Production of simulated caviar using Bigeye Tuna (*Thunnus obesus*) roe: Pilot scale study to promote fish roe based value addition sector in Sri Lanka

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Abstract **-** Simulated caviar also named as imitation caviar, is defined as salted roe that comes from a fish except sturgeon fish. Fish roe is removed as a by-product from processing plants and doesn't have a high demand in Sri Lanka at present. It is essential to add value to fish roe which is of low demand at present and enhance the income of fish processing industries and suppliers. Since Bigeye tuna (*Thunnus obesus*) is one of the most commercially important tuna fishery resources in Sri Lanka, Bigeye tuna was used as the resource species for the present study. Fish roe samples were subjected to "dry salting" method for different treatments with salt (g): fish roe (g) ratios as Treatment 01 - 0.25: 1.00, Treatment 02 - 0.50: 1.00 and Treatment 03 - 0.75: 1.00. Most appropriate treatment was assessed using sensory evaluation, proximate analysis, pH test and microbiological analysis. Highest average ash content (6.95 % ± 0.06) and maximum lipid content (11.89 % ± 0.88) were recorded for Treatment 03, while greatest protein value (25.65 % ± 0.11) was indicated by Treatment 01. Initial average pH values of the 03 treatments varied between 6.00 - 6.39 with significant difference for all 03 treatments. Treatment 03 (0.75 salt: 1.00 fish roe) has recorded lowest Total Plate Count (TPC) value for a period of 02 months with significant difference for all treatments ($P < 0.05$). Results of the sensory evaluation showed that best consumer preference for all parameters (color, texture, aroma, overall acceptability, mouth feel, and saltiness) were for treatment 01. Present study implicated that "dry salting" method with 0.25 salt: 1.00 fish roe by weight (Treatment 01), with proper nutritional quality (highest protein content: 25.65% / lowest lipid level: 11.50%) is most suitable processing method for simulated / imitation caviar production using roe samples of Bigeye tuna. Since Treatment 01 requires lowest salt level to process product, production cost is also minimum compare to other two treatments. Popularization of this edible source is useful to prevent mal-nutrition as nutritional rich source in rural and urban community of Sri Lanka.

Index Terms- Bigeye Tuna (*Thunnus obesus*), Imitation / Simulated Caviar, Dry Salting, Roe, Sri Lanka

I. INTRODUCTION

The roe is considered as an excellent raw material for the production of diverse delicacies that can be sold at good prices in many markets (Johansson, 2006). Caviar is one such delicious product and has a high demand among European countries. The term "caviar" refers to some of the processed fish roes, and is an expensive product high in nutrients (protein, lipid and ash), particularly B vitamin (Altug and Bayrak, 2003).
Caviar is defined as a product made from fish eggs of the Acipenseridae family (*Acipencer sp.*) by treating them with food grade salt (Johannesson, 2006).

Most caviar is produced in Russia and Iran by sturgeon fish harvested from the Caspian Sea, Black Sea, and Sea of Azov. Caviar can be classified as: "Beluga" - Obtained from *Huso huso* and caviar with coarse grained, black or dark gray, "Osetra" - Prepared by *Acipenser gueldenstaedtii colchicus* (Russian Strugeon) and fine grained gray green or brown color caviar that is lighter than beluga and Sevruga - Product is processed by *Acipenser stellatus*. The sevruga is smallest grains of greenish black (Inanli et al., 2010).

There are more than 20 species of sturgeon harvested for caviar (Al-Holy *et al*., 2005). Caviar is marketed through buyers who sell to exclusive restaurants, luxurious shops and mail-order retail outlets. The major problem for producers is to protect the raw material (roe samples) from spoilage by following clean working procedures. Freezing, various preservatives and packaging of caviar in anaerobic atmosphere are all methods that can help to limit spoilage of caviar. In addition to the salting process, freezing, smoking, canning, and sausage production technologies are also used in caviar production (Inanli *et al*., 2010).

According to the literature records, there is a severe depletion of sturgeon stocks due to pollution, overfishing and poaching. Especially over-exploitation of natural and enhanced sturgeon stocks for caviar production has led to drastic decreases in stocks (De Meulenaer and Raymakers, 1996). Therefore, it is advisable to diversify the fish used for producing this healthy, edible and tasty fish roe product using readily available marine and freshwater fish species other than sturgeon fish species.

Simulated caviar also can be named as imitation caviar and it is defined by United States Custom Service as roe that comes from a fish other than the sturgeon. Also it can be classified as a caviar substitute. Simulated caviar is not true caviar. Products from other fish species have to be labeled as "imitation / simulated caviar" or include the name of the fish before the word caviar in most markets, such as "lumpfish caviar" and "capelin caviar" (Sternin and Dore, 1993). There is a possibility to produce imitation caviar using roe of different fish species that are caught in the spawning season. For example, the eggs of lumpfish, whitefish and salmon may be prepared or preserved as caviar substitutes (Johansson, 2006). Products consumed as caviar but prepared from the eggs of fish other than sturgeon (e.g. salmon, carp, pike, tuna, mullet, cod, and lumpfish) are seasoned and colored to enhance consumer acceptance (Johansson, 2006). The imitation caviar prepared from salmon roe is described as ''keta caviar''. The product obtained from the roe of trout, carp, and grey mullet fish is known as ''red caviar.'' Mullets have gained importance for caviar production in recent years, especially with increased demand from France, Italy and the United States (Çelik *et al*., 2012). Smoked grey mullet caviar is used as a good appetizer.

Fish roe based aquatic products oriented for export or local market are rare in Sri Lanka. Nowadays, fish roe is removed as a by-product from fish processing plants and sometimes sold in the local market and doesn't have a high demand at present. Development of roe based new products targeting export or local market is important to add value to fish roe which is of low demand at present, to make them as a high demand product. Moreover, there is a potential in increasing the income of producers and farmers by introducing this kind of roe based new product in industrial sector. Popularization of this edible source is also useful to prevent mal-nutrition as nutritional rich source in rural and urban community of Sri Lanka.

Simulated Caviar (processed fish eggs) can be produced using readily available marine and freshwater fish species (Tilapia, Yellow fin tuna, Bigeye tuna, Catla, Common carp, Trevally) which are abundant in Sri Lanka. As literature reveals, several kind of research studies have been conducted related to the production of caviar and imitation caviar in countries such as Turkey, Iran, Russia and Germany, but there is no such research performed related to process of fish eggs in Sri Lanka. This is the first pilot scale study in focusing fish roe based product development using readily available fish species in Sri Lanka.

As resource species (marine) Bigeye tuna (*Thunnus obesus*) was selected for current study. It is a larger pelagic fish species in Family Scombridae. Bigeye tuna is morphologically distinguishable. Its body outline is rounded, forming a smooth dorsal-ventral arc between snout & caudal peduncle and eye diameter & head length is greater compared to other tuna species. Also pectoral fin of Bigeye tuna is thin, pointed and flexible and reaching beyond base of second dorsal fin.

There has a potential to use Bigeye tuna for simulated caviar production in Sri Lanka. Sri Lanka is one of the oldest and most important tuna producing islands in the Indian Ocean (Dissanayaka and Hewapathirana, 2011). The catches of tuna fishery resources of Sri Lanka are mainly, Yellowfin tuna (*Thunnus albacares*), Bigeye tuna (*Thunnus obesus*), Skipjack tuna (*Katsuwonus pelamis*), Kawakawa (*Enthynnus affinis*), Frigate tuna (*Auxis thazard*) and Bullet tuna (*Auxis rochei*) (Dissanayaka and Hewapathirana, 2011). Within them Bigeye tuna plays a major role as one of the most commercially important tuna fishery resource in Sri Lanka. All tuna species are processed before exporting, meanwhile belly flaps, guts and gonads with eggs are being marketed locally or discarded. Hence current study attempts to produce a nutritious product by adding value to Bigeye tuna gonads with egg (fish roe) which has a low value at present to high priced product. Since the raw material (fish roe) used for simulated caviar production is low demand or discarded fish roe of exploited fish, there will be no threat of overexploitation due to simulated caviar production. Moreover, fish roe is discarded to the environment in some cases. So, imitation caviar production is important alternative to prevent environmental contamination. Our main aim is to develop suitable methodology for simulated caviar (processed fish eggs) production using Bigeye tuna fish roe as an income generating nutritional rich value added product.

II. METHODOLOGY

Research was conducted in the Animal Science Laboratory of Uva Wellassa University, Sri Lanka.

Sample collection

According to the literature review, commercial value and accessibility, Bigeye tuna (*Thunnus obesus*) was selected as the marine finfish species. Egg sac samples of Bigeye tuna was purchased from fishermen in Negombo, Hambantota, Tangalle and Dondra fishery harbors and one processing plant: Ceylon fresh seafood (pvt) Ltd in Ja-Ela which is located between Colombo and Bandaranayke International Air port, as representing western and southern province. The samples were transported to the laboratory using cooler boxes to prevent spoilage of samples by decreasing the temperature.

Sample Pre-preparation

First, egg sacs were rinsed using clean water to remove adherent particles and impurities, prior to preparation of samples. Manual screening was followed to prepare the samples. The eggs were removed from the sac manually and separated from the connective tissue that surrounds them. There after the blood, connective tissues and other wastes on the roe were removed within 15 minutes while keeping eggs in 5% brine solution.

Preliminary trial

Caviar processing was carried out by using 'dry- salting' method. Then the surface moisture of the roe was removed with the help of a clean cloth. Iodized powder form salt packets were used in the study. Fish roe samples were put in plastic containers with one layer of salt and one layer of roe alternatively in dry - salting method. The best ratios of salt: fish roe were determined using different ratios in the preliminary study (Table 01). Similar weight of fish roe samples (250 g) were used for each trial and they were put in plastic trays with alternative salt layers keeping top and bottom layers as salt layers.

According to the weight (between 200 g-300 g) of the roe, the samples were let in salt, for 2.5 hours, while fish roe samples with 300g or over were required to keep for nearly 6 hours (Celik et al., 2012). During this period, they were pressed once using fingers and palm for five minutes for effective absorption of salt. Then they were dipped in tap water bath and covered with a wet, white cotton cloth for 4.5 hours to remove excess salt and moisture. After that, samples were left standing in a dry cool place at approximately 20 ^{0}C, to be dried. This procedure allows fish roe to be dried without being directly exposed to sunlight by preventing the oxidation and to be preserved for a longer time. Glass jars were filled manually with approximately 75 g of simulated caviar. Glass jars of caviar samples were kept in a hot water bath at 68 ^{0}C for 45 minutes for pasteurizing the product (US Customs and Border Protection, 2008). Processed samples were stored in a refrigerator (at 4±2 ^{0}C).

Table 1: Salt: Fish roe ratios used in the preliminary study

Trial No.	Trial 01	Trial 02	Trial 03	Trial 04	Trial 05
Salt proportion by weight	0.25	0.5	0.75	1.0	1.25
Big eye tuna fish roe proportion by weight	1.0	1.0	1.0	1.0	1.0

Sensory (organoleptic) evaluation of Preliminary study

The products were characterized by 30 untrained panelists in terms of color, texture, aroma, salty taste, mouth feel and overall acceptability on a hedonic scale of 1 to 5 points:

1 extremely dislikes
2 slightly dislike
3 neither like nor dislike
4 slightly like
5 extremely like

03 best ratios were selected for further experiments and analysis based on results of sensory evaluation.

Final experiment

The selected ratios of salt and fish roe (Table 02) were processed again by using same procedure to select most suitable ratio of caviar. 03 replicates were used in each experiment.

Table 2: Salt: Fish roe ratio used in the secondary trial

Treatment No.	Treatment 1	Treatment 2	Treatment 3
Salt proportion by weight	0.25	0.5	0.75
Big eye tuna Fish roe proportion by weight	1.0	1.0	1.0

Analysis of final products for chemical, biological and organoleptic properties

Final products were analyzed for sensory, chemical and microbiological characters to select the best treatment. Sensory evaluation was repeated as previously using 05 Hedonic scales for 30 untrained panelists.

Total Plate Count (TPC) was determined using plate cultures on nutrient agar following incubation at 37 °C for a period of 48 hrs. General Coliform and *E. coli* presence were examined at incubation temperature of 37 °C. The caviar samples were analyzed once in 14 days for 02 months of storage for their microbiological aspects. Initial pH values of the samples were determined with a pH meter.

Dry matter was determined by drying the samples at 105 ^{0}C to a constant weight (AOAC, 1990). The drying cabinet was used to determine the moisture content. Crude protein content was calculated by converting the nitrogen content determined by the Kjeldahl method (6.25xN) (AOAC, 1990). Lipid was determined by using the method described by Bligh and Dyer (1959). Ash was determined by drying sample at 600 ^{0}C for 04 hours.

Statistical Analysis

The MINITAB (version 14.0) program was used to test the differences between mean values of the different analyzed parameters. Differences between means of proximate composition, pH values and microbiological factors were analyzed by one-way analysis of variance (ANOVA), followed by Post Hoc test ($P < 0.05$). Friedman non-parametric test was used to analyze the results of sensory evaluation ($P < 0.05$).

III. RESULTS

According to the results of the proximate composition (Table 03) of the final products, lowest moisture content was recorded for the Treatment 03, while highest moisture content was recorded in the treatment 01. Maximum average ash and lipid content were detected for Treatment 03, while minimum lipid and ash level was for Treatment 01. Greatest protein value was indicated by Treatment 01.

There was no significantly difference between lipid and protein content of Treatment 02 and 03, while ash and moisture percentage was significantly different for all three treatments.

Table 3: Results of Proximate composition and pH values for treatments

Parameter	Treatment 1 1.0 (Fish roe): 0.25 (Salt)	Treatment 2 1.0(Fish roe) : 0.5(Salt)	Treatment 3 1.0 (Fish roe) : 0.75 (Salt)
Moisture %	52.80 ± 0.02^x	51.00 ± 0.13^y	50.09 ± 0.05^z
Protein %	25.65 ± 0.11^d	24.19 ± 0.05^c	24.11 ± 0.33^c
Lipid %	11.50 ± 0.08^a	11.85 ± 0.10^b	11.89 ± 0.88^b
Ash %	5.57 ± 0.03^e	6.48 ± 0.74^f	6.95 ± 0.06^g
pH	6.39 ± 0.01^p	6.00 ± 0.12^q	6.13 ± 0.21^r

Different superscript letters indicated that results were significantly different at 0.05 level ($P < 0.05$).

Highest pH value was recorded by Treatment 01. Average pH values of the 03 treatments were varied between 6.00 - 6.39 (Table 03).

Results of the sensory evaluation (Figure 1) showed greatest estimated median values and sum of ranks for all the six parameters for Treatment 01. Treatment 03 was indicated lowest estimated median and sum of ranks for all parameters. Therefore treatment 01 is considered as the best product according to consumer preference. All the parameters were significantly different at 0.05 level.

Total coliform and *E. coli* count were negative in all 03 products. Our research findings indicated that all final products are pathogenic free products up to two month period. Average values of the Total Plate Count (TPC) are given in Figure 2 for approximately 02 months of storage time period. Accordingly Treatment 03 records lowest TPC value for a period of 02 months.

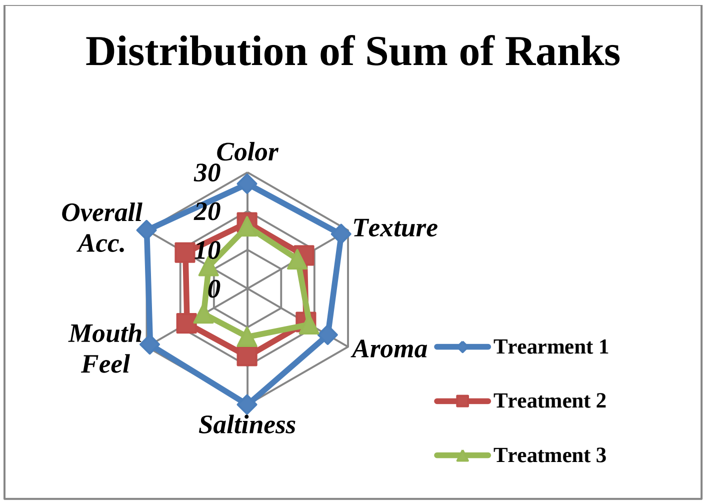

Figure 1: Results of the sensory assessment for organoleptic parameters

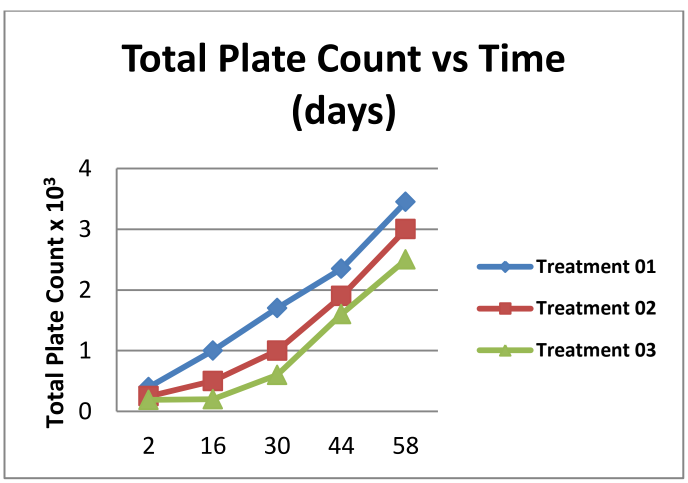

Figure 2: Results of the Total Plate Count (TPC) for final products in 58 days

IV. DISCUSSIONS

Salting is an ancient and popular procedure for preserving fish and it preserves the product by preventing the fish roe spoilage resulting from three basic mechanisms; enzymatic autolysis, oxidation and microbial growth. Sodium chloride has a capacity to inactivate autolytic enzymes in marine species (Ghaly et al., 2010). Klomklao et al. (2004) has conducted a research on proteolytic activities of spleenic extract from three tuna species; skipjack tuna (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*) and tongol tuna (*Thunnus tonggol*) and revealed that autolytic activities continuously decreased as NaCl concentration increased. Since Bigeye Tuna and previously studied Tuna species belongs to Family Scombridae, previous research findings are applicable to Bigeye Tuna also. Recent research findings suggests (Reddi et al., 1972, Siringan et al., 2006, Yongsawatdigul et al., 2000) that the ability of NaCl as inactivator of autolytic enzymes including proteolytic enzymes in both freshwater and marine fish. So, NaCl can be used as a preservative agent during flesh and by-product processing.

When salt content of the product is increased, water is drawn rapidly out of the product. Therefore, moisture content of the treatment 03 has decreased rapidly compared to the other treatments. The amount of salt applied and the duration of the processing affect the moisture content of the end product (Inanli et al., 2010).

After the salting, the mineral content of caviar increased above the levels in raw roe. Greatest average ash level was recorded for Treatment 03 (Table 03), since ash content is increased with increasing salt level. Inanli et al. (2010) also has revealed same finding. Their study has detected that average ash content as 2.21% in raw roe of Rainbow Trout, and at the end of salting, it was 6.38% in the 4% of salt group and 8.84% in the 8% salt group.

Maximum lipid content was recorded for Treatment 03, while minimum lipid content was Treatment 01. Himelbloom and Carpo (1998) have identified 11.00 % of average lipid content in the salmon caviar. The lipid levels found in the current study for three treatments were slightly similar to his findings. Maximum protein value (Table 03) was recorded for Treatment 01. Wirth, et al. (2000) found that the caviar obtained from sturgeon contained protein between 26.2-31.1%. Protein values of our study were slightly inferior to the protein content recorded for sturgeon caviar. This difference between our results and previous findings could be due to the fish species and processing method used in the present study.

Range of initial average pH values of the 03 treatments was between 6.00 - 6.39. According to research findings of Çelik et al. (2012) pH values of both fresh and dried samples of Flathead Grey Mullet (*Mugil cephalus*, Linnaeus 1758) ranged between 5.79 and 5.96 and did not change significantly with time. Bledsoe, et al. (2003) examined pH values for red caviar and black caviar as 5.80 and 5.45 respectively. There is a difference between pH values of processed products in the current study and previous findings. Inanli et al. (2010) has recorded that the chemical composition of the resulting caviar depends on fish species and processing techniques. Also the difference in chemical composition of various fish roe is mainly attributed to biological factors, including species, maturity stages, diet, season, harvest area and processing condition (Mahmoud *et al*., 2008).

According to results of the sensory evaluation, Treatment 01 is considered as the best product according to consumer preferences. The salt used in preparing caviar is a factor affecting flavor of the product. On the other hand, excessive salt disrupts the taste of the product. Therefore, the salt level should be determined with precision (Inanli, et al., 2010).

Coliform bacteria indicate the likely presence of pathogenic (disease-causing) bacteria or viruses, including *E. coli*. They are present in the intestinal tracts of all warm-blooded animals, including humans. Our research findings revealed that all final products are not pathogenic up to two month period. Himelbloom and Crapo (1998) also revealed that coliform contamination in caviar was not detected. It means that results of our study are analogous to their findings.

Microbial growth and metabolism is a major cause of fish spoilage which produce amines, biogenic amines such as putrescine, histamine and cadaverine, organic acids, sulphides, alcohols, aldehydes and ketones with unpleasant and unacceptable off-flavors (Dalgaard, et al., 2006, Emborg, et al., 2005, Gram and Dalgaard, 2002). For the growth of every microorganism, optimum moisture content is an essential factor same like pH and temperature. Salt content declines the moisture level of the product and creates a toxic condition for microbial populations. There is an inverse relationship between applied salt level and moisture percentage of the product. So with increasing salt level, moisture content of the product reduces rapidly and inhibits the favorable condition for microorganisms. Therefore Treatment 03 shows lowest TPC value for a period of 02 months. Total Coliform Count has increased with the time period due to the propagation of coliforms in all treatments within given period. In Sri Lanka, quality standards have not been yet recommended for fish roe based processed products. When compared with the Sri Lanka standard specification (2007) for salted, dried fish, all 03 final products of our study were always at accepted standard level (< 100TPC/ g) for the period of study.

Production cost of Treatment 03 is the highest, since this treatment requires highest salt percentage for processing. Also protein level is the lowest, while lipid content is the maximum for Treatment 03. Also consumer preference is least for all sensory qualities for Treatment 03. Although ash level is highest and microbial count is lowest for storage time period, Treatment 03 is not concerned as the best treatment. Treatment 02 requires intermediate salt amount for processing. Therefore production cost of Treatment 02 can be relatively high. As well as nutritional composition and consumer preference for Treatment 02 is not in satisfactory level, compared with Treatment 01. Treatment 02 is also not accepted as best treatment for processing of simulated caviar.

Consumer preference for all the sensory qualities (color, texture, aroma, mouth feel, overall acceptability and saltiness) is the greatest for Treatment 01 out of all treatments. Moreover nutritional composition of Treatment 01 is within acceptable level (highest protein percentage and lowest lipid content). Also production cost is the least, since Treatment 01 is required minimum salt content for processing. Therefore it can be recommended, Treatment 01 with 0.25 salt: 1.00 fish roe by weight is best treatment for processing of simulated caviar using Bigeye tuna fish roe. Bigeye tuna fish roe can be used in value addition sector of aquatic products by popularization the simulated caviar.

V. CONCLUSION

Big eye tuna fish roe is suitable for production of simulated caviar using dry salting method (0.25 salt: 1.00 fish roe by weight). This fish roe based product would be novel approach in roe based value addition sector. This product can popularize among coastal, rural and urban communities as a nutritional rich product with low production cost. Further experiments are needed to determine the shelf life of the products and enhance the product quality.

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