



PRODUCTION AND CHARACTERISTICS OF CARP AND SHARK FISH PROTEIN CONCENTRATE

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ABSTRACT

This work was performed for the production of fish protein concentrate (FPC) from underutilized fish species (shark and carp fish). Results showed that FPC is a dehydrated product with high protein content having up to (88%) and low-fat. FPC is virtually odourless and tasteless powder. Also, it is an excellent source of highly digestible amino acids. This protein concentrate was stored at room temperature for six months to evaluate its stability. FPC was free of any biogenic amines due to freshly fish and therefore it is a safe for human healthy and high in nutritional value. FPC samples showed very good functional properties, high emulsifying and water holding capacity. Water holding capacity of minced carp and shark were 390 and 391% at zero time and increasing to 400 and 405% after processing carp and shark fish protein concentrate, respectively. Protein solubility of carp and shark fish were 55.14 and 60.64% and decreased to 34 and 37% after processing of carp FPC and shark FPC. Emulsifying capacity of carp and shark fish were 38.19 and 48.12 g oil/g protein and decreased to 18 and 20 g oil/g protein after processing of carp and shark FPC. It can be concluded that, fish proteins may be used as functional ingredients. Shark and carp fish protein concentrate (FPC) that contain an excellent amino acid composition in which lysine is particularly high, together with good level of other essential amino acid. This indicate that the protein of shark and carp fish protein concentrate could be considered as a rich source for essential amino acids.

Key words: Fish protein concentrate, functional properties, biogenic amines, nutritional quality.

INTRODUCTION

Fish is one of the most important sources of available animal protein, and has been widely accepted as good source of protein and other elements for the maintenance of a healthy body (Arannilewa *et al.*, 2005). Many of the underutilized fish species are not used because of consumer unfamiliarity, boniness (Carp fish) and unpleasing looks as whole fish. As the process disguises the original nature of fish, the consumer may accept products made from mince. In the utilization of low-value fish, considerable progress has been made through the development of minced meat technology. These problems have stimulated extreme research in recent years. Common carp is the

most common fish species in country, and the cyprinid fish are the predominant fish in world aquaculture with 54% of total world fish production (Tacon *et al.*, 2006).

Shark fish (*Carcharodon carcharias*) as marine fish species that present in the red sea in large quantities involve the small and large sizes. Shark meat has an average proximate composition of 75% moisture, 23% protein, 1.6% lipids and 1.4% ash. The protein is an over-estimation of the true protein value since the meat contains significant amounts of non-protein nitrogen (NPN) in the form of urea which contributes to the N concentration. Shark fish is a good source of some essential amino acids, especially lysine and threonine (78% of the daily requirements for an adult in a 100 g

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portion), but low in minerals (Brennan and Gormley, 1999; Bosch, 2012).

Trimethylamine (TMA) levels are used universally to determine microbial deterioration leading to fish spoilage. Fish use trimethylamine oxide (TMAO) an osmoregulant to avoid dehydration in marine environments and tissue in fresh water. Bacteria such as *Shewanella putrificiens*, *Aeromonas* spp., *psychrotolerant*, *Enterobacteriaceae*, *p. phosphoreum* and *Vibrio* spp. can obtain energy by reducing TMAO to TMA creating the ammonia-like off-flavours (Gram and Dalgaard, 2002). Biogenic amines can be produced and degraded by normal metabolic activities in animals, plants and microorganisms. These amines are mainly produced by microbial decarboxylation amino acids in foods Brink *et al.* (1990), Hala'sz *et al.* (1994) and Kim *et al.* (2009). The most important biogenic amines as histamine, tyramine, tryptamine, putrescine, and cadaverine, are formed from free amino acids namely histidine, tyrosine, tryptophane, ornithine and lysine, respectively. Spermidine and spermine arise from putrescine Zarei *et al.* (2011). Presence of histamine in fish or fish products is the most important quality indicator as chemical indicators for spoilage of fish and is considered as a threat to public health bodies Etkind *et al.* (1987) and Morris (2001).

Early worldwide interest in the use of fish meat as protein supplements led to development of a product, known as fish protein concentrate (FPC). Fish protein concentrate has been defined as the product resulting by removing of water and oil from fish, thus increasing the concentration of the protein and other nutrient materials. The quality of the materials used to prepare FPC will influence the quality of the produced material (Sen, 2005). Also, Park and Lin (2005) reported that protein recovery in fish protein concentrate processing depends on fish freshness, the water/meat ratio, washing time, washing cycle, and pH of the washing solution. Fish protein concentrate has up to 90% protein and superior functional characteristics such as solubility and oil-emulsification capacity. Such powders have been prepared from fish species including capelin, threadfin bream, and shark (Venugopal, 1997). The powder obtained from threadfin bream was colourless and odourless and had a protein-efficiency ratio comparable with that of casein. Its oil-emulsification

capacity and water solubility were 2–3 times higher than those of conventional protein powder prepared by drying and grinding fish meat (Sathivel, 2005).

This work was carried out to produce fish protein concentrate from carp and shark fish and evaluation impact of storage at room temperature on physical, chemical and organoleptic properties of fish protein concentrate.

MATERIALS AND METHODS

Materials

Shark fish as marine fish species (*Carcharodon carcharias*) was obtained from Elobore market, Cairo and Common carp-fresh water fish (*Cyprinus carpio*) was obtained from the Central Laboratory for Aquaculture Research, Abbassa, Abu Hammad district, Sharkia Governorate. Protein concentrate were prepared from carp and shark fish at the laboratory, National Research Centre (NRC), Egypt.

Methods

Preparation of fish protein concentrate

Fish protein concentrate was prepared from shark and carp fish according to the flow diagram Fig.1 most of the process was done at low temperature to prevent protein destruction:

Physical and Chemical analyses

Colour was measured using L*, a* and b* CIE Lab scale according to the methods reported by Park, 1995 as well as Young and Whittle (1985), where L* is the parameter that measures lightness, b* the tendency towards yellow and a* the tendency towards red. Measurements were obtained in a Hunter Lab model D25-9 colorimeter (D45/2°) (Hunter Associates Laboratory Inc., Reston, VA, USA). Protein solubility (PS) was determined as follow: One gram of product powder was added to 40 mL of distilled water and 3% NaCl. A Vortex mixer (Thermolyne Maxi Mix II, USA) was used for 2 min., to homogenize the samples. Aliquots were centrifuged (Hettich Universal 30 RF) at 6280 g for 5 min., and the supernatants collected for protein estimation. The protein solubility was calculated on the basis of 100% solubility of the protein (Venugopal *et al.*, 1996).

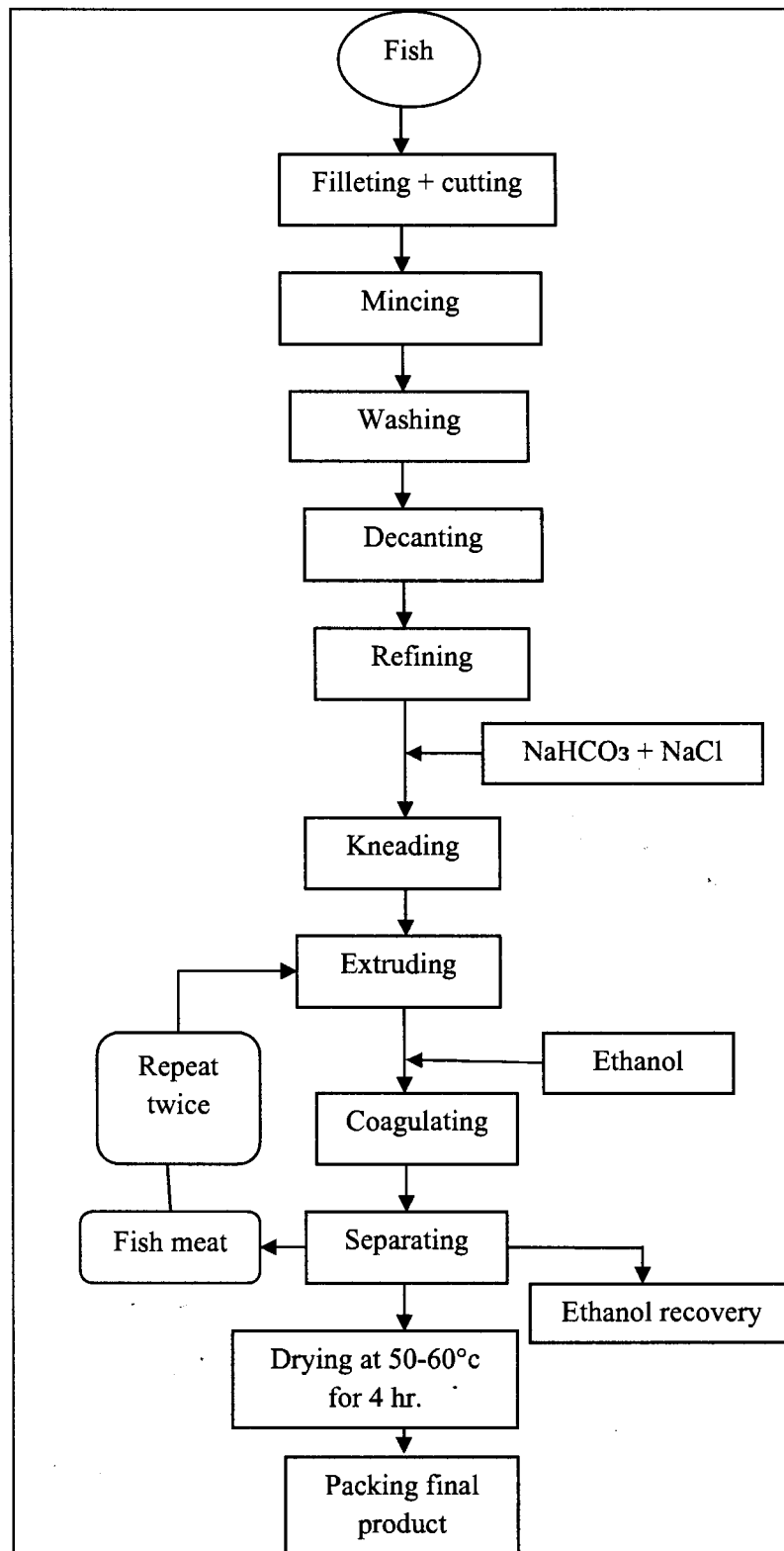


Fig. 1. Flow diagram of fish protein concentrate production (Morrissey, 1997)

Water holding capacity (WHC) was determined according to the method described by (Pipek *et al.*, 1999). Emulsifying capacity (EC) was determined according to the method described by (Yasumatsu *et al.*, 1972). Moisture, crude protein, ether extract and ash percentages were determined according to the method described by AOAC [2005]. Total volatile bases nitrogen (TVBN) and Trimethylamine nitrogen (TMAN) were determined according to Goulas and Kontominas (2005). Thiobarbituric acid value was determined according to the method described by Kirk and Sawyer (1991). Amino acids composition was determined using an automatic amino acid analyzer (LKB 4151 plus, Biochrom Ltd., Cambridge, UK) according to Bidlingmeyer *et al.* (1987). The degree of variability in different groups of amino acids (according to type of side chain) was expressed as percentages of total amino acids in each group and the ratio of essential amino acid was determined. Histamine, spermidine, cadaverine and spermine, were extracted and determined in all tested samples according to Majjala and Eerola (1993). The marked areas were determined in Microanalysis Centre, Faculty of Science, Cairo University using CS- 9000 Dual wavelength flying spot scanning densitometer (SHIMADZU) using wave length 254 nm. Standard curve of each dansylamine was used to calculate the concentrations of biogenic amines in the tested samples.

RESULTS AND DISCUSSION

Chemical Composition and Some Chemical Quality Attributes Of Raw Carp and Shark Fish

Table 1 shows the chemical composition and some chemical quality attributes of raw minced common carp (*Cyprinus carpio*) and Shark fish (*Carcharodon carcharias*). The fresh minced common carp contains 73.16% moisture, 15.64% protein, 10.07% fat and 1.14% ash. Also, the fresh minced shark fish contains 75.00% moisture, 23.00% protein, 1.50% fat and 1.40% ash. These values were in agreement with Lijubojevic *et al.* (2013). From the results it could be concluded that shark flesh contained low fat (1.5%), high protein content and low amount of trimethylamine. These values were in

agreement with Bosch (2012). The chemical composition of fish varies greatly from one species to another and from one individual to another depending on the diet, age, sex, environment and season (Guler *et al.*, 2008 ; Buchtova *et al.*, 2010).

The total volatile bases nitrogen (TVBN), trimethylamine nitrogen (TMAN) and thiobarbituric acid values (TBA) were 8.24 mg/100g, 1.08 mg/100g and 0.12 mg malonaldehyde/kg in raw minced common carp fish respectively and 11.80 mg/100g, 1.12 mg/100g and 0.08 mg malonaldehyde/kg in raw minced shark fish respectively (Table 1). These results are in agreement with Serkan *et al.* (2010).

Chemical Composition of Carp and Shark Fish Protein Concentrate

Table 2 shows the chemical composition of carp and shark fish protein concentrate. The results showed that the carp fish protein concentrate contained 9.10% moisture, 88.70% protein, 0.04% fat and 2.10% ash while the shark fish protein concentrate contained 8.55% moisture, 89.12% protein, 0.01 % fat and 2.20% ash. From the Table it could be observed that, the protein content in fish protein concentrate from both the two types of fish were nearly the same. On contrary, in raw materials, the protein content was higher in shark fish more than carp fish (Table 1). This difference could be related to the release of some soluble proteins with multiple washing processes during the preparation of the FPC. These values were in agreement with those of Bolly (2007).

Effect of Storage at Room Temperature on Functional Properties of Fish Protein Concentrate

Food protein additives are used because of their nutritional and/or functional properties. Functional properties include solubility, water holding capacity and emulsifying capacity. FPC can have a range of functional properties depending upon the used processing methods (Johnson, 1969).

Water holding capacity (WHC)

Data in Fig. 2 shows the changes in water holding capacity of minced fish and fish protein concentrate during storage at room temperature.

Table 1. Chemical composition and some chemical quality attributes of raw carp and shark fish

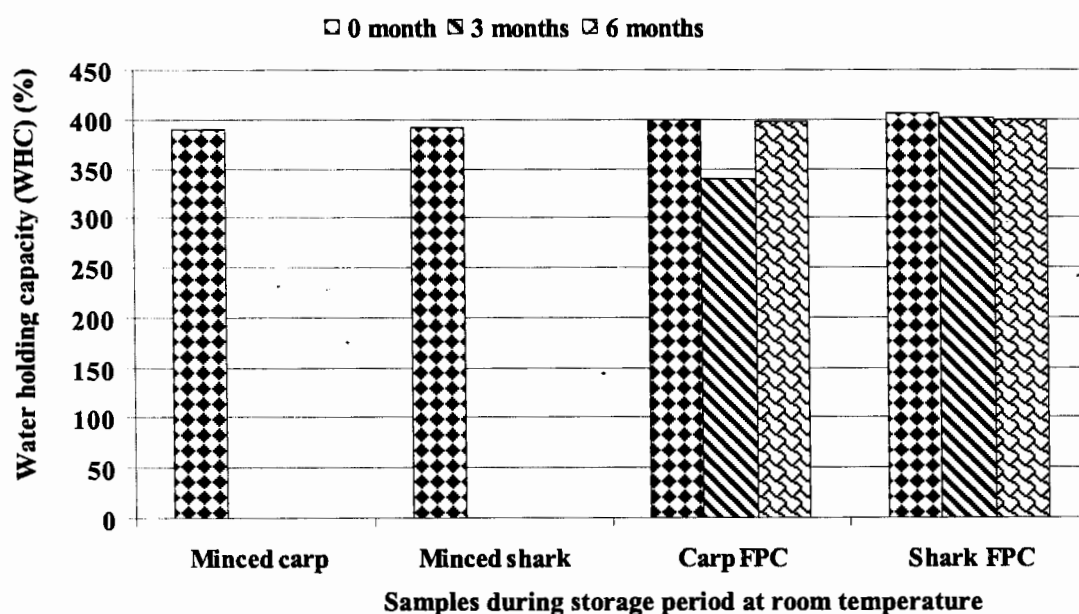
Items	Carp fish	Shark fish
Moisture content (%)	73.16	75.00
Crude protein (%)	15.64	23.00
Crude fat (%)	10.07	1.50
Ash content (%)	1.14	1.40
TVBN (mg/100 g)	8.24	11.80
TMAN (mg/100 g)	1.08	1.12
TBA (mg malonaldehyde/Kg)	0.12	0.08

On wet weight basis.

Table 2. Chemical composition of carp and shark fish protein concentrate

Items	Carp FPC	Shark FPC
Moisture content (%)	9.10	8.55
Crude protein (%)	88.70	89.12
Crude fat (%)	0.04	0.01
Ash content (%)	2.10	2.20

On wet weight basis, FPC: fish protein concentrate.

**Fig. 2. Water holding capacity of fresh minced fish and FPC samples**

The data shows that, water holding capacity of minced carp and shark were 390 and 391% at zero time and increased to 400 and 405% in carp and shark fish protein concentrate, respectively. These results are in agreement with Abou-Zaid and Elbandy (2014) who showed that protein extraction process caused the increase in the water holding capacity (WHC) value for crayfish protein concentrate powder (CPCP) sample when compared with the crayfish tail flesh sample. Also the most porous structure was formed during extraction as a result of removing some fatty matters and forming hydrogen bonds during the milling process of the CPCP. It may be the reason for more entrained water when compared with crayfish tail flesh sample. Nevertheless, this excess water may not be bound in tissue which can be checked during determination of water holding capacity (WHC) of both samples. Also, data showed that carp and shark fish protein concentrate were slightly decreased during six months of storage at room temperature, since was 400% and 405% at zero time, 399% and 402% after three months and decreased to 398% and 400% at the end of storage period (six months), respectively. These results are similar to that found by Ogunlade *et al.* (2005), who stated that the water holding capacity of the fish products are generally in the range of 280-404%.

Protein solubility (PS)

Protein solubility could be defined as the amount of protein or nitrogen constituent soluble in a particular buffer under specific conditions. It is a physicochemical property that is related to other functional properties and is influenced by amino acid composition and sequence, molecular weight of conformation and content of polar and non-polar groups in the proteins (Zayas, 1997). Data in Fig. 3 show the changes in protein solubility of minced fish and fish protein concentrate during storage at room temperature, Data shows that, protein solubility of carp and shark fish were 55.14 and 60.64% and decreased to 34 and 37% after processing of carp FPC and shark FPC, since they indicated that changes in solubility of muscle proteins under a variety of extracting conditions has been taken as a measure of change in protein conformation *i.e.*, denaturation, and has been

used as an indicator of the quality change that a stored muscle food has undergone. In addition, many functional properties of muscle food protein have been related to the solubilization of the protein in salt solution. Also, data show that protein solubility of fish protein concentrate were decreased during six months of storage, since it was 34% and 37% at zero time, 32% and 35% after three months and decreased to 30% and 33% at the end of storage period (six months), respectively. These results are in agreement with those found by Stefansson and Hultin (1994) and Srikar and Reddy (1991).

Emulsifying capacity (EC)

The emulsifying capacity is the maximum amount of fat emulsified by a protein dispersion. Oil is added at a given rate of a protein dispersion being constantly stirred, until the emulsion inverts into a water-in oil emulsion, as indicated by a sudden drop in emulsion viscosity (Van Hippel and Schleich, 1959).

Data in Fig. 4 shows the changes in emulsifying capacity of minced fish and fish protein concentrate during storage at room temperature. Data shows that, emulsifying capacity of carp and shark fish were 38.19 and 48.12 g oil/g protein and decreased to 18 and 20 g oil/g protein after processing of carp and shark FPC. Also, data shows that emulsifying capacity of fish protein concentrate from carp and shark fish was decreased during six months of storage, since was 18 and 20 g oil/g protein concentrate at zero time, 17 and 19 g oil/g protein concentrate after three months and decreased to 16.3 and 18.1 g oil/g protein concentrate at the end of storage period (six months) respectively. These results are nearly in agreement with those found by Dubrow (1973), Hermansson *et al.* (1971) and Srikar and Reddy (1991), since he stated that drying alcohol wet FPC solids at ambient conditions resulted in negligible loss in soluble protein and emulsifying capacity.

Amino acids of fish protein concentrate

Fish protein contains a well balanced amino acid composition. Fish is composed of 16-18 amino acids based upon the species type and seasonal variations (Haaland *et al.*, 1990). Data in Table 3 shows amino acids composition (%) of shark and carp fish protein concentrate (FPC) that contain an excellent amino acid composition in

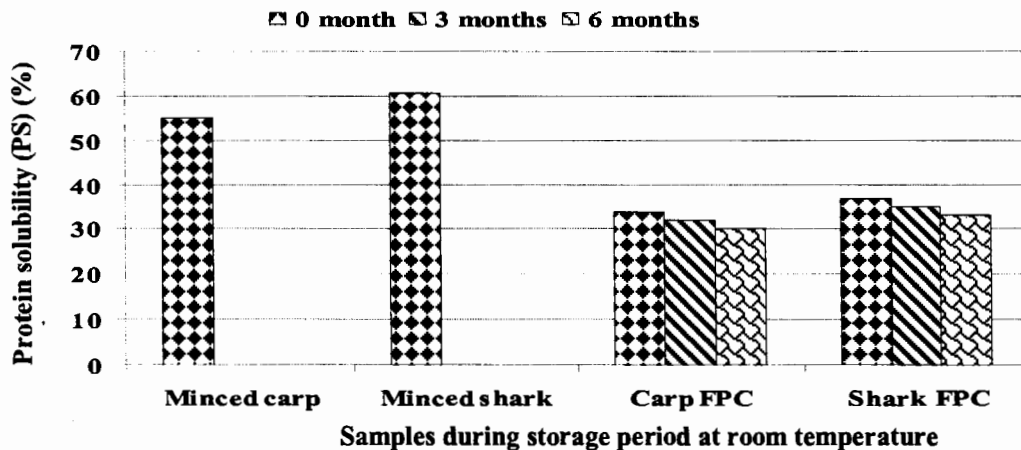


Fig. 3. Protein solubility of fresh minced fish and FPC samples

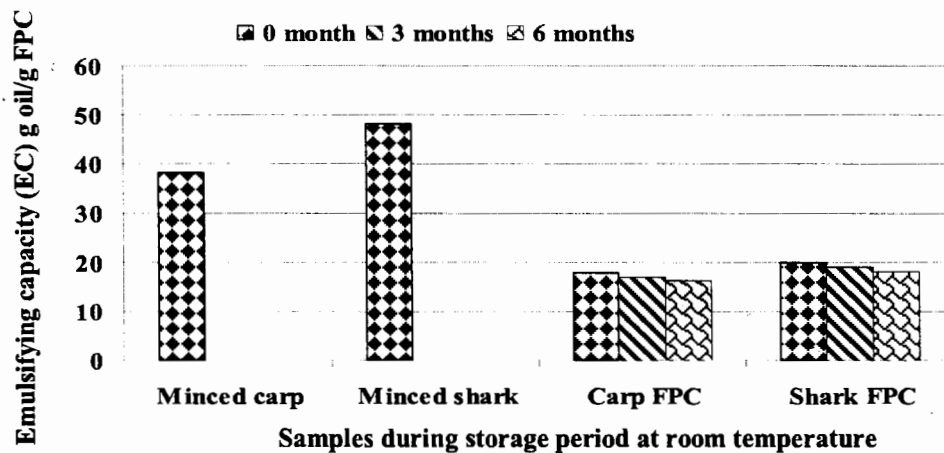


Fig. 4. Emulsifying capacity of fresh minced fish and FPC samples

Table 3. Amino acids composition (g/100 g sample) of carp and shark fish protein concentrate

Essential amino acids (%)	Carp FPC	Shark FPC	Non-essential amino acids (%)	Carp FPC	Shark FPC
Lysine	6.08	4.99	Aspartic	07.73	06.86
Therionine	3.38	2.77	Glutamic	10.51	10.98
Valine	3.61	3.33	Arginine	05.83	07.13
Methionine	2.05	2.06	Serine	02.93	02.79
Isoleucine	3.26	2.99	Proline	05.48	07.70
Leucine	5.66	4.63	Glycine	06.16	14.10
Phenylalanine	3.37	4.61	Alanine	05.34	07.76
Histidine	1.83	1.87	Cystine	1.22	1.07
			Tyrosine	2.46	2.11
Total EAAs	29.24	27.25	Total NEAAs	47.66	60.50
Total amino acids				76.90	87.75

EAAs: Essential Amino acids, NAAs: Non-Essential Amino acids.

which lysine is particularly high, together with good level of other essential amino acids. This indicates that the protein of shark and carp fish protein concentrate could be considered as a rich source for essential amino acids, this makes it a good supplement for food deficient in such essential amino acid. The results showed that, the fish protein concentrate of carp contained Glycine (6.16%) which was lower than that in shark fish (14.10), total amino acids found to be slightly lower than that of shark fish and Proline content of freshwater carps was 5.48% lower than that of shark 7.70%. These results are similar to that found by Sankar (2000) and Dust *et al.* (2005).

Biogenic amines (BA)

Biogenic amines are naturally occurring, low molecular weight organic bases, ubiquitous in animals and plants. Putrescine, cadaverine, spermidine and spermine have an aliphatic structure, histamine and tryptamine have a heterocyclic structure and tyramine and phenylethylamine have an aromatic structure. The formation of high levels of biogenic amines, especially histamine, in fish products may be rapid, and their development depends on the number of microorganisms present (Raslan and Hamed, 2012).

Data in Table 4 shows biogenic amine contents (mg/kg sample) of fresh minced fish as well as shark and carp fish protein concentrate. The results showed that, the fresh minced shark contain 4.315, 0.1375, 5.255 and 5.006 mg/kg of Histamine, Cadaverine, Spermidine and Spermine respectively. These results are nearly in agreement with those found by Bosch (2012).

Also, the results (Table 5) showed that the biogenic amine contents not detected in FPC of both two kinds of fish. Fish protein concentrate is free of biogenic amines meaning of product safety, freshly raw fish, high nutritive value and human healthy. The fish and its products are unsafe, if the amount of histamine in the muscular tissue of raw fish exceeded limit of 20 ppm and canned fish exceeded limit of 50 ppm (Maroni *et al.*, 1999 ; Arnold *et al.*, 1999). Product safety is not guaranteed by low histamine. It should be noted that the greater risk is produce of histamine in marine fish. Fresh water fish are not considered for the presence of

histamine as a threat to human health (Dalgaard and Rabi, 2009).

Colour Properties of Fresh Minced Fish and Protein Concentrate of Shark and Carp during Storage

Colour properties of shark and carp fish protein concentrate are shown in Table (5). There were significant differences in colour (L^* , a^* and b^*). It was observed that different treatment processing FPC affected the lightness, redness and yellowness of the samples. Comparison between the treatments showed that shark FPC had higher values of lightness (L^*) than the carp FPC. Generally, the lightness decreased with the advance of storage time for all treatments. Drying process reduced redness (a^*) value of samples for all treatments compared to control. This reduction in (a^*) values were very sharp for carp FPC. The significant decrease in (a^*) values indicated the change in colour from red to brown which could be due to the formation of metmyoglobin Devatkal *et al.* (2010).

Little differences in (a^*) values were observed for any samples during storage. Yellowness (b^*) values increased for all treatments after drying process and the b^* value of the shark FPC was lower than that for other treatments. In all cases as time of storage progressed, a slight increase in b^* values was noticed for all treatments. These results are nearly in a agreement with those found by Shahidi (1995) and Sequeira-Munoz *et al.* (2008).

Conclusion

Fish protein concentrate (FPC) is an animal protein with high quality, so it can be used as a protein supplement to increase nutritive value of foods. FPC is free of any biogenic amines due to freshly fish and therefore it is a safe, human healthy and high in nutritional value, FPC samples showed very good functional properties, high emulsifying and water holding capacity. Based on the results of this investigation, it maybe suggested that the production of shark and carp fish protein concentrate could be used safely for fortifying many types of food.

Table 4. Biogenic amines content of fresh minced fish as well as carp and shark fish protein concentrate (mg/kg sample).

Samples	Histamine	Cadaverine	Spermidine	Spermine
Minced carp	0.228	0.049	5.555	5.006
Minced shark	4.315	0.1375	5.255	5.006
Carp FPC	ND*	ND*	ND*	ND*
Shark FPC	ND*	ND*	ND*	ND*

ND*: not detected.

Table 5. Colour properties of fresh minced fish as well as shark and carp fish protein concentrate during storage at room temperature

Storage period (month)	Minced Carp			Minced Shark			Carp FPC			Shark FPC		
	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
0	46.50	5.39	14.80	55.42	3.72	16.50	56.11	2.69	25.09	68.34	1.42	19.84
	±0.15	±0.06	±0.19	±0.09	±0.28	±0.11	±0.23	±0.07	±0.14	±0.81	±0.04	±0.13
3	-	-	-	-	-	-	56.00	2.49	25.02	68.11	1.22	19.64
	-	-	-	-	-	-	±0.13	±0.007	±0.04	±0.01	±0.004	±0.03
6	-	-	-	-	-	-	55.12	1.69	26.22	67.13	0.99	20.54
	-	-	-	-	-	-	±0.22	±0.17	±0.14	±0.21	±0.04	±0.13

L* = lightness (100 = white; 0 = black), a* = redness (+100) to green (-80); b* = yellowness (70) to blue (-80).

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إنتاج وخواص مركز بروتين سمك المبروك والقرش

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تم تنفيذ هذا العمل لإنتاج مركز بروتين سمكي من أسماك القرش وأسماك المبروك، وتبين من النتائج أن المركز البروتيني ذو محتوى عالي من البروتين يصل إلى أكثر من (٨٨%)، كما أنه مصدر ممتاز للأحماض الأمينية، تم تخزين المركز البروتيني على درجة حرارة الغرفة لمدة ستة أشهر لتقييم درجة ثباته، وأظهرت النتائج أن المنتج خالي من الأمينات الحيوية وبالتالي فإنه ذو قيمة غذائية عالية وأمنة صحياً وأظهرت عينات المركز البروتيني خصائص وظيفية جيدة جداً، من استحلاب أو القدرة على الاحتفاظ بالماء، حيث كانت القدرة على الاحتفاظ بالماء لمفروم سمك المبروك وسمك القرش ٣٩٠ و ٣٩١% على التوالي، وأظهر المركز البروتيني زيادة في قدرة الاحتفاظ بالماء فقد وصلت إلى ٤٠٠ و ٤٠٥% عند بدء التخزين لمركزي بروتين أسماك المبروك وأسماك القرش، على التوالي، وكانت درجة ذوبانية البروتين لمفروم سمك المبروك وسمك القرش ٥٥,١٤ و ٦٠,٦٤%، وانخفضت هذه القيم إلى ٣٤ و ٣٧% في مركزي البروتين السمك، على التوالي، كما أظهرت النتائج أن القدرة على الاستحلاب لمفروم سمك المبروك وسمك القرش كانت ٣٨,١٩ و ٤٨,١٢ جرام زيت/جرام من البروتين وانخفضت هذه القيم إلى ١٨ و ٢٠ جرام زيت/ جرام من مركزي البروتين، ويمكن أن نخلص إلى أن بروتينات الأسماك يمكن أن تستخدم كمكونات وظيفية، وأن المركز البروتيني لأسماك المبروك وأسماك القرش ذو محتوى عالي من البروتين ومستوى جيد من الأحماض الأمينية الأساسية، هذا يشير إلى أنه يمكن اعتبار المركز البروتيني للأسماك تحت الدراسة مصدراً غنياً للأحماض الأمينية الأساسية.

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