Antioxidant capacity and nutritional value of tempe yogurt

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Abstract. Tempe is a cheap and popular food that can be processed into yogurt as an alternative to cow's milk. Tempe yogurt, which enriched with probiotics is an alternative beverage that able to increase body health. This study was to investigate the nutritional characteristics of tempe and nutrient content of yogurt tempe products. The sample of this study consist of eight test groups, cow milk yogurt (C1), juiced tempe yogurt (C2) and tempe yogurt, which was made using cow milk and varying amounts of tempe flour, 2.5% (T1); 5% (T2); 7.5% (T3); 10% (T4); 15% (T5) and 50% (T6) respectively. Each samples were prepared to proximate compound and antioxidant analysis and organoleptic test to determine the highest nutritional yogurt and most preferred taste. According to the test results, the highest carbohydrate, protein and lipid content were found in the T3 group, which reached respectively 12.21%; 11.72%; 24.46% while the antioxidant value reached 53.93%. Meanwhile, based on organoleptic test using hedonic scale for the highest value of taste preferences, showed that the most preferred taste was T2 group (4.39 point). The addition of tempe flour increases the nutritional value of yogurt in general. It caused by fermentation process of tempe, which releases nutritional compounds in soybeans. According to panelist, the T2 sample became a favorite one, because it has a softer milk texture, a balanced and more fragrant flavor. The conclusion is, the additional tempe flour in yogurt can increase nutritional value and preferred taste.

1. Introduction
Tempe is a fermented food from soybean from Indonesia that is high in nutrition value and rich in bioactive compounds. In general, consumption of soybean has been linked with various health benefit mainly in preventing the development of obesity, diabetes, and cancer due to the presence of flavonoids, especially isoflavones. Fermentation during tempe making process has beneficial effect as it increases the bioavailability of not only isoflavones but also peptides. These are two bioactive compounds that responsible for the health benefit of tempe [1,2].

Tempe is made from whole soybeans which are fermented by R. oligosporus, R. oryzae, R. Stolonifer, R. chlamydosporus and R. arrhizus, although modification using other microorganism has also been explored [3]. The high content of nutrition in tempe makes it possible to be developed into other products that increases its both economical and acceptance values, such as yogurt. The use of tempe as a yogurt ingredient is an alternative vegetarian food choice and is expected to improve the quality and nutritional value compared to the ordinary yogurt product. Additional probiotics in tempe yogurt is also expected to improve the beneficial effects of yogurt and/or tempe by their own. Furthermore, tempe is relatively cheap and easily obtained, making it promising to be mass produced.

Therefore, the aims of this study were to determine the optimum composition of tempe yogurt, characterize the nutritional value and antioxidant capacity of newly developed tempe yogurt, as well as analyze its acceptability.
2. Methods
This study was an experimental study to determine the nutritional content of tempe yogurt made with variations in the concentration of tempe milk. The study was conducted in Biochemistry and Microbiology laboratory, Semarang State University (UNNES). The study was carried out in two stages. The first stage is tempe making and the second is the making of tempe yogurt.

Tempe production as Tempe yogurt's main ingredient is based on the method described in previous publications [4-6]. This method involves pasteurization with two stages of heating. The first heating step runs at 100 °C for 30 minutes, the second goes at 60-70 °C for 15-20 minutes. The pasteurization step uses a double pan where a small plastic pan is inserted into a larger metal container that has been filled with water.

2.1. Tempe yogurt preparation
Fresh tempe is dried, ground into flour and mixed with fresh milk. The mixture is filtered through a thin cloth and heated at 43-45 °C, while as much as 6.5% sugar from the total concentration is added. The milk mixture is heated to 85-90 °C and stored at room temperature for 30 minutes under constant stirring. After 30 minutes, the mixture is cooled to 45 °C and inoculated with a yogurt starter culture. This culture is made from Lactobacillus acidophilus and L. casei in a 50:50 milk mixture, incubated for 4-6 hours and cooled for 12 hours until the fermentation process stops.

The sample of this study consisted of eight experimental groups, cow milk yogurt (C1), yoghurt was made from tempe only (C2). Porridge and yogurt tempe, which were made using a mixture of cow's milk and tempe flour. The concentration of tempe flour was 2.5% (T1) respectively; 5% (T2); 7.5% (T3) 10% (T4); 15% (T5) and 50% (T6).

2.2. Proximate analysis
Proximate analysis includes analysis of total carbohydrate, fat and protein content. Carbohydrate measurements were carried out by the -by -difference method, the measurement of protein content was carried out by the Bradford method, while the fat content was measured using the method of sokletation. Analysis of water and ash was carried out by the thermogravimetric method and ignoring with furnace tools.

Measurement of total antioxidant capacity is carried out at the Food and Nutrition Laboratory at Gadjah Mada University. The results of this test were analyzed using SPSS values and p < 0.05 was considered statistically significant.

3. Result and Discussion
The use of tempe as an ingredient in yogurt is one of the alternative ways of food developing and diversifying. Soybean tempe is known to contain high nutrients and is easily absorbed by the body. Making tempe yogurt by mixing tempe flour and milk has different macromolecular content. Significantly, the highest carbohydrate and fat content was obtained in the T4 group, as seen in Table 1 below.

| Treatment | Carbohydrate g/100 g | Protein g/100 g | Fat g/100 g | Water (%) | Ash (%) |
|-----------|----------------------|-----------------|-------------|-----------|---------|
| C1        | 10.811<sup>bc</sup> | 7.938<sup>a</sup> | 14.22<sup>a</sup> | 47.109<sup>a</sup> | 19.428<sup>a</sup> |
| C2        | 11.99<sup>be</sup>  | 14.301<sup>bd</sup> | 13.04<sup>b</sup> | 63.533<sup>b</sup> | 33.333<sup>b</sup> |
| T1        | 11.512<sup>abe</sup> | 6.125<sup>c</sup>  | 21.16<sup>c</sup> | 44.271<sup>c</sup> | 19.382<sup>a</sup> |
| T2        | 9.932<sup>ef</sup>  | 13.542<sup>d</sup> | 15.36<sup>d</sup> | 54.377<sup>d</sup> | 14.742<sup>c</sup> |
| T3        | 6.376<sup>d</sup>   | 14.283<sup>bd</sup> | 13.22<sup>b</sup> | 48.538<sup>e</sup> | 12.042<sup>d</sup> |
| T4        | 12.207<sup>e</sup>  | 11.718<sup>e</sup> | 24.46<sup>e</sup> | 54.474<sup>d</sup> | 15.288<sup>c</sup> |
| T5        | 11<sup>bc</sup>     | 14.601<sup>b</sup> | 13.22<sup>b</sup> | 48.333<sup>f</sup> | 12.035<sup>d</sup> |
| T6        | 9.675<sup>f</sup>   | 14.484<sup>b</sup> | 24.24<sup>e</sup> | 56.56<sup>d</sup> | 25.536<sup>e</sup> |

<sup>a-f</sup>ANOVA and Tukey's analysis showed an unequal alphabet, indicating a significant difference.
Meanwhile, the highest protein content was obtained from T5 group, which was 14.601 g/100 g. Based on this, yogurt with additional tempe flour by 15% has a higher protein content and is significantly different than milk yogurt and or tempe porridge yogurt. This is different from the protein content which actually decreases in tempe because of the fermentation process. The fermentation process by *Rhizopus* sp, converts protein into amino acids as a source of N (nitrogen) for growth.

The soy fermentation process also results in a decrease in lipid levels. Soybeans contain 82% triacylglycerol, 13% phospholipids, about 1% sterols, and 4% unsaturated and saturated fatty acids and the rest are phospholipids [7]. Biochemical activity of lipase enzyme of *Rhizopus* sp during fermentation process produces free fatty acids through hydrolysis of triacylglycerol. The fatty acid, will be used by microorganisms contained in the tempe microhabitat to growth. However, based on the Soxletation analysis shown that the addition amount of tempe flour actually increases fat content. The provision of tempe flour is 10%, resulting in fat content is 24.46 g/100 g. These levels were higher than the control group both C1 which was 14.22 g/100 g and C2 which was 13.04 g/100 g.

The highest water content in tempe yogurt was found in group C2 (cow's milk) there was 63.53%. But giving 50% tempe flour in the T6 group had a water content of 56.56% which was higher than the C1 group which was only 47.109%. The highest water content in C2 is likely due to the yogurt content which is only from cow's milk without the addition of tempe flour. The C1 group also has a higher ash content than other groups. The amount of ash in a food indicates the high minerals contained in these foods.

The ash content which is an inorganic residue left after the organic molecules burns out indicates higher mineral content. The largest mineral content in soybeans is in the skin, so the process of soybean skin peeling is likely reducing mineral content [8-9]. But the fermentation process results in the release of minerals in soybeans and increases mineral availability [10].

### Table 2. The content of organic compounds in various concentrations of tempe yogurt

|            | Crude fiber (g/100 g) | Antioxidant capacity (%) | Reducing sugar (g) | Lactic acid (g/100 g) |
|------------|-----------------------|--------------------------|--------------------|-----------------------|
| C1         | 1.035<sup>a</sup>    | 51.653<sup>a</sup>      | 3.868<sup>a</sup>  | 1.989<sup>b</sup>    |
| C2         | 3.621<sup>b</sup>    | 31.405<sup>b</sup>      | 5.598<sup>b</sup>  | 1.675<sup>b</sup>    |
| T1         | 2.45<sup>c</sup>     | 33.056<sup>b</sup>      | 4.612<sup>ab</sup> | 3.555<sup>c</sup>    |
| T2         | 5.434<sup>d</sup>    | 50.207<sup>ac</sup>     | 1.821<sup>c</sup>  | 4.68<sup>d</sup>     |
| T3         | 5.567<sup>d</sup>    | 47.521<sup>c</sup>      | 3.615<sup>a</sup>  | 1.44<sup>b</sup>     |
| T4         | 1.815<sup>ac</sup>   | 53.926<sup>d</sup>      | 5.434<sup>b</sup>  | 2.385<sup>b</sup>    |
| T5         | 1.1938<sup>a</sup>   | 52.479<sup>ad</sup>     | 6.216<sup>d</sup>  | 4.725<sup>d</sup>    |
| T6         | 3.067<sup>bc</sup>   | 41.942<sup>c</sup>      | 3.977<sup>a</sup>  | 1.693<sup>b</sup>    |

<sup>a-d</sup>ANOVA and Tukey's analysis showed an unequal alphabet, indicating a significant difference

Organic compounds measured in this study were crude fiber, reducing sugar and lactic acid. In addition, measurement of antioxidant capacity was conducted to find out how much antioxidant content in tempe yogurt. Based on ANOVA analysis, the highest crude fiber content was obtained from tempe yogurt T3 there was of 5.567 g/100 g of tempe yogurt which was higher than C1 and C2 groups, respectively 1.035% and 3.621%, while the lowest crude fiber content obtained from T5 group that is equal to 1.1938 g/100 g.

The highest antioxidant capacity contained in tempe yogurt was obtained from the T4 group of 53.926%. This was more significantly higher than the C1 control group of 51.653% and C2 of 31.405%. In addition, C1 tempe pure yogurt was significantly different from C2 or milk yogurt. This result is in line with a study by Shori [9] and Durazzo [10] which revealed that soybean addition in both cow-milk yogurt and soy-based beverage drink increased its antioxidant capacity.

The content of soybean antioxidant compounds can increase due to cooking and fermentation processes [11]. In addition, the pressure heating process was significantly (p < 0.05) increasing the total phenol content and total antioxidant activity (p < 0.05).
Meanwhile, the highest content of reducing sugars and lactic acid was obtained from the T5 group, which were 6.216 g/100 g and 4.725 g/100 g respectively. Reduction sugar is a monosaccharide component which is obtained by enzymatic alteration of carbohydrates. The α-glucosidase enzyme secreted by mold and bacteria breaks down polysaccharides in soybeans into oligosaccharides and some disaccharides. In the further cooking process, the heating process will break down oligosaccharides and disaccharides into monosaccharide forms.

The content of latent acid in tempe yogurt is greater when compared to groups C1 and C2. In tempe T5 yogurt, it contained lactic acid of 4.725 g/100 g, followed by tempe yogurt T2 group of 4.68 g/100 g. these levels differed significantly when compared with the C1 group which was only 1.989 g/100 g and C2 at 1.675 g/100 g. Lactic acid content is higher in the treatment group or group given tempe flour, possibly because of the addition of tempe provides additional nutrients that can be used by lactic acid bacteria to grow better.

4. Conclusion
The addition of tempe flour can increase the nutritional content of tempe yogurt. Increasing carbohydrate, protein and lipid compounds is higher in tempe yogurt than yogurt which is only made from milk or soybeans. In addition, the addition of tempe flour also significantly increases total antioxidant capacity, sugar reduction, crude fiber and lactic acid.

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