

Effect of okra mucilage addition on antioxidant properties of purple okra (*Abelmoschus esculentus* L. Moench) pudding

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Abstract. Okra (*Abelmoschus esculentus* L. Moench) is a type of vegetable popular in various parts of the world. Okra has bioactive components such as flavonoids, polyphenols, and saponins that have the potential as antioxidants and anti-inflammatories [1]. Based on Utami (2018), purple okra has a higher phenol content and quercetin levels ($2,034 \pm 70.474$ mg GAE 100 g^{-1} and $3,965 \pm 0.449$ mg 100 g^{-1}) compared to green okra ($1,807 \pm 60.332$ mg GAE 100 g^{-1} and $1,849 \pm 0.449$ mg 100 g^{-1}) [2]. Not only okra pods, but okra mucilage is also proven to contain bioactive components that have health benefits for the human body. Bioactive compounds in purple okra mucilage, can be utilized in functional food. This study determined the effect of mucilage addition on the antioxidant properties of purple okra pudding. The addition of mucilage significantly ($p < 0.001$) affected the antioxidant activity tested using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Purple okra pudding with 20% mucilage has the highest antioxidant activity, which is 59.02% free radical inhibition that is equivalent with 24.95 mg vitamin C g^{-1} extract, and an IC_{50} value of 351.23. However, the addition of mucilage did not have a significant effect on total flavonoids and phenol content ($p > 0.05$).

1 Introduction

Molecules called antioxidants can stop or reduce the oxidation of substances that are prone to oxidation—compounds known as antioxidants shield cells from the damaging effects of free radicals [3]. The body's metabolism and other outside variables may produce these free radicals. Since proteins, lipids, and cell DNA are excellent sources of electron pairs, free radicals—unstable entities with unpaired electrons—seek them out in biological macromolecules [4], oxidizing environments can harm proteins and DNA, resulting in aging, cancer, and other illnesses [5]. Natural antioxidants can slow or prevent the progression of T2DM by reducing mitochondrial oxidative stress and preventing the formation of Advanced Glycation End-products (AGEs) [6].

Okra (*Abelmoschus esculentus* L. Moench), commonly known as lady's finger or gumbo, is a dicotyledonous plant from the Malvaceae family [7]. Okra has bioactive components

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such as flavonoids, polyphenols, and saponins that have potential as antioxidants and anti-inflammatories [1]. Because of its content, in China, okra is used as a traditional medicine to treat diabetes and obesity [8]. Based on research conducted by Astutiningsih (2021) shows that quercetine compounds in okra have insignificant inhibition of alpha-amylase enzyme activity compared to acarbose [9]. In addition, okra contains food fibers such as alpha-cellulose and hemicellulose, which are antidiabetic components [10]. Elkhalfa *et al.* (2021) showed that okra has bioactive components that are useful as antioxidants, antidiabetes, anticancer, antiproliferative, antifatigue, antibacterial, anti dyslipidemia, antihyperglycemic, and anti-inflammatory [11, 12]. Okra is identified to have several bioactive components including *quercetine-3-O-gentiobioside*, *quercetin-3-O-glucoside (isoquercitrin)*, rutin, *quercetine* derivatives, *protocatechic acid*, *catechin* derivatives and vitamin C [12].

Research conducted by Khodija *et al.* (2020) stated that giving 40 g of boiled okra for 14 days can reduce fasting blood sugar (FBG) in diabetics and hypercholesterolemia [13]. Besides okra in its whole state, okra extract also increases immunity and inhibits oxidative stress [14]. Based on research conducted by Tavakolizadeh *et al.* (2023), administration of okra powder 3 times 1000 mg per day for 90 days was shown to reduce fasting blood glucose (GDP), HbA1c, total cholesterol, and triglyceride levels in people with diabetes [15]. In addition, polysaccharides or mucilage derived from okra also have hypoglycemic abilities, lower cholesterol levels in the blood, and can prevent cancer through their ability to bind intestinal bile acids [16]. Based on Utami (2018), purple okra, also known as red okra, has higher phenol content and quercetin levels ($2,034 \pm 70.474$ mg GAE 100 g⁻¹ and $3,965 \pm 0.449$ mg 100 g⁻¹) compared to green okra ($1,807 \pm 60.332$ mg GAE 100 g⁻¹ and $1,849 \pm 0.449$ mg 100 g⁻¹) [2]. In addition, purple okra plants also have a longer production life than green okra plants [17].

However, okra's slimy characteristics and strong odor cause it to be less preferred by most people [18]. So that other processing efforts are needed on okra so that it can be better accepted. One of them is processing okra into pudding. Pudding is one of the desserts that is generally cooked with a boiling technique made from agar-agar and has a sweet taste with a soft texture so that almost everyone likes it. Fruits and vegetables are often added to pudding as additional ingredients to increase the nutritional content of a pudding [19].

In previous research, Fadilah *et al.* (2024) have developed pudding products by adding purple okra to improve acceptability and utilize the bioactive components found in okra plants with flavonoid content of 63.32 mg QE 100 g⁻¹, phenol of 12.42 mg GAE 100 g⁻¹, and IC₅₀ value of 543.79 mg L⁻¹ [21, 22]. The intervention results of purple okra pudding conducted by previous studies indicate that purple okra pudding can be used as a functional food that can maintain the balance of antioxidant status in healthy people. However, the test results were not significantly different between respondents who were given pudding and those who were not given pudding or the control group. Based on this background, in this study, the researcher intends to develop purple okra pudding products by adding purple okra mucilage to increase the content of the bioactive components of the product. Because based on Cahyana *et al.* (2017) stated that okra mucilage has a total flavonoid content of 72.17 mg quercetin L⁻¹ and an IC₅₀ value of 525.92 mg L⁻¹ [22].

2 Materials and methods

This study used an experimental method with a one-factor Completely Randomized Design (CRD) design using three replicates. The treatment given was the difference in the amount of mucilage used in each formula, which consisted of three levels, namely 10%, 15%, and 20% mucilage addition. The research took place from January to May 2024. Purple okra pudding was produced at the Food Experiment Laboratory of the Department of Community Nutrition, Faculty of Human Ecology, IPB University. Bioactive component analysis was

conducted at the Chemical and Food Analysis Laboratory of the Department of Community Nutrition, Faculty of Human Ecology, IPB University.

1.1 Pudding preparation

Pudding-making refers to Fadilah et al. (2024) with modifications [21]. Making pudding begins with blanching purple okra at 97°C for 30 seconds. Then, some okra was macerated using distilled water (1:3) for 12 hours, and some was mashed using a blender. The next step is to make a pudding batter by mixing agar powder, sorbitol, vanilla, milk, dragon fruit, strawberry fruit, and distilled water, then add smooth okra along with mucilage into the pudding batter. After the pudding mixture is finished cooking, the fire is turned off, and citric acid is added.

1.2 Sample preparation

Bioactive components were analyzed using methanol extract from purple okra pudding obtained by macerating purple okra pudding with methanol (1: 2.5) for 2 hours. Then, the maceration results were filtered using Watman filter paper no. 41 to separate the solution and pudding pulp. After that, the solution was dried using a rotary evaporator at a temperature of 60°C with a pressure of 72 mbar.

1.3 Analysis of bioactive components

1.3.1 Antioxidant activity

Antioxidant activity was measured using the DPPH method referring to Hwang (2009) in Fadilah et al. (2024) [21]. DPPH method is very commonly used in testing antioxidant activity due to its simplicity, cost-effectiveness, sensitivity, and versatility in applications in various fields [23]. The antioxidant activity measurement step begins with adding methanol to prepare the sample into several concentrations, namely 1000, 500, 250, 125, 62.5 to 31.25 ppm. DPPH radical was dissolved in ethanol to obtain a DPPH solution with a concentration of 0.2 mM. Next, 1000 µL of the sample was pipetted into a test tube. Then, the same amount of 0.2 mM DPPH solution was added to the test tube. The addition of DPPH radicals will change the color of the solution from purple to light purple, indicating the reaction between DPPH radicals and the antioxidant content of the sample. The solution was incubated at room temperature in the dark for 30 minutes, and then the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. The same was done for vitamin C standards, which were made in a concentration series from 1000 - 0.001 ppm to obtain a standard curve.

1.3.2 Total phenol analysis

The Folin-Ciocalteu method, as described by Fadilah et al. (2024) [21], was used to determine the total phenol content of purple okra pudding. The most popular technique for figuring out the total phenolic content is this one. It involves phenolic chemicals in an alkaline media reducing the Folin-Ciocalteu reagent to produce a blue-colored complex. This technique is highly sensitive, relatively easy to use, and suitable for a variety of matrices, including plant and food extracts [24]. The test began with pipetting 500 µL of the sample, followed by equal additions of folin solution and Na₂CO₃. After using a vortex to homogenize the solution, it was allowed to sit at room temperature in the dark for ninety minutes. The absorbance was

read using a UV-Vis spectrophotometer at a wavelength of 765 nm. The same was done for gallic acid standards with a concentration series of 12.5 - 3.125 ppm to obtain a standard calibration curve.

1.3.3 Total flavonoid analysis

Measurement of the total flavonoid content of the sample refers to Fadilah et al. (2024) with a modified colorimetric method [21]. In principle, AlCl_3 will form a stable acid with the C-4 keto group and the C-3 or C-5 hydroxyl group of flavones and flavonols into an aluminum chloride-flavonoid complex. This method one of the most widely used methods for determining total flavonoid content. It involves forming a complex between flavonoids and aluminium chloride, which results in a color change that can be measured spectrophotometrically. This method has simple and rapid procedure and suitable for various types of samples, including plant extract and food. The initial step of the test is to prepare a 1000 ppm sample solution, 5% NaNO_2 solution, 10% AlCl_3 , and 5% NaOH . Then, 500 μL of sample solution was pipetted into an Eppendorf tube, and added 30 μL of 5% NaNO_2 and incubated for 5 minutes at room temperature in the dark. Next, 30 μL of 10% AlCl_3 was added and incubated for 1 minute in the dark at room temperature. The next step is to add NaOH 5% as much as 200 μL and 240 μL H_2O . After homogenizing with a vortex, the solution was transferred into a microplate, and the absorbance was measured at a wavelength of 492 nm using a microplate reader. The same was done for quercetin standard. Standards were made into series with concentrations of 250 – 15.625 ppm to obtain a standard calibration curve.

1.4 Data analysis

The experiments were carried out as many as 3 replicates. Data processing in this study was carried out using Microsoft Excel 2016 software and SPSS version 26.0. Analysis of variance on the content of bioactive components was tested using *One way-ANOVA*. Furthermore, if there are significantly different analysis results, it will be continued using Duncan's Multiple Range Test with a significance value of less than 0.05 ($p < 0.05$).

3 Results and discussion

The results of the antioxidant activity test of purple okra pudding made with addition of okra mucilage showed a significant effect (Table 1). The more mucilage was added, the percentage of inhibition and the AEAC value of the pudding increased. In addition, antioxidant activity was also assessed based on IC_{50} analysis, which stated the ability of the sample to inhibit 50% of free radicals from DPPH, assuming that the smaller IC_{50} value, the higher ability to ward off free radicals. Addition of okra mucilage was able to reduce the IC_{50} value of purple okra pudding samples. The highest antioxidant activity was shown in the sample with addition of 20% mucilage, which amounted to 59.02% free radical inhibition, equivalent to 24.95 mg vitamin C g^{-1} extract, and an IC_{50} value of 351.23. The antioxidant activity of purple okra pudding in this study was better when compared to the pudding used in the antioxidant status improvement intervention by Damayanthi et al. (2024) [20]. In addition, the physical characteristics of the pudding produced were also better because it had a softer texture and a languorous aroma that was much reduced because mucilage has properties that can improve the stability, texture, and appearance of food, and can also act as a gelling agent and texture modifier [25, 26]. The addition of the mucilage can cause this, whereas based on Cahyana et al. (2017), mucilage has flavonoid content and antioxidant activity (IC_{50}) of 72.17 ± 0.36 mg

quercetine L⁻¹ and 525.92±40.75 mg L⁻¹ [22]. However, the antioxidant activity of purple okra pudding is still lower when compared to purple okra extract (IC₅₀ = 316.86 ppm), which can be caused by the cooking process of the pudding [27]. Based on research conducted by Amalia (2024) related to the relationship of the cooking process in jam made from mandarin oranges to antioxidant activity, the results show that there is a decrease in antioxidant activity along with the increase in cooking time. Where the best antioxidant activity is owned by jam with a shorter cooking time [28].

Table 1. Antioxidant activities of purple okra pudding as affected by addition of okra mucilage.

Formula	% inhibition	AEAC	IC ₅₀ (ppm)
F1 (10% mucilage)	31.41±4.79 ^a	0.18±0.15 ^a	16684.32±5557.84 ^a
F2 (15% mucilage)	53.14±1.65 ^b	8.36±2.40 ^b	694.22±254.33 ^b
F3 (20% mucilage)	59.02±1.093 ^b	24.95±4.83 ^c	351.23±25.70 ^b

Note: values with different superscript letters within the same column indicate significant differences (p<0.05)
1 g extract ≈ 20 g purple okra pudding
F: Formula; AEAC: Ascorbic Acid Equivalent Antioxidant Capacity; IC: Inhibition Concetration.

To prevent the appearance of oxidative stress and vascular complications associated with diabetes, it is necessary to eat foods containing antioxidants, which can also inhibit the production of intracellular free radicals or enhance the ability of defense enzymes against free radicals [29]. Antioxidant therapy can help people with diabetes mellitus with cardiovascular complications, both macrovascular and microvascular when combined with other drugs to optimize the control of blood pressure, dyslipidemic conditions, and glucose levels. Previous studies have shown that purple okra can increase the level of SOD in the body. SOD is essential for maintaining cell and tissue health and helps prevent several diseases, such as diabetes, cardiovascular and neurodegenerative diseases. Decreased SOD activity has been linked to an increased risk of these diseases [20].

Okra's antioxidant ability is mostly attributed to *quercetin-3-O-gentibioside*, which also inhibits digestive enzymes like lipase, alpha-glucosidase, and alpha-amylase [11]. *Quercetine* is a powerful antioxidant that can inhibit pro-oxidant enzymes and increase the expression of genes encoding enzymes involved in drug metabolism and antioxidant defense, such as γ -glutamylcysteine synthetase, a rate-limiting enzyme in glutathione synthesis, is one of the potent antioxidants quercetin can block, as well as boost the expression of genes encoding enzymes involved in drug metabolism and antioxidant defense. Reduced glutathione (rGSH) concentrations as an antioxidant are typically low in diabetics, which may be due to increased oxidative stress levels. The redox state of cells is impacted by GST, which in turn impacts biochemical processes. Reduced or absent rGSH can lead to oxidative cell damage, physiological imbalance, and ultimately problems from diabetes [30].

In different to antioxidant activity, the addition of okra mucilage did not have a substantial effect on total flavonoid and phenol content of okra pudding (Fig. 1). Although the effect was not statistically significant, there was slightly increasing trend in total flavonoids and phenols in okra pudding along with the addition of okra mucilage. This can be caused by okra mucilage which contains other compounds such as alkaloids, saponins, tannins, and terpenoids which also contribute to antioxidant activity [28, 29]. Total flavonoids and phenols content in okra pudding with 10%, 15%, and 20% mucilage addition are 14.59±0.46 mg QE g⁻¹ and 5.34±1.94 mg GAE g⁻¹ extract, 15.10±0.90 mg QE g⁻¹ and 5.40±1.87 mg GAE g⁻¹ extract, and also 15.84±0.44 mg QE g⁻¹ and 5.67±1.83 mg GAE g⁻¹ extract, respectively.

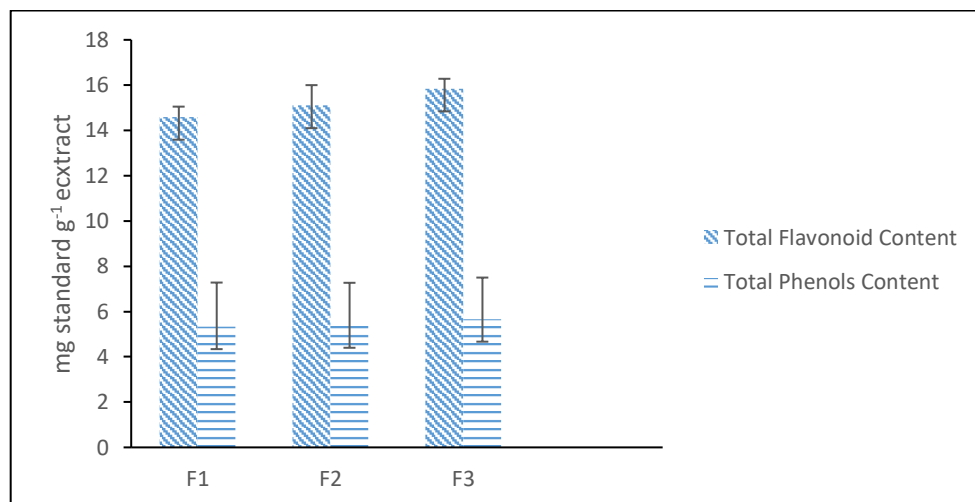


Fig. 1. Total flavonoid and total phenols content of purple okra pudding with addition of okra mucilage.

Pudding with the highest flavonoid and total phenol content is owned by purple okra pudding with the addition of 20% mucilage. The highest flavonoid content in purple okra pudding was 15.84 mg QE g⁻¹ extract or 79.20 mg QE 100 g⁻¹ pudding. This result is higher than the purple okra pudding developed by Fadilah *et al.* (2024), with flavonoid content equivalent to 63.32 mg QE 100 g⁻¹ [21]. The highest total phenol content in purple okra pudding was 5.67 mg GAE g⁻¹ extract, equivalent to 28.35 mg GAE 100 g⁻¹ pudding. This result was more significant than the purple okra pudding used in the antioxidant status improvement intervention, which had a total phenol content of 12.42 mg GAE 100 g⁻¹ pudding [20]. This could be due to the purple okra pudding developed in this study having more mucilage components. Based on research conducted by Gemede *et al.* (2018) on several types of okra, it is known that mucilage has an average total flavonoid and phenol content of 13.67 mg CE g⁻¹ and 37.42 mg GAE g⁻¹ [33]. Research conducted by Moradi *et al.* (2020) found that giving okra powder containing flavonoids at 2.6 mg 100 g⁻¹ for 8 weeks can reduce fasting blood glucose, triacylglycerol, and total cholesterol in adults with type 2 diabetes [34]. When it is compared, the purple okra pudding developed in this research has a greater content, so it is expected to have a faster effect on reducing fasting blood glucose, triacylglycerol, and total cholesterol.

Polyphenols, phenolic acids, and flavonoids are compounds in many vegetables and fruits that can function as antioxidants [29]. Flavonoids as antioxidants can suppress the formation of ROS (Reactive Oxygen Species) by inhibiting enzymes in the formation of ROS and increasing the regulation and protection of antioxidants. Flavonoids can also protect lipid membranes from oxidative damage so that lipid peroxidation can be inhibited and increased Melanoaldehyda (MDA) levels can be prevented. Based on research conducted to prove the hypoglycemic effect of flavonoids, it is concluded that plants containing flavonoids can reduce blood sugar levels [35].

However, it turns out that this study differs from several previous studies, which state that the antioxidant activity of a compound is positively correlated with the total phenolic compound component [36]. This can be caused by other bioactive mucilage compounds such as alkaloid, saponin, tannin, and terpenoid groups [27, 28].

4 Conclusion

Okra mucilage addition in purple okra pudding increased its antioxidant activity. The addition of 20% okra mucilage has the potential to improve antioxidant activities in functional food to increased the health status. Future studies can uncover antioxidant factors other than flavonoids and phenols that may be present in mucilage.

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