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Original Article

Development of a Protein- and Calcium-Rich Snack Food Made From a Local Anchovy (Stolephorus spp) Flour, Soy Protein Isolate and Bambara Groundnut (Vigna subterranea) Flour

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ABSTRACT

Background and Objectives: School children have a high snacking frequency. However, it is poor in nutrients. It is important to provide nutritious snack to support their growth and development. This study aimed to evaluate the nutrient content and sensory properties of a protein- and calcium-rich snack made from a local anchovy (LA), soy protein isolate (SPI), and Bambara groundnut flour (BGF)**.**

Materials and Methods: The formulas consisted of LA, SPI and BGF including F1 ((0.0:22.9:11.5), F2 (17.2:5.7:11.5) and F3 (22.9:0.0:11.5). Proximate and calcium content analyses were carried out in an accredited laboratory, while sensory properties (descriptive test) were evaluated by 25 semi-trained panelists.

Results: The protein content, in mean (standard deviation), of F0, F1, F2 and F3 was 33,57 (0,05), 25,72 (0.02), 20,64 (0,04), and 14,91 (0,05) respectively. The calcium content, in mean (standard deviation), of F0, F1, F2 and F3 was 57,58 (0,09), 164,18 (0,04), 188,78 (0,05), and 181,31 (0,05) respectively. Only F1 and F2 fulfilled the protein-rich claim based on Indonesian Food Drugs Administration standard (BPOM). All formulas fulfilled the 'good source of calcium' claims if compared with BPOM standard. However, F3 was the most preferred by the panelist (highest hedonic score for all parameters).

Conclusions: The new developed product could be an alternative snack which contains a good source of protein and calcium in order to alleviate protein-malnutrition among children, particularly in developing nations.

Keywords: *Stolephorus spp*, *Vigna subterranea*, Calcium-source snack, Protein-rich snack

Introduction

Two-third of Indonesia's region consists of marine which is high in biodiversity, particularly fish products. These products can be the main source of protein and long chain polyunsaturated fatty acids (LC-PUFAs) which can be beneficial for pregnancy (1), children growth and cognitive function(2,3) as well as for lowering the risk of Alzheimer among the elderly(4). However, consumption of such products in Indonesia is lower than their availability and lower if compared with the other ASEAN (Association of Southeast Asian Nations) countries such as Singapore, Malaysia and also Japan and Korea. Fish consumption among Indonesian population was 38.14 kg/cap/year, far below its availability (51.80 kg/cap/year)(5). LA together with Bambara groundnut were highly available in west Java, Indonesia and have the potential to be used as raw material for a healthy snack. A snack in the shape of 'stick' is often a choice of school children. As school children have a high snacking frequency(6,7), it is important to provide alternative nutritious snack to support their growth and development. The anchovy-based snack could be an answer to this issue, since it contains protein, LC-PUFA and minerals, particularly calcium(8). In addition, the use of Bambara groundnut, also named as 'Bogor nut', is another advantage, since it is locally available and is a good source of protein, calcium, phosphorus and iron(8) to promote the children growth and development.

The present study aimed to develop and evaluate the nutritional composition and sensory properties of a snack food for school children based on a locally

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available anchovy. The snack was planned to fulfill 'high protein' and 'high calcium' claims based on BPOM regulation (9).

Materials and Methods

Materials: Fish stick was formulated by mixing fresh anchovy (Jengki *anchovy*) from West Java - Indonesia, soy protien isolate (Manufactured by Shandong Crown Protein) and Bambara groundnut from West Java – Indonesia. Other ingredients as complement were egg, wheat flour (Bogasari Kunci Biru), pumpkin form Jakarta, Indoensia, tapioca flour (Rosebrand, Indonesia), margarine (Blue band, Indonesia), salt, pepper (Koepo-Koepoe) and baking powder (Koepoe-Koepoe, Indonesia). Chemical reagents for proximate and calcium analyses were provided by Saraswanti Indo Genetech Lab. Utensils used for processing the fish stick were blender (Philips, China), Oven (Modenna, Italy), food weighing scale (Camry electronic kitchen scale, China).

Methods: This study was conducted in December 2016-January 2017 and comprised of two main phases. The first phase consisted of the trial and error of the formulation, while the second phase included the evaluation of nutrient content and sensory properties. The trial and error resulted in the formulation of the materials as presented in Table 1.

Product preparation: The procedure for fish stick processing consisted of six steps. This procedure was a modification of a previous study(10). First, the tools and materials were prepared and the basic materials were weighed. Second, yellow pumpkin (YP) and Bambara groundnut (BG) were cleaned and chopped, while local anchovy (LA) was mashed using a meat grinder. Next, YP was steamed and mashed with a blender. Cleaned BG was boiled until cooked, then mashed with a chopper. Third, all the used materials were mixed and kneaded. Fourth, the dough was flattened with a rolling pin, then cut into the 'stick' form. The stick was 7 mm - 1 cm in width, 10-12 cm in length and 2-3 mm in height. Fifth, the fish stick was sun-dried for 3 hours. Later, the stick was baked in an oven at a temperature of 150-160 °C for 15-20 minutes. The baked sticks were removed from the oven and cooled down for 10-15 minutes before being packaged as the last step. The fish sticks were packaged in food grade plastic standing pouch with the code of 5 (polypropylene).

Chemical composition: The fish sticks were analyzed for chemical composition, namely water, ash, protein, and fat contents by AOAC developed methods(11). Carbohydrate content was determined by difference (100%-percentage of water, ash, lipid and protein), while calcium content was analyzed by the methods developed by Puwastien(12). The laboratory analysis was conducted in a national accredited laboratory named Saraswanti Indo Genetech Lab. The fresh baked stick was delivered to the laboratory in a wrapped and vacuum container in duplicate samples.

Table 1. Fish stick formulation based on trial and error

Ingredients	F0	F1	F2	F3
Anchovy, g (%)	0 (0.0)	50 (11.5)	75 (17.2)	100 (22.9)
Soy-protein isolate, g (%)	100 (22.9)	50 (11.5)	25 (5.7)	0 (0.0)
Bambarra groundnut, g (%)	50 (11.5)	50 (11.5)	50 (11.5)	50 (11.5)
Egg, g (%)	60 (13.8)	60 (13.8)	60 (13.8)	60 (13.8)
Wheat flour, g (%)	100 (22.9)	100 (22.9)	100 (22.9)	100 (22.9)
Pumpkin, g (%)	30 (6.9)	30 (6.9)	30 (6.9)	30 (6.9)
Tapioca flour, g (%)	65 (14.9)	65 (14.9)	65 (14.9)	65 (14.9)
Margarine, g (%)	25 (5.7)	25 (5.7)	25 (5.7)	25 (5.7)
Salt, g (%)	3 (0.7)	3 (0.7)	3 (0.7)	3 (0.7)
Pepper, g (%)	1.5 (0.3)	1.5 (0.3)	1.5 (0.3)	1.5 (0.3)
Baking Powder, g (%)	1.5 (0.3)	1.5 (0.3)	1.5 (0.3)	1.5 (0.3)
Total dough weight, g	436	436	436	436

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Sensory evaluation: Sensory properties of the fish sticks were evaluated by 25 semi-trained panelists aged 20-25 years old. They were asked to assess the acceptance and quality of the fish stick parameters, namely taste, color, aroma and texture. A Visual Analog Scale (VAS) questionnaire, a linear line scale, was employed which comprised a straight line 100 mm in length with specific descriptors on its edges(13). For acceptance, the panelists were asked to write their perception in a range of extremely dislike (0) and extremely like (100) for all parameters. For the quality parameters, they were was asked to mark on the line for each parameter. The descriptors were as follows: extremely not savory (0) and extremely very savory (100) for taste, dark brown (0) and yellowish gold (100) for color, extremely not real (0) and extremely real (100) for aroma, and extremely not crunchy (0) and extremely very crunchy (100) for texture property(13).

Statistical analysis: All data were expressed in mean (standard deviation) and statistically analyzed by qone-way ANOVA. The statistical analysis was conducted using Statistical Package for the Social Science (SPSS) version 21.0 software (SPSS Inc., Chicago, IL, USA). In addition, the protein and calcium contents were compared with BPOM standard(9), then classified as either high or source the nutrien claims'.

Results

Fish stick formulation: The fish sticks were formulated in three trials. The final formulation is presented in Table 1. In short, the formulas for the first trial were based on modifying the previous study(10). We removed cheese from Pratiwi's original formula and modified it by adding SPI, LA, YP and BG. This trial resulted in a hard texture determined by the research team members. The second trial, we modified the ratio between SPI and LA as 0:150 (F0), 120: 30 (F1), 130:20 (F2) and 150: 0 (F3). Both trials still resulted in the hard texture of fish stick. Next, on the third trial, the ratio of SPI to LA was again modified as 0: 100 (F0), 50: 50 (F2), 75: 25 (F3) and 100: 0 (F3). These ratios resulted in a preferable texture. Next the formulas was prepared for sensory evaluation by 25 semi-trained panelists. In addition, we also learned that the maximum amount of anchovy to be added was about 22.9% to have desirable textural properties if compared with commercial products.

Sensory properties of fish stick: Table 2 shows that in general, all the formulas had a hedonic quality score above 69 for all the parameters. Only F3 was perceived as the formula with the highest score for all the parameters by the panelists. This formula obtained scores of taste, color, aroma and textural properties above 80 mm. Based on statistical analysis, there was a significant difference of the mean score for all the parameter across the formulas implying that the ratio of SPI to LA can lead to different hedonic qualities of the fish stick. According to Table 3, F2 was the formula with the highest score for all the parameters. Except for taste with a score of 79, the other parameters were scored above 80. Statistical analysis showed a significant difference in the panelists` perceptions of the formulas color and aroma . It means that the ratio of SPI to LA may have affected the semi-trained panelists color and aroma perceptions.

Table 2. Fish stick's hedonic quality score assessed by 25 semi-trained panelists

Variable(s)[1]	Mean (SD)				p-value[2]
	F0	F1	F2	F3	
Taste[3], mm	76 (8)^{a}	76 (7)^{ac}	81 (7)^{ad}	82 (7)^{bd}	0,002
Colour[4], mm	72 (6)^{a}	77 (7)^{ad}	78 (6)^{bd}	80 (7)^{c}	0,002
Aroma[5], mm	69 (8)^{a}	72 (7)^{ad}	78 (6)^{bd}	80 (4)^{c}	0,001
Texture[6], mm	78 (7)^{a}	83 (4)^{b}	82 (4)^{abc}	84 (5)^{cb}	0,003

[1]Tested by visual analog scale (VAS) questionnaire expressed in 0-100 mm; [2]One-way ANOVAs test, significant at $p<0.05$; [a-d]Bonferonni test, significantly differ for different alphabets; [3]extremely not savory (0) and extremely very savory (100); [4]dark brown (0) and yellow gold (100); [5]extremely not real (0) and extremely real (100); [6]extremely not crunchy (0) and extremely very crunchy (100). F0-F3 are formulas with ratio of soy protein isolated (SPI): local fish anchovy (LA), F0= 100 g (SPI): 0 g (LA), F1 = 50 g (SPI): 50 g (LA), F2 = 25 g (SPI): 75 g (LA), F3 = 0 g (SPI): 100 g (LA)

Table 3. Fish stick's acceptance score assessed by 25 semi-trained panelists

Variable(s)[1]	Mean (SD)				p-value[2]
	F0	F1	F2	F3	
Taste[3], mm	78 (6)	77 (8)	79 (7)	77 (7)	0,835
Colour[4], mm	73 (6)[a]	77 (9)[ab]	80 (6)[bc]	75 (8)[ac]	0,007
Aroma[5], mm	71 (9)[a]	73 (10)[ab]	83 (7)[c]	75 (7)[ad]	0,001
Texture[6], mm	81 (8)	79 (7)	84 (7)	81 (4)	0,146

[1]Tested by visual analog scale (VAS) questionnaire expressed in linear line 0-100 mm; [2]One-way ANOVAs test, significant at p<0.05; [a-d]Bonferonni test, significantly differ for different alphabets; [3-5]extremely dislike (0) and extremely very like (100). F0-F3 are formulas with ratio of soy protein isolated (SPI): local fish anchovy (LA), F0= 100 g (SPI): 0 g (LA), F1 = 50 g (SPI): 50 g (LA), F2 = 25 g (SPI): 75 g (LA), F3 = 0 g (SPI): 100 g (LA)

Chemical composition of fish stick: The chemical compositions of the new developed fish sticks are presented in **Table 4**. it was observed that the energy contents of all the formulas were almost similar. The snack can be classified as high energy food, since it contains more than 4 kcal/gram. Except protein and calcium, the table shows a fluctuating moisture, ash, lipid and carbohydrate content of the fish sticks. In fact, the ratio of SPI to LA tended to increase the protein and calcium contents of the formulas. If compared with BPOM regulation (9) related to nutrition and health claim, all the formulas already fulfill the claim of 'good source of calcium'. F0 and F1 both fulfilled the nutrition claim of 'high protein' snack. However, statistical analysis showed a significant difference in the energy and nutrients content of the formulas.

Final price estimation of fish stick formulas: The price of the fish stick formulas is mostly determined by the quantity of LA. Higher LA content will make the formula costly. It can be seen in **Table 5** that the most expensive formula is F3 which contains the highest LA content. However, the price of all the formulas is not more than 1 USD for a 50-gram serving size.

Table 4. Nutritional composition of new developed fish stick in 100 g

Energy/Nutrients[1]	F0	F1	F2	F3	p-value[2]
Energy, kcal	421 (2.8)[a]	441 (2.5)[b]	434 (2.4)[b]	424 (2.2)[a]	0.004
Moisture, %	2,10 (0.04)[a]	3,33 (0.01)[b]	2,38 (0.07)[c]	2,78 (0.05)[d]	0.001
Ash, %	4,62 (0.01)[a]	2,81 (0.02)[b]	3,71 (0.04)[c]	2,14 (0.04)[d]	0.001
Lipids, %	9,58 (0.11)[a]	13,03 (0.07)[b]	11,72 (0.04)[c]	8,81 (0.04)[d]	0.001
Crude Protein, %	33,57(0.05)[a,□]	25,72 (0.03)[b,□]	20,64 (0.04)[c,£]	14,91 (0.05)[d,£]	0.001
Total carbohydrate, %	50,58(0.04)[a]	55,11(0.05)[b]	61,55(0.04)[c]	71,36(0.03)[d]	0.001
Calcium, %	57,58 (0.09)[a,¥]	164,18 (0.04)[b,¥]	188,78 (0.06)[c,¥]	181,31 (0.06)[d,¥]	0.001

[1]analyzed in accredited laboratory, [2]One-way ANOVAs test, significant at p<0.05; [a-d]Bonferonni test, significantly differ for different alphabets; [□,£,¥]nutrition claim for product if compared to BPOM (Indonesian FDA, 2016), □= high protein, £= good source of protein; ¥ = good source of calcium; F0-F3 are formulas with ratio of soy protein isolated (SPI): local fish anchovy (LA), F0= 100 g (SPI): 0 g (LA), F1 = 50 g (SPI): 50 g (LA), F2 = 25 g (SPI): 75 g (LA), F3 = 0 g (SPI): 100 g (LA)

Table 5. Final price estimation of fish stick formulas

Formulas	Estimated Price in USD/serving size*
F0	0.34
F1	0.94
F2	0.97
F3	0.98

*each formula serves about 50 gram/package; the price calculated by USD-IDR conversion rate of 1 USD equal to IDR 14435,-; F0-F3 are formulas with ratio of soy protein isolated (SPI): local fish anchovy (LA), F0= 100 g (SPI): 0 g (LA), F1 = 50 g (SPI): 50 g (LA), F2 = 25 g (SPI): 75 g (LA), F3 = 0 g (SPI): 100 g (LA)

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Discussion

Fish stick formulation: The present study indicated that anchovy substitution more than 22.9% of the total dough weight may result in a hard texture of the fish stick which may not be acceptable for school children. The hard texture of the fish stick may have occurred because of the collagen content found in the flour containing anchovy bone(14). The collagen found in the anchovy served as a binder, so the dough was more cohesive, strong and not easily broken(15).

Sensory properties of fish stick: It was found out that the ratio of SPI to LA in the developed formulas might have resulted in significantly different hedonic qualities among all the parameters. Food taste is identified and distinguished by the sprinkling papilla's buds. Several factors may affect the taste perception such as chemical compounds, temperature, consistency and food interactions with other components(16). Amrullah, Harmain and Dali (2015) realized that the processed and mixed food materials can also influence the taste perception of fish stick(17). Addition of salt, spices and anchovy distinctive aroma as a main ingredient, created a preferred taste from the panelists` point of view. Therefore, this study confirms that the addition of anchovy may increase the savory taste of fish stick. The present study, which used SPI and LA flour, is in line with Kristiastuti (2016) who found out that substitution of a specific flour may result in differences in taste perception(18). Related to fish stick color, it is important that a product have a good appearance or color, since it may be the first 'eye-catching' parameter. A food product which may be considered nutritious and tasty with excellent texture will not be eaten if the color or physical impression is deviated from desirable color(16). Different colors among the formulas may have been influenced by the color of the anchovy flour. In addition, other materials as well as the roasting process may have contributed to the color gradient of the fish stick. Handayani reported that the roasting process may affect the color of roasted food(19). The fish stick aroma was compose of the evaporation of various mixtures or mixtures of four smells (fragrant, sour, rancid and charred)were sensed by the (16). Since aroma is a result of mixing several ingredients, the fish stick aroma may have been produced from the mixture of ingredients, particularly LA, soy protein isolate and pepper(18). Fish stick texture results from the sensation of pressure that can be felt by mouth (bitten, chewed, swallowed) or touch (finger). Texture and consistency of a material may influence the taste and aroma of food. Changes in both of the properties may alter the taste and odor that arise, because it can affect the rate of stimulation of olfactory receptor cells and salivary glands. The thicker the material, the lower the taste and aroma acceptance(16). However, among the formulas, only color and aroma differed significantly. It demonstrates that the ratio of SPI to LA may have affected the color and aroma, but not the taste and texture. A product should have specific characteristics to be accepted by panelists or consumers. In our study, acceptance of the fish stick product was dominantly determined by its taste and texture.

Chemical composition of fish stick: All the formulas can be classified as energy-dense snack(20), since their energy content reached more than 4 kcal/gram. Energy-dense food can be an alternative to improve children nutritional status, particularly those with undernutrition. Carbohydrate content values were in the range of 50.5 to 71% and tended to increase as LA content was raised (from F0 to F3). It means that LA was the ingredient responsible the most for the carbohydrate content of the formulas. Among other macronutrients, carbohydrate contributed the most to total energy content (in the range of 48 to 67%). Carbohydrate may play an important role in enhancing the sensory properties, particularly the texture of the fish stick(21). Protein is the other nutrient that contributed the most to the total energy content. The protein values lay in the range of 14.9% to 33.5% which contributed to 14 to 31% of the total energy content. In contrast to carbohydrate, we found a reduction in the protein content from F0 to F3. This reduction was in line with the decrease in SPI. This trend shows us that SPI was the ingredient that contributed the most to the formulas total protein content. However, combination of the vegetable protein (SPI) and animal protein (LA) may have improved the protein quality of the formulas. The combinations may have increased the levels of

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limiting amino acids, thus elevating the protein quality(22).

Related to lipid content, F1 contained the highest lipid content, while F3 contained the lowest. It seems that combination of SPI and LA at the ratio of 50:50 brought about a higher lipid content. Energy from lipid accounts for about 18 to 26% of the total energy and is included in the safe range of lipid for snacking food(23). Using LA as an ingredient, may have contributed to high quality essential fatty acids in the fish stick formulas(24). These essential fatty acids may provide considerable benefits to support good children cognitive function(4). Another interesting nutrient in the formulas is calcium. it was observed that the calcium content tended to increase from F0 to F3. The increment was in line with of the increase in LA. The increment indicated that LA was responsible for the calcium content of the formulas, since it is one of the most important calcium-rich sources(24). If compared with BPOM regulation(9), related to nutrition and health claims, all the formulas already fulfill the claim of 'good source of calcium'. However, regarding the protein claim, only F0 and F1 fulfilled the nutrition claim of 'high protein' snack.

The high content of nutrients in a product does not mean high utilization in our body. Nevertheless, in the case of our product, the high content of protein and calcium will be in line with the high utilization of the nutrients in our body. This assumption is based on animal and isolated protein sources used as the main ingredients. Some studies have shown that the use of animal and isolated protein sources as ingredients would contribute to the high content and high digestibility of the protein. Singh et al (2008) reported that isolated or concentrated soy proteins were at high disgestibility and almost equal to the protein quality of milk, meat, and egg(25). Uran, H. and Gokoglu, N., (2014) found moderate protein and mineral contents of baked anchovy as compared with other cooking methods (frying and grilling). Even more, they observed a higher fatty acid content (mono- and polyunsaturated) in the baked anchovy and recommended baking as the best method in anchovy processing for healthy consumption(26). Finally, A study performed by Tengeni (2015) clarified that anchovy still had good bioavailibity (mean protein digestibility value of 73%) during four weeks of storageafter being processed by three different methods (traditional sun drying, improved sun drying and improved hot smoking) (27). Related to calcium, the digestibility will be high among people with low dietary intake of calcium(28). Therefore, the fish stick can be an alternative to combat low calcium intake among indonesian children(29).

Except for high energy-density, protein and calcium source claims, the present developed fish stick has another advantage. The price for developing a 50-gram portion size of the fish stick (packaged with food grade plastic, polypropylene) does not exceed 1 USD /package.

Conclusions

Our study have succesfully developed formulas made from LA and SPI as alternative snacks for school children. Among the formulas, only F2 was the most preferred by the panelists and classified as a good source of protein and calcium.

Authors' contributions: WK, DA and RF conceived and designed the experiments. WK performed the experiments. DA and RF were involved in data interpretation and statistical analysis. DA wrote the primary draft of the manuscript. DA and RF critically revised the manuscript for intellectual content. DA and RF were the guarantors of the paper. All the authors read and approved the final manuscript.

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