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RESEARCH ARTICLE

Study on caviar substitute production using readily available freshwater fish species: *Cirrhinus mrigala* (Hamilton, 1822)

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Abstract: Fish egg is considered as one of the by-products with low demand at present, therefore adding value is important to increase the demand of fish roe in the fishery sector in Sri Lanka. Mrigal (*Cirrhinus mrigala*) which is a commercially important freshwater fish species in inland fishery sector was selected as the resource fish species for the present study. Key objective of the current study was to identify the most suitable methodology for production of simulated caviar using Mrigal roe. Mrigal fish egg samples were subjected to dry-salting technique. Three ratios of salt: fish eggs were used as 0.05:1 (S1), 0.25:1(S2) and 0.45:1(S3) to determine the best ratio. Proximate composition evaluation, sensory assessment, microbiological tests and pH analysis were carried out to identify the most suitable roe processing method. Protein level of S1 and Lipid content of S3 sample were significantly different from other two samples ($p<0.05$). Highest protein percentage was reported from caviar produced by the S2 treatment (6.58 %), although maximum crude lipid level was recorded from the product of the S1 treatment (11.30 %). Moreover, moisture contents of three samples were statistically different when compared with each other. pH values of three products changed significantly within the period of 28 days of storage time ($p<0.05$). After evaluation of four sensory parameters (Overall consumer preference, Mouth feel, Odor, and Salty taste) highest consumer acceptance was received for the outcome of the S2 treatment. Finally, as the caviar produced by theS2 treatment preserves the highest protein value in the final product, moderate salt requirement and the best sensory qualities, it could be considered 0.25g of salt: 1g of fish eggs is the most appropriate treatment to process simulated caviar by Mirigal fish roe, among all three treatments.

***Keywords**: Caviar Substitute, Fish roe-based product, Dry salting, Value addition, Consumer preference*

Introduction

Seafood, including fish eggs, is very expensive product, since there are limited production and processing prospects (Gulyavuz and Unlusayin, 2008). Sea food is also accepted as a major component in a balanced diet due to its high nutritional value (Inanli *et al*., 2010). The salted fish roe that is known as ‘‘caviar’’ in English is defined as the preserved eggs or roe of sturgeon with salt(Inanli *et.al*., 2010). Caviars are processed using sturgeon eggs by preserving as salt-cured eggs which have been pulled out and screened or otherwise separated from the supporting connective tissue (Duyar*et al*., 2008). The caviar is a highly expensive, but delicious product rich with superior nutrient factors, vitamin A and vitamin B complex, which can be afforded only by millionaires (Altug and Bayrak, 2003).

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According to records of Mims *et al*, (2002), 90% of the sturgeon caviar comes from Caspian Sea. There are three main types of Caspian Sea caviar include beluga, osetra and sevruga in the world market. The most delicately flavored caviar is beluga sturgeon caviar which comes from the largest egg producing sturgeon species, *Husohuso* (Monfort, 2002). Sevruga sturgeon caviar offers the briniest taste; the smallest of eggs and the best price among other caviar types. It is the most abundant type out of the three caviar varieties and is produced by *Acipenserstellatus*. Osetra sturgeon caviar is produced from the eggs of Russian sturgeon fish (*Acipensergueldenstaedtii*) and Persian sturgeon species (*Acipenserpersicus*) that are medium sized sturgeon fish varieties. Colour of the osetra caviar varied from dark brown to golden yellow (Monfort, 2002).

Most of sturgeon fish varieties have high demand for their eggs which are processed as caviar (Mims *et al*, 2002). As a result, sturgeon populations are declining due to overexploitation by as much as 70% in the last century. Moreover, destruction and alteration of sturgeon habitats is another key reason for depletion of sturgeon stocks (Mims *et al*, 2002). Availability of raw material for original caviar production is restricting nowadays, because of shrinking the sturgeon wild fish population. Production of simulated caviar is one of the best alternatives instead of true caviar for conservation of that sturgeon species, by overcoming the aforesaid issue.

Caviar substitute is also called as simulated caviar or imitation caviar. According to the report of United States Custom Service (2008), caviar substitute is defined as the processed fish roe other than the sturgeon fish. Simulated caviar is processed using fish roe other than the sturgeon eggs (e.g. Tuna, Salmon, Cod, Pike, Lumpfish, and Mullet). Fish roe is thoroughly washed, separated of adherent organs, salted and pressed or dried as preferred during processing. Such kind of processed fish roe may be colored and also seasoned using spices to enhance the quality of the product (Johannesson, 2006). Resulting product consists with its own characters and different compared to Sturgeon caviar.

Both caviar and simulated caviar are not so popular among Sri Lankans. In Sri Lanka, there is no high demand for fish roe-based products in either local or export market. Therefore, current study focused on value addition to fish eggs and makes them a high demand value added product. Moreover, simulated/imitation caviar is possible to produce using fish eggs with low demand or disposal roe samples of harvested fish. As a result, potential risk on fish biodiversity would be minimized due to overharvesting of target fish for processing of imitation caviar. Also, local people, especially rural communities can acquire nutritional benefits from the simulated caviar since it has a high nutrient content.

Past studies revealed potential of processing simulated caviar using highly abundant marine and freshwater fish species. Fresh water fish: Mrigal (*Cirrhinusmrigala*) which is one of the major cultivable Indian carp species widely distributed in Southeastern and Sothern Asian region including Sri Lanka was selected as the resource fish species of the present study. Mrigal has a good taste as a food fish species. In Sri Lanka, it plays a vital role as a commercially important freshwater species in aquaculture and capture fishery sectors. Mrigal roe is usually removed as a by-product or sold in the local market at very low price. Value addition to Mrigal roe allows further enhancing the demand for this freshwater resource. Promotion of Mrigal roe-based products is important to increase the income of farmers, while adding value to local aquatic resources.

Therefore, main objective of the present study was to identify the most suitable processing method for caviar substitute production by Mrigal (*Cirrhinusmrigala*) fish roe.

Materials and Methods

Matured roe samples of *C. mrigala* were collected from Weerawila reservoir in Hambanthota district-Southern province of Sri Lanka. Samples were immediately transported to the animal science laboratory of UvaWellassa University using cooler boxes with ice (ice/roe ratio - 2/1) following recommended protocol (Celic*et al*., 2012). Fish roe were separated from the ovary sac using manual screening method. Adhered connective tissue and blood on the roe samples were separated by washing using 5%of brine solution. After removing blood and connective tissues, fish roe was sieved using the sieve set.

Surface moisture of the fish egg samples were removed using clean cotton cloth. Prepared fish egg samples were processed using dry salting technique with three different salt ratios. Fish egg samples were placed in plastic boxes as Iodized powder form of salt layer and fish roe layer respectively. Three different salt: fish roe ratios (0.05:1 (S1), 0.25:1(S2) and 0.45:1(S3)) were used in triplicates to determine the best ratio. All salted roe samples with weight at the range of 200g -300g per sample were kept at room temperature approximately for 2½ hours. Samples were compressed manually once using the palm for five minutes to increase the absorption rate of salt, at the initial stage. Then samples were immersed in water bath at room temperature approximately for 30 minutes. Finally, these processed roe samples were carefully covered using wet, white cotton cloth and allowed to keep for approximately four hours. It allows for absorption of excess moisture in the final products. As the next step, without using sun drying, processed caviar was allowed to dry in a cold dry place at 20^0C for 24 hrs to enhance the shelf life of the final products (Celic*et al*., 2012). Finally, the product was filled into glass jars manually and pasteurized by dipping the jars with simulated caviar products in the hot water bath at temperature of 155-160 ^{0}F (68- 71 ^{0}C) for the containers with 115g at 60 minutes (U.S. Custom & Border Protection, 2008).

Final products were analyzed for different parameters. Nitrogen content of each sample was assessed by Kjeldahlmethod (AOAC, 1990) and crude protein content of each sample was determined. Moisture content was determined according to AOAC (1990) and Lipid contents of samples were determined by Soxhlet method.

The pH value of each sample was determined at room temperature using a pH meter (Eutech pH meter, 310, USA) at seven-day intervals up to 28 days. Total Plate Counts (TPC) of the final products were tested using spread plate technique to determine the microbiological quality. TPC values were recorded at the initial stage and then subsequently once in 07 days for 28 days of storage period.

Final products were analyzed using 30 untrained male and female panelists for the overall consumer preference, odor, salty flavor, texture, mouth feel and color within a hedonic scale at the range of 1 to 5 scores: 1 highly dislike, 2 slightly dislike,3 neutral,4 slightly preferable and 5 highly preferable. Samples which were stored at 4^0C, allowed warming up to the room temperature before start the sensory evaluation process.

Variation of proximate composition, microbiological count and pH values of final products were analysed using one-way ANOVA followed by Tukey HSD for Post-Hoc analysis at the level of $P<0.05$. Results of the sensory evaluation were analyzed using non-parametric test (Friedmann) at the significance level of $P<0.05$using MINITAB (version16.0) statistical software.

Results and discussion

Results have shown potential of processing caviar substitute using Mrigal fish roe. Significant variation between the protein level of S2 and S3 treatments was not recorded, when compared to the protein level of S1 treatment ($p<0.05$) (Table 01). Lipid content of S1 and S2 treatments are not significantly different, when compared to the S3 treatment. But the highest protein content (6.58±0.02) was recorded from S2 treatment, while the highest lipid percentage (11.30±0.26) was recorded from S1 treatment (Table 01). As revealed by Himelbloom *et al,* (1998), total fat level for Salmon caviar (locally called as "ikura") was recorded as 11%, which is relatively similar to the highest fat content of final product in the current study. Further, Wirth *et al,* (2000) revealed that the protein level of sturgeon caviar was at the range of 26.2 - 31.1 %. Protein contents of salted and waxed flathead mullet caviar were 35.38 % and 40.83 % respectively (Sengor*et al*, 2000). Protein level of Mrigal caviar is significantly lower compared to past findings. Moisture contents of three samples were significantly different when compared to each other. Applied salt content and the moisture level of the final caviar products were inversely related to each other. As a result, moisture content of S3 treatment recorded the minimum moisture percentage (72.53±0.33). Water of the final product was drained by adding salt to the processed caviar and preserves the product. Different salt ratios of each product could be the main reason for the variation of final moisture contents of caviar samples. Moreover, bio-chemical compositions of the final products vary according to type of fish species and production techniques (Inanli *et al*., 2010).

The lowest pH value was recorded for S3 treatment (5.55±0.02) at the initial stage and highest value was observed for the S1treatment (6.59±0.09) at the 28^{th} day (Table 02). Increasing trend of pH value was observed in all three treatments and pH values of the three treatments were changed significantly with the time. According to Parkin and Brown (1983), autolytic reactions such as denaturation and polypeptide bonds cleavage cause increasing of pH, after glycolysis of the final products. However, pH values of black caviar and red caviar products were reported as 5.45 and 5.80 respectively (Bledsoe *et al*., 2003). The pH variability recorded among aforesaid caviar products may be due to different fish types used as raw materials and different treatments applied for simulated caviar production (Inanli *et al*., 2010).

At initial stage, lowest TPC was observed in the S2 treatment (4.69×10^4cfu/g) and highest was in the S3 treatment (4.76×10^4cfu/g). At the final day, highest count was observed in the S1 treatment (5.69×10^5cfu/g) while S3 treatment (5.40×10^5cfu/g) indicated the lowest value (Table 02). TPC values of S1 and S2 treatments have changed significantly with time and there is no significant change observed in theS3 treatment with 7^{th}, $14^{th}$and 21^{st}time frequency. Salting avoids deterioration of final product by inhibition of microbial growth, propagation, autolysis of enzymes and oxidation. As a result, shelf life of the caviar substitute is prolonged with incorporation of salt into the roe product. According to the study conducted by Jelodar and Safari (2006), *E. coli*, *Clostridium botulinum*, *Staphylococcus aureus* and *Listeria* sp. were zero in all processed caviar samples collected from Iranian processing plants. Moreover, they have emphasized that Total Viable Count (TVC) in the processed caviar products were significantly higher compared to the raw fish roe samples. Thus, it is suggested to follow standard hygienic practices, when processing caviar using any kind of fish roe. Still, quality standards and accreditations haven't been recommended for the fish egg-based value added products in Sri Lanka.

Table 01: Mean values of proximate composition (mean±SD as Dry Matter Basis) for the final treatments

Salt ratio	Protein%	Lipid%	Moisture%
0.05 (S1)	4.74±0.57^{b}	11.30±0.26^{a}	77.82±0.02^{a}
0.25 (S2)	6.58±0.02^{a}	10.90±0.10^{a}	75.02±0.01^{b}
0.45 (S3)	6.35±0.49^{a}	10.20±0.36^{b}	72.53±0.33^{c}

Results were significant different for each treatment ($P<0.05$).

Table: 02 pH and TPC values (mean±SD) of final products with storage time

Period	pH			TPC (cfu/g)		
	S1	S2	S3	S1	S2	S3
0 day*	5.56±0.005^{a}	5.59±0.07^{a}	5.55±0.02^{a}	4.74×104^{a}	4.69×104^{p}	4.76×104^{a}
7 days	5.80±0.09^{b}	5.79±0.07^{b}	5.74±0.06^{b}	5.10×105^{b}	4.92×105^{q}	5.15×105^{b}
14 days	6.03±0.23^{c}	6.02±0.21^{c}	6.05±0.23^{c}	5.46×105^{e}	5.10×105^{b}	5.15×105^{b}
21 days	6.28±0.13^{d}	6.26±0.14^{d}	6.29±0.11^{d}	5.71×105^{f}	5.60×105^{r}	5.15×105^{b}
28 days	6.59±0.09^{e}	6.53±0.14^{e}	6.50±0.13^{e}	5.69×105^{f}	5.63×105^{r}	5.40×105^{c}

* 0 day - Initial values of three treatments processed with different salt ratios
Different superscript letters denote that there is a significant difference of TPC and pH value with the storage time in each final product ($p<0.05$).

Based on the results of sensory analysis test, highest estimated median and sum of rank for four sensory parameters (Overall consumer preference, Odor, Mouth feel and Salty flavor) were recorded for S2 treatment. Based on the values of sum of ranks, significant greatest consumer preference for only color and texture was recorded for S1 product ($p<0.05$). So it can be concluded S2 treatment as the best among other samples (Figure 01). Salt level of the product is a key factor affecting on the taste of the final product, thus consumer acceptance is changed with taste level of the final product. As the records of Inanli, *et al*, (2010), it is strictly recommended to detect the accurate salt level suitable for final product during processing, since excess salt level interrupts the flavor and consumer preference of the final products.

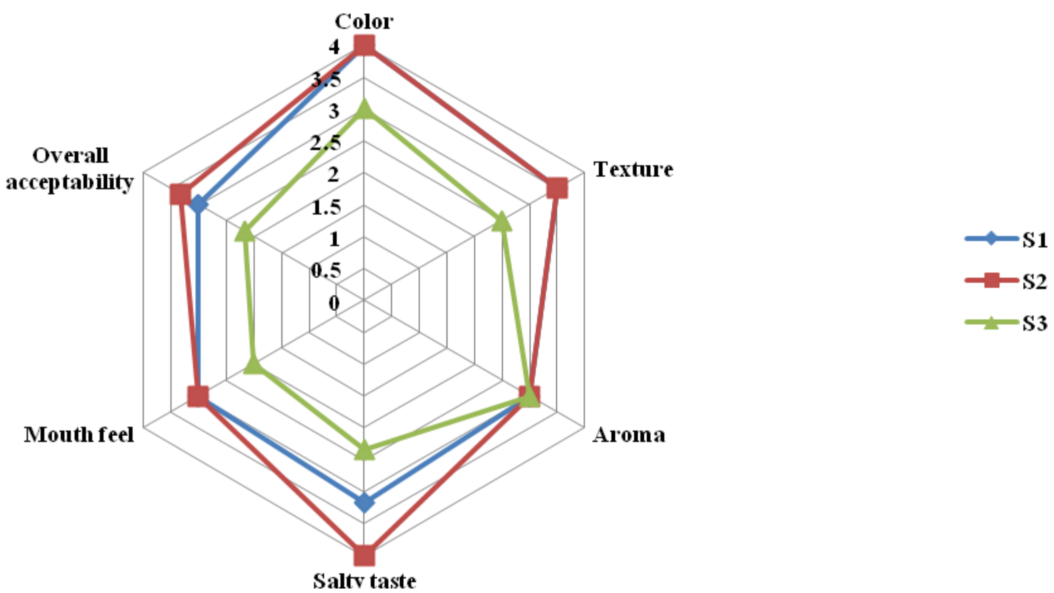

Figure 01: Results of the sensory evaluation test for organoleptic parameters for final products

Although lowest lipid percentage and lowest microbial count is recorded from the S3treatment, during storage time period, it received the lowest customer satisfaction for all the sensory parameters. Since this treatment requires the greatest salt concentration out of all three treatments, production cost of the product is comparatively high (Table 03). Therefore, S3 treatment was not considered as the best treatment. The product of the S1 treatment did not exhibit higher consumer acceptability for all the organoleptic parameters during sensory evaluation. Also highest microbiological count was recorded from this product duringthe storage time periodand nutritional composition was not in a satisfactory level (lowest protein level and highest lipid content). Therefore, product of the S1 treatment could not be accepted as the best treatment, even though it reduces the production cost with lowest salt level (Table 03). Among all three treatments, product of the S2 treatment records the highest customer preference as it shows the best sensory qualities for four organoleptic parameters. On the other hand, it presented the highest protein level and intermediate lipid level by the results of proximate analysis and indicates moderate microbiological count for storage time period. Moreover, as this treatment requires the intermediate salt concentration, it relatively reduces the production cost of the product (Table 03). Finally, outcome of the S2 treatment is considered as the best product for simulated caviar production using Mrigal roe as an alternative for original caviar. In Sri Lanka, caviar is not highly popular roe product among Sri Lankans, thus Mrigal roe can be used to promote simulated caviar production locally. However, final products of the current study could not compare with the original caviar product, since caviar is not commercially available in the local market of Sri Lanka.

Table: 03 Production cost per 1 kg of final product for each treatment

	Treatment : S1	Treatment : S2	Treatment : S3
Initial Capital Cost :Equipments, consumables, storing containers	5,000.00	5,000.00	5,000.00
Salt / for processing 1 kg	5.00	25.00	48.00
Mrigal Roe/kg	450.00	450.00	450.00
Electricity (Water heating and refrigerator usage)	2500.00	2500.00	2500.00
Water (For all purpose of water usage per 30 units)	200.00	200.00	200.00
Total Production Cost	**8,155.00**	**8,175.00**	**8,198.00**

Conclusion

Mrigal roe can be used as raw material for simulated caviar production in Sri Lanka. Greatest protein percentage was recorded from the caviar produced by the S2 treatment. Results of sensory assessment also revealed S2 treatment as the best treatment according to the consumer preference. Dry salting method with 01: 0.25- fish roe: salts by weight can be recommended as the most suitable method among the tested roe: salt ratios for production of simulated caviar using roes of Mrigal fish.

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