

Effect of beetroot (*Beta vulgaris* L.) and cinnamon (*Cinnamomum burmannii*) addition on total phenol, vitamin C and antioxidant activity in yogurt

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Abstract

Yogurt is a functional food made from fermented milk that usually uses lactic acid bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Beetroot and cinnamon contain bioactive substances that are beneficial for health, including phenol, antioxidants, and vitamins. Beetroot and cinnamon can be added to yogurts to increase their nutritional content through the fermentation process. This study tested five yoghurt formulations: plain yoghurt (K0), yoghurt with 5% beetroot and 0.5% cinnamon (K1), yoghurt with 5% beetroot and 1% cinnamon (K2), yoghurt with 10% beetroot and 0.5% cinnamon (K3), and yoghurt with 10% beetroot and 1% cinnamon (K4). Total phenolics were analyzed by the Folin-Ciocalteu method, vitamin C by iodine titration and antioxidant activity by the DPPH method. The addition of beets to yogurt increases the vitamin C and antioxidant content, with p-values of 0.008 and <0.001, respectively. But the addition didn't increase total phenol content with p = 0.101. However, there is no antioxidant activity differences between beetroot and cinnamon yogurt and vitamin C in orange juice.

1. Introduction

The Primary Health Research 2018 in Indonesia showed an increase in the incidence of degenerative diseases such as hypertension, diabetes mellitus, chronic kidney failure, and stroke (Arifin *et al.*, 2022). The causes of degenerative diseases vary, from free radicals to unhealthy lifestyles and diets. A healthy diet with enough antioxidants is needed to protect the body's cells from oxidative damage caused by free radicals (Grajek *et al.*, 2015). Functional foods such as yogurt can be an alternative to improve health of the human body.

Beetroot (*Beta vulgaris* L.) is type of root crop that contains rich antioxidants. Beetroot contains vitamin C and betacyanin/betalain, which has high antioxidant activity (Novatama *et al.*, 2016). Cinnamon (*Cinnamomum burmannii*) is a native plant of South Asia, China, and Southeast Asia including Indonesia. The high content of trans-cinnamaldehyde in cinnamon (68.65%) also acts as an antioxidant compound that can inhibit free radicals (Tasia and Widyaningsih, 2014).

Yogurt is fermented milk produced by fermentation using *Lactobacillus bulgaricus* and *Streptococcus*

thermophilus (Fisberg and Machado, 2015). The fermentation process of yoghurt increases the nutrient content and makes it easier for the body to absorb nutrients. The increase in antioxidants in yogurt is caused by lactic acid bacteria, which are able to break down proteins into bioactive peptides that have antioxidant activity (Rosiana and Amareta, 2016). The increase in phenolic compounds during fermentation is caused by microorganisms producing proteolytic enzymes that hydrolyze the phenolic complexes to become free phenols. Meanwhile, the increase of vitamin C is caused by biosynthesis of vitamin C by the microorganism in a suitable environment (Adetuyi and Ibrahim, 2014). Information about beetroot and cinnamon yogurt is limited. The development of functional food products for beetroot and cinnamon yogurt has never been done before. The purpose of this study was to determine the total phenol, vitamin C, and antioxidant activity in beetroot and cinnamon yogurt with various formulations.

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2. Material and methods

2.1 Yogurt making process

Beetroot was purchased from beetroot shop in Bantul, Yogyakarta, Indonesia while the cinnamon was purchased from Kranggan Market, Yogyakarta, Indonesia. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were obtained from The Center for Food and Nutrition Studies, Gadjah Mada University and maintained at the Faculty of Animal Science, Gadjah Mada University. The formulation of yoghurt with the addition of beetroot and cinnamon was carried out at the Nutrition Analysis Laboratory, also the Dietetics and Culinary Laboratory of the Faculty of Medicine, Public Health, and Nursing, Gadjah Mada University.

The beetroot was prepared by blanching in a closed pot for 3 minutes. After that, beetroot was extracted using a juice extractor from Cosmos CJ-355. Approximately, 30 grams of cinnamon stems were boiled for 15 minutes and then filtered with filter paper.

This study involved five yoghurt formulations with the addition of beetroot and cinnamon, designated as K0, K1, K2, K3, and K4. K0 served as the control (yoghurt without beetroot or cinnamon). K1 and K2 contained 5% beetroot combined with 0.5% and 1% cinnamon, respectively, while K3 and K4 contained 10% beetroot combined with 0.5% and 1% cinnamon, respectively.

The yogurt was prepared by mixing the beetroot juice, cinnamon extract, UHT milk, 5% of sugar, and 6% of skim milk using a National MX-T3GN blender, followed by pasteurization at 72°C for 15 seconds, then cooled down until ±45°C. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were inoculated to the mixed substance and incubated at 37°C for 18 hours.

2.2 Chemical analysis

2.2.1 Total phenol

The total phenol of the sample was analyzed using Folin-Ciocalteu method according to the method described by Das *et al.* (2012). This method uses yogurt extract dissolved in distilled water. To 1 mL of sample, 5 mL of Folin-Ciocalteu reagent (then diluted 1:10 with distilled water) and 4 mL of sodium carbonate solution (7.5%) were added. The tubes were then left to incubate for 2 hours at room temperature before the tubes were placed on the spectrophotometer to measure the total phenol in the sample. Different concentrations of gallic acid were used to create standard curves, and then the total phenolics were calculated.

2.2.2 Vitamin C

The vitamin C content was analysed using the iodine titration method, as described by Sudarmadji (2007). Ten grams of material was measured into a

100 ml flask and topped up to the mark with distilled water. The filtrate was separated. Between 5 ml and 25 ml of the filtrate was taken and added to a 125 ml flask, then 2 ml of a 1% amylose solution (a soluble starch) was added. The iodine standard was titrated to 0.01 N and the end point was marked by blue iodochrome. Vitamin C content was calculated with a standardized iodine solution.

2.2.3 Antioxidant activity

The antioxidant activity of yogurt samples and orange juice was analyzed using the DPPH method according to Yen and Chen (1995). This analysis was performed by adding 2 mL of a homogeneous solution of the test sample to 2 mL of 0.16 mM DPPH methanol solution. The mixture was vortexed for 1 minute and then left at room temperature for half an hour in the dark. The color was measured at 517 nm. The ability of the test sample to scavenge DPPH radicals was calculated using the following equation:

$$\text{Scavenging effect: } \frac{(1 - (A_{\text{sample}} - A_{\text{Sample blank}}))}{A_{\text{control}}} \times 100$$

With:

A Control = the control absorbance (DPPH solution without sample)

A Sample = the sample absorbance (DPPH + sample)

A sample blank = the sample blank absorbance (sample without DPPH)

2.5 Statistical analysis

All the results were represented by means ± standard deviations (SD) and analyzed with SPSS version 23. Total phenols were analyzed by Kruskal Wallis test, while vitamin C and antioxidant activity were analyzed by one-way Anova test. Antioxidants of beetroot and cinnamon yogurt compared to antioxidants of orange juice were analyzed by Mann-Whitney test. The significance level in all tests was accepted at $p < 0.05$.

3. Result and discussion

3.1 Total phenol analysis

Table 1 showed that the addition of beetroot and cinnamon did not affect the total phenol of the yogurt because the test result between groups showed no significant differences ($p > 0.05$). There is no increase in the total phenol in this study and this could be due to the facts that the phenolic compounds were still attached to the fibre matrix or sugars. This result was in agreement with the study by Tolonen *et al.* (2002), where kaempferol, a phenolic compound, was constant throughout the fermentation and indicates that the flavonoids remain in the cabbage fiber matrix. Beetroot

is a good source of sugar, so the more beetroot added to the yogurt, the more sugar content in the yogurt. The phenolic compound may bind to sugar, making the phenolic compound more stable (Kaprasob *et al.*, 2017). The high sugar content in beet can reduce fermentation rate and inhibit starter activity by causing plasmolysis of the cell wall. This can lead to a longer fermentation time and inhibit the release of phenolics (Silalahi *et al.*, 2010).

Table 1. Total phenol of beetroot and cinnamon yogurt.

Groups	Total phenol (%)
K0	0.0157 ± 0.0002
K1	0.0108 ± 0.0002
K2	0.0103 ± 0.0003
K3	0.0107 ± 0.0005
K4	0.0095 ± 0.0003
P	0.101

3.2 Vitamin C analysis

Table 2 showed that the addition of beetroot and cinnamon increase vitamin C level significantly ($p < 0.05$). Adding more beetroot increased vitamin C levels because beetroot is a good source of vitamins and minerals, including vitamin C. The results of this study are similar to those of Aufa *et al.* (2020) and Sutedjo and Nisa (2015), which showed that the addition of red guava juice and star fruit juice increased the vitamin C content of yogurt, as red guava and star fruit juices are high in vitamin C content. The process of vitamin C biosynthesis is catalysed by microorganisms during the fermentation process. Milk and beetroot are a good source of carbon and nitrogen so it's a suitable medium for the biosynthesis of vitamin C.

Table 2. Vitamin C of beetroot and cinnamon yogurt.

Groups	Vitamin C (mg/100 g)
K0	5.48 ± 1.55 ^a
K1	9.89 ± 1.55 ^b
K2	14.08 ± 1.53 ^c
K3	14.12 ± 1.54 ^c
K4	14.27 ± 1.55 ^c
P	0.008

Values with the same superscripts indicate no significant difference

3.3 Antioxidant activity analysis

Table 3 showed that the addition of beetroot and cinnamon increased antioxidant activity ($p < 0.05$). Further test results using the post hoc DMRT test showed that all treatment groups had significantly different antioxidant activity ($p < 0.05$). According to the research of Kusumawati *et al.* (2019), adding beetroot and cinnamon resulted in increased antioxidant activity. Beetroot and cinnamon have antioxidant activity from beta-carotene, transinamaldehyde, and vitamin C. The addition of antioxidant-rich foods and the fermentation process can increase the antioxidant activity of yogurt (Guruh *et al.*, 2017). Besides, there is also proteolysis of milk protein by lactic acid bacteria which produces

bioactive peptides and hydrolysis of whey and casein proteins so that the antioxidant activity of yogurt can be increased (Fardet and Rock, 2018).

Table 3. Antioxidant activity of beetroot and cinnamon yogurt.

Groups	Antioxidant activity (%)
K0	16.90 ± 0.22 ^a
K1	21.09 ± 0.51 ^b
K2	22.40 ± 0.25 ^c
K3	23.58 ± 0.11 ^d
K4	24.50 ± 0.11 ^e
P	<0.001

Values with the same superscripts indicate no significant difference

Although adding beetroot and cinnamon can increase the antioxidant content of yoghurt, Table 4 showed no differences in antioxidant activity between beetroot/cinnamon yoghurt and vitamin C in orange juice ($p > 0.05$). The results of this study were consistent with Hidayah *et al.* (2015), who found that the antioxidant activity of tomato yogurt was 0.0258% lower than that of vitamin C. This difference can be due to the fact that the antioxidant activity in plants is a mixed phenomenon, which can vary depending on the interaction between antioxidants, resulting in higher or lower amounts than the total amount of antioxidants.

Besides, inhibitory concentrations (IC_{50}) of beetroot and cinnamon (79.73 ppm and 27.97 ppm) is higher than IC_{50} of vitamin C (2.54 ppm) (Novatama *et al.*, 2016). This shows different antioxidant strength because the smaller the IC_{50} value, with lower values indicating higher antioxidant activity, and higher values indicating lower activity. This is because IC_{50} is defined as the amount of antioxidants required to reduce the initial DPPH concentration by 50%, which is calculated using a graph by plotting the percentage inhibition against the extract concentration (Wijaya *et al.*, 2023).

Table 4. Antioxidant activity of beetroot and cinnamon yogurt compared to antioxidant activity of vitamin C (orange juice).

Groups	Antioxidant activity (%)		p
	Groups	Vitamin C (Orange juice)	
Are K0	16.90 ± 0.22	43.24 ± 0.11	0.121
K1	21.09 ± 0.51	43.24 ± 0.11	0.121
K2	22.40 ± 0.25	43.24 ± 0.11	0.121
K3	23.58 ± 0.11	43.24 ± 0.11	0.121
K4	24.50 ± 0.11	43.24 ± 0.11	0.121

Conclusion

Addition of beetroot and cinnamon on yogurt increase vitamin C content and antioxidant activity but do not increase total phenol content of the yogurt. More addition of beetroot resulted in the increase of vitamin C level, while more addition of beetroot and cinnamon resulted to the increase of antioxidant activity. There is no antioxidant activity differences between beetroot and

cinnamon yogurt and vitamin C in orange juice.

Conflict of interest

The authors declare no conflict of interest.

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