Analysis of Macro and Micronutrient Content in Protein-Multivitamin Beverage Supplement for the Elderly

Mauren Gita Miranti¹,⁎ Tukiran Tukiran² Indah D. Wati³ Fauzia I. Sabila⁴

¹ Culinary Departement, Universitas Negeri Surabaya, Indonesia
²,³,⁴ Chemistry Departement, Universitas Negeri Surabaya, Indonesia
⁎Corresponding author. Email: maurenmiranti@unesa.ac.id

ABSTRACT
The elderly is susceptible to various diseases due to decreased immunity and physical changes, such as gastrointestinal (GI) disorders that cause and deficiency the nutrients. The elderly need to consume supplements as nutritional intake additional. This study aims to analyze the macro and micro nutrition content in beverage supplements with the basic raw materials of almond and tempeh filtrate. The research was a research experiment to study the effect of the proportion of almond filtrate and tempeh filtrate on the macro and micro nutritional content of beverage supplements. The research design used was a random design complete with two factorials, namely F1 (75% raw almond filtrate: 25% tempeh filtrate) and F3 (25% raw almond filtrate: 75% tempeh filtrate). Each treatment was replicated three times. The results showed there were differences in the content of carbohydrates (p=0.00), fat (p=0.002), vitamin B9 (p=0.000), and vitamin E (p=0.01) between the F1 and F3 formulations. Meanwhile, the protein (p=0,300) and crude fiber content (p=0,054) showed no difference between the F1 and F2 formulations in beverage supplements.

Keywords: Almond, Beverage supplement, Macro nutrition, Micro nutrition, Tempeh.

1. INTRODUCTION
Elderly or advanced age is the age of a person with 60 years of both men and women [1]. Various physical and psychological changes occur in the elderly that affect the health and ability of the body's metabolism, for example the state of cells in the elderly will change, such as a decreased number so that cell repair will be disrupted. This can affect the body's immune ability which causes a decrease in the body's immunity so that it is susceptible to disease. Other physical changes that occur in the elderly are gastrointestinal (GI) disorders characterized by decreased appetite, frequent constipation, decreased saliva production (saliva) and decreased intestinal peristaltic motion (saliva) [2]. Gastrointestinal (GI) disorders cause digestive disorders and nutritional deficiencies so that the elderly need to consume supplements as additional nutritional intake.

Beverage supplement are energy-boosting drinks that are included in the category of food supplements, which are products that complement the nutritional needs of foods, containing one or more ingredients in the form of vitamins, minerals, amino acids or other ingredients that have nutritional value and or physiological effects in concentrated amounts [3]. Most beverage supplement have the main substance in the form of protein. Protein is one of the important macronutrients for the body, because some of the functions of protein are as antibodies and repair and maintenance of body tissues. Generally, protein comes from milk and nuts, such as almonds and soybeans. The use of almond milk and tempeh milk as a source of protein as well as the main substance in the manufacture of beverage supplement product formulas. The main substance chosen came from nuts with the consideration that not everyone can consume animal milk because they suffer from lactose intolerance, especially the digestion of people who are classified as elderly.

Almond (Prunus dulcis) is known as one of the foods rich in antioxidants in the form of tocopherol and rich in Mono Unsaturated Fatty Acid (MUFA). A portion of 100 g of almonds contains about 50 g of healthy fats, most of which (40 g) are MUFA and PUFA, along with 4 g of saturated fat [4]. In addition, almonds also contain vitamin E, biotin, manganese, copper, fiber, protein, phosphorus, selenium, iron,
Riboflavin, potassium, tryptophan, magnesium, vitamin D, and calcium [5]. Almond milk has a balanced composition in terms of protein, fat, fiber and vitamins and minerals and contains no lactose [6]. Thus, it is suitable for those suffering from lactose and dairy protein intolerance [7].

Tempe is a processed soybean product formed by the type of mold Rhizopus sp. Tempe has higher quality and nutritional value than pure soybean. The amino acid content in tempeh is 24 times higher than soy milk. The fermentation process can increase levels of vitamin B2 (riboflavin), vitamin B6 (pyridoxine), folic acid, pantothenic acid, and nicotinic acid. The fermentation process in tempeh also activates the phytase enzyme which will decompose phytic acid (which binds several minerals) into phosphorus and isotol. With the breakdown of phytic acid, certain minerals such as iron, calcium, magnesium, and zinc become more available for use by the body [8]. Tempe milk is a product of tempe extraction with water so that a solution with dissolved solids is obtained. Tempe milk is one of the second-generation products of tempe [9]. The advantages of tempeh milk compared to cow's milk are that tempeh milk contains isoflavones, native phytoestrogens, lecithin, saponins and the amount of vitamins A and C is higher than cow's milk. In addition, tempeh milk does not contain cholesterol, but the calcium content of tempeh milk is lower than cow's milk [10].

In addition to protein, micronutrients also function as a booster for the body's immune system. Micronutrients are found in many vegetables, fruits, and medicinal plants. The addition of beetroot extract in beverage supplements is used as a natural dye in the manufacture of food products because of the betacyanin pigment content in beets [11]. Therefore, beverage supplement produced from the milk of almonds and tempeh by the addition extract some vegetables consumed elderly recommended to improve nutritional intake in preventing degenerative diseases.

Based on the analysis, there needs to be research to know the nutritional content of macro and micro in beverage supplements substituted from almond milk and tempeh. Macronutrient research covering testing carbohydrate, protein, fat, and fiber content. Micronutrient research covers testing the content of vitamins A, B9, C, and E.

2. METHODS

The research was a research experiment to study the effect of the proportion of almond filtrate and tempeh filtrate on the macro and micro nutritional content of beverage supplements. The research design used was a random design complete with two factorials, namely F1 (75% raw almond filtrate; 25% tempeh filtrate) and F3 (25% raw almond filtrate; 75% tempeh filtrate). Each treatment was replicated three times, so the total experiment was six experimental units. The manufacture of beverage supplements consisted of several stages, as follows:

2.1 Making raw almond filtrate and tempeh filtrate

Almond slices are soaked in water for 8 hours to soften the texture of almonds, improve digestion and absorption of nutrients by the body. Next, the almonds are drained and mashed with a slow juicer with added water at a 1:3 ratio between almonds and water. The resulting filtrate was filtered through a 100 mesh sieve to produce almond filtrate without sediment. The almond filtrate was packed and stored in the refrigerator.

Tempeh cut into cubes then steamed for 15 minutes. Steaming serves to stop the fermentation of tempeh. Furthermore, the steamed tempeh was crushed using a slow juicer with added water at a 1:3 ratio between tempeh and water. The resulting filtrate was filtered with a 100 mesh sieve to produce tempeh filtrate without sediment. The tempeh filtrate was packed and stored in a refrigerator.

2.2 Vegetable extract manufacture

Vegetables used as extracts are Moringa leaves, beetroot, and broccoli. Each vegetable is washed, cut into small pieces, aerated so that the water content is reduced, and mashed into a powder sample. Furthermore, each sample powder of 100 grams was macerated with 250 mL of sterile aqua for 4 hours and filtered with a vacuum pump so that the macerate filtrate and yield were produced. The macerate filtrate was evaporated with a rotatory vacuum evaporator at a temperature of 50 °C with pressure adjusted for water solvent so that a thick extract was produced.

2.3 Making Beverage Supplement

Making beverage supplement done with combined almonds and tempeh filtrate by comparison 75% filtrate raw almonds: 25% filtrate Tempe to formulation F1 and 25% filtrate raw almonds: 75% filtrate Tempe to formulation F3. The addition of extracts to beverage supplements is maximized as much as 1 gram of each vegetable extract and dates as much as 30% in 100 mL of a mixture of almond filtrate and tempeh filtrate.

2.4 Beverage Supplement Macro and Micro Nutrient Test

Macronutrient testing on beverage supplements was carried out by testing the protein content analyzed using the Kjeldahl test method, fat content was analyzed using the Weibull test method, carbohydrate content was analyzed using the Luff school test method, dietary fiber content was analyzed using the Gravimetric method.
Meanwhile, micronutrient testing was carried out by testing the content of vitamins A, C and E analyzed by HPLC and vitamin B9 analyzed by UPLC.

### 2.5 Data Analysis Technique

The data obtained the results of macronutrient content (carbohydrates, protein, fat, and fiber) and micronutrient content (vitamins A, B9, C, and E) were analyzed using the T-Test test with the SPSS version 17 program. Data interpretation if \( p < 0.05 \) indicates H0 is rejected and showed the difference between the F1 and F3 formulations. Meanwhile, if \( p > 0.05 \) indicates H0 is accepted and showed no difference between the F1 and F3 formulations.

### 3. RESULTS AND DISCUSSION

The nutritional value of food is the chemical bonds contained in food or drink that the body needs to perform its functions, such as producing energy, building and maintaining tissues, and regulating body cell metabolism. Determination of the nutritional value of protein-multivitamin beverage supplements carried out by proximate analysis of the macronutrient and micronutrient content. The results of the analysis of the nutritional value of protein-multivitamin beverage supplements based on almond filtrate and tempeh filtrate with different concentrations compared to determine the best formulation.

#### 3.1 Carbohydrate Content

Carbohydrates are found in plant foods in the form of simple carbohydrates and complex carbohydrates. Simple carbohydrates or simple sugars are easily digested to produce energy that can be used directly by the body. While complex carbohydrates such as glycogen and starch are energy reserves that are easily digested when needed at any time by the body. Complex carbohydrates play a role in controlling the body's blood sugar levels [12]. The results of the carbohydrate content test in beverage supplements are listed in Table 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average Carbohydrate Content (%)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>18.32 ± 0.0036</td>
<td>0.005</td>
</tr>
<tr>
<td>F3</td>
<td>14.37 ± 0.0020</td>
<td></td>
</tr>
</tbody>
</table>

Based on the data obtained with t-test analysis, it showed that the carbohydrate content of the beverage supplement had a value of \( p=0.005 \) (\( p < 0.05 \)). This indicated that there was a significant difference in carbohydrate content between the F1 and F3 formulations. The difference in carbohydrate content indicated that the addition of almond filtrate affects the carbohydrate content in the beverage supplement. The more concentration of almond filtrate made the carbohydrate content in beverage supplements even higher. This can be influenced by the processing of raw materials. In the manufacture of the product, there was a heat treatment on tempeh when making tempeh filtrate to reduce the unpleasant aroma. The existence of heat treatment can reduce the water content which can affect the results of the measurement of carbohydrate values the same as other proximate levels [13]. In addition, the treatment of soaking and boiling soybeans in the manufacture of tempeh caused a reduction in the sugar content, namely stachyose, raffinose, and sucrose to 51%, 48%, and 41% of the initial levels, respectively [14].

#### 3.2 Fat Content

Fat is the second macronutrient that produces energy after carbohydrates. Basic components of fats are fatty acids and triglycerides. Fat derived from food serves to absorb fat-soluble vitamins, provide essential fatty acids and provide energy for the body [12]. The results of the fat content test in beverage supplements are listed in Table 2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average Fat Content (%)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>8.68 ± 0.0024</td>
<td>0.002</td>
</tr>
<tr>
<td>F3</td>
<td>4.69 ± 0.0016</td>
<td></td>
</tr>
</tbody>
</table>

Based on the results of the analysis with the t-test, the fat content of the beverage supplement has a value of \( p=0.002 \) (\( p < 0.05 \)). This indicates that there was a significant difference in fat content between the F1 and F3 formulations. The difference in fat content indicated that the proportion of the addition of almond filtrate affects the fat content in the beverage supplement. The more concentration of almond filtrate made the fat content in beverage supplements even higher. Almonds are one of the nuts that contain high fat in the form of mono unsaturated fatty acids (MUFA), which is 67% of the total fat [5]. Meanwhile, in tempe, the fat content decreased due to steaming in the process of making tempeh filtrate. The presence of heating can affect the fat content in tempeh, because the protein in tempeh is coagulated so that a lot of water and fat comes out [15].
3.3 Protein Content

Proteins are formed from amino acids joined together into several peptide chains. Amino acids that can be synthesized by the body are called essential, while amino acids that are not synthesized by the body are called non-essential and must be obtained from food intake. The main function of protein for the body is to help and maintain body tissues, maintain immune function, and as a source of energy [12]. The test results for protein levels in beverage supplements are listed in table 3.

Table 3. Protein Content in Beverage Supplement

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average Protein Content (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1.88 ± 0.0004</td>
<td>0.300</td>
</tr>
<tr>
<td>F3</td>
<td>1.83 ± 0.0004</td>
<td></td>
</tr>
</tbody>
</table>

Based on the analysis by t-test showed the protein content of the supplement beverage has a value of p = 0.3 (p > 0.05). This indicates that there is no significant difference in protein content between the F1 and F3 formulations. The results of the analysis of protein content showed that the proportion of addition of almond filtrate and tempeh filtrate did not significantly affect the protein content. However, the protein content in the F1 formulation was more than F3. This is influenced by the fermentation process in tempeh, where many nutrients in soybeans undergo changes to become more soluble in water. Nearly half of the protein content of soybeans during fermentation is broken down into smaller, water-soluble products such as peptides and amino acid [16].

3.4 Fiber Content

Fiber is a residue of food ingredients consisting of cellulose and lignin. Fiber has no nutritional value for humans because it does not have cellulase enzymes to digest it. However, fiber plays a role in preventing constipation, diluting toxic substances in the colon and absorbing carcinogenic substances in digestion [17]. The results of the crude fiber content test in beverage supplements are listed in table 4.

Table 4. Crude Fiber Content in Beverage Supplement

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average Crude Fiber Content (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1.88 ± 0.0011</td>
<td>0.054</td>
</tr>
<tr>
<td>F3</td>
<td>1.65 ± 0.0006</td>
<td></td>
</tr>
</tbody>
</table>

Based on the results of the analysis with the t-test, the fiber content of the beverage supplement has a value of p = 0.054 (p > 0.05). This indicates that there is no significant difference in fiber content between F1 and F3 formulations. The results of the analysis of fiber content showed that the proportion of addition of almond filtrate and tempeh filtrate had no effect on crude fiber content in beverage supplements. Almonds include nuts that contain insoluble dietary fiber. Tempe also contains crude fiber which is included in the polysaccharide group (cellulose, hemicellulose, pectin, lignin). These polysaccharides cannot be absorbed by the body but can stimulate digestive enzymes [18]. Fiber can function as an antioxidant because it can simultaneously reduce low density protein (LDL) cholesterol and increase high density lipoprotein (HDL) cholesterol. Fiber in the colon will produce short chain fatty acids (SCFA) which can prevent colon cancer [19].

3.5 Micronutrient

In this study, vitamins A, B9, C and E were tested in beverage supplements. However, assay vitamins A and C were not detected in both formulations and vitamin B9 was not detected in the F1 formulation. This is due to the presence of limit detection in testing vitamins A, C and B9 in beverage supplements. Therefore, it is necessary to improve the formulation by adding the concentration of Moringa leaf extract selected as a source of vitamin A, beetroot extract as a source of vitamin B and broccoli extract as a source of vitamin C. The results of testing for vitamins B9 and E in beverage supplements are shown in table 5.

Table 5. The Content of Vitamins B9 and E in Beverage Supplement

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Sample</th>
<th>Average Crude Fiber Content (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B9</td>
<td>F1</td>
<td>Not detected</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>F3</td>
<td>381 ± 2.6458 mcg</td>
<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td>F1</td>
<td>1.59 ± 0.0265 mg</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>F3</td>
<td>0.64 ± 0.0207 mg</td>
<td></td>
</tr>
</tbody>
</table>
Based on the results of the t-test analysis, the content of vitamin B9 with a value of p = 0.000 (p < 0.005) and the content of vitamin E with a value of p = 0.01 (p <0.05). This shows that there is a significant difference in the content of vitamin B9 and vitamin E between the F1 and F3 formulations. The results of the analysis of vitamin B9 content showed that tempeh filtrate had an effect on vitamin B9 content in beverage supplement. The more concentration of tempeh filtrate made the folic acid (vitamin B9) content in beverage supplement even higher. Tempe is the result of soybean fermentation using Rhizopus oligosporus inoculum for 48 hours which has the potential as a natural folic acid (vitamin B9) fortification [20]. While the results of the analysis of vitamin E showed the concentration of almond filtrate affected the content of vitamin E in beverage supplement. Almond is one of the nuts that has a high vitamin E content [21].

4. CONCLUSION

Based on research data, the formulation of beverage supplement made from the almond filtrate and tempeh filtrate showed that there were differences in the content of carbohydrates (p=0.00), fat (p=0.002), vitamin B9 (p=0.000), and vitamin E (p=0.01) between formulations F1 and F3. Meanwhile, the protein content (p=0.300) and crude fiber (p=0.054) showed no difference between the F1 and F2 formulations in beverage supplements.

AUTHORS CONTRIBUTION

All authors conceived and designed this study. All authors contributed to the process of revising the manuscript, and at the end all authors have approved the final version of this manuscript.

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