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Physicochemical Properties and Nutrient Content of Tempe Flour Enriched Eel Flour

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Abstract

AIM: This study aims to evaluate the nutritional content and physicochemical aspect of the combination of eel and tempe flour.

METHODS: Samples of swamp eel and tempe flour were combined in a ratio of 1:1 (A), 1:2 (B), 1:3.5 (C), and 1 kilogram of Asian swamp eel meat without additional tempe (D), respectively. Homogenization was done using a sinmag planetary mixer. The sample analysis was performed at the SIG Laboratory (PT. Saraswanti Indo Genetech) on November 16, 2021. The samples were analyzed triple (triplicates). Statistical analysis was performed using GraphPad Prism version 9.3.0 for Mac OS. Variables were analyzed using multivariate ANOVA with 95% CI (0.05).

RESULTS: There was a significant difference between Samples A, B, C, and D for every proximate analysis parameter ($p = 0.000$) $p < 0.05$. The results showed a significant difference in the unsaturated fatty acid parameter between the groups ($p < 0.001$). Sample C has the highest B9 (folic acid) content compared to other samples (1258.53 ± 1.39 mcg/100 g) and a significant difference was found between samples ($p < 0.0001$).

CONCLUSION: Tempe flour enriched eel flour can act as a source of folate, due to its high folate content. Tempe flour enriched eel flour could be used as a flour mixture in any food products that require flour to increase folate content.

Introduction

The Asian swamp eel (*Monopterus albus*) is a species that is widely spread across tropical Asian regions (Herdiana *et al*., 2017) [1]. The Asian swamp eel is a source of protein with various mineral contents (Herawati *et al*., 2018) [2]. 100 g of Asian swamp eel meat contains 70 kilocalories, 14.60 g of protein, 1 g of carbohydrate, 0.8 g of fat, 169 mg potassium, 155 mg phosphorus, 55 mg sodium, 49 mg calcium, and 1.5 mg iron (Kementerian Kesehatan RI., 2018; Widiany, 2019) [3], [4]. Asian swamp eel could be processed in powder form by being processed in a blender (Herdiana *et al*., 2017) [1]. Eel flour is not much different than actual swamp eel meat; it is supposed to be rich in protein and minerals. Nevertheless, there are only a very few researches that are discussing eel flour, and even fewer when it comes to the physicochemical and nutritional aspects of the eel powder.

Tempe is a local plant-based protein source from Indonesia that is affordable and widely available (Kadar *et al*., 2020) [5]. Tempe has higher protein content compared to other plant-based protein sources (Widiany, 2019) [4]. A 100 g of tempe contains 201 kilocalories, 20.8 g of protein, 13.5 g of carbohydrate, 8.8 g of fat, 326 mg phosphorus, 234 mg potassium, 9 mg sodium, and 4 mg iron (Ministry of Health the Republic of Indonesia., 2018) [3]. Tempe has a relatively short shelf life (Astawan *et al*., 2016) [6]. Preserving tempe can be done by processing it into flour (Bintanah and Hendarsari, 2014) [7]. Tempe flour contains 46.5% protein, 24.7% fat, 13.5% carbohydrate, 2.5% fiber, and 2.3% ash (Fawzia and Marliando., 2013) [8]. The nutritional content of tempe flour is only assessed from proximate analysis, and there is still very little research that has been done to analyze the detailed nutritional composition and physicochemical aspect of tempe flour.

Eel and tempe flour are two foodstuffs that are rich in nutrients. Nevertheless, there were only very limited studies that analyzed the nutritional content, furthermore, the physicochemical aspect. Based on that fact, research is needed to analyze the nutritional content and physicochemical aspect of the combination of both eel and tempe flour. By doing so, it opens more possibilities for other researchers to follow through for a functional food product development deriving from eel and tempe flour. Therefore, this research is aimed to analyze the nutritional content and physicochemical aspect of eel and tempe flour combinations.

Methods

Asian swamp eel sample preparation

One thousand grams of Asian swamp eel (*M. albus*) were obtained from a local market located in Jakarta in November 2021. The entire sample was cleaned, steamed for 10 min, and separated from the bones. Before analysis, the sample was dried using an oven with a temperature of 60°C for 12 h, and then ground. The ground sample was then filtered with a 60 mesh filter.

Tempe sample preparation

One thousand grams of soybean (*Glycine max* (L.) Merr.) tempe were obtained from a local market located in Jakarta in November 2021. The entire sample was sliced into a thin square shape and then steamed for 20 min. After steaming, the sample was placed in a 60°C oven for 12 h and then ground. The ground sample was then filtered using a 60 mesh filter.

Mixing or combination of flour

Swamp eel and tempe flour samples were combined in the ratio of 1:1 (A; 1 kg of Asian swamp eel meat and 1 kg of tempe), 1:2 (B; 1 kg of Asian swamp eel meat and 2 kg of tempe), 1:3.5 (C; 1 kg of Asian swamp eel meat and 3.5 kg of tempe), and 1 kg of Asian swamp eel meat without any additional tempe (D). Homogenization was done using a sinmag planetary mixer.

Samples analysis

Sample analysis was done in SIG Laboratory (PT. Saraswanti Indo Genetech) on November 16, 2021. Analysis certificate numbers are listed below: SIG.LHP.XI.2021.161435112 (Sample A); SIG.LHP.XI.2021.161435113 (Sample B); SIG.LHP.XI.2021.161435114 (Sample C); and SIG.LHP.XI.2021.170834221 (Sample D). The proximate analysis consists of protein content, ash content, calories from fat, total fat, moisture content, total calories, and carbohydrate content using Kjeltec, Weibull, Food and Agriculture Organization method and Indonesian National Standard (SNI) for food products and derivatives. Fatty acid analysis was done using gas chromatography. Amino acid analysis was done using liquid chromatography-mass spectroscopy, ultra-performance liquid chromatography (UPLC), and high-performance liquid chromatography. Vitamin B analysis was done using UPLC and LCMS/MS methods. The samples were analyzed 3 times (triple/triplicates).

Data analysis and management

Statistical analysis was done using GraphPad Prism version 9.3.0 for Mac OS. The variables were analyzed using multivariate ANOVA with a 95% CI (0.05).

Results

Proximate analysis

The proximate analysis result of each sample (A, B, C, and D) is presented in Table 1.

Table 1: Proximate analysis of Sample A, B, C, and D

Parameter	A	B	C	D	p-value*
Protein content (%)	70.27 ± 0.01	62.06 ± 0.08	57.08 ± 0.08	84.42 ± 0.04	0.000
Ash content (%)	4.30 ± 0.01	3.33 ± 0.04	2.61 ± 0.06	2.51 ± 0.08	0.000
Calories from fat (kcal/100 g)	157.05 ± 0.13	175.14 ± 0.64	225.41 ± 0.83	63.81 ± 0.76	0.000
Total fat (%)	17.45 ± 0.01	19.46 ± 0.07	25.05 ± 0.09	7.09 ± 0.08	0.000
Moisture content (%)	6.43 ± 0.01	6.07 ± 0.06	5.90 ± 0.02	5.39 ± 0.05	0.000
Total calories (kcal/100 g)	444.33 ± 0.04	459.74 ± 0.04	491.23 ± 0.12	403.87 ± 0.57	0.000
Carbohydrate (%)	1.54 ± 0.05	9.10 ± 0.25	9.38 ± 0.26	0.60 ± 0.01	0.000

*p-value of one-way ANOVA test (95% CI, 0.05) between Samples A, B, C, and D.

The highest protein content was recorded in Sample D or control sample with no additional tempe (84.42 ± 0.04%), while the lowest was found in Sample C (57.08 ± 0.08%). The highest ash content was estimated in Sample A (4.3 ± 0.01%), while the lowest was found in Sample D (2.51 ± 0.08%). The highest total calories from fat were found in Sample C (225.41 ± 0.83 kcal/100 g), while the lowest was found in Sample D (63.81 ± 0.76 kcal/100 g). Moreover, the highest fat percentage was found in Sample C (25.05 ± 0.09%), while the lowest was found in Sample D (7.09 ± 0.08%). The highest moisture content was found in Sample A (6.43 ± 0.01%), while the lowest was found in Sample D (5.39 ± 0.05%). The highest total calories were recorded in Sample C (491.23 ± 0.12 kcal/100 g), while the lowest was found in Sample D (403.87 ± 0.57 kcal/100 g). In addition, the highest carbohydrate content was found in Sample C (9.38 ± 0.26%), while the lowest was estimated in Sample D (0.60 ± 0.01%). There was a significant difference between Samples A, B, C, and D for every proximate analysis parameter ($p = 0.000$) $p < 0.05$.

Fatty acid test

Apart from proximate analysis, a fatty acid percentage test was done on the percentage of saturated and unsaturated fatty acids. The result is presented in Figure 1.

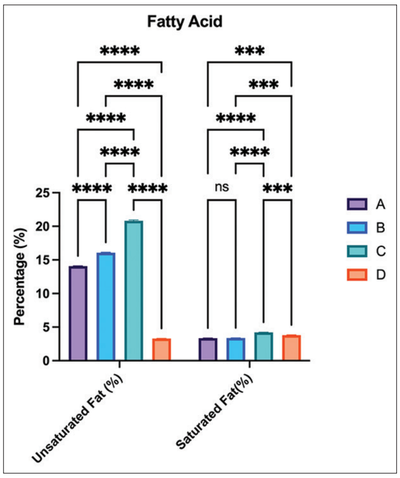

*Figure 1: Fatty acid percentage analysis result of each sample. ****p < 0.0001; ***p = 0.0003; [ns]Not significant p > 0.05 (p = 0.9666)*

There was a significant difference in unsaturated fatty acid parameters between groups ($p < 0.001$). The amount of unsaturated fatty acid from the highest to the lowest order is as follows: C (20.83 ± 0.12%), B (16.07 ± 0.03%), A (14.08 ± 0.03%), and D (3.30 ± 0.04%).

On saturated fat parameter, the highest percentage was found in Sample C (4.22 ± 0.03%). The fat content of the rest of the sample is as follows: Sample A 3.37 ± 0.01%, B 3.40 ± 0.01%, and D 3.80 ± 0.05%. Results show a significant difference between samples ($p < 0.05$), except for Samples A and B ($p > 0.05$).

Amino acid test

In amino acid parameters, the highest essential and non-essential amino acid content were estimated in Sample D (pure eel flour without additional tempe flour). Meanwhile, Sample C has the lowest amino acid content. Each sample has a significant difference according to the one-way ANOVA test. The exact amino acid amount in each sample are shown in Table 2.

Vitamin B9 (folic acid) test

Sample C has the highest vitamin B9 or folic acid content compared to other samples (1258.53 ± 1.39 mcg/100 g). The vitamin B9 content of the rest of the sample is as follows: Sample A 823.26 ± 10.50 mcg/100 g, B 1039.72 ± 8.41 mcg/100 g, and the lowest in-Sample D 128.52 ± 1.14 mcg/100 g. A significant difference was found between samples ($p < 0.0001$) (Figure 2).

Discussion

This research aims to analyze the physicochemical properties and nutrient content of flour mixture made up of soybean (*G. max* (L.) Merr.) tempe and eel (*M. albus*). The sample will be analyzed with methods that include proximate analysis (protein, ash, calories from fat, total fat, moisture, total calories, and carbohydrate), fatty acid test (unsaturated and saturated fatty acid content), amino acid test (essential, conditionally essential, and non-essential), and vitamin B9 test. Four samples were analyzed, Sample D is made from pure Asian swamp eel meat, while Samples A, B, and C were given additional tempe flour. Sample C has the highest tempe flour content, followed by Sample B, then Sample A.

Each parameter of proximate analysis is compared within the samples. It was found that the more tempe flour added, the lower the protein content. In the ash content parameter, the result seems to be unclear, Sample A with the least amount of tempe added has the highest ash content, the more tempe flour added, the lower the ash content, but Sample D (pure Asian swamp eel) has the least ash content. In calories from fat parameter, it was found that the more tempe flour added, the more the calories from fat. The result correlates with total fat, it was found that the more tempe flour added, the more the total fat. For moisture content parameter, the result is similar to ash content, Sample D (pure Asian swamp eel) has the lowest moisture content, while in Samples A, B, and C, it seems that the more tempe flour added, the lower the moisture content. For the total calories' parameter, it was found that the more tempe flour added, the higher the total calories. For carbohydrate content, the result is similar, the more tempe flour added, the higher the carbohydrate content. Sample A, B, and C fit into the Indonesian national standard for fish flour, except for the fat content parameter, in which the samples contain more fat than required. Sample D fits into the standard well, achieving Grade I (one) quality for moisture, protein, ash, and fat content parameters (Badan Standardisasi Nasional., 1996) [9].

Unsaturated and saturated fatty acid content are compared within samples. It was found that the more tempe flour added, the higher the unsaturated fatty acid content. The addition of tempe flour increases the unsaturated fatty acid content since tempe's fatty acid content is 80% unsaturated (Utari., 2010) [10]. In the saturated fatty acid parameter, the result seems to be unclear, the trend was not found. In Samples A,

Table 2: Amino acid test

Parameter	A	B	C	D	p-value*
Essential and conditionally essential					
L-Cysteine (mg/kg)	10261.62 ± 0.64	9213.42 ± 14.31	6999.23 ± 4.91	24415.82 ± 7.74	0.000
L-Methionine (mg/kg)	3428.83 ± 2.67	3333.71 ± 1.03	2586.08 ± 0.71	5874.25 ± 2.18	0.000
L-Phenylalanine (mg/kg)	41981.74 ± 206.62	31577.31 ± 85.51	31422.06 ± 156.91	44817.57 ± 181.53	0.000
L-Isoleucine (mg/kg)	30855.02 ± 62.88	25431.38 ± 53.36	23968.22 ± 52.86	39079.73 ± 139.68	0.000
L-Valine (mg/kg)	32082.52 ± 186.01	26394.83 ± 143.20	24808.54 ± 177.79	40704.56 ± 257.56	0.000
L-Arginine (mg/kg)	54371.94 ± 167.37	41167.50 ± 114.92	39409.52 ± 201.39	65369.49 ± 332.41	0.000
Glycine (mg/kg)	45502.48 ± 129.45	33400.93 ± 95.22	29863.68 ± 136.47	64468.97 ± 309.51	0.000
L-Liysine (mg/kg)	42294.51 ± 191.92	35069.44 ± 149.59	31249.15 ± 179.35	61345.93 ± 368.75	0.000
L-Leusin (mg/kg)	53658.79 ± 123.62	44369.21 ± 141.76	41816.71 ± 169.84	67780.58 ± 321.71	0.000
L-Tyrosine (mg/kg)	28962.30 ± 102.35	21138.03 ± 9.01	20545.63 ± 91.08	34494.15 ± 107.62	0.000
L-Proline (mg/kg)	30879.89 ± 52.75	26047.49 ± 38.80	24809.83 ± 78.15	38178.15 ± 142.45	0.000
L-Threonine (mg/kg)	37724.62 ± 140.23	28495.21 ± 118.15	26498.19 ± 71.32	47776.02 ± 166.16	0.000
L-Histidine (mg/kg)	20743.95 ± 19.87	15565.82 ± 19.28	15231.22 ± 50.06	23188.40 ± 66.30	0.000
L-Tryptophan (mg/kg)	5818.29 ± 4.85	5380.92 ± 12.38	5314.78 ± 11.48	6633.73 ± 5.91	0.000
Non-essential					
L-Serine (mg/kg)	37200.03 ± 94.39	30389.61 ± 74.23	29646.04 ± 146.12	42557.38 ± 205.56	0.000
L-Glutamic Acid (mg/kg)	94825.08 ± 431.44	81291.85 ± 367.55	77122.75 ± 304.93	114867.03 ± 643.34	0.000
L-Alanine (mg/kg)	35157.85 ± 95.92	27880.19 ± 64.75	24979.24 ± 106.30	49030.67 ± 197.74	0.000
L-Aspartic Acid (mg/kg)	56590.34 ± 146.25	48658.25 ± 164.90	46405.7 ± 175.86	68074.28 ± 337.61	0.000

*p-value of one-way ANOVA test (95% CI, 0.05) between Samples A, B, C, and D.

B, and C, it seems that the more tempe flour added, the higher the saturated fatty acid, but Sample D has the second-highest saturated fat content. Saturated and unsaturated fatty acid intake is associated with cardiovascular diseases. It was found that although there is no significant evidence that indicates the association between dietary saturated fat and risk for cardiovascular diseases, replacing saturated fat with mono and polyunsaturated fatty acid could potentially lower coronary heart disease events (Siri-Tarino *et al*., 2010; Clifton and Keogh., 2017) [11], [12]. It was also found that reducing saturated fatty acid intake could reduce combined cardiovascular events (Hooper *et al*., 2015) [13]. Therefore, adding tempe flour will result in higher unsaturated and lower saturated fatty acid percentages, which is desired.

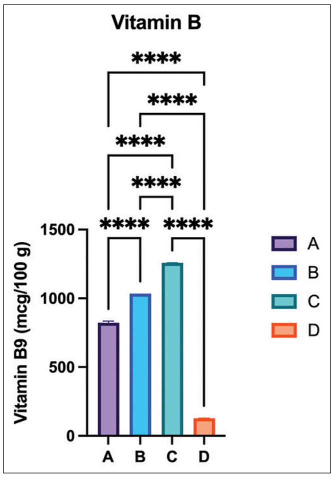

*Figure 2: Vitamin B9 content analysis result of each sample. ****p < 0.0001*

Essential, conditionally essential, and non-essential amino acid content are compared with samples. It was found that the more tempe flour added, the lower the essential, and conditionally essential amino acid content. The result is the same for the non-essential amino acid. This result applies to every specific amino acid tested. Amino acids are required by the body to produce hormones, neurotransmitters, muscle growth, and other cellular processes (Wu *et al*., 2013) [14]. The difference between essential and non-essential amino acids is the ability to be synthesized (Choi and Coloff., 2019; Combs and DeNicola., 2019) [15], [16]. Essential amino acids can't be synthesized, by the body, while non-essential amino acids can (Choi and Coloff, 2019; Combs and DeNicola, 2019) [15], [16]. Hence, the essential amino acid has to be acquired from dietary intake (Lopez and Mohiuddin, 2021) [17]. This means that the addition of tempe flour might not be optimal for amino acid content, both essential and non-essential. Nonetheless, the protein and amino acid content of the samples is still high.

Vitamin B9 content is compared within samples. It was found that the more tempe flour added, the higher the vitamin B9 content. Vitamin B9 is also known as folate, often confused with folic acid (Mikkelsen and Apostolopouluos, 2019) [18]. Folate is naturally occurring, while folic acid is its synthetic form (Naderi and House, 2018) [19]. Sources of folate include broccoli, spinach, lettuce, milk, liver, and eggs (Aslam *et al*., 2017) [20]. Regular daily intake of folate from these sources is usually lower than recommended. Folate deficiency could potentially cause megaloblastic anemia (Hariz and Bhattacharya, 2021) [21]. In the case of pregnancy, folate deficiency could cause neural tube defects (NTD) in infants (Seidahmed *et al*., 2014) [22]. Folate intake could reduce the risk of megaloblastic anemia, NTD, and also lower homocysteine levels to prevent detrimental cardiovascular effects (Merrell and McMurry, 2020; Chan *et al*., 2013) [23], [24]. Therefore, the addition of tempe flour works in favor of increasing vitamin B9 content which is beneficial for health. Tempe flour enriched eel flour can act as a source of folate, due to its high folate content. Tempe flour enriched eel flour could be used as a flour mixture in any food products that require flour to increase folate content.

Conclusion

Each parameter of proximate analysis was found that the more tempe flour added, relate with the lower the protein content. Sample D fits well into the standard, achieving Grade I quality for parameters of moisture, protein, ash, and fat content. The highest essential and non-essential amino acid content was found in Sample D (pure eel flour without additional tempe flour). Furthermore, Sample C has the highest vitamin B9 or folic acid and unsaturated fatty acid content compared to other samples. Tempe flour enriched eel flour has the potential to be a functional flour in the development of several functional food products rich in vitamin B9 and unsaturated fatty acids.

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