Effect of Fruit Lemon Juice Addition to The Content of Protein, Fat, Lactose and Probiotic on Soy Yogurt

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Abstract. This research aimed to determine the effect of lemon juice to the content of protein, fat, lactose and probiotics, in beverages soy yogurt. Soy yogurt which produced was a multifunction yogurt drink high levels of antioxidants, contains probiotics and can be used by people with lactose intolerance. The research method includes the production of fortified soy yogurt with lemon juice, we made with the ratio between the lemon juice and soy yogurt were 0:10 (L0) ; 1 : 9 (L1) ; 2 : 8 (L2) ; and 3 : 7 (L3). Analysis of the results include the content of protein by Kjeldahl method, the content of fat by Soxhletasi method, lactose test by Luff Schoorls method and content of probiotics with total plate count enumeration techniques. The results showed fortified yogurt had a protein content greater than before fortification (L3 > L2 > L1 > L0); The fat content L0 > L1 > L2 > L3. Fortified yogurt lactose content is smaller than before fortification (L0 > L1 > L2 > L3). The content of probiotic yogurt fortified L1 > L3 > L2. From this research can be concluded that the yoghurt fortified (L3) is the best, with the highest protein content, low fat, low lactose than L1 and L2, and had probiotics content. It is advised to conduct further research on the expired time of fortified soy yogurt products.

1. Introduction
Yogurt in the market usually comes from cow's milk. Related Research yogurt production uses cow's milk has been done by some previous researchers. The addition of star fruit extracts in the manufacture of yogurt drinks can be used as a flavor enhancer and to assist the growth of lactic acid bacteria. Fortified soy yogurt lemon juice is useful as a source of protein drinks, containing probiotics, low-lactose, and high levels of antioxidants. Drinks can be used as a functional beverage, the nutritional content needs to be known. According to Jannah, some additional research on the fruit in yogurt have been done like Strawberry, oranges and grapes, cerry, peach, raspberry, blueberry, coconut water. The fortification is done using yogurt were made from cow's milk. While the manufacture of yogurt using soy milk has not been done. Soy milk has a protein content of 4.4% is higher than cow milk is 2.9%. Lee et al., do the research by adding mango extract soy milk to streng the content of vitamins A, C and minerals. The addition can provide sweetness and reduce the unpleasant taste of soy milk. Soy milk and cow's milk have similar amino acid composition, so that soy milk can be used as a substitute for cow's milk for those who are allergic to animal proteins. Soy yogurt was fermented using lactic acid bacteria. Vij et al. states fermentation can increase the presence of isoflavones, help cutting peptide bonds of proteins, increases the solubility of calcium, improve health and help the immune system. Fermented milk requires bacteria, one of which is lactic acid bacteria. Lactic acid bacteria are added into the production of yogurt can act as probiotics. Havenar, states that probiotics are single or mixed cultures of microbial life is consumed by humans.
or animals, beneficial to the host to maintain the balance of microflora in the digestive tract. Donkor et al.\textsuperscript{10} states that the proteins in fermented soy milk can promote the growth of various strains of probiotics such as, \textit{Lactobacillus casei} and \textit{Streptococcus thermophillus}.

Fortification has been done on other sources of protein foods such as milk, soy milk and yogurt\textsuperscript{11}. According to Santoso\textsuperscript{12}, in general, have soy milk contains vitamin B1, B2, niacin, pyridoxine and vitamin B group is high. Other vitamins that are contained in sufficient quantities of vitamin E and K, while vitamin C is contained in small amounts. Micronutrients such as vitamins in addition to functioning in helping work the various types of enzymes are also some of which act as antioxidants\textsuperscript{13}. Vitamin C may act as an antioxidant. According to the USDA National Nutrient data base in 100 grams Lemon (Citrus limon) vitamin C contained as many as 53 mg, or about 88%. In this study produced soy yogurt fortified with vitamin C from lemon juice.

Soy yogurt can be used by people with lactose intolerance, which in his body does not have the enzyme lactase. In yogurt contains bacteria that can produce the lactase enzyme. This enzyme can convert sugar contained in milk is lactose into glucose and galactose, which causes lactose content is reduced, so the yogurt can be consumed by people with lactose intolerance. Research on the effect of the addition of lemon juice on the content of protein, fat, lactose and the amount of probiotics in yogurt fortified soy vitamin C has not been done before.

This study aims to determine the content of protein, fat, lactose and the amount of soy yogurt probiotic before and after fortification using lemon juice. This research made some yogurt with a variety of ratios between lemon juice and soy yogurt, ie 0:10 (L0); 1:9 (L1); 2:8 (L2); and 3:7 (L3), and then analyzed the content of protein, fat, lactose and probiotics. Products of yogurt fortified vitamin C each have a data content of protein, fat, lactose and probiotics. So, the yogurt can to be used as a multi functional drinks, which contain probiotics, with a low lactose content and high antioxidant.

2. Methods

2.1. Material

The materials was used in the research were Soybean (Bandung, Indonesia), lemon fruit (Bandung, Indonesia), \textit{Lactobacillus bulgaricus} and \textit{Streptococcus thermopillus} (Biofarma, Bandung, Indonesia), CuSO$_4$·5H$_2$O, K$_2$SO$_4$, sulfuric acid (H$_2$SO$_4$,98% Bratachem, Indonesia), sodium hydroxide pellets pure (NaOH) (s), Boric acid GR (H$_3$BO$_3$) (s), indicator Tashiro, Hydrochloric acid (HCl) 25%, Sodium thiosulfate 5-hydrate (Na$_2$S$_2$O$_3$·5H$_2$O), Potassium iodate GR (KIO$_3$), and Aquades.

2.2. Method

2.2.1. The production of fortified yogurt lemon juice

Production of fortified yogurt lemon juice preceded by the production of soy milk and soy yogurt with appropriate procedures performed in previous studies\textsuperscript{14}. Yogurt fortification with vitamin C was resulted from lemon juice obtained three variants: L1; L2 and L3. As for the variation of the ratio between lemon juice and soy yogurt is 1:9 (L1); 2:8 (L2) and 3:7 (L3). As a control use soy yogurt without the addition of lemon juice 0:10 (L0). Lemon juice sterilized using a 0.2 μm Millipore size.

2.2.2. Analysis of the results of fortified soy yogurt

2.2.2.1. Test of the protein content by Kjeldahl method

In the determination of protein content, we use the Kjeldahl method (material was used consisted of CuSO$_4$·5H$_2$O and K$_2$SO$_4$ mass ratio of 1:3, added 10 mL of concentrated H$_2$SO$_4$, NaOH 30%, 10 mL H$_3$BO$_3$ 3%, HCl 0.1 N and indicators Tashiro).

2.2.2.2. Test of the fat content by Soxhlet method

Determination of the fat content we use Soxhletation methods (materials used include 25% HCl and petroleum ether).
2.2.2.3. Test of lactose content using Luff Schoorls methods
Lactose content analysis used Luff Schoorl method, (the material used solution of KI 20%, 26.5% H₂SO₄, sodium tio sulfate 0.1 N and 1% starch indicator.

2.2.2.4. Probiotic content test using total plate count enumeration techniques.
Probiotics test performed using total plate count enumeration techniques with materials Lactobacillus bulgaricus and Streptococcus thermopillus and Luria Berthani media (LB).

3. Findings and Discussion

3.1. Production and characterization of Fortified Yoghurt
Fortified soy yogurt lemon juice has been successfully made in three variants. In Figure 1. we analyzed the protein content (blue line), fat (red line), and lactose (green line) yogurt before and after fortification. We analyze the difference between the ratio of lemon extract and soy yogurt: 0:10 (L0); 1:9 (L1); 2:8 (L2); and 3:7 (L3). In comparison to the raw material used we also tested against soy milk (LK).

![Figure 1](image)

Figure 1. Results of the analysis of protein, fat and lactose yogurt before and after fortification

From the experimental data obtained that the protein content of soy milk is 3.87%, while the protein content of soy milk theoretically is 3.6 to 4.5% \(^7\). It shows that based on the protein content of soy milk used meets the criteria as soy milk. While the protein content in soy yogurt without lemon juice extract is 4.47%. It shows that without the addition of lemon juice protein content of yogurt (fermented milk) which is produced in accordance theoretical data is 4.44\(^5\). From the experimental data obtained that more lemon juice are added, the protein content of yogurt fortified products is higher, it is supported by the opinion starting theoretically lemon juice contains 1.1% protein\(^5\). So the protein content of yogurt fortified sortable L1 (4.45%) <L2 (4.82%) <L3 (5.36%). Fortified yogurt product was selected with the highest protein content is L3.

From the experimental data obtained that the fat content in soy milk 1.87% while the fat content in soy milk theoretically is 2.5%, it is influenced by the quality of soymilk in the production, which is of course influenced also by the quality of soybean seed. If we look at the fat content of yogurt before fortification was 1.85%, and the fat content decreased with the increase of lemon juice, then the decline will affect the smell of rotten on soy yogurt, because the taste and the smell was caused by work lipoxygenase enzyme present in soybean seeds. Enzymes that react with fat in soybean grinding
time, particularly if used in cold water. The results of the reaction at least in the form of volatile compounds is ethyl-phenyl-ketone. While the lemon juice has a low fat content. In lemon juice contains omega-3 fatty acids a total of 26 mg / 100gr, omega-6 fatty acid total of 63 mg / 100g lemon juice. From the research results when sorted the fat content, the fortified yogurt lemon juice L1 (1.74%) > L2 (1.71%) > L3 (1.64%).

Figure 1, shows that more of lemon juice added, lactose content decreases. Known as milk sugar lactose, in the presence of the enzyme lactase, lactose is converted into glucose and galactose. Lactase enzyme production decreases with age a person, then the consumption of milk can cause diarrhea. L0 ie without fortification yogurt with lactose content of 1.35%; L1 with a lactose content of 0.99%; while the L2 with a lactose content of 0.77%; and L3 is 0.67%. With the addition of lemon juice can be concluded that the more lemon juice added lactose content decreases, L3 has a low lactose content.

3.2. Analyzed the number of Probiotics

Figure 2, we analyze the number of probiotics contained in yogurt before and after fortification. Probiotics are live microorganisms, when obtained in sufficient quantities would be beneficial to the health of the host\cite{15}. In the production of soy yogurt's probiotics used were *Lactobacillus bulgaricus* and *Streptococcus thermophilus* both types of bacteria are used to accelerate the process of acid production, increase the amount of lactic acid and improve aroma\cite{7}.

![Figure 2. Content of probiotics in yogurt before and after fortification](image)

Figure 2, the data content of probiotics in yogurt fortified lemon juice obtained all products containing probiotic. The highest content of probiotic products obtained at L3, followed by L1 and L2, or if sorted L3 > L1 > L2. In this study used two types of probiotics namely *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Evangelista, et al,\cite{16} stated that among the various lactic acid bacteria potential, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* traditionally used, the bacteria can be produced quickly in acid medium, through the formation of organic acids, especially lactic acid, and compounds such as acetic acid, ethanol, aromatic hydrocarbons, exopolysaccharide and various enzymes that are very important.

3.3. Antioxidant Activity of fortified soy yogurt
From previous studies\textsuperscript{14}, obtained antioxidant activity to control 40.04% of soy yogurt and yogurt products fortified with lemon juice, soy yogurt comparison: lemon juice 9: 1 (1) was 77.13%; ratio 8: 2 (2) was 93.88% and ratio of 7: 3 (3) was 88.97%.

The addition of lemon juice into the yogurt can increase the antioxidant content, because the lemon juice contains ascorbic acid (vitamin C) that can function as an antioxidant. In fortified yogurt product number antioxidant content of 40.04%. Increased antioxidant occurred after fortification lemon juice. The highest amount of antioxidants obtained in yogurt fortified L2, ie 93.88%. From the research it can be seen that the addition of lemon juice can produce multifunctional soy yogurt, that contain probiotics, high levels of antioxidants, and has a lactose content and lower fat and higher protein content than yogurt without fortification.

4. CONCLUSION
This study succeeded in making yogurt fortified fruit juice lemon as a multifunctional beverage. The drink containing the probiotic, with a high antioxidant, can be used by people with lactose intolerance. Yogurt fortified fruit juice are known high protein and low fat. We Obtained 4 variations of yogurt products with the ratio between lemon juice and soy yogurt are 0:10 (L0) ; 1 : 9(L1) ; 2 : 8 (L2) ; and 3 : 7 (L3) . Analysis of the results includes protein, fat, lactose, and probiotics. The results showed fortified yogurt had a protein content greater than before fortification (L3 > L2 > L1 > L0) ; The fat content of L3 < L2 < L1. The content of lactose yogurt fortified smaller than before fortification (L3 < L2 < L1 < L0) . The content of probiotic yogurt fortified L3 > L1 > L2.

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