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Development and Quality Evaluation of Fish Protein Concentrate Incorporated Value Added Products

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Abstract: **The present study entitled 'Development And Quality Evaluation Of Fish Protein Concentrate Incorporated Value Added Products' was aimed to develop and evaluate the quality and shelf life of Tilapia (*Oreochromis niloticus*) fish protein concentrate (TFPC) incorporated value added products like cookies, soup powder and flakes. TFPC was prepared by physical method and incorporated into value added products in different variation provided by adding TFPC in different proportions (3%,6%,10%) respectively. Fortification of 6% TFPC cookies, 3% TFPC soup powder and 6% TFPC flakes had the highest scores in the 9 point hedonic scale. The proximate analysis of TFPC showed a protein content of 75.02%, fat 0.018%, moisture 15.98% and ash content of 3.02%. The TFPC incorporated products were found to meet more than half of the RDA of protein and one third the RDA of Energy ,Calcium and Iron for a preschooler aged 7-9 years. The shelf life study of the products were done by biochemical and microbiological analysis. The results of biochemical test was found to have its peroxide value, total volatile base nitrogen within the accepted limits, while free fatty acid formation and trimethyl amine was above the accepted limit. Microbiological analysis by Total Plate Count resulted that both TFPC incorporated cookies and flakes were devoid of any colonies, and plate having soup powder showed few countable colonies. Therefore it can be assumed to be microbiologically safe for consumption upto two weeks. In conclusion, Tilapia Fish Protein Concentrate incorporated products can be considered as a valuable source of protein supplementation to the vulnerable population.**

Index Terms: **Tilapia fish, fish protein concentrate, sensory evaluation, nutritive evaluation, shelf life study, value added products.**

I. INTRODUCTION

Fish plays an important role in fighting hunger and malnutrition. It is not only a source of proteins and healthy fats, but also a unique source of essential nutrients, including long-chain omega-3 fatty acids, iodine, vitamin D, and calcium. An increased focus on fish and nutrition aids both developing countries and the developed world in recent years. In many developing countries, fish is the main or only source of animal protein, and is essential for providing micronutrients to vulnerable populations. (FAO, 2014). In addition aquatic protein is highly digestible and rich in several peptides and essential amino acids that are limited in terrestrial meat proteins, as for example methionine and lysine (Tilami & Sampels, 2017). But unfortunately fish comes under an underutilized animal protein because it is not economically available to all people. Also the perishable nature of product and Protein quality considered more important than protein quantity which takes into account essential amino acid composition and digestibility. In India, where rice and millet are staple foods, protein malnutrition is common among infants. . Protein deficiency can cause retarded growth and development, fatigue, and nutritional edema, and even might be life threatening. (Roy & shaikh, 2018).

India having a long coastline of about 8118 km and an economic zone of approximately 2.02 million square kilometers makes the fisheries sector an instrument of livelihood for large section of economically backward populations of the country (Economic review, 2016). So far work done in India on utilization of surplus fish has mainly been confined to production of fish meal.

Fish protein concentrate was widely publicized during the late 1960s as fix to eradicate malnutrition worldwide. (Pariser & Wallerstein,1980). Fish protein concentrate has all the characteristics of a food widely used around the world, solving problems of the use of fish and / or its waste. It is a concentrated product containing proteins (75%) with the following basic characteristics: low cost, low fat and moisture contents, deodorized, high digestibility, easy storage, does not require refrigeration and long shelf life. The large capacity of hydration and functional properties facilitate the preparation of various foods products. (Brasileiro *et al*, 2012).

II. RELEVANCE OF THE STUDY

Fish protein concentrate has all the characteristics of a food widely used around the world, solving problems of the use of fish and / or its waste. Value addition is the most promising sector in food processing industry. Value addition is an addition activity that in one way or the other changes the nature of a product thus adding to its value at the time of sale. The dual advantage of this approach is finding ways for better utilization of low valuable fish species and providing protein rich convenience foods. Hence studies conducted on incorporation of FPC in food products suitable for supplementing diets of vulnerable population like PEM children assumes significance.

Most of the preparation efforts of FPC have utilized enzymatic, chemical or solvent extraction process. However the physical method of FPC preparation which was not much utilized in previous studies was opted for the preparation to minimize chemical residues. In the present study, FPC incorporation in various food products was proposed to be done.

III. AIMS AND OBJECTIVES

The specific objectives of the present study were the following:

- To develop Fish Protein Concentrate (FPC) by physical Method.
- To standardize and develop value added products from Fish Protein Concentrate.
- To assess the sensory evaluation of the value added products.
- To determine the proximate and nutritive analysis of the FPC and value added products.
- To assess the shelf life by biochemical and microbiological analysis of the developed products .

IV. METHODOLOGY

A. Selection of fish

Two popular and economically viable fish were selected for checking the feasibility of producing the FPC, Pink Perch (*Nemipterus japonicas*) and Tilapia (*Oreochromis nicotilicus*).

Tilapia was selected for producing Fish Protein Concentrate because of its high biological value protein content (18.83%), with good amounts of essential fatty acids (IFCT,2017). It was available and economically viable . Tilapia has white flesh and bland flavour which is an important criteria for producing Fish Protein Concentrate.

B. Preparation of FPC

FPC is a dry, light coloured, tasteless, odorless, protein rich powder obtained from fish meat .

1) Physical method of FPC preparation

Fish meat is cooked to coagulate proteins and to release fat and water. The material is then repeatedly washed with freshwater to remove most fat., odorifies compounds and color pigments. The slurry is pressed to squeeze out water along with the impurities. The solid matter is finally dried to a stable level. Washings and drying help to increase protein content of the product.

C. Proximate analysis of FPC

Proximate analysis, a system for analysing forages and other feedstuffs, also referred to as the *Weende system*, utilizes wet chemistry laboratory procedures In a complete proximate evaluation, analyses are made for (1) Moisture (2) Crude protein (CP) ($N \times 6.25$); (3) Ether extract (EE) (a measure of lipids and fats); (4) Ash (the residue after burning made up of mineral content.)by AOAC(2005) procedures.

D. Value added product development

FPC was incorporated into food products in order to enrich them with proteins, without affecting the sensory quality characteristics of the product.The process of developing a value added product included the following steps:

1) Selection of products

To utilise the nutritional benefits of Fish Protein Concentrate (FPC) and products like cookies, flakes and soup powder were selected. These products were selected because of their high acceptability among consumers.

2) Study of the ingredient characteristics

- For soup powder preparation the major ingredient was corn flour along with spices like garlic powder, onion powder, and mixed herbs. Corn flour has characteristics like gluten free nature, anti-caking, thickening agent and bland flavour.
- For flakes preparation, important ingredients used were tapioca flour and corn flour in the ratio 2:1. Their favourable characteristics were bland flavour, odorless, gluten free, easy digestibility and water absorbing capacity .They also imparted crisp and crumb texture which is important for flakes. Since these flours are tasteless and odorless, other spices can be added to improve the taste.
- For preparation of cookies, all purpose flour was selected as they absorb less moisture, which improves flavour and keeping quality. As major aim of the present study was to incorporate FPC , ingredients other than sugar like vanilla extract , which could mask the fishy flavour of FPC were selected.

3) Product standardization

The different variations were tried out several times to obtain uniform yield in terms of sensory attributes. Incorporation of TFPC at 3%, 6%, 10% were done to obtain variations of cookies, soup powder and flakes. Trial and error method was used to arrive at the final recipe. Recipe for preparation of standard products (cookie, flakes, soup powder) was repeated twice to obtain the uniform yield.

E. Sensory evaluation

The organoleptic characteristics of the products (cookies, flakes and soup) viz., odour, taste, texture, appearance and overall acceptability, were assessed by a panel of 8 judges using a 9-point of hedonic scale.

F. Shelf life study of the value added products

After development of the products, the accepted proportion of the products (cookie, flakes and soup powder) was stored under room temperature and samples were tested for following:

1) Biochemical analysis

a) Peroxide value

Maximum permissible limit of peroxide value in oil/fats is specified to be 10 mEq/Kg of oil, as per FSSAI regulation.

b) Free fatty acids

The level of FFA depends on time, temperature and moisture content because the oils and fats are exposed to various environments such as storage, processing, heating or frying. The accepete limit of FFA is 3-4 (FSSAI, 2018).

c) Total volatile base nitrogen (TVB-N)

The homogenized fish muscle is extracted with trichloroacetic acid (TCA) which extracts water soluble components such as TMA and ammonia (major components of TVB-N).((Lakmanan , Mathew, Anandan, Asha, & Chatterjee, 2013)

d) Trimethyl amine

TMA also most widely used and internationally accepted chemical indices of fish quality. For the Europian economic committee (EEC) the limits suggested for TMA mg/100= 12. (Lakmanan, Mathew, Anandan, Asha, & Chatterjee, 2013). Gradual accumulation of the fishy odor is due to the compound TMA trimethyl amine from bacterial reduction of (TMAO) trimethyl amine oxide is one of the characteristic chemical changes attributed to fish spoilage.

2) Microbiological analysis

a) Total plate count

The total plate count is the enumeration of aerobic, mesophilic organisma that grow under aerobic conditions under moderate of 20^{o}-45^{o} C . All the media and the diluents used for microbiology tests were sterilized by autoclaving at a temperature of 121^{0}c for 15 min and all the glassware at 160C in hot air oven for 2 hour

V. RESULTS

A. Yield and evaluation of FPC

Fish Protein Concentrate from Tilapia (*Oreochromis niloticus*) and Pink Perch (*Nemipterus japonicas*) were prepared. FPC prepared from 1kg of pink perch resulted a yield of 100gm and had a fishy flavor and odor hence it was not accepted for incorporating into food products. While yield of FPC from Tilapia was higher than Pink Perch (ie 106gm) it had a bland taste and no odor hence could be easily incorporated into food products.

B. Proximate analysis

Table 1 : Proximate analysis of TFPC

Proximate analysis of TFPC	Contents %
Protein	75.02%
Fat	.018
Moisture	15.98
Ash	3.02

C. Sensory evaluation of value added products

The products developed were analyzed using a nine point hedonic rating test by a panel of 8 untrained judges. Different proportions of TFPC were incorporated into standardized recipe of butter cookies, soup powder and flakes. By trial and error method, the best possible proportions of TFPC incorporated product was assessed.

Table 2:Mean Sensory Score of Cookies Incorporated With TFPC

Cookies	Taste (9)	Texture	Flavour	Appearance	Overall acceptability	Mean score
Cookie (control)	8	8	8	8	8.12	8
Cookie (3%)	8	7	7	7.5	6.12	7.37
Cookie (6%)	8.5	8	8	8	8.3	8.3
Cookie (10%)	6	7	7	6	6.12	6.2

TFPC was incorporated at 3%, 6%, 10% respectively. An incorporation of 6% of TFPC was found to have the highest sensory scores. These results are in agreement with those reported by Mohammed *et al*, (2014) on Fortification of Biscuits with Fish Protein Concentrate and Evaluation of Production and Quality of Salt-Biscuits Supplemented with Fish Protein Concentrate by Ibrahim (2009).

Plate 2 :Cookies incorporated with TFPC

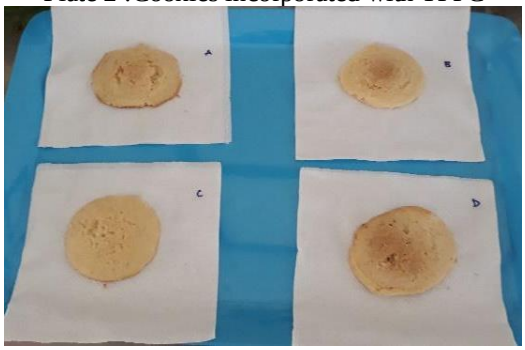


Table 3 :Mean sensory scores of TFPC incorporated soup powder

Samples	Taste	Texture	Flavour	Appearance	Overall Acceptability	Mean score
Soup powder (sp) (control)	7	7	8	8	6.2	7.5
SP(3%)	7.2	8	8	7	6.3	7.5
SP(6%)	8.2	8	9	8	8.2	8.3
SP(10%)	6.2	8.5	7	7	7.2	7.1

TFPC was incorporated at 3%, 6%, 10% respectively. An incorporation of 3% was found to have highest sensory scores. These results were similar to studies of Wartha *et al* (2013) on Preparation of fish soup powder from Tilapia fish and Rahman *et al* (2012) on Fish powder in instant fish soup mix. There studies successfully incorporated 25% and 10% fish powder respectively.

Plate 3 soup powder incorporated TFPC

Table 4: Mean sensory score of flakes incorporated with TFPC

Sample	Taste	Texture	Flavour	Appearance	Overll acceptability	Mean score
Flakes (control)	7	8	7	7	7.3	7.2
Flakes(3%)	6.5	6	7	8	6.5	6.8
Flakes(6%)	8.25	7.2	9	9	8.1	8.3
Flakes(10)	6	6.7	8	7	5.2	6.9

TFPC was incorporated at 3%,6% and 10% respectively. An incorporation of 6% was found to have higher sensory scores. These results were in agreement with reports provided by Moorjani in 1982 on Research and development work on fish-enriched protein foods from inexpensive varieties of fish and Rodrigues *et al* in 2017.

Plate 3 :TFPC incorporated flakes

D. Nutritive value calculation of value added products

In the present study macronutrients of the TFPC like proteins and fat were estimated by biochemical methods and rest of the nutrients like energy, minerals etc are calculated by using information from reference studies by Kazosi *et al* (2018). Nutritive value calculations regarding the other ingredients of the food products (cookies, soup, flakes) were calculated using IFCT,2018.

Table 5 : Nutritive Value of TFPC Incorporated Value Added Products

Sample	Energy (kcal)	Protein (gm)	Fat(gm)	Calcium (mg)	Iron (mg)
Cookies(6%)	338	16.5	28.8	10.4	3
Soup powder(3%)	103	15.4	.06	1.0	3.2
Flakes(10%)	164	29.5	30	600	16

Table 6 Percentage of RDA met by per serving of TFPC incoroporated value added products (cookies(6%), soup powder(3%), flakes(10%).

Sample	Energy	Protein	Fat	Calcium	Iron
Cookies	20%	56.2%	.21%	.173	20%
SP	6.12%	52.2%	96%	1.73%	18.87%
Flakes	9.70%	53.01%	96%	4.34%	21.25%
RDA(Age 7-9)ICMR, 2010	1690	29.5	30	600	16

E. Shelf life study of value added products

1) Biochemical analysis

a) Peroxide value

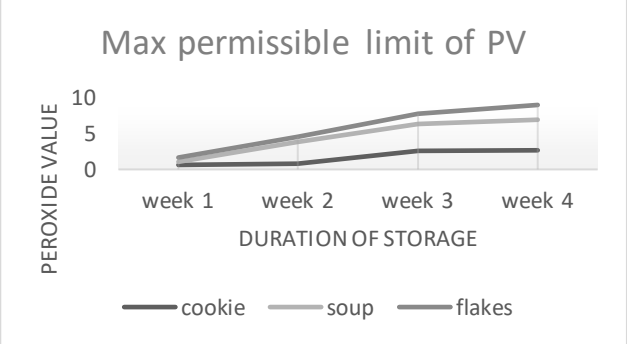
Peroxide value was found to gradually increase towards the end of the storage time with a value of 4.22 meq/ kg of sample.

But it was within the accepted limit of PV ie 10meq/ kg of sample. Hence PV of the products were in safe limits.These findings were similar to the results obtained by Mohhamad et al (2009)who did a study on Fortification of Biscuits with Fish Protein Concentrate.

Table 7: PV formation in TFPC incorporated products on storage

sample	Storage Period (weeks)				Remarks	Limit of PV
	1 week	2 week	3 week	4 week		
Cookie (6%)	.62	.73	2.48	2.64	Safe for consumption	10 meq/kg sample
Soup powder (3%)	.43	3	3.8	4.22	Safe for consumption	10 meq/kg sample
Flakes (6%)	.59	.7	1.36	2.04	Safe for consumption	10 meq/kg sample

Figure 2 Peroxide Value of TFPC incorporated products on storage



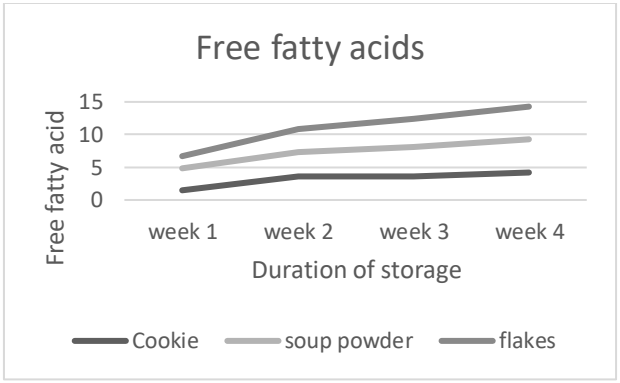
b) Free fatty acids

Free fatty acid was expressed as percentage of oleic acid ,formed as a result of lipid hydrolysis. The level of FFA depends on time, temperature and moisture content because the oils and fats are exposed to various environments such as storage, processing, heating or frying. The acceptable limit of FFA is 3-4 (FSSAI, 2018).

Table 8: FFA formation of TFPC incorporated products on storage

Samples	Storage period (weeks)				Remarks	Limit
	1 week	2 week	3 week	4 week		
Cookie (6%)	1.48	3.64	3.64	4.2	Un safe for consumption	3-4
Soup powder (3%)	3.36	3.64	4.48	5.08	Unsafe for consumption	3-4
Flakes (6%)	1.86	3.5	4.20	4.2	Un safe for consumption	3-4

Figure 3: FFA of TFPC incorporated products on storage

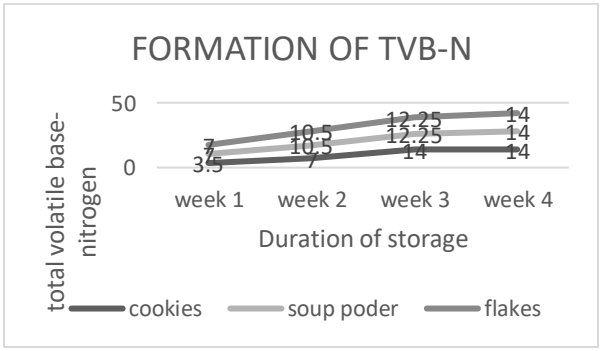


c) Total volatile nitrogen (TVB-N)

Volatile bases are formed in fish or fish products mainly on account of bacterial activity. The volatile compounds produced in fish muscle postmortem are TMA and ammonia.

Table 9: Total Volatile Base Nitrogen (TVB-N) Of TFPC Incorporated Products On Storage

Samples	Storage period (weeks)				Remarks	Limit
	1 week	2 week	3 week	4 week		
Cookies (6%)	3.5	7	14	14	Safe for consumption	30-35
Soup powder (3%)	7	10.5	12.25	14	Safe for consumption	30-35
Flakes (6%)	7	10.5	12.25	14	Safe for consumption	30-35



d) Trimethyl amine

TMA also most widely used and internationally accepted chemical indices of fish quality. Gradual accumulation of the fishy-odor compound tri methylamine (TMA) from bacterial reduction of tri methylamine oxide (TMAO) is one of the characteristic chemical changes attributed to fish spoilage.

2) Microbiological analysis

a) Total plate count

All the media and the diluents used for microbiology tests were sterilized by autoclaving at a temperature of 121^0c for 15 min and all the glassware at 160C in hot air oven for 2 hour. Total plate count was determined according to the BAM method.

Microbiological analysis by Total Plate Count resulted that both TFPC incorporated cookies and flakes were devoid of any colonies, and plate having soup powder showed few countable colonies. Therefore the products can be assumed to be microbiologically safe for consumption upto two weeks.

VI. SUMMARY AND CONCLUSION

The food products like cookies, soup powder and flakes were developed and added with different proportions of FPC. The best variation of the product were selected through sensory evaluation and further standardized. Proximate analysis of FPC and nutrient analysis of the products were done to assess shelf life. Biochemical tests like Peroxide value, Free Fatty Acid, Tri Methyl Amine, Total Volatile Base Nitrogen were carried out. Microbiological safety was assessed by Total Plate Count.

The following were the main findings of the study:

- FPC yield from Pink Perch was 10 % whereas from Tilapia it was 10.6%.However FPC from Tilapia (TFPC)was bland and odourless.
- The proximate analysis of TFPC showed a protein content of 75.02%, fat 0.018%, moisture 15.98% and ash content of 3.02%.
- Sensory evaluation by 9 point hedonic scale indicated 6% TFPC cookies, 3% TFPC soup powder and 6% TFPC flakes to be the best accepted samples.
- The nutrient analysis of per serving of the selected TFPC incorporated products were found to meet more than half of the RDA of protein and one third the RDA of Energy ,Calcium and Iron for a pre schooler aged 7-9 years.
- The shelf life study of the products were done by biochemical and microbiological analysis.
- Peroxide value was found to gradually increase towards the end of the storage time with a value of 4.22 meq/ kg of sample. But it was within the accepted limit of PV ie 10meq/ kg of sample. Hence PV of the products were in safe limits.
- Free fatty acid formation increased towards the end of the 4th week showing a max of 5.08% oleic acid. By the 3rd week the FFA formation was exceeding the accepted limit that is 3-4%.Hence the TPFC incorporated products had unsafe level of FFA by the 4th week.
- Similarly Trimethyl amine was also exceeding the normal limit that is 12 by the 3rd week of storage. Hence the TFPC incorporated products had an unsafe level of TMA resulting unfit for consumption.
- Total Volatile Base Nitrogen gradually increased towards the end, but doesn't exceed the limit towards the end. TVB-N value of the TFPC incorporated products were 14 which was within the limit and thus it was fit for consumption.
- Microbiological analysis by Total Plate Count resulted that both TFPC incorporated cookies and flakes were devoid of any colonies, and plate having soup powder showed few countable colonies. Therefore it can be assumed to be microbiologically safe for consumption upto two weeks.

Therefore it can be concluded that TFPC incorporated products namely cookies, soup powder and flakes were acceptable, nutritionally excellent and had a shelf life of upto two weeks.

RECOMMENDATIONS FOR FURTHER STUDY

- Addition of preservatives to improve quality and shelf life of products can be taken up.
- Supplementation studies using FPC incorporated products to vulnerable populations like PEM children, pregnant women, burns patients, Trauma patients etc could be carried out.

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