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EFFECT OF NISIN ON OXIDATIVE STABILITY AND MICROBIAL QUALITY OF ALMOND- AND WALNUT-ENRICHED CHEVON NUGGETS

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ABSTRACT

A study was undertaken to assess the efficacy of nisin on oxidative stability and microbial quality of chevon nuggets supplemented with almond and walnut at 5 and 10% level, respectively. Chevon nuggets were aerobically packaged in low density polyethylene (LDPE) pouches along with control and evaluated for storage quality for 21 days under refrigerated conditions (4±1°C). The products were analyzed for various physico-chemical, microbial and sensory parameters. The study revealed that ith increase in storage period the free fatty acid (FFA) content, thiobarbituric acid reactive substances (TBARS), total plate count and psychrophillic count showed significant increase; whereas sensory parameters showed significant decrease. Nisin treated preparations had significantly low TBARS and FFA values than non-nisin treated preparations. Nisin-treated preparations had significantly low total plate- and psychrotropic-counts. The nisin treated products were successfully stored for 14 days under refrigerated conditions (4±1°C) in LDPE pouches and the product showed better acceptability.

Key words: Almond, chevon, nisin, nuggets, refrigerated storage, walnut

INTRODUCTION

Meat has always been appreciated as a food of great nutritional value and its consumption is associated with good health and prosperity. The novel idea of using plant products in meat industry is an uncommon practice though various types of plant-based ingredients have been used to harvest technological, sensory, economic and nutritional benefits. Most importantly, lipids are amongst the bioactive components that have received much attention particularly with respect to the development of potential meat-based functional foods (Jimenez-Colmenero, 2007). The meat products are highly perishable due to easy nutrient availability for microbial growth (Vernozy-Rozan *et al.*, 2002). The special characteristics of fruit nuts, therefore, offer great potential to develop functional meat products. Until now the approaches to seek improved food safety have relied either on the use of chemical preservatives or antibiotics or some drastic physical treatments like high temperatures or refrigeration. However, these methods have many drawbacks *viz*., carcinogenic and toxicological concerns besides adverse changes in organoleptic characteristics and loss of nutrients (Rasooli, 2007). Since food safety is a health concern, therefore antimicrobial peptides from lactic acid bacteria (LAB) that target food pathogens without adverse effects have often been exploited (Reham, 2012).

Bacteriocins, the antimicrobial proteins or peptides, are ribosomally synthesized which kill closely related bacteria (Klaenhammer, 1993. Nisin is a polycyclic bacteriocin produced by *Lactococcus lactis*. Numerous reports have revealed that the addition of nisin to intact or processed meat products inhibit pathogenic and spoilage bacteria (Chung *et al*., 1989; Vignolo *et al*., 1996). Chevon nuggets are one of the most acceptable value added comminuted emulsion-based products that is either baked (steam cooked) or consumed after frying. The present study was aimed to evaluate the efficacy of nisin as a preservative in chevon nuggets treated with standardized levels of almond and walnut during refrigeration storage.

MATERIALS AND METHODS

Leg cut of adult Bhakarwal goat carcass, slaughtered by ritual method, was purchased from a local market of Jammu (J&K). The lean meat was obtained after deboning and trimming the fats manually. Lean meat was packed in low density polyethylene (LDPE) pouches and stored at freezing temperature (-18±2°C) till use. Commercially available refined cholesterol-free vegetable oil containing 900 k cal energy 100 g^{-1} and 14 g% saturated fatty acids was used for emulsion preparation. Condiments paste used contained fresh onion, garlic and ginger in 3:2:1 ratio. The spice mix formula was standardized in laboratory. The dried almond and walnut were obtained from a local market in Srinagar (J&K). The shells of these nuts were removed manually and kernels powdered, packed in polythene bags and refrigerated at 4±1°C. Nisin was procured from Hi-Media, Mumbai (India). The minimum potency of nisin used was 900 IU mg^{-1} containing NaCl (≥50%).

Preliminary trials were conducted to optimize the optimum levels of almond and walnut for preparation of reformulated chevon nuggets. Based on preliminary trials, 5% level of almond and 10% level of walnut were considered optimum for the preparation of almond- and walnut-enriched chevon nuggets. These optimized almond- and walnut-enriched chevon nuggets were selected for storage study to assess the oxidative stability and microbial quality with and without the addition of nisin (500 IU g^{-1}). The formulation of chevon nuggets that served as control was 68% lean meat, 9% added water , 8% vegetable oil, 3% condiment mixture, 4% refined wheat flour, 2.5% whole egg liquid, 2.5% spice mixture, 2% common salt, 0.5% sugar, 0.3% monosodium glutamate, 0.2% sodium hexametaphosphate and 120 ppm sodium nitrite.

Meat emulsion for the preparation of chevon nuggets was prepared by using a bowl chopper (Marsango, Italy). The batter obtained was moulded in oil-coated rectangular stainless steel boxes and cooked for 30 min after 1^{st} steam. The boxes were allowed to cool at room temperature after their removal from pressure cooker. The brick shaped chevon blocks so obtained were cut into nuggets. The pH of chevon nuggets was determined as per Keller *et al*. (1974) using a digital pH meter. Free fatty acid content was determined by US Army Laboratories (Natick) method [Koniecko, 1979]. Thiobarbituric acid reactive substances (TBARS) value of cooked products during storage was determined as per Witte *et al*. (1970).

Total plate-, psychrophillic-, coliform- and yeast and mold-counts of cooked chevon nuggets were determined as per APHA method (1984). Readymade media (Hi-Media) were used for microbial analysis. Plate count agar was used for the estimation of total plate count and psychrotropic count, violet red bile agar was used for the estimation of coliform count and potato dextrose agar was used for the estimation of yeast and mold count. Meat sample (10 g) was taken aseptically and blended with 90 mL 0.1% sterile peptone. Ten-fold serial dilution was done in pre-sterilized tubes containing 9 mL 0.1% peptone water. Sample preparation was done under laminar flow (Thermo Electron Corporation, Germany). The sensory evaluation of products was done for colour, appearance, flavour, juiciness, texture and overall acceptability by a panel of seven trained judges based on a eight point hedonic scale, wherein 8 denoted excellent, 7- very good, 6- good, 5- fair, 4- slightly poor, 3- poor, 2- very poor and 1- extremely poor (Keeton *et al.*, 1983). The data was statistically analyzed by employing the analysis of variance and least significant difference tests (Snedecor and Cochran, 1980). Pair's 't' test was employed to assess significance between the means.

RESULTS AND DISCUSSION

The mean values of various physicochemical parameters studied to predict oxidative stability of cooked chevon nuggets supplemented with either 10% walnut or 5% almond, with and without nisin during refrigerated storage (4±1°C) are given in Table 1. A significant decrease in the pH of products was observed on 7th day of storage in all the preparations; thereafter a progressive increase was noticed in all chevon nuggets. The addition of nisin insignificantly affected pH. Decrease in pH observed on 7th day of storage may be due to lactic acid production during anaerobic glycolysis (Raham, 2012). The increase in pH on extension of storage period may be due to the formation of volatile basic nitrogen under low temperature (Ibrahim and Desouky, 2009) and also due to the microbial load which appears to incite protein hydrolysis (Yassin, 2003). Koplay and Sezer (2013), however, did not notice any remarkable change in pH between nisin-treated and control groups during storage. Behnam *et al.* (2013) reported similar findings in case of rainbow trout stored under refrigeration. Contrarily, Reham (2012) reported a significant uniform decrease in minced beef. In present study, free fatty acids (FFA) depicted a significant increase from initial day to 21st day in all

Table 1: Effect of nisin (500 IU g^{-1}) on oxidative stability of chevon nuggets incorporated with almond and walnut aerobically packaged in LDPE films and stored in refrigerators

Treatments	Storage period (days)			
	0	7	14	21
		pH		
Control	6.17 ± 0.033^{Aa}	6.01± 0.014^{Ab}	6.19± 0.034^{Aa}	6.42±0.018^{Ad}
AL	6.19±0.020^{Aa}	6.05± 0.018^{Ab}	6.27±0.013^{ABCc}	6.41±0.017^{Ad}
ALN	6.18 ± 0.021^{Aa}	6.05± 0.016^{Ab}	6.25± 0.020^{ABc}	6.41±0.024^{Ad}
WL	6.19± 0.020^{Aa}	6.07± 0.027^{Ab}	6.34± 0.034^{Cc}	6.52± 0.015^{Bd}
WLN	6.20 ± 0.02^{Aa}	6.05 ±0.020^{Ab}	6.20±0.008^{ABa}	6.36± 0.028^{Ac}
	Thiobarbituric acid reactive substances (TBARS) [mg malonaldehyde kg^{-1}]			
Control	0.127±0.007^{Aa}	0.407±0.018^{Ab}	0.82 ± 0.017^{Ac}	1.28±0.079^{Ad}
AL	0.121±0.002^{Aa}	0.367 ± 0.02^{Ab}	0.72 ± 0.019^{Bc}	1.16 ±0.057^{Ad}
ALN	0.115±0.001^{Aa}	0.235±0.011^{Bb}	0.40 ± 0.019^{Cc}	0.728 ±0.01^{Bd}
WL	0.115±0.008^{Aa}	0.37±0.024^{Ab}	0.82±0.024^{Ac}	1.21± 0.113^{Ad}
WLN	0.116±0.002^{Aa}	0.22±0.012^{Bb}	0.30 ± 0.015^{Dc}	0.71 ±0.017^{Bd}
	Free fatty acids (% oleic acid)			
Control	0.080±0.004^{Ada}	0.131 ± 0.002^{Ab}	0.233 ± 0.011^{Ac}	0.354 ± 0.006^{Ad}
AL	0.075 ±0.003^{Aa}	0.128 ± 0.009^{Ab}	0.210± 0.009^{Ac}	0.349 ± 0.006^{Ad}
ALN	0.067 ±0.009^{Aa}	0.080 ± 0.003^{Bb}	0.110± 0.005^{Bc}	0.148 ± 0.01^{Bd}
WL	0.071±0.003^{Aa}	0.121±0.002^{Ab}	0.218±0.007^{Ac}	0.359± 0.003^{Ad}
WLN	0.068± 0.003^{Aa}	0.080 ± 0.002^{Bb}	0.118 ± 0.005^{Bc}	0.159 ± 0.003^{Bd}

*Mean±SE with different superscripts in a row (small alphabet) and a column (capital alphabet) differ significantly (p<0.05) from each other. AL = Optimized almond-enriched nuggets, ALN = optimized almond-enriched nuggets with nisin (500 IU g^{-1}), WL = Optimized walnut enriched nuggets; and WLC = Optimized walnut enriched nuggets with nisin (500 IU g^{-1})

the preparations. Nisin-supplemented chevon nuggets had significantly low FFA than those without nisin on 7th, 14th and 21st day of storage. On day 0, the FFA values were comparable in both nisin-treated and untreated preparations. Increase in FFA appears due to the hydrolysis and oxidation of fat. Our findings are supported by Raju *et al.* (2003) in case of nisin-treated fish sausage.

In present study TBARS values showed significant increase with the advancement of storage period. TBARS were significantly low in nisin treatments on all storage intervals and were comparable with the findings of Rahem (2012) and Behnam *et al.* (2013). The increase in TBARS may be attributed to the lipid hydrolysis, oxidative rancidity and secondary products formation at refrigeration temperature (Forrest *et al.*, 1975). Low TBARS values in nisin treated optimized chevon nuggets may possibly be due to the chelation of metal ion or scavenging of reactive oxygen species caused from antioxidant activity of nisin (Lin and Yen, 1999).

The microbiological characteristics of cooked chevon nuggets supplemented with 10% walnut or 5% almond with and without nisin revealed increase in total plate count (TPC) with increase in storage period in all preparations (Table 2). TPC of nisin treated almond-chevon nuggets decreased significantly on 7th day as compared to the control but was comparable with control on 14th day. Addition of nisin in optimized nuggets reduced TPC which is very much as per expectations. Nisin is a broad spectrum bacteriocin with bactericidal activity, even in low concentrations, towards a wide range of Gram-positive bacteria including *Staphylococcus aureus* and *Listeria monocytogenes* (Parada *et al*., 2007). Similar effects on total colony count due to the addition of nisin have previously been reported (Reham, 2012). Behnam *et al.* (2013) reported that nisin affected the total viable count (TVC) in vacuum packaged rainbow trout.

Psychrotropic colonies were not observed on 0 and 7th day in any of the chevon nugget preparations. Psychrotrophic counts were significantly reduced in nisin-treated walnut and almond optimized nuggets both at 14th and 21st day of storage period. Kim *et al*. (2006) and Behnam *et al*. (2013) observed similar trend in psychrotropic count of nisin-treated Korean seasoned beef and vacuum

Table 2: Effect of nisin (500 IU g^{-1}) on microbial characteristics of chevon nuggets supplemented with almond and walnut, aerobically packaged in LDPE films and stored in refrigerator

Treatments	Storage period (days)			
	0	7	14	21
	Total plate count (log_{10} cfu g^{-1})			
Control	2.53 ± 0.087^{Aa}	3.67 ± 0.131^{Ab}	4.64 ± 0.181^{Ac}	6.24 ± 0.194^{Ad}
AL	2.62 ± 0.067^{Aa}	3.78 ± 0.205^{Ab}	4.39 ± 0.212^{Ac}	5.91 ± 0.191^{Ad}
ALN	2.25 ± 0.094^{Ba}	1.53 ± 0.132^{Bb}	2.11 ± 0.071^{Ba}	2.98 ± 0.066^{Bc}
WL	2.67± 0.096^{Aa}	3.40 ± 0.131^{Ab}	4.65 ± 0.162^{Bc}	6.03 ± 0.178^{Ad}
WLN	2.17 ± 0.037^{Ba}	1.26 ± 0.120^{Bb}	1.72 ± 0.162^{Bc}	2.78 ± 0.108^{Bd}
	Psychrotrophic count (log_{10} cfu g^{-1})			
Control	nd	nd	2.21± 0.144^{Aa}	2.44 ± 0.205^{Aa}
AL	nd	nd	2.35 ± 0.187^{Aa}	2.75 ± 0.200^{Aa}
ALN	nd	nd	0.47 ± 0.216^{Ba}	1.27 ± 0.125^{Bb}
WL	nd	nd	2.41± 0.112^{Aa}	2.71± 0.118^{Aa}
WLN	nd	nd	0.10 ± 0.017^{Ba}	1.36 ± 0.124^{Bb}
	Yeast and mould count (log_{10} cfu g^{-1})			
Control	nd	nd	nd	1.93 ± 0.196^{A}
AL	nd	nd	nd	1.73 ± 0.152^{A}
ALN	nd	nd	nd	1.92 ± 0.157^{A}
WL	nd	nd	nd	2.25 ± 0.145^{A}
WLN	nd	nd	nd	1.98 ± 0.167^{A}

*Mean±SE with different superscripts in a row (small alphabet) and a column (capital alphabet) differ significantly from each other; AL = Optimized almond-enriched nuggets, ALN = optimized almond-enriched nuggets with nisin (500 IU g^{-1}), WL = Optimized walnut enriched nuggets; and WLC = Optimized walnut enriched nuggets with nisin (500 IU g^{-1}); Coliform count was not within detection range; nd = not detected

Table 3: Effect of nisin (500 IU g^{-1}) on the sensory characteristics of chevon nuggets incorporated with optimum levels of almond and walnut aerobically packaged in LDPE films and stored in refrigerator

Treatments	Storage period (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}
ALN	7.01 ± 0.097^{Ba}	6.15 ± 0.067^{Bb}	5.94± 0.067^{Cb}	5.03 ± 0.128^{Bc}
WL	7.08 ± 0.062^{Ba}	5.91 ± 0.112^{ABb}	5.52±0.116^{ABc}	4.14 ± 0.130^{Ad}
WLN	7.02 ± 0.084^{Ba}	6.16 ± 0.091^{Bb}	5.83±0.105^{BCc}	5.05 ± 0.110^{Bd}
		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
ALN	7.02 ± 0.092^{Ca}	6.44 ± 0.095^{Bb}	4.86 ± 0.154^{Bc}	Not tasted
WL	6.55± 0.095^{Aa}	5.89 ± 0.099^{Ab}	3.79±0.120^{Ac}	Not tasted
WLN	6.86 ± 0.105^{BCa}	6.46 ± 0.123^{Bb}	4.87 ± 0.100^{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}
ALN	6.98 ± 0.060^{Ba}	6.53 ± 0.115^{Bb}	6.21 ± 0.137^{Ab}	6.39 ± 0.122^{Bb}
WL	6.84 ± 0.063^{Ba}	6.63± 0.094^{Ba}	6.21±0.120^{Ab}	6.30 ± 0.104^{Bb}
WLN	6.83 ± 0.090^{Ba}	6.64 ± 0.142^{Ba}	5.98 ± 0.150^{Ab}	6.57 ± 0.073^{Ba}
		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^{ABa}	5.33 ± 0.084^{Ac}	Not tasted
ALN	6.82 ± 0.085^{Ba}	6.39 ± 0.101^{Cb}	5.96 ± 0.079^{Bc}	Not tasted
WL	6.89 ±0.085^{Ba}	6.12 ± 0.085^{ABCb}	5.24 ±0.106^{Ac}	Not tasted
WLN	6.96 ± 0.085^{Ba}	6.27 ± 0.098^{BCb}	5.79 ± 0.074^{Bc}	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}
ALN	7.00 ± 0.069^{Ba}	6.48 ± 0.074^{Cb}	5.80 ± 0.097^{Bc}	5.19 ± 0.125^{Bd}
WL	6.95 ± 0.068^{Ba}	6.22 ± 0.061^{Bb}	4.71 ± 0.132^{Ac}	4.09 ± 0.153^{Ad}
WLN	6.98 ± 0.070^{Ba}	6.50 ± 0.079^{Cb}	5.94 ± 0.075^{Bc}	5.21 ± 0.137^{Bd}

*Mean± SE with different superscripts in a row (small alphabet) and a column (capital alphabet) differ significantly from each other; AL = Optimized almond-enriched nuggets, ALN = optimized almond-enriched nuggets with nisin (500 IU g^{-1}), WL= Optimized walnut enriched nuggets; and WLC= Optimized walnut enriched nuggets with nisin (500 IU g^{-1})

packaged rainbow trout, respectively. No coliform colonies were detected any of the preparation during storage period. The absence of coliforms during storage depicts that heat processing and subsequent hygienic handling and packaging effectively restricted coliform growth in chevon nuggets. Pardi *et al.* (2001) reported that coliform in food is indicative of failures during processing, heat treatment or inadequate hygiene. Yeast and mould count was observed on 21st day. No significant effect of nisin was seen in treated products as compared to untreated ones, since nisin is ineffective against yeast and fungi (Hampikyan and Ugur, 2007; Boziaris and Adams, 1999).

The mean sensory scores of aerobically packaged nisin (500 IU g^{-1}) treated walnut- and almond-enriched chevon nuggets revealed a decrease in the scores of appearance, colour, flavour, juiciness and overall acceptability with increase in storage period ((Table 3). A non-significant effect of nisin was observed on appearance and colour. However, nisin imparted a favourable impact on flavour, juiciness and overall acceptability especially on 14th day of storage as compared to other preparations. Li Wei (2012) reported that nisin could get more obvious fresh-keeping effect on chilled meat and could prolong freshness and shelf.

Conclusion: The study revealed that in preparation of chevon nuggets, nisin (500 IU g^{-1}) can be used in meat emulsion with beneficial impacts on physicochemical and sensory qualities of product. Addition of nisin in optimized chevon nuggets depicted significantly low microbial profile, TBARS and FFA values and better sensory acceptability upto 14^{st} day of refrigeration storage (4±1°C).

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