Fishery Technology 57 (2020) : 22 - 27

Effect of Chitosan Hydrogel inclusion on Centesimal Composition of Pangasius (*Pangasianodon hypophthalmus*) (Sauvage, 1878) Emulsion Sausages under Refrigerated Storage

Kasturi Chattopadhyay, K. A. Martin Xavier*, P. Layana, A. K. Balange, B. B. Nayak
ICAR-Central Institute of Fisheries Education, Mumbai - 400 061, India

Abstract

Chitosan is a diverse polymer of biological origin having a widespread application in food industry. The cross linking properties of chitosan beneficially affects the protein-carbohydrate interactions which in turn enhances the functional properties in emulsion based meat products. In this context, the present study was conducted to incorporate chitosan hydrogel in varying ratio (0, 0.125, 0.25, 0.375 and 0.5%) into fish mince (*Pangasianodon hypophthalmus*) (Sauvage, 1878) sausages in order to develop a functional sausage products upon storage. Samples without chitosan additions showed a significant and rapid decline in the crude protein content after the 24th day of storage in contrast to the chitosan treated groups where a slower rate of decline was found only after 48th days of storage. Fat content also showed greater stability in the values in case of chitosan added samples upto 48th days of storage in comparison to the control samples where a rapid decrease in the contents were observed. However, ash and carbohydrate content showed minor variations among the treatment and control groups though all were showing decreasing trend upon storage. The comparative analysis of centisimal composition of the produced sausages showed that inclusion of chitosan gel had a significant effect ($p<0.05$) on the keeping quality of the product when compared with control and can be presumed as a superior ready-to eat quality product.

Keywords: Chitosan, emulsion sausage, centesimal composition

Received 23 May 2019; 15 January 2020; Accepted 30 January 2020

**E-mail: martinxavier@cife.edu.in*

Introduction

Modern day consumer's demands for ready to eat food products which are nutritionally rich and have better sensory attributes. Several food additives are used as ingredients during manufacturing process that adds to the taste, appearance, flavour and colour of the product. But in terms of human health, an additive used must be safe in preserving its nutritional as well as desirable quality parameters in the food product. Biopolymers serve as an interesting additive than harmful synthetic ones in terms of food safety which have prompted the industrial sectors to utilize them in formulating healthy functional ready-to-eat meat products. In this regard, chitosan being a copolymer of â-(1'!4)-linked 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose, possess many valuable properties that make it an interesting functional and technological additive in several agricultural, poultry, food and seafood industries. Chitosan has favourably influenced the physical, chemical and functional properties of meat-based products *viz.,* enhancing fat and water retention, stabilization, emulsification and gel formation by facilitating strong crosslinking interactions (covalent bond, hydrogen bond and hydrophilic bond) with meat proteins (Darmadji & Izumimoto, 1994; Draget, 1996; Lin & Chao, 2001; Kamil et al., 2002; López-Caballero et al., 2005; Mao & Wu, 2007; Amiza & Kang, 2013;).

Emulsion based meat products are prepared from a wide range of sources by comminuting and emulsification of meat protein where proteins act as emulsifier between water and fat phases (Peng et al., 2009). Addition of fat as an ingredient gives an emulsion product their characteristic flavor and mouth feel. Moisture contributes to the consistency and juiciness of the product. Among the fish species, Pangasius (*Pangasianodon hypophthalmus*) mince urge for special attention to make it a popular raw material for formulation of emulsion sausages due to its higher productivity, low cost, natural fat content and harder consistency. During storage, the nutrients present in the product get degraded and its content gets reduced. As an additive, chitosan inclusion can improve the binding of moisture and fat molecules forming homogenized lipid droplets maximizing the product integrity, shelflife and maintaining the nutritional profile of the final product (Sheard et al., 1989; Cho et al., 1999; Sayas-Barberá et al., 2011).

In this context, the present study was performed to evaluate the usefulness of chitosan inclusion in the form of a hydrogel at different ratios (0, 0.125, 0.25, 0.375 and 0.5%) into the emulsion batter of Pangas (*Pangasianodon hypophthalmus*) meat sausages and comparative assessment of its centesimal composition was done under refrigerated storage (3 ± 1°C).

Materials and Methods

Sutchi catfish (*Pangasianodon hypophthalmus*), having a total length of 40-45 cm and an average weight of 700-800 g were purchased from a local fish farm at Panvel, Mumbai, India. The fish were brought to the processing laboratory of CIFE in a chilled condition with 1:1 fish to ice ratio. All chemicals used in the experiment were of analytical grade supplied by Merck (Germany). Chitosan (100 cps viscosity, 92% deacetylation degree, 75.6 kDa molecular weight) used for the experiment was supplied by the M/s. India Sea Foods (Cochin, India). Salt, sugar, corn flour, vegetable oil etc. were purchased from local supermarket.

Before preparation of mince, fishes were cleaned, beheaded, gutted and washed thoroughly to remove blood and slime. After, they were splitted dorso-ventrally into two halves manually by a knife. The splitted fish was further deboned in a mechanical deboning machine (Baader694, Lubeck, Germany) by drum mechanism with a counter rotating belt. Mince was prepared by feeding the dressed fishes into the drum sieve having 5 mm Ø holes. The temperature was maintained below 10°C throughout the process. The final mince obtained was sealed in polyethylene pouches (LDPE) and kept in a refrigerator till the preparation of sausage.

Chitosan gel at concentrations of 0.125, 0.25, 0.375 and 0.5% (w/v) in acetic acid aqueous solution were prepared following Xavier et al. (2017) with slight modifications. Briefly, 0.125, 0.25, 0.375 and 0.5 % (w/v) of chitosan powder was dissolved separately with calculated amount of water containing acetic acid (0.4 mL g^{-1} chitosan powder) under continuous stirring and the resulting viscous solution was kept in chilled condition till usage. Chilled chitosan gel was added to the pre-emulsified batter in a silent cutter for preparing the final emulsion batter used for stuffing.

The minced meat separated from the meat bone separator was mixed with solution containing 0.25% (w/w) sodium tri-polyphosphate (STPP), 2.0% (w/w) iodised common salt (Tata Chemicals Ltd., Mumbai, India), and 1% (w/w) sugar. The ingredients were blended using a Hobart mixer (Hobart AE 200, London, UK) and kept in chilled condition (4°C) for 60 min. The mixture was initially comminuted using a food grade stainless steel silent cutter (Stephan UMC 5 Electronic cutter, Germany) for 3 min followed by slow addition of sunflower oil (Gold Winner-Kaleesuwari Refinery Pvt. Ltd. Chennai) and the mixing was continued for further 3 min. Different concentrations of prepared chitosan gel (0, 0.125 0.25, 0.375 and 0.5% codes as CS-0, CS-1, CS-2, CS-3 and CS-4 respectively) and corn starch were added to respective treatment batters as ingredients followed by blending each batter for 3 min temperature of the batter was maintained below 15°C by adding ice-water. The sausage batters thus obtained were stuffed manually into polyvinylidene casings (diameter: 4 cm, length: 17.5 cm) by Stainless Steel Sausage Stuffer (Kitchener 5-Lb, China) and linked at two ends. The stuffed sausages were cooked at 40°C for 30 min followed by 90°C for 20 min in a thermostatically controlled water bath (Strike 300, Steroglass, Perugia, Italy). They were further cooled in ice-water and stored overnight at 4°C in refrigerator for quality evaluation.

Proximate parameters like moisture (%), protein (%), fat (%) and ash (%) of emulsion sausage with different levels of chitosan (CS-0, CS-1, CS-2, CS-3 and CS-4) were determined according to the AOAC methods (AOAC, 2005). Moisture content of the sausage samples was determined by direct heating (100 ± 2°C) of the homogenised sample in a hot air oven for 18-24 h. Crude protein content of sausage samples was evaluated by estimating the total nitrogen content (micro-Kjeldahl method) using a distillation unit (Kelplus Pelican, India). Crude fat content of sausage samples was measured using petroleum ether (60-80 BP) as solvent according to the Soxhlet method. Ash content of sausage samples was determined by incinerating the sample at a temperature of 600±5ºC for 6 h using Phoenix Microwave furnace (CEM, UK). The carbohydrate content of each sample was calculated by difference. Each parameter were analysed in three replicates and the mean values expressed as gram per 100 g of sample.

The data generated for centesimal parameters were statistically analysed by statistical package SPSS version 22 (SPSS Inc., USA) in which data were subjected to two-way ANOVA factorial design considering two factors: Storage period (Factor 1) and CS (Factor 2) along with Duncan's multiple range test for determining the significant differences between the means. Comparisons were made at the 5% probability level. P value below 0.05 was considered statistically significant. The results were expressed as mean±S.D. (Chattopadhyay et al., 2019a).

Results and Discussion

Centesimal analysis is an important factor for quality assessment of sausage products during storage. Changes in the individual component under refrigerated storage signify different types of changes in the sausage quality *viz.,* increase in moisture content helps in microbial growth whereas reduction in protein and fat content indicates protein degradation and lipid oxidation respectively. The centesimal composition like moisture (%), protein (%), fat (%), ash (%) and carbohydrates (%) of freshly prepared emulsion sausage with different levels of chitosan (CS-0, CS-1, CS-2, CS-3 and CS-4) in the batter mix are depicted in Table 1. Among the proximate parameters the moisture, protein and carbohydrate contents showed significant difference between different treatment groups while, the ash and fat values are non-significant.

In the present study, the initial moisture levels in the Pangas mince sausages after preparation were similar to those reported for fresh fish sausages (Rahmanifarah et al., 2015). However, moisture content varies significantly ($p<0.05$) between the treatments groups and control samples during storage. Moisture content in the chitosan incorporated sausage samples (CS-1, CS-2, CS-3, CS-4) remains stable during storage in comparison to the control group (CS-0) where moisture content shows significant reducing trend (Fig. 1). This fact can be correlated with the ability of chitosan to absorb water by hydrogen bonding with water molecules through its hydroxyl and amine groups, thereby increasing the moisture content of the chitosan added sausage samples (Sormoli et al., 2012). These results are also in agreement with work reported by Chattopadhyay et al. (2019b) where chitosan hydrogel additions showed an increasing trend in water holding capacity in fish emulsion sausages on storage. However, the moisture content decreases significantly in the sausages with 0.50% chitosan content (CS-4) that can be related to the fact that higher amount of acid in gels might have interfered with the water holding capacity and simultaneously releases water leads to reduced moisture content. Experiment done by Andres et al. (2006) on chicken sausages reported that same level of ascorbic acid incorporation as an additive showed decreasing trend in moisture content when increasing levels of different fats were used. Work reported by Soultos

Table 1. Centesimal composition of emulsion sausages prepared from fish mince with different levels of chitosan gels (g 100 g^{-1} sample on wet basis)

Treatment	Moisture content	Protein (N x 6.25)	Crude lipid	Ash	Carbohydrate
CS-0	69.48 ± 0.67^{a}	13.95 ± 1.55^{c}	6.32 ± 0.20^{a}	2.95 ± 0.06^{a}	8.33 ± 1.29^{ab}
CS-1	68.32 ± 0.60^{a}	13.53 ± 0.10^{b}	6.33 ± 0.08^{a}	2.97 ± 0.05^{a}	8.85 ± 0.56^{abc}
CS-2	69.13 ± 0.44^{a}	13.91 ± 0.69^{b}	6.33 ± 0.02^{a}	2.99 ± 0.04^{a}	7.64 ± 1.07^{a}
CS-3	69.99 ± 1.17^{a}	13.70 ± 0.40^{a}	6.33 ± 0.07^{a}	2.99 ± 0.11^{a}	7.98 ± 1.29^{b}
CS-4	70.02 ± 0.23^{b}	13.64 ± 0.79^{a}	6.33 ± 0.07^{a}	3.04 ± 0.02^{a}	6.97 ± 0.68^{a}

Values are presented as the mean ± SD of three independent replications (n=3). A one-way ANOVA ($p<0.05$) and Duncan multiple range test (homogenous subsets indicated by alphabets) was performed to determine statistical significance between the groups.

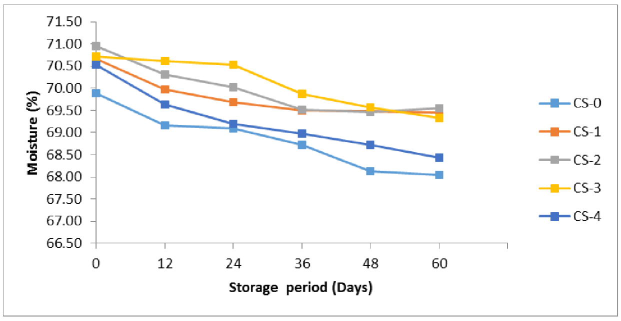

Fig. 1. Change in moisture content of the refrigerated emulsion sausages prepared with different concentrations of chitosan gel

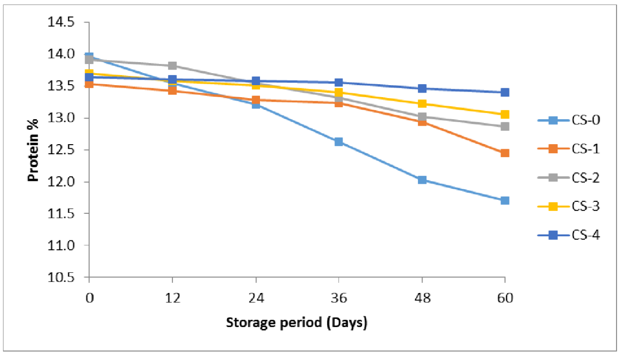

Fig. 2. Change in protein content of the refrigerated emulsion sausages prepared with different concentrations of chitosan gel

et al. (2008) showed that chitosan at 0.5 and 1 % concentration produced sausages with pH range 6.1 to 6.3 initially that increased with storage period and maintained its moisture intact.

Protein is the basic composition in fish meat that is responsible for the final texture as well as shelf life of any product. During storage, degradation of protein releases free amines that can favour microbial proliferation and can thus reduce the shelf life. In this storage study a significant decline in crude protein content in control sample after the 24th day of storage as compared to the treatment groups where, the protein content decreases slightly only after 48th days of storage irrespective of chitosan concentration (Fig. 2). Likewise, the results obtained for the protein content, also revealed that addition of chitosan limited the protein degradation process of sausages when subjected to refrigerated storage. The decline in the protein level as a function of the storage time in the control sample are in conformity with works of some authors where they indicated a possible correlation between protein and lipid oxidation parameters which resulted into reduction of protein content throughout the storage period in pork liver pâté and hamburger (Estévez & Cava, 2004; Ganhão et al., 2010). Due to the antimicrobial activity of chitosan lower total viable count (TVC) and the psychrophilic count (PPC) was observed in our previous study (Chattopadhyay et al., 2019a), which might have minimised the degradation of protein components during the storage period. This substantiated the effects of chitosan in increasing the shelf-life of the sausage (Chattopadhyay et al., 2019a).

Change in fat content of the refrigerated emulsion sausages prepared with different concentrations of chitosan gel is depicted in Fig. 3. There was significant ($p<0.05$) decrease fat content in the control sample during storage whereas the treatment groups showed stability in the fat content upto 48th days of storage after which slight decrease in the contents were noticed. The reduction in fat content in control samples with respects to the chitosan added samples are in line with the work of Sayas-Barberá et al. (2011) and Amaral et al. (2015) where they reported addition of chitosan in pork sausage gives stability to the fat content of the sausage under storage condition. Also, it can be assumed from this phenomenon that there is rapid lipid oxidation in the control sausage whereas addition of chitosan somewhat restricted the oxidation process under similar storage condition. It can be concluded that chitosan proved to be a better additive in giving stability to the fat content in the emulsion sausages. Better sensory attributes in terms of flavour were found till 48th day of storage for chitosan treated samples in contrast to control samples where a detectable fishy odour prevailed throughout the study period (Chattopadhyay et al., 2019a).

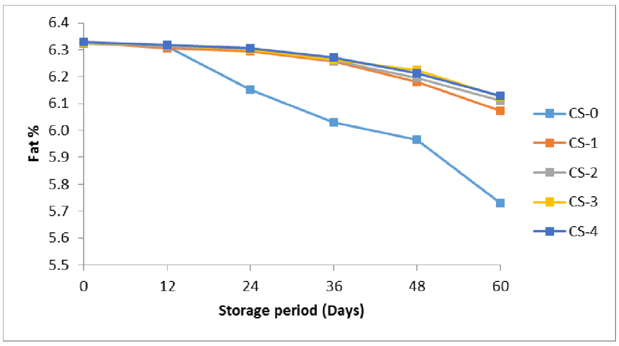

Fig. 3. Change in fat content of the refrigerated emulsion sausages prepared with different concentrations of chitosan gel

The results displayed in Fig. 4 shows the variation in the ash content in different sausage samples during refrigerated storage. Although there was little variation in the ash content among chitosan treated sausage groups at all-time points, the control samples showed a sharp reduction in ash content during subsequent sampling days. There was significant ($p<0.05$) decrease ash content in the control sample after 24th day of storage whereas the treatment groups showed stable composition upto 48th days of storage after which slight decrease in the contents were noticed. All treatment showed reduction in mineral content of sausage samples during storage. This reduction occurred due to the purge loss from sausage samples during refrigerated storage. Cooking loss will lead to leaching of salt and other mineral components. So if the product can retain water better, mineral composition will not be affected and thus nutritional quality will be maintained.

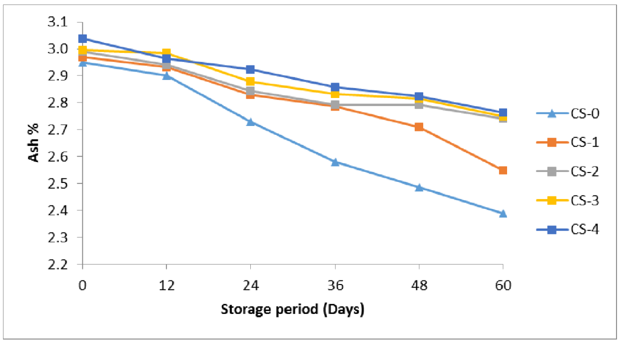

Fig. 4. Change in ash content of the refrigerated emulsion sausages prepared with different concentrations of chitosan gel

Carbohydrate content is less significant ingredient in emulsion sausages. Different types of starch are used as a filler source. In general, 5-10% starch is incorporated as filler in sausage manufacturing process. In terms of carbohydrate content, the present results showed that there was no significant difference among different sausage samples during storage, but were found highest in the control group in compared to the various chitosan incorporated sausage groups. As the chitosan gel concentration increase, the starch level is slightly reduced.

Based on this study, it can be concluded that the inclusion of chitosan hydrogel at 0.25% chitosan concentration (CS-2) in emulsion sausage helps in giving better retention in its proximate composition and extended shelf life. Thus, it can be validated that chitosan addition can improvise the final sausage

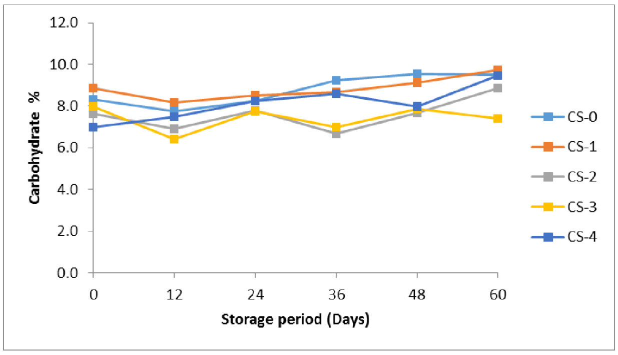

Fig. 5. Change in carbohydrate content of the refrigerated emulsion sausages prepared with different concentrations of chitosan gel

quality by extending its shelf life and can enhance its acceptability in the consumer domain as a highly popular food product.

Acknowledgements

The authors wish to thank the Indian Council of Agricultural Research (Ministry of Agriculture, Govt. of India) for their financial support of this study. The authors also express sincere thanks to the Director, ICAR-Central Institute Fisheries Education for providing necessary facilities for carrying out this work.

References

Amaral, D. S., Cardelle-Cobas, A., Nascimento, B. M. S., Monteiro, M. J., Madruga, M. J. and Pintado, M. M. E. (2015) Development of a low fat fresh pork sausage based on chitosan with health claims: impact on the quality, functionality and shelf-life. Food Funct. 6: 2768-2778

Amiza, M. A. and Kang, W. C. (2013) Effect of chitosan on gelling properties, lipid oxidation, and microbial load of surimi gel made from African catfish (*Clarias gariepinus*). Int. Food Res. J. 20: 1585-1594

Andres, S. C., García, M. E., Zaritzky, N. E. and Califano, A. N. (2006) Storage stability of low-fat chicken sausages. J. Food Eng. 72: 311-319

AOAC (2005) Official methods for analysis of AOAC International, 18th edn., Gaithersburg, Maryland, USA: AOAC International

Cho, Y. W., Cho, Y. N., Chung, S. H., Yoo, G. and Ko, S. W. (1999) Water-soluble chitin as a wound healing accelerator. Biomaterials. 20: 2139-2145

Darmadji, P. and Izumimoto, M. (1994) Effect of chitosan in meat preservation. Meat Sci. 38: 243-254

Draget, K. I. (1996) Associating phenomena in highly acetylated chitosan gels. Polymer Gels and Networks. 4: 143-151

Estévez, M. and Cava, R. (2004) Lipid and protein oxidation, release of iron from heme molecule and colour deterioration during refrigerated storage of liver pâté. Meat Sci. 68: 551-558

Ganhão, R., Morcuende, D. and Estévez, M. (2010) Protein oxidation in emulsified cooked burger patties with added fruit extracts: Influence on colour and texture deterioration during chill storage. Meat Sci. 85: 402-409

Kamil, J. Y. V. A., Jeon, Y. J., and Shahidi, F. (2002) Antioxidative activity of chitosans of different viscosity in cooked comminuted flesh of herring (*Clupea harengus*). Food Chem. 79: 69-77

Chattopadhyay, K., Martin Xavier, K. A., Amjad Balange, Porayil Layana and Binaya Bhusan Nayak (2019a) Chitosan gel addition in pre-emulsified fish mince - Effect on quality parameters of sausages under refrigerated storage LWT - Food Sci. Technol. 98: 295-301

Chattopadhyay, K., Martin Xavier, K. A., Amjad Balange, Porayil Layana and Binaya Bhusan Nayak (2019b) Chitosan hydrogel inclusion in fish mince based emulsion sausages: Effect of gel interaction on functional and physicochemical qualities. Int J. Biol. Macromol. 134: 1063-1069

Lin, K.W. and Chao, J.Y. (2001) Quality characteristics of reduced-fat Chinese-style sausage as related to chitosan's molecular weight. Meat Sci. 59: 343-351

López-Caballero, M. E., Gómez- Guillén, M. C., PérezMateos, M., and Montero, P. (2005) A chitosan-gelatin blend as a coating for fish patties. Food Hydrocolloids. 19: 303-311

Mao, L., and Wu, T. (2007) Gelling properties and lipid oxidation of kamaboko gels from grass carp (Ctenopharyngodon idellus) influenced by chitosan. J. Food Eng. 82(2): 128-134

Peng, W., Xu, X. L., and Zhou, G. H. (2009) Effects of meat and phosphate level on water-holding capacity and texture of emulsion-type sausage during storage. Agr. Sci China. 8: 1475-1481

Rahmanifarah, K., Shabanpour, B. and Shabani, A. (2015) Effect of thermal microbial inactivation and washing on quality properties of fish sausage during cold storage (4°C). J. Aquat. Food Prod. Technol. 24: 386-396

Sayas-Barberá, E., Quesada, J., Sánchez-Zapata, E., Viuda-Martos, M., Fernández-López, F., Pérez-Alvarez, J. A. and Sendra, E. (2011) Effect of the molecular weight and concentration of chitosan in pork model burgers. Meat Sci. 88(4): 740-749

Sheard, P. R., Nute, G. R. and Chappel, A. G. (1989) The effect of cooking on the chemical composition of meat products with special reference to fat loss. Meat Sci. 49: 175-191

Sormoli, M. E., Islam, M. I. U., and Langrish, T. A. G. (2012) The effect of chitosan hydrogen bonding on lactose crystallinity during spray drying. J. Food Eng. 108(4): 541-548

Soultos, N., Tzikas, Z., Abrahim, A., Georgantelis, D. and Ambrosiadis, I. (2008) Chitosan effects on quality properties of Greek style fresh pork sausage. Meat Sci. 80: 1150-1156

Xavier, K. A. M., Hauzoukim, Kannuchamy, N., Balange, A. K., Chouksey, M. K. and Gudipati, V. (2017) Functionality of chitosan in batter formulations for coating of fish sticks: Effect on physicochemical quality. Carbohydr. Polym. 169: 433-440