

Studies on Preparation of Functional Lipid and Micronutrient Enriched Bhola Bhetki Fish (*Nibea Soldado*) Spread

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Abstract

Fish spread products known as surimi products are regularly consumed as food products and are made from underutilized fish species like tilapia, atlantic menhaden, catfish, mackerel etc. Most of these fish spreads do not contain functional lipid or micronutrients like oryzanol, squalene and lignans. There is therefore need for proper selection of functional food materials and for developing process steps for making such nutritive spreads. The main objective is to produce nutritionally enriched Bhola bhetki (*Nibea soldado*) fish spread products by adding deoiled soya flour that contains good amount of protein, fiber, and phytonutrients like isoflavone. The objective of the study is also to use blended vegetable oils as the Bhola bhetki fish contains negligible amount of fat. Rice bran, sesame and soya bean oils are used in a varied proportion so as to obtain more linoleic and linolenic along with lignans like sesamol and sesamolone (in case of sesame oil) and oryzanol (in case of rice bran oil) and alfa linolenic acid (ALnA) from soyabean oil along with various tocopherols and tocotrienols.

Nadia Bhola bhetki fish was filleted and dipped in sodium tripolyphosphate solution. The fillets were finely minced and marinated with vinegar, salt and black pepper. The fish minces were then centrifuged at 5000rpm for 20 minutes. Then the supernatant rich with lipid layer was discarded and the sediment was taken and autoclaved at 121°C and pressure 15lb/sq" for specific time limits. Then cholesterol free egg lecithin, sodium benzoate, citric acid, sorbitol, monoglyceride and soluble starch were added and homogenized. Fortification and enrichment of the spread was done by adding blend of rice bran oil, soya bean oil, sesame oil, EPA and DHA through fish oil, vitamin E capsules and Soya Deoiled Cake (soya DOC).

Several chemical analyses of the fish spread products were investigated for determination of the contents of protein, carbohydrate, lipid, ash, fatty acid composition, oryzanol and isoflavone. Microbial analysis i.e. Total plate count was done for food safety. Tests for *E.coli*, *Salmonella sp* and *Staphylococcus sp* were also performed. Reduction of fishy odour of the fortified fish spreads due to autoclaving was noted. The composition data indicated that both protein and fiber increased from 15.5% to 22.4% and 2% to 3.8% respectively. Oryzanol content in fish spreads by weight was 0.1% to 0.4%. Fortified products showed absorbance and colour reaction for isoflavone detection. The spreads enriched with only soya bean oil contained moderate amount of palmitic acid, very high amount of linoleic acid (46.2%) and low amount of linolenic acid and stearic acid where as spreads containing blend of sesame, rice bran and soya bean oil were highly rich in oleic and linoleic acids. The EPA and DHA contents were as high as 2.8% and 9.2%. The Total Plate Count was within the acceptable limit. All the products gave negative results for *E.coli*, *Salmonella sp* and *Staphylococcus sp*. Fish spreads containing macronutrients like carbohydrate, protein, lipid and micronutrients such as antioxidants like oryzanol, squalene, β -sitosterol and lignans namely sesamol and sesamolone can be prepared from Bhola bhetki (*Nibea soldado*) fish by the above outlined process. The nutritionally enriched fish spreads can be utilized in various forms. They can be baked into fish cakes, fish balls, imitation crab meat analogues. The fish spread products can also be extruded.

Keywords: *Nibea soldado*; Spread; Functional lipids; Micronutrients

Abbreviations: SoyDOC: Soybean deoiled cake; RBO: Rice bran oil; SMO: Sesame oil; SBO: Soybean oil; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid; TPC: Total plate count

Background

The fish paste products or fish based spreads generally resembling surimi. These types of products are regarded as a kind of protein rich food. Generally such products are made from the fish species that are relatively less known or unutilized. Some of the fish species used to make surimi products are, big eye snapper, tilapia, alaskan pollock, cod, atlantic croaker, atlantic menhaden, catfish, mackerel [1-8].

The technology of production of fish spread products comprises a number of steps in sequence; at first fishes are minced and washed with cold water (5°C) and screened and dehydrated and cryoprotectants are added and frozen into blocks [9].

Further development includes the preparation of fish paste products from fish protein isolates [10].

In previous reviews it has been observed that food technologists prepared surimi by washing fish mince with ice-cold water for 25 minutes for four times every time changing the water.

Again washing is also done with 0.2% salt solution for two to three times. Then fillet mince was filtered and dehydration was realized in a cheese cloth by using wood press for 30min. By adding 4% sugar, 0.2% sodium polyphosphate, 2.5% salt, the dehydrated surimi was mixed for

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five minutes by a mixer and homogenized and stored in a freezer at -35°C [11].

Surimi is also prepared by adding ground ice to big eye snapper fish species [12].

The fillets were minced and used for surimi preparation as described by Benjakul, et al. Big eye snapper surimi stored at -18°C was thawed to obtain the core temperature of -21°C and used for Som-fug production following the method of Riebroy et al. [13].

Surimi was mixed with minced garlic, ground steamed rice, and salt for 15 min using a mixer (Model EC-20 Crypto Peerless, Birmingham, England). Different Lactic Acid Bacteria (LAB) starters at varying levels involving *L. plant arum* at 104CFU/g (LP104) and 106CFU/g (LP106), *P. acidilactici* at 104CFU/g (PA104) and 106CFU/g (PA106), and *P. pentosaceus* BT520 at 104CFU/g (PP104) and 106CFU/g (PP106) were added into the mixture and mixed thoroughly for 5 min. The mixture containing no LAB starters was used as the control. The mixture referred to as 'Som-fug raw mix' was then stuffed into a polyethylene casing with a diameter of 2.0 cm. Both ends were sealed tightly with rubber bands and the samples were incubated at 30°C in an incubator (Mammert BE400, Schwabach, Germany). The fermentation was conducted until the pH of Som-fug reached 4.60. Fermentation in surimi preparation is also carried out by varying slat and ginger composition in some cases [14].

Some times surimi products are used as gel like substance which can be prepared from more than two types of fish minced muscles [15].

Objective

Bhola bhetki (*Nibea soldado*) is an edible salt water fish in West Bengal, India. The fish contains about 18% protein, 0.03% total fat, Vitamin A 47.4IU, Niacin is also present but in negligible amount, 11-14mg Calcium and 0.28mg iron and 44mg sodium. The main objective is to produce nutritionally enriched fish based spread products which can be beneficial to school going children and can be affordable to all class of people. This type of product can be fortified by adding soy protein; that contains good amount of protein, fiber, essential fatty acids, antioxidants, and also phytonutrients like isoflavone. The objective of the study is also to use blended oils as the bhola bhetki fish contains negligible amount of fat (in this context fatty acid profile is studied) [16]. Rice bran, sesame and soy bean oils are used in a varied proportion so as to obtain more linoleic and linolenic along with lignans like sesamol and sesamoline (in case of sesame oil) and oryzanol (in case of rice bran oil) and ALnA from soybean oil along with various tocopherols and tocotrienols.

In this paper an approach is made with Bhola bhetki fish which is so far unused to process the production of fish paste like products. Further some fortification of products is also done.

Methods

Materials

Raw material: Nadia Bhola Bhetki fish is brought from local market.

Other ingredients: Cholesterol free Egg lecithin (extracted from raw egg), Refined Soybean oil and Refined Sesame oil were bought from local market, refined rice bran oil (supplied by Sethia Oil mill, Burdwan, W.B, India), fish oil (containing EPA and DHA from Maxepa (E.Mark))

Chemicals: Cornstarch, sorbitol, soluble starch, and Vitamin E

are E-Merck Products Vinegar (4% acetic acid), sodium benzoate and citric acid are provided by Kundu chemicals, Kolkata. Monoglycerides provided by Oil Technology Division, Chemical Technology Department Calcutta University), Salt (2%), Black pepper are purchased from local market.

Procedures

Production of the fish fillets: Nadia bhola bhetki fish is taken. Then it is descaled and deboned and washed for three or more times in cold water. Then the filleting is done from the dorsal side. These fillets are then dipped in sodium tripolyphosphate solution.

Preparation of fish mince: The fillets are finely minced and then marinated with vinegar. The marinated fillets are then centrifuged at 5000rpm for 20 to 25 minutes. Then the upper supernatant rich with lipid layer is discarded and the sediment is taken for autoclaving for reducing fishy odour and lowering microbial infestation.

Preparation of cholesterol free lecithin from egg yolk: The egg yolk is separated from the whole eggs and then weighed and extracted with the mixture of chloroform and methanol (2:1) for 2 hours. Then the extract is set aside until the sediments settled down and a clear solution is seen on the upper surface. The clear solution is first decanted followed by filtration using Whattmann No.1 filter paper. The filtrate obtained is next distilled to separate the solvents used. The residue obtained after solvent removal is washed 3 times with acetone to remove cholesterol and other lipids [17]. The cholesterol -free lecithin obtained as insoluble mass finally is used as emulsifier in the fish paste based spread.

Preparation of fish paste based spread: The fish fillets autoclaved to reduce odour are taken, mixed with cholesterol free lecithin as isolated from egg lipid, sodium benzoate, citric acid and sorbitol and passed through a blender and finally homogenized in a homogenizer.

Process of preparation of fish spread with Rice bran oil, Soy bean Oil, Sesame oil, EPA-DHA rich fish oil, Vitamin E and Soy Deoiled Cake: Bhola Bhetki fish contains only 0.03% oil. Fortification and enrichment of the spread is done by adding blend of Rice bran oil and sesame oil. From these blended oils we get good amount of linoleic acid along with some antioxidants like tocoferols, tocotrienols, squalene and oryzanol from rice bran oil and lignans like sesamol and sesamoline from sesame oils. The type of fish fillets also lack in EPA and DHA in them as most of these acids are present in organs like eye and digestive tract of the fish [16]. Thus EPA and DHA through fish oil are added which are good for brain and eye development. As it is discussed earlier that Vit A and minute quantities of Niacin are only present, vitamin E capsules are added. Then another type of fish spread is made by enriching it with Soybean Deoiled Cake (soy DOC) which is mixed with fish in varying ratio [fish fillets: Soy DOC= 60:40, fish fillets: SoyDOC=80:20 and fish fillets: Soy DOC= 20:80]. The SoyDOC rich fish paste is designed for more protein and fiber content and also for phytonutrients like isoflavone to the spread.

Chemical analysis: Several chemical analyses of the fish spread products developed were investigated that included protein, carbohydrate, lipid, solid content, total soluble solid, fatty acid profile, fiber, oryzanol, isoflavone content and ash. Protein was determined by Lowry's Method. (Lowry et al. (1951)). The carbohydrate content was determined by Anthrone's method. Lipid was extracted by Soxhlet method. Ash for each treatment was determined by placing the residue from moisture determination in a muffle furnace.

At 600°C for 8hrs till the material became white. Fatty acid composition was determined by Gas Liquid Chromatography. Estimation of oryzanol was done by the method of Khattoon et al. by measuring absorbance at 315nm of the lipid solution in spectroscopic n-hexane. Isoflavone was analyzed by extraction with 70% aqueous alcohol and then measuring by absorbance at 350 nm in spectrophotometer.

Microbiological analysis: Fish spreads were analyzed for microbial population. Serial dilutions of the samples were used for microbial enumeration with the following media : Standard plate count agar for Total Plate Count (TPC) and Baird Parker agar medium for *Staphylococcus aureus*, Tergitol -7 or Sodium heptadecyl sulphate agar for E.coli and Lactose broth, Selenine Cysteine Broth, Tetra thionate broth, Rappaport Vasilidia , HEA , XLDA, BSA , LIA and TSI (agar) for *Salmonella sp.* Portions (0.1 ml) of appropriate dilution were spread plated in triplicate. Counts on Standard plate count agar, were obtained after incubation for 48 h at 25°C, while Baird Parker agar plates were inoculated aerobically at 37°C for 24 h. Results were calculated as a mean of three determinations. Similarly Tergitol -7 agar plate inoculated aerobically at 37°C for 24 h. Again a mean of three determinations was considered. There is a vast process in case of *Salmonella sp.* At first pre - enrichment is done on a non selective media like Lactose broth. *Salmonella sp* to be analyzed is transferred in the broth and incubated for 24 hrs at 37°C . Then selective enrichment is done by transferring a loopful of the inoculums into Selenine Cysteine, Tetra thionate broth and Rappaport Vasilidia media. Further incubation is done in each case for 24 h at 37°C. The plates are examined for suspected species. Then a loopful of the suspected species is then transferred from each

inoculums to BSA and further incubated for 24 hrs at 37°C . Then for confirmation the suspected species are again transferred to LIA and TSI agar slant and further incubated for 24 hrs at 37°C and examined.

Quality assessment test: To assess the quality of the products a quality taste panel was prepared so as to determine appearance, rancidity, flavor, general acceptability and texture. Each score indicates the quality of the products in Table 1.

Results

The results include the effects of deodorization on the odour of the products, proximate composition of products, detection of isoflavone and quantification of oryzanol, fatty acid profiles of the lipid part, sensory evaluation and microbiological count of the products prepared.

The effect of deodorization on the odour characteristics of the products under the condition investigated is tabulated in Table 2.

Product 1 containing only Soybean Oil (SBO) made of Fish mince which is autoclaved for 3 min.

Product 2 containing blend of Soybean Oil (SBO) and Rice Bran Oil (RBO) made of Fish mince which is autoclaved for 3 min.

Product 3 containing Rice Bran Oil (RBO) only made from Fish mince which is autoclaved for 5 min.

Product 4 containing Rice Bran Oil (RBO), Sesame Oil (SMO), soybean oil, and EPA and vitamin E capsules is made from Fish mince which is autoclaved for 7 min.

Product 5 containing Rice Bran Oil (RBO), Sesame Oil (SMO),

| Factor | Score 1 | Score 2 | Score 3 | Score 4 | Score 5 |
|-----------------------|--------------|-----------------|----------------------|---------------------|-----------|
| Appearance | unacceptable | fair | medium | Good | Very good |
| Rancidity | extremely | highly | medium | Slightly detectable | no |
| Flavor | undesirable | extremely fishy | slightly undesirable | Good | excellent |
| General Acceptability | Very bad | Bad | Fair | Fair | Excellent |
| Texture | Not good | average | appropriate | Good | Excellent |

Table 1: Quality Assessment score sheet used by taste panel.

| Sl.no and products | time of deodorization | temperature and pressure | effect on odour |
|------------------------------|-----------------------|--------------------------|-----------------|
| 1) Fish mince Product 1 | 3mins | 121°C at 15lb/sq" | Extremely fishy |
| 2) Fish mince Product 2 | 5 mins | 121°C at 15lb/sq" | slight fishy |
| 3) Fish mince Product 3 | 7 mins | 121°C at 15lb/sq" | slightest tinge |
| 4) Fish mince Products 4,5,6 | 7 mins | 121°C at 15lb/sq" | slightest tinge |

Table 2: Deodorization effect on products.

| Compositions (% w/w) of components of the products | Spread using Soybean Oil only | Spread using blend of Sesame Oil & Rice bran oil | Spread using Rice bran oil only | Spread using Sesame Oil & Rice bran Soybean Oil ,and fish oil to provide EPA &DHA, and vitamin E | Spread using Sesame Oil, Rice bran oil, and EPA & DHA and vitamin E | |
|--|-------------------------------|--|---------------------------------|--|---|-------------------------------|
| | | | | | fish fillets: Soya DOC= 60:40 | fish fillets: Soya DOC= 80:20 |
| Protein | 15.5 | 15.5 | 15.5 | 15.5 | 22.3 | 21.4 |
| Carbohydrate | 5.8 | 5.8 | 5.8 | 5.8 | 14.45 | 11.7 |
| Total Fat | 30 | 33 | 30 | 32 | 25 | 28 |
| Moisture | 32.6 | 31.9 | 30.6 | 28.6 | 28.6 | 29.8 |
| Ash | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |
| Fiber | 2 | 2 | 2 | 2.4 | 3.8 | 2.9 |
| Oryzanol | - | 0.48% | 0.8% | 0.18* | 0.11* | 0.15* |
| Other additives(black pepper, salt) | 10.6 | 7.82 | 11.8 | 12.02 | 2.09 | 2.7 |

Values are mean±SD, n=5

*Evaluated on the basis of oryzanol content of Rice bran oil (0.8%)

Table 3: Proximate composition of paste products analyzed :(per100gm of product).

soybean oil, and EPA and DHA and vitamin E Capsules and Soya Deoiled Cake (DOC) [fish fillets: Soya DOC= 60:40] made from Fish mince which is autoclaved for 7 min.

Product 6 containing Rice Bran Oil (RBO), Sesame Oil (SMO), soybean oil, and EPA and DHA and vitamin E Capsules and Soya Deoiled Cake (DOC) [fish fillets: Soya DOC= 80:20] made from Fish mince which is autoclaved for 7 min.

Proximate composition of products (protein, carbohydrate, total fat, moisture, ash, fiber, oryzanol) is in the Table 3 below.

Isoflavone detection by UV absorbance and colour reaction

The use of soy DOC is expected to incorporate isoflavone in products and the tests performed are shown in the Tables 4 and 5.

Isoflavones are detected by reaction with alkali or acid as indicated below in Table 5.

Fatty acid profiles (composition) occurring in various fish-based spreads as determined by GLC analysis of the total fatty acids of the lipids as their methyl esters are shown in Table 6.

SBO- Soy bean oil, RBO-Rice bran oil, SMO-Sesame oil, DHA-Docosahexaenoic acid, EPA-Eicosapentaenoic acid and Soy DOC-Soya Deoiled cake.

From the above Table 6, it is clearly noted that spread containing having only Soya bean Oil contains moderate amount of Palmitic acid, very high amount of linoleic acid (46.2%) and low amount of Linolenic acid and Stearic acid where as spreads containing blend of SMO and RBO, RBO and SBO are highly rich in oleic and linoleic acids. The spreads are also fortified with EPA and DHA along with the blend of

other vegetable oils, and they contain EPA as high as 2.8% and DHA as high as 9.2%.

It is observed from the Table 7 that the spread which is prepared from undeodorized fish fillets containing only soybean oil is not at all acceptable whereas product 5 which contains 40% soya DOC is much acceptable while the other 4 products also can be accepted as spreads for consumption.

For TPC dilution factor of 10^{-6} dilution is chosen as the plate count colonies is within 30-300 which the palatable range for food products is.

TPC* (Total Plate Count) = (No. of colonies x dilution factor)/weight of sample cfu/gm.

The above table indicates that 10^{-2} dilution is chosen as in case of *E.coli* detection for Bhola bhetki fish industrial microbiologists do suggest choosing that dilution factor (Ref.IFB manual).

Discussions

The paper reports on the development of nutritionally superior quality fish based spreads. Some new approaches are made like the minimization of strong fishy odour of the products so as to make it acceptable, by deodorization by autoclaving for varying the time duration. It is observed that odour can be minimized by extending the deodorization to a certain length of time.

These very spreads contain beneficial nutrients and the microbiological limit is within limit (as it is in between 30-300cfu/gm).

Bhola bhetki fish contains minute amount of DHA in fish muscle whereas it contains large amount of EPA. DHA content is much high in

| Products | 250nm(O.D) | 350nm(O.D) | Result |
|--|------------|------------|---|
| Products using blended oils &Soya Deoiled Cake(DOC)[fish fillets: Soya DOC= 60:40] | 0.22 | 0.11 | Absorbance detects presence of isoflavone |

Table 4: U.V Spectrophotometric reading for detection of Isoflavone.

| Sample | Sodium hydroxide | Sulphuric acids | Result |
|--|------------------|-----------------|------------------------------------|
| Products using blended oils &Soya Deoiled Cake(DOC)[fish fillets: Soya DOC= 60:40] | Yellow to orange | Yellow to red | Presence of isoflavone is detected |

Table 5: Colour reaction for detection of Isoflavone.

| Spread with SBO | Spread with RBO | Spread with RBO+SBO | Spread with SMO | Spread with RBO+SMO | Spread +EPA +DHA (RBO+ SMO) | Spread +EPA+DHA +RBO+SMO+ 40% soy DOC | Spread +EPA+DHA +RBO+SMO +20% soy DOC |
|-----------------|-----------------|---------------------|-----------------|---------------------|-----------------------------|---------------------------------------|---------------------------------------|
| - | - | - | - | 0.2 | - | - | - |
| 13.8 | 18.5 | 15.8 | 8.3 | 13.0 | 12.5 | 16.0 | 13.2 |
| - | - | - | - | - | 7.6 | 7.6 | 1.7 |
| 5.2 | 3.4 | 4.2 | 2.7 | - | 2.3 | 3.3 | 3.3 |
| 29.1 | 36.1 | 43.1 | - | - | 34.67 | 32.67 | 28.67 |

| Fatty acids (%) (w/w) | Spread with SBO | Spread with RBO | Spread with RBO+SBO | Spread with SMO | Spread with RBO+SMO | Spread +EPA +DHA +RBO +SMO | Spread +EPA +DHA +RBO +SMO +40% Soya DOC | Spread +EPA +DHA +RBO +SMO +20% Soya DOC |
|-----------------------|-----------------|-----------------|---------------------|-----------------|---------------------|----------------------------|--|--|
| C 18:2 Linoleic | 46.2 | 34.1 | 40.1 | 42.3 | 31.8 | 0.8 | 0.37 | 0.33 |
| C18:3 Linolenic | 5.5 | 0.9 | 3.2 | 0.3 | 0.8 | 1.9 | 17.7 | 17.7 |
| C20:0 | - | - | - | 0.6 | - | - | - | - |
| C20:5 EPA | - | - | - | - | - | 2.2 | 2.5 | 2.5 |
| C22:6 DHA | - | - | - | - | - | 9.2 | 7.5 | 0.5 |

Table 6: Fatty acid composition (%w/w) of the total lipid material of Fish-based spread products.

| Sensory Evaluation | Product using SBO only | Product using blend of SBO and RBO | Product using (RBO) only | Product using (RBO) (SMO), SBO, EPA and DHA vit E capsules | Products using blended oils & Soya Deoiled Cake(DOC) [fish filllets: Soya DOC] | |
|-----------------------|------------------------|------------------------------------|--------------------------|--|--|-------|
| | | | | | 60:40 | 80:20 |
| Parameters | score | score | score | score | score | score |
| Odour | 1 | 2 | 3 | 4 | 5 | 4 |
| Texture | 1 | 2 | 5 | 5 | 4 | 5 |
| Taste | 1 | 4 | 5 | 3 | 5 | 4 |
| Spread ability | 1 | 3 | 4 | 4 | 4 | 5 |
| Rancidity | 1 | 2 | 3 | 4 | 5 | 4 |
| General Acceptability | 1 | 2 | 3 | 4 | 5 | 4 |

Table 7: Evaluations duly assessed have been recorded by those who tasted the products. (Table.7 Sensory evaluation scores of the spread products).

| Sl.no. | Tube dilution | Volume dilution | No. of colonies | Bacterial count TPC* |
|----------|------------------|-----------------|-----------------|----------------------|
| Product1 | 10 ⁻⁶ | 1ml | 80 | 0.000080 |
| Product2 | 10 ⁻⁶ | 1ml | 40 | 0.000040 |
| Product3 | 10 ⁻⁶ | 1ml | 20 | 0.000020 |
| Product4 | 10 ⁻⁶ | 1ml | 18 | 0.000018 |
| Product5 | 10 ⁻⁶ | 1ml | 12 | 0.000012 |
| Product6 | 10 ⁻⁶ | 1ml | 13 | 0.000013 |

Table 8: Total Plate Count of the Fish Based spread products.

| Sl.no. | T-7 Agar plates | Observations | Result |
|-----------|------------------|--|----------------------|
| Product 1 | 10 ⁻² | No yellowish tinge and no large mucoid colonies observed | <i>E.coli</i> absent |
| Product 2 | 10 ⁻² | No yellowish tinge and no large mucoid colonies observed | <i>E.coli</i> absent |
| Product 3 | 10 ⁻² | No yellowish tinge and no large mucoid colonies observed | <i>E.coli</i> absent |
| Product 4 | 10 ⁻² | No yellowish tinge and no large mucoid colonies observed | <i>E.coli</i> absent |
| Product 5 | 10 ⁻² | No yellowish tinge and no large mucoid colonies observed | <i>E.coli</i> absent |
| Product 6 | 10 ⁻² | No yellowish tinge and no large mucoid colonies observed | <i>E.coli</i> absent |

Table 9: Results of the analytical tests for Bacteria *E.coli*.

| Sl.no | Vol of inoculated lactose broth | BSA agar plate | Result |
|-----------|---------------------------------|-------------------|------------------------------|
| Product 1 | 1ml | No brown colonies | <i>Salmonella sp.</i> absent |
| Product 2 | 1ml | No brown colonies | <i>Salmonella sp.</i> absent |
| Product 3 | 1ml | No brown colonies | <i>Salmonella sp.</i> absent |
| Product 4 | 1ml | No brown colonies | <i>Salmonella sp.</i> absent |
| Product 5 | 1ml | No brown colonies | <i>Salmonella sp.</i> absent |
| Product 6 | 1ml | No brown colonies | <i>Salmonella sp.</i> absent |

Table 10: Analytical tests for *Salmonella sp.*

brain, eyes and liver which are discarded thus fortification is necessary and done by adding them through fish oil so that proportionate amount is retained in the paste products. Bhola bhetki of fish has of low lipid content and thus additions of blended oils highly enhance the nutritional property of the final paste products.

Due to the presence of micronutrients and essential fatty acids these types of spread products can serve as a whole meal for children providing expected developing beneficial effects in their brain and eye. Due to the use of RBO and the blended oils the products can be regarded as beneficial to the elderly people also for providing squalene, oryzanol and lignans that are known for their free radicals scavenging activity and thereby reducing free radicals initiated diseases and adverse health effects.

Fish spreads with macronutrients like carbohydrate, protein and lipid and micronutrients such as antioxidants like oryzanol, squalene and β -sitosterol from rice bran oil and lignans namely sesamol and sesamolone from sesame oil are also unique. Some of the products contain fiber certain vitamins and minerals that can serve as ideal meal. The products are highly proteinaceous especially when soya DOC is added, that increases the protein content to 27.3% from 15.5% and is

also calorie rich. Though high calorie the products impart essential fatty acids due to the use of blended oils. Since soya deoiled cake is used the spread also is expected to contain isoflavones.

The cholesterol free egg lecithin used as emulsifier makes the products also safe for those who have cholesterol problem.

The fish paste spreads can be utilized in various forms. It can be baked into fish cakes, fish balls, imitation crab meat analogues. The fish paste products can also be extruded [18].

Conclusion

From the paper a conclusion can be drawn that Bhola Bhetki fish can be utilized in making nutritionally enriched ready to eat fish spreads containing macronutrients like carbohydrate, protein, lipid and micronutrients such as antioxidants like oryzanol, squalene, β -sitosterol and lignans namely sesamol and sesamolone can be prepared from Bhola bhetki (*Nibeas soldado*) fish by the above outlined process. The nutritionally enriched fish spreads can be utilized in various forms. They can be baked into fish cakes, fish balls, imitation crab meat analogues. The fish spread products can also be extruded.

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