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Antioxidative properties of purple okra (*Abelmoschus esculentus l. moench*) pudding

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ABSTRAK

Latar Belakang: *Stres oksidatif merupakan gangguan keseimbangan pro-oksidan dan antioksidan dalam tubuh yang dapat mengarah pada perkembangan penyakit tidak menular. Pada kondisi stres oksidatif, antioksidan eksogen yang berasal dari asupan makanan sangat dibutuhkan untuk mempertahankan fungsi seluler. Okra ungu telah banyak diteliti karena senyawa antioksidannya yang melimpah, seperti flavonoid, yang lebih tinggi dibandingkan dengan okra hijau. Komponen bioaktif pada okra ungu dapat dimanfaatkan dalam bentuk pangan fungsional salah satunya puding karena okra menghasilkan mucilago yang dapat berperan sebagai gelling agent dalam memperbaiki tekstur makanan karena memiliki sifat hidrokoloid.*

Tujuan: *Penelitian ini bertujuan untuk mengembangkan puding okra ungu varietas zahira hasil biofortifikasi sebagai pangan fungsional dan menganalisis kandungan zat gizi, sifat antioksidatif, dan karakteristik mikrobiologinya.*

Metode: *Penelitian ini merupakan penelitian eksperimen laboratorium. Pembuatan puding okra ungu menggunakan metode blanching dan perebusan. Metode analisis terdiri dari AOAC, BPOM, dan SNI untuk uji proksimat, DPPH untuk uji aktivitas antioksidan, AlCl3 untuk uji kandungan total flavonoid, dan HPLC untuk uji kandungan kuersetin, serta metode cawan tuang untuk uji mikroba.*

Hasil: *: Puding okra ungu memiliki kadar air 92,86 g/100 g, kadar abu 0,4 g/100 g, lemak total <0,02 g/100 g, protein 0,91 g/100 g, karbohidrat 5,84 g/100 g, serta energi 26,98 kkal/100 g. Sifat antioksidatif yang teridentifikasi pada puding okra ungu adalah aktivitas antioksidan sebesar 53,66% inhibisi, total flavonoid 31,66±0,92 mg QE/g ekstrak, dan 1,01±0,04 mg/g ekstrak terduga turunan kuersetin. Karakteristik mikrobiologi puding okra ungu telah memenuhi standar BPOM untuk batas maksimal cemaran mikroba pada pangan olahan.*

Kesimpulan: *Puding okra ungu yang diformulasikan menujukkan potensi sebagai pangan fungsional dengan sifat antioksidatif.*

KATA KUNCI*: antioksidan; kuersetin; pangan fungsional; puding Okra ungu*

ABSTRACT

Background: *Oxidative stress is a disturbance in the balance of pro-oxidants and antioxidants in the body that can lead to the development of NCDs. In the condition of oxidative stress, exogenous antioxidants coming from dietary intake are needed to maintain cellular function. Purple okra has been widely studied for its abundant antioxidant compounds, such as flavonoids, which were higher compared to green okra. The bioactive components in purple okra can be utilized in the form of functional food, one of which is pudding since it produced mucilage that can act as a gelling agent in improving food texture because it has hydrocolloid properties.*

Objectives: *This study aimed to develop biofortified zahira variety purple okra pudding as a functional food and analyze its nutrient content, antioxidative properties, and microbiological characteristics.*

Methods: *This study was a laboratory experimental study. The making of purple okra pudding used blanching and boiling method. The analysis methods consisted of AOAC, BPOM, and SNI for the proximate test, DPPH for the antioxidant activity test, AlCl3 for the total flavonoid content test, and HPLC for quercetin content, as well as pour plate method for microbial tests.*

Results: *Purple okra pudding has a water content of 92.86 g/100 g, ash of 0.4 g/100 g, total fat of <0.02 g/100 g, protein of 0.91 g/100 g, carbohydrate of 5.84 g/100 g, as well as energy of 26.98 kcal/100 g. Antioxidative properties identified in purple okra pudding were 53.66% inhibition of antioxidant activity, 31.66±0.92 mg QE/g extract of total flavonoid, and 1.01±0.04 mg/g extract of suspected quercetin derivative. Microbiological characteristics of purple okra pudding were in accordance with BPOM standards for maximum limits of processed food microbial contamination.*

Conclusions: *The formulated purple okra pudding showed its potential as a functional food with antioxidative properties.*

KEYWORD*:* *antioxidant; functional food; purple okra pudding; quercetin*

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INTRODUCTION

Oxidative stress is a disturbance in the balance of pro-oxidants and antioxidants in the body (1). Modern lifestyles associated with unhealthy eating patterns, lack of exercise, and exposure to combinations of chemicals from various sources are external factors that contribute to increased oxidative stress which leads to the development of Non-Communicable Diseases (NCDs) (2,3). Globally, the World Health Organization (WHO) states that NCDs were responsible for 41 million deaths out of 57 million (71%) deaths in 2016. In Indonesia, NCDs became the highest cause of death as well, reaching 73% of all causes of death in 2016 (4).

Nowadays oxidative stress is also the main target for the development of new prevention and therapeutic strategies for NCDs (5). Under normal conditions, the increased free radicals can be controlled by the body's various antioxidant defenses. However, in conditions of an imbalance in the production of free radicals and their absorbers, exogenous antioxidants are needed to maintain cellular function (6). Bioactive components obtained from dietary intake such as flavonoids contained in fruits and vegetables can protect the body from oxidative stress. Flavonoids can play a role in various biological activities, including antitumor, anti-inflammatory, antioxidant, and antimicrobial activity (7). One of

the vegetables that has been widely studied for its bioactive components is okra. Okra contains various antioxidant compounds including polyphenols, hyperosides, quercetin, coumarin, uridine, and phenylalanine (8).

Purple okra or red okra (Abelmoschus esculentus L. Moench) is a superior variety identified as having better anti-diabetic bioactive components compared to green okra (9,10). Purple okra extract has an antioxidant capacity and antioxidant activity of 417.54 mg AEAC/100 g and 316.86 ppm respectively which acts as a free radical scavenger (9). Most of the antioxidant activity of purple okra (70%) comes from quercetin as the main flavonoid with a content of 0.45 mg/g which is higher than that of green okra 0.27 mg/g (9,11).

The bioactive components in purple okra can be utilized in the form of functional food, one of which is pudding. Pudding is usually consumed as a dessert and is in great demand by the public because of its sweet taste and soft texture (12). Okra produces mucilage which also contains antioxidants, has α-glucosidase inhibitory activity, and can act as a gelling agent in improving food texture because of its hydrocolloid properties (13,14). Hence, this study aimed to develop purple okra pudding as a functional food and analyze its nutritional content, microbiological characteristics, and bioactive components.

MATERIALS AND METHODS

This study was a laboratory experimental study. The ingredients used in the production of purple okra pudding were biofortified zahira variety purple okra which was developed at the Leuwikopo Experimental Garden of Bogor Agricultural University by Prof. Muhammad Syukur from the Agronomy and Horticulture department, water, skimmed milk, red dragon fruit extract, lemon juice, agar powder, sorbitol, and vanilla essence. Optimization of the formulation and process of making purple okra pudding was determined by trial and error. The method used was boiling which began with blanching (at 97°C for 30 seconds) of purple okra which has previously been washed. Some of the blanched okra was extracted for its mucilage using water as the solvent (1:3) at room temperature for 12 hours (14). The other blanched okra was pureed using a blender until it was smooth. The okra mucilage and pureed okra finally be mixed with the other boiled ingredients.

Table 1. Purple Okra Pudding Composition

Ingredients	Composition (%)
Water	43.4
Pureed purple okra	21.7
Purple okra mucilage	13.0
Skim milk	13.0
Dragon fruit extract	4.3
Lemon juice	2.6
Sorbitol	1.7
Agar powder	0.9
Vanilla essence	0.2
Total	100

Nutrient content analysis was carried out at Saraswanti Indo Genetech (SIG) Laboratory, Bogor. The analysis of proximate including ash, water, and fat was referred to SNI 01-2891-1992, while protein content was done according to AOAC 2001.11. 2005 and SNI 01-2891-1992. Carbohydrate content was determined using calculation by-difference methods, while the total calories were done through a calculation based on BPOM (National Agency of Drug and Food Control) formula (15).

Bioactive components of purple okra pudding were analyzed quantitatively, including antioxidant activity, total flavonoid, and quercetin content. The analysis was done at the Nutrient and Biochemistry Analysis Laboratory, IPB University. The sample used for bioactive component analysis was methanol-extracted purple okra pudding. The pureed purple okra pudding was firstly macerated using methanol (1:2.5) for 2 hours at room temperature to then centrifuged at 2500 rpm. The supernatant was dried using a rotary evaporator (60°C, 72 mbar).

The measurement of antioxidant activity was carried out using the DPPH method (2,2-Diphenyl-1-picrylhydrazyl) referring to Hwang (2009) in Cahyana (2017) with slight adjustments in terms of concentration of ascorbic acid as standard and the λ (14). In this study, the absorbance was measured using a microplate reader at the λ of 492 nm. The value of antioxidant activity using the DPPH method was expressed as % inhibition

which referred to the ability of the antioxidant compounds in the sample to scavenge free radicals. The total flavonoid content analysis procedure referred to Ghasemi et al. (2009) procedure using the colorimetric method with modification in terms of concentration of quercetin as a standard solution, and the wavelength (16). This study used a microplate reader at the λ of 492 nm to measure the absorbance. Total flavonoids were calculated as mg quercetin equivalent per gram of purple okra pudding extract (mg QE/g).

The Quercetin content of purple okra pudding was quantified using the High-Performance Liquid Chromatography (HPLC) method. Analysis was performed using an LC-20 AD from Shimadzu (Japan), equipped with a quaternary pump and autosampler. Quercetin was separated from the sample solutions using a C18 column (4.6×125 mm I.D., 5 µm particle size, Shim-pack GIST), with a mobile phase consisting of methanol (B) and water (A), at 25℃. The flow rate was 1.0 mL/min and injections were of 20 µL in volume. A low-pressure gradient was used as follows: 3 min 100% B; 3 min 95% B; and 3 min 90% B. Chromatograms were collected in the λ of 370 nm. The prepared sample was diluted in methanol and filtered through a durapore 0.45 µm PVDF membrane filter prior to injection.

Furthermore, microbial analysis was carried out to analyze the safety aspect of purple okra pudding. The microbial tests were done at Saraswanti Indo Genetech (SIG) Laboratory, Bogor S, and carried out using the pour plate method consisting of the Total Plate Count (TPC) (SNI ISO 4833-1:2015), Enterobacteriaceae (SNI ISO 21528-2:2017), and Salmonella sp. (ISO 6579-1:2017/Amd 1:2020).

RESULTS AND DISCUSSIONS

Nutrient Content

Table 2 displayed the result of the nutrient content analysis of purple okra pudding. The water contained in purple okra pudding was greater than in green okra pudding developed by Giyatmi et al. 2022 (82.0-84.3 g/100 g) (17). Other than water, the other liquid-form ingredients, namely skimmed milk, lemon juice, dragon fruit extract, as well as okra mucilage also constituted the high amount of the pudding's water content. Okra pod itself has high water content ranging from 87.98-90.60 g/100 g (18).

Table 2. Nutrient content of purple okra pudding

Parameter	Unit	Amount
Water	g/100 g	92,86
Ash	g/100 g	0,40
Total fat	g/100 g	<0,02
Carbohydrate	g/100 g	5,84
Protein	g/100 g	0,91
Energy	kcal	26,98

Therefore, there was a need to store purple okra pudding at a cool temperature to prevent spoilage.

The ash content of purple okra pudding was lower compared to green okra pudding (0.6-0.7%). The higher composition of okra the higher the ash content (17). Ash content represents the total mineral content in a product remaining after the combustion or complete acid-facilitated oxidation in food (19). Okra contains minerals including Ca, Cu, K, P, Na, Mg, Zn, Fe, and Mn that possibly decrease due to the cooking process (20). During the making of purple okra pudding, the blanching step possibly reduced all levels of mentioned minerals except Mg (21).

Purple okra pudding has a lesser fat content than green okra pudding (2.7-2.9 g/100 g) (17). This was because of the use of different kinds of milk as an ingredient in each product. Purple okra pudding used skimmed milk while green okra pudding used whole milk that has higher fat content (22). From the okra itself, the seed was the contributor to fat contained in okra as it was constituted by oil reaching 20-40 g/100 g of its total composition (23). According to BPOM (2022), purple okra pudding met the standard of a fat-free product that made it a healthier snack alternative for low-fat diets, such as hyperlipidemia and diabetes relevant to okra's proven hypolipidemic effect in preclinical and limited clinical studies (24,25).

The carbohydrate contained in purple okra pudding was almost half of the green okra pudding (11.7-14.0 g/100 g). This was due to the form and the amount of sweetener used in the products. The granulated sugar added to the green okra pudding by Giyatmi et al. 2022 has higher carbohydrate levels compared to sorbitol used for the purple okra pudding (17,26).. In addition, sorbitol consumption will result in incomplete absorption in the small intestine and lead to eliciting low glycemic and insulinemic responses (26). This made the use of sorbitol a choice for diabetics and low carbohydrate diets people (26). The okra itself contributed to the total carbohydrate of the pudding due to its carbohydrate content, primarily galactose (25%), galacturonic acid (27%), as well as rhamnose (22%) (21).

The protein contained in purple okra pudding (**Table 2**) came from skimmed milk and the okra itself. Okra has protein content reaching as much as 2 g/100 g raw okra (21). This formulated purple okra pudding was lesser in protein than green okra pudding (17). Purple okra pudding's total calorie was counted using BPOM's formula energy and could contribute up to 1,25% of general daily needs (15).

Bioactive Components

Table 3. Bioactive components of purple okra pudding

Component	Unit	Amount
Total flavonoid content	mg QE/g extract	31.66±0.92
Quercetin	mg/g extract	1.01±0.04
Antioxidant activity	%inhibition	53.66

1 g extract ≈ 50 grams of purple okra pudding

Antioxidant activity

A systematic review proposed that okra parts and products including powder, ethanolic or aqueous extract, subfractions, and antioxidants ingredients may have advantageous effects on health including hyperglycemia and hyperlipidemia conditions due to their antioxidant compounds and other components (25). Therefore, purple okra pudding was evaluated for its antioxidant activity using the DPPH assay to see the antioxidative potential. The result showed that purple okra pudding could inhibit 53,66% DPPH radical at 1 mg/mL extract concentration. In comparison, the variability in antioxidant activities of fresh immature okra fruit using the same method ranged from 60.40% to 92.71% at 1 mg/mL (27). This was relevant to the percent DPPH inhibition of Indiana accession of okra that reached 55.97% with a correspondent antioxidant activity of 1829.58±438.00 mg/g (28). Other than that, defatted okra seeds recorded a %Inhibition of 46.38-64.00% at 50 µg/mL concentration (29). In addition, an extract product of red okra pods has an inhibition percentage of 184.93%, 59.67%, and 57.96% for ethanol, n-hexane, and ethyl acetate extract consecutively at a concentration of 150 µg/mL (30).antioxidant activity of 1829.58±438.00 mg/g (28). Other than that, defatted okra seeds recorded a %Inhibition of 46.38-64.00% at 50 µg/mL concentration (29). In addition, an extract product of red okra pods has an inhibition percentage of 184.93%, 59.67%, and 57.96% for ethanol, n-hexane, and ethyl acetate extract consecutively at a concentration of 150 µg/mL (30).

The biggest compositions of purple okra pudding after water were purple okra fruit and its extracted mucilage that contributed to its antioxidant activity. Okra is a rich source of flavonoid compounds, namely hyperoside, coumarin scopoletin, hydroxycinnamic derivatives, oligomeric catechins, and flavonols that lead to its antioxidative characteristic (25). These antioxidants scavenge radicals and inhibit chain initiation or break chain propagation (31). A study found that the administration of different

doses of peel and seed powder of okra significantly increased liver, kidney, and pancreas superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione levels, and decreased thiobarbituric acid reactive substances (TBARS) ($P < 0.001$) levels in diabetic rats compared to diabetic control rats (32).

Total Flavonoid Content

Flavonoids are phenolic compounds that are highlighted for their antioxidant activity as well as the most abundant polyphenolic compounds in okra (33,34). A qualitative-quantitative study using LC-DAD-MS by Panighel et al. has identified that glycosylated flavonoids were found in both okra leaf and fruit (35). The most abundant flavonoid compounds of okra are flavonols, specifically quercetin and its derivatives (36). Moreover, red/purple okra pods have also xenobioticsidants (anthocyanin) that are responsible for the pods' red color. Purple okra extract contains anthocyanin with higher antioxidant and quercetin content than green okra (30,37).

In this study, a content of 31.66±0.92 mg quercetin equivalent/g purple okra pudding extract was identified. One gram of purple okra pudding extract was equivalent to 50 grams of purple okra pudding. Okra powder used in a Randomized Controlled Trial (RCT) contained 2.6 mg/100 g of flavonoid resulting in a significant decrease in fasting plasma glucose, homeostatic model of assessment for insulin resistance, quantitative insulin sensitivity check index, triacylglycerol, total cholesterol, as well as low-density lipoprotein cholesterol after 8 weeks intervention (38).

Quercetin Content

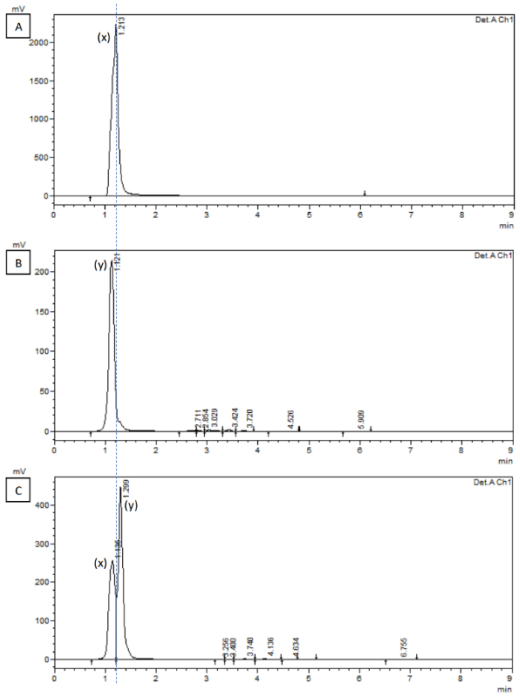

Figure 1. Chromatogram using HPLC of (A) Quercetin standard, (B) Purple okra pudding, (C) Spiked purple okra pudding by standard, with x = quercetin standard peak and y = suspected quercetin derivative peak.

Other studies indicated that okra fruit extract contained 0.8 mg/g of glycosylated flavonoids, while extracted okra leaf has a content of 70-210 mg/g (35). Yang et al. also found that the number of total flavonoids contained in hydro-ethanol extracted okra pod ranged from 4.12664−4.87355 mg/g dry weight (34).

Flavonoids play some roles in preventing injury induced by free radicals. Flavonoids stabilize the reactive oxygen species by being oxidized by the radicals that result in stable and less reactive radicals (39). Other than that, flavonoids exhibited an additive effect on endogenous scavenging compounds when the production of free radicals increased and caused the depletion of the compounds, called flavonoid-protein interactions (40). Flavonoids can also promote the activity of antioxidant enzymes through enzyme gene activation with various signaling cascades involving phytochemicals from red okra that induces the Keap1/Nrf2/ARE pathway (41,42). On the other hand, flavonoids can avoid the formation of free radicals due to their ability to inhibit the enzymes involved in the production of the free radicals or directly chelate the involved metal ions involved (43).

Studies have identified quercetin derivatives and epigallocatechin as major antioxidant compounds in okra (31). The fact was that 70% of the total antioxidant activity comes due to the quercetin derivatives (11). In this study, after spiking the sample with the quercetin standard, the identified compound was suspected to be the quercetin derivative whose peak appeared prior to the quercetin standard. A content of 1.01±0.04 mg/g purple okra pudding extract of the compound was expected to be quercetin-3'-O-sulphate which was identified to appear before quercetin with a 1.117 min retention time difference in taxifolin sample (44). According to another study, it could be expected as quercetin rhamnoside-(feruloyl-hexoside) which took shape 0.6 min retention time difference before quercetin in a combination of habanero white and capsicum annuum peppers sample (45). USDA stated that the quercetin content of okra was 5.75 mg/100 g (46).

Some studies discovered main individual flavonoid in okra was quercetin-3-O-gentiobioside (34,47,48), followed by isoquercetin, quercetin-3-sambubioside (Q3S), quercetin-3-malonylglucoside (Q3M), rutin, quercetin-7-glucoside (Q7G) consecutively (34). An in vitro microtetrazolium experiment indicated that okra's four main flavonoids (Q3G, Q3S, ISO, and Q3M) performed good inhibitory effects on the proliferation of several tumor cell lines that were associated with their glycoside derivatives (34,49). Other than that, the high content of quercetin3-O-gentiobioside and catechin derivative in okra plays an important role in the a-amylase and a-glucosidase inhibition effect (36).

Purple okra quercetin content (0.45 mg/g extract) was higher than that of green okra (0.27 mg/g extract) (50). The administration of purple okra extract with doses of 5 and 10 mg quercetin/kgBW was found to improve malondialdehyde and blood glucose levels of diabetic mice significantly (50). Another study identified a content of 147 mg quercetin/g of dry okra extract and suggested a potential inhibition of PPAR-α and PPAR-γ in the pancreas (51). Another study indicated that ethanol-extracted okra could improve serum lipid levels in diet-induced obese mice through its flavonoids, isoquercitrin, and quercetin-3-O-gentiobioside (52).

Microbiological properties

The microbial analysis was conducted in regard to the food safety aspect as required by BPOM. Based on the result presented in Table 4, purple okra pudding was in accordance with BPOM standards for maximum limits of microbial contamination in processed food (53).

Table 4. Microbiological properties of purple okra pudding

Parameter	Unit	Result	Standard
Enterobacteriaceae	colony/g	<10	$5x10^2$
Salmonella sp.	/25 g	negative	negative
TPC	colony/g	$1x10^1$	$5x10^5$

CONCLUSIONS AND RECOMMENDATIONS

Purple okra pudding has a water content of 92.86 g/100 g, ash of 0.4 g/100 g, total fat of <0.02 g/100 g, protein of 0.91 g/100 g, carbohydrate of 5.84 g/100 g, as well as energy of 26.98 kcal/100 g. The microbiological characteristics of purple okra pudding were in accordance with BPOM standards for maximum limits of microbial contamination in processed food. Antioxidative properties identified in purple okra pudding were 53.66% inhibition of radical scavenging activity, 31.66±0.92 mg QE/g extract of total flavonoid, and 1.01±0.04 mg/g extract of suspected quercetin derivative that made purple okra pudding a potential antioxidative functional food. It was recommended to continue further research to prove the antioxidative properties of purple okra pudding both in vitro and in vivo.

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