EFFECT OF THE ADDITION OF FISH PROTEIN ISOLATE ON BISCUITS' PHYSICOCHEMICAL AND SENSORY PROPERTIES

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ABTRACT

Biscuits are among the most popular baked goods. However, traditional biscuits are frequently poor in protein and low in the content of the essential amino acids. Meanwhile, fish protein isolate (FPI) is proven to contain all the essential amino acids. The addition of FPI to biscuits can improve their nutritional value but may change their physicochemical and sensory properties. The present study was, therefore, conducted to investigate the effect of the addition of different levels of FPI prepared from trimmings of yellowfin tuna on the physicochemical properties and sensory acceptability of biscuits. The results showed that the moisture and water activity of biscuits with 1.6-5.4% FPI added significantly decreased compared with the control biscuits without FPI. The hardness of the biscuits with FPI added compared to the control biscuits, however, was not significantly different. The results of the sensory evaluation also showed that the appearance, texture, color, aroma, taste, mouthfeel, and overall acceptability of the biscuits with FPI added and the control biscuits did not differ significantly, except for the color and aroma of the biscuits with 3.8-5.4% FPI added. The addition of 2.7% FPI to biscuits was found to have no negative impact on the biscuits' quality. These findings suggest that FPI prepared from the trimmings of yellowfin tuna is a potential protein source that can be used to improve the nutritional value of biscuits.

***Keywords**: Protein-enriched biscuit, Thunnus albacares, fish protein isolate, value-added products, malnutrition*

I. INTRODUCTION

Among Vietnam's principal seafood export items, tuna is one of the most important. The remaining raw materials in the tuna processing industry include the head, skin, dark muscle, viscera, bones, and so on, which account for around 50-70% of the fish's body weight and are called by-products [1][2]. In tuna by-products, meat trimming is a protein-rich part that can be utilized to prepare fish protein isolate (FPI) by using a pH shift method, and the FPI prepared from yellowfin tuna's dark muscle showed considerably greater amounts of total amino acids and total essential amino acids than the dark muscle [3]. As a result, it can be used to supplement foods with additional protein and nutritional value.

Baked goods can easily be supplemented with nutrients such as protein, vitamins, and minerals to meet the needs and satisfaction of consumers worldwide [4]. Biscuits are outstanding instant baked goods widely consumed by various consumer groups due to their distinct taste, good eating quality, convenience, and long shelf life [5][6]. Because of their low moisture level and thinness, biscuits are typically hard and crunchy, which is greatly enjoyed when eating. However, traditional biscuits are frequently poor in protein and low in the content of the essential amino acids because they are commonly made from grain products [7]. The addition of FPI to biscuits can improve their nutritional value in terms of protein. However, the addition of ingredients derived from seafood may have a detrimental influence on sensory properties despite boosting the nutritional and functional qualities of the food [8]. Previous studies have found that baked products added with fish protein powder at level of 3-10% has no significant effect on their physicochemical and sensory properties [4,9,10]. The level of fish protein powder acceptable for food supplements depends on its characteristics, such as the raw fish species used as raw material, the production method, etc. Therefore, the present study was conducted to investigate the physicochemical and sensory properties of biscuits with different FPI contents added in order to determine the appropriate level of additional FPI.

II. MATERIAL AND METHODS

2.1. Preparation of fish protein isolate

Yellowfin tuna (*Thunnus albacares*) trimmings supplied by a seafood company in Khanh Hoa Province, Vietnam, were used to prepare the fish protein isolate (FPI) by the pH-shift method as described by Nguyen *et al.* [3] with slight modifications. The trimmings were washed twice with cold tap water at 4 °C and pressed manually to remove excess water using a nylon monofilament filter bag (mesh size: 25 micron; Dong Son Ltd., Vietnam). The washed trimmings were minced with a food processor, and the minced material was mixed with cold distilled water at a ratio of 1:10. Subsequently, the mixture was homogenized at a speed of 3500 rpm for 2 min using a homogenizer (ULTRA- TURAX® T18 basic, IKA, Germany). Then, the homogenate was adjusted to pH 12 by adding 2 M NaOH and stirring for 4 hours at room temperature, followed by centrifugation at 3000 rpm for 20 min at 4 °C (MF 600, Biobiz, Incheon, Korea). The supernatant containing solubilized proteins obtained after centrifugation was separated from the floating fat layer and sediment layer. The protein solution was adjusted to pH 5.5 by adding 2 M HCl and then incubated on ice for 10 min to precipitate the protein. The precipitated protein was decanted through the filter bag and washed with cold distilled water to a neutral pH. The washed protein was then centrifuged at 3000 rpm for 20 min at 4 °C to dewater and remove the NaCl to obtain FPI with a proximate composition of 77.1 ± 0.41 % moisture, 0.93 ± 0.02 % ash, 19.27 ± 0.34 % protein, and 2.64 ± 0.05% lipid. The FPI was steamed for 10 min and then dried in 3 stages in a hot air oven: first at 45-50 °C for 6 hours, then at 60 °C for 2 hours, and finally at 40 °C for 2 hours. The moisture content of dried FPI was 10-12%. The FPI powder used for the addition of biscuits was prepared by grinding the dried FPI using a cutting mill (CM 120M – Vibrotechnik, Russian Federation) with a sieve size of 0.25 mm.

2.2. Preparation of biscuits

The ingredient formulation of the biscuits added with FPI and the control biscuits based on dough weight followed the description of Bao [11] with slight modifications and are presented in Table 1.

Table 1. The ingredient formulation of biscuits added with FPI and the control biscuits based on dough weight

Samples	Ingredients (g)								
	Wheat flour	FPI	Butter	Sugar	Edible oil	Salt	Cilantro	Baking powder	Water
Control	53.9	0.0	16.2	6.5	1.6	0.6	1.6	1.3	18.3
1.6% FPI	52.3	1.6	16.2	6.5	1.6	0.6	1.6	1.3	18.3
2.7% FPI	51.2	2.7	16.2	6.5	1.6	0.6	1.6	1.3	18.3
3.8% FPI	50.1	3.8	16.2	6.5	1.6	0.6	1.6	1.3	18.3
5.4% FPI	48.5	5.4	16.2	6.5	1.6	0.6	1.6	1.3	18.3

The mixture of ingredients was well kneaded and then incubated for 15 minutes at room temperature. Subsequently, the dough was sheeted to a thickness of 3 mm and cut into a square shape (30 mm × 30 mm). The shaped biscuit dough was baked in an electric oven at 175 °C for 25 minutes. The baked biscuits were cooled to room temperature and packed in high-density polyethylene ziplock bags for evaluation of their physicochemical and sensory properties.

2.3. Methods of analysis

Moisture content was determined following the procedure of the AOAC [12], and water activity was measured by a Rotronic HYGROLAB C1 (Switzerland). The hardness of biscuits was measured by a Sun Scientific Rheometer CR – 500DX (Japan). The color (L*a*b*) of both the bottom and upper surfaces of the biscuits was measured by a Konica Minolta CR-400/CR-40 (Japan). The whiteness index was calculated according to the following formula: $W = 100 - [(100-L)^2 + (a^2 + b^2)]^{1/2}$. The sensory quality of biscuits was evaluated according to the sensory scheme (the score ranged from 1 for the worst quality to 5 for the best quality) described by Bao [11], using a nine-member sensory panel that was trained to evaluate presenting samples in two preliminary evaluation sessions.

2.4. Statistical analysis

Microsoft Excel 2013 was used to calculate the means and standard deviations for all multiple measurements and to generate graphs. Analysis of variance (ANCOVA) was applied to the data using R software version 4.2.2 (http://cran.R-project.org). Significant differences were determined by a one-way ANOVA, and Tukey's Multiple Comparisons of Means was used to determine the statistical difference between samples at $p < 0.05$.

III. RESULTS AND DISCUSSION

3.1. Physicochemical properties of biscuits added with FPI and the control biscuits

The moisture content and water activity (a_w) of biscuits added with FPI and control biscuits are shown in Figure 1.

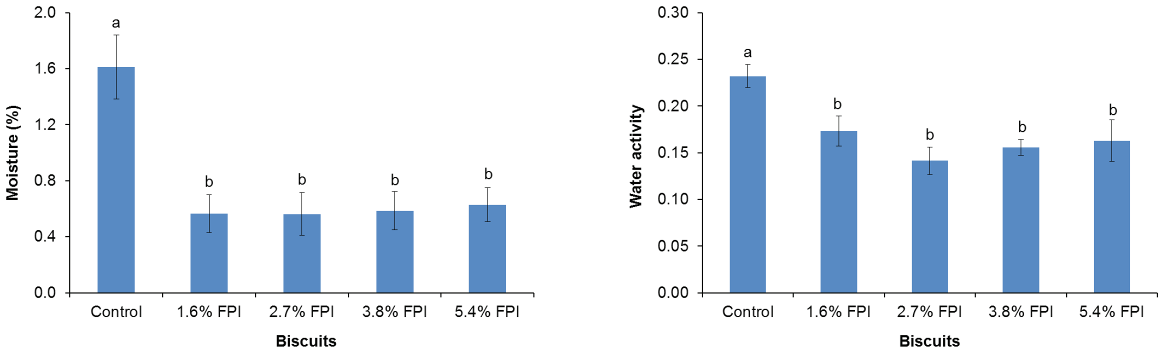

Figure 1. Moisture content (a) and water activity (b) of biscuits added with FPI and the control biscuits. Data are provided as mean ± SD (n = 9). Columns with different letters indicate a significant difference ($p < 0.05$).

Moisture content and a_w are always important factors influencing biscuit stability during storage, especially microbial resistance and rheological properties [13]. The typical biscuit has a low a_w value as well as a low moisture content. As shown in Figure 1, the biscuits added with FPI had a significantly lower ($p < 0.05$) moisture content and a_w value than the control biscuits. These findings back up those of Ervenka et al. [13], who discovered that the main ingredients in biscuits influenced their a_w value. However, different levels of FPI added to biscuits, ranging from 1.7 to 5.4%, did not result in significant differences ($p > 0.05$) in moisture content or water activity. These findings are consistent with those of Parate et al. [14], who discovered that the moisture contents of biscuits fortified with soy nugget powder were significantly lower than those of control biscuits, and that the moisture contents of biscuits added with 10-20% soy nugget powder did not differ significantly. The decrease in moisture content and a_w value of biscuits added with FPI was probably due to the poor moisture retention capability of the formulation of the biscuits added with FPI.

According to Arimi et al. [15], the crispiness of biscuits is affected by a_w, and the hardness of biscuits with a_w lower than 0.6 increases proportionally to a_w. The increase in hardness of the biscuits with increasing a_w may be due to the antiplasticization of the biscuits by water [16]. Free volume theory is used to explain a proposed mechanism for antiplasticization. In a glassy state, there exist low- and high-density domains. As a_w increases, the low-density domains are filled with water, increasing the density at the molecular level and leading to hardening [17][18]. The biscuits added with FPI and the control biscuits in the present study had a_w ranging from 0.14 to 0.23, and their hardness is shown in Figure 2.

The hardness of biscuits added with FPI varied from 59.4 ± 11.4 N to 73.7 ± 10.2 N and did not differ significantly (p > 0.05) from the control biscuit (67.2 ± 7.2 N). This finding is in line with that of Abraha et al. [9], who found that control biscuits and those added with various concentrations of fish fillet protein concentrate (ranging from 5 to 10%) did not significantly differ in terms of hardness. These results suggest that FPI can replace some of the wheat flour used to make biscuits without compromising the hardness of the final product.

Color is also considered a major quality attribute for the biscuits. Therefore, the effect of the addition of FPI on the color of biscuits was investigated, and the results are presented in Figure 3.

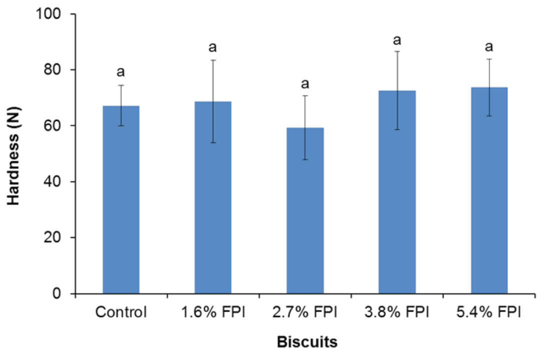

Figure 2. The hardness of biscuits added with FPI and the control biscuits. Data are provided as mean ± SD (n = 9). Columns with different letters indicate a significant difference (p < 0.05).

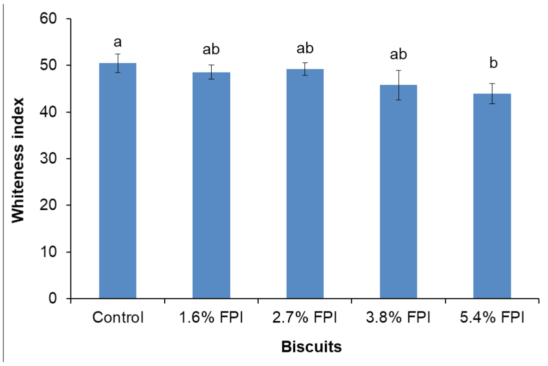

Figure 3. The whiteness index of biscuits added with FPI and the control biscuits. Data are provided as mean ± SD (n = 9). Columns with different letters indicate a significant difference (p < 0.05).

The whiteness index is commonly used to compare the difference in color between nutrient-added biscuits and their controls. As shown in Figure 3, all the biscuits added with FPI had a slightly lower whiteness index when compared with the control biscuit. As the percentage of FPI addition increased, the whiteness index decreased. The whiteness index of biscuits fortified with 5.4% FPI was significantly lower (p < 0.05) than that of the control biscuit. This is probably due to the fact that the color of FPI was darker than the wheat flour.

3.2. Sensory properties of biscuits added with FPI and the control biscuits

Sensory score of biscuits fortified with different level of FPI and the control biscuits as shown in Figure 4.

The results of the sensory evaluation (Figure 4) showed that the sensory scores on appearance, texture, color, aroma, taste, and mouthfeel of the biscuits added with FPI and the control biscuits did not differ significantly (p > 0.05) except for the color and aroma of the biscuits with 3.8 and 5.4% FPI addition. This is probably due to the fishy odor and darker color of FPI.

Although only the biscuits added with 5.4% FPI had significantly lower (p < 0.05) overall acceptance scores than control biscuits, those added with 3.8% FPI had significantly lower (p < 0.05) aroma scores than control biscuits, suggesting that biscuits with 2.7% FPI added are a proper level to improve protein nutrition without significantly affecting the physicochemical and sensory properties of biscuits.

The appearance of biscuits added with different levels of FPI is shown in Figure 5.

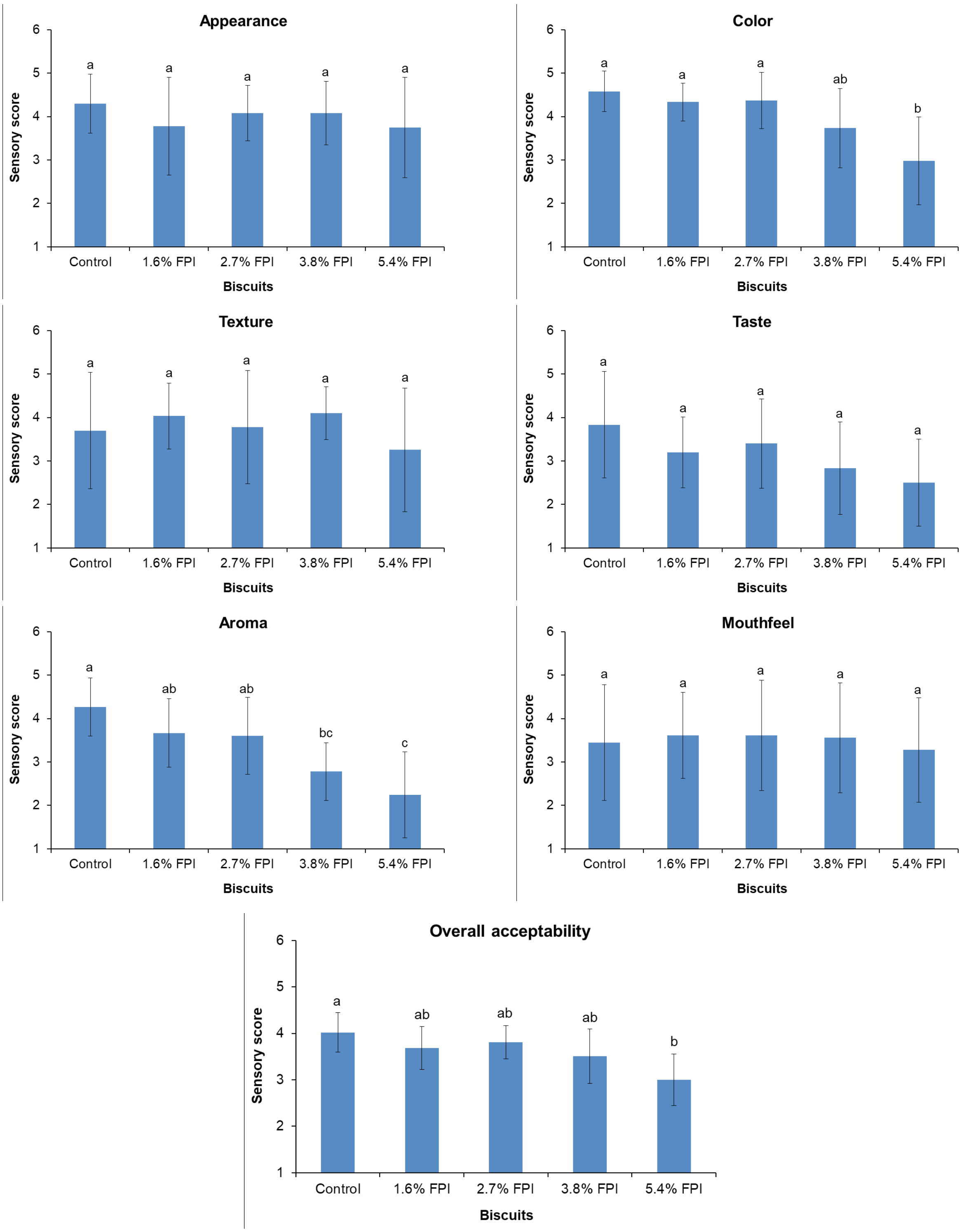

Figure 4. Sensory score of biscuits added with FPI and the control biscuits. Data are provided as mean ± SD (n = 9). Columns with different letters indicate a significant difference ($p < 0.05$).

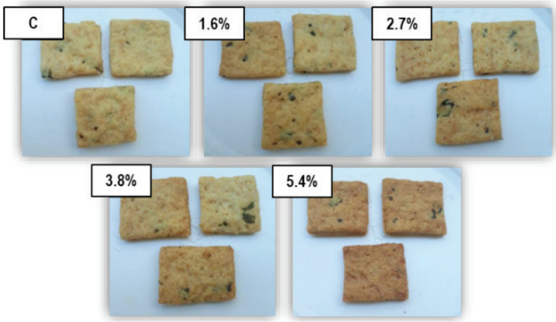

Figure 5. The appearance of biscuits fortified with different levels of FPI after 1 day of baking.

IV. CONCLUSION

The physicochemical and sensory properties of the biscuits with 2.7% FPI added were not significantly different from those of the control biscuits without FPI, except for their moisture and water activity, which were lower in the biscuits with FPI added. These findings suggested that FPI prepared from yellowfin tuna trimmings is a potential protein source that might be added to biscuits to improve nutritional value and that the addition of FPI also enhances the biscuit shelf life by lowering moisture and water activity.

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