Physical properties of cobia (*Rachycentron canadum*) surimi: effect of washing cycle at different salt concentrations

N. Hamzah · N. M. Sarbon · A. M. Amin

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Abstract This study aimed to determine the effects of 2–5 wash cycles and the addition of tetrasodium pyrophosphate (TSPP) (0 %, 0.05 Surimi% and 0.1 % w/w)—with or without the addition of 0.4 % calcium chloride (CaCl_2)—on the physical properties such as texture, colour, expressible moisture and microstructure of Cobia (*Rachycentron canadum*) surimi gel. The highest breaking force (484.85 g) was obtained with the addition 0.1 % TSPP alone on the fifth wash. However, a combination of 0.1 and 0.4 % CaCl_2 in surimi gels at wash cycle 5 resulted in the highest degree of whiteness (86.8 %), as well as total expressible moisture (2.785 %) and deformation (17.11 mm). The highest surimi gel strength (6,923 g.mm) was obtained after three wash cycles with the addition of 0.1 % TSPP +0.4 % CaCl_2 . The physical properties of Cobia fish surimi gels were affected by the number of wash cycles and treatments with TSPP and CaCl_2 .

Keywords Cobia · Surimi gel · Wash cycle · Tetrasodium pyrophosphate · Calcium chloride

Introduction

Surimi is the Japanese term for the stabilized myofibrillar protein obtained from mechanically deboned fish flesh that is washed, mixed with cryoprotectant, and then frozen for storage. In recent studies it was used as raw material for the production of a large variety of products such as fish and octopus balls, crab flavored claws, shrimp chips and cuttlefish balls. Surimi serves well for a variety of products and has become increasingly popular due to its unique textural

properties which include high nutritional value (Bourtoon et al. 2008).

The unique textural characteristic of surimi gel is due to the gelling property of myofibrillar proteins for which wash cycles are a crucial factor which determines the quality of the final product. Washing not only removes fat and undesirable substances such as blood, pigments and odorous contents, but also increases the concentration of myofibrillar protein (actomyosin). Carvajal et al. (2005) stated that the washing process improves the quality of surimi by concentrating its myofibrillar protein. However, the number of required wash cycles depends on species, condition, type of wash, and the desired quality of the surimi end product (Carvajal et al. 2005). Karthikeyan et al. (2004) stated that after repeated washing, ionic strength decreased while the addition of salt during processing solubilizes myofibrils and improves the facilitation of gel formation. Based on previous research by Venugopal (2006) stated that NaCl is commonly added in surimi to obtain optimum gelling formation of surimi. Since the catch of marine species is decreasing, manufacturers have to find potential alternative source for surimi production which is freshwater fish (FAO 2007).

Cobia (*Rachycentron canadum*) is a freshwater fish for which aquaculture is expanding globally, most notably in Taiwan, mainland China and Vietnam because of its rapid life cycle and high quality flesh (Yang et al. 2008). Recently, this species has been cited for producing high quality fillets that are suitable for sashimi and restaurant menu (Craig et al. 2006). It is a little known but good tasting and fast growing species of fish. There are, however, no studies reported on the production of surimi that utilizes Cobia to date. Furthermore, Cobia is a fast growing species that is readily available in Malaysia despite its present underutilization. In addition, thus far, no data has been reported on the effect of washing on the physical properties of Cobia surimi. This study is therefore of some significance in that it generates new knowledge

N. Hamzah · N. M. Sarbon (✉) · A. M. Amin
School of Food Science and Technology, Universiti Malaysia
Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia
e-mail: norizah@umt.edu.my

regarding the effects of wash cycles on the physical properties of Cobia in order to evaluate its potential for the production of surimi. Surimi production from Cobia shows promising potential for a variety of value added products such as fish balls, fish cakes, fish nuggets and, indirectly, the increased utilization of a local freshwater species. As treatment with wash cycles may increase the quality of surimi in terms of gel strength, this study investigates the effects of wash cycles on gel strength, color, expressed moisture, and the microstructure of the gel produced from Cobia surimi. In addition, this study also aimed to determine the effects of salt adjuncts at different concentrations of tetrasodium pyrophosphate (TSPP) with calcium chloride (CaCl_2) on the physical properties of Cobia surimi.

Materials and methods

Materials

About one-hundred-eleven (111) kg of fresh Cobia (*Rachycentron canadum*) were obtained from a local freshwater fish supplier in Langkawi, Kedah, Malaysia, with the mean weight is 4–5 kg and 58–60 cm length, transported in ice to the laboratory. Chemicals used such as TSPP, CaCl_2 and sodium chloride (NaCl) were analytical grade.

Methods

Preparation of surimi

The Cobia fish was headed, gutted and cleaned to remove skin and bone in order to obtain the flesh with a mechanical deboner (W 250, China). Frozen minced fish flesh was stored in block form at $<10^\circ\text{C}$ until used. Fish mince was washed with cold water ($<10^\circ\text{C}$) at a minced fish to water ratio of 1:3 (w/v). The mixture was stirred gently for 10 min and allowed to precipitate for another 10 min. Four samples of surimi were prepared and grouped into two, three, four and five wash cycles, respectively. Surimi was prepared according to the method of Department of Fisheries Malaysia (2009). The Cobia mince was washed with cold water with 0.2 and 0.3 % NaCl for 2 cycles. The washed Cobia mince was then filtered to remove excess water with a hydraulic press machine (Sakaya 11, Thailand) before being mixed with a cryoprotectant (3 % sugar and 0.3 % sodium tripolyphosphate) prior to freezing at -18°C . Similar procedures were repeated for cycles 3, 4 and 5, excepting for different wash steps as described below.

1. Mince was then washed twice with cold water with 0 % NaCl, 0.2 % NaCl and 0.3 % NaCl, for three wash cycles, respectively;

2. Mince was then washed twice with cold water followed by cold water with 0 % NaCl, 0 % NaCl, 0.2 % NaCl and 0.3 % NaCl for four wash cycles, respectively;
3. Mince was then washed twice with cold water followed by cold water with 0 % NaCl, 0 % NaCl, 0 % NaCl, 0.2 % NaCl and 0.3 % NaCl for five wash cycles, respectively.

All lots described were then mixed with cryoprotectant prior to freezing before further processing.

Preparation of surimi gel

Surimi gel preparation was conducted according to the method by Julavittayanakul et al. (2006). In order to prepare surimi gels, the frozen mince was thawed in running tap water for 30 min. Each lot was then mixed with 2.5 % NaCl using a cutter-mixer (Blixer®, Robot Coupe USA, Inc., Jackson, Mississippi, USA) for 5 min. until the salted combine resembled a minced batter. These batters were then stuffed into plastic cylindrical casings (diameter 2.5 cm, height 15 cm). The stuffed batters were cooked at 90°C for 20 min. before immediate placement in ice for 20 min. The resultant gel was stored at 4°C for 24 h prior to analysis.

Six different surimi gels were prepared from treatments with different numbers of wash cycles (2, 3, 4 and 5 cycles) with salt adjuncts [0%TSPP (control), 0.05 %TSPP, 0.10 %TSPP, 0 %TSPP + 0.4 % CaCl_2 , 0.05 %TSPP + 0.4 % CaCl_2 , 0.10 %TSPP + 0.4 % CaCl_2], respectively. To prepare the gels, frozen samples from different wash cycles were thawed at 4°C for 3–4 h until the core temperature reached 0°C . The samples were then cut into small pieces. 2.5 % (w/w) NaCl was added to the samples and blended for 5 min. at 4°C to obtain a homogeneous sol.

Next, TSPP and CaCl_2 were blended with the sol. The sol was then stuffed into a cellulose casing with a diameter of 2.5 cm; both ends of the casing were tightly sealed. The sol was then incubated at 40°C for 30 min. followed by heating at 90°C for 20 min. Each gel sample was then immediately cooled in iced water for 30 min. and stored for 24 h at 4°C prior to further analysis.

Texture analysis

The prepared surimi gels were equilibrated for about 2 h and tested at room temperature. Samples were removed from their casing and cut into cylinder-shaped discs (25 mm in length and 25 mm in diameter). Texture analysis was performed with a texture analyzer (model TA-XT2i, Stable Micro System Ltd, UK). A spherical gel was measured using a cylindrical stainless-steel probe (5 mm diameter) attached to a 5 kg load cell on the analyzer. The breaking force and deformation at fracture were recorded. The gel strength (g.mm) of the surimi produced was determined by multiplying the breaking force

with the degree of deformation. The breaking force (g) and breaking deformation (mm) were measured and results obtained were recorded and gel strength was calculated using the following formula (Cardoso et al. 2008). All determinations were performed in duplicate.

Colour determination

The surimi gels were then subjected to a colorimeter (Model CR-400, Konica Minolta Sensing, Inc., Osaka, Japan). The colorimeter was initially calibrated with a Minolta standard-white reflection plate. The measurement of L^* (lightness), a^* (red to green), and b^* (yellow to blue) were performed in triplicate. The whiteness of each sample was calculated as suggested by Park (2000) using the equation for whiteness indexing as follows:

$$\text{Whiteness index} = 100 - \left[(100 - L^*)^2 + a^{*2} + b^{*2} \right]^{1/2}.$$

Expressible moisture

Expressible moisture content was measured according to the method described by Arfat and Benjakul (2012). The expressible moisture was determined by cutting the cylindrical gel samples to a thickness of 5 mm which were then weighed as (X) g. The cylindrical gel was placed between two pieces of filter paper: one at bottom and one on top. A standard weight (5 kg) was placed on top of the sample for 2 min. The sample was then removed from the papers and weighed again as (Y) g. The total expressible water was calculated and expressed as a percent of the sample's weight as follows:

$$\text{Expressible moisture content(\%)} = [(X - Y) / X] \times 100.$$

Microstructure

Microstructure determination was conducted according to the method as described by Julavittayanakul et al. (2006) with some modification. For microstructure determination, samples of approximately 2–3 mm thickness were fixed with 2.5 % (v/v) glutaraldehyde in a 0.2 M phosphate buffer (pH 7.2) for 2 h. 0.2 M phosphate buffer was prepared by mixing 17.5 ml of 0.2 M sodium hydroxide (NaOH) with 25 ml of 0.2 M monobasic potassium phosphate (KH_2PO_4) diluted to 100 ml with distilled water. Samples were then rinsed for 1 h in distilled water before dehydration in ethanol in serial concentrations of 50, 70, 80, 90 and 100 % (v/v). Specimens were then viewed under a tabletop microscope (TM-1000, Japan) with magnification of $\times 600$.

Statistical analysis

Data was subjected to the two-way ANOVA using MINITAB Statistical Software (version 16.0). The two-way ANOVA table indicated whether or not an interaction was extant between factors; e.g. concentration of salt and number of washing cycle. If no interaction between factors was apparent, then a one-way ANOVA was run separately for each factor. If an interaction between two factors was indicated, all treatments were then analyzed together using the one-way ANOVA. The level for significance was set at $p < 0.05$ and significant differences from the mean were determined by Tukey's Multiple Comparison.

Results and discussion

Yield of surimi production

The yield of minced Cobia fish after mechanical deboning was 46.94 %, nearly half the weight of the whole fish. This weight was in agreement with Angela (1996) who found that cobia had a high yield of dressed fillets at 50–60 % of total body weight, compared to Sarker et al. (2012), who found a yield of minced flesh for Javanese carp and tilapia at 36.67 and 42.50 %, respectively. Cobia has a greater yield (46.94 %) than Javanese carp and tilapia. This may be due to larger size as well as less bone and fat contents. In addition, correctly headed and gutted carcasses give higher yields of minced flesh according to Santana et al. (2012). Filleting the fish and deboning the fillet resulted in higher yields obtained. Dey and Dora (2011) suggested that to obtain maximum yields, removing the head and thoroughly cleaning the gut and belly walls should be conducted prior to deboning process.

Table 1 presents the effect of wash cycles on the yield of surimi from minced Cobia flesh. These results showed that yields of surimi from the minced flesh decreased with the increased number of wash cycles. The yield of surimi was 61.53 % after the fourth and fifth wash cycles; lower than that obtained from the wash cycles two and three (71 %) (Table 1). This was due to the removal of undesirable components such as fat, blood, pigments, malodorous compounds and other impurities as wash levels increased (Karthikeyan et al. 2004). In addition, Karthikeyan et al. (2004) found that washing in higher concentrates of NaCl solution reduced the loss of myofibrillar proteins also causing a reduced yield of surimi. The addition of 0.2 and 0.3 % of NaCl used in the wash was specifically to extract myofibrillar proteins and ease the separation of undesirable matter. The results of additional washing with 0 % NaCl caused lower yields of surimi from cycles 4 and 5, respectively (data not shown). Although 10 % yield loss at the end of 4th and 5th washing cycle, this resulting in the increased of physical properties such as gel strength and whiteness of the surimi produced. A study by Amiza and Nur Ain (2012), found that

Table 1 Yield of surimi for each wash cycle before the process of surimi gel formation

No of wash cycles	Weight before (kg)	Weight after (kg)	Yield of surimi from minced fish (%)
2	6.5	4.8	73
3	6.5	4.8	73
4	6.5	4.0	61
5	6.5	4.0	61

the yield of surimi obtained from silver catfish mince after four wash cycles treated with 0.2 and 0.3 % NaCl was 60 %. This finding shows that cobia mince produces a slightly higher yield of surimi (61.53 %) than silver catfish (60 %) after 4–5 wash cycles in the same NaCl concentration. Sarker et al. (2012), found yields from minced flesh at 56.16 % for grass carp, 43.00 % for sin croaker, and 45.00 % for mackerel; all of which were lower than the results obtained.

In addition, a study by Nishioka and Shimizu (1984) stated that subsequent washing removed residual sarcoplasmic proteins along with a small amount of myofibrillar proteins. Consequently, after sarcoplasmic proteins are completely removed, further washing may cause severe loss of myofibrillar proteins. Hence, excessive washing results in a loss of myofibrillar proteins and decreases the yield. Thus also, the water-to-meat ratio and wash cycle duration are important factors that determine the yield of surimi from minced fish.

The total yield of surimi production obtained from cobia was 29 %. According to Sarker et al. (2012), yields from tilapia were 18.99 %, from grass carp 18.26 %, and 24.00 % from Alaskan Pollack. This shows that our total yield of surimi obtained from cobia is significantly higher. Lower surimi yields may be due to a loss of water soluble proteins during washing and also of insoluble proteins while draining the slurry (Yathavamoorthi et al. 2012). The large volume of water used to remove sarcoplasmic proteins, blood, fats, and other nitrogenous compounds from the minced flesh, is a factor affecting the yield of surimi. Karthikeyan et al. (2004), suggested that the best water-to-mince ratio was 3:1. This is because increasing the amount of water used for washing usually causes greater protein loss and decreased surimi yield. Sarker et al. (2012), found a lower surimi yield for tilapia (18.99 %) when the ratio of water to mince was 4:1 with four wash cycles. Thus, a logical approach to achieving optimal wash cycle effects and maximal surimi yield is to lower the water-to-mince ratio while increasing wash duration and the number of cycles.

Breaking force

Figure 1a presents significant ($p < 0.05$) relations between the number of wash cycles and the addition of TSPP and 0.4 % CaCl_2 affecting the breaking force of surimi gels. From all tested gels, surimi gel from five wash cycles with 0.05 % TSPP produced the highest breaking force (484.98 g). This

was followed by gels from four wash cycles with addition of 0.05 % TSPP (482.37 g) ($p > 0.05$). A study by Nishioka and Shimizu (1984) indicated that a greater number of wash cycles with the addition of salt to the mince produced stronger gel-forming ability in surimi yield. The textural properties of surimi gels are the result of the internal structure of the gel which is comprised of myofibrillar proteins. This is to be expected as the wash process most likely concentrates myofibrillar proteins causing a stronger breaking force due to a stronger protein network (Chen 2002). Park (2005) stated that the breaking force of surimi gels made from raw fish increased up to three wash cycles. Moreover, his results also indicated that protein-to-protein interactions were stabilized by strong bonds established in surimi gels. Figure 1 shows that breaking forces increased as wash cycles increased. An effective washing process must be able to remove undesirable sarcoplasmic proteins because sarcoplasmic proteins decrease gelation by obstructing the cross-linkage of actomyosin (Babji and Gna 1994). Washing decreases sarcoplasmic protein content and increases salt-soluble proteins (myofibrillar proteins) (Babji and Gna 1994).

Figure 1b presents significant ($p < 0.05$) relations between the number of wash cycles and the addition of TSPP with 0.4 % CaCl_2 that affected the breaking force of surimi gels. There is no significant difference in breaking force between wash cycle 3 and 4 at 0.1 % TSPP + 0.4 % CaCl_2 . Generally, the breaking force decreased as wash cycles and the addition of (TSPP + 0.4 % CaCl_2) increased. The decreased breaking force in the presence of higher phosphate content might be due to the chelation of calcium ions required for TGase activity (Nozawa et al. 1997). A study by Amiza and Nur Ain (2012), found a higher breaking force (600 g) for silver catfish gel with the addition of 0.05 % PP after three wash cycles. Compared to this study, the breaking force of surimi gel at similar wash cycles and salt concentrations was 500 g. Although cobia surimi showed a lower breaking force than that of silver catfish surimi, the results still indicate that the addition of TSPP at 0.05 % enhanced the effect of gel formation. Phosphate compounds might form a calcium ion complex—to a greater extent—with additional amounts. As a consequence, this could more effectively impede the activity of endogenous TGase (a Ca^{2+} dependent enzyme), which was evident by the lowered breaking force (Arfat and Benjakul 2012).

Fig. 1 a Breaking force of surimi gels for different wash cycles and the addition of TSPP at different concentrations: N (0 % TSPP); A (0.05 % TSPP); B (0.1 % TSPP). **b** Breaking force of surimi gels for different wash cycles and salt addition at different concentrations: C (0 % TSPP + 0.4 % CaCl_2); D (0.05 % TSPP + 0.4 % CaCl_2); E (0 % TSPP + 0.4 % CaCl_2)

Deformation

Since interactions between the number of wash cycles and the addition of TSPP did not significantly affect the breaking force of surimi gels ($p > 0.05$), evaluations were conducted separately (From two way ANOVA conducted, data not shown). The deformation of surimi gels as a result of different wash cycles is shown in Fig. 2, which demonstrates that deformation increased with the increased number of wash cycles. The highest deformation was obtained with wash cycle 4 (13.538 mm), followed by wash cycle 5 (12.812 mm) with no significant difference between them ($p > 0.05$). Moreover, wash cycle 3 presented an increased breaking force of 3.6 %

Fig. 2 Deformation of surimi gels for different wash cycles

compared to wash cycle 2. This result was the reverse of a study conducted by Amiza and Nur Ain (2012), which found that the deformation of surimi gel decreased as washing cycles increased and that only the first wash resulted in higher deformation. However, a study by Torigai and Konno (1997) showed that increased wash cycles resulted in higher deformation of surimi gel as the wash process concentrated myofibrillar proteins that enhanced gel strength.

The deformation of surimi gels which conducted in this study were increased as the concentration of TSPP increases (0, 0.05 and 0.1 % TSPP), as presented in Fig. 3. However, we must note that there were no significant differences in the deformation of surimi gels between different concentrations. The addition of TSPP at 0.1 % shows the maximum enhanced effect on gel deformation (2.492 mm). This finding agrees with a study by Torigai and Konno (1997) who reported that the addition of PP resulted in increased deformation because PP causes the dissociation of the actinomyosin complex to actin and myosin; thus, the enhanced effect on gel formation. Furthermore, the addition of phosphate at 0.05 % has also been reported to increase deformation values of threadfin bream and bigeye snapper surimi gels with optimal concentrations of added phosphate causing the formation of strong gel networks as a result of actinomyosin dissociation (Ellinger 1975).

Fig. 3 Deformation of surimi gels for with the addition of TSPP at different concentrations

The relationship between the number of wash cycles and the addition of TSPP with 0.4 % CaCl_2 (as shown in Fig. 4) significantly affected ($p < 0.05$) the deformation of surimi gels. Deformation of surimi gels increased with wash cycles as the addition of salt concentrations increased. The highest deformation (17.11 mm) was observed at the fifth wash cycle with added 0.1 % TSPP combined with CaCl_2 . This was followed by 0.4 % CaCl_2 at wash cycle 3 (15.35 mm). The addition of chloride salts such as CaCl_2 alters protein function during gelation (Mathew et al. 2009). Several chemical interactions are involved during the formation of network structures in surimi gels. These include non-covalent cross-links such as hydrophobic interactions, hydrogen bonds, ionic bonds, and covalent interactions such as disulfide bonds (Mathew et al. 2009). Generally, the development of a gel network involves two steps. First, interactions of the tail portion of myosin molecules occur; this is followed by hydrophobic interactions at the head portion (Sano et al. 1990). Because of hydrogen bond instability during heating, alpha-helices unfold which expose hydrophobic amino acids causing further hydrophobic interactions (Niwa 1992). Thus, the results we obtained suggest that the addition of TSPP and CaCl_2 enhanced protein function which then leads to gel formation and the increased deformation of surimi gel.

Fig. 4 Deformation of surimi gels at different wash cycles with the addition of salt at different concentrations: C (0 % TSPP + 0.4 % CaCl_2); D (0.05 % TSPP + 0.4 % CaCl_2); E (0.10 % TSPP + 0.4 % CaCl_2)

A previous study reported surimi gels from several fish species that had higher deformation because of protein–protein bonds, including hydrophobic interactions and disulfide bonds established at cooking temperatures which served to strengthen networks previously formed during setting (Arfat and Benjakul 2012). They also reported that an increased CaCl_2 concentration resulted in increased gel deformation in bigeye snapper, threadfin bream, barracuda, and bigeye croaker.

Gel strength

The interaction between the number of wash cycles and the addition of TSPP significantly affected gel strength of surimi gels as shown in Fig. 5a ($p < 0.05$). Gel strength gradually increased up to the fourth cycle and began to decrease with the fifth wash. This showed that the fourth wash cycle, with added 0.1 % TSPP, produced the highest gel strength (5,831 g.mm); followed by surimi gel with added 0.05 % TSPP (5,818 g.mm) at the same number of washes. However, no significant difference was found between them ($p > 0.05$). TSPP is added to improve the gel strength of cobia fish surimi gels. This agrees with the report of Julavittayanakul et al. (2006): that the addition of TSPP at 0.05 % increases gel strength. Moreover, Huda et al. (2011) reported that the gel strength of commercial surimi increased with the addition of phosphate blends. However, their results did not agree with a study by Amiza and Nur Ain (2012) who found that the highest gel strengths were obtained without the addition of either PP or CaCl_2 (6,100 g.mm), and that a lower gel strength (4,000 g.mm) was obtained from surimi gel containing 0.1 % PP. This was likely due to the fact that the addition of salt did not improve the gel strength of silver catfish surimi.

Figure 5b demonstrates significant relationships between wash cycles and the addition of TSPP + 0.4 % CaCl_2 affect in the gel strength of surimi gels ($p < 0.05$). Results showed that gel strengths gradually increased until wash cycle 3 and

Fig. 5 **a** Gel strength of surimi gels for different wash cycles with the addition of TSPP at different concentrations: N (0 % TSPP); A (0.05 % TSPP); B (0.1 % TSPP). **b** Gel strength of surimi gels at different wash cycles and salt treatments

decreased at cycles 4 and 5, respectively. Surimi gel containing of 0.1 % TSPP showed the highest gel strength with added 0.4 % CaCl_2 at wash cycle 3 (6,923 g.mm) compared to surimi gel without the addition of TSPP (5,280 g.mm).

Endogenous enzymes in fish muscle, namely proteinases and transglutaminase (TGase), are important factors that partly govern the gel-forming ability of fish protein. Proteinases contained in the sarcoplasmic fraction can be effectively eliminated by washing and dewatering as typically practiced in surimi processing. Nevertheless, some proteinases are tightly bound to myofibrils and cannot be removed by washing because washing removes soluble proteins and concentrates myofibril-bound proteinases. Based on our results, decreased gel strength at wash cycles 4 and 5 may have been caused by the loss of proteinases contained in myofibrillar proteins due to excessive washing. Fish TGase has been found to be Ca^{2+} dependent; however, this varies among fish species (Nozawa et al. 1997). In addition, Ca^{2+} can possibly activate the endogenous transglutaminase (endo-TGase) activity of surimi, thus catalyzing an acyl transfer reaction between α -carboxamide groups of glutamine and the ϵ -amino groups of lysine, causing the formation of lysine cross-links and thus, yield stronger gels (Arfat and Benjakul 2012).

Determination of colour

The relationship of wash cycle numbers and the addition of TSPP significantly affect the whiteness ($p < 0.05$) of surimi gels as presented in Fig. 6a. The whiteness of gels increased with the number of wash cycles and TSPP concentrations. Based on these results, surimi gels with added 0.1 % TSPP showed the greatest degree of whiteness at wash cycle 4 (80.77 %) compared to wash cycle 5 (80.34 %), but with no significance difference ($p > 0.05$). This might be associated with the ‘non-significant’ increase in expressible moisture of the gel, especially with increases in added phosphate (Fig. 7). The free water released to the gel’s surface contributed to the scattering of light, resulting in increased whiteness (Arfat and Benjakul 2012). This result agrees with Julavittayanakul et al. (2006) who reported that all surimi gels increased in whiteness as phosphate concentrations increased. As wash cycles remove sarcoplasmic proteins, including myoglobin, and to some extent blood residues containing hemoglobin that contribute to the colour of surimi gel (Julavittayanakul et al. 2006), thus, the effect of washing on whiteness was most remarkable at cycles 4 and 5, respectively.

Fig. 6 a The whiteness values of surimi gels for different wash cycles with the addition of TSPP at different concentrations: N (0 % TSPP); A (0.05 % TSPP); B (0.1 % TSPP). **b** The whiteness values of surimi gels for different wash cycle with salt addition at different concentrations: C (0 % TSPP +0.4 % CaCl_2); D (0.05 % TSPP +0.4 % CaCl_2); E (0.1 % TSPP +0.4 % CaCl_2). Different letters indicate significant differences between treatments ($p < 0.05$)

Figure 6b presents significant relationships between the number of wash cycles and the addition of TSPP and CaCl_2 ($p < 0.05$) that affect the whiteness of surimi gels. Gel whiteness increased with the number wash cycles with added TSPP concentrate. Wash cycle 5 with 0.1 % TSPP +0.4 % CaCl_2 showed the highest degree of whiteness (86.80 %), followed by surimi gel with 0.05 % TSPP +0.4 % CaCl_2 (80.91 %). According to Arfat and Benjakul (2012), these values are most likely the result of scattered light from the formation of insoluble CaCl_2 complexes with anions in the muscle.

Total expressible moisture

Figure 7 shows the effect of wash cycles on the total expressible moisture from surimi gel. There is no significant was found between the number of wash cycles and the addition of TSSP ($p > 0.05$). The obtained results demonstrated that total expressible moisture decreased as the number of wash cycles increased. The highest value of expressible moisture was obtained from wash cycle 2 (6.92 %). Nevertheless, there were no significant differences ($p > 0.05$) found in expressible moisture between cycles 3, 4 and 5. The process of washing

removes fats and sarcoplasmic proteins that can destabilize the protein network. Hence, increased washing produces gels with higher water retention which then leads to higher water retention of the surimi gel (Baxter and Skonberg 2007). The capacity for water retention has been shown to increase with increased numbers of wash cycles, such that the myofibrillar

Fig. 7 Total expressible moisture of surimi gels for different wash cycles

protein network is strong enough not to release the water held under applied pressure, resulting in a decrease in expressible moisture (Mathew et al. 2002). Since water holding capacity is directly correlated with myofibrillar protein content (Smith 1991), this capacity increases with increasing numbers of washes as washing has been shown to increase the concentration of myofibrillar protein. A study by Karthikeyan et al. (2004), found that the unwashed proteins of minced bigeye snapper flesh had poor gel forming ability because the strength of the myofibrillar protein network thus formed is related to the retention of water inside the gels.

Figure 8 shows that the addition of TSPP at different levels of concentration to Cobia surimi gels resulted in no significant difference ($p>0.05$) in expressible moisture content for all gels. These results also indicated that lower expressible moisture levels were obtained at added levels of 0.1 % TSPP (4.65 %), followed by 0.05 % TSPP (4.78 %), which demonstrated that the total expressible moisture decreased with increased TSPP concentration. The lower expressible moisture content indicates that more water is retained in the gel network due to higher water holding capacity (WHC) obtained by the gels. Phosphate has a negative surface charge which affects the surface charges of proteins, thus granting a strong influence on the protein's water binding capacity. Pyrophosphate has a specific effect on the water retention of actomyosin in meat and on the gel formation of casein in milk products (Ellinger 1975). In addition, phosphate anions increase ionic strength resulting in increased water holding capacity via the direct binding of water to phosphate anions and by the repulsion of protein groups due to the predominance of their negative charge. This repellent effect opens the protein structure and increases the number of available binding sites for water, which, in turn, allows for greater water retention in the meat (Xiong 2005). Therefore, this study suggests that the addition of 0.1 % of TSPP improves the water holding capacity of the protein network.

Figure 9 presents the relationship between the number of wash cycles and the addition of TSPP that significantly

Fig. 8 Total expressible moisture of surimi gels at different TSPP concentrations

decreased ($p<0.05$) the expressible moisture of surimi gels with increased wash cycles and the addition of TSPP +0.4 % CaCl_2 . The decreases in total expressible moisture show an increase in water retention in surimi gel. Lower expressible moisture was noticeable in surimi gel at wash cycle 5 in which was added the combination of 0.1 % TSPP and 0.4 % CaCl_2 , resulting in a greater water holding capacity of the protein network. Furthermore, lower expressible moisture was generally found in samples containing 0.1 % TSPP and 0.4 % CaCl_2 concentrations compared to surimi without added TSPP. The increased breaking force of the surimi gel generally correlated with a lowered level of expressible moisture content. These results show that cobia surimi gel with added 0.1 % TSPP in combination with 0.4 % CaCl_2 at wash cycle 4 demonstrated the highest gel strength (6556.5 g.mm) (Fig. 5a), along with the lowest level of expressible moisture (2.63 %). Hence, in the presence of PP and CaCl_2 at optimal concentrations, a three-dimensional organized matrix of gel was formed with the capability of holding water.

Microstructure

The structure of surimi gels from different wash cycles containing TSPP and/or CaCl_2 was treated at 600 MPa and examined via table-top microscopy. The microstructure of surimi gel from two wash cycles with TSPP added at 0, 0.05 and 0.1 %. However, only 0.05 % TSPP was added together with 0.05 % CaCl_2 . Surimi gel (2N) (Figure not shown), without additives at wash cycle 2, showed greater numbers of voids and porosity throughout a loose protein structure when compared to gels prepared with additives. This could well be due to the interaction of cross-links between sarco-plasmic and myofibrillar proteins in the surimi-gel structure can be improved although without additive added (Maltais et al. 2005).

Surimi gel (2B) with 0.1 % of added TSPP showed a finer structure with smaller pores and less roughness. Surimi gel (2E), with added TSPP at 0.1 % +0.4 % CaCl_2 , showed a rough structure that was better than gel produced without additives. These findings demonstrated that the addition of TSPP enhances the formation of a more ordered structure. Sample 2E gel also yielded a higher total of expressible moisture (6 %), which suggests lower water retention due to a less compacted and rough structure with decreased strength, thus making for easier water loss. Surimi gel 2E also showed lower deformation (11.4 mm) and breaking force (460 g) due to a less compact structure that is more porous and rough. This result contrasts with surimi gel 2B (with added TSPP at 0.1 %) which showed lower total expressible moisture (4.9 %) and a high breaking force (470 g). This gel, with added 0.1 % TSPP, possessed a finer structure with smaller pores due to TSPP's enhancement of cross-linkages between myofibrillar and sarco-plasmic proteins.

Fig. 9 Gel strength of surimi gels for different wash cycles with added salts at different concentrations: C (0 % TSPP + 0.4 CaCl_2); D (0.05 % TSPP + 0.4 CaCl_2); E (0.1 % TSPP + 0.4 CaCl_2). Different letters indicate significant differences between treatments ($p < 0.05$)

We know that protein microstructure is related to water holding capacity (Han et al. 2009), and that the addition of CaCl_2 causes changes in its conformation as well as in protein-to-protein and protein-to-water interactions (Maltais et al. 2005). Moreover, CaCl_2 addition might also accelerate the rate of both protein denaturation and gelation, yet the rate of gelling we observed was quickened and thus gave rise to a coarse microstructure and formations of inhomogeneous porosity. These latter developments altered the exposed protein surfaces along with the water-binding ability of polar amino

acids and water distribution. These changes were in addition to the size and homogeneous distribution of pores in the gel, all of which contributed to the gel's water holding capacity (Ahmad et al. 2007).

Surimi gel wash cycle 3 (3N) (Figure not shown), without added TSPP and CaCl_2 at wash cycle 2, presented as less rough and with small pores compared to gel with added TSPP and CaCl_2 at wash cycle 2. These results suggest that increasing the number of wash cycles minus additives results in a smoother and finer texture. Surimi gel with added 0.1 % TSPP

Fig. 10 Microstructure of surimi gel at wash cycle 5 with different concentrations of TSPP plus 0.4 % CaCl_2

(3B) also showed a fine, smooth structure compare to other samples. The addition of TSPP results in the formation of an ordered structure that enhances myosin and actin crosslinks. In addition, surimi gel with 0.05 % TSPP added at wash cycle 3, demonstrated a higher degree of interconnected, three-dimensional protein networks. The more compact and denser gel network with a finer texture, as observed in the 3B sample, was also coincidental with a lower level of expressible moisture due to increased water retention (4.8 %). This suggests that the addition of 0.05 % TSPP results in the formation of an ordered structure. Thus, finely adjusted and more orderly gel networks with fewer voids were observed in gels of the highest strength compared to the very loose networks with more voids as observed in gels of lower strength (Balange and Benjakul 2009). The addition of TSPP with CaCl_2 wrought a larger number of pores and a loose structure, indicating that the addition of CaCl_2 did not enhance the formation of an ordered structure. This agreed with a previous study which found that at wash cycle 3, gel formed from 0.05 % TSPP combined with 50 mmol of CaCl_2 had more voids when compared with 0.05 % TSPP and 50 mmol CaCl_2 respectively.

The addition of TSPP together with CaCl_2 improved the texture for wash cycle 4 (Figure not shown). This is significant due to the induction of protein cross-links via calcium ions. The cross-linking of proteins via calcium bridges was also previously suggested by Park (2005). Furthermore, the addition of 0.4 % CaCl_2 to surimi gel for all fourth-wash-cycle samples caused decreased porosity and finer structuring. Nevertheless, Fig. 10 shows that the addition of TSPP and CaCl_2 did not affect either the ordering or fine texturing of the structure much further at wash cycle 5; although the addition of CaCl_2 did increase deformation, gel strength, and improve the gel's water holding capacity. These results further indicate that excessive washing causes the loss of myofibrillar proteins which significantly contribute to the strength of the surimi gel network. Surimi gels at wash cycle 3 (with added 0.1 % TSPP) showed a finer, smoother, and more compact (denser) structure than did other surimi gels, even though the addition of CaCl_2 did little to improve the ordered texture of the protein structure.

Conclusion

Overall, this study demonstrates that surimi gel can be effectively produced from Cobia fish. To obtain good quality surimi, cobia fish requires five wash cycles and the addition of TSPP and CaCl_2 . Generally, the highest breaking force is obtained from surimi gel when 0.1 % TSPP is added on its own rather than in combination with 0.4 % CaCl_2 during wash cycle 5. This study found that the presence of CaCl_2 decreased

the gel's breaking force. However, the combined addition of 0.1 % TSPP with 0.4 % CaCl_2 during wash cycle 5 results in the highest degree of whiteness and total expressible moisture and deformation than gels treated with TSPP alone (i.e., without 0.4 % CaCl_2). Treatment with CaCl_2 during wash cycle 5 lowers whiteness, expressible moisture, and deformation. The gel strength of surimi gel was highest after three wash cycles with the addition of 0.1%TSPP and 0.4 % CaCl_2 . This study shows that the number of wash cycles and the addition of TSPP plus CaCl_2 affect the properties of Cobia fish surimi gels.

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