Effect of high pressure processing on the quality characteristics and shelf life of low-sodium re-structured chicken nuggets

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Summary

In this study, effect of high pressure processing (HPP) at 200 MPa, 400 MPa and 600 MPa (5 min, 15 °C) on the physico-chemical and sensory attributes of low-sodium re-structured chicken nuggets, containing potassium chloride as a partial sodium chloride replacer (approx. 50% replacement), was investigated. HPP had little or no effect on colour and sensory properties, while other properties like texture, water holding capacity and cooking yield were improved with increasing pressure levels. Changes in the microstructure as a result of pressure applied were observed by scanning electron microscopy. Differential scanning calorimetry analysis showed that, with increasing pressure levels, proteins became denatured and endothermal peaks decreased in size or were absent in the thermogram. The shelf life of HPP-treated nuggets was studied under refrigeration (4 °C ± 1 °C). HPP treatment suppressed microbial growth in the nuggets. Samples treated at 600 MPa were microbiologically stable even after 60 days of refrigerated storage. As for lipid oxidation, a slight increase in thiobarbituric acid-reactive substances (TBARS) values during storage was observed with increasing pressure levels. However, this did not cause any rancidity in the product. Therefore, it can be concluded that application of HPP can improve the quality characteristics and shelf life of low-sodium chicken nuggets.

Keywords

low salt/sodium; high pressure processing; NaCl replacement; chicken nugget; shelf life

Re-structured meat products have garnered immense interest among consumers and meat researchers in India in recent times as they are an excellent approach to utilize less valuable meat cuts and convert them into low-cost, innovative and palatable product [1]. However, the shelf life of such products is limited. This is because of processing steps, such as comminution or mixing, in the preparation of re-structured products, which disrupt the muscle structure, increase the surface area and expose the material to oxygen and microorganisms that promote deteriorative changes [2–4]. Common salt or sodium chloride (NaCl) is a vital ingredient in re-structured meat products, but its excessive intake is known to coincide with hypertension and cardiovascular diseases [5]. Several studies have established potassium chloride (KCl) as the most effective common salt replacer that can be used in meat products to reduce salt level up to 50% [6–11]. However, the product stability and quality during storage could be compromised as NaCl acts as a preservative as well.

The use of high pressure processing (HPP), as a post-packaging non-thermal decontamination technology for fresh and cured meat products, has been extensively studied in recently and is found to have higher consumer acceptance than other non-thermal decontamination technologies like irradiation [12, 13]. Several researchers have suggested the application of HPP to develop products with low salt contents. In this regard, a considerable number of studies was carried out and produced promising results [14–16]. In general, studies on the application of high pressure were carried out on fresh intact muscles, raw meat batters or cured meat products. A limited number of

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studies examined the application of HPP in extending the shelf life of ready-to-eat cooked meat products [17–19].

The objective of this study was to evaluate the effect of HPP at different pressure levels (200 MPa, 400 MPa and 600 MPa) on re-structured chicken nuggets with a low content of sodium chloride and containing potassium chloride as a partial NaCl replacer.

MATERIALS AND METHODS

Raw materials

Chicken breast meat (from 40 days old broiler birds of Ross 308 breed) was purchased from a local supplier in Mysore, India. Good quality spices (MTR Foods, Bangalore, India) and commercial brand black gram (*Vigna mungo*) flour (7 Hills, Mysore, India) were obtained from local market. Commercial brand common salt (Tata Salt, Tata Chemicals, Mumbai, India) containing 99.7% sodium chloride (38.7 g sodium per 100 g) was used for product preparation. Potassium chloride (food grade, 99.5% purity) was obtained from SV Scientific (Bangalore, India) and whey protein concentrate (80% protein) was obtained from Pristine Organics (Bangalore, India). All chemicals and media used for analytical purposes were procured from Himedia (Mumbai, India).

Experimental design

Four treatments, consisting of non-pressure-processed nuggets (C), which served as the control, and nuggets subjected to high pressure processing at 200 MPa, 400 MPa and 600 MPa (N-200, N-400 and N-600, respectively) were studied. Physico-chemical properties, such as water holding capacity (*WHC*), cooking loss, water activity, Hunter colour values and texture profile, were initially evaluated for the control and treated products. Analyses of lipid oxidation and microbiological safety were conducted after 15, 30, 45 and 60 days of refrigerated storage.

Preparation of nuggets

Preliminary studies were conducted to optimize the basic formulation and processing conditions for the preparation of re-structured chicken nuggets. The standardized formulation contained lean meat 75%, common salt 0.7%, potassium chloride 0.7%, whey protein 1.2%, black gram flour 2.5%, spice mix (red chilli, turmeric, coriander and black pepper powders) 2.5%, condiments (ginger and garlic paste) 2.5% and ice/chilled water 15%. Chicken breast meat was cleaned, trimmed off of excessive fat and connective tissues, and cut into cubes of about 3 cm. The lean meat was minced in a meat grinder (Sirman, Pieve di Curtarolo, Italy) using 8-mm plate. Ground lean meat was blended with common salt, potassium chloride and half of the ice at a low speed setting for 1 min. This was followed by 1 min resting time for protein extraction. Black gram flour and whey protein concentrate were evenly sprinkled, spices, condiments and remaining ice were added, and blending continued at high speed setting for another 2 min till a viscous batter was formed. Final batters temperature did not exceed 15 °C. Batter thus obtained was placed in aluminium mould, packed compactly, covered and cooked in steam without pressure for 30 min. The internal temperature of the cooked block was 72 °C, measured using a probe-type thermometer (HTA Instrumentation, Bangalore, India). The meat block was cooled to room temperature, chilled overnight at 4 °C and cut into nuggets of 4 cm × 2 cm × 2 cm. The nuggets were packed in polypropylene bags (thickness 80 μm) and then subjected to high pressure treatment.

High pressure treatment

A laboratory-scale high pressure food processing system (ISO-LAB FPG9400; Stansted Fluid Power, Stansted, United Kingdom) consisting of a high pressure vessel (2 l capacity) with dual high pressure pumps and pressure intensifiers, which work simultaneously, was used to achieve and maintain the desired pressure in the pressure vessel. The maximum operating pressure of the system was 1000 MPa. The high pressure vessel was surrounded by a liquid circulating jacket connected to a heating-cooling system. The pressure transmitting fluid used was 30% mono-propyleneglycol (supplied by M/S Hydraulicon Systems, Ahmedabad, India). Nuggets were divided into four batches, a control non-high-pressure-processed batch (C), and pressure-treated at 200 MPa 400 MPa, and 600 MPa for 15 min at 15 °C. Pressure and temperature were constantly monitored and recorded (at 1 s interval) during the process using a SCADA-based software (Stansted Fluid Power).

Sample storage and analyses

Immediately after HPP, the samples along with control were stored at 4 °C. Physico-chemical analyses and sensory evaluation were carried out to compare the quality characteristics of HPP-treated samples with control. Scanning electron microscopy (SEM) images of the samples were obtained to determine the effect of HPP on the

microstructure of nuggets. Samples were analysed for pH, microbial growth and lipid oxidation for up to 60 days.

Analytical procedures

Estimation of sodium and potassium content in low-sodium nuggets was carried out by inductively coupled plasma optical emission spectrometry (ICPOES) by Ultima 2 (JY-Horiba Instruments, Singapore) using the method described by WADIKAR et al. [20]. Briefly, (5 ± 0.05) g samples were calcinated at 550 °C for 6 h. The ash obtained was diluted in 100 ml 0.5 mol·l^{-1} nitric acid and filtered. For analysis, standard operating protocol for ICPOES was used with Win-IMAGE software (JY-Horiba Instruments) for quantitative analysis.

The control and high-pressure-treated nuggets were subjected to physico-chemical analyses.

pH was measured by homogenizing a 5 g portion of the sample in 10 ml distilled water at 20 °C using a digital pH meter (Cyberscan 510; Eutech Instruments, Klang Selangor, Malaysia).

Water activity was determined using Labmaster Aw (Novasina, Lachen, Switzerland) with the help of Novalog software (Novasina).

WHC was determined by the methods described by HONG et al. [21]. Five grams of the sample were placed in a centrifuge tube along with cotton wool as an absorbent. The samples were then centrifuged at 1 000 ×g for 15 min in a refrigerated centrifuge (Sorvall RC-5C; Dupont Instruments, Bishops Stortford, United Kingdom). The samples were removed from the tubes and re-weighed. The percentage *WHC* was calculated using the following equation;

$$WHC = \left(1 - \frac{m_0 - m_1}{W}\right) \times 100 \quad (1)$$

where m_0 is weight of sample before centrifugation, m_1 is weight of sample after centrifugation and W is total water content in the sample.

Cooking loss was determined by the method described by SHEARD et al. [22].

Colour of the nuggets was measured using a colorimeter (Hunter Associates Laboratory, Reston, Virginia, USA) and expressed as L^* (lightness), a^* (redness), and b^* (yellowness).

Texture measurement

Texture profile analysis (TPA) of nuggets was conducted using Texture Analyzer (TA Plus, Lloyd Instruments, Bognor Regis, United Kingdom) as per the method described by BOURNE [23]. Samples were compressed twice to get an imitation of mastication, which included first and second bite. Chilled samples were allowed to come to room temperature (27 °C). Uniformly sized pieces (4 cm × 2 cm × 2 cm were used as the test samples. These 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^{-1}. The maximum clearance between the moving crosshead and sample was maintained at 3 mm throughout the study. The measured and derived parameters were estimated using Nexygen texture analysis software (Lloyd Instruments) like:

- hardness (maximum force required to compress the sample, expressed in newtons),
- springiness (the height the sample springs back after initial compression, expressed in millimetres),
- cohesiveness (extent to which sample could be deformed prior to rupture, calculated as A_2/A_1, where A_1 is total energy required for first compression and A_2 is total energy required for the second compression), and
- chewiness (work required to masticate a sample for swallowing, calculated as springiness × hardness × cohesiveness, expressed in newton millimetres).

Scanning electron microscopy

Samples were frozen at -50 °C and lyophilized at 0.013 kPa for 48 h. SEM images of the samples were obtained using EVO LS10 microscope (Zeiss, Jena, Germany). The samples were sputter-coated with gold-palladium alloy and the morphological analysis was carried out at high vacuum using an operating voltage of 10 kV.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimeter (DSC 2010; TA Instruments, New Castle, Delaware, USA) was used to study the thermal denaturation of protein in the pressurizd and non-pressurized products. Samples of (15 ± 1) mg were weighed into aluminium pans and sealed. The scanning rate was 10 °C·min^{-1} over the temperature range of 20–100 °C. Three runs per sample were carried out using an empty pan as a reference. Thermal denaturation of chicken breast meat cooked in steam without pressure for 30 min and whey protein were also analysed. Temperatures (in degrees Celsius) and enthalpies of denaturation, ΔH (in kilojouls per kilogram) were reported within 1 °C and 5%, respectively.

Sensory evaluation

The samples were evaluated using nine point hedonic scale rating method for sensory prefer-

ences, where 1 = dislike extremely and 9 = like extremely, for attributes of colour, taste, odour, texture, saltiness and overall acceptability. Nuggets were deep-fried in refined sunflower oil (180 °C for 2 min) and served warm to a semi-trained panel of 10 judges. Water was provided for rinsing.

Shelf stability of re-structured nuggets

For storage studies, the samples were packed in polypropylene bags (80 μm thickness) and kept at (4 ± 1) °C for 60 days. Changes during the storage period were monitored by carrying out microbiological and sensory evaluations, and by determining the thiobarbituric acid reactive substances (TBARS) number at intervals of 15 days. Microbiological evaluation in terms of total counts, yeasts and moulds, psychrotrophic and coliform bacteria counts were determined as per the methods described by Downes and Ito [24]. The bacteriological media were obtained from Himedia. The average number of colonies for each species was expressed as logarithm of colony forming units per gram of sample. The extent of lipid oxidation during storage was determined by estimating the TBARS number as milligrams of malonaldehyde per kilogram sample by following the distillation method described by Taraldgis et al. [25].

Statistical analysis

The experiments were conducted with three replicates and Duncan's test was used to evaluate the statistical significance at $p < 0.05$ (Coplot:2003; CoStat version 6.204, CoHort Software, Monterey, California, USA) using ANOVA. Data were subjected to one way analysis of variance for comparing the means and two way analysis of variance for storage study.

RESULTS AND DISCUSSION

Sodium and potassium contents in low-sodium chicken nuggets

Sodium and potassium contents in the prepared nuggets were estimated by ICPOES and found to be 307 mg and 520 mg per 100 g nuggets (one serving), respectively.

Effect of high pressure processing on physico-chemical properties

In general, HPP had no significant influence on the colour, pH and water activity of nuggets (Tab. 1). This type of processing is known to cause dramatic changes in the colour of fresh meat [12]. However, these changes are not significant in cured meat [26] and cooked products, as seen in our study and also in a previous study on cooked ham [19]. The colour of meat generally depends on the myoglobin content. In cooked meat products like nuggets, myoglobin is denatured and hence application of high pressure is less likely to induce any further changes in colour. Other studies on low-salt meat products also reported no significant effect of pressure treatment on the

Tab. 1. Effect of high pressure treatment on physico-chemical properties of chicken nuggets.

Parameter	C	N-200	N-400	N-600
WHC [%]	58.5±0.4^{b}	60.3±0.6^{a}	60.5±0.5^{a}	60.6±0.5^{a}
Cooking loss [%]	16.2±0.2^{a}	15.5±0.5^{ab}	15.4±0.5^{ab}	14.9±0.1^{b}
Water activity	0.960±0.001	0.968±0.003	0.973±0.002	0.974±0.001
pH	6.12±0.11	6.1±0.1	6.09±0.0	6.1±0.12
Colour values				
*L**	63.71±0.91	64.25±1.40	64.74±0.59	65.67±0.66
*a**	5.97±0.29	6.23±0.17	6.33±0.28	6.06±0.11
*b**	31.09±0.55	30.17±0.57	31.33±0.33	31.35±0.94
Texture Profile Analysis				
Hardness [N]	8.10±0.32^{a}	7.66±0.28^{b}	7.28±0.14^{c}	7.00±0.24^{d}
Cohesiveness	0.31±0.04	0.35±0.05	0.33±0.05	0.33±0.02
Springiness [mm]	15.40±0.7^{a}	11.29±0.5^{b}	9.18±0.32^{c}	6.84±0.24^{d}
Chewiness [N·mm]	39.14±1.7^{a}	30.46±1.5^{b}	25.14±1.2^{c}	22.92±0.9^{c}

Means with different superscripts in the same row indicate significant difference ($p < 0.05$).
WHC – water holding capacity (expressed as percentage of bound water), C – non-pressure-processed nuggets (control); N-200, N-400, N-600 – nuggets subjected to high pressure processing at 200 MPa, 400 MPa and 600 MPa, respectively.

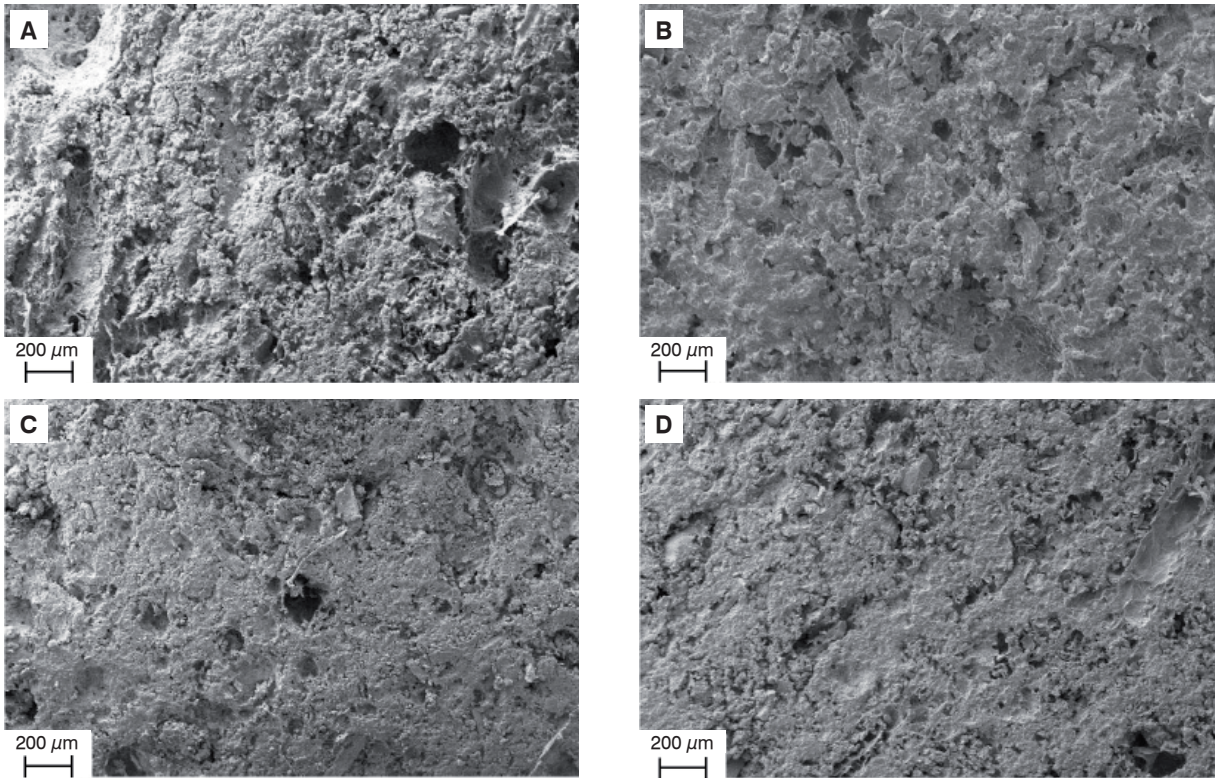

Fig. 1. Microstructure of low-sodium chicken nuggets at 100× magnification.

A – non-pressure-processed nuggets, B – high pressure processed at 200 MPa, C – high pressure processed at 400 MPa, D – high pressure processed at 600 MPa.

colour values and pH [16, 27–29]. However, Grossi et al. [30] reported an increase in lightness value and decrease in redness of low-salt pork sausages containing carrot fibre and potato starch subjected to high pressure treatment at 40 °C, which they explained to be due to a higher degree of changes induced by pressure and heat in the pigments, myosin and structural proteins, leading to a whitening effect.

Water holding capacity of nuggets increased with increasing levels of pressure, although the increase seen among pressure-treated samples was not significant. Actin and myosin, which are generally affected by pressure treatment, will be heat denatured on cooking. However, pressure treatment is said to induce solubilization of other myofibrillar proteins such as titin or connectin. Changes in gelation properties of these proteins after pressurization may have contributed to the slight increase in *WHC* as seen in this study. *WHC* can also be effected by F-actin that can survive cooking temperature and influence gel formation during subsequent pressure treatment [13].

Cooking loss observed during frying was reduced significantly in pressure-treated samples when compared to control. Increase in pressure levels from 200 MPa to 400 MPa did not bring about significant reduction in cooking loss. Conformational changes in meat protein caused by pressurization can force water into the protein matrix and thus affect protein hydration [31]. The residual protein that survived steam cooking may have undergone unfolding during pressure treatment resulting in improved binding of water and, consequently, lower cooking loss during frying of nuggets. Sikes et al. [32] and Crehan et al. [29] made use of high pressure to improve water binding and reduce the cooking loss of low-salt beef sausage batter and low-salt frankfurters. Other studies in recent years also described successful application of HPP in improving the water retention properties of raw materials used for the production of meat products with reduced salt content [30, 15]. However, O'Flynn et al. [28], and Cheftel and Culioli [33] established that there was no effect of HPP in the range of 25–150 MPa on water holding capacity of low-salt meat products.

Effect of HPP on textural properties

Texture profile of low-salt re-structured nuggets is shown in Tab. 1. It was seen that pressure

treatment significantly reduced the hardness, springiness and chewiness of the nuggets. In previous studies [28, 29, 34, 35] it was reported that pressure in excess of 150 MPa can significantly affect hardness and springiness of low-salt meat products. Reduction in chewiness observed in the present study may be due to disruption of intact muscle fibres upon the application of pressure as seen in SEM images. Cohesiveness was not significantly affected by pressure treatment, although a slight decrease in this parameter was observed at 400 MPa and 600 MPa. Cohesiveness is improved by proper protein extraction before cooking [36]. Hence, pressure treatment after cooking will have little effect on this parameter. O'Flynn et al. [28] and Crehan et al. [29] also found no significant affect of HPP on gumminess and cohesiveness of low-salt meat products. In most of the studies, the effect of HPP on textural properties was evaluated in products subjected to pressure treatment before cooking. Effect of pressure treatment on the texture of cooked meat product was not reported so far in any study. Heat treatment such as cooking causes hydrogen bonds to break, which results in texture variation in meat products. Subsequent application of pressure in such products will not cause any more textural alterations as myosin would have already been extracted and denatured, and gel-like structure formed. Hydrogen bonds that maintain the secondary, tertiary, and quaternary structures are heat-labile but unaffected by pressure [13]. Hydrophobic and electrostatic interactions, if present, may get disrupted by high pressure, affecting certain parameters like juiciness and springiness to some extent. The present study demonstrates that high pressure treatment, as a post processing decontamination technique, can be used for reduced-salt meat products and is helpful in improving the texture of such products.

Effect of HPP on microstructure

SEM images obtained for all the samples are shown in Fig. 1. In control sample, the matrix appeared more aggregated, less smooth, with big irregular holes and the muscle fibres were long and more discrete. Samples processed at 400 MPa and 600 MPa showed highly interconnected network with numerous small pores and more regular network structures. Occurrence of long fibres was reduced in the nuggets with increasing pressure levels. These observations are in concordance with those of TPA, which showed that pressure treatment significantly ($p < 0.05$) affected the textural parameters of low-sodium nuggets. This is in agreement with the findings of Trespalacios and Pla [34, 35] and Hong et al. [37] who reported that pressure treatment resulted in more compact structures with smaller pores in low-salt chicken gels and re-structured pork, respectively. Recently, Møller et al. [38] and Tokifuji et al. [39] studied the effect of high pressure treatment on pork products and found pronounced changes in the microstructure, which favoured improvement in textural properties.

Effect of HPP on thermal behaviour

Fig. 2 shows DSC thermograms of control and pressurized samples. Moisture content of nuggets subjected to DSC analysis was 74.0% ± 0.2%. Cooked chicken meat (75.4% moisture) showed transitions at 60 °C (myosin), 71 °C (sarcoplasmic and connective tissue proteins) and 81 °C (actin) with melting enthalpy of 1.16 kJ·kg^{-1}, 2.8 kJ·kg^{-1} and 2.5 kJ·kg^{-1}, respectively. The typical transition temperature for myosin is 59 °C, for sarcoplasmic proteins is 69 °C and for actin is 80 °C, as observed by other researchers in chicken muscle [34–35, 40–42]. In control nuggets, peaks at 55 °C, 62 °C and 70 °C with an associated melting enthalpy of 1.2 kJ·kg^{-1}, 0.7 kJ·kg^{-1} and 2.1 kJ·kg^{-1}, respectively, which tentatively could be attributed to the thermal denaturation of native proteins myosin (first transition) and actin (last transitions) of residual actomyosin complex, left over after heating. The intermediate transitions are mainly due to sarcoplasmic and connective proteins. Several fused peaks around 70–90 °C were recorded with a maximum transition temperature of 85.93 °C and ΔH of 1.02 kJ·kg^{-1}. The shift in myosin and

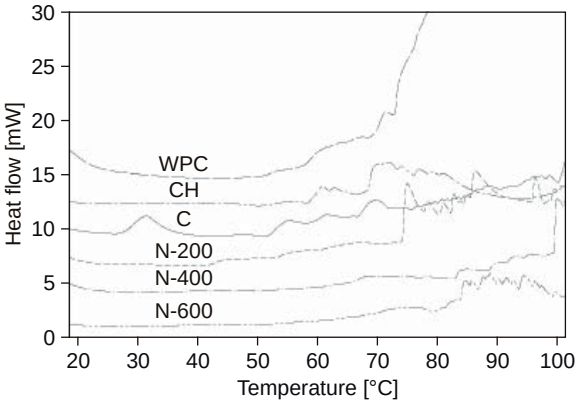

Fig. 2. Differential scanning calorimetry profiles of low-sodium re-structured chicken nuggets subjected to high pressure treatment.

WPC – whey protein concentrate, CH – cooked chicken breast meat, C – non-pressure-processed chicken nugget (control), N-200, N-400, N-600 – chicken nuggets subjected to high pressure processing at 200 MPa, 400 MPa and 600 MPa, respectively.

actin peaks observed in the nuggets could be due to destabilizing effect of sodium chloride and potassium chloride on myofibrillar proteins [43]. It was reported that actin shows high sensitivity (reduction in melting temperature up to 16 °C) in the presence of salts like NaCl [44]. Moreover, interaction between whey protein and myofibrillar proteins during heating can cause a downward shift in myosin and actin endothermic peaks and, in some cases, complete disappearance of the actin peak [45]. With increasing pressure levels, proteins became denatured, endothermal peaks decreased in size or were absent in the thermogram. In 200 MPa processed sample, these peaks were seen at 53.39 °C, 60.95 °C and 69.8 °C but with reduced enthalpies of denaturation (0.8, 0.6 and 1.25 kJ·kg^{-1}, respectively). Only sarcoplasmic and actin peaks (above 70 °C) were seen in 400 MPa processed sample (with melting enthalpy of 0.98 kJ·kg^{-1}), while the endothermic peaks disappeared in 600 MPa processed nuggets. Actin is generally considered to be more stable to heating and pressure treatments. Similar results were earlier reported in studies on pressure treatment of pork and duck meat batters after heating [46, 47]. It can be noted that endothermic events centred around 80–90 °C seen in all samples, which can be assigned to β-lactoglobulin and α-lactal-

Tab. 2. Effect of high pressure treatment on sensory quality of chicken nuggets.

Parameter	C	N-200	N-400	N-600	*LSD*
Colour	8.05±0.55	8.02±0.51	7.90±0.46	7.99±0.74	0.52
Taste	8.15±0.47	8.00±0.82	8.00±0.67	7.80±0.58	0.59
Odour	8.05±0.51	7.52±0.48	7.75±0.44	7.50±0.47	0.43
Texture	7.55±0.55^{b}	7.86±0.40^{b}	7.95±0.55^{b}	8.08±0.34^{a}	0.42
Saltiness	8.00±0.35	7.91±0.32	7.85±0.46	8.16±0.29	0.33
Overall acceptability	7.92±0.29	7.65±0.47	7.90±0.52	8.00±0.33	0.38

Means with different superscripts in the same row indicate significant difference ($p < 0.05$).
C – non-pressure-processed chicken nuggets (control); N-200, N-400, N-600 – chicken nuggets subjected to high pressure processing at 200 MPa, 400 MPa and 600 MPa, respectively, *LSD* – least significant difference.

Tab. 3. Microbiological evaluation during cold storage of chicken nuggets.

Microbiological parameter	Day of storage	C	N-200	N-400	N-600
Aerobic counts [log CFU·g^{-1}]	0th	2.83 ± 0.03^{Aa}	2.3 ± 0.04^{Ba}	1.61 ± 0.02^{Ca}	0.79 ± 0.04^{Da}
	15th	4.37 ± 0.15^{Ab}	2.6 ± 0.02^{Bb}	2.52 ± 0.07^{Cb}	2.37 ± 0.06^{Db}
	30th	6.13 ± 0.64^{Ac}	5.80 ± 0.01^{Bc}	4.66 ± 0.06^{Dc}	4.37 ± 0.15^{Ec}
	45th	7.3 ± 0.10^{Ae}	7.03 ± 0.06^{Bd}	5.95 ± 0.01^{Cd}	4.97 ± 0.15^{Ed}
	60th	6.57 ± 0.12^{Cd}	7.30 ± 0.04^{Ae}	7.13 ± 0.06^{Be}	6.52 ± 0.08^{De}
Yeast and mould counts [log CFU·g^{-1}]	0th	1.30 ± 0.05^{Aa}	0.2 ± 0.01^{Ba}	0	0
	15th	2.06 ± 0.11^{Ab}	0.90 ± 0.015^{Bb}	0.80 ± 0.05^{Ca}	0.58 ± 0.04^{Da}
	30th	2.82 ± 0.08^{Ac}	2.75 ± 0.05^{Bc}	2.77 ± 0.09^{Cb}	1.35 ± 0.02^{Db}
	45th	3.20 ± 0.06^{Ad}	3.08 ± 0.38^{Bd}	2.93 ± 0.12^{Cc}	2.31 ± 0.01^{Dc}
	60th	3.00 ± 0.14^{Ad}	3.30 ± 0.05^{Bd}	2.84 ± 0.08^{Cc}	2.57 ± 0.06^{Dc}
Psychrotroph counts [log CFU·g^{-1}]	0th	1.00 ± 0.05^{Aa}	0.50 ± 0.05^{Ba}	0.32 ± 0.06^{Ca}	0.30 ± 0.01^{Da}
	15th	1.88 ± 0.34^{Ab}	0.7 ± 0.05^{Bb}	0.5 ± 0.05^{Cb}	0.46 ± 0.06^{Db}
	30th	2.52 ± 0.03^{Ac}	1.48 ± 0.15^{Bc}	1.10 ± 0.10^{Cc}	0.82 ± 0.06^{Dc}
	45th	4.30 ± 0.18^{Ad}	2.92 ± 0.14^{Bd}	2.80 ± 0.09^{Cd}	1.33 ± 0.08^{Dd}
	60th	5.08 ± 0.49^{Ae}	4.57 ± 0.06^{Be}	3.98 ± 0.32^{Ce}	2.63 ± 0.12^{De}

Storage temperature: (4 ± 1) °C. Means with different uppercase letters in superscripts in the same row indicate significant difference ($p < 0.05$). Means with different small letters in superscripts in the same column indicate significant difference ($p < 0.05$).
C – non-pressure-processed chicken nuggets (control); N-200, N-400, N-600 – chicken nuggets subjected to high pressure processing at 200 MPa, 400 MPa and 600 MPa, respectively.

bumin, the two major whey protein components [45], survived pressure treatment. This was confirmed by the DSC trace of whey protein concentrate (4.1% moisture) in our study, which showed a maximum transition temperature at 87.99 °C (ΔH = 151 kJ·kg^{-1}). Signals below 40 °C are relative to fat melting and are not shown as they are not relevant to the study.

Effect of HPP on sensory properties

The effect of pressure treatment on the sensory parameters of nuggets was recorded (Tab. 2). Application of pressure up to 600 MPa had no significant effect on colour, taste, odour, saltiness and overall acceptability of the product. HPP is seen to retain sensory attributes like flavour and odour as it affects only non-covalent bonds and keeps covalent bonds intact [29, 48]. In a recent study by Grossi et al. [30], assessment of salt-reduced sausages by napping technique showed no significant change in sensory attributes upon HPP. O'Flynn et al. [28] also reported that organoleptic properties of low-salt sausages were maintained after high pressure treatment. Results from the instrumental analyses of colour and texture were in concordance with those of the sensory analyses. In this study, pressure-treated samples showed a slightly higher texture scores. The instrumental hardness values showed a negative correlation of 0.97 with the sensory scores for texture. The texture of nugget is influenced by its structure. The results indicate that pressure treatment brought about an increase in tenderness mainly due to disruption of muscle fibres and an increase in the moisture retention during cooking, which had positive effects on the sensory evaluation scores.

From the sensory evaluation it was seen that, in general, pressure treatment up to 600 MPa did not affect sensory properties like colour, taste, odour and overall acceptability, while there was a positive alteration in texture. These findings agree with other studies on the favourable effects of high pressure treatment on the shelf life without significantly affecting sensory properties of meat products [11, 19, 39, 49].

Effect of HPP on microbiological growth during refrigerated storage

Application of pressure reduced the microbial load of nuggets and the most striking effect was seen on nuggets subjected to 600 MPa, which diminished the counts of aerobic microorganisms, yeasts, moulds and psychrotrophs to less than 10^1 CFU·g^{-1} (Tab. 3). The samples had an initial count of $10^{2.8}$ CFU·g^{-1} for aerobic micro-organisms, $10^{1.3}$ CFU·g^{-1} for yeasts and moulds, and 10^1 CFU·g^{-1} for psychrotrophs. The increase of pressure also had an effect on the microbiological profile of chicken nuggets during the refrigerated storage.

Aerobic counts gradually increased in all samples during the 60 day refrigerated storage and exceeded the threshold level of 10^7 CFU·g^{-1} in control, N-200 and N-400 samples. Although the aerobic counts were reduced, C nuggets developed an offensive odour by 60th day of storage.

A similar trend was observed for yeasts and moulds except that only control and N-200 nuggets were found to contain more than 10^3 CFU·g^{-1} on 45th and 60th day of storage, respectively. Coliforms were not detected in any of the samples during the storage study, which indicated proper sanitary measures undertaken during preparation and processing of nuggets.

Psychrotroph counts were reduced to less than 10^1 CFU·g^{-1} after HPP and remained so in both N-200 and N-400 samples up to 15 days and up to 30 days in N-600 nuggets. Treatments up to 400 MPa were therefore not adequate to significantly reduce microorganisms in nuggets and prevent spoilage during the storage period.

All microbial counts of nuggets processed at 600 MPa were within the standards specified for cooked meat products throughout the storage period [50]. Hence, treatment of 600 MPa was found to be effective in delaying the proliferation of microorganisms and in extension of the shelf life of nuggets to 60 days. Several recent studies demonstrated high pressure treatment at 600 MPa and above to be effective in reducing bacterial spoilage and extending shelf life of cooked ham [19, 51], NaCl-free dry cured hams containing KCl and potassium lactate [52], chicken breast fillets [53] and beef stored at –18 °C [54]. In line with the findings of previous studies, our study confirmed that HPP at 600 MPa has an inhibitory effect on the growth of spoilage microorganisms in chicken nuggets.

Effect of HPP on lipid oxidation and pH during refrigerated storage

Lipid oxidation affects processed meat quality and shelf life. It is generally regarded that HPP induces lipid oxidation in fresh and cured meat products [12, 51, 55]. The nuggets subjected to refrigerated storage had a moisture content of 74% ± 0.2%. In the present study, a slight increase in lipid oxidation was seen only in 600 MPa treated sample on 0th day compared to control (Tab. 4). Previously, Wiggers et al. [56] reported that HPP treatment at 600 MPa caused a significant increase in lipid oxidation in cooked chicken product, but the effect of lower pressure levels on TBARS

Tab. 4. Effect of high pressure treatment on TBARS of chicken nuggets.

Day of storage	C	N-200	N-400	N-600
	TBARS [mg·kg^{-1}]			
0th	0.07±0.0^{a}	0.07±0.0^{a}	0.07±0.0^{a}	0.08±0.0^{a}
15th	0.23±0.01^{Ab}	0.24±0.02^{ABb}	0.25±0.01^{Bb}	0.26±0.02^{Cb}
30th	0.38±0.00^{Ac}	0.32±0.07^{Bc}	0.33±0.03^{BCc}	0.33±0.05^{Cb}
45th	0.40±0.02^{Cd}	0.41±0.00^{Bd}	0.35±0.01^{Dd}	0.43±0.02^{Ab}
60th	0.60±0.05^{Ae}	0.49±0.04^{Be}	0.40±0.01^{Ce}	0.46±0.06^{ABb}

Means with different uppercase letters in superscripts in the same row indicate significant difference ($p < 0.05$). Means with different small letters in superscripts in the same column indicate significant difference ($p < 0.05$).
TBARS – thiobarbituric acid reactive substances (expressed as milligrams of malonaldehyde per kilogram of sample), C – non-pressure-processed chicken nuggets (control); N-200, N-400, N-600 – chicken nuggets subjected to high pressure processing at 200 MPa, 400 MPa and 600 MPa, respectively.

values was insignificant. TBARS values of all samples increased significantly ($p < 0.05$) during the storage period. Up to 45 days, TBARS values did not exceed 0.5 mg malonaldehyde per kilogram of sample, which is considered to be acceptable for cooked meat products. TBARS values of 0.6–2 mg can result in off-flavour in cooked meat products. ANOVA on the results of TBARS estimation showed that the effect of HPP treatment was not significant ($p > 0.05$). Hence, pressure treatment was not found to trigger lipid oxidation to an extent that causes rancidity in the product. It is also believed that low contents of fat and salt in the product could have contributed in inhibition of lipid oxidation during storage. Also, refrigeration is said to retard fat rancidity as hydroperoxides are more stable at low temperatures [57].

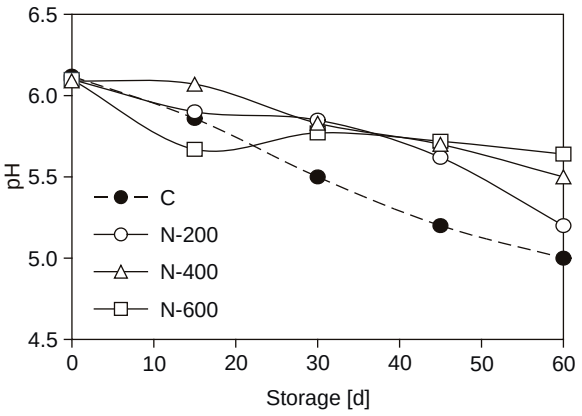

Fig. 3. pH of high-pressure-treated nuggets during refrigerated storage (4 °C ± 1 °C).

C – non-pressure-processed chicken nuggets (control); N-200, N-400, N-600 – chicken nuggets subjected to high pressure processing at 200 MPa, 400 MPa and 600 MPa, respectively.

HPP had no influence on the pH value of the product on the 0th day as no significant changes in pH values were observed. However, a steady decrease in pH values was seen during the storage period (Fig. 3). In control sample, which had started giving offensive odour due to excessive microbial proliferation, pH dropped from 6.12 to 5 in 60 days. At any sampling day throughout the storage period, 600 MPa treated sample recorded the highest pH among all four samples, while the pH of pressure-treated samples remained higher than control. Decrease in pH was in positive correlation with the increase in microbial counts during storage.

CONCLUSION

Application of HPP was successful in extending the shelf life of low-sodium chicken nuggets. HPP also imparted desirable effects on the physicochemical properties, while retaining the sensory quality of the product. It was found that, with increasing pressure levels, water holding capacity of nuggets increased, while hardness and cooking loss decreased. Non-pressure-treated nuggets were acceptable for at least 30 days at refrigerated storage. Shelf stability of low-sodium nuggets under refrigerated conditions was considerably improved with 600 MPa HPP treatment. The product was found to be microbiologically safe, without any detection of rancidity even after 60 days of refrigerated storage (4 ± 1 °C). To sum up, the present study demonstrated, for the first time, that safe replacement of about 50% NaCl with KCl in re-structured chicken nuggets is possible with the combination of HPP. Application of this technology should be considered and evaluated for development of low-salt/low-sodium cooked meat products.

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Received 19 April 2015; 1st revised 7 July 2015; accepted 31 July 2015; published online 19 October 2015.