Mineral bioavailability in jelly drink made of green okra (Abelmoschus esculentus) and strawberry (Fragaria ananassa) extract

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Abstract. Okra jelly drink is a food product that uses gelling agent to form jelly texture that can be consumed using a straw, and the use of okra and strawberry in jelly drinks can increase its nutritional contents. This study aims at determining the effect of green okra (Abelmoschus esculentus) and strawberry (Fragaria ananassa) extract ratio on the bioavailability of calcium, phosphorus, potassium, and sodium contents. The bioavailability of calcium, potassium, and phosphorus is analyzed using in vitro analysis. In addition, the identification of calcium, potassium, and sodium contents using Atomic Absorption Spectrophotometer and phosphorus analysis using Spectrophotometer are conducted. The study found that the difference of okra and strawberry extracts ratio does not affect the calcium content (p=0.224) but it affects the phosphorus (p=0.005), potassium (p=0.041), and sodium (p=0.001) contents in jelly drink. Moreover, the treatment of various okra and strawberry extracts ratio does not affect the bioavailability of calcium (p=0.394) and potassium (p=0.666) but it affects the bioavailability of phosphorus (p=0.007) in green okra jelly drink. Mineral bioavailability is influenced by several inhibitory factors, such as oxalate, phytate, tannin, and dietary fiber that can decrease the mineral bioavailability.

1. Introduction
Vegetables and fruits are the sources of fiber, antioxidants, vitamins, and minerals that can be beneficial to people’s health. Some of them are contained in okra and strawberry. Okra is a vegetable with the Latin name Abelmoschus esculentus that belongs to the family of Malvaceae plants [1]. Fruit also contains various types of nutrients that can be beneficial to people’s health, which include the contents of strawberries or Fragaria ananassa. This fruit is usually conical to round shaped and has a refreshing sweet and sour taste. Strawberries contain phenolic compounds such as flavonoid, anthocyanin, and tannin [2]. Anthocyanin is a compound that acts as a natural colorant on strawberries. The high level of natural colorant in strawberries can improve the organoleptic aspect in food products.

Vegetables and fruits serve as good sources of minerals. The examples include the calcium, phosphorus, potassium, and sodium contained in okras and strawberries. Okra pods, per 100 g of edible portion, contains 84 mg of calcium, 90 mg of phosphorus, 299 mg of potassium, and 7 mg of sodium [3,4]. Strawberries, per 100 parts of edible portion, contains 16 mg of calcium, 24 mg of phosphorus, 153 mg of potassium, and 1 mg of sodium [4].

The availability of minerals in the human body is essential for physiological functions. However, the consumption of vegetables and fruits, which are the sources of minerals, is still low. Riskesdas identifies the lack of 93.5% vegetables and fruits consumption in Indonesia [5]. To address this issue,
several efforts can be made to increase the consumption of vegetables and fruits. For example, nutrition education and food product diversification with vegetables and fruits. One of the ways to diversify food product is producing jelly drinks with okras and strawberries. The use of okras and strawberries in the production of jelly drinks can increase the nutritional contents of the jelly drinks.

Minerals from the food being consumed cannot be entirely absorbed by the human’s body. The amount of minerals that can be absorbed and utilized in metabolic processes or stored by the human’s body is called mineral bioavailability [6]. The results of a research conducted by Amalraj and Pius shows that calcium bioavailability is influenced by inhibitory factors and the availability of other minerals [7]. Different food sources have different mineral bioavailability. Vegetables and fruits have lower mineral bioavailability than dairy produce. Mineral bioavailability in fruits and vegetables varies from 5% to 50% [8]. Therefore, the researchers are interested in identify the effect of green okra (Abelmoschus esculentus) and strawberry (Fragaria ananassa) extracts ratio on the bioavailability of calcium, phosphorus, potassium, and sodium contents in green okra jelly, which is a food product made from okras and strawberries.

2. Method
2.1. Design, location and time
This study is an experimental study on jelly drinks with completely randomized design with two repetitions. The formulation and production of the jelly drinks were conducted at the Food Experiment Laboratory; the analysis of the mineral contents and bioavailability were conducted in the 2nd Floor of Nutrition Biochemistry Laboratory, Department of Community Nutrition; and the AAS identification was conducted in the Integrated Chemistry Laboratory, Department of Chemistry, Bogor Agricultural University. The study was conducted from January to June, 2017.

2.2. Material and equipment
The materials used in the production of jelly drinks were green okras, strawberries, lemons, jelly powder (a combination of carrageenan and konjac), potassium citrate, and sucralose. The green okras were from the Agribusiness Development Station of Bogor Agricultural University and the strawberries were from the supermarket. The gel-forming materials i.e. jelly powder and potassium citrate as well as the sucralose were from Setia Guna Chemical Store. The materials for the analysis of mineral contents and bioavailability consisted of deionized water, HCl, pepsin enzyme, pancreatic enzyme, bile extract, NaHCO₃, NaOH, oxalic acid, dialysis pouches, H₂SO₄, HNO₃, KH₂PO₄, ammonium molybdate, and ammonium metavanadate.

The tools used for the formulation of the jelly drinks were thermometers, basins, gas stoves, knives, pans, scales, blenders, glasses, bowls, strainers, and cups. The tools used in mineral bioavailability analysis were glassware, glass flasks, measuring cylinders, scales, incubators, Mohr pipettes, dropper pipettes, volumetric pipettes, test tubes, aspirators (bulbs), mouthpieces, stirrer glasses, ashing cups, blenders, burettes, measuring glasses, Erlenmeyer flasks, spray bottles, plastics, scissors, dialysis pouches, Whatman 42 filter paper, dialysis pouches, and water baths.

2.3. Analysis process
2.3.1. Formulation of green okra jelly drinks with strawberries. The jelly drinks formulation begins with a trial and error on the use of gelling agent and sweetener types. Based on an organoleptic test with limited panelist, the use of gelling agent in this research was 0.4% of carrageenan-konjac combination and the sweetener used was sucralose. The followings are the trial and error results of the jelly drinks formulation.
Table 1. Green okra jelly drinks formula.

| Formulation | Okra extract: strawberry extract (%)<sup>a</sup> | Jelly powder (%)<sup>b</sup> | Potassium citrate (%) | Sucralose (%) | Lemon extract (%) |
|-------------|-----------------------------|-----------------|----------------------|--------------|------------------|
| F1          | 100:0                       | 0.4             | 0.15                 | 0.02         | 3                |
| F2          | 80:20                       | 0.4             | 0.15                 | 0.02         | 3                |
| F3          | 70:30                       | 0.4             | 0.15                 | 0.02         | 3                |
| F4          | 60:40                       | 0.4             | 0.15                 | 0.02         | 3                |
| F5          | 50:50                       | 0.4             | 0.15                 | 0.02         | 3                |

<sup>a</sup>measured from the amount of okra and strawberry extract per 100 ml - the extract is made with drinking water with 1:5 ratio

<sup>b</sup>The gelling agent is a combination of carrageenan and konjac

2.3.2. Production of jelly drink. The production of the extracts was done by blending the okras, with the ratio of green okras and water equal to 1:5 (w/v). Previously, the okras were washed and water blanched. Blanching is a process of heating fruits and vegetables using steam or hot water with the temperatures ranging from 90-95°C for one or two minutes [9]. The okra blanching process in this jelly drink production used hot water with the temperature of 90-95°C for two minutes. The same procedure was carried out in the process of making the strawberry extract, but without the blanching process. The blanching process of okras aims at improving the organoleptic aspect, because blanching process can reduce unpleasant aroma in food [10]. Blanching process was not carried out on strawberries to prevent the loss of compounds contained in the strawberries. The process of water blanching can cause the loss of nutrient contents in food due to the dissolution in water and the heating process [11]. The okra and strawberry extracts was then filtered using food strainers. Afterward, the green okra extract was added with strawberry extract in accordance with the ratio in the formulation and additional ingredients were added accordingly. The mixture was then boiled and poured into a container. Afterward, the jelly drinks are stored in the refrigerator and ready to evaluate the quality using national standard [12]

2.3.3. Mineral contents analysis. The analysis of mineral contents carried out on the sample consisted of the analysis of calcium, potassium, and sodium contents using Atomic Absorption Spectrophotometer method and the analysis of phosphorus using Spectrophotometer method [13].

2.3.4. Mineral bioavailability test. Bioavailability is the amount of nutrients that can be absorbed by the human body and are ready to be used for metabolic processes. In vitro mineral bioavailability analysis is imitating the condition of digestive process that occurs in the gastrointestinal, which is done by setting the pH with dialysis process and the use of commercial enzyme. The mineral bioavailability testing was conducted using in vitro analysis with dialysis method [13]. This method consists of three stages: sample preparation stage; in vitro digestion simulation stage using pepsin enzyme, pancreatic enzyme, and dialysis pouch; and calcium and potassium contents identification with Atomic Absorption Spectrophotometer and phosphorus content identification with Spectrophotometer.

2.4. Processing and analysis of data
The processing and analysis of the data were presented in the form of description and statistical analysis. All of the data that has been obtained was tabulated using Microsoft Excel 2013 and processed using SPSS 16.0 for Windows. The data of mineral analysis results on each formula was tested using One-Way ANOVA with 95% confidence level (α=0.05), and further tests were conducted using Duncan.
3. Result and discussion

3.1. Formulation of green okra jelly drink with strawberries

The formulation of jelly drinks aims at making the jelly drinks to have a semi-solid form and a chewy texture. The formulation of green okra jelly drinks using jelly powder as the gelling agent is to form a gel structure that is strong but can be consumed with a straw. Jelly powder used in this study was a white flour food material consisting of hydrocolloid ingredients—a combination of carrageenan and konjac. Jelly drinks have a texture that, if consumed using a straw, can be destroyed easily, but they still have chewy gel form [14]. The results of the trial and error in making jelly in this study reveals that the use 0.4% jelly powder can form a structure of jelly that can be consumed using straws. Furthermore, the formation of this gel structure was also aided by the addition of 0.15% of potassium citrate. Potassium citrate can form a buffer system that helps maintain the pH of the jelly drinks so that the structure of the jelly drinks remains stable [15]. The addition of potassium to carrageenan formed a strong jelly structure due to the addition of potassium cation. However, the addition of too much potassium disrupts the balance of ions, so that the structure of the jelly becomes brittle and prone to syneresis [15].

The formulation of green okra jelly drinks used sucralose to give a sweet taste. This formulation used 0.02% of sucralose. Sucralose is an artificial sweetener that belongs to the Food Additive category. Food Additive refers to the ingredients added to food to affect the nature or form of the food. The maximum limit of the use of sucralose in the jelly food category is 400 (mg/kg) [16]. The purpose of the use of sucralose that replaces sugar (sucrose) in this formulation is to provide a sweet taste and form a jelly structure. The treatment in this study is the difference of okra and strawberry extract ratio. The addition of strawberries aims at improving the product of jelly drinks in terms of the organoleptic and nutritional contents. Strawberries have a sweet and fresh taste. In addition, strawberries can also be used as a natural red colorant. This can improve the product of jelly drinks in terms of organoleptic aspect. Strawberries are very good for health and contain lots of phosphorus, calcium, iron, bromide, salicylic acid, and vitamins B, C, E, and K [17].

3.2. Mineral contents and bioavailability

Mineral contents in food is very diverse [18]. Moreover, mineral bioavailability is the percentage of minerals that can be absorbed and utilized in the metabolic processes or stored in the body [6]. The following table presents the results of the mineral contents and bioavailability measurement.

| No | Sample  | Potassium content (mg/100g) | Potassium bioavailability (%) | Sodium content (mg/100g) | Sodium bioavailability (%) |
|----|---------|-----------------------------|-------------------------------|--------------------------|----------------------------|
| 1  | F1(100:0) | 83.8994 ± 0.896a | 34.3840 ± 3.832 | 22.3700 ± 0.368b | - |
| 2  | F2(80:20) | 86.9207 ± 3.187a | 35.6439 ± 2.442 | 18.0735 ± 1.179a | - |
| 3  | F3(70:30) | 83.0272 ± 2.892ab | 34.1753 ± 3.205 | 16.9400 ± 0.411a | - |
| 4  | F4(60:40) | 86.7823 ± 1.550a | 32.0251 ± 0.947 | 18.4410 ± 0.327a | - |
| 5  | F5(50:50) | 78.2173 ± 0.521b | 32.7839 ± 0.707 | 16.1745 ± 0.23a | - |

The different letters after the results represent significant differences (p<0.05)
3.3. Calcium content and bioavailability
Calcium is the basic constituent mineral element of bones and teeth [6]. Calcium is responsible for impulse delivery of the muscular and nervous system, normal function of the heart, hormone secretion, blood clotting, cell membrane permeability, and activation of various enzymes [19]. The results of the calcium mineral content analysis on the strawberry okra jelly drinks show ranges between 4.1750 mg/100 g for F5 (50:50) to 5.4382 mg/100 g for F1 (100:0). This corresponds to the initial contents of the okra and strawberries in which the calcium content of the okras is higher than the calcium content of strawberries. Therefore, the greater the ratio of okra extract in the formula, the higher the calcium content. Based on [3], the calcium content of 100 g of okra is 84 mg, whereas every 100 g of strawberries contain 16 mg of calcium [4].

The results of the statistical tests with ANOVA showed that the treatment of various okra and strawberry extracts ratio does not affect the calcium mineral content in green okra jelly drinks (p=0.224). This can be caused by the dilution in the process of extracting the okras and strawberries, so that there is a decrease in the concentration of calcium in each extract. The blanching stage on the okras can also decrease the calcium content in them so that the calcium content in the okra extract can also decrease [20].

The measurement of calcium bioavailability in jelly drinks was done with in vitro analysis. The results of the statistical test with ANOVA showed that the treatment of various okra and strawberry extracts ratio does not affect the bioavailability of calcium mineral in green okra jelly drinks (p=0.394). This can be caused by the blanching process in the early stages of extracting the okras. Blanching can reduce the antinutrients contents on food [10]. Furthermore, the extraction of okras and strawberries contains dregs, resulting in decreased fiber content in the okra and strawberry extracts. In addition, the addition of water to the extraction process can reduce the concentration of antinutrients in the green okra jelly drinks. Calcium bioavailability is influenced by factors such as inhibitory factors and the effects other minerals. The in vitro analysis results of the influence of inhibitory factors on calcium showed that high inhibitory factors, such as oxalate, phytate, tannin, and dietary fiber, can decrease calcium bioavailability [7]. The existence of oxalic acid in food can form an insoluble oxalate salt, which inhibits the absorption of calcium. Phytic acid and fiber can also inhibit the absorption of calcium [21].

3.4. Phosphorus content and bioavailability
Phosphorus is the second most plentiful mineral in the body, which covers 1% of the body weight. Approximately 85% of the phosphorus in the body is calcium phosphate found in bones and teeth, while the rest is in muscles and extracellular fluids [21]. The average phosphorus content of the green okra jelly drinks is 3.7204 mg/100 g. The results of the statistical test with ANOVA on the phosphorus content showed that the treatment of various ratio of okra and strawberry extracts influence the phosphorus mineral content in the strawberry okra jelly drinks (p=0.005). The following Tukey test results showed significant differences between F1 (100:0) and F2 (80:20) (p=0.021), F2 (80:20) and F3 (70:30) (p=0.005), F3 (70:30) and F4 (60:40) (p = 0.011), and F2 (80:20) and F5 (50:50) (p=0.040).

The results of the statistical test with ANOVA on the phosphorus bioavailability show that the treatment of various okra and strawberry extracts ratio influences the phosphorus mineral bioavailability in the strawberry okra jelly drinks (p=0.007). The following Tukey test results showed significant differences between F1 (100:0) and F2 (80:20) (p=0.020), F2 (80:20) and F3 (70:30) (p=0.006), F2 (80:20) and F4 (60:40) (p = 0.035), and F2 (80:20) and F5 (50:50) (p=0.010). Similar to the calcium bioavailability, the phosphorus bioavailability is also influenced by dietary fiber and inhibitory factors, such as phytate, tannin, and oxalate. The occurrence of bonds between phosphorus and phytate forms phytate phosphorus that cannot be absorbed by the body [3].
3.5. Potassium content and bioavailability

Potassium plays a very important role in the human body. The function of potassium is similar to sodium, which is maintaining the osmotic pressure and the acid-base balance together with chloride. The difference of potassium is that it is more specific to keep the osmotic pressure in the intracellular fluid [22]. The measurement results showed that the potassium content of each formula ranges from 78.2173 mg/100 g to 86.9207 mg/100 g. The results of the ANOVA test show that the treatment of various okra and strawberry extracts ratio has significant effect on the potassium content in okra jelly (p=0.041). The following Tukey test results showed that the potassium content of F2 (80:20) is not significantly different (p>0.05) from the potassium content of F1 (100:0), F3 (70:30), and F4 (60:40). Moreover, the potassium content of F5 (50:50) is not significantly different from the potassium content of F3 (70:30). Based on USDA, the potassium content of okras is 299 mg/100 g and the potassium content of strawberries is 153 mg/100 g [4]. In comparison to the results of the study, there is a decrease in potassium content in green okra jelly drinks. This can be caused by the processing of the materials that reduce the potassium content of jelly drinks, which includes water blanching, filtration, and dilution. This is in line with the other research that states that the decrease in potassium content can be caused by water blanching treatment, so that some of the potassium in okras are dissolved into the water used for blanching [23].

The results of potassium bioavailability analysis show that the highest potassium bioavailability among the five formulas is the F2 (80:20) and the lowest among the five formulas is the F4 (60:40). Based on the ANOVA test results, the treatment of various okra and strawberry extracts ratio does not significantly affect the potassium bioavailability (p=0.666). Potassium is absorbed through the intestinal tract by diffusion through the capillary walls and active absorption. Potassium enters the body cells also by diffusion and requires active metabolism [23].

3.6. Sodium content

Sodium is a micro nutrient that has an important role in the human body [24]. Almatsier states that sodium is the main cation in the extracellular fluid and a small part is in the intracellular fluid [25]. The results of the analysis of sodium content in green okra jelly drinks show ranges between 16.1745 mg/100 g for F5 (50:50) to 22.3700 mg/100 g for F1 (100:0). The ANOVA test results showed that the treatment of various okra and strawberry extracts ratio has an effect on the sodium content on okra and strawberry jelly drinks (p=0.001). The following Tukey test results showed that the sodium content of F1 differs significantly from F2, F3, F4, and F5 (p <0.05).

Sodium content in okra and strawberry-based jelly drinks tends to decrease as the number of okra used decreases and the number of strawberries used increases. This corresponds to the initial sodium content found in okras and strawberries based on USDA data, which is the sodium contained in okras are greater than the sodium content contained in strawberries [4]. This causes the sodium content of F1 to F5 are decrease.

4. Conclusion and recommendation

Based on organoleptic characteristics and nutrients content the best formula is 80:20 (F2) formula for potassium bioavailability or 50:50 (F5) formula for phosphorus bioavailability. The difference of okra and the strawberry extracts ratio does not affect the calcium content in green okra jelly drinks. However, the difference in okra and strawberry extracts composition affects the phosphorus, potassium, and sodium contents in green okra jelly drinks. The treatment of various okra and strawberry extracts ratio does not affect the bioavailability of calcium and potassium in green okra jelly drinks. However, the difference in okra and strawberry extracts influences the bioavailability of phosphorus in green okra jelly drinks. Blanching and boiling process in the making involved that purposed to reduce unwanted odor and aroma, also to form jelly texture. However, this process reduced mineral content also. Another method is still needed to be developed by reduce unwanted aroma and taste but not the mineral content.
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