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# Development of low salt restructured chicken nugget by response surface methodology and its quality evaluation

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**Abstract**

Central composite design of Response Surface Methodology (RSM) was used to study the effect of sodium chloride; NaCl (0.5-1%) and salt replacer potassium chloride KCl (0.5-1%) in combination with whey protein concentrate (0.5-1.5%) and black gram flour (2-4%) on the overall acceptability, saltiness and instrumental hardness of low salt restructured chicken nuggets. Reduction of NaCl content to 0.75% in the product with the addition of 0.70% KCl, 1.18% whey protein and 2.5% black gram flour showed a desirability of 97%. The physico-chemical, sensory and textural properties as well as stability at refrigerated temperature (4±1°C) of low salt nuggets developed with optimized levels of the four factors were compared with control nuggets containing 1.5 % NaCl. Although the keeping quality of low salt nuggets was lower than control, a shelf life of at least 25 days was achieved under refrigerated condition.



## Introduction

In recent times, concerns about dietary sodium intake and its correlation with increased risks of hypertension and cardiovascular diseases have propelled meat researchers to develop newer strategies to reduce sodium content in meat products. Common salt is the major contributor of sodium in diet and also a vital ingredient in the development of meat products especially restructured and reformed meat products like steaks, roll, meatballs, nuggets, patties etc. Salt helps in the extraction of myofibrillar proteins to meat surface which on heat coagulation help in binding of adjacent meat pieces together (Hamm, 1986; Offer and Knight, 1988; Ruusunen and Puolanne, 2005; Doyle and Glass, 2010). Moreover, salt brings about conformational changes in myofibrillar proteins, which ultimately enhance the ability of protein to bind water (Offer and Trinick, 1983; Terrel, 1983). Reduction in salt content in such products will therefore, inevitably result in not just reduction of saltiness perception but also loss of desirable properties like water binding, tenderness and juiciness.

Strategies to reduce common salt in meat products include the use of salt replacers like potassium chloride, magnesium chloride, calcium chloride etc. potassium chloride (KCl) is the most common salt substitute used in low salt meat products, but at higher concentrations can confer a bitter taste to the product (Gou *et al*., 1996; Colmenero *et al*., 2005; Desmond, 2006; Guàrdia *et al*., 2008; Horita *et al*., 2011; Canto *et al*., 2014). Incidentally, potassium has a negative effect on blood pressure and at equal ionic strength potassium chloride interacts with meat proteins in a similar way as sodium chloride (Doyle and Glass, 2010). Addition of carbohydrates like starch flours and non meat proteins can compensate for losses owing to reduction of salt in meat products (Verma and Banerjee, 2012). Several studies have reported that whey protein can improve water binding, cooking yield and texture of meat products (Hongsprabhas and Barbut, 1999; Ulu, 2004; Pietrasik *et al*., 2007; Szerman *et al*., 2007, 2008, 2012) and thus, can be utilized in developing low salt meat products. Legume flours like black gram flour reportedly increase moisture retention and yield in meat products (Modi *et al*., 2003; Serdaroglu *et al.*, 2005; Bhat and Pathak, 2011).

Although a number of studies have focused on the development of low salt meat products using various salt replacers and extenders, these studies have not examined the simultaneous effect salt replacement and incorporation of non meat proteins and binders (Gou *et al*., 1996; Tseng *et al*., 2000; Gelabert *et al.*, 2003; Colmenero *et al*., 2005; Guàrdia *et al*., 2008; Askin and Kilic, 2009; López-López *et al*., 2010; Horita *et al*., 2011; Szerman *et al*., 2012). Researches undertaking the development of low salt restructured meat products are scarce (Cofrades *et al*., 2011;

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Canto *et al*., 2014) and studies employing statistical analysis to investigate the interaction of salt and salt replacer in meat products are even lesser (Pietrasik and Li-Chan, 2002; Saricoban *et al*., 2009). Response Surface Methodology (RSM) is an effective statistical tool for optimizing ingredient levels as well as process parameters in products. A central composite design of RSM makes it possible to study the effect of more than one variable simultaneously and obtain products with desired responses (Hunter, 1959; Gupta and Premavalli, 2012).

Therefore, the main objectives of this research was (i) to study the effect of variables namely common salt and salt replacer (KCl) levels in conjunction with non meat protein (whey protein concentrate) and black gram flour on the overall acceptability, saltiness and hardness of low salt restructured chicken nuggets; (ii) to optimize the levels of the four variables in order to obtain a product with maximum desirability and (iii) to compare the physico-chemical, sensory and textural attributes of low salt and control nuggets and to assess their shelf life at refrigerated temperature (4±1$^{o}$C).

## Materials and Methods

Fresh chicken meat was purchased from a local meat market (Mysore, India). Good quality spices, black gram flour and common salt were obtained from local market. Potassium chloride (food grade) was obtained from SV Scientific, Bangalore and whey protein concentrate (80% protein) was obtained from Pristine Organics, Bangalore. All chemicals and media used for analytical purpose were procured from Himedia (Mumbai, India).

### *Experimental design*

The statistical software Design Expert 6.09, Stat-Ease Inc., Minneapolis, USA, was used to set up the experimental design and to analyze data. A central composite design was used without any blocking. Four ingredient factors viz. common salt (sodium chloride, NaCl), potassium chloride (KCl), whey protein concentrate (WPC) and black gram flour were considered and the codes for these four independent variables were A, B, C and D, respectively. The ranges for the selected factors were chosen based on their satisfactory results from preliminary sensory trials. The minimum and maximum limits for the factors were selected as, 0.5 to 1% for NaCl, 0.5 to 1% for KCl, 0.5 to 1.5% for whey protein concentrate and 2 to 4% for black gram flour. Three responses were taken namely over all acceptability (OAA), saltiness and hardness, as these parameters influenced the product quality and acceptability. OAA and saltiness were evaluated using nine point hedonic scale rating test for likability and hardness measured using Texture Analyser (TA Plus, Lloyd Instruments, Hampshire, UK). The experimental design consisted of 14 factorial points, 8 axial points and 6 replicates at the centre. A total of 30 design points were generated for the four factors and the responses were evaluated.

### *Nugget preparation*

All separable subcutaneous fat and connective tissues were removed; the muscle was then cleaned and cut into cubes of about 3 cm. The lean meat was minced in a meat grinder (Sirman, Italy) using 8mm plate. Ground lean meat (75%) was blended with common salt, potassium chloride and half of the ice at low speed setting for 1 minute. This was followed by 1 minute resting time for protein extraction. Black gram flour and whey protein concentrate were evenly sprinkled on the meat mixture. Spices (2.5%; red chilli powder, coriander powder and cumin powder), condiments (2.5%; ginger-garlic) and remaining ice were added and blending continued at high speed setting for another 2 minutes till a viscous batter was formed. Final batters temperature did not exceed 15$^{o}$C. Batter thus obtained was placed in aluminum mold, packed compactly, covered and cooked in steam without pressure for 30 minutes. The internal temperature of cooked block was 72°C, measured using a probe type thermometer (HTA Instrumentation Pvt. Ltd., Bangalore). The meat block was cooled to room temperature, chilled overnight at 4°C and cut into nuggets of 4 x 2 x 2 cm$^{3}$. The nuggets were packed in 200 gauge polypropylene bags prior to sensory and physico-chemical analysis. The responses obtained for the 30 runs were analyzed using statistical software of the best-fit design and the optimized compositions for the four factors were obtained. Low salt nuggets were prepared with the optimized levels of whey protein concentrate, black gram flour, sodium chloride and potassium chloride. Control samples (1.5% NaCl) were prepared following the same method without adding potassium chloride and whey protein concentrate.

### *Sensory evaluation*

The nugget samples (30 combinations) were evaluated using nine point hedonic scale rating method for sensory preferences where 1= dislike extremely and 9=like extremely. Nuggets were deep fried in refined sunflower oil (180$^{o}$C for 2.5 minutes) and served warm to a semi-trained panel of 10 judges. Water was provided for rinsing. The results of saltiness and over all acceptability for the 30 samples were presented as mean of 10 evaluations. Sensory evaluation was also carried out in order to draw comparison between the low salt nuggets prepared using optimized levels of ingredients and control samples.

*Texture measurement*

Texture profile analysis of nuggets was conducted using Texture Analyser (TA Plus, Lloyd Instruments, Hampshire, UK) as per the method described by Bourne (1978). Samples were compressed twice to get an imitation of mastication which included first and second bite. Samples were placed on the fixed platform and compressed to 85% of their original height. A 5 mm cylindrical probe was connected to the moving crosshead which was cycled at a pre and post test speed of 30 mm/ min. The maximum clearance between the moving cross head and sample was maintained at 3 mm throughout the study. The measured and derived parameters like hardness (in Newton) springiness (in millimeter), cohesiveness, gumminess (in Newton) and chewiness (in Newton*millimeter) were estimated using Nexygen software.

*Analytical procedures*

Low salt nuggets were prepared using the optimized product formulation and their proximate composition and physico chemical characteristics were compared to those of the control nuggets. Moisture (hot air oven), protein (Kjeldahl), fat (solvent extraction) and ash (muffle furnace) contents of the nuggets were estimated as per the standard procedures of AOAC (1995). pH was determined using a digital pH meter (Cyberscan 510, Eutech Instruments, Malaysia). Water activity was determined using Labmaster. Aw (Novasina instrument, Switzerland) with the help of Novalog software. Water holding capacity, cooking loss and % shrinkage on frying were determined by the methods given by Lianji and Chen (1991), Sheard *et al.* (1998) and Modi *et al*. (2003) respectively.

*Shelf stability of restructured nuggets*

For storage studies the samples were kept at 4±1°C and examined at intervals of five days for 30 days. Changes during storage period were monitored by carrying out microbiological and sensory evaluations and by determining the TBARS (Thiobarbituric Acid Reactive substances) number. Microbiological evaluation in terms of total plate, yeast and mold, psychrotrophic and coliform bacteria counts of the samples were determined as per the methods described by Downes and Ito (2001). The bacteriological media were obtained from M/s. Himedia, Mumbai, India. The average number of colonies for each species was expressed as log10 cfu/g sample. The extent of lipid oxidation during storage was determined by estimating the TBARS number as milligram of malonaldehyde per kilogram sample by following the distillation method described by Taraldgis *et al.* (1960).

*Statistical analysis*

The experiments were conducted in triplicates and results of storage studies were evaluated statistically using Student Newman Keuls test at $p<0.05$ (Coplot:2003. CoStat version 6.204) using ANOVA. Critical difference and students t-test was used to compare the means of the data obtained from the proximate and physico-chemical analysis of low salt and control nuggets.

## Results and Discussions

*Optimization of low salt nuggets*

Reduction of salt in meat products to reduce or delay the risk of cardio vascular diseases is a recent concept which has received immense attention from meat researchers. Salt being an essential ingredient for the production of restructured meat makes the development of low salt products a difficult task. In our study, low salt restructured chicken nuggets were prepared by replacing common salt with potassium chloride and by adding non meat protein i.e. whey protein concentrate and black gram flour as binder. The optimized low salt restructured nuggets were developed using Central Composite Design with minimum possible number of points. The experimental design with the four factors and the three responses is presented in Table 1. The responses selected namely OAA, saltiness and hardness directly influenced the quality of the product and their values were dependent on the four factors chosen for the design. OAA and saltiness for the 30 runs ranged from 6.4 to 8.16 and 6 to 8.45 respectively on nine point hedonic scale, while hardness ranged from 5.63 to 13 N.

The results of the central composite design were analyzed for second order polynomial equation and regression analysis of the responses carried out found quadratic model to be best fit with $R^2$ values greater than 0.90 and non significant lack of fit for all responses. The predicted $R^2$ was in agreement with adjusted $R^2$. Analysis variance carried out for each response found the model F-values to be 35.13, 34.58 and 25.84 for OAA, saltiness and hardness respectively, which implied that the models were significant. The polynomial equations obtained for

Table 1. Experimental design for optimization of low salt restructured chicken nuggets

| Run | Factor A WPC | Factor B BGF | Factor C NaCl | Factor D KCl | Response 1 OAA | Response 2 Saltiness | Response 3 Hardness (N) |
|---|---|---|---|---|---|---|---|
| 1 | 1.00 | 1.00 | 0.75 | 0.75 | 7.52 | 7.93 | 7.88 |
| 2 | 1.50 | 2.00 | 0.50 | 1.00 | 7.20 | 8.20 | 6.28 |
| 3 | 1.00 | 3.00 | 0.75 | 1.25 | 6.40 | 7.43 | 6.50 |
| 4 | 0.50 | 4.00 | 0.50 | 1.00 | 7.10 | 6.50 | 5.63 |
| 5 | 1.00 | 3.00 | 0.75 | 0.75 | 7.97 | 8.00 | 7.83 |
| 6 | 1.00 | 3.00 | 1.25 | 0.75 | 7.20 | 7.25 | 9.00 |
| 7 | 1.00 | 5.00 | 0.75 | 0.75 | 7.05 | 7.17 | 13.00 |
| 8 | 0.50 | 2.00 | 0.50 | 0.50 | 7.74 | 7.10 | 7.78 |
| 9 | 1.00 | 3.00 | 0.75 | 0.25 | 7.00 | 7.25 | 8.00 |
| 10 | 1.50 | 4.00 | 0.50 | 1.00 | 6.50 | 7.34 | 6.87 |
| 11 | 1.00 | 3.00 | 0.75 | 0.75 | 8.16 | 8.37 | 7.94 |
| 12 | 1.00 | 3.00 | 0.75 | 0.75 | 8.01 | 8.43 | 7.39 |
| 13 | 1.00 | 3.00 | 0.75 | 0.75 | 8.14 | 8.53 | 7.43 |
| 14 | 1.50 | 4.00 | 0.50 | 0.50 | 7.15 | 7.17 | 9.60 |
| 15 | 1.50 | 4.00 | 1.00 | 1.00 | 7.19 | 7.22 | 10.73 |
| 16 | 0.50 | 4.00 | 1.00 | 1.00 | 7.06 | 6.85 | 7.79 |
| 17 | 1.50 | 4.00 | 1.00 | 0.50 | 7.37 | 7.72 | 10.76 |
| 18 | 0.00 | 3.00 | 0.75 | 0.75 | 7.68 | 6.50 | 5.79 |
| 19 | 1.50 | 2.00 | 0.50 | 0.50 | 7.60 | 8.20 | 6.22 |
| 20 | 1.00 | 3.00 | 0.25 | 0.75 | 7.00 | 6.94 | 6.45 |
| 21 | 0.5 | 2.00 | 1.00 | 0.50 | 7.47 | 7.46 | 7.61 |
| 22 | 0.50 | 4.00 | 1.00 | 0.50 | 7.10 | 7.00 | 8.97 |
| 23 | 0.50 | 2.00 | 1.00 | 1.00 | 7.00 | 7.17 | 7.29 |
| 24 | 1.00 | 3.00 | 0.75 | 0.75 | 7.97 | 8.43 | 7.15 |
| 25 | 1.00 | 3.00 | 0.75 | 0.75 | 8.06 | 8.45 | 7.68 |
| 26 | 0.50 | 4.00 | 0.50 | 0.50 | 7.40 | 6.00 | 9.42 |
| 27 | 0.50 | 2.00 | 0.50 | 1.00 | 7.20 | 7.26 | 6.55 |
| 28 | 1.50 | 2.00 | 1.00 | 1.00 | 7.26 | 7.06 | 7.45 |
| 29 | 2.00 | 3.00 | 0.75 | 0.75 | 7.58 | 7.72 | 6.92 |
| 30 | 1.50 | 2.00 | 1.00 | 0.50 | 7.60 | 7.86 | 7.08 |

the three responses are given below;

$$OAA = 3.638 + 0.395A + 0.743B + 3.252C + 6.172D - 0.087AB + 0.890AC - 0.110AD + 0.246BC + 0.146BD + 0.864CD - 0.375A^2 - 0.180B^2 - 3.623C^2 - 5.223D^2$$

$$Saltiness = -0.527 + 4.444A + 0.180B + 9.005C + 8.128D + 0.096AB - 1.335AC - 0.675AD + 0.747BC + 0.237BD - 2.570CD - 1.285A^2 - 0.211B^2 - 5.190C^2 - 4.220D^2$$

$$Hardness = 17.045 - 3.451A - 3.822B - 8.645C - 1.805D + 1.043AB + 2.388AC + 2.093AD + 1.032BC - 1.655BD + 6.518CD - 1.261A^2 + 0.705B^2 + 0.449C^2 - 1.454D^2$$

Response surface plots of the quadratic model were plotted as a function of two factors while keeping the other two factors at their optimum levels. The variation of responses with respect to different factor levels can be seen in the surface plots for each response. The variations in OAA, saltiness and hardness with reference to common salt and potassium chloride, keeping black gram flour and whey protein concentrate at constant levels have been plotted. As NaCl content increased from 0.5 to 1%, all three responses namely OAA, saltiness and hardness were found to increase. With increase in the level of KCl added to the product, OAA increased up to a certain extent and then showed a decrease with further increase in the KCl level (Figure 1a). Saltiness was found to increase with the addition of KCl (Figure 1b). However, addition of KCl, did not influence hardness of the product as can be seen in Figure 1c. Variations in responses were also seen keeping NaCl and KCl at optimum levels while varying the levels of black gram flour and whey protein concentrate (surface plots not shown). OAA of the product was found to increase with increasing levels of black gram flour and whey protein up to a certain extent and then reduced with their increasing contents. Saltiness acceptability was not directly influenced by binder but was seen to slightly decrease with increasing levels of black gram flour and increase with increasing levels of non meat protein. Hardness of the product increased at higher levels of black gram flour.

The final solution for optimized design was obtained by keeping all four factors namely whey protein, black gram flour, NaCl and KCl and the response hardness in range. The other two responses namely overall acceptability and saltiness were maximized. The optimized values for whey protein concentrate, black gram flour, NaCl and KCl in low salt restructured chicken nuggets were obtained as 1.18%, 2.49%, 0.75% and 0.69% respectively. The response values for the optimized factors were 8.10, 8.53 and 7.18 N for OAA, saltiness acceptability and hardness respectively. Numerical solution showed

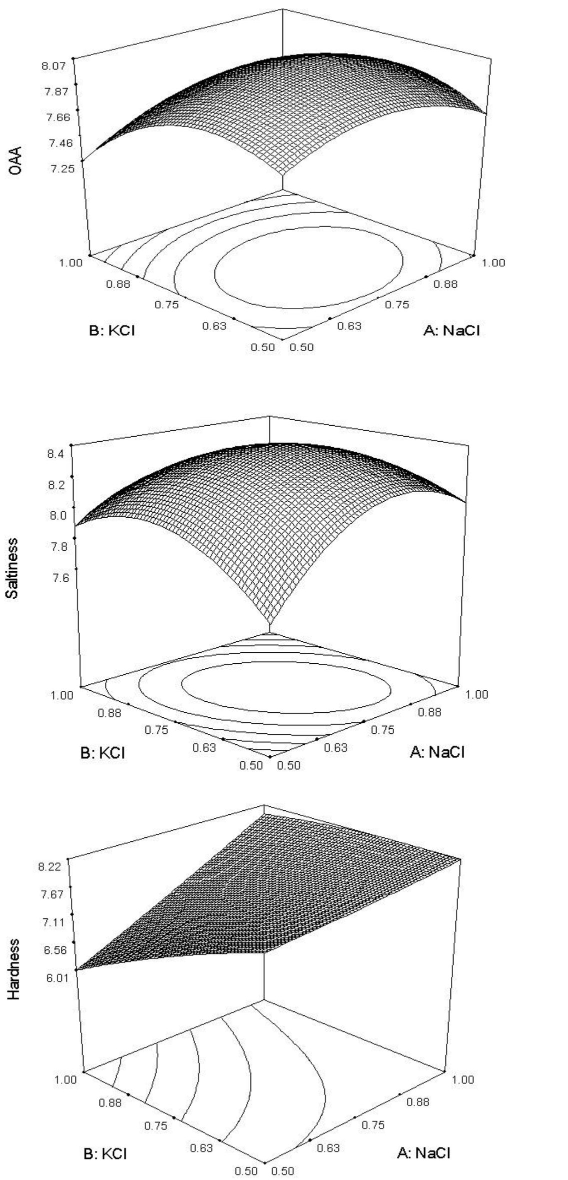


Figure 1. Response surface plot depicting variation of OAA (a) , Saltiness (b) and Hardness (c) with respect to variables NaCl and KCl

that the optimized factors had a desirability of 97.8%.

Next, using the optimum formulation, low salt restructured nuggets were prepared and all the response variables of this product were analyzed. Experimental values of each response were compared to the predicted values. The sensory scores for OAA and saltiness for low salt nuggets were 7.92 and 8.25 respectively and instrumental hardness was recorded to be 7.15. The predicted response values from the optimized solutions for maximum desirability were 8.1 for OAA, 8.53 for saltiness and 7.18 for hardness which were in concurrence with the actual values. Thus, the chosen mathematical model adequately predicted the studied attributes.

Previously, Saricoban *et al*. (2009) used RSM to study the effects of fat, wheat bran and salt (NaCl) on the quality characteristics of cooked beef patties and optimized fat level at 11.89%, wheat bran at 7.55% and NaCl at 0.28% to achieve highest acceptability for the product. Pietrasik and Li-Chan (2002) used RSM to study the interaction of NaCl, microbial transglutaminase and heating temperatures on the textural and color characteristics of pork batter gel. Ruusunen *et al.* (2003) also used RSM to develop low salt-phosphate free frankfurters. Several other studies have used RSM to optimize the levels of different ingredients in various meat products like cured pork (Schilling *et al*., 2004), frankfurters (Pereira *et al*., 2011), low fat sausages (Marchetti *et al*., 2013) and pork patties (Jung and Joo, 2013). Thus RSM has been employed as an effective statistical tool for optimizing ingredients in product formulations and can be of particular use in developing designer meat products.

*Physico-chemical characteristics of nuggets*

Low salt nuggets were prepared using the optimized levels of NaCl, KCl, whey protein and black gram flour and their quality characteristics were compared with control nuggets (prepared using 1.5% NaCl and 2% black gram flour, without the addition of KCl and whey protein). The proximate compositions and physico-chemical properties of optimized low salt restructured nuggets and control nuggets estimated are shown in Table 2. The low salt restructured nuggets had significantly higher moisture ($p<0.05$) and protein ($p<0.05$) contents. The higher moisture content is due to the addition of binders that retained water during cooking and hence, increased the moisture in product. Addition of non meat protein (whey protein) led to an increase in the protein content of low salt nuggets. The fat ($p>0.05$) and ash ($p>0.01$) content did not differ significantly among the two samples. The fat content (4.20%) of the product being low makes it suitable for consumers who desire low fat meat products. pH of the low salt and control nuggets did not differ significantly ($p>0.01$) with low salt nuggets reporting a pH of 6.19±0.02 and control nuggets 6.24±0.04. Replacement of sodium chloride by other chloride salts like calcium and potassium chloride tends to decrease the pH of the meat products and the same has been reported in other studies as well (Gimeno *et al*., 1999; Horita *et al*., 2011).

Water holding capacity of low salt nuggets was significantly higher than ($p<0.05$) control nuggets. Earlier, Hongsprabhas and Barbut (1999) had reported that addition of whey protein can improve water binding in low salt (≤ 1.5% NaCl) poultry meat batters. Higher water holding property indicates that

Table 2. Proximate composition and physico-chemical parameters of low salt and control restructured nuggets

| Parameter | Low salt nuggets | Control Nuggets | *t*-Value |
|---|---|---|---|
| Moisture (%) | 74.04±0.14 | 73.68±0.13 | 3.31* |
| Protein (%) | 15.58±0.21 | 14.45±0.21 | 6.58* |
| Fat (%) | 4.20±0.20 | 4.35±0.35 | 0.64* |
| Ash (%) | 2.87±0.16 | 2.63±0.06 | 2.45* |
| pH | 6.18±0.02 | 6.24±0.04 | 1.77** |
| Water holding capacity (% bound water) | 58.49±0.38 | 56.95±0.23 | 6.04* |
| Cooking loss (%) | | | |
| On Steaming | 7.50±0.5 | 7.78±0.48 | 0.70* |
| On Frying | 16.02±0.22 | 19.61±0.16 | 22.49* |
| Water activity | 0.975±0.001 | 0.968±0.000 | 10** |
| % shrinkage | 6.63±0.15 | 8.16±0.30 | 7.86* |
| OAA score [a] | 7.92±0.27 | 8.21±0.15 | 1.26* |
| Saltiness [a] | 8.25±0.35 | 8.5±0.47 | 1.34* |
| Hardness (N) | 7.15±0.05 | 7.25±0.43 | 0.40* |
| Cohesiveness | 0.36±0.02 | 0.25±0.01 | 10.75* |
| Springiness (mm) | 8.45±0.33 | 13.33±0.70 | 10.91* |
| Gumminess (N) | 2.57±0.13 | 1.80±0.13 | 7.28* |
| Chewiness (Nmm) | 21.72±0.77 | 24.03±1.98 | 1.88* |
| Adhesive Force (N) | 0.22±0.03 | 0.56±0.05 | 9.86* |

n=3; [a] $n_1$= 10
*$p<0.05$, **$p<0.01$

moisture retention during subsequent frying would be higher for low salt nuggets, as was seen from the results of cooking loss studies. Though cooking loss during steam cooking did not differ significantly ($p>0.05$) among the two products, loss during frying was found to be significantly higher ($p<0.05$) for control nuggets when compared to low salt nuggets. A high degree of correlation was found between water holding property and total cook loss of the product. Same trend was observed by Dzudie *et al*. (2002), Pietrasik and Li-Chan (2002) and Youssef and Barbut (2011) in different meat products. The results of the present study was in agreement with previous studies that reported improved yield with the addition of whey protein concentrate and black gram flour to reduced salt (1.25% NaCl) sous vide cooked beef muscles (Szerman *et al*., 2012) and buffalo meat burgers (Modi *et al*., 2003) respectively. Horita *et al*., (2011) reported that replacement of NaCl by KCl and other chloride salts such as magnesium and calcium chloride can mitigate losses incurred during cooking of low fat mortadella. Water activity was found to be significantly ($p<0.01$) higher in low salt nuggets than control nuggets, mainly due to greater retention of moisture during cooking in low salt nuggets. However, this could be detrimental to the keeping quality of the product. Percent shrinkage during frying was found to be significantly higher ($p<0.05$) for control nuggets when compared to low salt samples.

Table 2 shows the results of texture profile analysis of low salt and control nuggets. It is clearly seen that low salt and control nuggets differ significantly ($p<0.05$) in all parameters except hardness and chewiness. This indicates that hardness and chewiness, two important textural attributes that influence the consumer's perception of meat product quality were not compromised in low salt nuggets. The interaction between non meat proteins and binders can influence gel formation, thereby, affecting textural properties of low salt meat products

(Szerman *et al*., 2008; 2012) and this may be the reason for differences observed in certain textural parameters in our study. Gou *et al*. (1996) and Guàrdia *et al*. (2008) and did not find any significant effect of KCl substitution (up to 60%) of NaCl on the texture profiles of dry cured pork loin and fermented sausages respectively, while Colmenero *et al.* (2005) reported a decrease in hardness of frankfurters incorporated with KCl and microbial transglutaminase as salt replacers but found no difference in other textural parameters such as springiness, chewiness and

Table 3. Effect of refrigerated storage (4±1ºC) on sensory quality, lipid oxidation and pH of restructured nuggets

| Days | 0 | 5 | 10 | 15 | 20 | 25 |
|---|---|---|---|---|---|---|
| *Appearance* | | | | | | |
| Low salt | 7.8±0.23[a] | 7.75±0.28[ab] | 7.60±0.44[abc] | 7.4±0.41[bc] | 7.33±0.30[c] | 7.25±0.23[c] |
| Control | 8.1±0.74[a] | 8.01±0.62[ab] | 7.77±0.56[ab] | 7.5±0.42[ab] | 7.5±0.45[ab] | 7.32±0.41[b] |
| *Taste* | | | | | | |
| Low salt | 7.95±0.37[a] | 7.65±0.46[a] | 7.55±0.37[ab] | 7.47±0.44[ab] | 7.15±0.52[bc] | 7.03±0.17[c] |
| Control | 8.5±0.56[a] | 8.45±0.50[a] | 8±0.47[b] | 7.92±0.29[bc] | 7.7±0.42[bc] | 7.45±0.39[c] |
| *Odour* | | | | | | |
| Low salt | 7.95±0.25[a] | 7.55±0.44[b] | 7.4±0.29[b] | 7.05±0.12[c] | 7.02±0.13[c] | 6.88±0.31[c] |
| Control | 8.1±0.21[a] | 7.98±0.32[a] | 7.51±0.47[b] | 7.5±0.43[b] | 7.29±0.37[bc] | 7±0.26[c] |
| *Texture* | | | | | | |
| Low salt | 8±0.23[a] | 7.8±0.22[ab] | 7.64±0.18[b] | 7.3±0.32[c] | 7.29±0.27[c] | 7.2±0.23[c] |
| Control | 8.1±0.39[a] | 7.99±0.21[ab] | 7.97±0.34[ab] | 7.75±0.48[ab] | 7.5±0.58[b] | 7.48±0.43[b] |
| *OAA* | | | | | | |
| Low salt | 7.92±0.55[a] | 7.72±0.5[ab] | 7.5±0.48[abc] | 7.47±0.67[abc] | 7.2±0.42[bc] | 7.0±0.4[c] |
| Control | 8.21±0.47[a] | 8±0.29[ab] | 7.8±0.26[bc] | 7.75±0.29[bc] | 7.55±0.37[c] | 7.45±0.31[c] |
| TBARS | | | | | | |
| Low Salt | 0.07±0.001[a] | 0.09±0.001[b] | 0.15±0.01[c] | 0.23±0.01[d] | 0.29±0.01[e] | 0.32±0.01[f] |
| Control | 0.09±0.001[a] | 0.10±0.01[b] | 0.21±0.01[c] | 0.27±00.01[d] | 0.30±0.01[e] | 0.38±0.01[f] |
| pH | | | | | | |
| Low Salt | 6.12±0.11[a] | 6.15±0.01[a] | 6.01±0.01[b] | 5.85±0.01[c] | 5.81±0.02[c] | 5.64±0.02[d] |
| Control | 6.24±0.02[a] | 6.2±0.01[a] | 6.08±0.07[b] | 5.97±0.02[c] | 5.9±0.01[d] | 5.84±0.01[e] |

Means with different superscripts in the same row indicate significant difference ($p<0.05$)

cohesiveness. Horita *et al*. (2011) also reported that 50% replacement of NaCl by KCl did not affect the textural properties of mortadella.

Sensory scores for OAA and saltiness did not differ significantly ($p>0.05$) between the two products. Hence, replacement of NaCl by KCl with the addition of binder and non meat protein did not significantly affect the perception of saltiness by the panelists in the product and at the same time maintained its overall sensory quality. Previously, Gou *et al*. (1996), Gelabert *et al*. (2003), Guàrdia *et al*. (2008) and Horita *et al*. (2011) reported that up to 40% substitution of NaCl by KCl was possible in meat products without compromising the saltiness of the product. Substitution at 50% or higher conferred a bitter flavor as well as decreased the saltiness of the products.

*Shelf stability of low salt restructured chicken nuggets*

TBARS is most commonly used method to assess the extent of lipid oxidation in foods (Ladikos and Lougovois, 1990; Ross and Smith, 2006). TBARs values of both low salt and control nuggets increased significantly ($p<0.05$) during storage period (Table 3). Although a steady increase was seen, the TBARs values did not exceed 0.5 mg which is considered to be the acceptable limit of TBARS number for cooked meat product. TBARs number of 0.6 to 2 mg can result in off flavor in meat products. Refrigeration retards fat rancidity as hydro peroxides are more stable at low temperatures (Libby, 1975). In the present study, lipid oxidation took place at a higher rate in control samples than in low salt nuggets. However, the difference in the TBARS number between the two samples was not significant (($p>0.05$). Horita *et al*. (2011) also reported lower values of TBARs number during cold storage in mortadella prepared using salt substitutes like KCl and calcium chloride than in control products containing 1.5 % NaCl. Other studies also reported similar trends in TBARS values in refrigerated meat products (Modi *et al*., 2003; Naveena *et al*., 2006; Thomas *et al*., 2006, 2007; Ahamed *et al*., 2007). A significant decrease ($p<0.05$) in pH was seen after 10 days of storage in both the samples (Table 3). pH dropped to 5.63 and 5.84 on the 25$^{th}$ day in case of low salt and control nuggets respectively. Decrease in pH was in positive correlation with the increase in microbial count throughout the storage period.

Production of restructured meat products involves a great deal of handling and consequently higher risks of contamination, which must be controlled for better stability of the product during storage. Reduction of sodium chloride in such products can further hamper

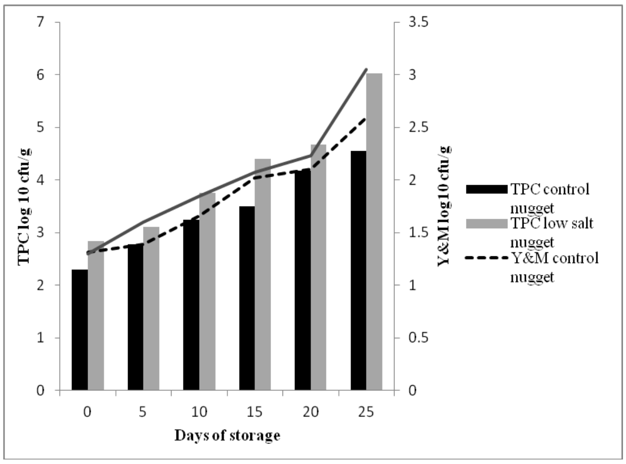


Figure 2. Effect of refrigerated storage (4±1°C) on Total plate counts (TPC) and Yeast and Mould (Y&M) counts of Control and Low salt nuggets

shelf life (Cofrades *et al*., 2011). The mean total plate count increased significantly ($p<0.05$) during storage for both the samples (Figure 2). The count for low salt nuggets was higher than control nuggets at any point of storage. This could be attributed to the higher moisture content and water activity of low salt nuggets. An increase in the bacterial counts was reported by Tejasvi *et al*. (2013) in chicken nuggets, Thomas et al. (2006, 2007, and 2013) in pork nuggets and buffalo meat nuggets, Ahamed *et al*. (2007) in buffalo meat cutlets and Anandh *et al*. (2008) in buffalo rolls during cold storage. Yeast and mold count also increased gradually during storage for both the samples, with low salt nuggets showing a growth of 3.05 $log_{10}$ cfu/g and control nuggets showing 2.59 $log_{10}$ cfu/g at the end of storage period (Figure 2). Anandh *et al.* (2008) also reported increase in yeast and mold counts with storage days in refrigerated buffalo rolls. Coliforms were not detected in any of the samples during the storage study indicating proper sanitary measures undertaken during preparation and processing of nuggets. Psychrotrophs were not detected in both the samples up to 15 days of storage. The mean psychrotrophic bacterial count reached 2 $log_{10}$ cfu/gm for low salt nuggets and less than 1 $log_{10}$ cfu/gm for control nuggets on the 25$^{th}$ day of storage. An increase in psychrotrophic bacterial counts during later stages of refrigerated storage were observed by Thomas *et al*. (2006 and 2007) in restructured buffalo meat nuggets. Absence of psychrotrophs in the initial stages of cold storage may be due to cooking of the product to high temperature followed by storage at low temperature resulting in retardation of microbial growth due to temperature shock.

Throughout storage, all microbial counts of both low salt and control restructured nuggets were within the standards specified for cooked meat products (Jay, 1996). Visible slime and off-odor were observed on the 30$^{th}$ day of storage in low salt nuggets suggesting that the nuggets kept well without microbial spoilage up to 25 days under refrigerated conditions.

OAA scores reduced from 7.92 on 0$^{th}$ day to 7.01 on 25$^{th}$ day for low salt nugget and for control nugget the score came down to 7.45 on 30$^{th}$ day from an initial 0th day score of 8.21 (Table 3). A significant ($p<0.05$) decrease in OAA scores were found after 10th day of storage in case of both the samples. Control nuggets were found to be more acceptable than low salt nuggets during storage period. Thomas *et al*. (2006 and 2007) reported decrease in OAA scores in restructured buffalo meat nuggets with advancement in refrigerated storage period. The reduction in overall acceptability scores may be attributed to degradation in flavor and textural properties due to microbial growth, oxidation of fat and loss of firmness during storage period. Other studies have also reported decrease in OAA scores during refrigerated storage of meat products (Ahmed *et al*., 2007; Alakali *et al*., 2010; Biswas *et al*., 2011).

## Conclusion

Low salt restructured nuggets developed after optimization contained only 0.75% NaCl as opposed to control nuggets containing 1.5% NaCl. The total salt level was also reduced to less than 1.5% by the addition of 0.7% KCl, used as a salt replacer in combination with whey protein and black gram flour. The results of this study found the physico-chemical and sensory properties of the optimized product to be similar to control nugget, although, faster degradation in the keeping quality under refrigerated condition was observed for low salt product compared to control. Nevertheless, the low salt nuggets developed had a shelf life of at least 25 days under refrigerated conditions (4±1°C). The results of this work can be useful to meat industries for developing reduced salt restructured meat products by exploiting the synergistic effect of salt replacer (KCl), non meat protein (whey protein concentrate) and binder (black gram flour).

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