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Production and Quality Evaluation of Biscuit Incorporated with Fish Fillet Protein Concentrate

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Abstract

This research was aimed to evaluate the physico-chemical and nutritional qualities of biscuit produced from sturgeon fillet protein concentrate (SFPC), based on gross chemical composition, effects of SFPC incorporation, and storage stability for six months. SFPC was used in production of biscuits by replacing low gluten wheat flour by 5%, 7% and 10% to ensure the quality and acceptability of the biscuit. The obtained results for biscuits fortified with SFPC revealed that the nutritional and proximate composition of biscuits were significantly ($P \leq 0.05$) improved: moisture (4.75 ± 0.08-4.76 ± 0.11), protein (14.63 ± 0.12-19.52 ± 0.12), fat (16.20 ± 0.06-16.50 ± 0.17), ash (1.53 ± 0.04-1.66 ± 0.12). Total amino acids were: 6.93, 13.15, 13.93, and 17.20; and essential amino acids: 2.43, 5.29, 5.87, and 7.48 gram/100 gram, for 0%, 5%, 7% and 10% SFPC, respectively. Leucine was the major amino acid in 5% and 7% SFP, while Leucine, Phenylalanine and lysine were the major amino acid in 10% SFPC. The obtained results also showed the produced fish biscuit and SFPC had a good physico-chemical quality. In addition that fish biscuit protein characterized with a good nutritional protein quality as it composed of all essential amino acids. Microbial and physico- chemical results revealed that the shelf life of biscuit supplemented with SFPC were more than 6 months. The obtained results also showed the supplementation of SFPC into the wheat flour mix ratio used for making biscuits at level up to 10% improved the most nutritional content, rheological properties of prepared biscuits dough batches and physical, and sensory quality characteristics of the final biscuit products. Therefore, the present results recommended that it should be directed towards the utilization of SFPC products in food products fortification, especially cereal products up to the concentration of 7-10% depending upon the fortified food product.

Keywords: Sturgeon fish; Nutritive value; Low gluten wheat flour; Supplementation; Chemical and sensory quality

Introduction

Fish and fish products are rich in protein (18-20%), essential amino acids, and good sources of vitamins and minerals for maintenance of healthy body, comparing to terrestrial animals and agricultural products [1-4].

Sturgeon fish is the largest, popular and valuable fish in the estuaries of Yangtze river of China, as well as in the world, ranging from 2 to 5 m and 200 to 500 kg in length and weight, respectively [5-8]**.** Despite of an excellent source in nutritional composition (**15-21% protein, 0.13-4.5% fat, and 1-2.8% ash content**) and tasty flesh, sturgeon fish has been largely used for commercial luxury food products, such as caviar since 2000 [9-**13**].

With the trends toward malnutrition, nutritious, healthy eating and developments of nutritional food, the application of fish protein in biscuit baking is a viable current alternative. In the protein content, in particular, it can improve the nutritional value of the therein often poor products and play crucial role in alleviating malnutrition throughout the world [1,14]. According to Chari and Sreenivasan [15] and Syahrul [14] fish proteins are highly nutritive and well balanced with respect to the essential amino acids and rich in lysine than cereals and other staple food items. Enrichment of cereal foods with fish and fishery products even at low levels, greatly improves their overall growth promoting property, and help to combat world protein malnutrition.

Biscuit is prominent ready-to-eat baked snack form of bakery products which has unique taste, readily available, long shelf-life and good eating quality, enhanced its consumption widely by different group of consumers [16,17]. Given the low moisture content associated with the thinness, biscuits are characteristically hard and crunchy, properties that are much appreciated upon eating. However, compared to fish products its nutritional content is very low, being widely prepared from cereal products, which have low protein content and essential amino acids like lysine as well as other micronutrients [18]. Such a product represents a useful means of supplementing the diet of any section of the people, especially to meet the requirement of protein to a greater extent. As Mohamed et al. [1] reported, bakery products can be easily supplemented with protein, vitamins and minerals in order to meet and satisfy consumer all over the world. Therefore, there is a need to find an alternative which can increase nutritional content by substitution with some level of fish fillet protein concentrate.

Fish protein concentrate (FPC) can be defined as a product flour from fish flesh with concentrated high quality protein (between 75

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and 95%) than the original material aimed for human consumption, made by eliminating most of the fat and water content. It can be used as a substitution for wheat flour while manufacturing bakery products to increase nutritional content of the developed product [14,19,20]. In addition to its high protein content, FPC can be stored at room temperature for long time without undergoing many changes [14]. In recent years, there have been a number of applications of fish protein concentrate in bakery food products (such as biscuit, cake, bread) aiming to increase their protein content and improving their nutritional value by increasing the content of essential amino acids [1,21-23]. According to previous studies, FPC (6-10%) is an excellent dietary supplement and can be used to fortify diverse range of cereal products, such as, ice cream, mayonnaise, corn snacks, to increase protein content, improve flavor, taste and nutritive value and provide a healthy source of easily digested proteins [24-28].

Production of any food product depends on consistent supply of a raw material to assure reliability and profitability; ultimately to meet and satisfy consumers. The Yangtze River is rich in such type of fish species [6], which can supply consistently to both wholesale and retail buyers, so sturgeon would be a consistent source of aquatic protein for people who are eating more fishery products and producing bakery products for its health benefits. The well balanced nutritional composition and desirable sensory quality attributes of sturgeon could make it a viable source for production of biscuits. Because production of biscuit from sturgeon freshwater fish has not existed on the market and consumers have not had opportunities to obtain benefits from it. Taken the nutritional benefits in to account and nourish consumer in improved value added biscuit with substantive health benefits, SFPC was produced and used as a substituent of wheat flour for making biscuits.

To the extent of the authors knowledge, there is no published report on production of biscuit enriched with SFPC. The present work is further studies of our previous studies that report on development of wheat flour biscuits fortified with sturgeon fillet powder [29]. Therefore, the present investigation is the first of its kind on development of sturgeon freshwater fish fillet protein concentrate based biscuit. With the above views in consideration, the current investigation was carried out to evaluate the physico-chemical and nutritional qualities of biscuit produced from SFPC, based on gross chemical composition, microbiologic analyses, effects of SFPC incorporation, and storage stability for six month.

Materials and Methods

The investigation was carried out in the collaborative innovation laboratory of science and technology in aquatic products of the department of food science and technology, Jiangnan University, China.

Source and treatment of raw materials

Sturgeon fish (*Acipeneser sinensis*) was obtained from the Yangtze River by Hua Da Aquatic Products Science and Technology Industry Co., Ltd. and transported to the laboratory of department of food science in an insulated ice box. Low gluten wheat flour, sugar, margarine, salt, vanilla and baking powder were procured from local market. The high density polyethylene bags and required chemicals were used from the laboratory stock. The wheat flour was passed through 0.4 mm sieve No. 2 screen and packed in polyethylene pouches by vacuum package machine (Intelligent vacuum package machine, S/N: 0595-26398866, Made in Guangzhou, China) and stored at room temperature for further use and analysis.

Technological methods

Preparation of fish sample and fish protein concentrate: The fish sample collected was cleaned with potable water, filleted and trimmed. Fish protein concentrate was prepared from sturgeon fillet based on the method described by Chattopadhyay et al. [19], in boiling water for 15 minutes under frequent agitation till the whole mass was completely disintegrated. Then, the slurry was allowed to settle by cooling so that the oil floats up the oil water emulsion is then decanted of by tilting the vessel. The operation was repeated once more.

The solid mass was squeezed and pressed manually using a clean muslin/cheese cloth. The pressed mass was fragmented, washed with running warm water and squeezed and pressed again. The squeezing, pressing and washing of the boiled mass was done ten times to remove excess fat and water. Thereafter, the pressed mass was put on aluminum foil trays and was dried by oven dryer (Air blast dryer, Box Model: DHG-9070A, China) at 60°C for 12 hours. The dried sample was ground and turned to powder using a blender (Joyoung, JYL-D025, S/N: 141102470279D:03) 3 times for 2-3 minutes in each time. It was then screened by fine mesh 0.4 mm sieve size No. 2 screen [29]. The SFPC was packed in polyethylene pouches and stored at -18°C for further use and analysis.

Preparation of mix ratios used in the production of fortified biscuits

Fish protein concentrate was made from sturgeon fillet and mixed with low gluten wheat flour at three levels (0%, 5%, 7% and 10%) to be produce blends in the production of fortified biscuit.

Preparation of biscuits incorporated with sturgeon fillet protein concentrate

Dough strength was determined by farinograph (S/N: 072112, Brabender® GmbH, Germany) using the standard method of American Association of Cereal Chemists [30] of constant dough weight method at 25 ± 0.2 using a 300 g mixing bowl on a 13.24% moisture basis was weighed and placed into the corresponding Farinography mixing bowl. Water from the burette was added to the flour and mixed to form a dough. As the dough was mixed, the farinogram consistence (FU) versus time (min) was recorded for 20 min. Farinograph values of biscuit dough such as water absorption, dough development time, dough stability, weakening and mixing tolerance index were evaluated.

The formulation used for production of biscuits supplemented with 5%, 7%, and 10% are outlined in Figure 1. All the ingredients were weighed accurately. Margarine and sugar were creamed in mixer with flat beater for two min at a slow speed. The pre-weighed low gluten wheat flour with baking powder, SFPC and cream were taken in the bowl of dough mixer and dough was prepared using appropriate amount of water (20 ml) with proper mixing and kneaded by hand for 2 minutes. The kneaded dough was divided into equal portions and then the dough pieces were sheeted to a thickness of 0.3 ± 0.5 cm with the help of rolling pin and aluminum frame of standard height. Each biscuit dough was shaped using a circular biscuit cutter (4.5 cm inside diameter) and transfer to a lightly greased baking tray. They were then baked at 175°C for 10-12 min in a pre-heated baking oven. After baking, the biscuits were cooled to room temperature for 20 min, packed in polyethylene poushes and sealed until analysis.

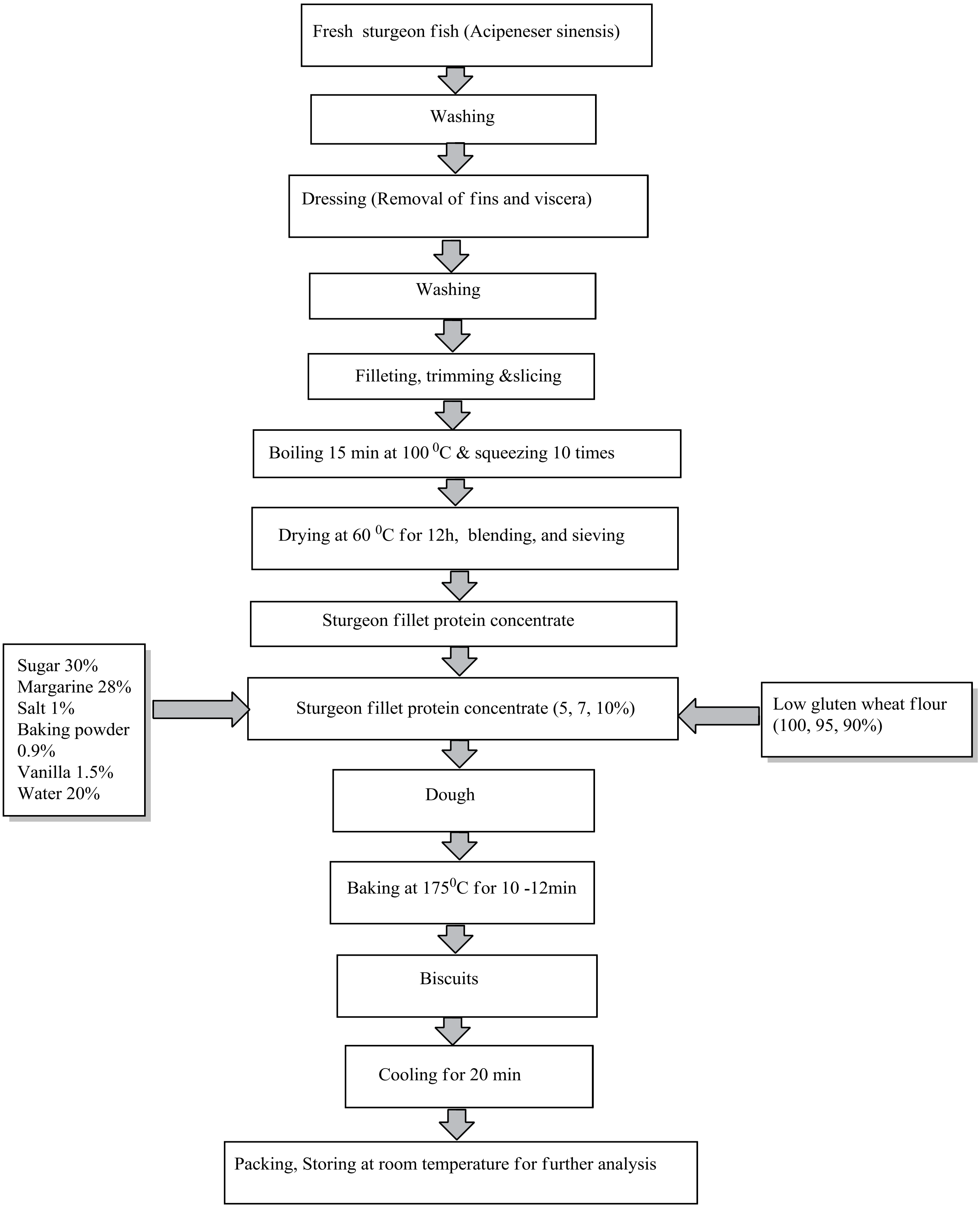


Figure 1: Flow chart of formulation and production of biscuit incorporated with sturgeon fillet protein concentrate (0, 5, 7, 10%).

Determination of physical characteristics of produced biscuits

The baking quality of prepared biscuits such as, diameter and thickness were measured using the standard method described by American Association of Cereal Chemists [30]. Diameter and thickness were measured using vernier caliper. Spread ratio (D/T) for produced biscuits was calculated by dividing the average value of biscuit diameter (D) on the average value of biscuit thickness (T).

Biscuits texture: The texture (hardness and fracturability) characteristics of biscuits were measured by TA-XT plus Texture Analyzer (Stable Micro Systems Texture Analyzer, Model: TA-XT, S/N: 12828, Godaiming, Surrey Gu7 1YL, UK). Biscuits were placed on the platform and the cylinder probe 2 mm was attached to the crosshead of the instrument. The analyzer was set at a return to start cycle, pre-test speed, test speed, post-test speed and distance to contact the biscuit with a 50 kg load cell: 1 mm/s, 1 mm/s, 10 mm/s and 5 mm, respectively. A force plot was made for every test sample. Measurements were conducted in triplicates and results are expressed as mean ± S.D values.

Color determinations of biscuits: Objective evaluation of biscuits both bottom and upper surfaces color was measured using UltraScan PRO (Hunter Lab, Made in U.S.A) in the reflection mode. The color assessment system is based on the Hunter L^*, a^* and b^* coordinates. L^* representing lightness and darkness, + a redness, and + b yellowness according to the standard method described by American Association of Cereal Chemists [30]. The instrument was standardized with white and black tile of Hunter Lab Color Standard.

Physico-chemical quality, microbial and nutritional evaluation of sturgeon fillet protein concentrate and biscuits incorporated with sturgeon fillet protein concentrate

Proximate composition of low gluten wheat flour (LGWF), raw sturgeon fillet (RSF), sturgeon fillet protein concentrate (SFPC), supplemented biscuits and wheat flour biscuit as control was determined as crude protein [31], crude lipid [32], ash [31] and moisture [31], Free fatty acid (FFA) and peroxide values (PV) [31], and thiobarbutric acid (TBA) (expressed as mg malonaldehyde/ kg of dry matter) [33]. The total volatile base nitrogen (TVB-N) were estimated by Conway's micro-diffusion analysis [34] and the obtained results were expressed as mg N/100 g of dry matter. The Total amino acid was determined following the method of Bidlingmeyer et al. [35] and the current findings were expressed as g/100 g of protein. The pH value was estimated directly by using a digital METTER TOLEDO pH meter (S/N:B60299458) according to the standard method of the [31]. Microbial analysis of total plate count (TPC) and total fungal count (TFC) were analysed according to ISO [36].

Energy value (total calorific value) of SFPC was calculated by the addition of protein calorific value and fat value (protein calorific value=90.42 × 4 and fat caloric value=5.34 × 9). High amount of energy (409.74 kcal) in the SFPC could be supplemented in the biscuit and other food items effectively.

Sensory evaluation

The consumer's acceptability of fish base biscuit were evaluated through a taste testing panel of 100 testers selected from post graduate students of Jiangnan University Department Food Science. All the panelists were briefed and made well acquainted with sensory attributes before evaluation. The test was conducted while the samples were fresh. The panellists were provided with clean water to rinse their mouth after tasting each biscuit. Biscuits were evaluated for their color, texture, flavor, taste, appearance and overall acceptability. The samples were coded with different letters and served to the panellists at random to guard against any bias. The panellists asked to rate the given samples a nine point hedonic scales with the ratings of: 9=Like extremely, 8=Like very much, 7=Like moderately, 6=Like slightly, 5=Neither like nor dislike, 4=Dislike slightly, 3=Dislike moderately, 2=Dislike very much and 1=Dislike extremely [37].

Statistical analysis

The obtained results were statistically analyzed using statistical program (SPSS 20.0) for Windows. All analytical determinations were performed two times in triplicates each and the mean values were reported. All the data obtained from the experiments were statistically compared by one way variance analysis (ANOVA). Least significant difference (LSD) between mean were reported at a significant level, $p \leq 0.05$. Descriptive analysis were also used in analysis of sensory evaluation.

Results and Discussion

Physico-chemical quality, chemical composition (on wet basis) and microbial of RSF, SFPC and LGWF

Physico-chemical quality, chemical composition and microbial load of RSF, SFPC and LGWF are depicted in Table 1. The results indicated that SFPC had significantly higher content of protein, fat and low moisture compared to RSF and LGWF. The drying process significantly reduced moisture (1.96%) content of SFPC which resulted in increasing protein (90.42%), ash (1.77%) and fat (5.34%) content. The results of this study are in harmony with Wu and Mao [38] who found that drying of grass carp fish significantly increase protein content and lower moisture content. Akhade et al. [2] also reported similar results of fish protein concentrate from ribbon fish, *Lepturacanthus savala* were 86.80%. In addition, Beltagi et al. [39] reported that dried fish has 77-87% protein. Omotosho et al. [40] highlighted that ash content is a good indicator of the mineral content in fish and fish products. Regarding ash content in the present study, SFPC is significantly ($P \leq 0.05$) higher in ash content compared to RSF and LGWF. This may be due to the fact that fish is an excellent source of amino acids and minerals. Akhade et al. [2] has been reported similar results of ash content of FPC extracted from eviscerated meat of ribbon fish were found to be 1.87%. Jeyasanta et al. [41] highlighted that moisture content below 10% level is good for microbial safety of the fishery products. In this study, low moisture content was recorded in SFPC which indicates its microbial stability and extended shelf life.

Table 1 also indicates the levels of FFA (1.13% as oleic acid), PV (0.67 meq.pv/kg fish oil), TVB-N (1.76 mg N/100), TBA (0.07 mg malonaldehyde/kg) of RSF were significantly higher than SFPC and LGWF. Lipid Hydrolysis was slightly higher in RSF with release of FFA 1.13% as oleic acid, but in SFPC (0.37% as oleic acid) was lower because most of the lipid was already extracted. Primary lipid oxidation was

Parameters	RSF	SFPC	LGWF
Moisture (%)	69.96 ± 1.32[a]	1.96 ± 0.06[c]	13.24 ± 0.04[b]
Ash (%)	1.04 ± 0.04[a]	1.77 ± 0.01[b]	0.49 ± 0.05[c]
Crude Protein (%)	21.63 ± 2.12[b]	90.42 ± 0.76[a]	12.47 ± 0.54[c]
Fat (%)	2.51 ± 0.27[b]	5.34 ± 0.10[a]	0.75 ± 0.04[c]
pH	6.34 ± 0.04[b]	7.01 ± 0.06[a]	6.04 ± 0.02[c]
FFA	1.13 ± 0.14[a]	0.37 ± 0.01[b]	0.05 ± 0.03[b]
PV	0.67 ± 0.08[a]	0.40 ± 0.10[a]	0.05 ± 0.02[b]
TVB-N	1.76 ± 0.25[a]	1.14 ± 0.13[b]	0.47 ± 0.20[b]
TBARS	0.07 ± 0.06[a]	0.14 ± 0.01[a]	0.00 ± 0.00[a]
TPC (cfu/g)	4.3×10^3 ± 0.25[a]	Absent	Absent
TFC (cfu/g)	Absent	Absent	Absent

[*]The values in the table are the mean of triplicates with standard deviation (±). Mean values followed by the same letter in the row are not significantly different (P ≥ 0.05).

Table 1: Physico-chemical quality, chemical composition (on wet basis) and microbial of RSF, SFPC and LGWF.

calculated by means of PV. Even though PV value was very low in all samples but it was slightly higher in RSF which indicates primary lipid oxidation occurs initially. However, the physico-chemical parameters of all samples were below the maximum allowable value of fresh fish. Fish and fishery products could show off odor, taste rancid and no nutritional values when the values are above the suggested maximum limit. Acceptable limit 30-35 mgN/100 g for TVB-N [42],10-20 meq of PV/kg of fish oil for PV and for TBA, 10-20 mg malonaldehyde/fish lipid [43] have been reported by the aforementioned authors. In this regard, all samples were in fresh condition.

The pH of fish and fish product is one of the important factor governing the quality of fish. The pH values of RSF, SFPC and LGWF were in a fresh condition. Freshness of fish and fish products can be influenced by pH value because loss of their freshness can in turn affect bacterial growth [41,44]. The microbiological safety of the RSF, SFPC and LGWF was ascertained through the assessment of microbiological parameters, including TPC and TFC (cfu/g), as indicated in Table 1. TFC and TPC growth were not detected in all samples, but TPC (4.3 × 10^3 cfu/g)) was obtained in RSF. However, the recorded result of this study was within the acceptable limit. From the obtained results of the present study, the microbiological assessment of the all samples indicates that they were of high quality and safe for utilization. The border for total plate count (TPC) is 1 × 10^5 cfu/g in the fish and fishery products [45].

Rheological properties of prepared biscuits dough

The results of the effect of incorporating of low gluten wheat flour with sturgeon fillet protein concentrate on rheological properties of dough are summarized in Table 2. The water absorption (61.25) of mix ratio 10% SFPC was the highest value obtained amongst all samples. The obtained data indicates that, WA, DS and MTI increased progressively as the incorporation level was increased and this could be due to higher protein content in the blends compared to control sample. No significant difference in WA (Table 2). The results of this study showed that the blends would be useful in biscuit products where hydration to improve handling is desired. The results of the present study are in accordance with Abou-Zaid and Elbandy [22] and David et al. [46] who reported that protein content can hold more water due to the hydroxyl groups which allows for the interactions that enhanced to increase water absorption.

Kulhomaki and Salovaara [47] stated that dough stability (DS) indicates the time when the dough maintains maximum consistency and is a good indication of dough strength. Good wheat quality dough has stability of 4-12 min. In our findings, the mix ratio of 10% SFPC was significantly ($P \leq 0.05$) higher in DS, while wheat flour control (0%) was the lowest. By the addition of SFPC the dough rheological properties are positively affected. Similar results of DS and WK was reported by Abou-Zaid and Elbandy [22] and Mohamed et al. [1].

The time required for dough development or time necessary to reach 500 FU of dough consistency (DDT) slightly decreased by the addition of SFPC. Wheat flour with 10% of SFPC showed the lowest DDT (1.10), but wheat flour control (0%) was the highest, DDT (2.35). Decreased DDT could be explained with differences in chemical composition, as protein content increases DDT, AT and WK were decreased. The results, WK, and AT are in agreement with result reported by Ammar et al. [48], Abou-Zaid and Elbandy [22] and Mohamed et al. [1] but obtained results of this study for DDT were not comparable with the result obtained by Abou-Zaid and Elbandy [22] which could be due the differences in raw materials used and treatments applied in the study.

Biscuit physical analysis

Physical properties are important and helpful to judge consumer acceptability. The average value of physical characteristics of biscuits such as diameter, thickness and spread ration are presented in Table 3. No significant ($p \geq 0.05$) difference in physical characteristics of the biscuit. The diameter and spread ratio of biscuits were increased, whereas thickness was decreased slightly as inclusion of SFPC increased in the biscuit formulation. From the current result, it can be noticed that diameter and spread ration of biscuit incorporated with 10% SFPC were the highest among the other experimental biscuits. The increase in diameter and spread ration could be attributed due to difference in their fat, protein and moisture contents which affect the water absorption capacity. Moreover, the aggregation of SFPC induced formation of protein network, which helped increase the elasticity and extensibility of the dough, thus leading to increased spread ratio. The findings of this study is in harmony with earlier work reported [23,49]. This assertion is also confirmed by sensory data in Figure 2. Biscuit spread ratio stand for a ratio of diameter to thickness. Biscuits having higher spread ratio are considered most desirable quality attributes [23,50].

Hardness and fracturability are textural quality of biscuit that are very important and desirable quality attributes for bakery products. Hardness is the textural property which attracts more attention in evaluation of quality characteristic baked goods, because of its close association with human perception of freshness [50]. Fracturability measure the resistance of a biscuit to fight to regain its original status or form. Distance at which the biscuit breaks is noted as fracturability. Biscuit that breaks at shorter distance has higher fracturability [17,51]. According to the present study, no significant differences ($P \geq 0.05$) in hardness and fracturability (Table 3). The value of hardness and fracturability were higher in control biscuit comparing to the experimental biscuits. From the experimental biscuit samples, biscuits with inclusion of 10% had slightly lower values in textural properties. Hardness and fracturability of SFPC supplemented biscuits decreased as the proportion increased which could be due to high protein content. However, textural properties of biscuits did not affect as protein and fat content increases. Similar results were reported by Chauhan et al. [50] and Tang and Liu [52]. It was found that hardness of biscuit decreased

Mixed Ratios	WA(%)	AT(min)	DDT(min)	DS (min)	WK(FU)	MTI (FU)
0%	60.75 ± 1.06^a	1.3 ± 0.14^a	2.35 ± 0.35^a	1.2 ± 0.14^c	111.5 ± 6.35^a	18.5 ± 0.71^d
5%	60.05 ± 0.07^a	1.25 ± 0.07^a	1.9 ± 0.07^{ab}	1.95 ± 0.07^c	78.5 ± 7.78^b	24.5 ± 0.71^c
7%	59.6 ± 0.14^a	1.15 ± 0.07^a	1.55 ± 0.07^{bc}	6.25 ± 0.07^b	42 ± 4.24^c	35.5 ± 0.71^b
10%	61.25 ± 0.35^a	1.14 ± 0.07^a	1.10 ± 0.01^c	8.7 ± 0.42^a	20 ± 3.75^c	47.5 ± 0.58^a

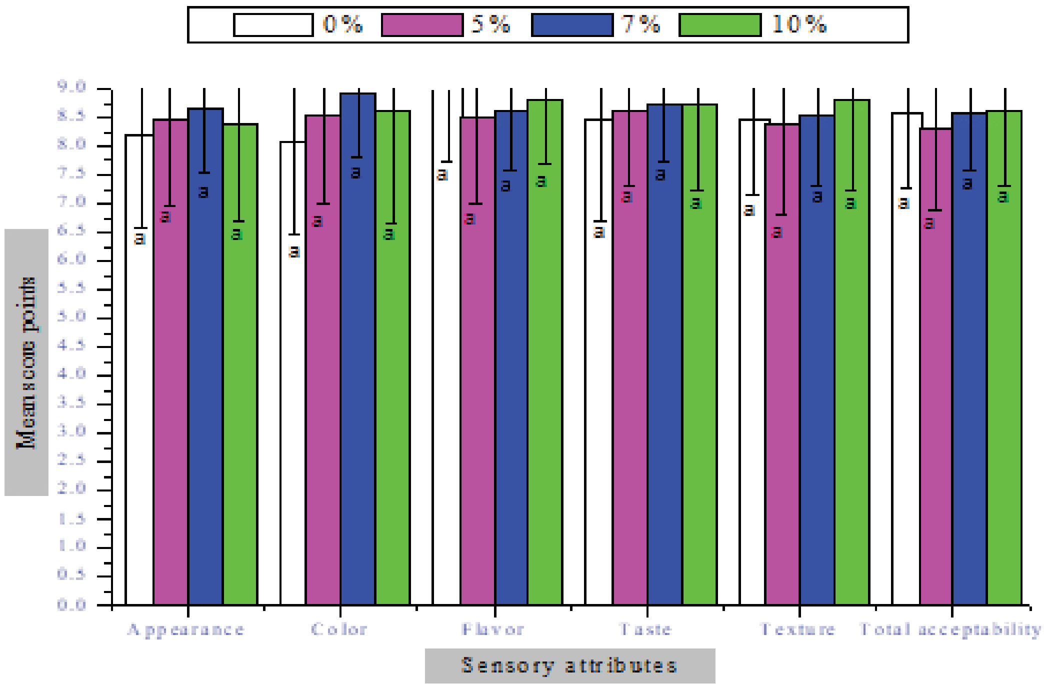
[*]The values in the table are the mean of triplicates with standard deviation (±). Mean values followed by the same latter in the column are not significantly different ($P \geq 0.05$)
[*]FU, Farinaceous units; WA, Water absorption; AT, Arrival time; DDT, Dough development time; DS, Dough stability; WK, weakening; MTI, Mixing tolerance index.

Table 2: Effect of incorporating of low gluten wheat flour with sturgeon fillet protein concentrate on rheological properties of dough.

as addition of soy protein and amaranth flour increases, respectively.

Physico-chemical quality, chemical composition and microbial load of biscuit control and fortified LGWF biscuits with SFPC (on dry weight basis)

In this study, fish protein concentrate was produced from sturgeon fillet due to its nutritional characteristics and its potential for human consumption, aimed to produce biscuits. Fish protein concentrate is a low cost animal protein with high quality protein contain between 75 and 95%, therefore it can be used as a protein supplement to enhance nutritive value of agricultural foods and improve shelf life of final baked products [20]. Protein content among the produced biscuits



*The values in the figure are the mean of triplicates with standard deviation (±). Mean value with the same letters are not statistically different ($p \geq 0.05$).
Figure 2: Effect of blending ratio on sensory characteristics of SFPC enriched biscuits.

Parameters	0%	5%	7%	10%
Diameter (cm)	4.13 ± 0.0.05[a]	4.32 ± 0.04[a]	4.33 ± 0.07[a]	4.34 ± 0.09[a]
Thickness (cm)	0.42 ± 0.05[b]	0.42 ± 0.04[b]	0.4 ± 0.00[b]	0.4 ± 0.00[b]
Spread ratio (D/T)	9.83 ± 1.22[c]	10.37 ± 1.00[c]	10.82 ± 0.18[c]	10.85 ± 0.22[c]
Hardness (g)	1777.32 ± 1695.55[d]	1455.41 ± 1259.65[d]	1334.87 ± 1171.17[d]	1254.96 ± 1094.95[d]
Fracture ability (g)	784.63 ± 445.76[e]	998.09 ± 383.33[e]	974.41 ± 739.34[e]	635.41 ± 105.57[e]

*The values in the table are the mean of triplicates with standard deviation (±). Mean values followed by same superscript letters in a row are not significantly different ($P \geq 0.05$).

Table 3: Physical analysis of biscuits.

Parameters	0%	5%	7%	10%
Moisture (%)	4.73 ± 0.14[a]	4.75 ± 0.08[a]	4.75 ± 0.10[a]	4.76 ± 0.11[a]
Ash (%)	0.78 ± 0.06[b]	1.53 ± 0.04[a]	1.60 ± 0.01[a]	1.66 ± 0.12[a]
Crude Protein (%)	9.50 ± 0.18[d]	14.63 ± 0.12[c]	17.88 ± 0.01[b]	19.52 ± 0.12[a]
Fat (%)	15.58 ± 0.05[b]	16.20 ± 0.06[a]	16.35 ± 0.03[a]	16.50 ± 0.17[a]
pH	6.56 ± 0.03[b]	6.66 ± 0.02[a]	6.73 ± 0.00[a]	6.67 ± 0.02[a]
FFA	0.19 ± 0.11[a]	0.20 ± 0.05[a]	0.18 ± 0.08[a]	0.18 ± 0.13[a]
PV	0.04 ± 0.01[a]	0.04 ± 0.01[a]	0.04 ± 0.00[a]	0.04 ± 0.01[a]
TVB-N	1.58 ± 0.00[a]	1.32 ± 0.12[a]	1.32 ± 0.12[a]	1.45 ± 0.13[a]
TBARS	0.03 ± 0.01[b]	0.08 ± 0.03[ab]	0.10 ± 0.01[a]	0.13 ± 0.01[a]
TPC (cfu/g)	Absent	Absent	Absent	Absent
TFC (cfu/g)	Absent	Absent	Absent	Absent

The values in the table are the mean of triplicates with standard deviation (±). Mean values followed by the same letter in the row are not significantly different ($P \geq 0.05$).

Table 4: Physico-chemical quality, chemical composition (on dry basis) and microbial of biscuits.

varied from 9.50% to 19.52% (Table 4). Highest protein content was for biscuit fortified with 10% SFPC. This data can be highlighted that composite flours, obtained by enriching the LGWF with SFPC at 10% depending on the type of protein source, have given successful results due to the high levels of protein in SFPC, which, mixed with LGWF, have the advantage of improving the nutritional value of prepared biscuit because of the better composition of amino acids. The results showed that biscuits fortified with SFPC had higher protein content than control biscuit, however, the protein content of all biscuits were significantly different ($p \leq 0.05$). Protein of biscuits were increased as incorporation level increased, thus enhancing nutritive value of biscuits, because fish and their derivatives contain a high biologic protein with a balanced amino acid profile and a good proportion of methionine and cysteine, which are the main limiting amino in cereal protein sources [3,28]. The results of this study are in compliance with those reported by Nurul et al. [53], Mohamed et al. [1] and Chambo et al. [4]. Ibrahim [21] described in the findings that, fortification of household common foods, such as biscuit, by fish protein concentrate of 6% and 10% is the best level for increasing its nutritional and satisfaction values. In addition, Venugopal [28] reported that nutritive value of cereal proteins can be increased when fortified with fish protein concentrate.

The biscuit with 10% SFPC had slightly higher moisture content (4.76%) of all samples. There were no significant difference in moisture content among biscuits produced. Generally, moisture content recorded for all biscuits were very low which indicates stability, quality and concentrate nutrients. Eneche [54] reported 5.0 to 6.6% moisture content of biscuit and recommended that these values are better for shelf life stability of biscuits. Moisture content obtained in this study was slightly lower than moisture content (5.30-7.50%) reported by **Youssef and Mousa [49]** and slightly higher than moisture content (3.56-4.26%) reported by [1]. This might be attributed due to difference in raw materials, fish species and processing techniques used in the study. The control biscuit had the lowest ash content of all samples. Significantly higher ash (1.66%), and fat content (16.50%) was recorded in biscuit with 10% SFPC. This helped to verify that with an increase in the levels of SFPC, the crude protein, fat and ash contents increased progressively. The increased ash content of the supplemented biscuits (from 0.78 to 1.66% for 0 to 10% addition of SFPC, respectively) can be considered beneficial, because aquatic animal food products are a richer source of most essential minerals and trace elements than cereal products [4,55]. Data obtained in ash and fat content in this study were slightly lower than those indicated by Mohamed et al. [1], whereby biscuit with 3% carp fish protein concentrate had ash content (1.88%) and fat content (21.33%). Species, raw materials and processing techniques used might be contributed for the differences.

The results in Table 4 also indicate the levels of quality criteria of wheat biscuit control and biscuits with inclusion of SFPC. All, these quality parameters play vital role for acceptance of fish products. Tsighe et al. [44] reported that free fatty acids are a product of hydrolytic rancidity, involving the breakdown of triglycerides into their components (fatty acids and glycerol). These liberated fatty acids can create a rancid odor and off-flavor in the fish, leading to rejection by the consumer. Results of all quality criteria were below the allowable limits suggested by previous researchers [42,43,56]. This might be due to low fat, moisture content and no microbial growth and enzymes activities. During the initial processing of SFPC heating and drying process presumably inhibited enzymatic lipolysis, and this finding is in agreement with the findings of Chattopadhyay et al. [19]. The values of FFA, PV, TVB-N did not differ ($p \geq 0.05$) among biscuits produced. Low significant variation was observed between biscuit control and SFPC based biscuits in their TBA value. The low variation could be attributed to fat content recorded in the present study. Nishimoto et al. [57], Beltagi et al. [39] and Tsighe et al. [44] highlighted that the TBARS value are used to estimate secondary oxidative products, which are more stable than the primary oxidative products and is a helpful indicator of fish quality that it mainly used for evaluation of oil stability and monitoring of deterioration during fish storage.

Table 4 also indicates microbial safety of wheat biscuit control and biscuits with inclusion of SFPC to wheat flour at 5, 7 and 10% levels. TPC and TFC were not detected in all biscuit samples, which could be due to low moisture content obtained and hygienic condition and drying applied during the process. Microbial assessment of the present study illustrated that all biscuits were of safe and high quality for consumption. The result of this study was comparable to the result reported by Beltagi et al. [39]. Microorganisms are highly dependent on moisture content, the most sensitive quality attributes, of foods to ensure their activities, thus the reduction in moisture can be important to increase the shelf life of the biscuit products. Moisture content above 10% could be an indicator of the susceptibility of the product to undergo microbial spoilage [41]. The pH value of biscuits were also a good indication for the freshness and safety of the produced biscuit products, which ranges 6.56 to 6.67 for biscuit control and biscuit with inclusion 10% of SFPC, respectively for which indicate that a good quality product. The results of the present study were higher compared with results indicated by Chambo et al. [4], the pH of bread were in a range of 5.89 to 6.04 for 0% and 15% addition Nile tilapia meal, respectively. The difference could be due to fish species, type of wheat flour used, and processing techniques applied during biscuit production.

Amino acids composition of LGWF, RSF, SFPC, biscuit control and biscuits fortified with SFPC

The results regarding the analysis of amino acid composition of biscuit control and experimental biscuit samples are presented in Table 5. The total EAAs were 2.21, 5.29, 5.87 and 7.48 g/100 g and the corresponding value of NEAAs were 5.00, 7.86, 8.06 and 9.72 g/100 g for biscuit control and fortified biscuits (5, 7 and 10%), respectively. Among the EAAs, Leucine, Lysine, Phenylalanine, Valine were dominant in fortified biscuit samples, whereas in biscuit control Isolucine and Tyrosine were the major once. Glutamic, Aspartine and Proline were the major NEAAs in supplemented as well as in control biscuit. The EAAs and NEAAs composition were significantly higher in SFPC among all samples. Biscuit samples with the inclusion of 10% SFPC were higher in EAAs and NEAAs contents compared with control biscuit and biscuit samples fortified with 5 and 7% SFPC. From the obtained findings, it could be noticed that fortified biscuits composed of all amino acids which were higher than the biscuit made of LGWF. Significant difference was observed in the amino acid composition between biscuit control and fortified biscuits, as well as RSF, SFPC and LGWF. The findings here show that addition of SFPC to LGWF increase the nutritional composition of supplemented biscuits. This could be due to fish contains higher amount of amino acids compared to cereal products. Moreover, Proteins derived from fish are nutritionally superior than those in plant sources. Generally, in this study amino acids composition of supplemented biscuits were increased, as the level of fortification increases. The results of the present study are in accordance with Cercel [58], reported that inclusion of fish protein into wheat flour bread improve nutritional quality of bread due to an excellent source of highly digestible amino acids. **Friedman [59] has also** reported that, fish has a better balance of dietary essential amino acids compared with all other animal protein sources which could play crucial role in protein enrichment of bakery products. Similarly, Rajor et al. [16] observed that addition of fish protein concentrate markedly increased amount of amino acids in the supplemented biscuits, particularly arginine, lysine, methionine and threonine. They reported that fortification of biscuits with 8% of FPC are acceptable in terms of texture and flavor, but higher levels resulted in darker color of biscuits and crackers. Based on the results obtained in the current study, SFPC could be used to increase nutritional composition of wheat flour based products, especially, essential amino acids which plays very significant roles in nutritional point of view, since the body cannot synthesize and should therefore be supplemented from the diet. The result of this is in harmony with those obtained by Ibrahim [21] and Mohamed et al. [1] highlighted that fish protein concentrate has a good nutritional protein quality. Sen [27] emphases that fortification cereal food products with fish protein concentrate is beneficial to increase nutritional value of the agricultural products.

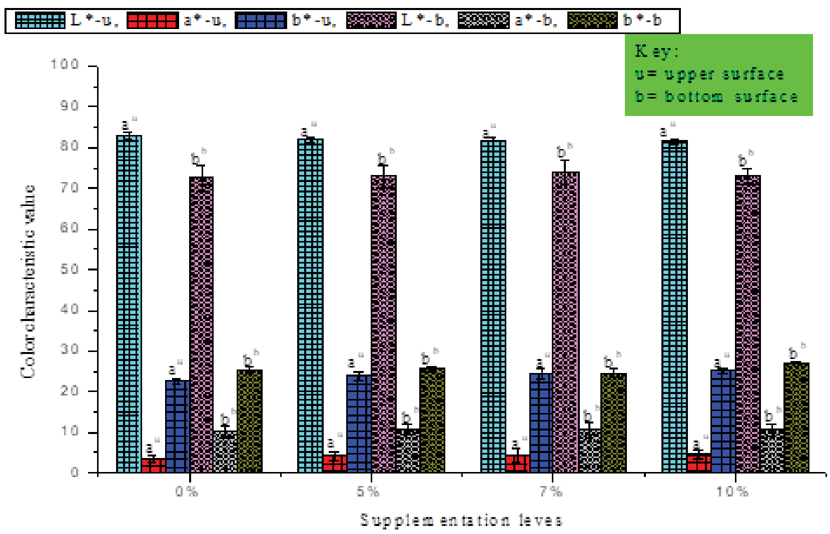
Sensory assessments

The effect of incorporation of SFPC at three levels (5, 7 and 10%) on the 100 judging scores of sensory attribute characteristics; appearance, color, flavor, taste, texture and overall acceptability of produced biscuits were studied and the obtained results are demonstrated in Figure 3.

EAAs	LGWF	RSF	SFPC	0%	5%	7%	10%
Lysine	0.17 ± 0.00^{d}	2.40 ± 0.71^{b}	10.12 ± 0.95^{a}	0.16 ± 0.02^{d}	0.57 ± 0.01^{c}	0.70 ± 0.02^{c}	0.94 ± 0.05^{c}
Therionine	0.22 ± 0.02^{d}	1.07 ± 0.28^{b}	3.71 ± 0.15^{a}	0.16 ± 0.01^{d}	0.50 ± 0.02^{c}	0.54 ± 0.02^{c}	0.69 ± 0.05^{c}
Valine	0.41 ± 0.00^{d}	1.48 ± 0.42^{b}	5.13 ± 0.03^{a}	0.30 ± 0.04^{d}	0.66 ± 0.01^{c}	0.71 ± 0.07^{c}	0.89 ± 0.02^{c}
Methionine	0.09 ± 0.02^{d}	0.52 ± 0.25^{b}	3.10 ± 0.12^{a}	0.07 ± 0.02^{d}	0.34 ± 0.02^{bc}	0.43 ± 0.06^{bc}	0.49 ± 0.03bc
Isolucine	0.36 ± 0.02^{d}	1.40 ± 0.40^{b}	5.24 ± 0.18^{a}	0.26 ± 0.02^{d}	0.61 ± 0.01^{c}	0.69 ± 0.01^{c}	0.85 ± 0.03^{c}
Phenylalanine	0.50 ± 0.08^{d}	1.07 ± 0.24^{b}	4.16 ± 0.23^{a}	0.34 ± 0.05^{d}	0.66 ± 0.05^{c}	0.72 ± 0.05^{c}	1.03 ± 0.18^{bc}
Histidine	0.21 ± 0.04^{d}	0.69 ± 0.04^{b}	2.16 ± 0.31^{a}	0.17 ± 0.02^{d}	0.42 ± 0.03^{c}	0.44 ± 0.02^{c}	0.50 ± 0.03^{bc}
Tyrosine	0.16 ± 0.09^{b}	0.63 ± 0.21^{b}	3.44 ± 0.40^{a}	0.21 ± 0.05^{b}	0.42 ± 0.08^{b}	0.45 ± 0.07^{b}	0.64 ± 0.03^{b}
Cystie	0.03 ± 0.01^{b}	0.06 ± 0.02^{b}	0.33 ± 0.17^{a}	0.05 ± 0.01^{b}	0.24 ± 0.01^{b}	0.24 ± 0.02^{b}	0.23 ± 0.00^{b}
Leucine	0.60 ± 0.03^{d}	2.09 ± 0.61^{b}	8.12 ± 0.33^{a}	0.49 ± 0.06^{d}	0.87 ± 0.02^{c}	0.96 ± 0.01^{c}	1.23 ± 0.05^{c}
Total EAAs	2.75	11.41	45.51	2.43	5.29	5.87	7.48
NEAA							
Aspartic	0.58 ± 0.10^{d}	2.59 ± 0.57^{b}	9.67 ± 0.45^{a}	0.51 ± 0.11^{d}	1.02 ± 0.12^{c}	1.10 ± 0.08^{c}	1.45 ± 0.16^{c}
Glutamic	3.35 ± 0.33^{b}	4.63 ± 1.22^{b}	17.19 ± 0.73^{a}	2.30 ± 0.19^{b}	2.97 ± 0.19^{b}	3.30 ± 0.54^{b}	3.70 ± 0.24^{b}
Arginine	0.47 ± 0.11^{d}	1.88 ± 0.23^{b}	6.59 ± 0.47^{a}	0.37 ± 0.08^{d}	0.75 ± 0.10^{c}	0.75 ± 0.06^{c}	0.99 ± 0.10^{c}
Serine	0.34 ± 0.05^{b}	0.84 ± 0.15^{b}	3.49 ± 0.44^{a}	0.27 ± 0.05^{b}	0.56 ± 0.05^{b}	0.56 ± 0.03^{b}	0.71 ± 0.07^{b}
Proline	1.49 ± 0.29^{c}	1.16 ± 0.14^{b}	3.64 ± 0.45^{a}	1.11 ± 0.25^{c}	1.40 ± 0.23^{b}	1.13 ± 0.15^{b}	1.30 ± 0.19^{b}
Glycine	0.34 ± 0.03^{c}	1.36 ± 0.07^{b}	4.24 ± 0.32^{a}	0.24 ± 0.01^{c}	0.57 ± 0.03^{c}	0.60 ± 0.01^{c}	0.74 ± 0.07^{c}
Alanine	0.29 ± 0.03^{d}	1.44 ± 0.31^{b}	5.47 ± 0.40^{a}	0.21 ± 0.01^{d}	0.59 ± 0.03^{c}	0.62 ± 0.02^{c}	0.82 ± 0.04^{c}
Total NEAAs	6.86	13.90	50.29	4.50	7.86	8.06	9.72
Total AAs	9.61	25.31	95.80	6.93	13.15	13.93	17.20

The values in the table are the mean of triplicates with standard deviation (±). Mean values followed by the same letter in the row are not significantly different (P ≥ 0.05).
*EAAs: Essential Amino acids, NEAAs: Non- Essential Amino acids, AAs: Amino acids

Table 5: Amino acids composition (g/100 gm).



*The values in the figure are the mean of triplicates with standard deviation (±).Values with the same letters are not statistically different (p ≥ 0.05).

Figure 3: Color parameter for the upper and bottom surface biscuits analyzed.

The results of this study showed that biscuits produced from SFPC had very high acceptable values of all the sensory attributes comparing with biscuit control, even though biscuit prepared from wheat flour had slightly higher scores in terms of flavor (9.00). All the sensory attributes were non-significant difference ($p \geq 0.05$) compared to wheat control biscuit as well as among inclusion levels. However, biscuits supplemented with 10% SFPC were the most liked and their overall acceptability (8.60) was the highest among the others. The results of this study are in agreement with Abou-zaid and Elbandy [22] reported that 6-10% of crayfish tail powder and protein concentrate should be used in biscuit fortification of wheat flour blends. In previous study, addition carp fish protein concentrate up to 3% at the expense of wheat flour did not cause any significant deleterious effect on the overall acceptability of produced biscuits [39]. Inclusion of SFPC to wheat flour did not affect the appearance index of biscuits, which could be due to reduction of fat content, as outlined in Figure 4. Addition of SFPC to wheat flour improved all organoleptic characteristics as well as nutritive values of wheat biscuits. Akhtar et al. [60] mentioned that substitution of cereal products with fish protein concentrate, which is rich in protein can increase their nutritional value and sensory attributes.

Biscuit samples with 7% SPFC had slightly higher mean scores in terms of appearance (8.67) and color (8.93), while biscuit samples with 10% SFPC had slightly higher scores in terms of taste (8.73), texture (8.80) and overall acceptability. From this data, it can be noticed that, incorporating of SFPC 10% had slightly lower score on color of biscuits comparing with 7% SFPC, that could be due to protein and fat content differences. The results of the present study are comparable to

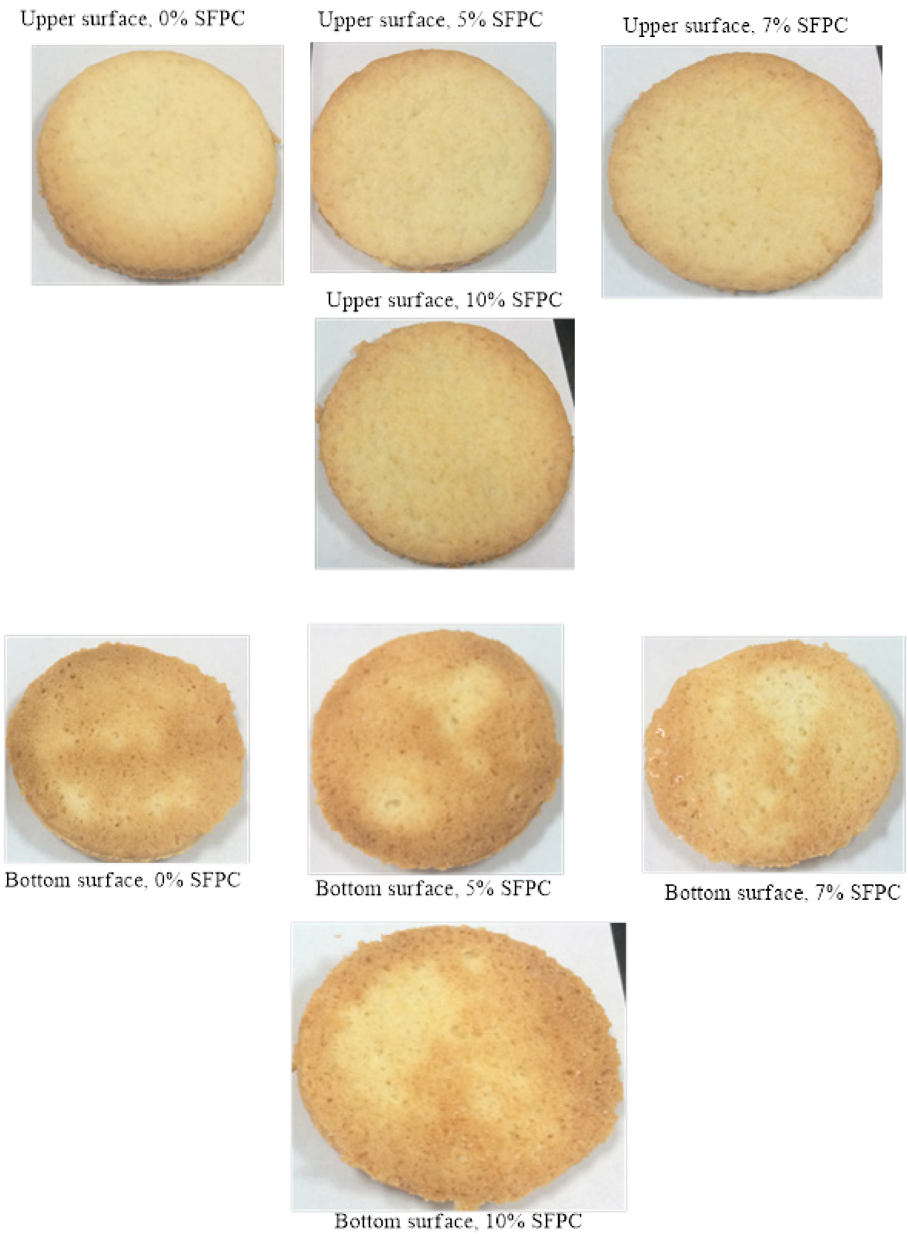


Figure 4: Photographs of upper and bottom surface of biscuit control (0% SFPC) and biscuits fortification with SFPC at three levels (5, 7 and 10%).

Mohammed et al. [61], reported that increasing levels of fish protein concentrate resulted in decreased color scores which might be due to maillard reaction. Texture is considered one of the most important sensory attributes for the acceptance of biscuits, determining their quality, since it affects consumer acceptance. Regarding texture, biscuits supplemented with 7 and 10% were most liked by the panelists. The results of this study confirms results reported by Khan and Nowsad [62], flour with protein content of fisher product, 7-10%, tends to yield biscuit with a better and longer -lasting crustiness. Taste is an important sensory attribute in acceptance of any food product. The taste result of this study showed that fortified biscuits with 7 and 10% SFPC the highest mean score of 8.73. This was closely followed by that of 5% and 0% substitutions with SFPC with mean score of 8.60 and 8.47, respectively and there was no significant difference between them. This result has proved that these biscuits supplemented with 7 and 10% SFPC were better accepted than control biscuit (0%) and biscuit fortified with 5% SFPC in terms of the taste of the biscuit samples. The taste of the samples increased with the increasing addition of SPFC. From taste results it was noted that, inclusion of SFPC upto 10% did not affect taste of the prepared biscuits. Probably, reduction of fat might solve and minimize to the lowest level of the fishy odor. Normally fish has a fish odor which can contribute a taste and flavor of fish for a product which mixed with it. Among the biscuit samples supplemented with SFPC, biscuit sample fortified with 5% SFPC was least preferred in terms color (8.53), flavor (8.50), taste (8.60), texture (8.40) and overall acceptability (8.30), while the one fortified with 10% was the most preferred in all sensory attributes except in terms of appearance and color. Sensory data indicated that biscuit supplemented with 10% SFP did not affect all sensorial attributes of the produced biscuit and moreover biscuit was appreciated by panelists for its pleasant taste and good texture.

Effect of mixing ratio on color parameters of upper and lower surface of biscuits

The color of upper and bottom biscuits were depicted in Figure 4. L^* value represented the lightness of biscuits by level 0-100. The b^* values suggest the yellowness of biscuits and redness is donated by a^* values. Color is very important attribute because it can arouse consumer's appetite and play crucial role in acceptance of food products [52]. Several factors have been reported to affect the development of color on the product surface of biscuits, including amino acids, ingredients, temperature, air velocity, moisture and heat transfer into the sample. The L^* value of upper surfaces wheat biscuit control was higher comparing biscuits fortified with SFPC, that could be due to lower protein and fat content of wheat flour biscuit than biscuits supplemented with SFPC. Mamat et al. [63] stated in the findings that lightness has inverse relationship with protein content. a^* and b^* values of biscuit samples were higher for the bottom than upper surface. Probably this effect is due to browning and maillard reaction in which the browning reactions are more marked at the bottom of the biscuits, indicating that the tray had a higher temperature than the air in the oven. Whereas, L^* value was higher for the upper than bottom surface, being lighter on the upper surface. However, color of biscuits did not show significant difference ($p \leq 0.05$) in all the color parameters. With increase in SFPC, lightness was slightly decreased while both redness and yellowness were increased which was mainly attributed to maillard reactions between sugar and free amino acids provided by SFPC. The amount of inclusion levels of SFPC on LGWF slightly affecting the color of produced biscuits (Figure 4). The current result is in harmony with other workers [1,52,53,63].

Shelf life of biscuits

This study further assayed of biscuits produced by incorporating of SFPC during storage in a polyethylene package for six months under ambient temperature. There are three observation points, namely 2, 4 and 6 months. This study analyzed alteration of the biscuit during storage at room temperature at interval of 2 months based on the physico-chemical, chemical and microbiologic analyses. The results of physico-chemical and chemical parameters during storage can be seen in Table 6.

The biscuit products were acceptable more than six months of storage. The moisture content of the biscuits were low and varied between 4.73-5.50% (0%), 4.75-5.70% (5%), 4.75-5.54% (7%) and 4.76-5.45% (10%) from day zero up to six months storage period. No significant difference was observed among the produced biscuit in terms of moisture content. Moisture content is an exact indicator of the susceptibility of the food product to undergo microbial spoilage. Moisture content above 10% is good and conducive for the growth of microorganisms in dry fish and their products [64]. Results of the present study shows moisture content were below the recommended levels, so microbial load were also limited throughout the storage period in all biscuit products. According to Surpalekar and Bretscheider [65] fortified biscuit with groundnut protein can be stored for 5 months at a temperature of 25-30°C. Similarly, biscuit supplemented with 8% FPC was successfully stored for 161 days at 22, 32, 42 and 52°C without any change in flavor [66].

There was no significant variation in crude protein, lipid and ash contents during the storage in all biscuit samples. FFA increased, from 0.12 to 0.15 for biscuit control, 0.11 to 0.14 for 5%, 0.10 to 0.15 for 7%, 0.09 to 0.17 for 10% as oleic acid and TVB-N increased from 0.04 to 0.17 mgN/100 g for 10%, from zero day up to six months, which was well within the limit of acceptability [42,67]. There was no significant changes in FFA, PV, TBA and pH throughout the storage time in all biscuit samples. All the quality criteria were not increased up to the 4 months of storage. According to other study [64] supporting to the storage stability of biscuit, FFA values in food product has direct relation with microbial activity: high level of free fatty acids is an indication of microbial spoilage activity.

FFA is an initial step in fat degradation, development of hydrolytic rancidity and off flavors in fatty foods. It forms by the hydrolysis of lipid in the fish and their products [44,67,68]. FFA formation could be inhibited by heating and it may be due to the hydrolysis of lipids by phospholipids. Similar results have been reported by Chattopadhyay et al. [19] and Kaneniwa et al. [69]. During initial processing of the SFPC heating and drying process presumably inhibited enzymatic lipolysis. During the 6^{th} month of storage FFA of biscuit fortified with 10% SFPC increased from 0.09 to 0.17 and in 6 months of storage period it did not exceed the acceptable limit. Most of the acidity of fish and fish products begins to be noticeable to the palate when the free fatty acid values calculated as oleic acid is about 0.5-1.5% [67]. Rancidity developments is a major problem in the storage of fishery products but in our study, fat was already pressed out and so oxidative and hydrolytic rancidity were low within the acceptable limit in the SFPC supplemented biscuits during the period of storage which indicated that biscuits had shelf-life more 6 months.

Peroxide value (PV) is the indicator of primary lipid oxidation product and it indicates very early stage of development of oxidative rancidity. The present study prevails that the PV value of biscuits fortified with 5,7 and 10% SFPC increased up to 0.04 to 0.13, 0.04 to

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0.15 and 0.04 to 0.17 meq of PV/kg of fish oil, respectively throughout the storage period which was within the acceptable limit of 10–20 meq of PV/kg of fish oil [29,43,68]. The thiobarbituric acid reacting substance values increased very slightly from 4th to 6th month in case of both control and treated biscuits. Among treatments, the TBARS values were comparable during entire storage period and biscuit added with 10% SFPC showing slightly higher values in 6th month (0.15 mg malonaldehyde kg^{-1}) as compare to control (0.06) and SFPC added biscuits (0.09 mg malonaldehyde kg^{-1} for 5% and 0.12 mg malonaldehyde kg^{-1} for 7%). However, thiobarbituric acid value was well within the maximum acceptable limit which were much lower than the threshold values of 10-20 mg malonaldehyde kg^{-1} of sample which indicates good quality fishery products [43]. This indicates that the level of quality indicators such as hydrolysis of lipid (FFA), primary and secondary lipid oxidation does not increase during storage. Suggesting, there was low fat content in the samples and also there was increase in extraction of total lipids at the time of SFPC processing. The results of the current study agreed with the results of Olayinka et al. [70] who studied the nutritional composition, sensory evaluation and microbiological studies of fish cake made from shrimp bycatch. In contrast to our result, Raja et al. [71] observed rapid increase of TBAS from day zero to 28 in control and fish curls fortified with different flours, suggesting that due to differences in fish species and processing methods.

The pH is an important index for determining the quality of fish and fish products. The pH treatment can also alter quality of fish and its product [44]. In our study, during the storage period the pH values of biscuit samples decreased slightly in all biscuit samples in which the decrease was non-significant ($p \leq 0.05$), as outlined in Table 6. At the

Inclusion levels	Storage period (Month)	Physico-chemical & chemical parameters								
		Moisture	Ash	Fat	Protein	FFA	PV	TBA	pH	TVB
0%	0	4.73 ± 0.14^{a}	0.78 ± 0.06^{b}	15.58 ± 0.05^{b}	9.50 ± 0.18^{d}	0.12 ± 0.11^{a}	0.04 ± 0.01^{a}	0.03 ± 0.01^{b}	6.56 ± 0.03^{b}	0.48 ± 0.00^{a}
5%		4.75 ± 0.08^{a}	1.53 ± 0.04^{a}	16.20 ± 0.06^{a}	14.63 ± 0.12^{c}	0.11 ± 0.05^{a}	0.04 ± 0.01^{a}	0.05 ± 0.03^{ab}	6.66 ± 0.02^{a}	0.52 ± 0.12^{a}
7%		4.75 ± 0.10^{a}	1.60 ± 0.01^{a}	16.35 ± 0.03^{a}	17.88 ± 0.01^{b}	0.10 ± 0.08^{a}	0.04 ± 0.01^{a}	0.10 ± 0.01^{a}	6.73 ± 0.00^{a}	0.52 ± 0.12^{a}
10		4.76 ± 0.11^{a}	1.66 ± 0.12^{a}	16.50 ± 0.17^{a}	19.52 ± 0.12^{a}	0.09 ± 0.13^{a}	0.04 ± 0.01^{a}	0.13 ± 0.01^{a}	6.67 ± 0.02^{a}	0.54 ± 0.13^{a}
	2									
0%		4.79 ± 0.06^{a}	0.78 ± 0.02^{b}	15.50 ± 1.71^{b}	9.44 ± 0.58^{d}	0.12 ± 0.01^{a}	0.05 ± 0.03^{a}	0.04 ± 0.01^{c}	6.56 ± 0.03^{b}	0.48 ± 0.01^{a}
5%		4.81 ± 0.09^{a}	1.56 ± 0.07^{a}	16.16 ± 1.36^{a}	14.57 ± 0.02^{c}	0.11 ± 0.04^{a}	0.04 ± 0.06^{a}	0.05 ± 0.01^{bc}	6.66 ± 0.02^{a}	0.52 ± 0.09^{a}
7%		4.82 ± 0.06^{a}	1.60 ± 0.01^{a}	16.30 ± 0.04^{a}	17.82 ± 0.18^{b}	0.10 ± 0.01^{a}	0.04 ± 0.04^{a}	0.09 ± 0.05^{ab}	6.73 ± 0.00^{a}	0.52 ± 0.02^{a}
10		4.83 ± 0.13^{a}	1.66 ± 0.07^{a}	16.45 ± 0.22^{a}	19.46 ± 0.20^{a}	0.09 ± 0.01^{a}	0.04 ± 0.08^{a}	0.11 ± 0.00^{a}	6.67 ± 0.02^{a}	0.55 ± 0.99^{a}
	4									
0%		4.85 ± 0.07^{a}	0.77 ± 0.12^{a}	15.44 ± 0.06^{b}	9.37 ± 0.02^{d}	0.13 ± 0.01^{a}	0.07 ± 0.04^{a}	0.05 ± 0.03^{a}	6.53 ± 0.04^{a}	0.48 ± 0.18^{a}
5%		4.87 ± 0.64^{a}	1.53 ± 0.06^{a}	16.09 ± 0.17^{a}	14.51 ± 0.01^{c}	0.10 ± 0.02^{a}	0.10 ± 0.01^{a}	0.07 ± 0.01^{a}	6.64 ± 0.01^{a}	0.54 ± 0.02^{a}
7%		4.88 ± 0.12^{a}	1.57 ± 0.00^{a}	16.24 ± 0.44^{a}	17.75 ± 0.20^{b}	0.10 ± 0.00^{a}	0.12 ± 0.06^{a}	0.11 ± 0.01^{a}	6.70 ± 0.02^{a}	0.55 ± 0.01^{a}
10		4.90 ± 0.00^{a}	1.62 ± 0.04^{a}	16.39 ± 0.04^{a}	19.39 ± 0.18^{a}	0.10 ± 0.00^{a}	0.12 ± 0.02^{a}	0.13 ± 0.05^{a}	6.64 ± 0.01^{a}	0.57 ± 0.01^{a}
	6									
0%		5.50 ± 0.07^{a}	0.68 ± 0.12^{a}	14.44 ± 0.04^{b}	8.30 ± 0.01^{d}	0.15 ± 0.03^{a}	0.08 ± 0.03^{a}	0.06 ± 0.01^{a}	6.49 ± 0.01^{a}	0.51 ± 0.08^{a}
5%		5.70 ± 0.64^{a}	1.40 ± 0.06^{a}	15.73 ± 0.07^{a}	13.01 ± 0.03^{c}	0.14 ± 0.06^{a}	0.13 ± 0.07^{a}	0.09 ± 0.04^{a}	6.61 ± 0.06^{a}	0.57 ± 0.05^{a}
7%		5.54 ± 0.12^{a}	1.48 ± 0.00^{a}	15.88 ± 0.14^{a}	16.15 ± 0.10^{b}	0.15 ± 0.01^{a}	0.15 ± 0.04^{a}	0.12 ± 0.05^{a}	6.66 ± 0.09^{a}	0.62 ± 0.03^{a}
10%		5.45 ± 0.00^{a}	1.57 ± 0.04^{a}	15.99 ± 0.02^{a}	18.19 ± 0.08^{a}	0.17 ± 0.04^{a}	0.17 ± 0.01^{a}	0.15 ± 0.02^{a}	6.61 ± 0.03^{a}	0.65 ± 0.07^{a}

The values in the table are the mean of triplicates with standard deviation (±). Mean values followed by the same letter in the column are not significantly different ($P \geq 0.05$)

Table 6: Effect of storage period on physico-chemical and proximate composition of control biscuit and sturgeon fillet protein concentrate biscuit supplemented with 5%, 7% and 10%.

Storage period (Month)	0%	5%	7%	10%
Total plate count				
0	NG	NG	NG	NG
2	NG	NG	NG	NG
4	NG	NG	NG	NG
6	NG	NG	NG	NG
Fungal growth				
0	NG	NG	NG	NG
2	NG	NG	NG	NG
4	NG	NG	NG	NG
6	NG	NG	NG	NG

*NG= no growth.

Table 7: Effect of storage period on bacteriological aspect (cfu/g) of control biscuit and sturgeon fillet protein concentrate biscuit supplemented with 5%, 7% and 10%.

6 months of storage period biscuits incorporated with 5, 7 and 10% SFPC had pH of 6.61, 6.66, 6.61, respectively in good condition which indicated a good quality product. In another study [72] demonstrating the effect of replacing pork lard with carrot and onion on the quality of Chinese style sausage, a decrease in pH during storage in sausages supplemented with carrot and onion was reported, and these values of pH decrease are similar with the decrease in pH values of biscuit samples found in the present study.

From the investigated TPC and TFC were not detected throughout the storage period in all biscuit samples, as depicted in Table 7. This might be due to low moisture content throughout the storage period in biscuit samples and good packaging material used. Direct correlation was observed between bacterial count and pH value of the product. Previous work have been highlighted that dry food and food products owe to their durability of storage but get rapidly attack by moulds and bacteria when exposed to moist air with subsequent absorption of water and hence good packaging is essential to retain the original quality of food products [73]. This fact is duly supported by Chattopadhyay et al. [19] where authors have reported that high density polyethylene pouches are good packaging material for food products. A high density polyethylene pouch which was readily available packaging material preserve the quality of biscuits fortified with SFPC without any adverse effect more than six months at 20-35°C. Biscuit enriched with fish protein was stored for a period of more than 6 months in polyethelene bags at 21-32°C found by Gajera [74] confirmed the storage time, good packaging material and quality of prepared biscuits found in the present study.

Conclusion

This study revealed that the sturgeon fillet protein concentrate is a good source of protein, ash, fat, and amino acids as compared with low gluten wheat flour that could play a vital role in improving nutritional and sensorial attributes of biscuit and could be used for enriching the protein content of cereal based biscuit at large improve health of consumers. Sensory characteristics of the biscuits were not significantly influenced by the addition of SFPC. The SFPC-formulated biscuit with 10% were well accepted by their sensory characteristics. So, the use of SFPC in biscuit was effective for technological and nutritional advantages of biscuits. Biscuit incorporated SFPC could be conveniently packed in high density polyethylene pouches for a period of more than 6 months in ambient conditions without any marked loss of physicochemical, microbial and sensory quality. Therefore, the current study assures that the nutritional value of biscuit can be improved by supplementing its flour with protein-rich sources, such as fish protein concentrate.

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