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Consistent effect of Nile tilapia (*Oreochromis niloticus*) fortification in biscuit on nutritional composition, consumer perception and shelf life properties

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Abstract

Fish can be regarded as a promising protein ingredient for food fortification and enrichment. This study aimed to investigate the potential application of Nile tilapia fish as a dietary supplement. Fish powder was fortified in 0 (control), 5, 10 and 15% levels in dry ingredients by replacing wheat flour and other ingredients were kept constant. Then, biscuits were prepared and the proximate composition, physical and texture properties were determined, as well as sensory acceptance. The addition of fish had a statistically significant ($p < 0.05$) effect on moisture, ash, fat, protein, carbohydrate, total energy, color, water activity, hardness and sensory acceptance. The protein content increased as much as 2.4-4.6 times as more fish was added. Results show that up to 5% fortification level, the biscuits were acceptable with improved functional properties compared to the control. There were significant changes in physical transformation in the biscuits packaged with polyethylene and aluminum foil at 25°C and 35°C, respectively. Fortification of fish powder could be used in the production of high-protein biscuits.

1. Introduction

The high demand for health-promoting products leads to the fortification of nutritional protein ingredients, fiber, antioxidants and other active compounds in several food products. Biscuits are baked food produced from flour and are commonly consumed by many people because of their varied flavors, long shelf life, and fairly low price (Kishor *et al.*, 2017). A biscuit is one of the well-known products and represents the largest category of snacks. Numerous supplementary ingredients are fortified into biscuits; for example, sidr leaf and flaxseed (Seedy *et al.*, 2021), protein hydrolysate for white shrimp (Sinthusamran *et al.*, 2019), and herb, spices, millet and oil seeds (Agrahar-Murugkar, 2020).

Fish and fish products have gained considerable attention as an important source of nutrition in the human diet. Nile tilapia (*Oreochromis niloticus*) is an economical fish, which has high market value and wide acceptance. This fish is a popular substitute for quality animal protein sources for human consumption in many tropical countries, because of being fast-growing, efficient conversion of a low-protein diet, and toleration in a wide range of environmental conditions (Shiau and Shue, 1989). Nile tilapia contains nearly 13.86-17.12% proteins, 1.73-3.17% fat, and 1.76-3.30% ash (Jim *et al.*, 2017). Due to tilapia's whiteness and lack of odor, it could be an ideal choice for the development of fish products such as fish curry, sandwiches, burgers and sticks (Dhanapal *et al.*, 2010).

Generally, commercial bakery products are mainly composed of a high content of carbohydrates, fats, and calories, but are low in fiber and protein (Mishra and Chandra, 2012). Thus, the supplement of bakery products with Nile tilapia meat might increase the bakery's nutritional value, especially protein content. In consideration of nutritional benefits and sensory properties, the current investigation was carried out to produce biscuits from Nile tilapia as a partial substitute for wheat flour. The biscuits forming these fortifications were evaluated for nutritional, physico-chemical and texture properties and sensory acceptance. In addition, the performance of packaging, including polyethylene (PET) and aluminum foil, on the physical transformation

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was also evaluated.

2. Materials and methods

2.1 Fish biscuits preparation

Fresh fish, Nile tilapia, was purchased from a local fish market, Surin, Thailand. The fish was gutted, cleaned, and deboned to obtain fillets, as shown in Figure 1a. The fish fillet was then streamed for 15 mins and ground to paste, roasted until dried, and sieved by 60 mesh (250 µm) before being packed into an aluminum foil bag and stored at 4°C. The Nile tilapia powder compose of 14.56±1.03% protein, 1.94±0.16% fat, 5.53±0.88% moisture, 0.57±0.06% fiber, and 2.21±0.47% ash.

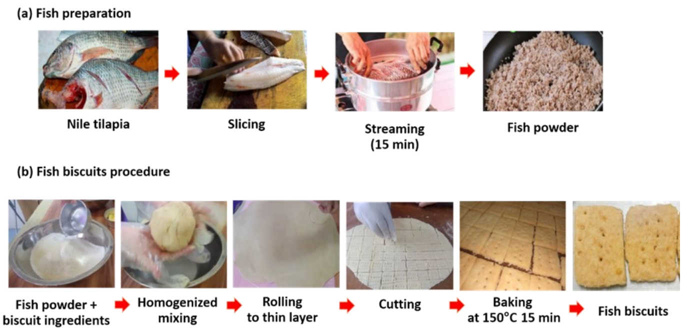

Figure 1. The diagram of (a) fish powder preparation and (b) fish biscuits preparation.

The formulations for the production of biscuits supplemented with 5, 10, and 15% Nile tilapia are represented in Figure 1b. All ingredients, including sugar 1.8%, salt 1.1%, margarine 16.4%, milk 35.0%, egg 18.3%, and wheat flour 27.4%, were weighed and mixed in a dough mixture. The dough was sheeted to the thickness of 0.5±0.1 cm with a rolling pin and then cut to biscuit shape. They were then baked at 150°C for 15 mins in a baking oven. The biscuits were cooled at room temperature for 20 mins before being packed in polyethylene bag and sealed until analysis.

2.2 Proximate composition and total energy

Moisture, ash, fiber, and fat contents were assayed by the methods of Association of the Official Analytical Chemists (AOAC, 2005). The protein content was determined by multiplying the total nitrogen content estimated by the Kjeldahl method by 6.25. The carbohydrate content was obtained by subtracting the sum of protein, fat, ash, and fiber from a hundred per cent. Water activity (a_w) was carried out using 4TEV water activity meter (Aqualab, Pullman, WA, USA). Total energy was calculated based on protein, fat, and carbohydrate contents using the formula described by Merrill and Watt (1955). Energy value (kcal/100 g) = 4 × protein (%) + 9 × fat (%) + 4 × carbohydrate (%).

2.3 Color measurement

The color of fish biscuits was determined using Hunter Color Measurement Spectrophotometer (UltraScan VIS, HunterLab, Virginia, USA). The results were recorded in terms of color parameters, L^*, a^* and b^*. The L^* represents the lightness of the sample, where a positive value indicates whiteness and zero indicates black. The positive a^* value (above zero) represents product redness whereas the negative values represent green shade. The b^* values above zero signify yellow hue while blue hue for below zero. The change in color was determined by calculating the color difference (ΔE) using the following equation:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

Where ΔL: L^*sample-L^*control, Δa: a^*sample-a^*control, and Δb: b^*sample-b^*control

Yellowness index (YI) was calculated using the following formula:

$$YI = \frac{142.86b^*}{L^*}$$

2.4 Texture properties

The texture (hardness and fracturability) of biscuits was measured by TA-XT plus Texture Analyzer (Model: TA-XT, Godalming, Surrey, UK). The analyzer was set at pre-test speed, test speed, post-test speed, and distance to contact the biscuits with a 5 kg load cell: 1 mm/s, 3 mm/s, 10 mm/s, and 100 mm, respectively. Ten biscuits were used for each treatment.

2.5 Sensory acceptance

The acceptance of fortified fish biscuits was evaluated by a group of 50 testers in an age group of 20-50 years old. The panelists were asked to rate on a sheet of 9-point hedonic rating tests accordingly for overall acceptability (Meilgaard, 1999). All experiments were carried out according to the relevant guidelines and regulations.

2.6 Shelf life study

The packed products (30.0 g) with PET and aluminum foil by vacuum seals were maintained in a storage chamber under 50% relative humidity at 25°C and 35°C in the absence of light. The biscuits at 0, 1, and 2 weeks were evaluated for their moisture content, a_w, color, hardness, and fracturability, according to the above methods.

2.7 Statistical analysis

All experiments were done in triplicate. The data were expressed as mean values ± standard deviation

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(SD) derived from triplicate determinations. Comparisons were performed by one-way analysis of variance (ANOVA) with the Duncan multiple range test for significance at $p < 0.05$ using SPSS software (Version 17, SPSS, Inc., USA).

3. Results and discussion

3.1 Proximate composition and water activity

The proximate composition and water activity (a_w) of fish-supplemented biscuits are presented in Table 1 in comparison with biscuits without Nile tilapia powder (control). Carbohydrate (52.64%) was the major component of control, followed by fat (29.33%), and protein (12.75%) with minor amounts of fiber and ash. Fortification of tilapia fish markedly increased the protein content of biscuits in a dose-dependent manner ($p < 0.05$) and the highest protein content was obtained from 15% of fish fortification (24.46%) by a 2-times increase over the control. Apart from protein content, a significant amount of fat, ash, and total energy were also observed ($p < 0.05$). The lower carbohydrate content was found in fish fortification, which indicated the dilution effect of biscuit content with the increasing of dry matter from fish powder. The increase in protein, ash, and lipid was consistent with pasta fortification with tilapia protein concentrate (Goes *et al.*, 2016) and tilapia flour (Monteiro *et al.*, 2016). The increase of energy value in biscuits with fish powder might be due to the inclusion of polyunsaturated fatty acids and essential amino acids (de Oliveira *et al.*, 2015). The decrease of a_w in fish biscuit formulation compared to control might be due to the attribution of protein-water binding and protein-polysaccharide-water complex (Rosenvold and Andersen, 2003). The increase in moisture content in biscuits fortified with tilapia powder could be due to the hydroxyl group of protein interacting with water, which results in more water (Abou-Zaid and Mohamed, 2014).

3.2 Color

The color of food products is a major criterion affecting overall quality and acceptability by consumers. The L*, a*, and b* values were measured as the surface of biscuits, and are presented in Table 1. The L*, a*, and b* values were significantly different between control and fortified biscuits ($p < 0.05$). The lower L*, a*, and b* values of control were due to the lower protein and fat contents (Abraha *et al.*, 2018) and the Millard reaction (Mohammed *et al.*, 2016). However, the sample with the addition of 10% and 15% Nile tilapia powder also had a similar color value ($p > 0.05$). The YI of the control biscuit was significantly different ($p < 0.05$) from those supplemented with 5%, 10%, and 15% Nile tilapia powder. The obtained results of ΔE and YI values confirm the different color of the control in contrast to the fortified biscuits.

3.3 Texture properties

The texture properties including hardness and

Table 1. Properties and proximate composition of biscuits fortified with tilapia fish.

Properties	Fish content (%)			
	0	5	10	15
Color				
L*	44.56±0.62[c]	51.50±0.33[b]	52.74±0.70[a]	53.34±0.73[a]
a*	6.61±0.31[c]	7.48±0.19[b]	7.72±0.31[a]	7.93±0.35[a]
b*	20.47±0.95[a]	16.62±0.41[c]	19.53±0.36[b]	20.04±0.42[a]
ΔE	-	7.98±0.56[ab]	8.31±0.86[a]	8.89±0.48[a]
YI	65.63±1.25[a]	46.10±0.97[c]	52.90±1.65[b]	53.67±1.29[b]
Physical				
a_w	0.34±0.01[a]	0.19±0.00[c]	0.24±0.00[b]	0.24±0.00[b]
Texture				
Fracturability[ns]	11.60±3.37	12.30±2.67	13.10±2.88	14.00±3.79
Hardness (N)	98.13±1.19[a]	3.13±1.56[c]	6.93±1.67[b]	7.01±2.86[b]
Proximate (%)				
Moisture	2.82±0.28[c]	2.71±0.11[c]	3.31±0.12[b]	3.80±0.03[a]
Ash	0.86±0.06[c]	0.95±0.02[c]	1.07±0.06[b]	1.27±0.35[a]
Fat	29.33±0.11[d]	30.29±0.08[c]	31.12±0.36[b]	32.48±0.49[a]
Protein	12.75±0.87[d]	31.17±2.32[c]	45.58±6.87[b]	52.46±6.87[a]
Fiber[ns]	1.60±0.62	1.44±0.98	1.66±1.24	1.77±1.22
Carbohydrate	54.22±1.02[a]	33.03±1.65[b]	18.90±2.06[c]	8.86±2.27[d]
Total energy (kcal/100 g)	700.26±3.21[d]	782.68±8.46[c]	844.83±27.89[b]	995.91±40.81[a]

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different for each parameter ($p < 0.05$).

fracturability of fortified fish biscuits were measured and are presented in Table 1 in comparison with control. With the addition of tilapia powder, there was no significant difference between the samples with respect to fracturability ($p > 0.05$). The hardness value of biscuits significantly decreased with the addition of Nile fish powder by 2-5 times. This might be due to the decrease in starch content and high amount of protein, which do not interact with the gluten network (Desai *et al.*, 2018).

3.4 Sensory acceptance

The sensory evaluation of biscuits fortified with fish was carried out to evaluate the acceptability and determine at which ratio the produced biscuits are most liked by consumers (Table 2). The investigated attributes, such as appearance, color, flavor, taste, and overall acceptability of control (0%) and biscuits fortified with three levels (5%, 10% and 15%) of Nile tilapia, were evaluated by panelists consisting of 50 judges. The results showed significant differences between control and experimental samples ($p < 0.05$). The biscuits made with 5% fortification were the most liked, and their overall acceptability was the highest (8.30±0.95) among the treatments. As can be seen in Table 2, biscuits made with 5% fish powder addition were the best among the fortified fish biscuits for all the sensory attributes investigated, except color attributes. The results show that the increase of the additional level of fish powder by more than 5% decreases color of the biscuits, which could be due to the Millard reaction (Mohammed *et al.*, 2016). Sensory analysis showed that biscuits fortified with 5% Nile tilapia powder enhanced the sensory attributes due to their pleasant taste, flavor, and good appearance.

3.5 Shelf life study

The physical transformation such as color, fracturability, hardness, moisture, and a_w of biscuits packaged in aluminum foil and PET at 25°C and 35°C are presented in Table 3. There were significant changes in all investigated parameters in biscuits packaged in aluminum foil and PET at both 25°C and 35°C ($p > 0.05$). However, the biscuits kept under 25°C showed greater physical transformation than at 35°C. The change of L*, a, and b* values might be due to the inclusion of polyunsaturated fatty acids and essential amino acid (de Oliveira *et al.*, 2015) with the protein-polysaccharide-water mechanism (Rosenvold and Andersen, 2003). The fracturability was reduced 2-fold due to the sucrose reduced expansion mechanism (Barrett *et al.,* 1994). The increase in hardness values was due to the increase in protein in biscuits (Gallagher *et al.*, 2005). An increase in both moisture and a_w of the biscuits packaged in aluminum foil and PET was found throughout the storage, which is in agreement with the results of sweet potato chips in different packaging systems (Marangoni *et al.*, 2018).

4. Conclusion

Nile tilapia powder could be used in the fortifying of biscuits. The addition of fish powder influenced the physical, texture, and quality properties of biscuits, depending on the level used. According to sensory analysis, the addition of 5% Nile tilapia powder was the most liked, compared to other levels. Based on the performed measurement, it can be assumed that a reasonable addition of Nile tilapia at 5% improves the overall quality of biscuits. Therefore, Nile tilapia fortified biscuits could be a new product rich in protein and enhanced sensory properties. During storage, it is much more important to prevent or delay the physical transformation. Regardless of the packaging material, the quality loss of fish biscuits is unavoidable; however, the change of texture properties is markedly higher in aluminum foil. In addition, the microstructure of supplemented biscuits as well as the physico-chemical texture properties between Nile tilapia composite and biscuit.

Conflict of interest

The authors declare no conflict of interest.

Table 2. Sensory acceptance of biscuits fortified with tilapia fish.

Attributes	Fish content (%)			
	0	5	10	15
Appearance	7.50±0.86^a	5.63±0.85^b	8.03±0.49^a	5.37±1.03^b
Color	7.10±0.80^a	7.10±0.80^a	5.90±0.80^b	5.63±0.61^b
Flavor	7.93±1.08^a	5.67±0.80^b	7.97±0.61^a	5.10±0.66^c
Taste	7.20±0.76^a	5.33±0.66^b	7.70±0.46^a	5.13±1.27^b
Texture	7.43±0.72^a	5.57±0.67^b	7.37±0.80^a	5.70±1.05^b
Overall acceptability	7.47±0.93^a	5.50±0.77^b	8.30±0.95^a	5.47±0.93^b

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different for each parameter ($p < 0.05$).

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Table 3. Changes in color, texture and moisture content of fish biscuits during storage at 25 and 35°C.

Properties	Periods in weeks	Aluminum foil*		PET *	
		25°C**	35°C**	25°C**	35°C**
L*	0	51.84±1.44[a]	51.84±1.44[ns]	51.84±1.44[a]	51.84±1.44[ns]
	1	49.67±1.80[a]	51.40±1.60	44.64±1.55[b]	52.31±1.96
	2	47.25±0.71[ab]	51.38±0.72	43.30±1.56[b]	52.64±1.55
a*	0	8.40±0.30[a]	8.40±0.30[a]	8.40±0.30[a]	8.40±0.30[a]
	1	7.03±0.18[b]	6.97±0.22[b]	6.97±0.22[b]	6.11±0.22[b]
	2	6.30±0.13[c]	6.78±0.43[b]	6.78±0.43[b]	5.34±0.20[c]
b*	0	19.53±1.34[ns]	19.53±1.34[ns]	19.53±1.34[a]	19.53±1.34[a]
	1	18.87±0.50	19.49±0.51	15.47±0.48[b]	19.40±0.60[a]
	2	17.55±0.13	19.47±0.48	15.27±1.03[b]	17.47±0.48[b]
Fracturability (N)	0	13.10±2.88[a]	13.10±2.88[a]	13.10±2.88[a]	13.10±2.88[a]
	1	5.63±1.51[b]	5.69±1.97[b]	10.53±4.80[b]	10.66±3.35[b]
	2	5.81±2.42[b]	5.16±0.91[b]	5.21±2.03[c]	5.11±1.27[c]
Hardness (N)	0	6.93±1.67[b]	6.93±1.67[b]	6.93±1.67[b]	6.93±1.67[b]
	1	8.50±1.90[b]	5.90±1.44[b]	11.50±4.99[a]	6.10±1.10[b]
	2	13.60±4.00[a]	11.60±4.16[a]	12.30±4.90[a]	13.40±7.01[a]
Moisture (%)	0	3.31±0.12[a]	3.31±0.12[b]	3.31±0.12[a]	3.31±0.12[ns]
	1	2.86±0.72[ab]	3.58±0.38[a]	2.53±0.40[b]	3.34±0.12
	2	2.53±0.31[b]	3.88±0.09[a]	2.28±0.48[b]	3.41±0.06
a_w	0	0.19±0.00[b]	0.19±0.00[c]	0.19±0.00[c]	0.19±0.00[c]
	1	0.21±0.00[b]	0.33±0.01[b]	0.25±0.00[b]	0.31±0.01[b]
	2	0.33±0.01[a]	0.42±0.01[a]	0.42±0.01[a]	0.46±0.00[a]

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different for each parameter ($p < 0.05$).

[ns] no statistically significant difference within the same column for each parameter at $p < 0.05$.

*Significant difference at $p < 0.05$ in comparison between aluminum foil and PET.

**Significant difference at $p < 0.05$ in comparison between 25°C and 35°C.

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