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Preparation and Characterization of a Novel Spread like Edible Formulation from Protein Fractions of Colossoma Macropomum (Rupchanda) Fish

Nandita Das, Mahua Ghosh

Department of Chemical Technology, University College of Science and Technology, University of Calcutta, Kolkata, West Bengal 700009, India

Corresponding Author: Nandita Das

ABSTRACT

In the present study, a novel spread like semi solid formulation was prepared primarily with isolated protein fraction (IPF) obtained from Colossoma macropomum (Rupchanda), a freshwater fish, and rice bran oil. The isolation of enriched IPF (having 97% protein) from rupchanda fish was carried out by extraction from raw fish and standardised for quality parameters such as protein content by Kjeldahl method, amino acid profiling by HPLC, moisture, oil holding capacity, water holding capacity etc. Serine was found to be the most abundant amino acid in IPF. The palatability and stability of formulated spread was assessed and it was found that the formulation is palatable as well as stable upto 60 days when refrigerated at 4°C.

Keywords: Isolated Protein Fraction (IPF), Spread, Stability, Amino acid profiling.

INTRODUCTION

Fish and seafood products, have a high nutritional value regarding beneficial amounts of protein, lipids as well as essential micronutrients. In many developing countries, fish is the main or only source of animal protein, and is essential for providing micronutrients to vulnerable populations. Fish protein has since long been considered having a high nutritional value due to higher protein content than most terrestrial meats. [(1)] In addition, aquatic protein has higher digestion rate and rich in several peptides and essential amino acids that are limited in terrestrial meat proteins. Fish proteins are a rich source of protein and have a lower caloric density, and have a high content of omega 3 long chain polyunsaturated fatty acids (n-3 LC PUFA) compared to land living animals. [(2)] Strong links between fish and seafood consumption and positive health effects, especially with the decreased risk of coronary heart and cardiovascular diseases, decreased inflammatory disease as arthritis and prevention of cancer have been shown by many researchers. [(3,4)]

During the polishing process of the rice, a unique vegetable oil rich in antioxidants produced from the outer layer of rice is called rice bran oil (RBO). RBO is more enriched with antioxidants like oryzanol, tocotrienol, tocopherol, squalene as compared to other edible oils. [(5)] This essentially results in health benefits like better skin, superior immunity system, prevention of certain types of cancer, maintaining healthy lipid level etc.

In the present study, a novel semi solid spread was prepared with IPF and RBO (1:1 w/w). This formulation is aimed for a balance mix of protein and fats which is crucial for well being of human health if consumed on daily basis. The main challenge for preparation of this spread was two folds: (i) to make the spread palatable and free from fishy smell and (ii) to prevent degradation and make it stable. The first one was achieved by addition of oregano herb

and this addition has not only omitted foul fishy smell and made the spread palatable, but also imparted additional health benefit coming from oregano, such as, anti-fungal and anti-bacterial properties. As the RBO is one of the key ingredients, the chances of becoming the formulation degraded are very high and so to combat the second challenge, butylatedhydroxytoluene (BHT), a known antioxidant, in small quantity (well below the permissible limit set by Food Safety and Standards Authority of India-FSSAI) was added in the formulation.

The stability study was performed for the assessment of organoleptic, chemical and safety aspect of the formulation by storing it at 4°C and 20°C for 60 days.

MATERIALS AND METHODS

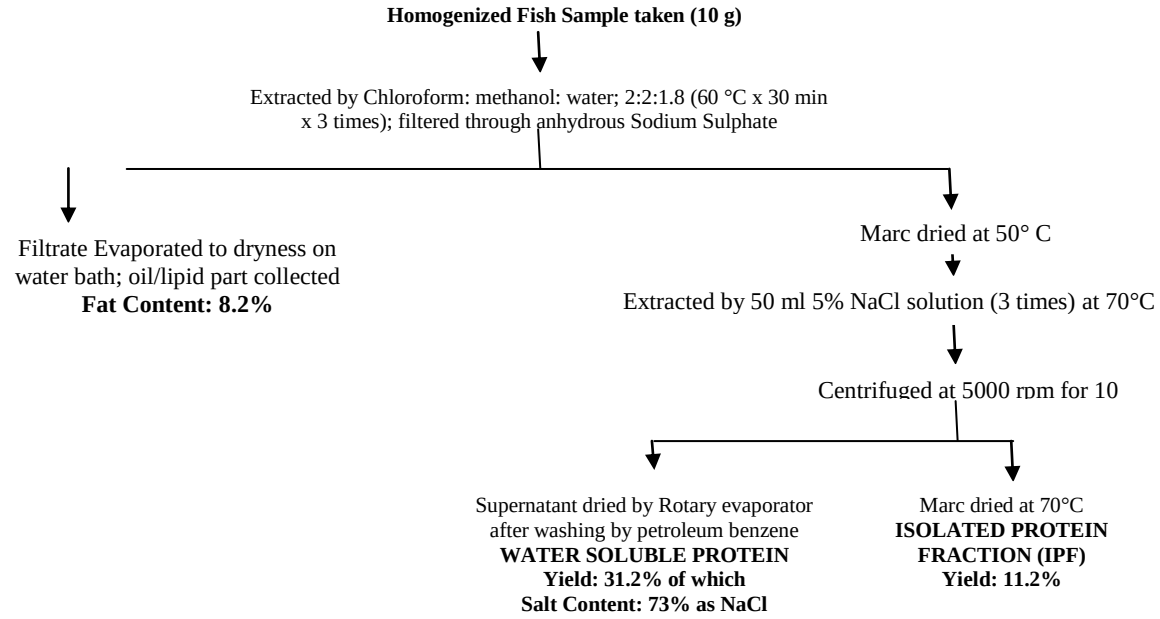
Ingredients:

Rupchanda fish, RBO and Oregano were procured from the local market of Kolkata. Other chemicals and reagents were procured from Merck India Ltd., Mumbai, India and were of AR grade.

Isolation of Protein Fraction:

After the collection of fresh sample, the head, viscera, scales, bones were removed and the obtained sample was cleaned by local fisher-folk to obtain raw, edible parts. To avoid metal contamination, non-metal equipment such as plastic cutting boards, buckets, strainers, and ceramic cutting knives were used in the whole process. Next, the samples were washed with de-ionized water. The fillet was scrapped with a knife into a mortar and mashed until a uniform mixture was produced.

The homogenized material was extracted by chloroform, methanol and water mixture (2:2:1.8; v/v/v) solvent. This extractive, consisting mostly lipid material, was discarded. The defatted material was dried and extracted by water having sodium chloride concentration of 5%. The extraction was carried out at 70°C. the solution was centrifuged. The marc was dried and considered as IPF. The flowchart of extraction procedure is given below.



Characterization of IPF:

Determination of total lipid content:

The lipid contents of the collected fish samples were determined using a rapid method of total lipid extraction and purification by Bligh, E.G. and Dyer, W.J. 1959. [(7)]

Estimation of Protein content:

Indirect protein determination (Kjeldahl method) was performed according to

method 981.10 of the AOAC International. [6]

Water Holding Capacity & Oil Holding Capacity:

Briefly, 1 g of IPF was taken in a small pre-weighed centrifuge tube and small quantities of water were added (0.1 ml at a time) slowly and absorbed in IPF by vortexing. Water was added till excess water was seen above sample layer and not absorbed after vortexing. The mixture was kept at room temperature for 1 hour. After 1 hour of incubation, the mixture was centrifuged at 5000 rpm for 5 minutes. Supernatant was discarded and weighed for the determination of water holding capacity. To determine oil holding capacity, rice bran oil was used in place of water.

Characterization of RBO:

Rice bran oil sample was analyzed following AOAC methods. Following parameters were performed: Butyro-refractometer reading at 40°C, moisture and volatile matter, saponification value, iodine value, acid value, unsaponifiable matter, oryzanol content, flash point, test for argemone oil.

Formulation of Spread:

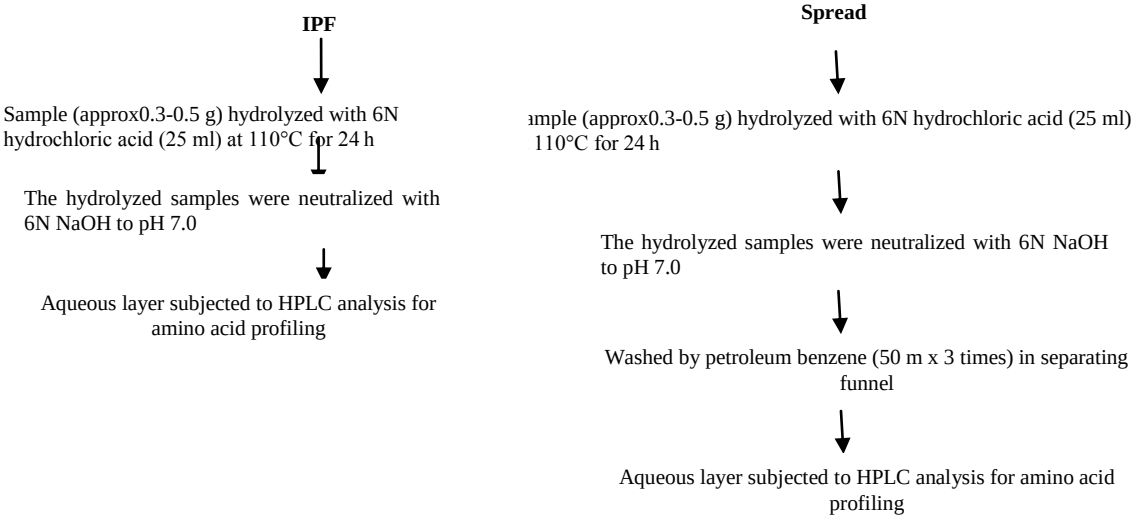
Isolated Protein Fraction (IPF) was taken in a mortar and pestle. Oil fraction was prepared separately with rice bran oil (RBO) and BHT mixed together. Then oil phase was added drop wise to IPF with vigorous trituration. Further, oregano powder was added, further triturated. Finally, the spread was poured to mould and refrigerated at 4°C for 3 hrs.

Characterization of Spread:

The prepared spread was characterized for the following parameters, namely, moisture content, peroxide value, serine content and microbiological screening (Total plate count, yeast &mould). All of the parameters except serine content were tested by standard method following AOAC. Total amino acid profile including serine content was determined by HPLC. As amino acids lack UV chronophers, analysis was carried out after derivatisation by orthopthalaldehyde (OPA) and Fluorenylmethoxycarbonyl protecting group (FMOC).

Estimation of serine content (amino acid profiling):

Sample preparation of IPF and Spread: Amino acids including serine content were determined following Ishida et al. [8] Briefly, sample (approx 0.3-0.5 g) was hydrolyzed with 6N hydrochloric acid (25 ml) at 110°C for 24 h. The hydrolyzed samples were neutralized with 6N NaOH to pH 7.0. In case of spread, the sample was transferred to a separating funnel and extracted by petroleum benzene (50 m x 3 times). Petroleum benzene layer (oil soluble part) was discarded. The aqueous part was filtered through Whatmann No. 1 filter paper in 100 ml volumetric flak. The volume was made up using distilled water.



HPLC Method: Shimadzu assembly (LC 2030), equipped with a UV detector, quaternary pump, injector, ZORBAX Eclipse-AAA [RP C18 150 x 4.6 mm; 3.5 µm] column were used, 40mM phosphate buffer having pH 7.8 (solvent A) and a mixture of Methanol: Acetonitrile: water (45:45:10) (Solvent B) were used as the mobile phase with a flow rate of 2.0 ml/min. the column temperature were kept 40°C. 5µl sample was injected and detection was done at 338 nm for OPA-amino acids and 262nm for FMOC-amino acids. The gradient was maintained as follows:

Time (min)	%A	%B
0	100	0
1.9	100	0
18.1	43	57
18.6	0	100
22.3	0	100
23.2	100	0
26.0	100	0

Sensory Evaluation of Spread by Panel Testing:

The sensory evaluation of food is carried out on a scientific basis to ascertain the product formulations or processing techniques that are anticipated to be successful in the market place. In the research and development process of a product, trained sensory panelists evaluate the samples and provide guidance in improvement of the product. This type of testing wherein the scores are determined by individual decisions based on the use of senses and do not rely on the mechanical devices, is known as sensory or subjective evaluation. Thus, sensory evaluation has been defined as a scientific method to evoke, measure, analyze and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing. [(9)] The taste panel was set up comprising of 10 persons. The sensory evaluation of spread was based upon flavor, texture, appearance and palatability. The scale was set up from 1 to 10 in the ascending order of preference and 70 was internally set as cut off.

Stability Study:

The stability study of the prepared spread was carried out as per below schedule.

Objective	To perform stability study of Protein based Spread	
Mfg. Date	August, 2019	
Study Tenure	**Condition**	**Duration**
	4ºC (Refrigerated)	60 days
	20ºC (Room Temperature; air conditioned room)	60 days

Stability study details:

Time Points (In Months)	Sampling plan		Details of samples
	4ºC (Refrigerated)	20ºC (Room Temperature; air conditioned room)	
Initial (Initiated on 19.08.19)	Peroxide value, Moisture, Serine Content, Microbiological Screening		Samples kept at refrigerator and RT.
1 day (20.08.19)	€	€	
3 day (22.08.19)	€	€	
7 day (26.08.19)	€	€	
15 day (03.09.19)	€	€	
30 day (19.09.19)	€	*	
60 day (19.10.19)	€	*	

€ All quality parameters to be analyzed except microbial screening and serine content

***** All quality parameters to be analyzed except microbial screening.

RESULTS

Characterization of IPF:

Table 1: Functional properties of IPF

Parameter	Results
Total Lipid Content on dry basis (%)	1.06
Protein Content on dry basis (%)	97.0
Water Holding capacity (ml/g)	2.74
Oil Holding capacity (ml/g)	1.87

Isolated protein fraction (IPF) was characterized and the results are incorporated in Table 1 and 2. It was found the enrichment of protein content was achieved as high as 97% when analysed by classical Kjeldahl method. Complete amino acid profiling was carried out by HPLC (Table 2).

Table 2: Amino acid profiling of IPF

Amino acid	Retention time	g/100g	g/100g on dry basis
Aspartic Acid	1.694	8.01	8.77
Glutamic Acid	3.43	8.19	8.97
Serine	6.006	39.48	43.25
Arginine	8.225	3.69	4.04
Alanine	8.827	3.92	4.31
Tyrosine	10.031	1.56	1.70
Glycine	11.086	2.81	3.07
Methionine	12.377	7.46	8.30
Leucine	13.876	3.82	4.19
LycinemonoHCL	15.461	2.79	3.06
Hydroxyproline	14.827	4.95	5.42
Proline	17.072	1.75	1.91

The chromatogram is given in Figure 1. It was observed that, moisture content and lipid content was kept relatively low to impart stability and prevent degradation of the spread prepared from IPF.

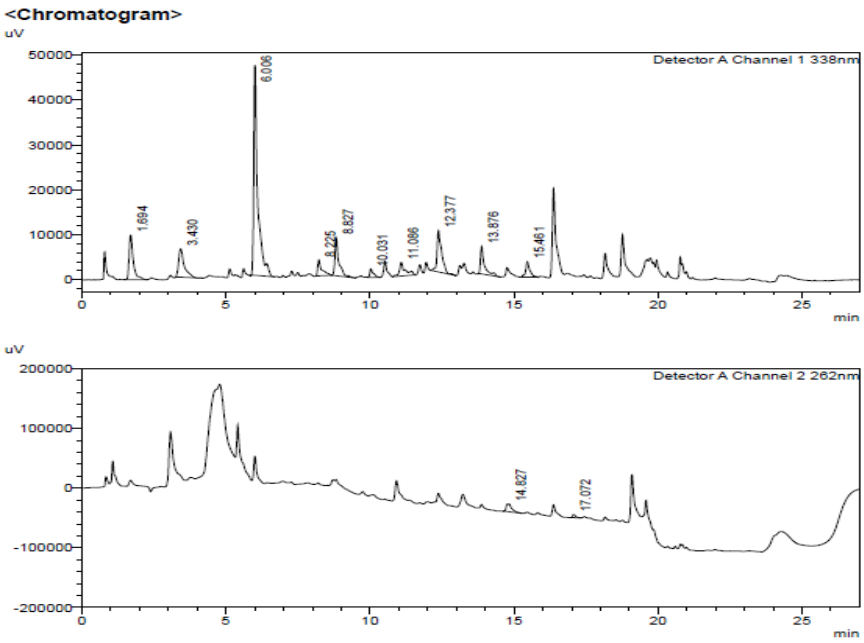


Figure 1: Chromatogram of Amino acid profile of IPF

Characterization of RBO:

Rice bran oil (RBO) sample was analyzed following AOAC methods. The results are incorporated in table 3. It was found that the samples were found to meet the quality specifications as per Food Safety Standard Authority of India (FSSAI) regulations. Thus, the sample was used for the formulation of spread.

Table 3: Characterization of RBO

Parameter	Specifications as per FSSAI	Results
Moisture and volatile matter (%)	Not More Than 0.1	0.004
Butyro-refractometer reading at 40°C	51.0-66.4	58.4
Saponification value	180-195	186.5
Iodine Value (Wij's method)	90-105	94.2
Acid Value	Not More Than 0.5	0.16
Unsaponifiable matter (%)	Not More Than 3.5	0.96
Oryzanol Content (%)	Not Less Than 1.0	1.34
Flash Point	Not Less Than 250°C	258°C
Test for argemone oil	Shall be negative	Negative

Characterization of Spread:

The prepared spread was characterized for different physico-chemical parameters. The results are incorporated in Table 4 and 5. It was observed that the spread was free from obnoxious

smell. The microbiological count was also relatively low for a non-sterile product. Thus, it was found to be safe and suitable for sensory evaluation by panel. Additionally, complete amino acid profiling (including Serine) was carried out by HPLC. The Chromatograms are given in Figure 2 and 3.

Table 4: Characterization of Spread

Parameter	**Results**
Physical Appearance	Brown colored Jelly having characteristic smell with visible particle of oregano
Moisture Content (%)	2.86
Peroxide Value (milliequivalent/kg)	4.53
Total Plate Count (cfu/g)	3500
Yeast &Mold (cfu/g)	200

Table 5: Amino Acid Profiling of Spread

Amino acid	**Retention time**	**g/100g**	**g/100g on dry basis**
Aspartic Acid	1.694	4.25	4.38
Glutamic Acid	3.414	4.97	5.11
Serine	6.022	20.96	21.58
Arginine	8.234	2.06	2.12
Alanine	8.838	2.12	2.18
Tyrosine	10.036	0.88	0.91
Glycine	11.091	1.29	1.34
Methionine	12.379	4.70	4.84
Leucine	13.877	2.12	2.18
LycinemonoHCL	15.462	1.68	1.72
Hydroxyproline	14.832	2.92	3.01
Proline	17.441	0.31	0.32

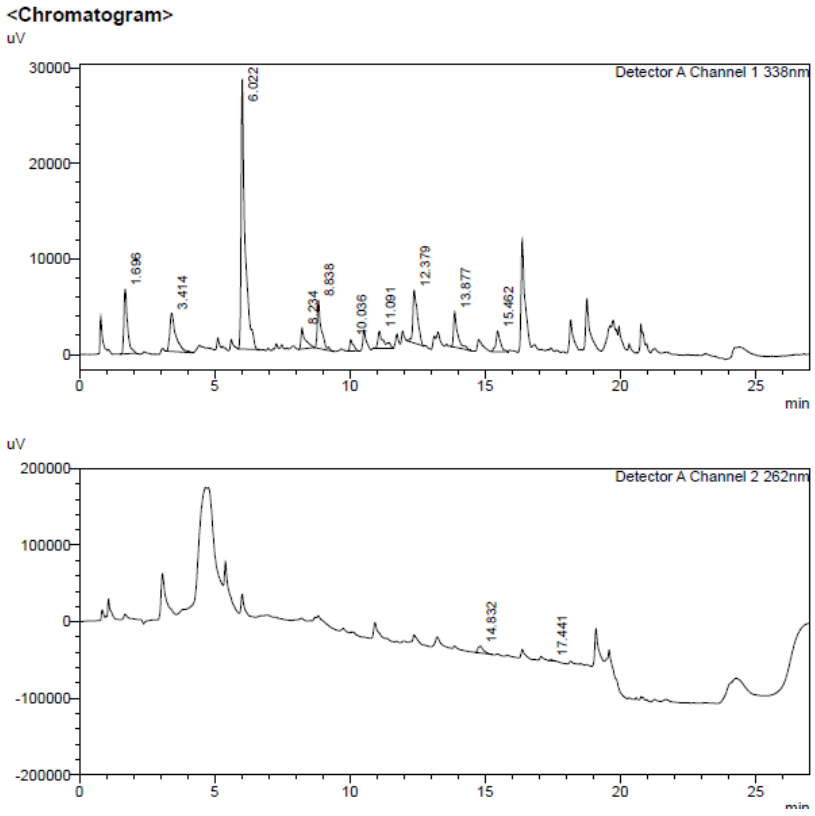


Figure 2: Chromatogram of Amino acid profile of Spread (Initial)

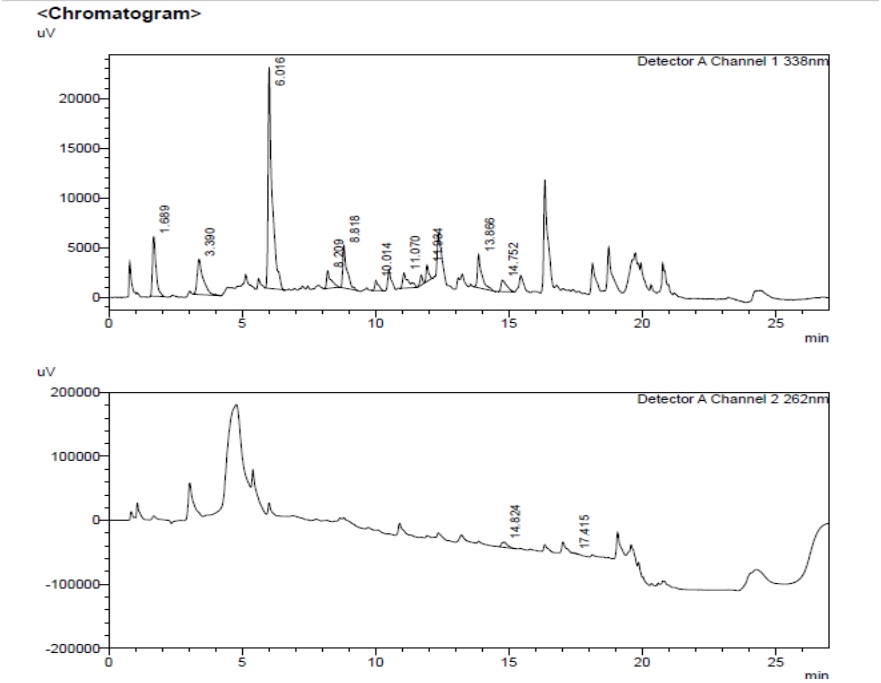


Figure 3: Chromatogram of Amino acid profile of Spread (after 60 days at 4°C)

Sensory Evaluation of Spread by Panel Testing:

Sensory evaluation was carried out by panel testing. Smokers were excluded from the panel. The results showed that the sample was well accepted by the panel. Hence, the sample was found to be palatable. The results were given in table 6.

Table 6: Sensory Evaluation of Spread by Panel Testing

Parameters / Panelists	Flavor	Texture	Taste	Appearance	Remarks (if any)
Panelist 1	7	8	6.5	7.5	None
Panelist 2	6	8	5.5	9	Fishy smell
Panelist 3	8	7	7.5	9.5	None
Panelist 4	6	7.5	8.5	8.5	Smell of fish
Panelist 5	8	7	7	8.5	None
Panelist 6	7	6	6.5	9.5	Appearance was good
Panelist 7	9	6.5	9	9.0	Gritty particle felt
Panelist 8	7	7.5	8.5	9.5	None
Panelist 9	8	8	7	8.5	None
Panelist 10	7	7.5	7.5	8.5	None
Total Score	73	73	73.5	88	-
Cut off for acceptance	70	70	70	70	-

Stability Study:

The stability study was carried out as per protocol and it was found that the product is stable upto 60 days at 4°C and at and 30 days at 20°C (Room Temperature; air conditioned room). The results were given in Table 7.

Table 7: Stability Datasheet

Sl No.	Parameters	In House Specification	Initial	1 day		3 days		7 days		15 days		30 days		60 days	
				4°C (Refrigerated)	20°C (Room Temperature; air conditioned room)	4°C (Refrigerated)	20°C (Room Temperature; air conditioned room)	4°C (Refrigerated)	20°C (Room Temperature; air conditioned room)	4°C (Refrigerated)	20°C (Room Temperature; air conditioned room)	4°C (Refrigerated)	20°C (Room Temperature; air conditioned room)	4°C (Refrigerated)	20°C (Room Temperature; air conditioned room)
1	Physical Appearance	Brown colored Jelly having characteristic smell with visible particle of oregano	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Brown colored Jelly having characteristic smell with visible particle of oregano upper layer becomes oily
2	Peroxide Value (milliequiv/1000g)	Not More Than 10.0	4.53	4.65	4.86	5.12	5.74	5.52	6.87	5.87	7.58	6.18	9.46	7.11	
3	Moisture (%)	Not More Than 8.0	2.86	2.92	3.15	3.01	3.54	3.11	3.74	3.13	3.92	3.42	4.52	3.71	
4	Serine Content on dry basis (%)	Not Less Than 15.0	21.58	NA	NA	NA	NA	NA	NA	NA	NA	17.57	NA	16.75	

DISCUSSION

IPF was found to have 97% (w/w) total protein content among which serine was found to be the most abundant amino acid accounting for 43.25% among total amino acid. L-Serine is involved in many biological processes of the human metabolism. For instance, it is essential to cell proliferation and brain development. [(10)] It also plays a crucial role in inhibiting pain signals. Thus, abundance of serine in IPF may add to an additional dimension on nutritional health benefits of consumption of IPF in palatable form.

The developed spread formulation was found to be palatable, safe and stable upto 30 days when kept at 20°C and up to 60 days when kept at 4°C after Sensory evaluation, microbiological testing and stability study. Moreover, the spread was found to have appreciable amount of serine content which was found to be stable and unhindered after 60 days of stability study.

CONCLUSION

The present study was conducted in view of developing a novel food formulation which shall satiate daily needs of protein and fat and at the same time achieve certain health benefits. In the present study, the formulated spread was found to be palatable, safe and stable. Moreover, the present of serine in abundance contribute to several health benefits. Thus, this formulation, prepared from isolated protein fraction of obtained from Colossoma macropomum (Rupchanda) and rice bran oil is unique, palatable, and nutritious and can be included in daily diet for optimum health benefits.

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