Tempeh and Vital Wheat Gluten based Analog Meat Development as Vegetarian Alternative Food

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Abstract: Vitamin B12 deficiency is one of the main problems in the vegetarian diet with prevalency from 11% to 90%. Tempeh and vital wheat gluten-based meat analog are expected to provide vitamin B12 for vegetarians. Completely Randomized Design was used as the experimental design. The organoleptic test was carried out by 25 semi-trained panelists and 30 consumers. The content of vitamin B12, protein, fat, carbohydrate, moisture, ash, Total Plate Count, and also production cost was analyzed. Organoleptic test result showed that F2 has the best acceptability with 6,67 mg/100g of vitamin B12, 9,25% protein, 2,94% fat, 28,32% carbohydrate, 56,72% moisture. 2,69% ash, and 2,3 x 104 cfu/g in Total Plate Count. The cost to produce one package of tempeh and vital gluten wheat-based meat analog is 9.171 rupiahs. Therefore, it is proved that tempeh and vital wheat gluten can produce a meat analog with high vitamin B12 content that also fulfills the minimum quality requirements in SNI 3818:2014. However, it was necessary to analyze the storability of the product, also analyze the protein content with the Kjeldahl method and analyze the pseudo vitamin B12 that might be contained in the product.

1 INTRODUCTION

The vegetarian diet is becoming more popular and growing, not only globally (Figus, 2014), but also in Indonesia (Siahaan, Nainggolan, & Lestrina, 2015). The growing population of vegetarians may cause by the benefits of the vegetarian diet compared to non-vegetarian diets, such as lower triglyceride, lower blood pressure, lower sugar blood level, and also higher HDL (High-Density Lipoprotein) (Setiyani & Wirawanni, 2012).

Vegetarian only eat plant-based food that is lack of vitamin B12, because vitamin B12 only can be found in animal-based food such as beef, so usually vegetarian-only get their vitamin B12 intake from a supplement (Pawlak, Lester, & Babatunde, 2014). Therefore, vegetarians usually suffer from vitamin B12 deficiency (Pawlak R., Is Vitamin B12 Deficiency a Risk Factor for Cardiovascular Disease in Vegetarians? 2015). The prevalence of vitamin B12 deficiency in vegetarians on every age group is described as follows, 62% on pregnant women, 25-86% on children, 21-41% on the teenager, and 11-90% on elder (Pawlak, Parrot, Cullum-Dugan, & Lucus, 2013). A study on the vegetarian community in Jogjakarta, Indonesia also showed there is a deficiency in vitamin B12, zinc, and folate acid intake (Anggraini, Lestariana, & Susetyowati, 2015). Vitamin B12 deficiency can cause megaloblastic anemia that can increase the chance of premature birth during pregnancy (Rogne, et al., 2017), and also skin pigmentation disorder (Rzepka, et al., 2018).

Early study found that vitamin B12 can be found in some of the plant-based food, such as fermented food, tea, mushroom, dried seaweed (nori) and algae (Rizzo, 2016). Tempeh is an Indonesian soy-based fermented food that also contained a considerable amount of vitamin B12 (4,6 mcg/100g) (Okada, 1989). The vitamin B12 in tempeh is produced by the activity of bacteria K. Pneumoneae (Mo, et al., 2013). Tempeh is a very popular plant-based protein source in Indonesia, and maybe can be made into analog meat. Analog meat is one of the vegetarian dishes with texture, taste, and color resembling animal meat (Sedgwick, 2013).

The objective of this research is to develop a tempeh and vital gluten wheat-based meat analog as an alternative vegetarian food.

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2 METHODS

This research used a Completely Randomized Design as the experimental design, with two factorials and four levels of treatment which will be compared to one control product. The main ingredients in this experiment are tempeh that was bought from a traditional market in Bekasi city, and vital gluten wheat from an online shop “Tokopedia”. Besides the main ingredients, side ingredients such as mushroom Boullion “Totole”, soy sauce “ABC”, water, instant yeast “Fermipan”, garlic powder “Koepoe-koepoe”, and powdered pepper “Ladaku”. And for the materials used in the chemical analysis were obtained from the Esa Unggul University chemistry laboratory.

2.1 Making Tempeh and Vital Gluten Wheat Based Analog Meat

The guideline in making tempeh and vital gluten wheat-based analog meat (will be referred to as product) was based on the experiment by Bintanah & Handarsari (2014) with modification. The formulation of the product is shown in Table 1. The ratio of vital gluten wheat and tempeh was chosen based on the formulation with the highest organoleptic score in an experiment by Bintanah & Handarsari (2014). Unlike the guideline experiment, the tempeh used in this research was fresh tempeh, not tempeh flour to avoid vitamin B12 loss because of excessive heat when making tempeh flour. Rice bran flour that used in the guideline experiment also replaced with vital gluten wheat to reach a meat-like texture. Analog meat “Rodeo” is used as control. Cookware like Phillips electric stove, steamer pot set, Phillips food processor, glass bowl, plate, and measuring spoon was used to make the product.

2.2 Product Organoleptic Test

The product was evaluated in an organoleptic test by 25 semi-trained panelists and 30 consumer panelists. Color, texture, taste, and aroma of products were rated using the Visual Analog Scale by semi-trained panelist and Likert Scale by consumer panelist. The participant for semi-trained panelists were students of the Nutrition department in Universitas Esa Unggul whose already passed organoleptic test course, in healthy condition, and was asked to feast (except for plain water) 1 hour before the test begins. Whilst participants for consumer panelists were people who have been on a vegetarian diet for at least 4 months and in good health. The test was carried out on Universitas Esa Unggul Organoleptic Laboratory, Bekasi. Tools that are used in the organoleptic analysis were organoleptic questionnaire, plate, toothpick, and ballpoint.

2.3 Vitamin B12 Analysis

Analysis of Vitamin B12 levels was carried out using HPLC (High-Performance Liquid Chromatography) method by MBRIO Food Laboratory. HPLC with the specification of UV detector, 265 nm wavelength, C18 25 cm Eurosphere, 1,0 ml/minute flow speed, Whatman no.41, and 0,45 mcg millipore shifter, also with 20 mcg injector volume was used.

2.4 Protein Analysis

In this study, analysis of protein content was carried out using the formol titration method, the analysis was carried out in Universitas Esa Unggul Chemistry Laboratorium. Tools that were used in this analysis were PYREX® glassware, digital scale (0,001 g accuracy), burette, Phillips electric stove, cooking pot, tongs, pipettes, measuring tube, and measuring glass.

2.5 Fat Analysis

The Soxhlet method was used to analyze the total fat content of the product. The tools used in this analysis were fat flask, desiccator, digital scale (0,001 g accuracy), Soxhlet extraction device set, Memmert laboratory oven, and tongs. The analysis was carried out in Universitas Esa Unggul Chemistry Laboratory.

Table 1: Tempeh and vital gluten wheat-based analog meat formulation.

Formula	Vital gluten wheat (g)	Tempeh (g)	Instant yeast (g)	Mushroom buillon (g)	Soy sauce (ml)	Garlic powder (g)	Powdered pepper (g)	Water (ml)
F1	90	10	4	5	10	2	1	40
F2	50	50	4	5	10	2	1	40
F3	40	60	4	5	10	2	1	40
F4	30	70	4	5	10	2	1	40

2.6 Carbohydrate Analysis

By difference, the method was used to calculate the carbohydrate total contained in the product. By calculating the remaining from 100 percent after deducted by the total of protein, fat, moisture and ash content that have been analyzed. Carbohydrate analysis was carried out in Universitas Esa Unggul Chemistry Laboratory.

2.7 Moisture Analysis

To analyze water content, the gravimetric method is used. Porcelain cup, digital scale (0,001 g accuracy), Memmert laboratory oven, and tongs were used in this analysis. The analysis was carried out in Universitas Esa Unggul Chemistry Laboratory.

2.8 Ash Analysis

The gravimetric method with dry ashing was used to analyze the ash content of the product. Ashing cup, saucer, digital scale (0,001 g accuracy), Barnstead Thermolyne 1300 furnace, and crucible tongs were used in this analysis. The analysis was carried out in Universitas Esa Unggul Chemistry Laboratory.

2.9 Total Plate Count Analysis

Calculation of the total plate count was carried out using the pour plate method and was done in Universitas Esa Unggul Microbiology Laboratory. The tools that were used to analyze total plate count are measuring glass cup (PYREX®), petri dish, and dilution bottle.

3 RESULT AND DISCUSSION

3.1 Product Description

At the time of the first trial, tempeh was only mashed with a food processor without any cooking process, after which it was mixed with other ingredients, the result was that the analog meat product which was already cooked had a bitter aftertaste and smelled unpleasant. In the next experiment, the tempeh was cut square with a thickness of 1 cm, and after that, it was steamed for 20 minutes on medium heat. After that, tempeh is allowed to stand for 5 minutes at room temperature to lower the temperature, then put into a food processor until smooth. After that, tempeh is mixed with other ingredients according to the product manufacturing flow. After the product is cooked, the product no longer has a bitter aftertaste and the unpleasant aroma has diminished.

The ingredients that are mixed first are instant yeast, garlic powder, mushroom bullion, and powdered pepper after that add soy sauce and water. After homogeneous, add tempeh and mix, finally add wheat gluten flour then stir and knead until mixed (no residue in the container). Mixing wheat gluten flour is done at the end so that tempeh can be mixed with gluten. Analog meat that has been cooked and cooled has brown colors, solid, tough and slightly fibrous, also has a slight aroma of tempeh.

3.2 Organoleptic Analysis

Organoleptic analysis in this research consisted of two assessment results, a hedonic quality test to determine the organoleptic characteristics of the product, and also hedonic test to determine the level of preference for panelists on products. There were five product variations tested on organoleptic analysis, namely F0 / control, F1, F2, F3, and F4. After organoleptic analysis, the assessment of the panelists is processed statistically with the One-Way ANOVA test and Duncan post hoc test.

The result of the hedonic quality test and hedonic test by semi-trained panelists also hedonic test by consumer panelist is shown respectively in Table 2, Table 3, and Table 4.

Table 2: Hedonic quality test score.

Parameter	Formulation					P-value
	F0	F1	F2	F3	F4	
Color	6,18 ± 2,47	4,30 ± 2,87	4,95 ± 2,69	4,91 ± 2,95	4,60 ± 3,03	0,172
Texture	$6{,}92 \pm 2{,}56^{b}$	$7{,}36 \pm 1{,}71^{b}$	$6{,}49 \pm 2{,}34^{b}$	$4{,}82 \pm 2{,}17^{a}$	$4{,}01 \pm 2{,}35^{a}$	0,001*
Taste	$3{,}96 \pm 2{,}07^{a}$	$7{,}70 \pm 1{,}42^{cd}$	$7{,}86 \pm 1{,}20^{d}$	$6{,}66 \pm 2{,}25^{bc}$	$6{,}10 \pm 2{,}53^{b}$	0,001*
Aroma	$4{,}16 \pm 2{,}18^{a}$	$5{,}83 \pm 2{,}63^{bc}$	$6{,}22 \pm 2{,}19^{c}$	$5{,}47 \pm 2{,}40^{abc}$	$4{,}54 \pm 2{,}49^{ab}$	0,013*

Note:
The data presented are mean ± Standard Deviation (scaled 0-10)
* There is a significant difference in these parameters with a value of $P \leq 0.05$
Values followed by different superscript letters have significant differences

Table 3: Hedonic test score by a semi-trained panelist.

Parameter	Formulation					P-value
	F0	F1	F2	F3	F4	
Color	5,08±2,20[a]	5,72±1,85[a]	6,91±1,91[b]	6,07±1,89[ab]	6,16±1,67[ab]	0,020*
Texture	5,03±2,12[a]	6,34±2,04[b]	7,69±1,42[c]	6,47±1,97[b]	5,22±2,26[a]	0,001*
Taste	2,97±2,19[a]	6,59±2,17[bc]	7,47±1,43[c]	6,68±2,27[bc]	5,75±2,28[b]	0,001*
Aroma	3,73±2,38[a]	6,17±2,41[b]	7,10±1,85[b]	6,33±2,34[b]	4,74±2,06[a]	0,001*

Note:
The data presented are mean ± Standard Deviation (scaled 0-10)
* There is a significant difference in these parameters with a value of P ≤ 0.05
Values followed by different superscript letters have significant differences

Table 4: Hedonic test score by consumer panelist.

Parameter	Formulation					P-value
	F0	F1	F2	F3	F4	
Color	2,00[a]	3,00[b]	3,00[b]	3,00[ab]	2,00[a]	0,001*
Texture	3,00[b]	3,00[b]	3,00[c]	3,00[b]	2,00[a]	0,001*
Taste	1,00[a]	3,00[b]	3,00[c]	3,00[b]	2,00[a]	0,001*
Aroma	2,00[a]	3,00[b]	3,00[b]	3,00[b]	2,00[a]	0,013*

Note:
The data presented are mean ± Standard Deviation (scaled 1-4)
* There is a significant difference in these parameters with a value of P ≤ 0.05
Values followed by different superscript letters have a significant difference

3.2.1 Color

In the assessment of color quality, the formula with the highest mean value is F0 or control formula, this is in accordance with the results of research by Dinata (2014) which stated that from the results of hedonic quality tests conducted on 6 analog meat formulations, there are similarities in appearance. When viewed from the mean values in the non-control formulation (F1, F2, F3, and F4), the color quality of the product is still not close to the desired color quality (light brown). But this value is not significant because of the results of the One-Way ANOVA test on the color indicator state that the value of P> α, which means there is no significant difference in the color indicators between formulations. This can be caused by the ratio of tempeh to wheat gluten which is not much different (Juliana, 2009).

On the other hand, the hedonic test results on the color parameters show a significant difference (P-value <α) after being tested statistically. When referring to Table 3, it is seen that there is a significant increase in the mean value of the color parameters from F0 to F2, and there is no significant difference between F2, F3 and F4. This means that the increase in color preference is obtained from the ratio of tempeh and wheat gluten to 50g: 50g, to the ratio of tempeh and wheat gluten to 70g: 30g, this can be due to the range of tempeh and wheat gluten use which does not differ greatly between F2, F3 and F4. Significant increase from F1 to F2 can also be caused by differences in the ratio of the use of tempeh and wheat gluten flour which is quite far (10g: 90g to 50g: 50g). The same is true of hedonic assessments by consumer panelists, where there was a significant increase from F0 to F1 and F2. This is also supported by the research of Bintanah & Handarsari (2014) which states that panelists rate very fond of the color indicators of vegetarian nugget formulations with a ratio of 40g flour: 60g rice bran. Thus, from the results of the organoleptic analysis of color parameters, it can be said that an increase in the use of tempeh with the right ratio will increase the preference of panelists to analog meat colors, even though the panelists have not been able to see the difference in color quality.

3.2.2 Texture

One Way ANOVA test results stated that there were significant differences in the texture quality between the five analog meat formulations. The highest texture quality values are owned by F0, F1, and F2. It can also be seen that there was a significant decrease in texture quality from the three best formulations to F3 and F4. This means that to obtain a texture quality that is close to tough, we need higher concentrations of wheat gluten flour compared to tempeh concentrations. This is supported by research by Mulyani, Rosida & Rahmadani (2012) which stated that the less amount of gluten in vegetarian meatballs, the resulting texture will be softer.

The hedonic test results by both trained panelists and consumers showed that there was a significant difference (P <α value) in the assessment of color indicators, where the highest mean value was owned by F2, this concluded that the panelists preferred the texture of analog meat which tends to be tough as in texture which is owned by F1 and F2. This is the same as research conducted by Susanti, et al. (2017) that panelists most like analogous meatball texture which is more resilient.

3.2.3 Taste

From the taste quality indicator, there were significant mean differences between formulas. Formula with the highest mean is F1 and F2, while the lowest mean is owned by F0, so it can be said that F2 tends to have a savory taste and F0 tends to have an unpleasant taste. If noted in Table 2 on the taste indicator, there is a significant increase from F0 to F1 to F4, but there is also a significant decrease in mean from F2 to F3 and F4. This concludes that the addition of a tempeh use ratio can increase the taste of analog meat savory, but if the use ratio is more than 1: 1 with a wheat gluten flour, it will reduce the savory taste and add the tempeh unpleasant taste in analog meat. But the low quality of taste F0 is not caused by the unpleasant taste produced by tempeh, because in F0 (analog meat brand "rodeo") it does not use raw materials of tempeh. This can be caused by the use of mushroom broth in F1, F2, F3, and F3 containing MSG (Monosodium Glutamate) (Tsai, 2007), which can increase the savory taste of food products (Thariq, Swastawati, & Surti, 2014), so that F0 products that did not use mushroom broth has a lower taste quality compared to other formulations.

From the hedonic assessment in Table 3 and Table 4, it can also be seen that F0 has the lowest taste preference compared to other formulations. Judging from the evaluation by the semi-trained panelists, the best taste acceptance is owned by F1, F2, and F3, while the panelists of consumers prefer the taste to F2 only. The difference between the mean value of F2 with the mean values of F0 and F4 also shows that the ratio of tempeh and wheat gluten to flour affects the acceptability of analog meat. This is in accordance with the research of Bintanah & Handarsari (2014), where there was a decrease in the taste preference value in vegetarian nuggets in formulas with the lowest tempeh concentration and also the highest concentration.

3.2.4 Aroma

It can be seen in Table 2 that in the color indicator there were significant differences between formulations. The mean value of the highest aroma quality is owned by F1, F2, and F3 with a slightly flavorful characteristic typical of processed meat. A significant decrease in the mean value between the highest mean formulation with F4 states that the use of a tempeh ratio that is too high can reduce the quality of the aroma becomes unpleasant. Like the taste quality, the low quality of aroma in F0 is not caused by the use of a high tempeh ratio and causes unpleasant aroma, but it can be caused by the fact that the F0 product does not smell anything.

Then it can be seen also in Table 3 and Table 4 that there were significant differences in the hedonic taste of analog meat products with the highest mean owned by F1, F2, and F3. Furthermore, the post-hoc test results in both assessments also showed that there was a significant decrease in the formulation with the highest mean on F0 and F4. So it can be concluded from the results of hedonic and hedonic quality assessments on analog meat taste indicators that the appropriate use ratio of tempeh and wheat gluten can maximize the quality value and taste of tempeh aroma, and the ratio of using tempeh that is too high or too low can reduce the quality and acceptability aroma indicator of analog meat products made from tempeh.

3.3 Selected Product

Based on the organoleptic analysis, it was concluded that the formula with the best organoleptic value was F2 (hereinafter referred to as the selected product/ formulation) with a ratio of tempeh to wheat gluten as much as 50g: 50g. When viewed from its quality, the chosen formulation has a neutral color (not pale or light brown), a slightly tough texture, a taste that tends to be savory and had a slightly distinctive aroma of processed meat. Then in terms of hedonics (by semi-trained panelists), it has a rather preferred color, preferred texture, preferred taste, and preferred aroma.

3.3.1 Hedonic Quality

Based on Table 5 it can be concluded that the value of P (sig) in the color and texture parameters is > α (0.050), which means there was no difference in the hedonic color quality values between the selected product with control and also there is no difference in the value of hedonic texture quality between tempeh-based analog meat and wheat gluten flour as a vegetarian alternative food with control. While the results of the Independent t-test on the taste and aroma parameters indicate that the P (sig) value is smaller than α (0.050). This suggests that there is a difference in the quality taste and aroma between a selected product with a control product.

The mean difference column presents the mean difference between the control product and the selected product. In the taste parameter, there was a difference between the control product and the selected product, where the selected product has a taste quality value of 3.896 ± 0.478 greater than the control product. As for the aroma parameters, the selected product has an aroma quality value of 2.068 ± 0.618 greater than the control product.

3.3.2 Hedonic

In both Table 6 and Table 7, there was a significant difference between the control product and the selected product which is marked by the value of P (sig) <α (0.050) on every parameter (color, texture, taste, and aroma). It also shows that all parameters (color, texture, taste, and aroma) have negative mean difference values, this shows that based on the evaluation of panelists who are somewhat trained and panelists of consumers, the selected products have higher hedonic (preference) values compared to control products. , each of which can be seen in the difference in the mean column.

3.4 Nutrition Value

Nutritional analysis was carried out on selected formulation with a ratio of tempeh to wheat gluten 50g: 50g. After the analysis of nutrients is carried out, the results of the analysis of nutrients will be compared with the control product (F0). Data on the nutrient content of control products is obtained from the nutritional value information table contained behind the product packaging. The result of the nutritional analysis and control product's nutrition value showed in Table 8.

Table 5: Independent t-test results in a hedonic quality score.

Parameter	Formulation			
	t	df	Sig (2-tailed)	Mean difference
Color	1,693	48	0,097	1,236±0,730
Texture	0,618	48	0,540	0,428±0,693
Flavour	-8,143	48	0,001*	-3,896±0,478
Aroma	-3,347	48	0,002*	-2,068±0,618

Note:
* There is a significant difference in these parameters with a value of P ≤ 0.05
Mean difference column showed the mean difference between F0 to the selected formulation

Table 6: Independent t-test results in a hedonic score by a semi-trained panelist.

Parameter	Formulation			
	t	df	Sig (2-tailed)	Mean difference
Color	-3,131	48	0,003*	-1,824±0,583
Texture	-5,224	48	0,001*	-2,664±0,510
Flavour	-8,609	48	0,001*	-4,500±0,523
Aroma	-5,572	48	0,001*	-3,360±0,603

Note:
* There is a significant difference in these parameters with a value of P ≤ 0.05
Mean difference column showed the mean difference between F0 to the selected formulation

Table 7: Independent t-test results in a hedonic score by consumer panelists.

Parameter	Formulation			
	t	df	Sig (2-tailed)	Mean difference
Color	-2,873	48	0,006*	-0,600±0,209
Texture	-3,319	48	0,002*	-0,567±0,171
Flavour	-10,518	48	0,001*	-1,833±0,174
Aroma	-3,479	48	0,001*	-0,633±0,182

Note:
* There is a significant difference in these parameters with a value of P ≤ 0.05
Mean difference column showed the mean difference between F0 to the selected formulation

Table 8: Nutrition value of selected product and control.

Parameter	F0*	Selected product	SNI**
Vitamin B12 (mg/100g)	-	6,68	-
Protein (%)	67	9,25	Min 8,0
Fat (%)	5,2	2,94	Max 10
Carbohydrate (%)	17	28,32	-
Moisture (%)	-	56,72	Max 70,0
Ash (%)	-	2,69	Max 3
Total Plate Count (cfu/g)	-	$2,3 \times 10^4$	Max 1×10^5

Note:
*Nutritional value table of analog meat "Rodeo"
**SNI 3818:2014

3.4.1 Vitamin B12

From the analysis of vitamin B12 in selected products, it was found that the selected product contained 6.67 mg / 100g of vitamin B12 or 6670 mcg / 100g of product. This amount is very high when compared to the vitamin B12 content in tempeh found by Yuniati & Almasyhuri (1989), which is as much as 1.8 mcg / 100g when analyzed by the Microbiological Assay method. When compared with the vitamin B12 content in beef (3.17 mcg / 100g) (Bennink & Ono, 1982), as well as beef liver (41.3 mcg / 100g) (Yuniati & Almasyhuri, 1989), analog meat products based on tempeh and wheat gluten flour also still contains vitamin B12 which is much higher.

The production of vitamin B12 in tempeh is caused by the bacteria K. Pneumoneae during the fermentation process, not because of mold/yeast (Areekul, et al., 1990), so it can be ascertained that high levels of vitamin B12 in selected products are not due to the use of instant yeast, This is also supported by the results of Kustyawati's research (2009), which states that the addition of yeast does not play a role in the formation of vitamin B12 in tempeh. Research conducted by Bennink & Ono (1982) also states that the amount of vitamin B12 in beef before and after cooking does not have a significant difference, in fact, there should be a possibility of a decrease of 27-33% after the beef is cooked. A temporary assumption that researchers can give is that tempeh used to make products already contain high vitamin B12, which can be caused by the high activity of the K. pneumoneae bacteria as stated by Areekol, et al., (1990). Another conjecture that led to the high analysis of vitamin B12 in selected products is the presence of pseudovitamin B12. Pseudovitamin B12 is a form of biologically inactive vitamin B12, which can be taken into account when analyzing vitamin B12 in protein sources using the UHPLC (Ultra-High-Performance Liquid Chromatography) method (Schmidt, Call, Macheiner, & Mayer, 2019). Therefore, it is necessary to conduct an analysis of vitamin levels with other methods to prevent overestimation of vitamin B12 content in analog meat products based on tempeh and wheat gluten. But it should also be noted that pseudovitamin B12 can increase the absorption of vitamin B12 by intrinsic intestinal factors (Toporek, 1960).

3.4.2 Protein

Even though it meets the requirements of SNI 3818:2014, the results of the analysis of protein content in selected products are very low, even though the product uses the main ingredients of protein sources, namely tempeh and gluten. This can be seen when comparing protein levels in selected products with "rodeo" control products. The low level of protein analysis results in analog meat based on tempeh and wheat gluten flour is caused by the use of formol titration method which is not suitable for analyzing protein content in solid and chewy foodstuffs such as as analog meat. This can be proven from the results of Mukhoyaroh's research (2015) where the results of the analysis of protein content in tempeh made with several types of soybean only ranged between 0.01-0.72%, whereas the protein content of tempeh according to the Indonesian Food Composition Table (2017) is as much as 20 8%. In contrast, the results of the analysis of protein content by the Kjeldahl method on vegetarian meatballs based on gluten and soy flour are in the range of 55,634-71,596% (Mulyani, Rosida, & Rahmadani, 2012). The process of heating (cooking) does not cause a decrease in protein levels, because according to research Sundari, et al., (2015), an increase in protein levels before and after boiling as much as 1- 3.2%. Therefore, it can be concluded that a more suitable method for analyzing protein content from analog meat products based on tempeh and wheat gluten flour is the Kjeldahl method, not the formol titration method.

3.4.3 Fat

Besides fulfilling SNI quality requirements, the fat content of selected products is lower when compared to "rodeo" control products. This is caused by the use of soy flour which has higher fat content compared to tempeh (U.S. Department of Agriculture, 2019). When compared with animal protein sources such as beef (low fat), the selected product has a much lower fat content (KEMENKES RI, 2017). Besides that, tempeh contains 50,12% w / w of linoleic acid (omega-6) unsaturated fatty acids from the total unsaturated fatty acid content of 80% w / w tempeh (Utari, 2010). Therefore, it can be assumed that the fatty acids contained in selected products are unsaturated fatty acids.

3.4.4 Carbohydrate

In SNI 3818: 2014, quality requirements for carbohydrate content for mixed meatball products are not regulated. But when compared to "rodeo" control products, the selected products have higher carbohydrate content, this is because the carbohydrate analysis method used in this study is by difference. The results of carbohydrate content by the by difference method are obtained by calculating the remaining 100% after subtracting the results of an analysis of protein content and fat content. Therefore, the results of the analysis of protein and fat levels will affect the results of the analysis of carbohydrate levels. Based on these principles and the discussion of protein content analysis, it can be concluded that the results of carbohydrate content will also be affected by the method used to analyze protein content in the product. By looking at data at the United States Ministry of Agriculture's Food Data Center (2019), it appears that tempeh (7.64g) carbohydrate content is lower than soybean flour (31.92g), thus supporting the assumption that carbohydrate content of selected products should be lower than with control products. This is because the method used to analyze protein content is the formol titration method, not the Kjeldahl method. In addition, soy flour also has starch levels high enough to cause high carbohydrate levels (Mulyani, Rosida, & Rahmadani, 2012). In addition to the by difference method, carbohydrate levels can also be analyzed by chromatographic methods such as HPLC or GC (Gas Chromatography) (BeMiller, 2017).

3.4.5 Moisture

In food products, water content affects the quality of the product because it affects the acceptability, freshness, and storability of the product (Utama & Anjani, 2016). Therefore the "rodeo" control product is sold in dry form to increase its shelf life. Then when compared with the quality requirements of SNI 3818: 2014, the water content of selected products still meets the requirements. In addition, the moisture content of the selected product (56.72%) is also not much different from vegetarian meatball products made from gluten and soy flour (53.813%) made in the research of Mulyani, Rosida, & Rahmadani, (2012). The amount of water content in selected products is also caused by the absorption of wheat gluten flour which can absorb 1.3-1.5 times the amount (Mühlenchemie, 2006). High water content in selected products can affect the shelf life of the product, this can be seen from the results of observations by researchers when the product is stored in a chiller for 3 days, white hyphae appear, but the appearance of hyphae (white fungus tissue in tempeh) does not change the aroma product.

3.4.6 Ash

What is meant by ash content in food is inorganic residues (such as minerals) that are left after the elimination of organic substances either by oxidation or combustion (Ismail, 2017). From the analysis of ash content, it can be seen that the selected product has an ash content of 2.69% which still meets the SNI 3818: 2014 quality requirements, which is a maximum of 3%. In a similar study, namely, vegetarian sausage made from tempeh and oyster mushroom, the results of the analysis of ash content were not much different from the selected product, which was 2.26% (Ambari, Anwar, & Damayanthi, 2014). The process of self-healing, especially boiling can reduce ash content in tempeh by 0.54%. In addition to minerals, heavy metal contamination also includes inorganic compounds that can be calculated in the analysis of ash content, so sometimes it can be said that ash content also reflects the amount of heavy metal contamination in food (Halagarda, Kędzior, & Pyrzyńska, 2017).

3.4.7 Total Plate Count

The results of the analysis of the total plate count can describe the contamination of a food product. From the analysis of total plate numbers in selected products, it can be said that analog meat products made from tempeh and wheat gluten flour are safe to consume because they still meet SNI 3818: 2014 requirements. One way to prevent/slow the increase in the number of total plates is by giving an edible coating layer that can be done by utilizing algae Caulerpa sp. (Mailoa, Tapotubun, & Marutty, 2017). In addition, the drying process also affects the total plate count (Ruga, 2011).

4 CONCLUSIONS

From the results of hedonic quality tests and hedonic tests that have been carried out, the best formulation in F2 is most preferred by panelists and has the best color, texture, taste and aroma quality compared to other formulations. The selected product has a vitamin B12 content of 6.67 mg / 100g, 9.25% protein, 2.94% fat, carbohydrate 28.32%, water 56.72%, ash 2.69%, the total plate count is 2.3 x 104. It is proved that tempeh and vital wheat gluten can produce a meat analog with high vitamin B12 content that also fulfills the minimum quality requirements in SNI 3818:2014.

In future research, we suggest to analyze the vitamin B12 levels using Microbiological Assay method and identify the content of Pseudovitamin B12 in selected products. Also, protein analysis should be using the Kjeldahl method or quantitative analysis for carbohydrate content analysis. We also suggest to Conduct an analysis to see the product storage time, factors that influence and also changes related to nutrients that occur. And lastly to intervene by giving products to increase plasma vitamin B12.

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