Comparative effect of High pressure and Conventional Heat Processing on the Development and Shelf life of Restructured Surimi balls during Chilled Storage

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Abstract

There is an increasing demand towards innovative fish products to cater the demand of modern market and restructured surimi products are such emerging products. Restructured surimi balls were developed by restructuring the washed and concentrated pink perch surimi, formed into shapes under traditional heat setting at 90°C for 60 min and pressure setting at 200 MPa for 15 min. Surimi was added with MTGase (0.5%) for enhancing the crosslinking between proteins and casein as a substrate for cross linking, so as to improve the physico-chemical properties of the balls. A comparative analysis on the storage characteristics of pressure set and heat set surimi balls were done under chilled conditions. When compared with heat set balls the texture characteristics and gel strength values were less, whereas elastic nature of the product was retained in high pressure processing. High pressure can retain the raw texture of the balls with softer and glossier appearance while heat causes texture to be harder and dry. The quality indices values were found within the limit even after 16 days of storage under chilled conditions. Even though microbial quality of both samples were comparable, higher sensory score was given for pressure-set sample. Also, MTGase addition had synergistic effect with high pressure in enhancing the overall quality of products. Present study exhibited the potential of high pressure technology in developing restructured surimi balls through pressure induced gelation similar to the traditional heat induced gelation.

Keywords: High pressure processing, restructured products, surimi balls, pink perch

Introduction

Fish product development in recent years have been driven mostly by the markets coupled with the drivers of health and technology. So the focus was towards satisfying the consumers shifting demands for new alternatives of fish and sea foods, which have led to more value addition and introduction of novel fish products. The demand for high quality minimally processed health food is on the raise globally. In order to address the modern consumer demand new alternative technologies and processes are being introduced which often adds quality and safety. These technologies are appealing to be utilized in replacing the conventional method or combination with themselves or with traditional ones (Barbosa-Canovas & Bermudez-Aguirre, 2011). The most extensively researched and promising non-thermal process for preservation of foods appears to be high pressure processing (HPP) (Ross et al., 2003) which is being commercially applied mostly for the processing of liquid based foods like milk, juices, shakes, smoothies etc. (Qiu et al., 1998; Jia et al., 1999; Leistner & Gould, 2002).

The perishability and the safety concerns with respect to fishery products have attracted detailed investigations on their amenability to high pressure. The high pressure effect on spoilage-causing and pathogenic microorganisms can be exploited for shelf life extension of fresh fish. Furthermore, high pressure processing offers novel texturized products making use of its effect on fish myofibrillar proteins (Venugopal, 2005). HPP has received increased attention in recent years as a possible way of improving functional properties of muscle proteins and also as a powerful tool for protein and enzyme modulation studies (Mozhaev et al., 1996). High

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pressure causes volume changes in the food or its constituent parts, resulting in protein denaturation, textural alterations, gelation, greater flavour and colour retention and enzyme modifications (Hugas et al., 2002) and having potential for the production of surimi, kamaboko or other minced products. The high pressure effect on restructured food proteins has been a current area of food research. Pink perch (Nemipterus japonicus), one of the important fish species of surimi manufacture for its abundance, low cost and white meat, is of great commercial value and a diversified market potential, was selected for developing restructured product.

Based on this background, the present study was designed at developing restructured surimi balls from pink perch using the non-thermal high pressure processing and compare its shelf stability against conventional heat processed balls under chilled storage conditions. The hypothesis was that application of pressure will also minimise protein denaturation, increase nutritional and sensorial quality and maintain inherent properties which could be lost in traditional pasteurisation process.

Materials and Methods

Pink perch (Nemipterus japonicus) with an average weight of 200 g was used as raw material for the preparation of restructured surimi balls. The raw material was procured from local fish market and transported to the lab under iced condition. The fishes were thoroughly washed with chilled potable water, weighed, descaled, headed and gutted. Mince was obtained using deboning machine (BAADER 694, Lubeck, Germany) equipped with a drum of holes 3 mm diameter in size. The mince then washed 3 times with ice cold water at the ratio 1:3 in order to avoid any debris, blood and water soluble extrac- tives from the meat. The mixture was stirred gently and the washed mince was filtered using a muslin cloth. The washing process was carried out thrice and the final washing was done with sodium bicarbonate to remove fat from mince. Thus the concentrated myofibrillar protein obtained after washing and pressing the mince is referred as surimi. Surimi was mixed thoroughly with 0.2% sodium tripolyphosphate (STPP), 4% sucrose and 4% sorbitol as cryoprotectant during frozen storage. Frozen surimi was used for the preparation of surimi balls added with standardized ingredients which is depicted in Table 1.

Table 1. Standardised ingredients for the preparation of restructured surimi ball

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surimi</td>
<td>1000 g</td>
</tr>
<tr>
<td>Corn starch</td>
<td>150 g</td>
</tr>
<tr>
<td>Transglaminase enzyme</td>
<td>5 g</td>
</tr>
<tr>
<td>Casein</td>
<td>10 g</td>
</tr>
<tr>
<td>Oil</td>
<td>50 ml</td>
</tr>
<tr>
<td>Salt</td>
<td>20 g</td>
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</tbody>
</table>

Surimi was thoroughly mixed with these ingredients in bowl chopper (Garant MTK661, MADO, Dornhan, Germany) for 10 min under low temperature (<15°C). The mix was filled immediately in spherical silicone mould having (40 mm diameter) for getting a proper ball shape. An incubation temperature of 25°C for 30 min was given for MTGase enzyme to act and make strong crosslinkings. In order to prevent the entry of water or pressure transmitting fluid into the sample, the silicone mould with mince was packed in polyester polyethylene laminated pouches under vacuum, using a vacuum sealer (VAC-STAR, Model No: 101051) before processing. Two sets of samples were made and processed under high pressure and conventional heat processing. After processing samples were demoulded, repacked and kept under chilled conditions (2 ± 1°C) for analysing the storage characteristics. Samples were designated as pressure set and heat set for high pressure and conventional heat processed ones and collected at every four-day interval for a period of 16 days.

High pressure processing of samples were done using High pressure equipment (Model No: FPG7100:9/2C, Stansted Fluid Power Ltd. Essex, United Kingdom). The instrument is equipped with a cylindrical pressure chamber, having 2L capacity and a length and diameter of 570 mm, 70 mm respectively. The pressure transmitting fluid used was distilled water. Adiabatic compression of pressure transmitting fluid causes a raise in temperature at a rate of 3-4°C for every 100 MPa increase in pressures. Samples were subjected to 200 MPa pressures at a constant temperature (38.7°C) and holding time (15 min). The, pressurized samples were taken and stored under iced condition for 16 days. Regular replacement of ice was ensured.
Traditional heat setting of vacuum-packed surimi balls were done in a water bath set at 90°C. Incubated samples were given a heating (90°C) for a period of 60 min, as surimi mix was packed inside the mould, an additional heating time was given for maintaining a proper core temperature. The heated samples were then immediately cooled down to 3-4°C by keeping in ice for 15 min. Processed samples were stored at 2-3°C under iced condition.

The amount of expressible moisture (Em) in each sample was measured according to the method developed by Jauregui et al. (1981) with slight modifications. Sample weight (W1) of 3 g (±0.2 g) was taken and kept between two layers of filter paper (Whatman No.1), which were then placed at the bottom of 50 ml centrifuge tube padded with extra filter paper and centrifuged at 5000 rpm for 10 min at 15°C. Immediately after centrifugation, the samples were reweighed (W2) and the Em % was calculated as follows:

\[ Em \% = \left( \frac{W1 - W2}{W1} \right) \times 100 \]

Instrumental Texture Profile Analysis (TPA) of samples was carried out after equilibrating the products at room temperature for 15 min. Analysis was done with a texture analyser (TA Plus, Lloyd instruments-Ametek, Hampshire, UK) equipped with a 50 kg load cell (Bourne, 1978). Samples were placed on the platform of the machine and subjected to analysis. TPA was done by using cylindrical plunger of 50 mm Ø. Texture parameters of hardness, cohesiveness, springiness, gumminess and chewiness were analysed.

Gel strength of the samples were calculated using a steel ball probe having diameter 10 mm. Samples were cut from the balls in 4 x 2 cm width x height and placed on the texture analyser (TA Plus, Lloyd instruments- Ametek, Hampshire, United Kingdom). Test speed was set at 50 mm/min and depression limit of 100 mm was used. The gel strength was expressed as the product of breaking force and breaking strain (g/cm) (Ko et al., 1990).

Folding test was conducted according to Suzuki (1981) by folding a 3 mm thick slice of fish ball in quadrants to examine the structural failure of the sample. The evaluation was performed in accordance with a five-point scale as follows: grade (5)- no crack when folded into quadrants; grade (4)- no crack when folded in half, but occurs on second folding; grade (3)- crack develops gradually when folded in half; grade (2)- crack develops immediately when folded in half; grade (1)- crumbles when pressed by finger.

TVB-N and TMA were analysed by the micro diffusion method as per Conway (1962) with slight modifications, and the values were expressed as mg 100 g–1 of the sample. TBARs was determined using distillation method described by Tarladgis et al., 1960.

The aerobic plate count was estimated by pour plate technique (Maturin & Peeler, 2001). Briefly, 50 g of each sample were drawn aseptically and homogenized with 450 ml of phosphate buffer in a filter stomacher bag using a Stomacher® 400 Circulator (Seward Limited, UK) for 2 min at 200 rpm. After decimal dilutions, duplicates of three consecutive dilutions were plated on Plate Count Agar (Difco). Plates were enumerated after incubation at 35 ± 2°C for 48 ± 2 h for obtaining aerobic plate count.

Statistical analysis of data was carried out using IBM-Statistical Package for Social Science software (SPSS VERSION 20.0, ChicagoIL. USA). Analysis of variance (ANOVA) was carried out with Duncan’s Multiple Range Test (DMRT), to determine the significant difference among the treatments and during the period of storage. A T-test was carried out to determine the significant difference between treatments. The level of significance was set up at pd”0.05. All the above experiments were carried out in triplicates and the results were expressed as Mean ± S.E.

Results and Discussion

The ingredients and process for developing the restructured surimi balls was optimized based on the preliminary experiments. Surimi added with salt (2%) solubilized the meat especially myofibrillar fractions and microbial transglutaminase enzyme (MTGase) 0.5% formed crosslinkings between proteins. A setting incubation at 25°C for 30 min was given for mince before processing in order to make transglutaminase mediated crosslinkings. MTGase is widely used enzyme in food industry as binding agent, which is capable of catalyzing acyl-transfer reactions forming covalent cross-links between the primary amines of various proteins as well as peptides, thereby improving the gel structure (Jiang et al., 1998). Casein added along with MTGase enhanced the physico chemical properties of the surimi balls. Earlier reports showed proteins have
been used as binding agents or as additives to improve mechanical and functional properties of fish products e.g., egg white (Yetim & Ockerman, 1995), casein and beef plasma thrombin (Baker et al., 2000), casein, whey protein concentrate and MTGase (Uresti et al., 2004) etc. Other ingredients like corn starch and vegetable oil was added at concentrations optimized based on the sensory acceptance. Even though high pressure had an effect on the gelatinization of starch, in the present study the applied pressure was low (200 MPa), which was not enough to undergo gelatinization of starch. So the pressure induced gelatinization was mainly contributed by muscle protein. Restructuring was done by mincing, mixing with other ingredients, grinding and forming of into shapes using ball shaped moulds.

The release of expressible moisture is an indicator of the water holding capacity of the product. More stable gels have less expressible water, indicates higher water holding capacity. A significant difference (p<0.05) in expressible moisture was observed between pressure set balls than heat set balls (Fig. 1). The pressure set balls had an initial expressible moisture of 30.08±0.81%, which slightly decreased on 4th day and then increased to 32.80±0.79% on 16th day, whereas heat set samples initially had 20.03±0.58% increased to 21.17±0.67% after storage. This might be due to sudden expulsion of entrapped water from the samples during cooking and further forming strong covalent crosslinking resulting from higher water protein interaction. Pressurization causes a significant reduction in water holding capacity which is attributed to muscle protein denaturation and reduces the water protein interactions. Gomez-Estaca et al., 2009 observed a reduction of water holding capacity in tuna, salmon and bacalao carpaccio at 200, 250 and 300 MPa pressure applied for 5 and 15-min holding time. Addition of MTGase enzyme increased the expressible moisture in surimi balls because MTGase forms intermolecular crosslinks between myosin, which was enhanced during heating. Also the added starch undergo gelatinization at high temperature. So, the release of expressible moisture was less during heat setting compared with pressure set ones. Unlike in heat setting, pressures of 200 MPa can cause partial denaturation of protein and form incomplete networks, the entrapped water will be lost during centrifugation. Trespalacios & Pla (2007) observed a lower release of expressible moisture in gels obtained from pressure without TCage than enzyme added ones. The pressure effect on the binding properties of meat depends on various factors like species, type of muscle, protein, ionic strength, level of fat and treatment conditions like pressure, time and temperature (Jimenez-Colmenero et al., 1997).

High pressure can induce modification in texture by affecting the myofibrillar protein structure and their gel forming properties. Changes in gel strength of heat and pressured induced surimi balls during storage are shown in Fig. 2. Significant difference (p<0.05) was observed in gel strength values among the means between treatments. A lower gel strength value was observed in pressure set samples (208.80±12.61 g cm) than in heat set samples (466.09±11.78 g cm) because at 200 MPa pressure gel network formed were softer and giving a glossier appearance than hard heat set ones. The difference in the gel forming ability is due to the differences in protein integrity and bonding formed during thermal processing (Benjakul et al., 2001). High pressures of 200-300 MPa can cause changes in tertiary and quaternary structures but changes in secondary structure takes place only above 700 MPa (Rastogi et al., 2007).

Pressure set samples form soft gels due to partial protein denaturation and aggregation that resulted in formation of gel network, which are sometimes reversible especially below 400 MPa. So the gel networks formed at 200 MPa pressure was not stable. This is because pressure induced gelation is formed due to the association of intermolecular hydrophobic associations whereas heat induced gelation is due to disulphide bonding (Lanier, 1996). Also pressure gels are mostly associated with non-covalent bonds, but heat gel associated with formation of covalent interactions. These interactions are caused by the denaturation due to violent

![Fig. 1. Changes of expressible moisture value in pressure set and heat set surimi balls under chilled storage](image-url)
movement of molecules (Barbosa-Canovas & Tabilo-Munizaga, 2004). Another important fact is that the addition of MTGase enzyme enhanced the gel networks in protein by catalysing the acyl reaction between lysine and glutamine. Also heat induced starch gelatinisation favours a good gel strength. During storage the gel strength showed an increase in both samples, but after 8 days of storage the gel strength decreased. This change might be due to the increased proteolytic activity and low temperature modification in protein network of the balls, when stored under chilled condition.

The textural parameters of surimi balls were affected by the application of heat and pressure. Table 2 shows the result of TPA analysis (hardness, cohesiveness, springiness, gumminess, chewiness) of restructured surimi balls. Statistically significant difference (p<0.05) was observed in all texture parameters tested after each sampling except for springiness between pressure set and heat set samples. An initial hardness value of 38.24 ±0.82 N was observed in heat set restructured balls increased to 66.32±1.50 N on 8th day and thereafter it decreased. Similarly, in pressure set restructured balls hardness increased from 14.75±1.68 to a maximum 23.86±1.44 N. The harder nature of heat set balls might be due to aggregation and water loss induced by denaturation of myofibrillar fraction (Lopez-Caballero et al., 2000) during heating whereas the tenderness was retained in pressure induced gels (<400 MPa) making the balls softer but stable. Tenderness is the most important criteria that characterise the eating quality of the product. Previous observations state that pressure induced gels were softer, glossier and stable than hard, dry heat induced gels (Kunnath et al., 2015, Van-Camp & Huyghebaert, 1995). The retention of water in the surface of balls have made them appear glossier. The cohesiveness values were more in pressure induced gels than heat induced ones and they changed from 0.71±0.03 to 0.64±0.02 whereas heat set restructured balls showed marginal difference (0.44±0.01 to 0.49 ±0.05) during storage. Macfarlane (1985) and Suzuki et al., 2006 reported that pressure treatment improved the cohesion between meat particles in reformed meat or fish type products. No significant difference was observed in springiness values (t-test; p<0.05) among the means between the heat set and pressure set samples, at all stages of samples. The gumminess value, of pressure set and heat set restructured balls increases from 10.57±1.51 to 12.58±1.16 and 16.78±1.29 to 21.11±1.03 and chewiness value, increases from 11.67±1.87 to 15.32±1.88 and 21.58±1.19 to 24.79±0.40 and respectively. Pressure treated texture was different from that seen in cooked, being softer, cohesive, less chewy and gummier.

Folding test is simple and fast method to measure the binding property of the restructured surimi balls. The pressure set surimi balls were graded 5 up to 8 days of storage and on 16th day a grade of 4 was given. On 0th day, heat set restructured surimi balls were graded 5 (no crack when folded in to quadrant, 8th day was graded below 3 and the 16th day surimi balls were graded 2. The folding tests clearly showed a more folding test value in pressure set samples due to the retention of elastic gel nature of the product than heat induced ones. Even after 9 days of storage the pressure set gels were showing superior elastic nature than heat induced ones. So, the application of pressure can create soft and elastic gels than when compared with traditional heating as it causes loss in the elastic nature of the product.

TVBN relates to the organoleptic score and is an index for spoilage of products. The changes in TVB-N values in both samples during chilled storage was shown in Table 3. Initially the TVB-N values were almost similar in both samples, but the increase in values were higher in pressure set samples during chilled storage. These changes in TVB-N values were significant in pressure set samples when compared to other during storage. This might be due to the presence of bacteria that survived after pressurization at 200 MPa might be responsible for the formation of volatile bases in the product. As previously described the application of 200 MPa can cause only small reduction of microbial count.

Fig. 2. Changes in gel strength value of pressure set and heat surimi balls under chilled storage

Table 2 shows the result of TPA analysis (hardness, cohesiveness, springiness, gumminess, chewiness) of restructured surimi balls. Statistically significant difference (p<0.05) was observed in all texture parameters tested after each sampling except for springiness between pressure set and heat set samples. An initial hardness value of 38.24 ±0.82 N was observed in heat set restructured balls increased to 66.32±1.50 N on 8th day and thereafter it decreased. Similarly, in pressure set restructured balls hardness increased from 14.75±1.68 to a maximum 23.86±1.44 N. The harder nature of heat set balls might be due to aggregation and water loss induced by denaturation of myofibrillar fraction (Lopez-Caballero et al., 2000) during heating whereas the tenderness was retained in pressure induced gels (<400 MPa) making the balls softer but stable. Tenderness is the most important criteria that characterise the eating quality of the product. Previous observations state that pressure induced gels were softer, glossier and stable than hard, dry heat induced gels (Kunnath et al., 2015, Van-Camp & Huyghebaert, 1995). The retention of water in the
Changes in TBARs value of the products during storage is shown in Fig. 3. and the two samples showed an increase in values during storage. In pressure-set restructured balls, the TBARs value increased from 0.68 to 1.39 mg MDA kg\(^{-1}\), whereas in heat-set restructured balls it is increased from 0.36 to 0.88 mg MDA kg\(^{-1}\) sample. Increase in TBARs values in pressure-set sample might be due to the pressure induced denaturation of haem proteins and membrane disruption causes the release of Fe\(^{2+}\) from haem group, which promote the oxidation of lipids (Bajovic et al., 2012). Similar observation was noticed by Bindu et al., 2013 in high pressure treated *Fenneropenaeus indicus* (100, 270, 435 and 600 MPa) and observed an increasing trend of TBA values from 0.32 to 0.65 at 270 MPa pressures. Maximum TBA values (1.07 mg MDA Kg\(^{-1}\)) noticed in pressure-set ones but was observed to be below the limit of acceptance. Addition of MTGase may trigger oxidative reaction in fish mince during chilled storage (Moreno et al., 2010; Kellerby et al., 2006) especially in pressure-set ones. A significantly higher rate of oxidation (peroxides and TBARS) was observed in pressure-set samples TMA detected after 4 days of storage. Small amount of TMA may be produced by the endogenous enzymes inside the fish. But main degradation of TMAO to TMA is due to enzymes of psychrotrophic bacteria that invade the meat during low temperature storage (Sen, 2005). All values were below the limit, showing an expected higher shelf life in chilled storage conditions. During storage TMA values were increasing in both samples. Increasing trend of TVB-N and TMA during chilled storage is due to endogenous enzyme activity, which was also noticed in pressure treated *Fenneropenaeus indicus* during chilled storage (Bindu et al., 2013).

Oxidative rancidity is an important organoleptic feature for acceptance or rejection of fish, especially when pressurized, due to the prooxidant effect of pressure on fish muscle (Montero et al., 2005).
pressure-set samples due to the cross-linking of protein at interface which causes changes in the protein structure that altered the iron binding site on protein, bringing iron and peroxides closer and trigger oxidation. Even though lipid oxidation increased with storage, all values were within limit even after 16 days of storage indicated a higher shelf life of the product during chilled storage.

Sensory evaluation was carried out with ten sensory panellists based on hedonic scale (Jones et al., 1955). Panellist evaluated the different sensory attributes like appearance, freshness, firmness, succulence, odour and their overall acceptability for both pressures set and heat set surimi balls and the average scorings of the panellist is shown by a spider web graph.

The sensory analysis helps to know the preference of the consumer towards traditional or pressure processed balls and also understand the reasons for liking and disliking of product (Lawless & Heymann, 1999). The spider web graphs clearly showed the descriptive analysis of sensory attributes. Sensory scores of both products were above acceptable level in hedonic scale. Even though the appearance of heat set balls were superior, the freshness and succulence retained higher in pressure-set balls. The pressure induced setting helped to form surimi ball with more fresh and natural. The succulent raw taste and texture of the meat gels was more preferred by the sensory panel than the hard cooked gels. So overall acceptability of panellist towards pressure set balls were higher than conventional heat set balls. A decreasing trend was noticed in the sensory scores during storage, but the observed difference among the two samples was insignificant after 8 days of storage. Moreover, sensory evaluation is a
subjective method and could not reveal the minute differences in textural attributes as in instrument analysis.

High pressure processing can be used to develop restructured surimi balls from pink perch mince through pressure induced denaturation, aggregation and gelation. When compared with conventional heat-set products pressure-set samples retained freshness and elasticity than heat-set samples and the pressure induced gels were softer and glossier than heat induced ones. A comparative storage analysis was done to analyse the effect of different processing (high pressure and heat treatment) on the shelf life of the product. The TVB-N, TMA and TBARs values were found to be below the limit which intend a more shelf life under chilled conditions. Reduction in microbial count in pressure treated samples were similar to cooking, but the recovery rate will be faster in pressure treated samples during storage. Even though sensory likings were higher for pressure-set balls, further study is required in textural and microbial quality enhancement of the pressure processed restructured balls.

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References

meat and myosin pastes. Nippon Shokuhin Kogyo Gakkaishi. 37: 637-642


