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RESEARCH ARTICLE

Study of the best washing cycle for processing of surimi from spotted sardinella (*Amblygaster sirm*): A preliminary study

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ABSTRACT

Amblygaster sirm is an abundant small pelagic fish species that can be used as an alternative ingredient for the processing of surimi. However, due to a lack of knowledge regarding the preparation of surimi from Amblygaster sirm, it is not yet used as a potent raw material in the processing of surimi. Therefore, this study aimed to investigate the effect of washing on the physico-chemical properties of Amblygaster sirm fish muscles and the processing of surimi thereby. Collected fish samples were analyzed for myoglobin content along with the number of washing cycles. Surimi was made out of the treatment that gave the lowest myoglobin content and was analyzed for the sensory attributes i.e. aroma, color, flavor, juiciness, tenderness and overall acceptability. As per the results, double washing showed the most extensive loss in myoglobin content and fat content (P<0.05) as well. Protein contents differed significantly (P<0.05) between treatments. Accordingly, double washing was the best number of washings that produced the best quality surimi with a least number of washing cycles leading to low wastewater and labor. However, the sensory evaluation results indicated that the overall acceptability of the product behaved at an average governed by the people's perceptions regarding surimi.

Keywords: *Myoglobin, surimi, washed mince, washing cycle*

INTRODUCTION

Surimi is a Japanese origin famous product which is considered in most of the countries. Processing of surimi is undertaken in an ancient Japanese way of preserving fish in the form of minced fish (Chen *et al*., 1996). For the preparation of surimi, a wide variety of animal protein sources have been tested by processors all over the world. Among them duck meat, pork, chicken meat and fish have mostly been tested. Tuna fish, milk fish, Atlantic cod and shark like fish species have been widely used as the possible sources for processing surimi (Bechtel *et al*., 1996; Sasidharan and Venugopal, 2020). Fish resources are limited and, thus, people are used to consuming highly valued fish directly. Therefore, food manufacturers move towards low valued fish protein sources for the preparation of value-added fish products (Rustad *et al*., 2011).

Low-value fatty pelagic fish species present a significant opportunity for surimi processing (Hultin and Kelleher, 2000; Priyadarshana and Walpita, 2019). However, high dark muscle content associated with elevated lipid and myoglobin content is one of the most difficult constraints to be overcome by processors who use small pelagic fish species, such as sardine and mackerel, for surimi processing. Ultimately, it has led many difficulties in the processing of quality surimi. Anyway, surimi manufacturers tend to use a pre-washing treatment to process high quality surimi from the low-quality pelagic fish species (Ochiai *et al*., 2001; Chen, 2002; Choi *et al*., 2007).

Since whiteness is considered to be one of the major quality parameters in the surimi industry, an effort should be made to maintain ideal whiteness. For instance, excessive development of myoglobin has to be avoided to achieve a favourable whiteness. Myoglobin was considered as the sarcoplasmic protein that determines the surimi whiteness, since it combines with other components to encourage formation of pigments. As mentioned earlier, pre-washing is a unique tactic used in the processing of surimi in order to get rid of excessive loads of myoglobin and fats (Ochiai *et al*., 2001). Higher gel strength together with optimum cohesiveness and chewiness in the final product enhance water holding capacity (WHC), pH value and, protein solubility which are subsidiary benefits of having a prewashing treatment (Kim *et al*., 2007). Extraction efficiency of myoglobin is mainly dependent on fish species, and especially the washing process (Jin *et al*., 2007). Therefore, many studies have been performed with different washing reagents and washing cycles; for a variety of meat types *i.e.* duck meat (Ismail *et al*., 2010; Ahmad *et al*., 2014), pork meat (Jin *et al*., 2009), chicken meat (Onodenalore *et al*., 1992; Yang and Froning, 1992), milk fish, mackerel meat and sardine meat (Benjakul *et al*., 2004; Benjakul *et al*., 2004; Chaijan *et al*., 2004). *Amblygaster sirm* is also a pelagic fish species that is predominantly available in the fish by-catch. Therefore, *Amblygaster sirm* can be used as a potent raw material in the processing of surimi due to its high availability (Conand, 1991; Karunasinghe and Wijeyaratne, 1991). Since the literature does not provide any information on the behavior of physicochemical properties of *Amblygaster sirm* fish along with prewashing as a treatment; this study was conducted to evaluate the effect of water washing on the physico-chemical properties of fish muscles and surimi thereby. This study will serve as a baseline for the utilization of *Amblygaster sirm* in the development of many more food products for human consumption.

MATERIALS AND METHODS

Fish sample collection

Amblygaster sirm fish samples were collected in fresh form, from a stall of Ceylon Fisheries Cooperation. Collected samples were then transported within 2 h under chilled conditions (<4 °C) to the Laboratory of Livestock Production, Sabaragamuwa University of Sri Lanka and stored at -18 °C. Then the fish samples were immediately rinsed using tap water at 4 °C (pH = 7.2). Digestive tract, gills and fins were removed from the whole fish and samples were washed again. After that the cleaned samples were filleted manually.

Preparation of washed fish mince

Washed fish mince was prepared in accordance with the procedure developed by Chaijan *et al*. (2004) with fewer modifications, as follows. Fish fillets were thoroughly minced using a mincer (Sirman-model TC 22 E, India) that exhibited a mincing diameter of 4 mm. Minced fish sample was then divided into five portions and thereby subjected to five treatments; (T0: Unwashed/Control, T1: fish mince prepared by washing one time (Single washed), T2: fish mince prepared by washing two times (double washed), T3: fish mince prepared by washing three times (triple washed), T4: fish mince prepared by washing four times (quadruple washed) separately. Each treatment was allocated in triplicates with 50 g of fish mince.

Tap water at 4 °C was used as the washing reagent for the preparation of washed fish mince using raw and minced fish muscles. For the preparation of single washed fish mince; 50 g of fish mince was placed inside a container and the tap water was incorporated with fish mince, wherein the fish mince: washing reagent ratio was 1:3 (w/v). Then, the mixture was gently stirred ensuring a rotational speed of 30 rotations per minute for 10 min. Temperature inside the mixture was regulated with the addition of iced cubes at 4 °C temperature. Mixture was then allowed to be settled for a time period of 10 min. After that, the settled mixture was filtered using a sleeve wherein the mesh size was 64 eyes per 1 cm^2. Finally, the washed fish mince samples were subjected to centrifugation at 700 g (700 x 9.8) for 15 min time period with the aid of a hand operating centrifuge. Dewatered sample was then tightly packed inside a polypropylene bag to ensure a proper sealing and was stored at -18 °C and the analysis was conducted thereby.

Aforesaid protocol was followed for the preparation of double washed, triple washed, and quadruple washed fish mince while repeating the procedure for two, three and four times respectively.

Determination of the myoglobin content in fish mince

In order to determine the ideal washing cycle fish mince samples from each and every treatment were quantified for intrinsic myoglobin contents. Myoglobin contents in the prepared samples were analyzed in accordance with the protocol suggested by Jin *et al*. (2007).

Two grams of sample was homogenized inside a 20 mL, 0.04 mol dm^{-3} sodium phosphate buffer solution (pH = 6.8) with a rotational speed of 13,500 rotations per minute for a time period of 20 s with the aid of a homogenizer. 10 g of the homogenized sample was then centrifuged with a rotational speed of 4,000 g for 30 min period using a centrifuge. Generated supernatant was then filtered using Whatman (No.1) filter papers and an amount of 0.2 mL of sodium dithionite (1% w/v) was incorporated into the filtrate. Finally, the myoglobin content was quantified using a spectrophotometer wherein the considered wavelength was at 555 nm. Quantification of the myoglobin content was performed along with a standard equation introduced by Basauri and Regenstein (1992) wherein the amounts were quantified in mg g^{-1} of fish mince.

Myoglobin content = Ab x 16,111 x D x Wt x 7.6

(Ab = absorbance, D = dilution factor, Wt = weight of mince in grams)

Proximate analysis

Washed fish mince samples from unwashed, double washed and quadruple washed groups were analyzed for crude protein using Kjeldahl unit (Nitrogen, N x 6.25) and crude fat using Soxhlet apparatus in accordance with the AOAC (2000) standards.

Determination of the pH value

pH values of the washed and unwashed fish mince samples were also measured in accordance with the protocol suggested by Jin *et al*. (2007) using a digital pH meter. Five grams of minced sample was thereby added into 45 mL of distilled water and homogenized. pH value was then recorded.

Preparation of surimi

As per the results obtained in the determination of the idealist washing cycle, rest of the unwashed fish mince was washed away with the idealist washing cycle *i.e.* double washing. For processing of surimi using washed fish mince, a protocol was followed with a recipe introduced by Chaijan *et al*. (2004) with some slight modifications, as follows. Ingredients *i.e.* washed fish mince (wet) (74%) or washed fish mince (dry) (50%) with ice and water (24%), starch (6%), sucrose (4%), fat (olive oil) (3%), egg white (10%), salt/sodium chloride (2.5%), sodium phosphate (0.5%) were obtained in the appropriate amounts, as described by the Chaijan *et al*. (2004).

Firstly, sucrose was incorporated into washed fish mince, and then the mixture was chilled at 4 °C temperature for a period of 24 h continuously. Followed by the chilling step, then the mixture was ground using a bowl chopper with the addition of ingredients *i.e.* olive oil, ice, and water (4 °C), salt, starch, egg white and sodium phosphate with the aforesaid amounts. Prepared batter was then stuffed into non-edible casings using a hand operating stuffer. Then, the prepared batter was incubated at 40 °C temperature for a period of 30 min and a cooking step was further continued thereby at 90 °C for a period of 30 min inside an oven. After the cooking was done, the non-edible casings were removed, and the cooked surimi samples were then stored at 4 °C temperature prior to the determination of the whiteness.

Determination of surimi whiteness

Surimi whiteness was quantitatively determined in accordance with the procedure described by Jin *et al*. (2007). Colour [L*(lightness), a*(redness), b* (yellowness)] was measured using a colourimeter (Minolta) for each samples separately. Surimi whiteness was then quantified with the use of the formula described by Park *et al.* (1996).

Whiteness = 100 - [(100 - L*) 2 + a*2 + b*2]1/2

Sensory evaluation

Sensory evaluation was performed in the Laboratory of Livestock Production, Sabaragamuwa University of Sri Lanka with the aid of a panel associated with 15 untrained tasters. Surimi was further cooked in cooking oil prior to the sensory evaluation. Moreover, the samples were cooled down to room temperature (28 °C) prior to the taste evaluation. Prepared surimi samples were trimmed down in to slices while maintaining a slice thickness of 1 cm and to retain a slice diameter of 1.8 cm and served for each panelist. Five-point scale-based evaluation procedure was used to evaluate the colour, flavor, aroma, tenderness, juiciness, and overall acceptability like attributes and was as follows.

*Colour (5 = extremely good and 1 = extremely poor), *flavor (5 = extremely good and 1 = extremely poor), *aroma (5 = highly intense and 1 = extremely weak), *tenderness (5 = extensively tender and 1 = poorly tender), *juiciness (5 = very juicy and 1 = very dry), *overall acceptability (5 = Extremely good and 1 = Extremely poor).

Statistical analysis

Data were subjected to analysis of variance and mean comparison was performed using Duncan's multiple range test (at 95% significance) for myoglobin content determination and proximate analysis. All statistical analysis was undertaken with SPSS software.

RESULTS AND DISCUSSION

Effect of the washing on myoglobin content of fish mince and colour characteristics of the final product

As shown in the Figure 1, the myoglobin contents of each treatment showed an extensive reduction along with the washing treatment. Moreover, the myoglobin content of the unwashed mince sample was 7.583 mg g^{-1} of mince. With a single washing, the myoglobin content was reduced to 7.191 mg g^{-1} of mince, with a myoglobin removal percentage of 5.16%. Surprisingly, the double washing treatment was able to remove an excessive amount of myoglobin while washing away down to 3.836 mg g^{-1} of mince leading a 46.65% reduction. However, washing three times has reduced the myoglobin content down to 3.216 mg g^{-1} of mince, accounting only 16.16% reduction. With respective to the quadruple washing, the myoglobin content in mince sample was reduced down to 2.289 mg g^{-1} of mince, with a rate of 28.82%.

As per the results, there was a significant difference (P<0.05) in myoglobin contents among each treatment. Moreover, the double and quadruple washings exhibited, the percentage wise, the highest reductions in myoglobin contents, wherein the double washing had the significantly highest *(P*<0.05) reduction. According to the mean comparison data, the greatest difference was found between the T2 means and the other treatment means (critical range = 0.2939).

These findings are in line with the results reported by Jin et *al.* (2007) and Ismail *et al*. (2010) on the myoglobin content of chicken breast meat and duck meat that have been subjected to four washing cycles.

Mancini and Hunt (2005) investigated that the metmyoglobin content could be extensively increased with pre-washing treatment. With regard to their findings, they have highlighted that the triple and quadruple washings led to much higher redness values and low whiteness values compared to the single and double washing treatments. They have stated that this effect was possible owing to the oxidation of myoglobin while turning into brownish met myoglobin. Moreover, increasing exposure of myoglobin accompanied by an increasing number of washings served as the prerequisite (Mancini and Hunt, 2005).

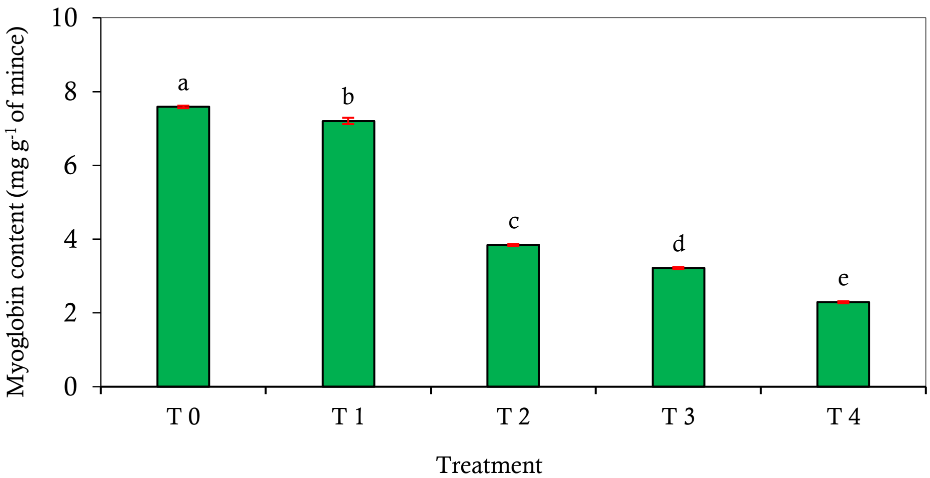

Figure 1: Behavior of myoglobin content (±SE) with the washing cycle: T0 - unwashed; T1 - single washed; T2 - double washed; T3 - triple washed; T4 - quadruple washed. Letters with different superscripts are significantly (P<0.05) different from each other.

Effect of washing on proximate composition of fish mince

The crude protein contents and crude fat contents of the samples that were subjected to quadruple washing, double washing and un-washing treatments are shown Figure 2 and Figure 3, respectively. The crude fat content (in wet basis) of the unwashed fish mince sample (2.66%) was significantly (P<0.05) reduced down to 2.08% with the double washing. However, from the double washing to quadruple washing the fat content was reduced down to 1.75%, which was not a significant (P>0.05) reduction.

As described by the Yang and Froning (1992), an effective pre-washing treatment will always an efficient way of removing excessive fat from the meat samples. Fat removing effectiveness was extremely affected by the two factors, the density and polarity differences in between the fish mince and the washing reagent. As per the results of this study, it can be also stated that tap water as the washing reagent was possible to reduce undesirable fat contents in fish muscles. The fat content showed a decreasing trend with the increasing washing cycles, and however, only the double washing accounted for a significant reduction (P<0.05) in the fat content. Minced fish meat allows a rapid removal of free fatty acids and thus, might be the possible cause that reduces fat extensively at two washing cycles (Suvanich *et al.*, 2000).

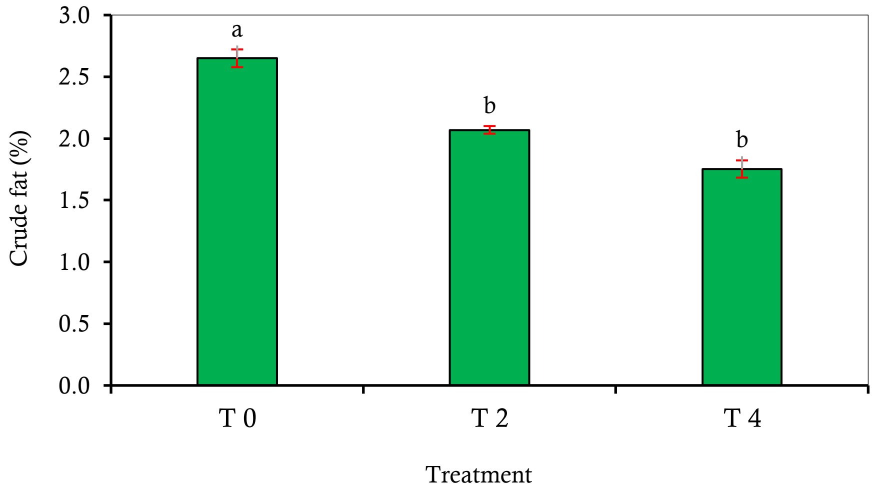

Figure 2: Behavior of crude fat content (±SE) with the washing cycle: T0 - unwashed; T2 - double washed; T4 - quadruple washed. Letters with different superscripts are significantly (P<0.05) different from each other.

As guided by the statistical analyzes results, crude protein contents of the washed fish mince remained significantly similar among the treatments. Crude protein content (in wet basis) of the un-washed mince (11.11%) was reduced down to 10.42% by double washing, and down to 9.88% with the quadruple washing where the reductions remained same.

Though the losses were significantly same, the degree of protein loss was gradually increased along with the washing treatment. As described by the Shahidi *et al*. (1992), the slight reduction in protein content was governed by the draining out of water, together with water soluble proteins. According to Baxter and Skonberg (2008), sarcoplasmic proteins were the possible portion that leached out during the pre-washing process. Anyway, the behavioral patterns of the fat and protein during the washing process were complement with the results reported by Jin *et al*. (2009) and Ismail *et al*. (2010).

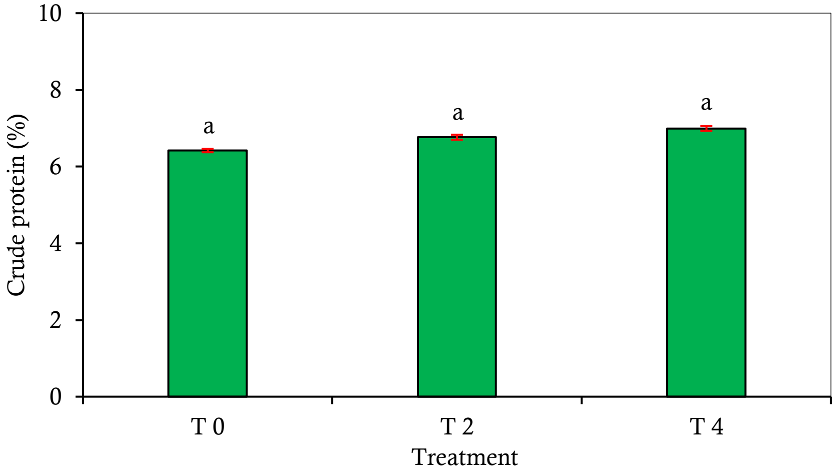

Figure 3: Behavior of crude protein content (±SE) with the washing cycle: T0 - unwashed; T2 - double washed; T4 - quadruple washed. Letters with different superscripts are significantly (P<0.05) different from each other.

Effect of the washing on pH values of fish mince

Behavior of pH values along with the quadruple washing, double washing and un-washing treatments are shown in Figure 4. pH values of the washed mince samples have been significantly (P<0.05) increased with the number of washing cycles.

As suggested by Ramadhan *et al*. (2014), pH value directly affects the physical properties such as water holding capacity, tenderness and colour attributes in the fish meat. High pH value has an ability to enhance the water holding capacity and gel strength. Therefore, high pH value has a few positive impacts on the processing of surimi.

Since, pre-washing has been positively affected due to increments in pH, reduction of undesirable myoglobin content and fat content a prewashing is beneficial in surimi processing from *A. sirm*. However, availability and cost for the washing reagent should be extremely taken into the consideration. Therefore, the washing treatment that can perform better results within a least number of washings has to be considered in the processing of surimi. In this study, the double washing outcome significantly results through the least number of washings and thus selected for the processing of surimi from *A. sirm*.

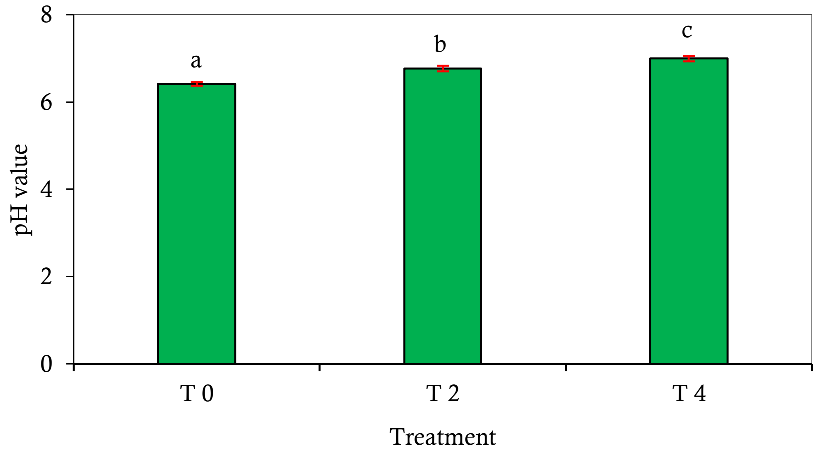

Figure 4: Behavior of pH value (±SE) with the washing cycle: T0 - unwashed; T2 - double washed; T4 - quadruple washed. Letters with different superscripts are significantly (P<0.05) different from each other.

Sensory evaluation

Due to the unavailability of a typical fish surimi in the Sri Lankan local market, comparison between the processed surimi and a commercially available surimi was unachievable, and therefore on behalf of the sensory evaluation, a self-evaluation was carried out for the final product.

Aroma: As represented by Figure 5, a majority of, 53% of total panelists suggested that the aroma was within an acceptable level in the processed surimi product.

Colour: As shown in Figure 6, the perception of the majority (53%) was that the colour is not enough. However, as per the analysed data, the whiteness factor of the prepared surimi was around 55; that is an ideal value in related to the surimi industry (Jin *et al.*, 2009). Since the surimi was not yet a popular food item in Sri Lanka, people always tend to mismatch surimi with sausages.

Flavour: As represented in Figure 7; only 33% of people declared that the flavour was good enough and the majority of people (47%) announced that the flavour was average. In here also, Sri Lankans prefer to consume sausages that are processed by adding adequate amounts of spices. Therefore, they search for the spicy taste even within the surimi, though the addition of spices was not practiced in the surimi processing.

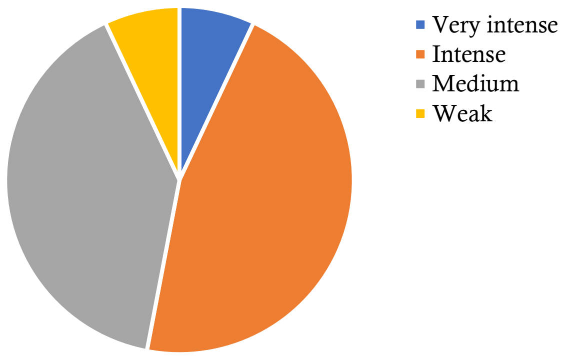

Figure 5: Sensory evaluation results for aroma.

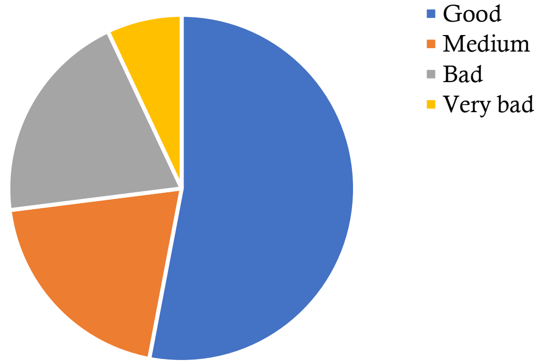

Figure 6: Sensory evaluation results for colour.

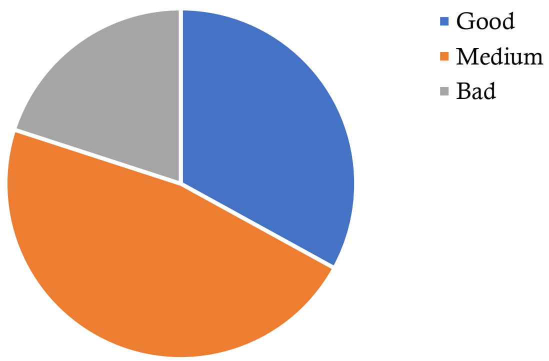

Figure 7: Sensory evaluation results for flavor.

Juiciness: 60% of the people (Figure 8) announced that the product had medium juiciness whereas only 7% said the product was juicy. Further, 33% declared that the product was dry. These effects were mainly due to the deep frying. Considerably higher moisture losses in the portions resulted poor juiciness, due to the shrinkage within cellular structures (fish muscles) (Yamsaengsung and Moreira, 2002).

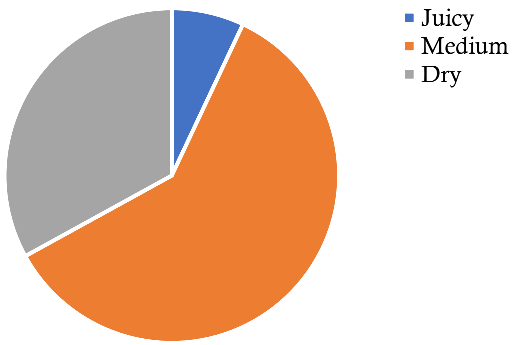

Figure 8: Sensory evaluation results for juiciness.

Tenderness: As in Figure 9, only 27% said that the product seemed to be tender and only 6% declared that the product was tough. However, a majority of the panelists (67%) announced that the tenderness was medium. Generally, sausages and surimi look like processed meat products that are comprised of a medium tenderness and therefore, the responses of the panelists to the tenderness were at an acceptable level (Jin *et al.,* 2007).

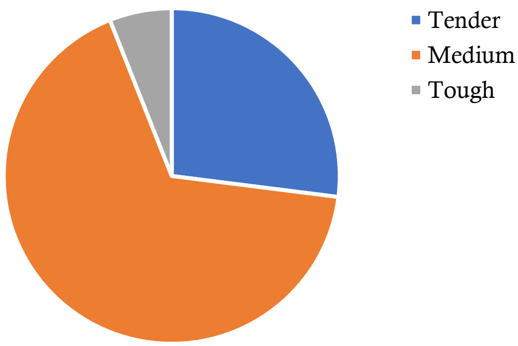

Figure 9: Sensory evaluation results for tenderness.

Overall acceptability: According to the majority of the people (53%), the overall acceptability was medium (Figure 10). Only 13% declared that the processed product was not of acceptable quality. Moreover, according to 34 % of the panelists the overall acceptability of the final product was at satisfactory level.

A considerable proportion of the panelists commented that the attributes of the product were poor while giving low scores on the colour and flavor like attributes. This was mainly because the surimi was generally lighter, and comprised of a totally different aroma and flavor, as well as somewhat lower juiciness compared to the sausages.

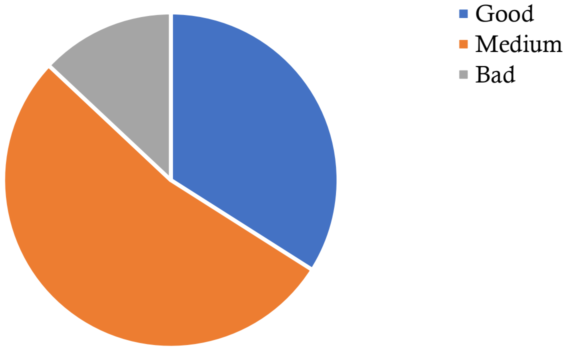

Figure 10: Sensory evaluation results for overall acceptability.

Though surimi products have a low acceptability among Sri Lankans, surimi made from common carp (*Cyprinus carpio*) (Elyasi *et al.*, 2010) and black mouth croaker (*Atrobucca nibe*) (Hosseini-Shekarabi *et al.*, 2018) look like fish that have a higher acceptancy among people in other countries. Moreover, as revealed by a survey, the consumption of crab meat is still remained extremely poor among the Sri Lankans guided by their attitudes and financial issues (Herath and Radampola, 2016). Surimi is consumed by humans as an alternative feedstuff for crab meat (Guenneugues and Ianelli, 2014) and therefore, the rejection of surimi by Sri Lankans is highly possible. For processing of surimi from *A. sirm* fish, it costs 710.00 Sri Lankan Rupees for one kilogram of the product. Compared with the prices of crab meat in Sri Lanka, the cost of surimi is quite reasonable, even in terms of financial aspects. Therefore, installation of a proper marketing strategy would be essential to popularize this product among the Sri Lankans.

CONCLUSIONS

In respect to the myoglobin removal and the fat removal, double washing was the most effective treatment that accounts for the removal of myoglobin and excessive fat along with the least number of washings. Therefore, washing two times is recommended to produce the best quality surimi from the *Amblygaster sirm* fish. In the sense of sensory attributes, the overall acceptability of the product was remained at an average level and therefore, a proper market strategy should be implemented for further commercialization.

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