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Study of the Correlation Between Vitamins and Iron on the Antioxidant Activity of Soy Milk Yogurt Enriched with Purslane (*Portulaca oleracea* L.)

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ABSTRACT

Soy milk yogurt is a functional food that has health benefits. To improve the nutritional (vitamin) and functional (antioxidant) properties of yogurt, purslane leaves are added. This study aims to determine the relationship between vitamins C, E and iron on antioxidant activity (AA) in soy milk yogurt substituted with purslane. This research method uses random group 1 factor with a ratio of soy milk: purslane (100:0; 92.5:7.5; 85:15; 77.5:22.5; 70:30). Data were analyzed using correlation-regression. The results showed that the higher the purslane enrichment, the higher the vitamin E and vitamin C and each had a strong positive correlation with AA with an influence of 88.97% and 89.16% respectively. On the other hand, the higher the addition of purslane, the lower the iron content, causing AA to increase, with an Fe effect of 91.54% and a strong negative correlation. Thus, enriching yogurt with purslane leaves only increases vitamins C and E which have a positive impact on its antioxidant activity.

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- Higher purslane substitution results in higher vitamin E and vitamin C content and increases strong antioxidant activity
- Higher purslane substitution reduces the iron content in soy milk yogurt thereby increasing antioxidant activity

Introduction

Demand for food products that can improve health has increased quite rapidly in recent years, such as the enrichment of bioactive compounds and increased nutritional value of food (1,2). One of the functional food products that is in great demand because it can reduce the risk of chronic disease is yogurt (3). Yogurt is a product resulting from fermentation of cow's milk with lactic acid bacteria (LAB) types *Streptococcus* subsp *thermophilus* and *Lactobacillus* subsp *bulgaricus* in the form of porridge or ice cream with a distinctive sour taste (4,5). During the yogurt fermentation process, LAB will break down lactose as an energy source for LAB into lactic acid, diacetyl and carbon dioxide, thereby lowering the pH with a sour taste (6).

Cow's milk is milk that is commonly used as a base for yogurt, but cow's milk cannot be consumed by lactose intolerant sufferers because the lactose content is 4.7% (7) so it must be replaced with lactose-free plant-based milk. Lactose intolerance is the inability of the digestive system in the intestine to digest lactose due to not having sufficient intestinal lactase enzyme activity when consuming dairy products (8). The solution for sufferers of lactose intolerance is to replace cow's milk with plant-based milk. One type of plant-based milk that has nutritional value similar to cow's milk is soy milk, whose nutritional value is comparable to dairy milk (9). Soy milk contains 3.24% protein, slightly lower than cow's milk, namely 3.5% (10). Soy milk has advantages over cow's milk with an Fe content of 0.16%, Zn 0.02%, vitamin C 0.04%, vitamin E 0.01%, vitamin A 0.003% and unsaturated fatty acid (PUFA) levels of 63.5% (10,11).

Yogurt can be made from soy milk so it is a source of antioxidant compounds such as polyphenolic compounds and vitamin C (12). Increasing the functional value of yogurt and other dairy products with the addition of bioactives has been carried out in yogurt which is substituted with rosella extract (13), moringa extract (14) including fortification of cow's milk yogurt with purslane extract (2). The same thing can also be done with soy milk yogurt by adding bioactive-rich components such as purslane leaves.

Purslane (*Portulaca oleracea* L.) is a herbaceous plant that is widely distributed in tropical and subtropical climates (15). Purslane can grow wild but is also cultivated intentionally for consumption as food or as a medicinal plant that can improve health such as antioxidant, anti-inflammatory, analgesic, antibacterial and antidiabetic (16,17). Purslane is a plant that is rich in bioactive components because it contains vitamin E which acts as an antioxidant in fighting free radicals which is important for preventing peroxidation and oxidative stress (18). The resulting yogurt is expected to contain higher antioxidants than just soy milk yogurt. Therefore, it is necessary to test whether soy milk yogurt enriched with purslane leaves will have high antioxidant activity, while the product also contains Fe which can trigger free radicals. Thus, it is necessary to analyze the relationship between vitamins (vit. E and vit. C) and Fe on the antioxidant activity of soy milk yogurt substituted with purslane leaves.

Methods

Research was conducted in the Lab. Food Chemistry and Lab. Food Design, Faculty of Agriculture, Tanjungpura University. Purslane plants were obtained from residents gardens in Pontianak and soybeans were obtained from the Pontianak Flamboyan market. The ingredients used are fresh purslane leaves, dried soybeans, skim milk and sugar. The yogurt bacterial starter used is from commercial Yogurmert yogurt seeds (*Streptococcus* subsp *thermophilus* and *Lactobacillus* subsp *bulgaricus*). The chemicals used for analysis are DPPH, petroleum ether, methanol, ammonium thiocyanate, nitric acid, alpha-tocopherol, iodine, starch and distilled water (all spec. pro analysis). Equipment used yogurt incubator, UV-Vis spectrophotometer, vortex, furnace, filter paper including glass equipment for analysis.

Purslane leaves are sorted from dirt then blanched for 3 minutes (90℃) and blended with the addition of 0.5:1 water. Making soy milk begins by soaking dry soybean seeds for 6 hours then blending them with a soybean water ratio of 1:4 and filtering with gauze until soybean juice is obtained, then boiled to 90 ℃. Preparation of soy milk yogurt begins by adding bacterial strain powder (3.6 mg) to soy milk, then the soy milk is substituted with purslane essence at the ratio of soy milk and purslane formulations according to the treatment (control 100:0; 92.5:7.5; 85:15; 77.5: 22.5 ; 70:30) and incubated at 42℃ for 12 hours in a yogurt incubator. The soy milk yogurt that has been incubated is then packaged into 100 gram containers (plastic) and cooled at 4℃. Soy milk yogurt samples were analyzed for pH (19), vitamin E content (20), vitamin C (21), Fe content (22) and antioxidant activity (2). Data analysis was carried out using the correlation-regression relationship method.

Results

Characteristics of Sample Treatment

The characteristics of the soy milk yogurt sample with purslane substitution showed differences from the control sample without purslane substitution. From the results of the analysis of the composition of purslane substituted soy milk yogurt, it was shown that there was an increase in the content of vitamin E, vitamin C and antioxidant activity, but the addition of purslane to soy milk yogurt showed a decrease in the iron content of soy milk yogurt. The results of sample characteristics for each treatment can be seen in Table 1.

Table 1. Compositional Characteristic of Sample Treatment

Compositions	Consentration of soy milk : Purslane (%)				
	Control	92.5 : 7.5	85 : 15	77.5 : 22.5	70 : 30
pH	4.72	4.65	4.67	4.74	4.80
Vitamin E (mg/100g)	2.4	2.8	7.5	8.6	13.5
Vitamin C (%)	27.50	41.14	44.88	49.50	57.42
Iron (mg/100g)	0.55	0.51	0.48	0.45	0.40
Aktivitas antioksidan (%)	9.63	19.60	40.15	47.90	53.45

Correlation Between Vitamin E, Vitamin C and Iron with Antioxidant Activity

The correlation results between vitamin E and Vitamin C content on antioxidant activity show that the higher the purslane substitution will increase the vitamin E content with indicators r = 0.943, r^2 =

0.8897 and vitamin C r = 0.944, r^2 = 0.8916 in soy milk yogurt purslane substitution will increase the antioxidant activity of yogurt. On the other hand, the higher the purslane substitution in soy milk yogurt, the higher the Fe content, reducing the antioxidant properties of yogurt with indicators r = -0.956, r^2= 0.9154. The correlation-regression results between vitamin E, vitamin C and Fe on antioxidant activity can be seen in Table 2 and Figures 1-3.

Tabel 2. Correlation between vitamin E, vitamin C, iron and antioxidant activity

Correlation	Correlation coefficient (r)	Regression (r^2)
Vitamin E vs. Antioxidant Activity	(+) 0.943	0.8897
Vitamin C vs. Antioxidant Activity	(+) 0.944	0.8916
Iron vs. Antioxidant Activity	(-) 0.956	0.9154

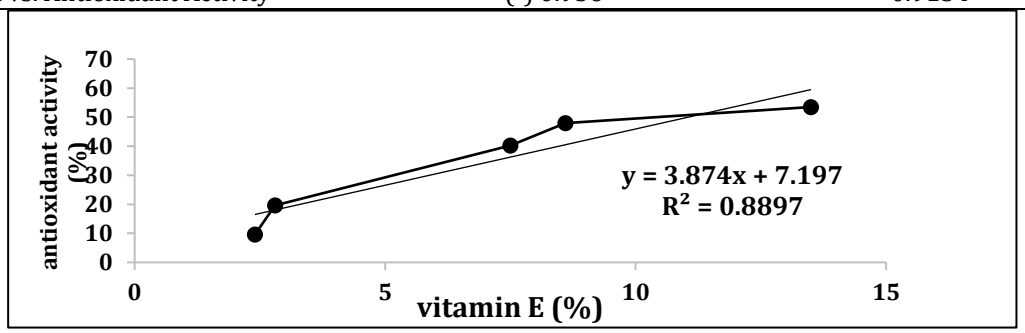

Figure 1. Correlation between antioxidant activity and vitamin E in soy yogurt subs. purslane

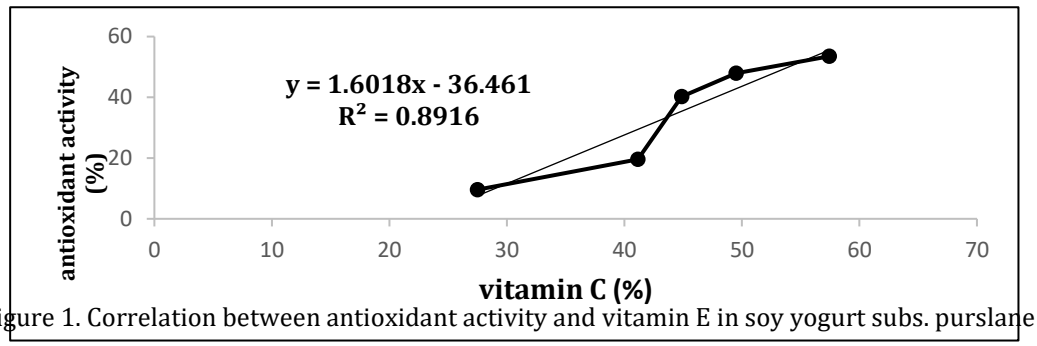

Figure 2. Correlation between antioxidant activity and vitamin C in soy yogurt subs. purslane

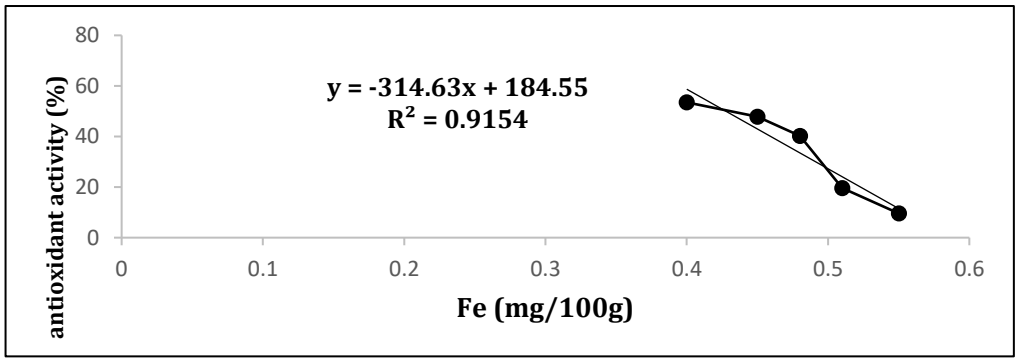

Figure 3. Correlation between antioxidant activity and iron in soy yogurt subs. purslane

Discussion

Based on the results of the analysis of soy milk yogurt substituted with purslane, it shows that the higher the vitamin E contained in the resulting yogurt product, the higher the antioxidant activity (AA). This can be caused because the vitamin E found in soy milk and purslane does not decrease during the yogurt fermentation process. Hydrolysis of carbohydrates, fats and proteins by LAB produces metabolites including vitamins and minerals and secondary forms such as antibiotics, enzyme inhibitors

and growth during fermentation (23). The addition of purslane to soy milk yogurt also causes an increase in vitamin E content, because vitamin E can also act as an antioxidant by inhibiting free radicals (24). The vitamin E content in the control sample (soy milk yoghurt without puslane) was 2.4 mg/100g (Table 1), showing that the higher the purslane substitution, the higher the vitamin E, AA showed a strong positive correlation with the indicator r = 0.943, while the effect of vit. E on AA can be seen with the regression indicator r^2=0.8897) meaning that the role of vitamin E is 88.97% on AA.

The antioxidant mechanism in tocopherol involves the transfer of one hydrogen atom from the 6-hydroxyl group to the chromane ring, inactivating singlet oxygen and other reactive species (18,25). The unique structure of vitamin E efficiently contributes to the antioxidant activity of tocopherols (25). Soybeans are high in oil, so soybeans contain vitamin E, which is found in soybean oil, namely 10.99 mg/100g (18). Research results (26) showed that the alpha-tocopherol yield from fermented tofu was higher (48.88 micrograms/g) than unfermented tofu (32.91 micrograms/g), while research results (23) showed that LAB during the fermentation period increased the vitamin E content in fermentation. Shrimp sausage with the highest vitamin E on day three was 4.98 mg/100g.

The same thing can also be seen from the correlation between vitamin C and AA. Vitamin C has been reported to have strong antioxidant activity because it can neutralize free radicals and can induce the transformation of $Fe3^+$ into $Fe2^+$by catalyzing $Fe2^+$ in the formation of reactive oxygen species via Fenton (24,29). Vitamin C activity comes from 2,3-enediol which functions as an electron donor to produce ascorbate free radicals (30). The addition of purslane to soy milk yogurt also causes an increase in the vitamin content. C while increasing AA. The vitamin C content in the control sample (soy milk yoghurt without purslane) was 27.5% (Table 1), thus showing a strong positive correlation with the correlation indicator of r = 0.944, while the effect of vit. C on AA can be seen with the regression indicator r^2=0.8916) meaning the role of vitamin C is 89.16% on AA.

On the other hand, the high level of iron in the soy milk yogurt produced causes a decrease in AA which is caused by auto-oxidation of iron which is influenced by pH and iron chelation, presumably because the high level of vitamin C in purslane can reduce $Fe3^+$ in soy milk yogurt (27). The results of Figure 3 and Table 2, which show the correlation-regression between iron and AA, show a correlation coefficient indicator (r = -0.956) so that the resulting correlation shows a very strong negative relationship. This explains that the higher the iron content in yogurt, the more it reduces antioxidant activity. Besides that, the indicator value r^2=0.9154 states that the regression value means 91.54% of the role of iron in reducing AA. It is suspected that the higher the amount of purslane leaves, the higher the PUFA content so that iron levels decrease, because the more PUFA, the more Fe will be used to trigger the formation of free radicles. Increasing the content of vitamin C and vitamin E with the addition of purslane leaves can stabilize iron in triggering the formation of free radicals, so that antioxidant activity is still high (24). This is proven by (27) which shows that the high level of iron in soy milk yogurt causes a decrease in antioxidant activity due to auto-oxidation of iron which is influenced by pH and iron chelation. Another study from (28) shows that the enhancing effect of vitamin C on iron absorption is largely due to its ability to reduce $Fe3^+$to $Fe2^+$but also due to its potential to chelate iron. Yogurt has an acidic pH, the higher the addition of pusrlane leaves will increase the pH and the higher the pH, the lower the iron chelation occurs.

Conclusion

Purslane enrichment in soy milk yogurt shows a strong positive correlation between vitamin E and vitamin C on the antioxidant activity of the soy milk yogurt produced. However, the relationship between iron and antioxidant activity shows a strong negative correlation. The higher the purslane substitution in soy milk yogurt, the lower the iron content and the higher the antioxidant activity.

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