

Fortification of Biscuits with Fish Protein Concentrate

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Abstract: This study was aimed to evaluate the impact of the fortification of biscuits with carp fish protein concentrate (CFPC) and shark fish protein concentrate (SFPC). The results show that the protein and total ash content increases by increasing the fortification level of both types of fish protein concentrate compared to the control sample. The crude fiber and carbohydrate of biscuit samples decreased by increasing the fortification levels compared to the control sample. Total Essential amino acids of biscuits were 2.99, 3.39 and 3.77% for biscuit control; biscuits fortified with 3% CFPC and 3% SFPC respectively. Besides, the corresponding value of Non-Essential amino acids were 4.22, 4.93 and 6.24 for biscuit control, biscuits fortified with 3% CFPC and 3% SFPC respectively. Acid value (AV), Peroxide value (PV) and Thiobarbituric acid number (TBA) increased gradually up to the end of the storage time in all samples. Biscuits prepared by fortification with CFPC and SFPC had a lower AV than control sample. From the obtained judging results, it could be observed that the fortification of CFPC and SFPC at levels 1, 2 and 3% into wheat flour blend used in biscuit making didn't cause any significant (at $p \leq 0.05$) deleterious effect on overall acceptability of produced biscuits which exhibited the hedonic scale of very good or excellent judging scores (7.5-9.5) and better acceptability.

Key words: Fish protein concentrate • Biscuits quality • Sensory quality • Nutritional quality

INTRODUCTION

Fish is one of the most important sources of animal protein in the range of 17-20% and other elements for the maintenance of a healthy body [1]. Farmed common carp production was nearly 14 percent of the total global freshwater aquaculture production in 2002 (33 138 962 tonnes). Common carp production increased by an average global rate of 9.5 percent/yr between 1985 (681 319 tonnes) and 2002. In the past decade (1993-2002) this has increased to 10.4 percent/yr. This is greater than the expansion rate of farmed grass carp (10.1 percent/yr), silver carp (8.8 percent/yr) and bighead carp production (7.2 percent/yr), but less than that for tilapias (11.8 percent/yr) during this decade. In Europe, common carp production was 144 602 tonnes in 2002. This represents a substantial reduction from peak production of over 402 000 tonnes in 1990, caused by changes in Eastern Europe. However, European production appears to be gradually increasing again; the 1993-2002 trough was 125 274 tonnes in 1997. Common carp production increased from (3 228 240 tonnes) in 2009 to (3 791 912

tonnes) in 2012 [2]. Mean while, the carp species are generally low in the food chain and have not been utilized widely due to a lot of inter-muscular bones [3]. The protein content of sharks forms approximately 22% of the flesh. Shark protein can be considered as a good source of essential amino acids and can serve as a cheap substitute to fulfill several amino acid deficiencies in protein-poor diets. In sharks, the largest fraction of this NPN consists of urea, which occurs not only in the liver (as in land animals), but all over the body [4, 5]. Fish Protein Concentrate (FPC) has been defined as the product resulting by removing of water and oil from fish, thus increasing the concentration of the protein, that it is an excellent source of highly digestible amino acids and other nutrient materials. The quality of the materials used to prepare FPC will influence the quality of the produced material [6, 7]. Bakery products in the Middle East especially in the Arab countries consider an important source of nutrients i.e. carbohydrate, protein, minerals and vitamins. Most bakeries products can easily be enriched and fortified with proteins, vitamins and minerals to meet specific needs of the target groups of the

population [8]. Good eating quality makes biscuits attractive for protein fortification and other nutritional improvements. Development of fortified biscuits or other composite flour bakery products is the latest trend in bakery industry. In the same time, most of the effort directed towards improving the diets of people is centered on the improvement of the protein content and quality of cereals by the addition of protein-rich foods, protein concentrates and/or essential amino acids. Also, it was found that 6% FPC is the best level for supplementation of biscuits [9] and 10% FPC increased nutritional value and percent of satisfaction values for some common foods [10]. The overall acceptability of salt biscuits was improved by the supplementation with 5% fish protein concentrate [11]. The present study was carried out to determine the possibility of producing high nutritional biscuits enriched with fish protein concentrate and the effect of storage on the quality of product.

MATERIALS AND METHODS

Biscuits ingredients (wheat flour (72% extract) - salt - sugar - shortening fat - vanilla - baking powder - eggs - milk) were purchased from local market, Cairo, Egypt. Fish protein concentrate was prepared from carp and shark fish at the laboratory, National Research Center (NRC), Dokki, Giza, Egypt as the following in Fig. 1.

Dough Characteristics: Wheat flour of 72% extraction was well mixed with CFPC and SFPC to produce individual mixtures containing 0, 1.0, 2.0 and 3.0% fortification levels to manufacture of biscuits (on flour weight basis). The rheological properties of dough was determined by Farinograph (Model Type No: 81010 (31, 50 and 63 rpm), Brabender, OHG, Duisburg, 1979, Germany) in department of food technology National Research Center (NRC) according to the standard methods (AACC) [12]. Parameters measured were water absorption, dough development time, dough stability, weakening and mixing tolerance index (MTI).

Preparation and Evaluation of Biscuits: Biscuits preparation was carried according to the method described by American Association of Cereal Chemists (AACC) [12]. The ingredients were weighed at the formula: 100 g of wheat flour of 72% extraction and CFPC and SFPC fortification at levels (0, 1.0, 2.0 and 3.0%); 50.0 g of fine sugar; 1.0 egg (≈ 50.0 g); 28.0 g of shortening; 0.93 g of salt; 1.11 g of sodium bicarbonate; 1.0 g of vanilla and 15 ml whole milk. The eggs were initially

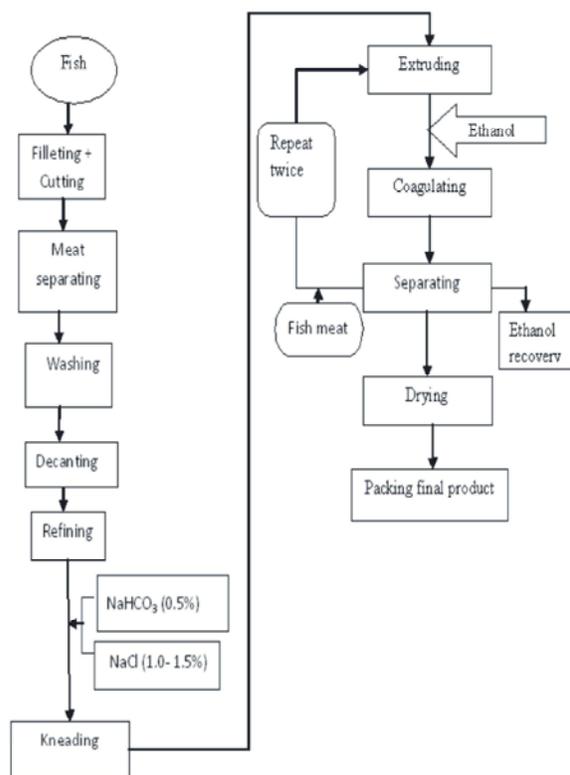


Fig. 1: Flow diagram of Marine beef (fish protein concentrate) production.

homogenized with a hand blender (Braun, Kronberg, Germany) for a few seconds. Then, add sugar and shortening and mixed for 20 sec. at speed 3, half of the flour and all other ingredients were then included and mixed for 20 sec. The remaining flour was added and mixed for 140 sec to give a total mixing time of 3.0 min. following a rest time of 20 min, then the dough was sheeted for 5 mm thickening with a diameter of 50 mm. Biscuits formed manually and baked at 180°C for 20 min. in a thermostatically controlled oven with air fan (Shel Lab 1370 FX, USA). The biscuits cooled at room temperature for 75.0 min.

Physical Characteristics of Biscuits

Baking Quality of Biscuits: Diameter (w) was measured by Boclase (HL 474938, STECO, Germany). Also, volume (v) and thickness (T) of biscuits were determined according to standard methods. The spread ratio W/T was calculated. Percent spread ratio was calculated according to standard methods described by American Association of Cereal Chemists (AACC) [12] by dividing the average value of diameter (w) by the average value of thickness (T) of biscuits.

Color Analysis: The color was measured by using a Hunter Lab. Model D25 color and difference Meter [13]. This color assessment system is based on the Hunter L*, a* and b* coordinates. L* representing lightness and darkness, + a redness, + b yellowness and - b blueness with white Tile of Hunter Lab color standard: (L=92.56, a=-0.87 and b= -0.15).

Major Chemical Composition: Moisture content, crude protein, crude fiber, fat content and total ash were determined according to the method described by AOAC [14]. Total carbohydrates were calculated by difference.

Amino Acids Composition: Amino acids composition was determined with an automatic amino acid analyzer (LKB4151 plus, Biochrom Ltd., Cambridge, UK) according to Bidlingmeyer *et al.* [15]. 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 species and the ratio of essential amino acid was determined.

Lipid Autoxidation: The acid value (AV), peroxide value (PV) and thiobarbituric acid value (TBA) were the parameters used for the assessment of lipid auto-oxidation. These three parameters were determined in the oil extracted from the samples of grounded biscuits using n-hexane [16]. The extracted oil was kept in a tightly closed dark bottle in a deep freezer at (-20°C) for subsequent analysis. The acid and peroxide values were determined according to the methods of AOAC [14]. Thiobarbituric acid value was determined as described by Kirk and Sawyer [17]. Stability of biscuit lipids were followed periodically at intervals of 1 month during storage for 3 months at ambient temperature.

Sensory Evaluation: Organoleptic properties of the biscuits were evaluated in National Research Center (NRC) department of food technology by 10 panelists according to Ogunjobi and Ogunwolu [18]. Five coded samples of biscuit were presented to each of the panelist and they were asked to assess the biscuits for color, flavour, taste, texture, appearance and overall acceptability using 10-point hedonic scale (0-2 = Dislike very much, 3-4.9 = Dislike moderately, 5-5.9 = Dislike slightly, 6-6.9 = Neither like nor dislike, 7-7.9 = Like slightly, 8-8.9 = Like moderately, 9-9.9 = Like, 10 = Like very much) for the assessment.

Statistical Analysis: The obtained results were statistically analyzed using SPSS statistical package (Version 9.05) according to McClave and Benson [19] analysis of variance (ANOVA), Duncan's multiple range test and least significant difference (LSD) was chosen to determine the significant difference among various treatments. Differences considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Fish protein concentrate (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 [20, 21]. Table 1 shows the chemical composition of wheat flour and carp fish protein concentrate and shark fish protein concentrate. From data presented in Table 1 it's clear that, the contents of moisture, protein, fat content, crude fiber and total ash were 11.2%, 12.00%, 1.56%, 1.02% and 0.86% in wheat flour, respectively. Similar results were obtained by Yassen *et al.* [22], who reported that the chemical composition (% on dry weight basis) of wheat flour (72%) were moisture 11.59%, protein 10.86%, fat 1.56% and ash 0.60%. Also, the results showed that the carp fish protein concentrate contained 9.10% moisture, 88.70% protein, 0.04% fat and 2.10% ash and the shark fish protein concentrate contained 8.55% moisture, 89.12% protein, 0.01% fat and 2.20% ash content. These values were in agreement with Bolly [23], who reported that the moisture content of (fish protein concentrate) marine beef in wet base is < 10% for good quality marine beef, protein content of marine beef was 60-89%, fat content was 0-3.71% and ash content was 2-3.5%. From these results, it could be concluded that the fish protein concentrate (FPC) are high protein content by removing maximum moisture and fat from fish and much lower in fat by removing with ethanol or isopropanol.

Rheological Properties of Dough Samples as Affected by Fortification FPC: Rheological properties of dough can be used as quality indicators for cereal products [24]. This fact has contributed to use rheological testing for following the changes in dough systems and mechanical properties. Farinograph parameters such as water absorption, arrival time, dough development time, dough stability, weakening and mixing tolerance index were different between control and blends congaing CFPC or SFPC at different levels are showed in Table 2. The water absorption was found in the range of 63.0% to 64.5% and

Table 1: Chemical composition of wheat flour, carp fish protein concentrate (CFPC) and shark fish protein concentrate (SFPC)

Compounds*	Samples		
	Wheat flour	CFPC	SFPC
Moisture content (%)	11.20	9.10	8.55
Crude protein (N% x 6.25)	12.00	88.70	89.12
Crude fat (%)	1.56	0.04	0.01
Ash content (%)	0.86	2.10	2.20

*On wet weight basis

Table 2: Effect of fortification of wheat flour with FPC on rheological properties of dough by farinograph

Parameters	Control	Carp fish protein concentrate (CFPC)			Shark fish protein concentrate (SFPC)		
		1%	2%	3%	1%	2%	3%
Water absorption (%)	63.5	63.0	63.5	64.5	64.0	64.5	65.0
Arrival time (min)	1.2	1.3	0.5	1.6	1.2	2.2	0.6
Dough development time (min)	1.75	2.00	1.50	2.00	1.75	3.50	1.20
Dough stability (min)	4.7	5.9	10.5	6.9	3.0	5.0	3.9
Weakening (BU)	55	40	20	30	110	30	30
Mixing tolerance index (BU)	40	30	10	20	60	30	20

Where: BU=Brabender units.

Table 3: Chemical composition of biscuit fortified with different levels of FPC

Chemical composition of samples	Control	Carp fish protein concentrate (CFPC)			Shark fish protein concentrate (SFPC)		
		1%	2%	3%	1%	2%	3%
Moisture	3.56±0.12 ^a	3.68±0.05 ^b	3.80 ±0.03 ^a	4.00 ±0.01 ^b	3.90±0.14 ^a	4.20 ±0.02 ^b	4.26 ±0.01 ^a
Protein	9.51±0.03 ^b	10.22±0.08 ^a	11.10±0.01 ^b	12.00±0.05 ^b	10.24±0.11 ^a	11.21±0.06 ^a	12.11±0.03 ^b
Crude fiber	0.60±0.03 ^b	0.59±0.01 ^a	0.58±0.14 ^a	0.57±0.05 ^b	0.59±0.03 ^b	0.58±0.04 ^a	0.57±0.14 ^a
Total ash	1.80±0.08 ^a	1.80±0.03 ^b	1.84±0.01 ^a	1.88±0.02 ^b	1.82±0.11 ^b	1.86±0.06 ^a	1.88±0.05 ^b
Fat content	21.30±0.06 ^a	21.31±0.11 ^a	21.32±0.03 ^b	21.33±0.08 ^a	21.30±0.01 ^b	21.31±0.04 ^a	21.32±0.08 ^a
Carbohydrate*	63.23±0.11 ^a	62.40±0.08 ^a	60.36±0.03 ^a	58.22±0.05 ^b	62.15±0.02 ^b	59.84±0.06 ^a	58.86±0.04 ^a

The values are mean ± S.D of three independent determinations. The values with different superscripts in a column differ significantly ($p \leq 0.05$). * Calculated by difference method

Table 4: color quality of biscuits from different formulas

Fortification treatment	Color characteristics value					
	Upper surface of biscuit			Bottom surface of biscuit		
	L*	a*	b*	L*	a*	b*
Control	60.52±0.05 ^{de}	11.25±0.06 ^a	25.52±0.14 ^b	65.41±0.03 ^a	10.52±0.11 ^c	26.00±0.14 ^{abc}
1% SFPC	58.82±0.08 ^d	12.68±0.14 ^a	22.10±0.05 ^a	65.71±0.08 ^{bed}	11.01±0.03 ^{ab}	25.72±0.11 ^{cd}
2% SFPC	57.50±0.02 ^a	12.99±0.16 ^c	25.14±0.06 ^a	66.21±0.11 ^{bc}	11.54±0.02 ^a	26.24±0.03 ^{ab}
3% SFPC	56.73±0.07 ^c	13.32±0.07 ^a	25.74±0.08 ^a	67.45±0.11 ^{bc}	11.80±0.05 ^a	26.99±0.07 ^{bed}
1% CFPC	58.71±0.11 ^c	12.39±0.03 ^a	21.93±0.11 ^c	65.58±0.06 ^{cd}	10.88±0.07 ^b	25.58±0.09 ^d
2% CFPC	57.32±0.08 ^c	12.75±0.09 ^c	22.99±0.06 ^a	66.05±0.02 ^c	11.32±0.08 ^a	25.02±0.12 ^c
3% CFPC	56.59±0.02 ^a	13.14±0.11 ^c	25.56±0.08 ^a	67.36±0.05 ^c	11.65±0.06 ^a	26.71±0.16 ^c

Means with the same superscripts at the same column are not significant at ($p < 0.05$), L = lightness (100 = white; 0 = black), a* = redness to a- = green; b* = yellowness to b- = blue.

Table 5: Amino acids composition (%) of biscuit control and fortified with 3% of FPC

Essential amino acids	Biscuit control	3% CFPC	3% SFPC	Nonessential amino acids	Biscuit control	3% CFPC	3% SFPC
Lysine	0.21	0.31	0.39	Aspartic	0.45	0.58	0.68
Therionine	0.23	0.29	0.34	Glutamic	1.84	2.02	2.09
Valine	0.40	0.41	0.50	Arginine	0.31	0.40	0.59
Methionine	0.14	0.15	0.19	Serine	0.38	0.43	0.51
Isolucine	0.29	0.31	0.38	Proline	0.71	0.76	1.02
Leucine	0.55	0.61	0.69	Glycine	0.26	0.38	0.79
Phenylalanine	0.51	0.56	0.56	Alanine	0.27	0.36	0.56
Tyrosine	0.28	0.32	0.32	--	--	--	--
Histidine	0.20	0.22	0.24	--	--	--	--
Cystine	0.18	0.21	0.16	--	--	--	--
Total EAAs	2.99	3.39	3.77	Total NAAs	4.22	4.93	6.24
Total AAs	--	--	--	--	7.21	8.32	10.01

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

64.0% to 65.0% in blends containing CFPC and blends containing SFPC. However, the blends containing SFPC showed higher water absorption due to more protein. Compared to the control, water absorption was increased by the addition of CFPC or SFPC. Similarly, the dough development time was higher in all blends compared to control. The addition of CFPC or SFPC increased the stability of dough blends compared to the controls at levels 1% and 2%. While weakening and mixing tolerance index decreased by addition CFPC or SFPC at different levels. These results are in agreement with those reported by Abou-Zaid and Elbandy [25].

Chemical Composition of Biscuit Fortified with Different Levels of FPC: The chemical composition for different samples of biscuits fortified with CFPC and SFPC at levels 0, 1, 2 and 3% are showed in Table 3. It was observed that, the protein and total ash content increases by increasing the fortification level of both types of FPC compared to the control sample. On the other hand, crude fiber and carbohydrate of biscuit samples decreased by increasing the fortification levels compared to the control sample. These results are in agreement with those obtained by Ibrahim [26].

Physical Characteristics of Biscuits

Color Values of Biscuits: The data presented in Table 4 shows the color attributes of the biscuits. In regard to the surface color, it was cleared that the addition of CFPC and SFPC decreased the color characteristics as CFPC and SFPC levels increased. The lightness (L^*) value of crust surface decreased, but the redness (a^*) value increased. These results also showed that, the highest increasing in redness (a^*) and yellowness (b^*) found in biscuit samples fortified with FPC at levels 2 and 3% from the control. These results were in agreement with those reported by Abou-Zaid and Elbandy [25].

Amino Acids Composition (%) of Biscuit Fortified with 3% of FPC: Table 5 shows the amino acids compositions (%) for biscuits (control) and fortified with 3% of CFPC and SFPC. Total Essential amino acids were 2.99, 3.39 and 3.77%, respectively. Besides, the corresponding value of non-essential amino acids were 4.22, 4.93 and 6.24, respectively. From the obtained results, it could be illustrated that the CFPC and SFPC composed of all essential amino acids which were higher than control. Therefore, FPC had a good nutritional protein quality. These results are nearly in agreement with those obtained by Ibrahim [26].

Chemical Quality Attributes: The least stable macro-components in food are the lipids. The biscuits become unacceptable and are rejected by the panelists as a result of rancidity development. To prolong the shelf-life of such foods and prevent the occurrence of rancidity, the presence of antioxidants is required. The changes in fat quality parameters, i.e. AV, PV and TBA of the biscuits fortified with SFPC and CFPC were followed throughout the storage period for 3 months at room temperature and the results obtained in Table 6. From the table, it could be noticed that AV, PV and TBA increased gradually up to the end of the storage time in all samples. Biscuits fortified with SFPC and CFPC had a lower AV than control. Similar findings were reported by Jeyasanta *et al.* [27] since they showed that peroxide values were within the acceptable limit of 10-20 mEq/kg of fat throughout the storage period. The Thiobarbituric acid value also was well with in the acceptable limit which was less than 3 mg malonaldehyde kg^{-1} of sample which indicates good quality fishery products. This indicates that the level of quality indicators such as hydrolysis of lipid (FFA), primary and secondary lipid oxidation does not increase during storage. This is because there was low fat content in the sample and also there was increase in extraction of total lipids at the time of processing.

Table 6: Effect of storage period on the chemical attributes of the stored biscuit

Treatments	Time of storage (month)											
	0			1			2			3		
	AV	PV	TBA	AV	PV	TBA	AV	PV	TBA	AV	PV	TBA
Control	0.62	3.20	0.010	0.92	5.20	0.021	1.15	7.5	0.030	1.46	9.36	0.092
1% SFPC	0.63	3.22	0.011	0.93	7.00	0.027	0.96	7.75	0.031	1.11	9.21	0.090
2% SFPC	0.64	3.24	0.012	0.94	7.30	0.028	0.94	7.66	0.032	1.10	9.21	0.092
3% SFPC	0.65	3.26	0.014	0.92	7.40	0.029	0.93	7.50	0.034	1.12	9.00	0.095
1% CFPC	0.64	3.23	0.013	0.92	7.10	0.028	1.11	8.23	0.032	1.30	9.40	0.092
2% CFPC	0.65	3.25	0.015	0.93	7.20	0.029	1.07	8.25	0.035	1.33	9.36	0.094
3% CFPC	0.67	3.27	0.017	0.94	7.30	0.030	1.11	8.21	0.038	1.29	9.33	0.096

*AV: acid value; PV: peroxide value; TBA: thiobarbituric acid.

Table 7: Sensory characteristics of produced biscuits as affected by the fortification of SFPC and CFPC

Samples	Appearance (10)	Color (10)	Texture (10)	Taste (10)	Flavor (10)	Overall acceptability (10)
Control	9.0±0.63 ^a	9.5±0.57 ^a	10.0±0.95 ^a	10.0±0.85 ^a	9.0±0.58 ^a	9.6±0.77 ^a
1% SFPC	8.5±1.25 ^a	9.5±1.60 ^a	8.6±0.71 ^b	8.5±0.70 ^b	9.0±0.95 ^a	9.3±0.79 ^a
2% SFPC	8.0±0.76 ^b	9.5±0.95 ^a	7.5±0.63 ^c	7.0±1.22 ^c	8.5±0.67 ^b	9.4±0.92 ^a
3% SFPC	7.0±0.88 ^c	9.3±1.00 ^a	7.5±0.67 ^d	8.5±1.52 ^d	8.0±0.79 ^c	9.2±1.30 ^a
1% CFPC	9.0±0.60 ^a	9.4±0.74 ^a	10.0±0.75 ^a	9.0±1.06 ^a	9.0±0.75 ^a	9.5±0.70 ^a
2% CFPC	8.8±1.14 ^a	9.5±0.96 ^a	7.5±1.52 ^c	7.0±1.06 ^c	9.0±0.84 ^a	9.4±1.03 ^a
3% CFPC	8.5±1.23 ^a	9.2±0.96 ^a	6.5±0.21 ^d	6.0±1.03 ^{cd}	8.4±0.67 ^b	9.4±1.34 ^a
L.S.D 0.05*	0.45	0.51	Ns	Ns	0.6	Ns**

*Means with the same superscripts at the same column are significant at (p<0.05), **Ns: not significant

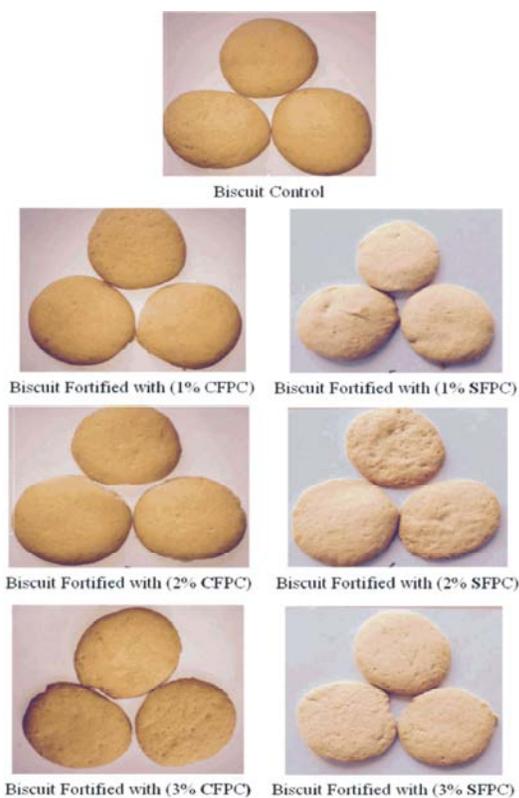


Fig. 2: Biscuits fortification with carp and shark fish protein concentrate.

Sensory Characteristics of Produced Biscuits as Affected by the Fortification of SFPC and CFPC: The effect of fortification of SFPC and CFPC at different levels (0, 1, 2 and 3%) on the judging scores of sensory quality characteristics; flavor, color, taste, appearance, texture and overall acceptability of produced biscuit was studied and the obtained results are shown in Table 7 and Fig. 2. From the obtained judging results, it could be observed that the fortification of SFPC and CFPC at levels 1, 2 and 3% into wheat flour blend used in biscuit making didn't cause any significant (at $p \leq 0.05$) effect on overall acceptability of produced biscuits which exhibited the hedonic scale of very good or excellent judging scores (7.5-9.5) and better acceptability. These results are in agreement with those reported by Abou-Zaid and Elbandy [25].

CONCLUSION

In conclusion the present work provides that raw carp and shark fish are generally considered as a good source to produce FPC. The FPC made from carp and shark fish had high nutritional value and it was more effective for fortification biscuits whereas, it maximizing the amount of protein content. Moreover, the level used (1, 2 and 3%) didn't affect on the appearance and other

sensory characteristics. So, the biscuit making potential increases in its nutritional characteristics with increasing levels of incorporation of protein concentrate.

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