

Nutritional Assessment of Crackers Supplemented with Tilapia Fish Protein Concentrate

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Abstract: This study was carried out to evaluate the chemical quality criteria and nutritional quality characteristics; with regards the gross chemical composition, essential minerals content and amino acids composition, of tilapia fish protein concentrate powder (FPCP) as well as to determine the effect of the incorporation of FPCP at different levels (0, 5, 10, 15 and 20%) as a source of animal protein, on quality characteristics of produced crackers. Results revealed that FPCP contained high level of protein 75.32% as dry weight and interesting amounts of ash 2.14%. Also, FPCP was rich in minerals such as P (695.35 mg/100g), K (596.76 mg/100g), Mg (249.58 mg/100g), Na (237.54 mg/100g), Ca (121.87 mg/100g) and Zn (18.899 mg/100g) as average. Also, FPCP contained high levels of essential amino acids such as lysine, methionine and cysteine content and a high digestibility and biological value. On the basis of nutritional value, crackers containing 20% FPCP had significantly higher protein and ash contents than the control samples. From the obtained judging results, it could be noticed that cracker containing 10% FPCP had the highest scores for color, texture and overall acceptability compared with control sample.

Key words: Tilapia Fish Protein Concentrate Powder • Crackers • Nutritional Quality • Sensory characteristics

INTRODUCTION

Fish can play a vital role as an important source of protein in human nutrition [1], they are source of vitamins, minerals and essential fatty acids [2, 3] and, therefore, capable of increasing the nutritional value of the diet and foods receiving their supplementation. Demands for fish protein ingredients including dried fish protein to develop functional food or ready-to-eat products are gradually growing in the world [4]. rotein concentrates are widely used as ingredients in food industry because of their high nutritional quality, functional properties, high protein level and low content of antinutritional factors [5].

Fish protein powder (FPP) is a dried and stable fish product, intended for human consumption, in which the protein is more concentrated than in the original fish flesh [6]. Global Food and Agriculture Organization (FAO) has defined Fish protein concentrate as a product of flour for human consumption made from whole fish flesh, by eliminating most of the fat and water content, so that the percentage of obtained protein content is higher than the

original raw material [7]. Fish powder is one type of fish and fishery product with concentrated protein content than in fresh fish [8].

Biscuits are widely accepted and consumed in many developing countries, so its need supplementation with another sources of proteins such as legumes and milk proteins to improve their nutritional values [9]. 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 and other nutrient materials [10] and FPC is a functional ingredient that can be used for enhancing the nutritional value of food products [11].

Biscuits are the most popular baker products consumed nearly by all sections of the society in Egypt. Some of the reasons for such wide popularity are low cost as comparing with other processed foods affordable cost, good nutritional quality and availability in different forms, varied taste and longer shelf life. Bakery products are sometimes used as a vehicle for incorporation of different nutritionally rich ingredients [12, 13].

The goal of the present study was to evaluate the chemical composition, minerals content, nutritional quality and sensory attributes of crackers containing tilapia fish protein concentrate powder (FPCP) prepared at different levels of FPCP.

MATERIALS AND METHODS

Materials: Fresh Nile Bolti fish (*Tilapia nilotica*), small and large size, were obtained from El-Burullus Lake and transported in an ice-box to the laboratory. The fish samples were washed by tap water and heads, heads, viscera and skin were removed, washed again and minced using a meat mincer and dried in hot air oven at 60°C.

The other ingredients including wheat flour (72% extraction ratio), salt, shortening, sodium bicarbonate, dried active bakers' yeast were all purchased from super markets, Alexandria, Egypt.

Methods

Preparation of Fish Protein Concentrate: Fish protein concentrate from Tilapia fish meat (o edible parts) was extracted by using solvent isopropyl alcohol 70 % (food grade) and dried in hot air dryer at 400C. Finally, it was milled using a grinder and was sieved through size of mesh 60 [14].

Preparation of Crackers Supplemented with FPC: The cracker formula included 100g wheat flour (72% extraction), 2g sugar, 5g whole egg, 2g salt, 2g dried active bakers' yeast and 60 ml water (Table, 1). Ingredients were mixed into cohesive dough, rolled into a consistent, thin sheet using a pasta roller and cut in pieces after proofing. The crackers were baked at 170°C for 15 min. then cooled at ambient temperature [15]. The fish protein concentrate powder (FPCP) was added to the crackers at levels of 5, 10, 15, 20% based on the weight of wheat flour.

Physical Characteristics of Cracker: Weight (g), diameter (cm) of crackers was measured by Boclase (HL 474938, STECO, Germany and thickness or height (cm) of crackers were determined according to standard methods. The spread ratio diameter /height was calculated. Percent spread ratio was calculated according to standard methods described by American Association of Cereal Chemists [16] by dividing the average value of diameter by the average value of thickness or height of crackers.

Table 1: Ingredients of crackers dough formula supplemented with FPCP

| Ingredient | Crackers | | | | |
|--------------------|----------|--------|--------|--------|--------|
| | Control | 5% | 10% | 15% | 20% |
| FPCP* | 0.0 | 5 | 10 | 15 | 20 |
| Wheat flour (72%) | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Salt | 2.02 | 2.02 | 2.02 | 2.02 | 2.02 |
| Shortening | 9.64 | 9.64 | 9.64 | 9.64 | 9.64 |
| Sodium bicarbonate | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 |
| Dry Yeast | 3.48 | 3.48 | 3.48 | 3.48 | 3.48 |
| Water | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 |
| Total | 165.3 | 170.3 | 175.3 | 180.3 | 185.3 |

*FPCP: Fish protein concentrate powder

Analytical Methods

Proximate Composition: The proximate analysis was determined according to AOAC [17]. The moisture content was determined by drying in a forced air convection oven at 105°C until a constant weight was obtained. Crude protein content (N×6.25) was determined using the Kjeldahl method. Crude fat was determined using petroleum ether (40 - 60°C) in Soxhlet apparatus. Ash was determined using a muffle furnace at 550°C. Total carbohydrates were calculated by the difference.

Minerals: All glassware was washed overnight in a solution of 10% HCl in deionized distilled water (ddH₂O, v/v) prior to use. Ashed samples were dissolved in 2mL of 70% nitric acid. The acidified samples were neutralized in 5 mL of ddH₂O and filtered through Whatman No.1 paper. Samples were diluted to volume with ddH₂O in a 50-mL volumetric flask [18]. Minerals content were determined using inductively coupled plasma optical emission spectrometry (model P400; Perkin Elmer, Shelton, Conn., U.S.A).

Amino Acids Analysis: Amino acids composition determination was performed according to the method of Duranti and Cerletti [19]. The dried samples were digested with HCl (6M) with 0.1% mercaptoethanol in an evacuated tube at 110°C for 24 h. After cooling at room temperature, the hydrolyzed samples were filtered through Whatman No. 1 filter paper and the filtrate was diluted with distilled water to 25ml in a volumetric flask, Five ml of the diluted filtrate was dried in a vacuum desiccator in the presence of potassium hydroxide. The resultant dried residue was dissolved in 1 ml of sodium citrate buffer (pH 2.2) and stored at 4°C until analysis by using Amino Acid Analyzer (Beckman amino acid analyzer, Model 119 CL). The amino acid composition was expressed as g of amino acid per 100g of protein.

Amino Acid Score (AAS): Amino acid score (AAS) was calculated according to Bhanu *et al.* [20] as the following equation:

$$AAS \% = \frac{\text{g amino acid of sample}}{\text{g same amino acid of FAO/WHO reference}} \times 100$$

In vitro Protein Digestibility: In vitro protein digestibility was carried out according to the method described by Elkhailil *et al.* [21] with slight modification. Twenty mg of samples were digested in 10 ml of trypsin (0.2 mg/ ml in 100 mM Tris-HCl buffer, pH 7.6). The suspension was incubated at 37°C for 2h. Hydrolysis was stopped by adding 5 ml 50% trichloroacetic acid (TCA). The mixture was allowed to stand for 30-35min at 4°C and was then centrifuged at 10000×g for 25 min. The resultant precipitate was dissolved in 5 ml of NaOH and protein concentrate was measured using the Kjeldahl method. Digestibility was calculated as follows:

$$\text{Protein digestibility (\%)} = \frac{(A - B)}{A} \times 100$$

where:

A= Total protein content (mg) in the sample

B=Total protein content (mg) in the TCA precipitate

Biological Value (BV): The (B.V.) was assayed according to the following equation which was recommended by Oser [22].

$$BV = 49.09 + 10.53 (\text{PER})$$

where:

BV = Biological value

PER = Protein efficiency ratio

Protein Efficiency Ratio (PER): The protein efficiency ratios of the tested samples were based on their amino acids contents according to the recommendations of Alsmeyer [23]. However, the following equations were used:

$$PER = -0.468 + 0.454 (\text{Leucine}) - 0.105 (\text{Tyrine})$$

Microbiological Analysis: Ten grams of tested samples were suspended in 90 ml sterilized peptone water. Decimal dilutions were plotted to determine total viable count as log10 CFU/g [24].

Sensory Evaluations: Cracker samples were subjected to the sensory evaluation test along with the control. Ten trained panelists were asked to evaluate the samples according to the method described by Hooda and Jood [25] on hedonic scale consisting of 9 points ranged from 1 (extremely dislike) to 9 (extremely like). Crust color, texture, taste, odor and overall acceptability of cake samples and color, texture, taste, odor and overall acceptability of cracker samples, were subjectively evaluated.

Statistical Analysis: The obtained data were statistically analyzed by SPSS computer software. The calculated occurred by analysis of variance ANOVA and follow up Duncan's multiple range tests by SPSS ver.11 according to Abo-Allam [26].

RESULTS AND DISCUSSION

Gross Chemical Composition of Tilapia Fish Concentrate and Wheat Flour: Proximate compositions of wheat flour and tilapia fish protein concentrate (FPCP) are shown in Table (2). Results indicated that protein content of FPCP is especially high, 67.0%. From the obtained data it's clear that the contents of moisture, crude protein, crude lipids, ash, crude fiber and total carbohydrates were 11.36, 11.75, 1.33, 0.74, 0.35 and 75.47% in wheat flour, respectively. These results are in accordance with Yaseen *et al.* [27] 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 tilapia fish protein concentrate contained 6.94% moisture, 75.32% protein 6.88% crude lipids, 2.14% ash, 0.02 fiber and 8.72% carbohydrates, respectively. In this respect, Mohamed *et al.* [28] reported that 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. Chalamaiah *et al.* [29] reported that total ash content of dehydrated and defatted protein concentrates was found to be 5.95 and 1.95 %, respectively.

Concerning bacterial load, the recorded TVC was 3.50 and 50.23×10^2 cfu/g in wheat flour and FPCP samples, respectively, while total moulds and yeasts were 0.38×10^2 cfu/g and 1.42×10^2 cfu/g, respectively. These results agree with Hammad [30] who found that wheat flour contain 3.50×10^2 cfu/g total bacteria count and 0.43×10^2 cfu/g moulds and yeasts.

Table 2: Chemical composition (%) and total microbial count ($\times 10^2$ cfu/g) of wheat flour and Tilapia fish protein concentrate (FPCP)

| Constituent | Wheat flour | FPCP* |
|--------------------------|-------------------------------|-------------------------------|
| Moisture | 11.36 \pm 0.23 ^a | 6.94 \pm 0.14 ^b |
| Crude protein | 11.75 \pm 0.12 ^b | 75.32 \pm 0.22 ^a |
| Crude lipids | 1.33 \pm 0.05 ^b | 6.88 \pm 0.13 ^a |
| Ash | 0.74 \pm 0.03 ^b | 2.14 \pm 0.09 ^a |
| Crude fiber | 0.35 \pm 0.01 ^a | 0.02 \pm 0.00 ^b |
| Carbohydrates** | 75.47 \pm 0.24 ^a | 8.72 \pm 0.04 ^b |
| Total viable count (TVC) | 3.50 \pm 0.04 ^b | 5.23 \pm 0.20 ^a |
| Moulds and yeasts | 0.38 \pm 0.11 ^b | 1.42 \pm 0.13 ^a |

*FPCP = Fish Protein Concentrate Powder

** Available carbohydrates were calculated by difference

Values are means of triplicates \pm standard deviationValues with the same superscript in a row are not significantly different ($P > 0.05$).

Minerals Content of Tilapia Fish Concentrate: Macro minerals such as sodium, potassium, magnesium, calcium and phosphorus and trace minerals (iron, manganese, copper and zinc) are needed for the regulation of healthy functions in the human body. In addition, calcium, phosphorus and magnesium are involved in bone health [31]. When studying Nile tilapia fillets, Marengoni and Santos [32] observed values were ranged from 1.33 to 1.56 g/kg of magnesium and 16.33 to 15.66 g/kg of zinc; this suggests that this species contains considerable amounts of these minerals.

Table (3) represents values of minerals content for tilapia fish protein concentrate powder. From the obtained results, it could be observed that FPCP was rich in P (695.76 mg/100g), K (596.35 mg/100g), Mg (249.58 mg/100g), Na (237.54 mg/100g), Ca (121.87mg/100g), Zn (18.90mg/100g), Fe (1.75 mg/100g), Cu (0.506 mg/100g) and Mn (0.468 mg/100g). Magnesium, manganese and zinc are responsible for regulating the activity of several

enzymes [33]. Therefore, the products which were prepared from tilapia fish concentrate are considered as a good source for minerals, where the major minerals were Na, K, P, Ca, Mg and Zn. These results agree with Sartori and Amancio [34] who reported that fish meat is a main source of calcium and phosphorus, but also contains iron, copper and selenium.

Specific Volume of Supplemented Crackers with FPCP: Data presented in Table (4) indicate the physical characteristics of produced crackers.

The results indicated that diameter and thickness of crackers were slightly increased within creasing supplementation percentage of tilapia fish protein concentrate powder (FPCP) compared with control cracker. Also, spread ratio was increased from 2.192 in control sample to 2.212 in sample containing 20%. These may be due to the weakening of gluten network. These are good results because crackers or cookies having high spread ratios are more desirable [35].

Chemical Composition of Crackers: Data given in Table (5) showed the proximate analysis of crackers supplemented by different levels of dried tilapia fish protein concentrate powder. Moisture contents of crackers ranged from 4.72 to 5011%. In this respect, Kure *et al.* [36] reported that 10% moisture was suggested as the upper limit needed for the biscuits to prevent spoilage by microorganisms and to increase the shelf life. It could be observed that moisture content of all cracker samples were below this level. In addition, the moisture content has an important effect on the shelf-life of the cracker and the type of cookie may influence the occurrence of spontaneous breaking or cracking [37].

Table 3: Minerals content of FPCP (mg/100g)

| Minerals (mg/100g) | Ca | Mg | K | P | Na | Fe | Mn | Cu | Zn |
|--------------------|--------|--------|--------|--------|--------|------|-------|-------|--------|
| FPCP | 121.87 | 249.58 | 596.76 | 695.35 | 237.54 | 1.75 | 0.468 | 0.506 | 18.899 |

Table 4: Specific volume of the control cracker and the crackers supplemented with different levels of FPC

| Treatment | Weight (g) | Diameter (cm) | Height (cm) | Spread ratio* |
|-----------|-------------------------------|------------------------------|------------------------------|--------------------------------|
| Control | 11.29 \pm 0.10 ^c | 5.48 \pm 0.11 ^c | 2.50 \pm 0.12 ^d | 2.192 \pm 0.08 ^d |
| 5% FPCP | 11.36 \pm 0.05 ^d | 5.56 \pm 0.11 ^d | 2.53 \pm 0.10 ^d | 2.198 \pm 0.04 ^d |
| 10% FPCP | 11.58 \pm 0.09 ^c | 5.64 \pm 0.02 ^c | 2.56 \pm 0.12 ^c | 2.203 \pm 0.07 ^{bc} |
| 15% FPCP | 11.69 \pm 0.12 ^b | 5.76 \pm 0.06 ^b | 2.61 \pm 0.18 ^b | 2.207 \pm 0.06 ^b |
| 20% FPCP | 12.45 \pm 0.11 ^a | 5.84 \pm 0.00 ^a | 2.64 \pm 0.05 ^a | 2.212 \pm 0.03 ^a |

* Spread ratio = Diameter / Height

Values are means of triplicates \pm standard deviationValues with the same superscript in a row are not significantly different ($P > 0.05$).

Table 5: Proximate analysis of crackers supplemented with fish protein concentrate powder (FPCP)

| Constituent (%) | Supplementation level (%) | | | | |
|-----------------|---------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | Control | 5 | 10 | 15 | 20 |
| Moisture | 5.11±0.07 ^a | 5.04±0.03 ^b | 4.88±0.15 ^{dc} | 4.76±0.01 ^d | 4.72±0.21 ^d |
| Crude protein | 10.23±0.11 ^c | 13.28±0.01 ^d | 15.33±0.13 ^c | 16.44±0.03 ^b | 18.54±0.11 ^a |
| Crude fat | 12.33±0.21 ^e | 13.41±0.11 ^d | 14.53±0.05 ^c | 14.89±0.09 ^b | 16.69±0.16 ^a |
| Ash | 2.08±0.06 ^c | 2.33±0.06 ^d | 2.54±0.16 ^c | 2.59±0.11 ^b | 2.64±0.03 ^a |
| Crude fiber | 0.37±0.12 ^{dc} | 0.39±0.04 ^d | 0.43±0.00 ^c | 0.56±0.07 ^b | 0.62±0.09 ^a |
| *Carbohydrates | 69.88±0.14 ^a | 65.55±0.13 ^b | 62.30±0.13 ^c | 60.77±0.03 ^d | 56.87±0.05 ^e |

*Carbohydrates were calculated by difference

Values are means of triplicates ± standard deviation

Values with the same superscript in a row are not significantly different ($P > 0.05$).

The data in the same Table (5) shows that protein content of crackers contained FPCP at levels 5, 10, 15 and 20% was significantly higher than that of control and it increased gradually with the increasing in the supplementation ratios from 10.23% in control to 18.54% in crackers contained 20% FPCP. In this respect, Venugopal [38] reported that addition of 3 % of FPP (fish protein powder) to wheat flour (protein content, 10.4 %) increased its protein content to 12.4 % with an increase of NPU from 50 to 67%. The addition of the FPCP increased the protein content of the crackers gradually, with significant differences being in the protein content of the cracker samples. Netto *et al.* [39] and Shaviklo [40] reported that fish is a rich animal protein source with high nutritive value; consumption of them can meet many nutritive requirements of body and is useful to improve human health at large.

The supplemented FPCP crackers showed significantly higher crude fat and ash contents than the control crackers. The addition of fish protein concentrate powder and other ingredients in crackers increased the ash and crude fiber content compared to the control sample. On the other hand, carbohydrates of crackers samples decreased by increasing the supplementation levels compared to the control sample. These results agree with Ibrahim [41] and Mohamed *et al.* [28] who reported that the protein and total ash content of biscuits increases by increasing the fortification level of both types of carp fish protein concentrate and shark fish concentrate compared to the control sample.

Amino Acid Composition: The concentration of dietary essential amino acids is a major factor determining the nutritional value of food protein. The fish muscle protein contains an excellent amino acid composition and is an excellent source of nutritive and easily digestible proteins [42].

The amino acids composition (g/100g) of tilapia fish protein concentrate powder and crackers supplemented with (FPCP) are shown in Table (6), along with the recommendation made by FAO/WHO/UNU [43] for essential amino acid composition. The obtained results showed that tilapia fish concentrate powder contains all the essential amino acids in good proportion as reported by Sathivel *et al.* [44]. Also, FPCP had a good balanced amino acid composition with higher level than FAO/WHO/UNU [43]. In this respect, Murueta *et al.* [45] recommended that fish powder can be used as a supplementary protein to increase nutritive value, as it contains high protein content.

The results indicated that 20% FPCP supplemented crackers increased all essential amino acids content compared to control. It could be noticed that supplementation increased isoleucine, leucine, methionine, threonine, valine and histidine as compared with the FAO/WHO [43] reference pattern, resulting in an improvement of the nutritive value of crackers. Akhade *et al.* [46] reported that use of this product is especially beneficial for children and pregnant women.

From the same table, crackers supplemented with FPCP at 5, 10, 15 and 20% level had high content of histidine (2.32, 2.34, 2.37, 2.40 and 2.46 g/100g, respectively) compared with FAO/WHO/UNU [43] reference pattern (1.6 and 1.5 for children and adult requirements), this amino acid is very important because it is essential for children.

Glycine is an important amino acid as it is one of the main components of human connective tissue [47]. Arginine is an essential amino acid for children growth [48] and it is relatively high in the fish samples, this is in agreement with the result of Adeyeye [49]. Glutamic acid and Aspartic acid are the most concentrated amino acid present in the fish, while the Lysine content of the fish can serve as a good source of protein for the fortification of cereal weaning foods.

Table 6: Amino acids composition (g/100g) of crackers supplemented with different levels of tilapia fish concentrate powder

| Amino acids | Supplementation levels (%) | | | | | | FAO/WHO/UNA* | |
|---------------|----------------------------|---------|-------|-------|-------|-------|--------------|-------|
| | FPCP | Control | 5 | 10 | 15 | 20 | Children | Adult |
| Isoleucine | 4.68 | 3.23 | 3.31 | 3.37 | 3.54 | 3.87 | 3.00 | 3.00 |
| Leucine | 7.91 | 6.25 | 6.27 | 6.35 | 6.55 | 6.64 | 5.00 | 5.90 |
| Lysine | 9.21 | 2.15 | 2.34 | 2.48 | 2.52 | 2.64 | 4.80 | 4.50 |
| Methionine | 2.66 | 3.11 | 3.20 | 3.19 | 3.19 | 3.19 | 2.30 | 1.60 |
| Phenylalanine | 4.06 | 3.43 | 3.46 | 3.51 | 3.54 | 3.65 | 4.10 | 3.80 |
| Threonine | 4.50 | 2.75 | 2.79 | 2.82 | 2.85 | 3.13 | 2.50 | 2.30 |
| Valine | 4.80 | 4.30 | 4.36 | 4.42 | 4.47 | 4.50 | 2.90 | 3.90 |
| Histidine | 2.68 | 2.32 | 2.34 | 2.37 | 2.40 | 2.46 | 1.60 | 1.50 |
| Tyrosine | 3.36 | 4.07 | 4.11 | 4.14 | 4.13 | 4.13 | - | - |
| Total EAA | 43.86 | 31.61 | 32.18 | 32.65 | 33.19 | 34.21 | 26.20 | 26.50 |
| Alanine | 5.76 | 3.46 | 3.48 | 3.51 | 3.55 | 3.58 | - | - |
| Arginine | 6.44 | 4.11 | 4.21 | 4.27 | 4.30 | 4.48 | - | - |
| Aspartic acid | 9.88 | 5.35 | 5.42 | 5.47 | 5.75 | 6.24 | - | - |
| Glutamic acid | 14.56 | 18.21 | 18.36 | 18.47 | 18.52 | 18.35 | - | - |
| Cysteine | 0.34 | 1.14 | 1.15 | 1.14 | 1.16 | 1.16 | - | - |
| Glycine | 5.16 | 3.26 | 3.68 | 3.73 | 3.89 | 4.45 | - | - |
| Serine | 4.18 | 4.52 | 4.67 | 4.84 | 5.28 | 5.79 | - | - |
| Proline | 4.02 | 5.43 | 5.44 | 5.49 | 5.55 | 5.77 | - | - |
| TNEAA | 50.34 | 45.48 | 46.41 | 46.92 | 48.00 | 49.82 | - | - |
| TAA | 94.20 | 77.09 | 78.59 | 79.57 | 81.19 | 84.03 | - | - |

* FAO/WHO/UNU energy and protein requirements (2007)

Table 7: Amino acid scores of crackers supplemented with FPCP

| Essential amino acids (g/100g) | Supplementation levels | | | | | | Ref. pattern (FAO/WHO 2007) |
|--------------------------------|------------------------|---------|--------|--------|--------|--------|-----------------------------|
| | FPCP | Control | 5 | 10 | 15 | 20 | |
| Isoleucine | 156 | 107.67 | 110.33 | 112.33 | 118.00 | 129.00 | 3.00 |
| Leucine | 158.20 | 125.00 | 125.40 | 127.00 | 131.00 | 132.80 | 5.00 |
| Lysine | 191.87 | 44.79 | 48.75 | 51.66 | 52.50 | 55.00 | 4.80 |
| Methionine | 115.65 | 135.22 | 139.13 | 138.69 | 138.69 | 138.69 | 2.30 |
| Phenylalanine | 99.02 | 83.88 | 84.39 | 85.61 | 86.34 | 89.02 | 4.10 |
| Threonine | 180.00 | 110.00 | 111.60 | 112.80 | 114.00 | 125.20 | 2.50 |
| Valine | 165.52 | 148.28 | 150.34 | 152.41 | 154.14 | 155.17 | 2.90 |
| Histidine | 167.50 | 145.00 | 146.25 | 148.13 | 150.00 | 153.75 | 1.60 |

Amino Acid Scores (AAS): A high correlation was obtained between AAS determination in products and values obtained by biological assays for protein quality [50].

The amino acid scores (AAS%) in crackers contains different levels of FPCP are given in Table (7). The results in this table show that lysine was found to be the first limiting amino acid, whilst phenylalanine and isoleucine was the second and third limiting amino acids. Also, it was found that phenylalanine, methionine and isoleucine were the first, second and third limiting amino acids in FPCP.

Nutritional Quality of Crackers Supplemented with Different Levels of FPCP: Nutrition parameters of FPCP and crackers supplemented with different levels of FPCP were calculated and results are showed in Table (8).

Table 8: Computed protein efficiency ratio (PER), biological value (BV) and *in vitro* protein digestibility of crackers supplement with FPCP

| Samples | PER | BV | <i>In vitro</i> protein digestibility |
|--------------------|------|-------|---------------------------------------|
| Casein reference * | 2.50 | 76.23 | 90.03 |
| FPCP | 2.77 | 78.25 | 93.14 |
| Control | 1.94 | 69.55 | 70.23 |
| 5% FPCP | 1.95 | 69.59 | 73.25 |
| 10% FPCP | 1.98 | 69.94 | 75.43 |
| 15% FPCP | 2.07 | 70.91 | 73.08 |
| 20% FPCP | 2.12 | 71.34 | 77.57 |

*Casein reference: FAO/WHO/UNU (1985)

PER: Protein efficiency ratio

BV: Biological value

FPCP showed to have PER, BV and *in vitro* protein digestibility (2.77, 78.25 and 93.13%, respectively) higher than those of casein reference [51] (2.55, 76.23 and 90.03%, respectively). This indicated that FPCP proteins are high nutritional value. The obtained results show that

Table 9: Sensory attributes of crackers supplemented with FPCP

| Samples | Taste | Color | Texture | Flavor | Appearance | Overall acceptability |
|-----------|------------------------|-----------------------|-------------------------|------------------------|-----------------------|------------------------|
| Control | 8.64±.67 ^a | 8.77±.93 ^a | 8.77±.75 ^{ab} | 8.91±.83 ^a | 9.23±.57 ^a | 8.82±.85 ^{ab} |
| 5% FPCP | 8.50±1.16 ^a | 8.45±.82 ^a | 9.09±.83 ^a | 8.68±.72 ^{ab} | 8.64±.33 ^b | 8.68±.87 ^{ab} |
| 10% FPCP | 8.55±1.04 ^a | 9.00±.50 ^a | 9.18±.60 ^a | 8.55±.69 ^{ab} | 8.43±.08 ^c | 8.95±.47 ^a |
| 15 % FPCP | 7.73±1.15 ^a | 8.64±.67 ^a | 8.49±1.20 ^{bc} | 7.64±1.36 ^c | 8.37±.27 ^c | 8.18±.72 ^{bc} |
| 20% FPCP | 7.59±1.36 ^a | 8.36±.81 ^a | 8.00±.45 ^c | 8.00±.89 ^c | 8.25±.61 ^d | 7.95±.96 ^c |

Values followed by the same letter in column are not significantly different at $p \leq 0.05$

in vitro protein digestibility increased with increasing FPCP level, which was 70.23% in control increased to 77.57% in crackers contain 20% FPCP. The digestibility is an important criterion that determines the availability of physiologically active amino acids and peptides and is affected by processing treatments [52]. The digestibility of fish meat is typically high, more than 95%, depending on the species and higher than the digestibility of meat and milk in general [53]. *In vitro* protein digestibility of cracker supplemented with different levels of FPCP was ranged from 70.23 and 77.57%. In this respect, Rani *et al.* [54] reported that the *in-vitro* protein digestibility of bread was 78.10%, cake 77.40% and biscuits were 75.05% on supplementation of soy flour. Also, Abdel-Aal [55] and Vitali *et al.* [56] showed that *in vitro* protein digestibility of wheat-based biscuits ranged from 44.3 to 68.9%, which is lower than that in this work.

The protein efficiency ratio (PER), net protein utilization (NPU), true digestibility (TD) and biological value (BV) were estimated for traditional Pakistani weaning food which was incorporated with different levels of fish protein powder (FPP). Values such as NPU, BV, PER and TD showed remarkable improvement in weaning food incorporated with 10 % FPP indicating that the addition of 10 % FPP to the prototype scanned result in superior nutritional quality and FPP could be an ideal source of protein for enriching the weaning food [57].

Sensory Characteristics of Biscuits as Affected by Addition of FPCP: No significant difference ($p \leq 0.05$) was observed among the cracker samples with FPCP for taste and color. The results in Table (9) show that crackers control gave the highest scores for taste, flavor and appearance, while crackers prepared using 10% FPCP had the highest scores for color, texture and overall acceptability which recorded 9.00, 9.18 and 8.95 respectively. Goes *et al.* [58] indicated that the addition of 20% of tilapia protein concentrate in fresh pasta is ideal for improving the nutritional quality of noodles without affecting the sensory properties.

Sensory evaluation showed good acceptability of the enriched crackers, receiving hedonic terms from 'liked moderately' to 'liked very much' and indications of good products for people specially children. The overall acceptability of salt biscuits was improved by the supplementation with 5% fish protein concentrate [59].

CONCLUSIONS

Crackers supplemented with tilapia fish protein concentrate could provide with high nutritional value, showing that tilapia fish is a good source of protein, amino acids and minerals for incorporation into baked products and solve malnutrition especially for children. The made crackers exhibited that the greatest color, texture and overall acceptability sensory characteristics among panelist were those containing 10% tilapia fish protein concentrate. So, the results of this study showed that the developed crackers were not only improved in terms of nutritional value and health benefits specially children which the major group consumed this products, but also highly accepted by various age of consumers.

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