

Development of High Nutritious Fish Powder Mixture Using *Chirocentrus dorab*, *Selaroides lepotolepis* and *Stolephorus indicus* for Bakery Industry

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ABSTRACT

The research was aimed to develop a highly nutritious fish powder mixture by incorporating dried fish powder of small fish, *Chirocentrus dorab* (Katuwalla), *Selaroides lepotolepis* (Suraparawa) and *Stolephorus indicus* (Hadella) to enhance the nutritional value. Raw fish was collected from the fishing area of Chillaw and Galle in Sri Lanka. The developed fish powder mixture is a potential ingredient to enrich the nutritional value of wheat flour mixtures or dough prepared in the bakery industry conveniently. The fish powder mixture formulae were developed by mixing with different ratios of selected small dry fish powder. The best fish powder formulation was selected using sensory evaluation conducted using fish powder incorporated wheat flour buns. Further, in order to enhance the odor of fish powder mixture, natural ingredients such as clove, almond essence and vanilla were separately incorporated into the selected fish powder formulation and they were evaluated by a

sensory panel. Of them, clove was selected as the best odor enhancer by the panelists. The proximate composition of selected fish powder showed 5.98% of moisture, 4.44% of ash, 6.98% of fat and 44.25% of protein, 9.23% of crude fiber and 29.16% of carbohydrates, respectively. In microbiological tests, no colonies of *E. coli* and *Staphylococcus aureus* were detected in fish powder. Further, to verify the shelf life of fish powder, Aerobic Plate Count was performed at ambient temperature and without adding any natural or artificial preservatives into fish powder. The shelf life of fish powder was three weeks at ambient temperature. In conclusion, the developed nutritious fish powder has high potential as a low cost, convenient ingredient to enhance the protein content in bakery products. Further studies on extended shelf life determination by adding natural preservatives could be recommended.

Keywords: *Chirocentrus dorab*, Fish powder, *Selaroides lepotolepis*, Shelf life, *Stolephorus indicus*

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INTRODUCTION

Economically developing countries like Sri Lanka has a long need for inexpensive sources of animal protein for human consumption as a help to prevent malnutrition (Abbey, 2017). The use of high protein fish offers a possible way of alleviating this problem. Many people are unable to afford for seafood products, especially in the areas where the seafood is not available. Therefore, as a solution to the economic and logistic challenges, increasing small fish consumption among

the poor will be essential and this product can be introduced to stimulate the demand.

In this study, three varieties of small fish were selected based on the high nutritious value according to a previous study conducted by Edirisinghe *et al.* (2000). The study exemplifies that the protein content of *Chirocentrus dorab* (Katuwalla), *Selaroides lepotolepis* (Suraparawa) and *Stolephorus indicus* (Hadella) are respectively, 17.6%, 15.7 % and 15.9% among many other small fish varieties in Sri Lanka. These small fish are abundant in coastal fishing areas, however, people have lesser consumption compared to the other fish species. The Food and Agriculture Organization of the United Nations has approved a high-quality fish powder product, which can be incorporated in quick bread, cakes, cookies, cereals, pastries, and baby foods without lowering the appealing of these foods to the consumer.

Therefore, in order to minimize the gap between the consumption of high nutritious small fish among the Sri Lankan community a fish powder mix incorporated with wheat flour was developed and evaluated for the application to the bakery industry, which was the main objective of the study.

MATERIALS AND METHODS

Sample Preparation

The selected fish varieties were collected from fish landing sites in Chillaw and Galle,

Sri Lanka and they were transported to the laboratory of Horizon Campus, Malabe, Sri Lanka in ice boxes. The fish were then washed, degutted, and de-headed, and the cleaned fish were dipped in pure lime extract for 10 minutes and arranged on perforated trays to oven-dry under 60 °C for one hour and 45 minutes separately for each fish type. Once the samples were dried, those were ground and sieved with a mesh sieve. The dried fish samples were measured and stored in airtight plastic bottles until next use under the room temperature.

Formulating Fish Power

A 50g of each fish type was collected and mixed with wheat flour with different ratios to make buns as shown in Table 1.

Table 1: Ratios of mixing fish powder and wheat flour for making buns

Fish flour (g)	Wheat flour (g)	Treatment no
10	20	T ₁
15	15	T ₂
20	10	T ₃

Separately, 5g of yeast was dissolved in 15ml of hot water and 10g of yeast was added and mixed thoroughly and allowed to react for 10 minutes and one-third of the yeast mixture was added to three batches of flour separately. A 3g of salt and 8g of margarine were also added to the mixture. Samples were mixed gently using clean

gloved hands and the dough was kept to rise for about 45 minutes. Then, approximately 10g of dough was taken and shaped to round and placed on the tray. This was then baked at 180°C for 25 minutes in a preheated electric oven.

A sensory evaluation was conducted with 30 untrained panelists. Further, the results of the five points hedonic scale sensory evaluation for overall color texture, fishy odor, taste and after taste of fish powder buns were analyzed by the Kruskal-Wallis nonparametric method using MINITAB statistical software version 18.1. As per the results, the best fish powder formula was selected for further development of appealing odor.

Formulating and Selecting Odor Enhancer

In order to develop an appealing odor to the powder mixture, three enhancers such as vanilla, cloves and almond essence were used. The batches were prepared according to the below table (Table2) using the best sample from the fish powder mixture in Table 1. A sensory evaluation was conducted with 30 untrained panelists using the same statistical method described

above. As per the results, the best odor enhanced fish powder formula was selected for the analysis.

Nutritional Analysis

The determination of moisture content was done according to the oven drying method of ISO 712:2009 (Sri Lanka Standards, 2009). Total ash content was determined using ISO 2171:2007 (Sri Lanka Standards, 2007) method. Crude fat content, crude protein content and crude fiber content were determined according to AOAC (2000) method. Further, the carbohydrate content was determined according to the Nitrogen free method described by AOAC (2000).

Calorific Value

The caloric value (CV) was calculated in kilocalories per 100g (kcal/100g) by multiplying the energy values for each nutrient such as crude fat, protein and carbohydrate values as follows, where, % CV = (% Protein × 4.36) + (% Lipid × 9.02) + (% Carbohydrate × 3.68) (USDA, 2019).

Table 2: Ratios of mixing selected fish powder (T₁) with odor enhancers for making buns

Fish powder (g)	Wheat Flour (g)	Enhancer	Quantity (ml)	Treatment No
10	20	Cloves	5	T ₄
10	20	Vanilla	5	T ₅
10	20	Almond essence	5	T ₆

Evaluation of Microbial Stability of the Fish Powder Mixture

Total Plate Count and Enumeration of Yeast and Mold

The samples were prepared according to the SLS 516:2013 standards (Sri Lanka Standards, 2013). The total plate count was determined according to the method described by SLS 516: 2013 Part 1 and Part 2 and the same method was used to determine the enumeration of yeast and mold. This was also used for the analysis of shelf life and was performed in two weeks' time intervals for 4 weeks and results were compared with SLS standards. Further, Enumeration of *Staphylococcus aureus* was performed according to SLS 516: 2013 Part 6 and detection and enumeration of coliform, faecal coliform and *Escherichia*

coli were done according to SLS 516: 2013 Part 3 (Sri Lanka Standards, 2013).

RESULTS

Selection of the Most Preferred Fish Powder Formula by Sensory Evaluation

The null hypothesis stated that the medians of all groups were equal whereas, the alternative hypothesis stated that at least one group was different from the population median. The comparison of H_{stat} and H_{table} values for sensory attributes showed that only texture was significantly different among the treatments and other sensory attributes had no significant differences among three different batches, T₁, T₂ and T₃. According to the average ranks of each sensory attribute, a WEB (Radar) presentation was created (Figure 1) and the analysis showed fondness for colour,

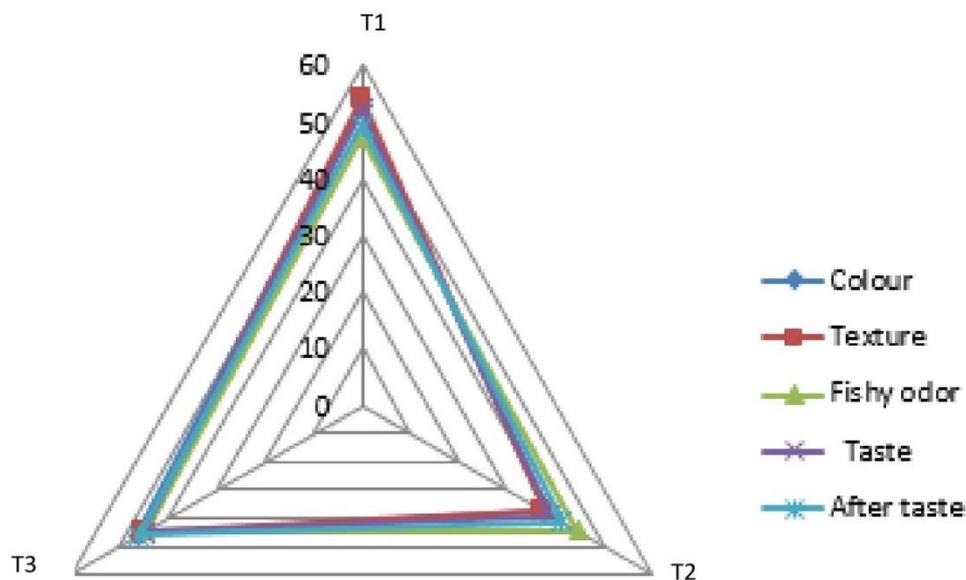


Figure 1: Radar chart for sensory attributes of each treatment with fish powder formula

texture, taste and after taste were relatively higher and fishy odour was relatively lower in sample T₁. Therefore, sample T₁ was selected as the most preferable fish powder formula and used for further development.

Selection of the Most Appealing Fish Powder Formula with Enhanced Odor by Sensory Evaluation

According to the analysis of the comparison between H_{stat} and H_{table} values for each sensory attribute, only mouth feels and overall likeness were significantly different ($p < 0.05$) and overall colour, overall flavour, overall odour and overall texture were not significantly different. According to the average ranks of each sensory attribute, a WEB (Radar) presentation was created (Figure 2) and the analysis showed

the fondness for overall flavour, overall odour, overall texture were relatively higher and overall mouthfeel and overall likeness were greater in sample T₄. Therefore, sample T₄ was selected as the most preferable fish powder formula with enhanced odour, which included clove and it was used for further nutritional and microbial analysis.

Nutritional Analysis; Proximate Composition of the Formulated Fish Powder

The observed results for the final fish powder formula (T₄) were ranged within the standard specification limits for dried fish products according to the specification for dry fish products of the SLS standards, SLS 643: 2007 (Sri Lanka Standards, 2007).

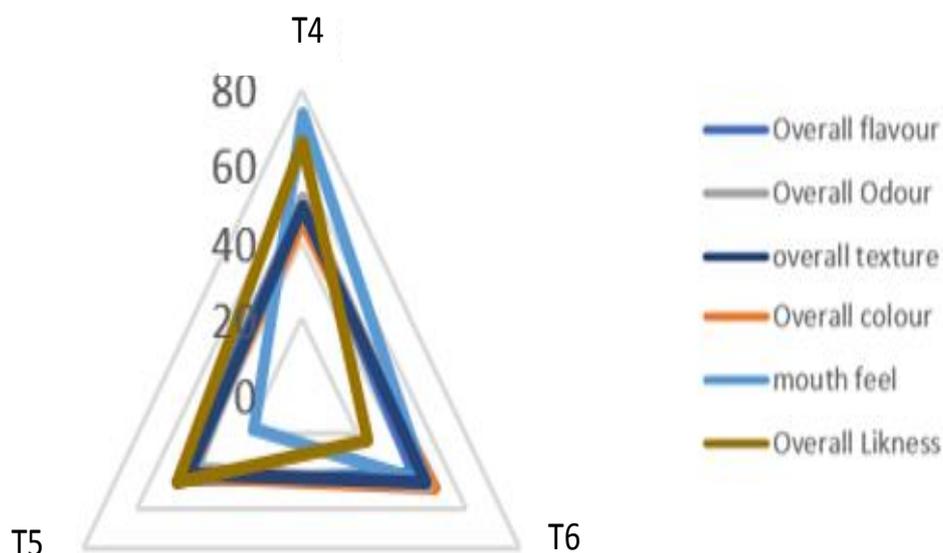


Figure 2: Radar chart for sensory attributes of each treatment with odor enhance

The proximate composition showed high content of crude protein whereas carbohydrate content was lower than protein. The calorific value was found to be 363.19 kcal/100g (Table 3).

Table 3: Proximate composition of the formulated fish powder by dry basis

Parameter	% by mass
Crude Protein,	44.25
Ash Content	4.4
Moisture	5.98
Crude Fiber	9.23
Total Fat	6.98
Carbohydrate Content	29.16

Microbial Analysis of Final Fish Powder Formula and Shelf Life Determination

The total aerobic plate count of the final fish powder was 2.0×10^5 CFU/g (Table 4). The SLS standard level for the dried fish products of total aerobic plate count was 5×10^5 CFU/g where the study result was less than the standard value. The yeast and mold value resulted in the final fish powder formula was 0.2×10^2 CFU/g (Data not shown) and according to the SLS standard SLS 643: 2007 (Sri Lanka Standards, 2007), yeast and molds should be less than 1×10^4 CFU/g. There was no detection recorded for *coliform*, *Fecal coliform*, or *Escherichia coli* and *Staphylococcus aureus*. Therefore, considering the microbial analysis results, the developed final fish powder could be accepted under the SLS standards.

The results showed that after one week, the aerobic plate count had been

reduced and then again increased after 4 weeks. According to the results after four weeks, total aerobic plate count was 5.2×10^5 and that was higher than the specified limit given by SLS 643: 2007 (Sri Lanka Standards, 2007) for dried fish products. Therefore, the developed fish product is considered safe to consume with in the first three weeks of preparation stored under room temperature.

Table 4: Change of total aerobic plate count over the weeks

Storage Period (Week)	Total aerobic plate count (CFU/g)
0	2.0×10^5
1	9.2×10^4
2	3.8×10^5
3	4.1×10^5
4	5.2×10^5

DISCUSSION

A fish powder mixture using *Chirocentrus dorab*, *Selaroides leptotelepis* and *Stolephorus indicus* was developed with further appealing odor-enhancer, vanilla. Nutritional analysis and microbial analysis were conducted to evaluate the nutritional composition and microbial parameters respectively.

The nutritional analysis is the key commercial concern as food manufacturing companies need to ensure that the product meet the appropriate nutritional composition. Moisture content is an indicator of shelf stability, thus increase

moisture content can enhance microbial growth, which leads to deterioration in foods. The moisture content of the product (5.98%) was lower than the SLS standards according to SLS 643: 2007 and low moisture content of the samples indicated the possibility of reducing the microbial growth.

Usually, 100g of wheat flour contains 13.7% protein content (Venugopal, 2006), however, the developed fish powder mixture contains 44.25% of proteins and that could be due to the appreciable amount of protein present in fish (Edirisinghe *et al.*, 2000). Therefore, the fish powder mixture can serve as an inexpensive source of protein to the consumers (Shaviklo, 2013). When the protein content is higher than 65%, fish protein powder can be classified as a high-level protein concentration (Shaviklo, 2013) and thus, the developed fish powder is considered as moderate level protein concentration. Also, in order to enhance the level of proteins, fish protein should be protected against denaturation during drying (Park and Lin, 2005). Comparatively fish protein contains a higher level of essential amino acids (Shaviklo, 2012) and particularly, they are rich in lysine and methionine (Venugopal, 2006). Therefore, the developed product is a valuable supplement to improve the protein quality and quantity of indigenous diets, such as diets of preschool children and other vulnerable groups. Such products have been used to increase the weight and height of the children (Owsu Amoako, 2001), and therefore the developed fish powder may possibly be used for treating

malnutrition. However, the effects of enriching foods with fish ingredients on sensory quality can give negative effects both on flavor and odor if they are used at inappropriate levels. Therefore, the level of enrichment should not affect the acceptance and sensory properties of the product.

Another advantage of developing fish powder is that fishery resources are limited and there is a necessity to optimize the utilization of the low-value fishes and by-products (Shaviklo, 2013). Adding value to the underutilized fish and fish by-products through developing fish powder mixture seems highly profitable (Shaviklo, 2012).

According to the sensory attributes, fishy odor is due to the dried fish odor and flavor, rancid odor and flavor, fish liver oil odor and flavor (Shaviklo, 2012). The developed fish powder contained 6.98% of fat content, and having higher fat content such as above 3% can lead to rancid odor and makes it unacceptable for use as reported by Khoshkhoo *et al.* (2012).

Further, sensory attributes of fish powder mixture are similar to dry fish (Venugopal, 2006), thus, the fish powder mixture can be successfully marketed in the areas, where fish powder from dried fish is used in tasty and spicy dishes consumed with the staple dish. Production of fish powder has two effects on food, first, it increases the content of nutrients in the diet, second, it improves the utilization of fishery resource and fish protein consumption per capita (Pauly *et al.*, 2001).

The crude fibre content of the wheat flour is 2.7 % and the developed fish powder mixture contains 9.98% of crude fibre content which is higher than wheat flour. The increased fibre content aids better bowel movement and it is necessary for foods generally as it reduces food retention time in the digestive tract (Akoja and Coker, 2018). Wheat flour contains 76% of carbohydrates, whereas, the developed fish powder mixture has 29.16% of carbohydrates. This is because fish contains more of the proteins and thus, proportionally, due to the availability of fish powder, overall carbohydrate content has been lowered. However, this is advantageous, as this can be an applicable food source for the dietary plans for lowering body weight.

The calculated calorific value for the fish powder mixture was 363.19kcal/100g. The whole grain wheat flour contains 340 kcal/100 g (Kumar *et al.*, 2011) which is slightly lower than the developed fish powder mixture. Generally, the recommended daily calorie intake is 2700 kcal per day for men and 2200 kcal per day for women, and this can vary according to the age and body weight, etc. (Brown *et al.*, 2016). It is also recommended that in order to maintain healthy body weight, individuals must be able to correctly match energy intake with energy expenditure (Brown *et al.*, 2016) and the developed fish powder mixture would be helpful in controlling daily calorie intake.

Foodborne pathogens have been extensively incriminated worldwide as

common causes of bacterial infections in humans (Pires *et al.*, 2012). Food poisoning is commonly manifested as diarrheal diseases, which are often triggered either by toxin production by the microbe or by the host's reaction to the infection. *Staphylococcus aureus* and *Escherichia coli* are important causes of enteric illness and thus known as "Hygiene indicator organisms" (Carpenter *et al.*, 1997). The main objective of using these bacteria as indicators is to reflect the hygienic quality of the fish powder mixture. *Escherichia coli* is commonly used as a faecal indicator organism and without having *Escherichia coli* indicates that there is no direct or indirect faecal contamination (Carpenter *et al.*, 1997). About 0.2×10^2 CFU/g of yeast and molds were detected in the developed fish powder and that was however, lower than the acceptable minimum count according to SLS standards. This might have occurred due to improper processing and partial dehydration of fish during drying. Therefore, the temperature can be increased by 10°C and the oven drying time can also be extended to 2 or 3 hours more. Even though the fish proteins can be denatured by exposing longer time to the higher temperature, it can be controlled by a sufficient concentration of sucrose along with a small amount of polyphosphate that can protect fish proteins from denaturation during drying and storage (Carvajal *et al.*, 2005) because of the formation of hydrogen bonds with proteins by providing a replacement for the hydration shell (Carpenter *et al.*, 1997).

The determination of shelf life using Aerobic Plate Count method is an indicator of quality assessment of food product and this method provides useful information about the general quality and shelf life of the food under the given storage conditions. Shelf life of the developed fish powder mixture was limited to 3 weeks. The moisture content of the developed fish powder was 5.98%. Moisture has a significant effect on crude protein, crude fat, mold growth and insect infestation and protein and fat can be decreased with a higher amount of moisture content (Muhammad *et al.*, 2003). Moisture content is not directly related to mold and microbial growth rates, but water activity affects the mold and microbial growth (Thomsan, 1994). Water activity is a measure of microbial susceptibility accepted by the United States Department of Agriculture and a key component in many hazard analysis and critical control point plans (Carter, 2016). Therefore, increasing the drying time can lower the water activity of the fish powder mixture, and thus extend the shelf life. There was no natural or artificial preservative added for extending the shelf life in the present product. Therefore, shelf life can also be extended using natural preservatives since the consumer preference is mostly towards natural preservatives due to safety concerns (Mei *et al.*, 2019). Bacteriocins, organic acids and plant-derived antimicrobials can extend fish shelf life and decrease lipid oxidation. Moreover, some algae and mushroom species can also provide a potential source of natural preservatives (Mei *et al.*, 2019). The plant-derived compounds could extend fish shelf

life by reducing the total aerobic plate count and they are also combined with nisin which is an antibacterial product synthesized by microbial culturing to extend the shelf life of fish and fish products (Mei *et al.* 2019). Some of the examples of plant-based preservatives are thyme essential oil, cinnamon oil, rosemary oil, oregano and rosemary extract with onion juice (Mei *et al.*, 2019). Adding plant-based natural preservatives also provides antimicrobial, antioxidant, antiviral, anti-inflammatory, and other health-promoting benefits (Mei *et al.*, 2019).

Moreover, Vilain *et al.*, (2016) suggest that beneficial micronutrients in fish are not widely available in other food items. Fish is among the best sources of micronutrients such as iron, zinc, calcium, vitamin A and iodine. Therefore, fish consumption is a better way to fill the micronutrients deficiency gap (Vilain *et al.*, 2016)

CONCLUSION

Incorporation of dried fish powder of *Chirocentrus dorab* (Katuwalla), *Selaroides lepotolepis* (Suraparawa) and *Stolephorus indicus* (Hadella) into wheat flour mixture has significantly improved the nutritional, microbial and sensorial quality of wheat flour-based bakery product. The product was developed using, two sensory evaluations conducted to select the best fish powder and wheat flour ratios and appealing odor enhancers. The developed fish powder mixture showed 3 weeks shelf stability under room temperature and it has

potential to be extended further by using natural preservatives. The level of moisture, ash, total fat, fibre, carbohydrate and protein content of the developed fish powder mixture were in the acceptable range. Microbial study showed that the microbial counts were within the accepted range and fish powder incorporated with wheat flour was safe to consume within 3 weeks of production under the storage of room temperature.

Further studies are recommended on the effects of different packaging materials, extending shelf life using natural preservatives and a better way to incorporate fish oil during the fish powder production.

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