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Effect of packaging materials on quality of fish protein concentrate extracted from ribbon fish, *Lepturacanthus savala* (Cuvier, 1829)

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SUMMARY :

Fish protein concentrate (FPC) is a healthy and highly nutritive product produced hygienically from fishes in which, protein and other nutrients are more concentrated than fresh fishes. In the present study an attempt was made to study the functional properties of FPC derived from ribbon fish, *Lepturacanthus savala* were investigated in HDPE packaging materials and their shelf-life. The changes in chemical analysis of FPC powder prepared by Canadian process and stored at room temperature were investigated. Moisture content was increased in the range from 10.78 to 12.72 per cent, whereas the ash content was decreased in the range from 1.87 to 1.08 per cent, protein content was decreased in the range from 86.80 to 83.04 per cent and also, the fat content was decreased in the range from 0.55 to 0.40 per cent during the storage period of 180 days. The changes in functional properties of FPC powder prepared by Canadian process stored at room temperature were also investigated. The viscosity was decreased in the range from 114.00 to 100.00cP; solubility was decreased in the range from 88.92 to 83.78 per cemt, emulsification capacity was decreased in the range from 67.66 to 65.08 per cent, emulsification stability was decreased in the range from 58.84 to 56.02 per cent foaming capacity was decreased in the range from 42.50 to 32.64 per cent foaming stability was decreased in the range from 26.50 to 22.56 per cent gelation was decreased in the range from 26.50 to 20.25g. cm and also, the water holding capacity was decreased in the range from 4.27 to 3.86ml/g during the storage period of 180 days. The TPC (Total Plate Count) of the FPC powder showed increasing trend throughout the shelf-life study. The TPC of the FPC powder in storage at ambient temperature was increased from $0.42x10^2$ to $1.48x10^3$cfu/g. As per microbiological quality of FPC extracted from ribbon fish, TPC value was within the acceptable limit during storage period of 180 days. The sensory qualities of FPC powder during storage at ambient temperature for 180 days were observed. For the FPC powder gradual reduction in the scores were observed with increase in the storage period. But, during storage period the sensory evaluation score indicated that the FPC extracted from ribbon fish was within the acceptable limit.

KEY WORDS : Ribbon fish, Fish protein concentrate, Functional properties, Packaging materials

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Fish is one of the most important sources of animal protein available worldwide and has been widely accepted as a good source of protein and other elements for the maintenance of healthy body. In recent years the importance of finfish in the diet has extended from its image as a cornerstone of a healthy diet to more specialized roles in the disease prevention (Sharmila, 2006). Fish is one of the most nutritious foods available for human consumption. Fish flesh on an average contains 18–20 per cent protein. Fish proteins are classified under three major categories, *viz*., myofibrillar, sarcoplasmic and stroma proteins (Balachandran, 2012). Globalization, industrialization, economic growth and transition in lifestyle patterns has greatly influenced the choice of food and the food consumption pattern of all age groups in developing countries like India (WHO, 2000). Adequacy in nutrient intake in terms of quantity and quality are major determinants of health of a nation. India is undergoing nutrition transition and is facing the dual burden of malnutrition *i.e.* problem of under-nutrition and micronutrient deficiencies. Fish is one food that can address this problem unswervingly.

FPC can play an effective role in decreasing protein deficiency in some crowded parts of the world that suffers from malnutrition. Studies have shown that adding FPC to human diets has positive effects especially for growing babies and pregnant women (FAO, 2006). FPC is a low cost animal protein with high quality, so it can be used as a protein supplement to increase nutritive value of foods (Cordova-Murueta *et al.,* 2007). Considerable works were done to develop FPC production methods and use it in different foods, but unfortunately there is little information about sustainability of FPC during storage at different environmental conditions (Rasekh *et al*., 2001).

Development of FPC represents the first concentrated effort to increase the use and the value of underutilized fish by converting it into a more readily acceptable form. However, FPC produced by these technologies was deficient in some of the functional properties and their cost of production was high. Recognizing this problem several investigations have aimed at improving the functional properties of FPC by rationally modifying the parameters of extraction and by employing enzymes to partially hydrolyze proteins (Sikorski *et al*., 1981).

Functional properties of food macromolecules including proteins are defined as a set of physico-chemical characteristics that contribute to the structural, mechanical, and other physico-chemical properties and determine the behaviour of food systems during processing, storage, preparation and consumption. The commonly used functional properties of proteins in foods include solubility, viscosity, water holding capacity, emulsification and foaming (Hamann, 1994).

Solubility is usually considered the premier functional property because of its relevance to other properties such as viscosity, foaming and emulsification. Solubility of the protein molecule is often as pre-requisite for these other properties to be observed. Several parameters are known to affect protein solubility. These include pH, temperature, ionic strength and the presence of other materials capable of binding of the protein. The universal solvent is water, and solubility can give an indication of the effect of processing conditions and the potential usage of the protein (Hermansson, 1973).

Emulsions occur in all types of food systems, and emulsifying agents are consequently the object of much attention (Dickinson and Stainsby, 1987). Emulsions are defined as dispersed immiscible droplets (the dispersed phase) within another liquid (the continuous phase) that are stabilized by interphase compounds. Examples of emulsions are oil-in-water or water-in-oil systems. Proteins stabilize emulsions by lowering the surface tension between the amino acid content. Emulsion are thermodynamically unstable because of the positive free energy, which causes interfacial tension and the emulsifying agent acts at the interface to reduce such tension to prevent droplets of the dispersed phase coming together (coalescence) to form larger droplets and eventually two separate phases (Parker, 1987).

Foaming is important in products incorporating air, such as meringues, cakes and soufflés. The foam consists of a gas, usually air, dispersed in and well-enclosed by a liquid and stabilized by the functional agent. The action of the protein is similar to that in emulsions but, in foam, the protein must also form a strong cohesive layer about the air pockets (Halling, 1981). Increased hydrophobicity and a degree of insolubility in proteins lead to increased foam stability; however, complete denaturation of the protein is not desirable as it results in a loss in elasticity. Foam capacity is a measure of the ability of the protein to form a gas-filled cellular system through the incorporation of the gas by whipping, agitation or other agitation under specified conditions.

Viscosity is the ratio of the shear stress to shear rate applied to a fluid and indicates the resistance to flow of the fluid. There are several means of measuring viscosity, depending on the nature of the liquid and the viscosity expected. The methods commonly use simple rotational viscometers (Hall and Ahmad, 1992).

The aim of present study was to extract FPC from minced meat of the ribbon fish (*Lepturacanthus savala*) as a raw material for the production of FPC and packed in HDPE bags of size 6 x 8 inch were used for packing of FPC during the storage period and study of the chemical analysis, functional properties, sensory and microbiological evaluation of FPC during storage at ambient temperature.

EXPERIMENTAL METHODS

Chemical analysis :

Chemical analysis of raw materials and extracted FPC powder were analyzed by measuring moisture, ash, protein and fat contents according to AOAC official methods (AOAC, 2005).

Packaging materials:

HDPE bags of size 6 x 8 inch were used for packing of FPC during the storage period.

Determination of viscosity :

FPC sample was determined according to the method of Cho *et al.* (2006). FPC solutions at the concentration of 6.67 per cent (w/v) were prepared by dissolving the dry powder in distilled water and heating at 60°C for the determination of viscosity. The viscosity (cP) of 10 ml of the solution was determined using Brookfield digital viscometer (Model DV – E Brookfield Engineering, USA) equipped with a number 1 spindle at 40°C ± 1°C.

Determination of solubility :

The solubility of FPC was determined according to Hoyle and Merritt (1994). About 500 mg of FPC sample was accurately weighed and dispersed in 50 ml of 0.1M NaCl at pH 7. The solution was stirred for 1 hr. and centrifuged for 30 min at 10,000 rpm. The supernatant was analyzed for nitrogen by the micro–Kjeldhal method (AOAC, 2005). The Nitrogen Solubility Index (NSI) was calculated as

$$\text{NSI (\%)} = \frac{\text{Supernatant nitrogen concentration (mg)}}{\text{Sample nitrogen concentration (mg)}} \text{ x } 100$$

Emulsifying capacity and stability :

The method of Butt and Batool (2010) was used to determine emulsifying capacity and stability. FPC sample (1.8 gm) was added to 25 ml of distilled water (pH 7) and dispersed at maximum speed in a homogenizer/ blender. Corn oil (12.5 ml) was added and homogenized/ blended at high speed for 1 min.; the emulsion formed was equally divided into two 12 ml centrifuge tubes and centrifuged for 5 min. at 5200 rpm. Emulsion capacity was calculated as follows:

$$\text{Emulsifying capacity (\%)} = \frac{\text{Height of emulsified layer}}{\text{Height of total contents of tube}} \text{ x } 100$$

Emulsion stability was determined in a similar way to that of emulsion capacity except that the emulsion was initially heated in a water bath at 85°C for 30 minutes and subsequently cooled to 25°C prior to centrifugation.

$$\text{Emulsifying stability (\%)} = \frac{\text{Height of emulsified layer after heating}}{\text{Height of total contents of tube}} \text{ x } 100$$

The FPC powder (1 g) was added to 100 ml of distilled water and homogenized for 1 min. at high speed. The mixture was carefully transferred into a 250 ml calibrated beaker for volume measurement. The foam was calculated as the volume of mixture after blending compared to the original volume. The foaming stability was the ratio of the foam capacity after 30 min. divided by the original foam capacity.

Estimation of water holding capacity :

Water holding capacity (WHC) was determined using the centrifugation method (Diniz and Martin, 1997). Duplicate samples (0.5 g) of FPC were dissolved in 20 ml of water in centrifuge tubes and dispersed with a vortex mixer for 30 sec. The dispersion was allowed to stand at room temperature for 6 hr., and then centrifuged at 2800 ×g for 30 min. The supernatant was filtered with Whatman number 1 filter paper and the volume recovered was measured. The difference between the initial volume of distilled water added to the protein sample and the volume of the supernatant was determined and the results were reported as ml of water absorbed per gram of FPC sample.

Statistical analysis :

The data were analysed to test significant difference by applying analysis of variances (ANOVA) tool available in MS-Excel 2010. The significant differences were

tested by 5 per cent level of significances and are mentioned as $p < 0.05$ for significances difference (Zar, 1999).

EXPERIMENTAL FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Storage study of Canadian process at ambient temperature :

Changes in moisture :

In present study the moisture content of FPC sample was found to be increased in trends during the storage study (0 to 180 days) in the range of 10.78 to 12.72 per cent (Table 1).

Chudasama *et al.* (2012) has observed similar results of changes in moisture content of edible fish powder during storage period at 0, 1st, 2nd, 3rd, 4th, 5th and 6th month were prepared by two methods *i.e.* without bone (T_1) and with bone (T_2) as 7.77, 7.82, 8.42, 9.27, 10.04, 11.14 and 11.50 per cent, respectively; and 8.10, 8.24, 8.32, 9.31, 10.19, 10.64 and 11.33 per cent, respectively. According to Syahrul (2015) has reported the lowest water content occurred in 45 day of period research as many as 8.24 per cent and 9.74 per cent, for steam and non-steam method, respectively. It means that the water content that was generated from non-steam method was higher than that of steam method. Water content from catfish FPC from both methods tends to increase during storage time. The water contents increased because FPC of catfish was defined as dry product, therefore, absorption of water vapor from surrounding air make the product turn into moist or there was a surge level of water content. This finding was similar to findings of Syarief and Halid (1993). They reported that decreasing or increasing of water content during storage time are caused by vapor processing and then, absorption on food source as the effect of air condition. Moreover, producing water content in this process was still below the quality standard guideline for FPC. It may be concluded that during 45 days of storage, FPC from catfish was acceptable to consume. Based on National Standard Board (1992), the maximum water contents for FPC was 10 per cent. However, Chari and Sreenivasan (1980) has observed changes in moisture content of FPC from Shark, *Carcharias* sp. during storage periods at 0, 1st, 2nd, 3rd, 6th, 9th and 12th month were found to be 5.5, 5.2, 5.7, 5.5, 5.8, 5.6 and 5.9 per cent, respectively. Chattopadhyay *et al.* (2004) has been reported similar values of changes in moisture content of edible fish powder from silver bellies during storage period at fresh powder, 1st, 2nd, 3rd, 4th and 5th month were found to be 5.55, 5.45, 6.01, 6.63, 6.76 and 7.00 per cent, respectively. Jeyasanta *et al.* (2013) has reported the similar results of changes in moisture content of edible fish powder during the storage period at fresh, 1st, 2nd, 3rd, 4th and 5th as 3.28, 5.55, 5.45, 6.02, 6.04 and 6.50 per cent, respectively.

Changes in ash :

In present study the ash content of FPC sample was found to be decreased in trends during the storage study (0 to 180 days) in the range of 1.87 to 1.08 per cent (Table 1). Chudasama *et al.* (2012) has observed the values of changes in ash content of edible fish powder during storage period at 0, 1st, 2nd, 3rd, 4th, 5th and 6th month were prepared by two methods *i.e.* without bone (T_1) and with bone (T_2) as 6.28, 6.17, 5.83, 5.73, 5.23, 5.16 and 5.22 per cent, respectively and 7.01, 6.69, 6.64, 6.30, 6.22, 5.94 and 5.90 per cent, respectively.

Changes in protein :

In present study the protein content of FPC sample was found to be decreased in trends during the storage study (0 to 180 days) in the range of 86.80 to 83.04 per cent (Table 1).

Chattopadhyay *et al.* (2004) has observed values of changes in crude protein content of edible fish powder from silver bellies during storage period at fresh powder, 1st, 2nd, 3rd, 4th and 5th month were found to be 62.52, 61.12, 60.67, 58.12, 58.25 and 58.98 per cent, respectively. Chudasama *et al.* (2012) has observed similar results of changes in protein content of edible fish powder during storage period at 0, 1st, 2nd, 3rd, 4th, 5th and 6th month were prepared by two methods *i.e.* without bone (T_1) and with bone (T_2) as 59.00, 58.31, 57.81, 56.75, 55.02, 53.13 and 53.00 per cent, respectively and 57.83, 57.54, 56.82, 56.13, 55.52, 54.69 and 52.24 per cent, respectively. Jeyasanta *et al.* (2013) has reported the similar results of changes in protein content of edible fish powder during the storage period at fresh, 1st, 2nd, 3rd, 4th and 5th as 55.60, 52.01, 51.52, 50.67, 48.99 and 48.72 per cent, respectively. According to Khoshkhoo *et al.* (2012) protein content of

FPC (91.2%), after 6 months at 35°C, was decreased to 73.6 per cent and 69.4 per cent in VP and MAP, respectively. It was due to the O_2 presence and aerobic bacterial reactions, but at 5°C, protein content in VP and MAP were decreased from 91.2 per cent to 88.4 per cent and 81.2 per cent, respectively.

Changes in fat :

In present study the ash content of FPC sample was found to be decreased in trends during the storage study (0 to 180 days) in the range of 0.55 to 0.40 per cent (Table 1).

Jeyasanta *et al.* (2013) has reported the similar results of changes in lipid content of edible fish powder during the storage period at fresh, 1st, 2nd, 3rd, 4th and 5th as 0.5, 0.3, 0.3, 0.3, 0.2 and 0.2 per cent, respectively. However, Chudasama *et al.* (2012) has observed similar results of changes in lipid content of edible fish powder during storage period at 0, 1st, 2nd, 3rd, 4th, 5th and 6th month were prepared by two methods *i.e.* without bone (T_1) and with bone (T_2) as 4.67, 4.88, 5.18, 5.44, 5.56, 6.02 and 6.10 per cent, respectively and 4.63, 5.00, 6.03, 6.43, 6.64, 6.90 and 6.95 per cent, respectively. Chattopadhyay *et al.* (2004) has observed values of changes in crude fat content of edible fish powder from silver bellies during storage period at fresh powder, 1st, 2nd, 3rd, 4th and 5th month were found to be 8.97, 8.93, 7.21, 8.12, 8.62 and 7.97 per cent, respectively. According to Khoshkhoo *et al.* (2012) lipid content of processed FPC of Kilka was evaluated 0.5 per cent, after six months of storage at 35°C, lipid content in VP changed to 0.45 per cent, so it did not show significant decrease; but in MAP, it was decreased to 0.36 per cent. It was because of O_2 presence in MAP package and oxidation of lipids. It was also detected that an increase of temperature induces and accelerates oxidation. Chen (2007) have investigated lipid oxidation of raw red claw crayfish tail meat in VP and MAP (80% CO, 10% O_2 and 10% N_2) during 14 days of preservation at 2°C, it was detected that lipid oxidation in VP occurred lower than in MAP. The oxidation related changes of lipid and cholesterol contents of milk powder stored in VP and MAP was reported by Cluskey *et al.* (1997) and the lowest lipid and cholesterol oxidation was observed in VP. In another research, decrease in extractable amounts of lipid in stored FPC with 0.5 per cent lipid after 6 months at 37°C and in 50°C (very significantly) was reported. Also the amount of neutralized lipids, free fatty acids, C20:5 and C22:6

Table 1 : Changes in proximate composition of FPC powder during storage at ambient temperature

Storage periods (Day's)	Moisture (%)	Ash (%)	Protein (%)	Fat (%)
0	10.78	1.87	86.8	0.55
30	10.78	1.74	86.48	0.52
60	11.2	1.74	85.59	0.51
90	11.65	1.64	85.08	0.48
120	11.98	1.36	83.86	0.45
150	12.18	1.25	83.42	0.42
180	12.72	1.08	83.04	0.4

Table 2 : Changes in functional properties and microbiological changes of FPC powder during storage at ambient temperature

Functional properties	Storage period (Days)						
	0	30	60	90	120	150	180
Viscosity (cP)	114.00	110.00	110.00	106.00	103.00	102.00	100.00
Solubility (%)	88.92	88.48	86.70	86.02	85.76	85.12	83.78
Emulsification capacity (%)	67.66	66.98	66.45	66.12	65.86	65.24	65.08
Emulsification stability (%)	58.84	58.84	56.84	56.82	56.48	56.12	56.02
Foaming capacity (%)	42.50	42.50	40.28	40.02	38.50	35.44	32.64
Foaming stability (%)	26.50	26.22	26.05	25.48	25.18	24.35	22.56
Gelation (g.cm)	26.50	24.50	23.50	23.50	21.50	20.50	20.25
Water holding capacity (ml/g)	4.27	4.18	4.18	4.07	4.02	3.98	3.86
Microbiological changes	$0.42x10^2$	$0.92x10^2$	$1.7x10^2$	$2.08x10^2$	$2.68x10^2$	$1.12x10^3$	$1.48x10^3$
TPC(cfu/ g)	(1.62)	(1.96)	(2.23)	(2.32)	(2.43)	(3.05)	(3.17)

polyunsaturated fatty acids were decreased (Medwadowski *et al.*, 1971).

Functional properties :

Changes in viscosity :

In present study the viscosity of FPC sample was found to be decreased in trends during the storage study (0 to 180 days) in the range of 114.00 to 100.00 cP (Table 2). The highly soluble, non-swelling proteins possess low viscosity (albumin and globulins) while soluble proteins with high initial swelling show a concentration depending decrease in viscosity (Kinsella, 1976). Change in apparent viscosity of muscle homogenates was related to changes in actomyosin (Borderias *et al.*, 1985). Consequently any factors *viz*., pH, temperature, concentration and ionic strength, which unfold protein, influence their viscosity (Mohan *et al.*, 2007). Viscosity was, therefore, considered as a more reliable index than solubility for protein denaturation (Colmenero *et al.*, 1988). The viscosity of protein solution markedly decreased during frozen storage due to protein denaturation and aggregation of protein molecules (Oguni *et al.*, 1987). The increase in apparent viscosity was an indication of protein-protein interaction and aggregation during thermal denaturation of proteins (Takashi *et al.*, 1993).

Partiban *et al.* (2005) has observed the results of changes in viscosity of Tilapia, *Oreochromis mossambicus* protein storage period were 0, 2, 4, 7, 10, 12 and 14 days as 3.25, 3.44, 3.55, 3.37, 3.23, 3.12 and 3.01 mm^2/sec, respectively. Bragadottir *et al.* (2007) apparent viscosity tended to decrease after storage at elevated temperature. Apparent viscosity was defined as the viscosity of a non-Newtonian fluid (Bourne, 2002). Protein solutions do not show Newtonian behaviour, especially when protein concentration was high, which results in shear thinning behaviour when protein molecules orient themselves in the direction of flow (Damodaran, 1996). Viscosity was mainly influenced by protein properties like molecular weight, size, axial ratio, hydration and frictional ratio and shape of the molecule, which also are influenced by temperature, pH and ionic strength (Kinsella, 1979). Roura *et al.* (1990) attributed the decrease in viscosity of whiting actomyosin with increase in surface hydrophobicity during storage in ice to the alteration in the protein structure and exposure of more hydrophobic patches.

Changes in solubility :

In present study the solubility of FPC sample was found to be decreased in trends during the storage study (0 to 180 days) in the range of 88.92 to 83.78 per cent (Table 2). FPC from whole fish prepared by isopropyl alcohol extraction has been described, which exhibited a decreased solubility (Sikorski *et al.*, 1981). The decrease in solubility attributed mainly due to the leaching out of the water soluble proteins and the aggregation and insolubalization of myofibrillar protein fractions. With increasing in temperature, the ionic bonding (including hydrogen bonds) holding the 3D structure of protein gets disrupted leading to protein-protein interaction affecting protein solubility. The thermal influence on protein leads to opening up of the native structure exposing the hydrophobic patches to the exterior, which effectively displaces water molecules from protein surfaces promoting aggregation (Kuntz and Kauzmann, 1974).

Bragadottir *et al.* (2007) protein solubility of saithe powder showed tendencies to increase with storage time, but only significantly at the higher storage temperature ($p<0.05$). The protein solubility was initially 45.2 per cent and ended in 46.9 per cent and 48.2 per cent at 0°C and 30°C, respectively. Partiban *et al*. (2005) has observed the results of changes in total soluble of protein (TSP) of Tilapia, *Oreochromis mossambicus* protein storage period were 0, 2, 4, 7, 10, 12 and 14 days as 61.58, 56.69, 56.62,

Table 3 : Sensory evaluation of FPC powder during storage at ambient temperature for 180 days

Storage period (day's)	Attribute			
	Appearance	Texture	Odour	Overall acceptability
0	8.5	8.3	8.0	8.2
30	8.2	8.1	7.9	8.1
60	7.8	7.8	7.6	7.6
90	7.6	7.6	7.4	7.5
120	7.2	7.3	7.1	7.2
150	6.8	6.9	6.8	6.9
180	6.5	6.6	6.5	6.5

56.28, 50.29, 48.95 and 46.95 g/100g fish meat basis, respectively.

Changes in emulsification capacity :

In present study the emulsification capacity of FPC sample was found to be decreased in trends during the storage study (0 to 180 days) in the range of 67.66 to 65.08 per cent (Table 2). The unfolding of the 3-D structure of protein at the storage temperature contributes to the increase in surface active SH groups, which facilitates the interaction of protein with non-polar portion leading to higher emulsion capacity Matsudomi *et al.* (1982). By the 30th day emulsification capacity was decreased and continued till the end of storage period. The decrease in emulsification capacity could be related to the formation of disulfide bond between the protein molecules by the oxidation of SH groups exposed during protein unfolding during the initial periods of storage. A decrease in the emulsion capacity from the first day has been reported in Pink perch and Sardine during ice storage (Sarma *et al.*, 1999).

Partiban *et al.* (2005) has observed the results of changes in emulsion active index (EAI) of Tilapia, *Oreochromis mossambicus* protein storage period were 0, 2, 4, 7, 10, 12 and 14 days as 124, 183, 191, 288, 190, 189 and 186 m^2/g, respectively. The Emulsifying properties of proteins are primarily due to their ability to reduce the interfacial energy at oil-water interphase. The emulsion capacity of a protein denotes the maximum amount of oil that can be emulsified under specified condition (Zayas, 1997).

Changes in emulsification stability :

In present study the emulsification stability of FPC sample was found to be decreased in trends during the storage study (0 to 180 days) in the range of 58.84 to 56.02 per cent (Table 2). Partiban *et al*. (2005) has observed the results of changes in emulsification stability of Tilapia, *Oreochromis mossambicus* protein storage period were 0, 2, 4, 7, 10, 12 and 14 days as 570, 730, 450, 620, 600, 610 and 600 sec., respectively.

Changes in foaming capacity :

In present study the foaming capacity of FPC sample was found to be decreased in trends during the storage study (0 to 180 days) in the range of 42.50 to 32.64 per cent (Table 2). The protein that retains their tertiary structure at the interface will maintain extensive protein-protein interaction forming strong foam. Partiban *et al.* (2005) has observed the results of changes in foaming efficiency of Tilapia, *Oreochromis mossambicus* protein storage period were 0, 2, 4, 7, 10, 12 and 14 days as 176.6, 180.0, 186.67, 200.0, 213.33, 160.0 and 133.33 per cent, respectively.

Changes in foaming stability :

In present study the foaming stability of FPC sample was found to be decreased in trends during the storage study (0 to 180 days) in the range of 26.50 to 22.56 per cent (Table 2). Owing to decrease the foam stability of FPC, the alteration of hydrophobicity and low molecular weight of peptide in protein hydrolysate were reported to improve foaming by forming stable interfacial layer (Wild and Clark, 1996). The protein that retains their tertiary structure at the interface will maintain extensive protein-protein interaction forming strong foam. The myofibrillar protein from tilapia was less effective as foaming agent comparing to whey protein (Webb *et al.*, 2002).

Changes in water holding capacity :

In present study the water holding capacity of FPC sample was found to be decreased in trends during the storage study (0 to 180 days) in the range of 4.27 to 3.86 ml/g (Table 2).

Bragadottir *et al.* (2007) water-holding capacity (WHC) of the saithe powder showed tendencies to decrease with storage time. At 30^{o}C the WHC decreased from 121 per cent to 99 per cent during four months storage ($p<0.05$), whereas after four months at 0^{o}C the WHC was 110 per cent. Water-holding capacity refers to the ability of the protein to absorb water (Damodaran, 1996). Water molecules bind to several groups in proteins and some of the factors that affect water-holding capacity of proteins are for example, protein concentration, pH, ionic strength, temperature, other food components like polysaccharides, lipids and salts, rate and length of heat treatment (Zayas, 1997). Increasing the temperature usually causes the water-holding capacity of proteins to decrease, but this was dependent on the degree of protein denaturation. Denaturation causes unfolding of the protein molecule and gives it more surface area where it exposes hydrophobic groups that were previously hidden. Denatured protein has approximately 10 per cent more water-holding capacity than the native protein, but when

it was allowed to aggregate, a loss in water-holding capacity can be observed because of protein-protein interactions (Damodaran, 1996). A decrease in WHC for the higher storage temperature in this study may, therefore, be regarded as an indication of protein denaturation. Partiban *et al.* (2005) has observed the results of changes in water holding capacity of Tilapia, *Oreochromis mossambicus* protein storage period were 0, 2, 4, 7, 10, 12 and 14 days as 2.8, 3.0, 3.1, 2.8, 2.7, 2.7 and 2.7 (unit/ g protein), respectively.

Microbiological analysis :

In present study the total plate count (TPC) of FPC sample was found to be increased in trends during the storage study (0 to 180 days) in the range of $0.42x10^2$ to $1.48x10^3$ cfu/g (Table 2) are considered as the TPC limit of acceptable. Jeyasanta *et al.* (2013) has reported the similar results of changes in total plate count (TPC) of edible fish powder during the storage period at fresh, 1st, 2nd, 3rd, 4th and 5th as $2.0x10^2$, $2.0x10^2$, $1.4x10^2$,$1.2x10^2$, $1.0x10^2$ and $1.0x10^2$ cfu/g, respectively. However, Chudasama *et al.* (2012) has observed similar results of changes in total plate count (TPC) of edible fish powder during storage period at 0, 1st, 2nd, 3rd, 4th, 5th and 6th month were prepared by two methods *i.e.* without bone (T_1) and with bone (T_2) as $1.39x10^3$, $1.51x10^3$, $2.03x10^3$, $2.35x10^3$, $2.92x10^3$, $3.10x10^3$ and $3.15x10^3$ cfu/g, respectively and $1.26x10^3$, $1.78x10^3$, $1.91x10^3$, $2.20x10^3$, $2.63x10^3$, $3.05x10^3$ and $3.09x10^3$ cfu/g, respectively.

Changes in sensory quality :

Table 3 shows the sensory qualities of FPC during storage at ambient temperature for 180 days. For the FPC gradual reduction in the scores was observed with increase in the storage period time but, during storage period the sensory evaluation score indicated that the FPC extracted from ribbon fish, *Lepturacanthus savala* was within the acceptable limit. According to Muraleedharan and Gopakumar (1998) the influence of storage conditions on colour and odour of the protein concentrate. It was observed that the product could be remained without any change in colour or odour at 10°C for 3 months. Even at ambient conditions these changes were negligible. The polyester/ polythene laminate having good barrier properties could effectively prevent quality deterioration of the spray dried protein concentrate during the storage period, at both conditions of storage. According to Jeyasanta *et al.* (2013) sensory scores of the edible fish powders storage period at ambient temperature the product had better texture, odour, taste, appearance and improved storage characteristics.

Conclusion :

The attempt was made in the present investigation to prepare FPC from ribbon fish, *Lepturacanthus savala* by Canadian process and. Then packed in HDPE stored at ambient temperature for 6 months and evaluate of proximate compositions, functional properties, microbiological analysis *i.e.* Total Plate Count and sensory evaluation. During the storage period the evaluated parameter was indicated that the FPC extracted from ribbon fish, *Lepturacanthus savala* was within the acceptable form up to 6 months.

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