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Effect of walnut (*Juglans regia*) on quality of chevon nuggets

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Abstract
The walnuts were found to be very useful for the development of value added healthier chevon nuggets and various sensory and storage properties studied showed a significant changes. A total of Four different levels of walnuts (*Juglans regia*) (0, 5, 10 and 15%) were selected to replace lean meat in the formulation. The chevon nuggets developed were assessed for various sensory parameters. Based on these parameters, chevon nuggets containing optimum (10%) levels of walnut were selected and further studied for its storage quality. The aerobically packed nuggets at the low density with the value of 0.930 g.cm^3 with control treatment of 0% levels of walnut were studied for microbiological and sensory values for a connective three weeks' time period under the temperature regime (4±1 ^{o}C) below room temperature to evaluate storage parameters.

Keywords: Chevon, nuggets, refrigerated storage, walnuts

Introduction
The potential for value-addition in meat can be well acknowledged considering that demand for meat has been growing strongly ever since the dawn of 21st century due to the factors like industrialization and globalization which stimulated growth of per capita income (Deogade *et al*., 2008) [22]. Epidemiological studies show that there is an inverse relationship between regular nut consumption and risk of hypertension as well as a lower prevalence for hypertension at high nut consumption (Wang *et al.*, 2013) [53].
These effects were often attributed to the favourable fatty acid profile and the dietary fibre content of nuts. Some studies also showed an increase in HDL-cholesterol (HDL-C) as well as a decrease in triglycerides (TG) and Apo lipoprotein B (Apo B) (Tay *et al*., 2011; Li *et al.*, 2010) [42, 35].
Vegetables like brinjal are also rich in antioxidants like phenols and its related compounds (Dar *et al*. 2015) [15] which provide antioxidant activity (Dar *et al*. 2014a, b; Dar *et al*. 2016a, b) [17, 18, 14, 16, 19] as honey (Tlak Gajger 2015) [46] and deterrence to insect pests (Dar and Mir 2016) [14, 16, 19]. Because of the evident health benefit, there is a need to study the importance of including a certain amount of walnutin human diets. In light of these considerations and the nutritional profile defined, the present study was undertaken to analyses the quality attributes of walnut enriched chevon nuggets and to study the effect of refrigeration on the storage quality of developed nuggets.

Material and Methods
Material Inputs
Leg cut of adult Bhakarwal goat carcass slaughtered by ritual method was purchased from the local market in the vicinity of SKUAST Jammu. The lean meat was obtained after deboning and trimming the fat manually. The lean meat so obtained was packed in LDPE pouches and stored at standard freezing temperature (-18 ± 2 ^{0}C) until it was used. Commercially available refined vegetable oil containing energy (900 Kcal/100 gram), saturated fatty acids (14g %) and cholesterol 0%was used in emulsion preparation. 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 walnuts 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 ^{0}C.

Preparation
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 cooked for 30±2 minutes after first steam. The boxes were allowed to cool at room temperature after removal from pressure cooker. The brick shaped chevon block so obtained was cut into nuggets.

Storage studies
The method described by US Army laboratories (Natick) (Koneicko *et al*., 1979) [34], was used for the estimation of Free fatty acids. Thiobarbituric acid reactive substances (TBARS) value of cooked products was determined using standard procedures (Witte *et al*., 1970) [54]. Total plate count, psychrophillic count, coliform count and yeast and mold count were determined by methods of APHA (1984) [3].

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*., 1979) [33]. The attributes of sensory evaluation included colour and appearance, flavour, juiciness, texture and overall acceptability

Statistical Analysis
The statistical analysis of data was carried out by analysis of variance and least significant difference tests (Snedecor and Cochran, 1994) [43]. In significant effects, least significant differences were calculated at appropriate level of significance for a pair wise comparison of treatment means.

Results and Discussion
Sensory attributes
The mean values of various sensory parameters of chevon nuggets fortified with 0, 5, 10 and 15 percent levels of walnut are presented in Table-2.
There was a gradual increase in sensory scores of appearance with increase in level of walnut. Flavour was significantly (P<0.05) lower at 15% level when compared to control, 5% and 10% levels. A similar influence of walnut on flavour of restructured beef steak has already been reported by Jimenez-Colmenero *et al*. (2003) [29]. Texture scores were comparable between control, 5 and 10% levels of walnut treatments and both control and 5% treatment scored significantly (P<0.05) higher than 15% level. In order to explain the effect of walnut on the texture properties of chevon nuggets, there are several contradicting factors to be considered that can influence the texture of walnut incorporated nuggets. One of the first aspects to consider is increased protein content, which in the given formulation conditions, is attributable to the walnut. Walnut protein is composed essentially of albumin (6.8%), globulin (17.6%), prolamin (5.3%) and glutelin (70.1%) (Karwai *et al*., 2000) [31]. Non-meat ingredients containing these kinds of proteins are commonly used in processed meats to improve textural (Sze-Tao *et al*., 2000) [44]. This behaviour can therefore encourage the formation of the kind of molecular associations implicated in protein gel network formation, thus producing harder textures. A second aspect to consider is the difference in fat/moisture and protein/moisture ratios. When walnut was added, the protein content of the walnut incorporated chevon nuggets increased, the moisture level decreased and the fat level increased ($P < 0.05$) with respect to control. Some authors have reported that these changes produce an effective concentration of muscle protein available for gel formation and thus related to harder structures (Claus and Hunt 1991) [11]. Again, previous studies (Cofrades *et al*., 2004), have suggested that walnut does not contribute to textural properties and they report softer structures when walnut content was increased and meat content reduced accordingly in the formulation of different meat products. This behaviour was attributed to a combination of several factors: primarily a reduced presence of myofibrillar proteins and a diluting effect of non-meat ingredients (walnut) in meat protein systems and the poorer gelling properties of walnut globular proteins at processing temperatures (70 °C), which interfered to some extent in myofibrillar meat protein interactions (Claus *et al*., 1990) [11]. The overall acceptability was significantly (P<0.05) higher at 10% level as compared to control, 5 and 10% levels. Several authors also reported that when walnut is added to frankfurters and restructured beef steak, some effects on sensory quality are perceived. In the reported cases addition of walnut was adjudged as slightly off-flavour (walnut-like) but this was not perceived as a negative element, since walnut incorporated products achieved positive scores(5.8 in comparision to the score of 5 for control) for overall acceptability (Jimenez-Colmenero *et al*., 2003; Cofrades *et al*., 2004) [29, 13],

Storage studies
The mean values of various storage parameters of cooked chevon nuggets incorporated with 0 and 10 percent level of walnut during refrigerated storage (4±1^{0}C) are presented in Tables 1 and 2.

pH
The pH values of both control and optimized walnut treated nuggets increased significantly (P<0.05) at the progressive storage intervals. The pH value of optimized walnut treated nuggets was significantly higher than control at day 7, 14 and 21 days of storage. 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 and to microbial load which may cause protein hydrolysis with the appearance of alkyl groups (Ibrahim and Desouky, 2009; Yassin 2003) [26, 55]. Aerobically packed products on refrigerated storage showed an increase in pH values due to more psychrotropic growth (Sahoo and Anjaneyulu, 1997) [39]. An increment of pH could be attributed to the modification of meat protein conformation during thermal denaturation (Ang and Hamm 1982) [1].

Free fatty acid (FFA)
FFA increased significantly (P<0.05) from day 0 to day 21 in all chevon nugget preparations. Walnut treated chevon nuggets maintained significantly (P<0.05) 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. Hydrolysis of proteins and other biochemicals in insects e.g. bees were hurdled during disease and pest attack. However some reports from European countries showed that protein digestion and metabolism is impaired due to various diseases (Tlak Gajger *et al*. 2011a, Tlak Gajger *et al*. 2014a,b) [47, 51, 48, 49] and pests in bees and other animals. The administration of the certain proteins, fatty acids and herbal preparations (Tlak Gajger. 2011b) [47, 51] were found suitable for midgut activity in bees (Tlak Gajger *et al*. 2013a,b; Jay singh and Cornforth, 2003) [50, 52, 28].

Thiobarbituric acid reactive substances (TBARS) value (mg malonaldehyde/kg) TBARS values followed a trend similar to FFA values with a significantly (P<0.05) increasing trend towards the advancement of storage period. TBARS values greater than 1 were observed in both control and optimized nuggets at day 14 and day 21 of storage period. It has been previously reported that TBARS values greater than 1 are usually associated with rancid flavour/odour by sensory panelists (Jay singh and Cornforth, 2003) [28].

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) [25]. Earlier it has been reported that TBARS values are affected by storage and increase gradually during storage period and increase in TBARS values on storage might be attributed to oxygen permeability of packaging material that led to lipid oxidation (Ferial *et al*., 2011; Brewer *et al*., 1992) [24, 8].

Total plate count (log cfu/g)

Total plate count (TPC) increased from day 0 to subsequent days of storage in all preparations. Optimized walnut treated nuggets recorded comparable values of total plate count 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. The results are in agreement with the previous findings of increase in total plate count of microwave cooked chicken seekh kababs under refrigerated storage (Bhat *et al.,* 2013) [7].

Psychotropic count (log cfu/g)

Psychotropic colonies were not observed on day 0 and day 7 in any of the chevon nugget preparations. There was a non-significant effect of walnut incorporation on the psychotropic count.

Psychotropic colonies appeared on day 14th of refrigeration storage. This appearance of Psychrotrophs after such a long gap might be caused by sufficient heat treatment during cooking, which drastically injured and killed the psychotropic population reducing the number of surviving injured and resistant ones to a non-countable limits (Jay, 1996) [27].

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) [27]. During storage, the environmental factors like temperature (5 ^{0}C), gaseous atmosphere and pH contribute to the growth of Psychrotrophs (Elisabeth-Borch *et al*., 1996) [23].

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 (Bhat *et al*., 2015) [6]. The presence of high concentration of coliforms in food is indicative of failures during processing, heat treatment or inadequate hygiene (Bhat *et al.,* 2015) [6]. Coliforms have been found to be be sensitive to heat treatment with a decimal reduction time under 2 minutes at 60^{0}C (Denis *et al*., 2006) [21].

Yeast and mould count (log cfu/g)

Yeast and mould colonies were not observed until 21st day of storage. Walnut treated optimized nuggets and control preparations maintained comparable yeast and mould counts throughout the storage period. The appearance of yeasts and moulds could be due to chemical and enzymatic activities which breakdown fat, protein and carbohydrates of meat product resulting in slime formation (Dave *et al.,* 2011) [20]. Earlier reports suggest that appearance of yeast and mould during the last day of storage of chicken snacks was due to availability of nutrients in meat (Singh *et al*., 2011).

Sensory parameters

The mean values of various sensory parameters of cooked chevon nuggets incorporated with 0 and 10 percent level of walnut during refrigerated storage (4±1^{0}C) are presented in Table-3.

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 aminoacids (Chandralekha *et al*., 2012) [9]. A decrease in appearance and colour scores of chicken meat patties under refrigerated storage were reported (Kala *et al*., 2007) [30]. Decrease in flavour scores might be correlated with the increase in TBA value in the meat products stored under aerobic conditions (Tarladgis *et al*., 1960). Studies 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 (Chandralekha *et al*., 2012) [9]. 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 flavor (Pearson and Gillet 1997) [38]. 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 21st day of storage might be attributed to proteolytic and disulphide bond changes taking place with progress of storage period (Santamaria *et al*., 1992) [40]. 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±1 ºC for more than 7 days. Hence reformulated chevon nuggets evolved in this study could be safely stored up to 7 days of storage at 4±1ºC

without any marked loss of physico-chemical, colour, microbiological and sensory quality.

Table 1: Effect of different levels of walnut on sensory attributes of cooked chevon nuggets (Mean ±SE)*

Sensory Attributes	Levels of walnut (%)			
	0	05	10	15
Appearance and colour	6.27±0.135^{a}	6.69±0.124^{b}	6.89±0.098^{b}	6.99±0.101^{b}
Flavour	6.83±0.106^{a}	6.79±0.113^{a}	6.89±0.076^{a}	6.42±0.129^{b}
Juiciness	6.35±0.132^{a}	6.64±0.112^{a}	6.938±0.090^{b}	6.91±0.093^{b}
Texture	6.64±0.080^{a}	6.67±0.085^{a}	6.50±0.134^{ab}	6.22±0.148^{b}
Overall acceptability	6.38±0.125^{a}	6.50±0.099^{a}	6.89±0.081^{b}	6.50±0.087^{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(twenty one) for each treatment.

Table 2: Effect of refrigeration storage on the microbiological characteristics of chevon nuggets incorporated with optimum levels of almond and walnut 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	2.59± 0.082^{Aa}	3.64± 0.152^{Ab}	4.79 ± 0.145^{Ac}	6.33 ±0.205^{Ad}
WL	2.66 ±0.095^{Aa}	3.41±0.143^{Ab}	4.65±0.181^{Ac}	6.033±0.191^{Ad}
Psychrotrophic count (log_{10} cfu/g)				
Control	ND	ND	2.23 ± 0.141^{Aa}	2.33±0.199^{ABa}
WL	ND	ND	2.46±0.302^{Aa}	2.78±0.115^{Aa}
Coliform count (log_{10}cfu/g)				
Control	ND	ND	ND	ND
WL	ND	ND	ND	ND
Yeast and Mould count (log_{10}cfu/g)				
Control	ND	ND	ND	2.17 ± 0.130^{A}
WL	ND	ND	ND	2.25±0.145^{A}

*Mean± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly (P<0.05).WL= walnut incorporated chevon nuggetsn=6 (six observations) for each treatment

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

Treatment	Storage period in days			
	0	7	14	21
Appearance				
Control	6.33±0.098^{Aa}	6.05±0.113^{Ab}	5.25±0.127^{Ac}	4.38±0.152^{Ad}
WL	6.92±.073^{Ba}	6.48±0.097^{BCb}	5.77±0.091^{Bc}	4.63±0.151^{Ad}
Flavour				
Control	6.75±0.123^{ABa}	6.15±0.148^{ABb}	3.97 ± 0.116^{Ac}	Not tasted
WL	6.61±0.112^{Aa}	5.98±0.092^{Ab}	3.82±0.118^{Ac}	Not tasted
Texture				
Control	6.61 ± 0.136^{Aa}	6.30 ± 0.119^{Aa}	6.26±0.121^{Aa}	5.13 ± 0.135^{Ab}
WL	6.71±0.069^{Aa}	6.27±0.113^{Ab}	6.01±0.124^{ABb}	5.60±0.121^{Bc}
Juiciness				
Control	6.39 ± 0.096^{Aa}	6.00 ± 0.079^{Ab}	5.76±0.093^{ABb}	Not tasted
WL	6.92 ±0.090^{BCa}	6.08 ±0.091^{ABb}	5.62 ±0.108^{Ac}	Not tasted
Overall acceptability				
Control	6.48 ± 0.108^{Aa}	6.03 ± 0.081^{Ab}	5.30±0.145^{Ac}	3.78 ± 0.05^{Ad}
WL	6.9 ± 0.065^{ABa}	6.30 ±0.076^{Bb}	5.19±0.121^{Ac}	4.37 ±0.171^{Bd}

*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 WL= walnut incorporated chevon nuggets. n=21 (twenty one observations) for each treatment.

Conclusions

The reformulation of chevon nuggets with added walnut in order to develop healthier cooked meat products showed a high level of acceptability. The storage quality of the developed products were found acceptable and it was further studied and both control and walnut treated optimized chevon nuggets maintained acceptable microbial profile, TBARS, FFA values and better sensory acceptability on 7th dayof refrigeration storage (4±1^{0}C). Incorporation of walnuts (10%) successfully improved the quality of chevon nuggets and could be used commercially to develop healthier functional meat poroducts**.**

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