± SE with different superscripts in a row differs significantly (P<0.05).n=6

Table 2: Effect of different levels of almond on sensory attributes of cooked chevon nuggets (Mean ±SE)*

SENSORY ATTRIBUTES	Levels of almond (%)			
	0	5	10	15
Appearance and colour	6.45±0.169^{a}	7.01±0.087^{b}	6.88±0.089^{b}	6.89±0.101^{b}
Flavour	6.44±0.152^{a}	6.94±0.097^{b}	6.63±0.115^{ab}	6.54±0.147^{a}
Juiciness	6.30±0.092^{a}	6.83±0.086^{b}	6.71±0.090^{b}	6.71±0.103^{b}
Texture	6.44±0.115^{a}	6.77±0.089^{b}	6.80±0.098^{b}	6.46±0.107^{a}
Overall acceptability	6.48±0.109^{a}	6.85±0.065^{b}	6.43±0.077^{a}	6.59±0.105^{a}

***Mean ±SE with different superscripts in a row differs significantly (P<0.05). Mean values are scores on 8 point descriptive scale where 1-extremely poor and 8- extremely desirable. n = 21 for each treatment.

There was no significant (P>0.05) difference in the ash content among the three treatments of almond in chevon nuggets, although the ash content at 15% level was significantly (P<0.05) higher as compared to control. The ash content of other two treatments of almond was comparable to control. Rajkumar *et al*. (2014) reported more or less similar results for ash in almond incorporated goat meat nuggets.

Emulsion stability and cooking yield

The effect of almond incorporation presented a favourable effect on the emulsion stability of chevon nuggets. Emulsion stability was significantly (P<0.05) higher in treated chevon nuggets compared to control but comparable between 5, 10 and 15% levels of almond treatment. Cooking yield followed a trend similar to that of emulsion stability. There was no significant (P>0.05) difference in cooking yield among the three treatments of almond, although all the almond treatments scored significantly (P<0.05) higher than control. Significantly (P<0.05) higher values of emulsion stability in almond incorporated chevon nuggets might be attributed to the ability of protein matrix in almond to bind fat and moisture. However, in contrast some authors have reported increased cooking loss as the fat content increases (Berry, 1994; Liu *et al*., 1991) but others have reported no such findings (Blackmer *et al*., 1997 and Trout *et al*., 1992). Rajkumar *et al*. (2014) observed slightly contrasting results and reported improvement in cooking yield up to a certain level of almond incorporation but loss in cooking yield at higher levels of almond incorporation.

Sensory attributes

Sensory evaluation of all treatments presented in the Table 2 indicated that, chevon nuggets incorporated with almond scored significantly higher (P<0.05) in appearance and juiciness as compared to control. Flavour recorded highest value at 5% treatment level and differed significantly (P<0.05) from control. All other treatments of almond were comparable to control in flavour scores. Increased juiciness in almond incorporated chevon nuggets can be attributed to higher levels of fat in the product. Decrease in flavour at higher levels of walnut incorporation may be attributed to intense almond like flavour in chevon nuggets. Texture was comparable between 5 and 10% levels of almond incorporation and differed significantly (P<0.05) from control and 15% level. Overall acceptability was significantly (P<0.05) higher for chevon nuggets incorporated with 5% almond level. Lower texture scores at higher levels of almond incorporation may be attributed to reduced presence of myofibrillar proteins and a diluting effect of non-meat ingredients (almond) in meat protein systems and the poorer gelling properties of almond globular proteins at processing temperatures, which interfered to some extent in myofibrillar meat protein interactions.

Rajkumar *et al*. (2014) reported that textural properties of chevon nuggets were not affected upto 5% of almond incorporation in chevon nuggets. On the basis of analysis of different physico-chemical, proximate and sensory parameters 5% percent of walnut was optimum for making designer chevon nuggets.

Table 3: Effect of refrigeration storage on the physico-chemical characteristics of chevon nuggets incorporated with optimum levels of almond aerobically packaged in LDPE films and stored in refrigerator. (Mean ±SE)*

TREATMENT	STORAGE PERIOD IN DAYS			
0	7	14	21	
		pH		
CONTROL	6.17 ± 0.033^{a}	6.01± 0.014^{b}	6.19± 0.034^{Aa}	6.42±0.018^{d}
AL	6.19±0.020^{a}	6.05± 0.018^{b}	6.27±0.013^{ABCc}	6.41±0.017^{d}
		TBARS (mg malonaldehyde/kg)		
CONTROL	0.127±0.007^{a}	0.407±0.018^{b}	0.82 ± 0.017^{Ac}	1.28±0.079^{d}
AL	0.121±0.002^{a}	0.367 ± 0.02^{b}	0.72 ± 0.019^{Bc}	1.16 ±0.057^{d}
		FFA(% oleic acid)		
CONTROL	0.080±0.004^{da}	0.131±0.002^{b}	0.233 ± 0.011^{c}	0.354 ± 0.006^{d}
AL	0.075 ±0.003^{a}	0.128±0.009^{b}	0.210± 0.009^{c}	0.349 ± 0.006^{d}

*Mean± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly (P<0.05).n=6 for each treatment. AL= Almond incorporated chevon nuggets.

Storage studies

Oxidative stability

The mean values of various parameters to determine oxidative stability (pH, FFA and TBARS) of cooked chevon nuggets incorporated with 0 and 10 percent level of almond during refrigerated storage (4±1ºC) are presented in Table 3.

pH

pH values of both control and optimized almond treated nuggets increased significantly (P<0.05) at the progressive storage intervals. The pH value of optimized almond treated nuggets was comparable to control at all intervals of storage period. The increase in pH on subsequent days of storage might be attributed to formation of volatile basic nitrogen components as affected by biochemical changes under low temperature (Ibrahim and Desouky, 2009) and to microbial load which may cause protein hydrolysis with the appearance of alkyl groups (Yassin, 2003). Aerobically packed products on refrigerated storage showed an increase in pH values due to more psychotropic growth (Sahoo and Anjaneyulu, 1997). Ang and Hamm (1982) reported that increment of pH could be attributed to the modification of meat protein conformation during thermal denaturation.

Free fatty acid (FFA)

FFA followed a significant (P<0.05) increasing trend from day 0 to day 21 in all preparations. Almond treated chevon nuggets maintained non-significantly lower FFA values throughout storage period as compared to control. The increase in FFA value of the chevon nuggets revealed that fat present in the system underwent hydrolysis and oxidation.

Thiobarbituric acid reactive substances (TBARS) value

TBARS values followed a trend similar to FFA values with a significantly (P<0.05) increasing trend towards the advancement of storage period. Almond treated chevon nuggets maintained lower but non-significant TBARS values as compared to control through the storage period. Regarding the increased TBARS values for all preparations with the advancement of storage time; it could be due to lipid hydrolysis, oxidative rancidity and secondary products formation at refrigeration temperature (Forrest *et al.*, 1975). A general trend of increased TBARS

Table 4: Effect of refrigeration storage on the microbiological characteristics of chevon nuggets incorporated with optimum levels of almond aerobically packaged in LDPE films and stored in refrigerator. (Mean ±SE)*

TREATMENT	STORAGE PERIOD IN DAYS			
0	7	14	21	
		Total plate count(log_{10} cfu/g)		
CONTROL	2.53 ± 0.087[a]	3.67 ± 0.131[b]	4.64 ± 0.181[c]	6.24 ± 0.194[d]
AL	2.62 ± 0.067[a]	3.78 ± 0.205[b]	4.39 ± 0.212[c]	5.91 ± 0.191[d]
		Psychotrophic count (log_{10} cfu/g)		
Control	ND	ND	2.21± 0.144[a]	2.44 ± 0.205[a]
AL	ND	ND	2.35 ± 0.187[a]	2.75 ± 0.200[a]
		Coliform count (log_{10}cfu/g)		
CONTROL	ND	ND	ND	ND
AL	ND	ND	ND	ND
		Yeast and Mould count (log_{10}cfu/g)		
CONTROL	ND	ND	ND	1.93± 0.196
AL	ND	ND	ND	1.73± 0.152

*Mean± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly (P<0.05).n=6 for each treatment. AL= Almond incorporated chevon nuggets

value during refrigerated and frozen storage of meat and meat products had also been reported by (Devatkal *et al*., 2004). Brewer *et al*. (1992) reported that increase in TBARS values on storage might be attributed to oxygen permeability of packaging material that led to lipid oxidation.

Microbiological characteristics

The mean values of various microbiological characteristics of cooked chevon nuggets incorporated with 0 and 10 percent level of almond during refrigerated storage (4±1°C) are presented in Table 4.

Total plate count

Total plate count increased from day 0 to subsequent days of storage in all preparations. Optimised almond treated nuggets recorded comparable values of TPC with respect to control. The increase in total plate count might be due to permissive temperature and relative availability of moisture and nutrients for the growth of mesophillic bacteria. Nath and Mahapatra (1995) also reported a linear increase in total plate count of chicken patties stored under refrigeration.

Psychotropic count

Psychotropic colonies were not observed on day 0 and day 14 in any of the chevon nugget preparations. A non-significant effect of almond was observed on the psychotropic count.

This appearance of Psychotrophs after such a long gap might be caused by sufficient heat treatment during cooking, which drastically injured and killed the psychotrophic population reducing the number of surviving injured and resistant ones and hence do no not form any colony.. A detectable count on day 14 onwards while nil on preceding observations might be attributed to the fact that bacteria generally need some lag phase before active multiplication is initiated (Jay, 1996). A gradual increase in psychotrophic counts during storage of meat products had earlier been reported by Sen (1993), Nag (1998) and Kalaikanan (1998).

Table 5: Effect of refrigeration storage on the sensory characteristics of chevon nuggets incorporated with optimum levels of almond aerobically packaged in LDPE films and stored in refrigerator. (Mean ±SE)*

TREATMENT	STORAGE PERIOD IN DAYS			
0	7	14	21	
		Appearance		
Control	6.71±0.124^{Aa}	5.72±0.110^{Ab}	5.33 ± 0.139^{Ac}	4.17 ± 0.151^{Ad}
AL	6.98 ± 0.078^{Ba}	6.05 ± 0.102^{Bb}	5.70±0.107^{BCc}	4.38 ± 0.133^{Ad}
		Flavour		
Control	6.59± 0.118^{ABa}	5.96 ± 0.121^{Ab}	4.15 ± 0.134^{Ac}	Not tasted
AL	7.03 ± 0.079^{Ca}	5.97 ± 0.078^{Ab}	4.63 ± 0.142^{Bc}	Not tasted
		Texture		
Control	6.40 ± 0.130^{Aa}	6.16 ± 0.090^{Aab}	6.22±0.061^{Aab}	5.94 ± 0.150^{Ab}
AL	6.82 ± 0.079^{Ba}	6.58 ± 0.113^{Bab}	6.72 ± 0.095^{Ba}	6.32 ± 0.136^{Bb}
		Juiciness		
Control	6.41 ± 0.093^{Aa}	5.93 ± 0.099^{Ab}	5.44 ± 0.113^{Ac}	Not tasted
AL	6.79 ± 0.097^{Ba}	6.09± 0.062^{ABb}	5.33 ± 0.084^{Ac}	Not tasted
		Overall acceptability		
Control	6.36 ± 0.102^{Aa}	5.95 ± 0.092^{Ab}	4.79 ± 0.146^{Ac}	3.75 ± 0.086^{Ad}
AL	6.83 ± 0.062^{Ba}	6.28± 0.093^{BCb}	4.69 ± 0.126^{Ac}	3.96 ± 0.142^{Ad}

*Mean± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly (P<0.05). Mean values are scores on 8 point descriptive scale where 1- extremely poor and 8- extremely desirable. n = 21 for each treatment. AL= walnut incorporated chevon nuggets.

Coliform count (log cfu/g)

No coliform colonies were detected in any of the preparations on any interval of storage period. The absence of coliforms during storage depicts that heat processing and subsequent hygienic handling and packaging was effective to control coliform growth in chevon nuggets. Pardi *et al*. (2001) reported that presence of high concentration of coliforms in food is indicative of failures during processing, heat treatment or inadequate hygiene.

Yeast and mould count (log cfu/g)

Yeast and mould count was observed on day 21. No significant (P>0.05) effect of almond was found in treated products when compared to control on the yeast and mould count. This appearance of yeast and mould count could be possibly due to post processing contamination. Singh *et al*. (2011) reported that yeast and mold appeared during the last day of storage of chicken snacks due to the availability of nutrients in meat.

Sensory parameters

The mean values of various sensory parameters of cooked chevon nuggets incorporated with 0 and 10 percent level of almond during refrigerated storage (4±1°C) are presented in Table 5.

A decreasing trend in the scores of appearance and colour, flavour, juiciness and overall acceptability was observed both in control and optimized nuggets at the progressive storage intervals. Decrease in colour scores with advancement of storage days might be attributed to oxidative fading, moisture loss) and non-enzymatic browning from reaction between lipid oxidation products and amino acids (Chandralekha *et al*., 2012). A decrease in appearance and colour scores of chicken meat patties under refrigerated storage were reported by kala *et al.* (2007). Decrease in flavour scores might be correlated with the increase in TBARS value in the meat products stored under aerobic conditions (Taraladgis *et al*., 1960). Study of Chandralekha *et al*. (2012) showed that reduction in flavour scores might be due to the overall reduction in the quantum of volatile flavour components from spices and condiments and due to fat oxidation during storage. The decline in flavour score in all products could be attributed to fat loss as fat content of meat product has greater role in development of flavour as reported by Pearson and Gillet (1997). Some dehydration of the product during refrigerated storage could be the reason for lower juiciness scores during refrigerated storage in low density polyethylene. Decline in textural scores on 21^{st} day of storage might be attributed to proteolytic and disulphide bond changes (Santamaria *et al*., 1992) taking place with progress of storage period. The observations from present study indicated that both control and walnut treated chevon nuggets retained acceptable physico-chemical characteristics, colour values, microbiological counts and good to very good sensory rating during storage in LDPE pouches under refrigerated storage at 4±1ºC for 14 days. Hence reformulated chevon nuggets evolved in this study could be safely stored up to 14 days of storage at 4±1°C without any marked loss of physico-chemical, colour, microbiological and sensory quality.

CONCLUSION

On the basis of analysis of different physico-chemical, proximate and sensory parameters 5% percent of walnut was optimum for making designer chevon nuggets. The storage quality of the developed products was further studied and both control and almond treated optimized chevon nuggets maintained acceptable oxidative stability, microbial profile and sensory acceptability upto 14^{th} day of refrigeration storage (4±1ºC).

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