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Effect of Walnut (*Juglans regia*) on Physico-chemical, Sensory and Storage Quality of Chevon Nuggets

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ABSTRACT

The present study was undertaken to evaluate the possibl utilization of walnuts in the development of value added healthier chevon nuggets and to study their physico-chemical, sensory and storage properties. Four different levels of walnuts (Juglans regia) (0, 5, 10 and 15%) were selected to replace lean meat in the chevon nugget formulation. The chevon nuggets developed were analysed for various physicochemical and sensory parameters. Based on various parameters viz. physicochemical and sensory parameters a 10% level of walnuts was adjudged as optimum for the preparation of walnut enriched chevon nuggets. Based on this study, chevon nuggets containing 10% levels of walnut was further studied for its storage quality. The optimized nuggets were aerobically packaged in low density polyethylene pouches with density value of 0.930 g/cm^3 along with control (0% levels of walnut) and evaluated for storage stability (physicochemical, microbiological and sensory parameters) for 21 days under refrigerated condition (4±1°C). Both walnut enriched optimized nuggets and control were successfully stored for 7 days under refrigerated conditions (4±1°C) without marked loss in storage quality.

Keywords: *Chevon, Nuggets, Refrigerated Storage, Walnuts*

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INTRODUCTION

The potential for value-addition in meat could 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). Diet is one of the most important factor determining well-being and health and therefore there is increasing demand for safer and healthier foods. A promising approach to improve health care would be to produce a healthier food as a preventive health care strategy (Decker and Park 2010).

There is a growing evidence that regular consumption of nuts is associated with a variety of health promoting effects and to date there are no adverse effects reported even with high nut intake (Fischer *et al.* 2013). Walnuts are rich in dietary fibre (6.7%), unsaturated fatty acids (70% of total fatty acids), minerals (2%) and polyphenols (51.4-58.2 mg gallic acid equivalents GAE/g FW) and are increasingly associated with an overall healthy lifestyle and reduced risk of disease (Akbaraly *et al.* 2011; Rabrenovic *et al.* 2008). Recent studies by Manchester *et al.* (2000) and Gonzalez Gomez *et al.* (2009) have indicated that walnuts (*Juglans regia*) contain healthful constituents like melatonin (100 ng/g) and serotonin (9.8ng/g). Melatonin, an indolamine is effective in regulating sleep-wake cycle so its consumption may be an effective and natural way to regulate sleeping disorders (Wassmer and White-house 2006). It is also a potent free radical scavenger and broad-spectrum antioxidant. Serotonin is a neurotransmitter that regulates mood, appetite, sleep and improve health benefits because of improvement in oxidation, inflammation and cardiovascular risk markers (Tulipani *et al.* 2011). Epidemiological studies show that there is an inverse relationship between regular nut consumption and risk of hypertension with a lower prevalence of hypertension at high nut consumption (Weng *et al.* 2013). These effects were often attributed to the favorable 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 apolipoprotein B (Apo B) (Tay *et al.* 2011).

Because of the evident health benefit, there is a need to study the importance of including a certain amount of walnut in human diets. In light of these considerations and the nutritional profile defined, the present study was undertaken to analyze the quality attributes of walnut enriched chevon nuggets and to study the effect of refrigeration on the storage stability of developed nuggets.

MATERIALS AND METHODS

Procurement of materials for the preparation of chevon nuggets: Leg cut of 12 months old 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) for 5 days. 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 3:2:1 ratio. The spice mix formula was standardized in the laboratory. The dried walnuts were obtained from local market of Srinagar. The shells of these walnuts were removed manually and the kernels were ground in powder form and packed in polythene bags and refrigerated at 4 ± 1^{0}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%,

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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 120 ppm. Meat emulsion was prepared by using Bowl chopper (Marsango, Italy). The emulsion so obtained was molded in oil coated rectangular stainless steel boxes with 15×7.5×15×7.5 cm dimension 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 (4×2cm).

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

Storage studies: The method described by US Army laboratories (Natick) (Koniecko *et al.* 1979) 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). Total plate count, psychrophillic count, coliform count and yeast and mold count were determined by methods of APHA (1984). Product stored at 4±1°C was evaluated at 7 days interval for the parameters described above.

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

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

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 walnut (*Juglans regia*) are represented in Table 1.

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

Parameters	Levels of walnut (%)			
	0	5	10	15
pH	6.17 ± 0.07^{a}	6.39 ± 0.13^{a}	6.37 ± 0.05^{a}	6.47 ± 0.13^{a}
Moisture	59.64 ± 0.53^{a}	57.81 ± 0.865^{ab}	56.15± 1.28^{bc}	53.31± 1.23^{c}
Protein	17.57± 0.26^{a}	18.47± 0.38^{ab}	18.81± 0.4^{b}	19.06 ± 0.52^{b}
Fat	9.76 ± 0.35^{a}	11.59 ± 0.53^{b}	13.09 ± 0.31^{c}	13.92 ± 0.54^{c}
Ash	1.55 ± 0.08^{a}	1.80 ±0.06^{ab}	2.14 ±0.15^{bc}	2.19 ±0.12^{c}
Emulsion stability	80.34±1.09^{a}	83.66±0.80^{b}	84.01±0.95^{b}	83.62±0.83^{b}
Cooking yield	81.10 ± 0.84^{a}	83.57 ± 0.54^{b}	84.08 ± 0.35^{b}	83.07 ± 0.54^{b}

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

pH and proximate composition: Addition of walnut resulted in non-significant (p>0.05) increase in pH values of cooked chevon nuggets. Similar results have been reported for the influence of walnut upon the pH of restructured beef steaks with added walnut (Jimenez-Colmenero *et al.* 2003). These results are also consistent with the findings in healthier frankfurters added with walnut (Ayo *et al.* 2005). It has also been reported that increasing the amount of walnuts, while keeping the meat protein content constant, had no significant effect on the pH of meat batters (Cofrades *et al.* 2006). There was a gradual decrease in moisture content (56.15±1.28%) at 10% levels of chevon nuggets with increasing levels of walnut incorporation. Mostafa (2013) on the basis of his findings on evaluation of selected nuts and their protein functional properties reported about 1.1% moisture level in walnut kernels. So, lesser moisture content in walnut incorporated chevon nuggets is most probably attributed to the reported lesser moisture values in walnut incorporated nuggets. Similar results were recorded in case of restructured beef steaks with added walnuts (Jimenez-Colmenero *et al.* 2003).

Protein content of chevon nuggets prepared by incorporating different levels of walnut followed a non-significant (p>0.05) variation but was comparable to that of control. The results obtained were in consonance to the results obtained in case of restructured beef steaks with added walnuts (Jimenez-Colmenero *et al.* 2003).

The fat content of chevon nuggets prepared by incorporating different levels of walnut was significantly (p<0.05) higher as compared to control. The increase in fat content of chevon nuggets incorporated with walnuts is attributed to the higher levels of fat (50-72%) in walnut kernels (Periera *et al.* 2008). Hence, increase in the levels of walnut incorporation in chevon nuggets is associated with increase in fat content of the products.

Ash content of chevon nuggets prepared by incorporating different levels of walnut was significantly (p<0.05) higher at 10% and 15% levels as compared to control. These results were consistent with

other findings in meat batters with different levels of walnut (Ayo *et al.* 2005). The increase in the ash content can be attributed to the fact that added walnuts contain appreciable amount of minerals (1.7%) as reported by Rabrenovic *et al* (2008).

Emulsion stability and cooking yield: Chevon nuggets prepared by incorporating different levels of walnut had significantly ($p<0.05$) higher values of emulsion stability and cooking yield than control. Walnut has been reported to increase the ability of the protein matrix to retain water and bind fat, hence providing a possible reason of improvement in emulsion stability and cooking yield in walnut enriched chevon nuggets (Jimenez-Colmenero *et al.* 2003). Earlier studies reported a reduction in cooking loss, but significantly only when 10% or more of walnut was added (Jimenez-Colmenero *et al.* 2003). However, in contrast Lui *et al.* (1991) have reported increased cooking loss as the fat content increases.

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.

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

Sensory Parameters	Levels of walnut (%)			
	0	5	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}
	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}
	6.64±0.080^{a}	6.67±0.085^{a}	6.50±0.134^{ab}	6.22±0.148^{b}
	6.38±0.125^{a}	6.50±0.099^{a}	6.89±0.081^{b}	6.50±0.087^{a}
acceptability				

*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.

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). 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). Non-meat ingredients containing these kinds of proteins are commonly used in processed meats to improve textural properties (Sze-Tao *et al.* 2000). 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). 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 variation 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.

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 (Cofrades *et al.* 2006; Claus and Hunt 1991).

In the reported studies 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 comparison to the score of 5 for control) for overall acceptability (Jimenez-Colmenero *et al.* 2003; Cofrades *et al.* 2004).

Storage study

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 3 and 4.

pH: pH values of both control and 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). Aerobically packed products on refrigerated storage showed an increase in pH values due to more psychrotropic growth (Sahoo and Anjaneyulu 1997).

An increment of pH could be attributed to the modification of meat protein conformation during thermal denaturation (Ang and Hamm 1982).

Table 3: Effect of refrigeration storage on the physico-chemical 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
	pH			
CONTROL	6.17 ± 0.027^{Aa}	6.26± 0.021^{Ab}	6.36± 0.015^{Ac}	6.47± 0.034^{Ad}
WL	6.20±.016^{Aa}	6.31±.107^{Bb}	6.43±0.010B^{Cc}	6.53±0.01^{Bd}
	TBARS (mg malonaldehyde/kg)			
CONTROL	0.347± 0.001^{Aa}	0.722±0.002^{Ab}	1.03 ± 0.016^{Ac}	1.29 ± 0.016^{Ad}
WL	0.335±0.008^{Aa}	0.708±0.005^{Bb}	1.01± .032^{Ac}	1.21±0.020^{Bd}
	FFA (% oleic acid)			
CONTROL	0.087±0.001^{Aa}	0.127±0.001^{Ab}	0.211±0.001^{Ac}	0.363±0.001^{Ad}
WL	0.074±0.001^{Ba}	0.116±0.001^{Db}	0.201±0.001^{Bc}	0.356±0.001^{Bd}

*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 nuggets. n=6 (six observations) for each treatment.

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.

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. 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 (Jaysingh and Cornforth 2003). 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). 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).

Table-4: 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 nuggets n=6 (six observations) for each treatment.

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).

Psychrotropic count (log cfu/g): Psychrotropic 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 psychrotropic count. Psychrotropic 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 psychrotrophic population reducing the number of surviving injured and resistant ones to a non-countable limits (Jay 1996). 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). 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).

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

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). 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.

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 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 aminoacids (Chandralekha *et al.* 2012). A decrease in appearance and colour scores of chicken meat patties under refrigerated storage were reported (Kala *et al.* 2007). Decrease in flavour scores might be correlated with the increase in TBA value in the meat products stored under aerobic conditions. 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). 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). Evaporative loss of moisture from 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). 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^{o}C for more than 7 days. Hence reformulated chevon nuggets developed in this study could be safely stored up to 7 days of storage at 4±1^{o}C without any marked loss of physico-chemical, colour, microbiological and sensory quality.

CONCLUSIONS

The present study showed that the formulation of chevon nuggets with added walnut in order to develop healthier cooked meat products, altered their physicochemical properties but the resulting product possessed acceptable physicochemical and sensory properties when compared with control nuggets. On the basis of analysis of different physico-chemical and sensory parameters 10 percent of walnut was optimum for making functional chevon nuggets. Chevon nuggets prepared with 10% levels of walnut had highest overall acceptability (6.89±0.081) based on sensory parameters. The storage quality of the developed products 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 day of 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 products.

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ETHICS STATEMENT: Not applicable

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