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Research Article

Intervention with Purple Okra Pudding and Supplement to Improve Antioxidant Status in Healthy Adults

Evy Damayanthi*, Mira Dewi, Muhammad Aries, Zuraidah Nasution
Department of Community Nutrition, Faculty of Human Ecology, IPB University, 16680 Bogor, Indonesia

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***Corresponding Author:**
tel: +628161933260
email: edamayanthi@apps.ipb.ac.id

ABSTRACT

This research aimed to analyze the potency of purple okra-based products in improving the antioxidant status of healthy adults. Thirty adults with high body fat percentages were allocated into three groups: the first group was treated with 100 g of purple okra pudding/day, the second group was provided with a purple okra extract supplement that contained 3.80 g of extract/day, and the third group was a control group. The intervention was carried out for 28 days. All subjects were exposed to nutrition education, and data on their characteristics, food intake, and physical activity level were collected. The results showed that purple okra pudding and supplement had antioxidant activity of 0.39 and 455.39 mg AEAC/g extract, IC_{50} of 543.79 and 71.78 ppm, and total phenol of 6.21 and 24.49 mg GAE/g extract, respectively. There were significant differences among subject groups in energy, protein, and fat intake as well as physical activity level. The group treated with purple okra pudding showed a significantly higher ΔSOD after the intervention, most probably due to the role of antioxidants contained in purple okra in upregulating antioxidant defense. In contrary, there was a declining trend of ΔSOD in the group treated with purple okra extract supplement. The different effects observed between the two groups might be due to the different phenol contents between the two intervention products. This study showed that purple okra has the potential as a functional food and health supplement in improving the antioxidant status of healthy adults with high body fat percentages as indicated by a higher change of SOD level (0.08 u/mL) in comparison to the control (-0.07 u/mL).

INTRODUCTION

Oxidative stress is a cellular condition that occurs as a result of a physiological imbalance between Reactive Oxygen Species (ROS) production and endogenous antioxidant defenses (Ighodaro & Akinloye 2018). Free radical molecules, particularly ROS, are derived from biotransformation of molecular oxygen and external factors, including unhealthy eating patterns, lack of exercise, and exposure to chemicals from various sources (Sharifi-rad *et al.* 2020). Increased oxidative stress is associated with high body fat percentage and several degenerative diseases that have become one of the highest causes of death (Fernández Sánchez *et al.* 2011; Ighodaro & Akinloye 2018; WHO 2018).

The interplay between the body's natural antioxidant defenses and free radicals is important in maintaining health, prolonging aging, and preventing age-related diseases. Superoxide Dismutase (SOD) is one of the three key enzymes and first-line defense antioxidants that acts to suppress or prevent the formation of free radicals in cells. SOD works by breaking down hydrogen peroxides and hydroperoxides into harmless molecules and neutralizing molecules that have the potential of developing into free radicals with the possibility to induce other radical production (Ighodaro & Akinloye 2018; Liu *et al.* 2023).

Efforts to maintain and increase the activity of endogenous antioxidants such as SOD are expected to reduce oxidative stress. Apart from that, consumption of exogenous antioxidants, such as those contained in antioxidant-rich

foods or supplements, will increase the total antioxidants that work to combat oxidative stress in the body so that optimal oxidative status can be obtained (Ighodaro & Akinloye 2018). Among the antioxidant-rich foods, okra (*Abelmoschus esculentus* L. Moench) is one of the vegetables widely studied for its bioactive components (Tyagita *et al.* 2019).

Okra, also known as lady's finger, is a type of vegetable that is popular in many parts of the world. It is usually green in color; however, there is also a purple variety known as purple okra (Manickavasagam *et al.* 2015). This vegetable contains bioactive compounds such as flavonoids and polyphenols, which have the potential as antioxidant and anti-inflammation (Majd *et al.* 2019). Biofortified purple okra is a superior variety identified as having higher amount of anti-diabetic bioactive components as compared to green okra (Anjani *et al.* 2018). Seventy percent of the antioxidant activity of purple okra comes from quercetin, which is the main flavonoid in purple okra, with a content of 0.45 mg g^{-1} (Roy *et al.* 2014; Anjani *et al.* 2018).

The extract of okra can be administered as an encapsulated supplement. The capsule protects the active substances contained from external factors and controls their release to the digestive system, thereby maintaining their functional stability. Purple okra extract has an antioxidant capacity of 4.17 mg AEAC g^{-1}, antioxidant activity (IC_{50}) of 316.86 ppm, total phenol content of 3.60%, and quercetin of 0.45 mg g^{-1} (Anjani *et al.* 2018). In extract form, it has more bioactive components, thereby allowing for a higher antioxidant content (Susanty & Bachmid 2016).

Bioactive components in purple okra can also be delivered in the form of pudding, which is used as a functional food. Pudding is usually consumed as a dessert and is well accepted because of its sweet taste and soft texture (Darmawan *et al.* 2014). Okra produces mucilage that also has an antioxidant activity and hydrocolloid properties that can act as a gelling agent in improving food texture (Cahyana *et al.* 2017). Measured by the DPPH method, a pudding with popping boba made from purple okra has an antioxidant capacity of 3.54 mg AEAC 100 g^{-1} (Dyastasari 2019). This research aimed to analyze the potential of purple-okra-based products in improving the antioxidant status of healthy adults.

METHODS

Design, location, and time

This study encompassed a laboratory phase and a quasi-experimental research segment employing a pre-posttest control parallel group design. Ethical clearance was obtained from the Commission on Ethics for Research Involving Human Subjects, IPB University, as per letter number 762/IT3.KEPMSM-IPB/SK/2022. The research was carried out of the IPB University, Darmaga campus, from November 2022 to April 2023.

Sampling

Subjects were students and staff of IPB University at Darmaga campus. The inclusion criteria were being in the adult age group (19–44 years), having a high Body Fat Percentage (BFP) of >28% for women and >20% for men, as measured with InBody 270 (Lee & Nieman 2007)), having a normal nutritional status (BMI 18.50–25.00 kg/m^2), not suffering from degenerative diseases as diagnosed by medical doctor, not smoking and consuming alcohol, not being pregnant or breastfeeding, having normal Fasting Blood Glucose (FBG) levels (70–100 mg dL^{-1}), having no milk allergy or intolerance, not currently taking antioxidant supplements and drugs, and willing to participate in the study by filling out the informed consent form.

Body fat percentage is directly related to higher markers of oxidative stress and lower antioxidant defenses (Fernández-Sánchez *et al.* 2011). We expected that those with high body fat percentages would be more responsive to antioxidants as given in the intervention.

The minimum number of subjects required was calculated using a sample size formula by Notoatmodjo (2012) as follows:

$$n = \frac{2\sigma^2 \times [Z_{1-\alpha/2} + Z_{1-\beta}]^2}{(\mu_1 - \mu_2)^2}$$

$$n = \frac{2(5.95)^2 \times [1.96 + 1.28]^2}{(85.38-76.19)^2}$$

$$n = \frac{2\ x\ 35.4025\ \times 10.496}{84.4561}$$

$$n = 8.79 \approx 9$$

Where n is the minimum sample size required, $Z_{1-\alpha/2}$ is the level of significance (Z=1.96, with a confidence interval of 95%), $Z_{1-\beta}$ is the test power (Z=1.28 with test power of 90%), σ is the standard deviation of the difference in mean SOD levels (Rosidi 2014), and μ1–μ2 is the significant

difference in mean SOD levels between two groups (Rosidi 2014). With an additional 10% of dropout possibility, the minimum number of subjects required was 10 per group. Subjects were divided into three groups, namely: 1) group that received a purple okra supplement that contained purple okra extract at 3.80 g/day; 2) group that was given 1 cup (100 g) of purple okra pudding per day, and; 3) control group.

Research stages. This research consisted of two stages, namely preliminary research and main research. The preliminary research began with the production of supplement and pudding from biofortified purple okra, which was grown at the Leuwikopo Experimental Farm of IPB University.

The purple okra supplement was prepared by extraction using 96% food-grade ethanol (3:1) for 3×24 hours and drying using a vacuum pan evaporator (60°C, 30 minutes). The dried okra extract was then powdered and packaged into capsules.

The preparation of the purple okra pudding used a boiling method, which began with blanching (at 97°C for 30 seconds) of freshly-washed purple okra. The blanched okra was then extracted using water as the solvent (ratio 1:3) at room temperature for 12 hours in order to get its mucilage (Cahyana *et al.* 2017). The other parts of the blanched okra was then pureed using a blender until it was smooth. The okra mucilage and pureed okra were next mixed with the other ingredients including water, skimmed milk, agar powder, lemon and dragon fruit extracts, sorbitol, and vanilla essence. The products were analyzed for total phenolic content, antioxidant capacity, and antioxidant activity.

The main research was a clinical trial. Subjects were determined by screening according to the inclusion criteria and divided into three groups purposively by considering the subject's ownership of a refrigerator for pudding storage and residence. The next step was blood collection and measurements of SOD and FBG before the intervention, followed by an intervention period of 28 days according to Basu *et al.* (2021) and Subawa *et al.* (2022) on human subjects that showed improvement in oxidative markers after 28 days of intervention. During the intervention, nutrition education was provided in week 1, while 2×24 hour food recall and physical activity data collection was performed in weeks 1–4. Blood collection and post-intervention SOD level measurement were conducted on the 29th day.

Data collection

Data was collected by laboratory analyses of total phenolic content using the Folin-Ciocalteu method, antioxidant capacity using the DPPH method with ascorbic acid as the standard, and antioxidant activity using the DPPH method. Meanwhile, data collected from the subjects includes subjects' characteristics (Body Mass Index and BFP) that was measured using a digital body scale, a stadiometer, and a Bioelectrical Impedance Analysis (BIA), as well as their dietary intake and physical activity, obtained by 2×24 hour food recall and a Physical Activity Level questionnaire, respectively (FAO 2001). In addition, FBG levels were measured using the Glucose Oxidase-Peroxidase Aminoantipyrine method, while SOD levels were analyzed using the Enzyme-like Immunosorbent Assay (ELISA) method (Cayman Chemical, Ann Arbor, MI, USA).

Data analysis

Data were processed using MS Excel and SPSS 23 software for statistical analysis. The normality test was carried out using the Shapiro-Wilk test. The difference in SOD and FBG levels between groups was analyzed using one-way ANOVA, followed by Duncan's multiple range test when there was a significant result ($p<0.05$).

RESULTS AND DISCUSSION

The bioactive components of purple okra pudding and supplement

The bioactive components of purple okra pudding and supplement are presented in Table 1.

The capacity of the antioxidant compounds in purple okra extract can be expressed as

Table 1. Bioactive component of purple okra pudding and supplement

Bioactive component	Unit	Mean values	
		Supplement	Pudding
Antioxidant capacity (AEAC)	mg AEAC g^{-1}	455.39	0.39*
Antioxidant capacity (IC_{50})	ppm	71.87	543.79
Total phenol	mg GAE g^{-1}	24.49	6.21*

*1 g of purple okra pudding extract is equivalent to 50 g of purple okra pudding; AEAC: Ascorbic Acid Equivalent Antioxidant Capacity

Ascorbic Acid Equivalent Antioxidant Capacity (AEAC). The AEAC value of the purple okra extract supplement was 455.39 mg AEAC g^{-1}, which is different from the value obtained in previous research on purple okra extract, namely 0.04 mg AEAC g^{-1} (Anjani *et al.* 2018). This difference might be due to differences in varieties, forms of okra before extraction, and the solvents used. Meanwhile, the AEAC value of the purple okra pudding was 0.39 mg AEAC g^{-1} extract. In other words, the antioxidant capacity of 100 g of purple okra pudding is equivalent to 0.78 mg of vitamin C. The AEAC value of pudding sample found in this study is different from that obtained in previous research on boiled okra, which showed a value of 1.3 mg AEAC 100 g^{-1} (Utami 2018). With the use of 25 g of purple okra and an extra 15 g of purple okra mucilage extract, the antioxidant activity of purple okra pudding was more than half of the antioxidant activity of 100 g of boiled okra.

The antioxidant capacity that produces the % inhibition is used to obtain the IC_{50} value, which is defined as the concentration required to inhibit 50% of DPPH free radicals. Antioxidant activity of the purple okra extract supplement analyzed in this study was 71.87 ppm, which showed strong antioxidant activity (Santoso *et al.* 2022). A different value was obtained in a previous study performed on the purple okra extract, which was 316.86 ppm (Nabila *et al.* 2018). For purple okra pudding, the value of antioxidant activity (IC_{50}) found in this study was 543.79 ppm. A different value was obtained from purple okra extract analyzed in a previous study, which was 316.86 ppm (Nabila *et al.* 2018). The lower IC_{50} value in purple okra extract means that the antioxidant activity of purple okra extract was higher than that of purple okra pudding. This was due to the extraction process that resulted in a higher concentration of antioxidant compounds, leading to a higher antioxidant activity (Susanty & Bachmid 2016).

Several studies on okra have shown the presence of phenolic compounds, which are expressed as Gallic Acid Equivalents (GAE). The total phenol found in purple okra supplement used in this study was 24.49 mg GAE g^{-1}. Another study found that the strong antioxidant effect in okra sample comes from the seed extract of okra, while the peel extract shows almost no presence of these compounds. Total phenol content of okra seed extract was reported to be 56.66 mg GAE g^{-1} (Chaemsawang *et al.* 2019). Meanwhile, the pudding sample used in this study was found to have total phenol of 6.21 mg GAE g^{-1} extract. A different value was obtained from analysis of fresh purple okra in a previous study, which was 20.3 mg GAE g^{-1} (Utami 2018). The difference could occur because the previous study used fresh okra, allowing it to maintain its bioactive components, which are thermolabile (Rifkowaty & Wardanu 2016). The mechanism of antioxidant action can be due to stabilization of ROS initiators, increasing enzymatic endogenous antioxidants, and chain termination (Kurutas 2015).

Subject characteristics

A total of 30 subjects who met the inclusion criteria were included in the study. The subject characteristics are shown in Table 2.

At baseline, all groups shared the same characteristics in terms of normal nutritional status (BMI of 18.7–25.0 kg/m^2), normal FBG levels (73–99 mg/dL), but high body fat percentages (>28% for women, >20% for men). There was a significant age difference between the supplement and pudding groups due to a wider range of age of the pudding group. In adults, free radicals begin to accumulate from the beginning

Table 2. Subject characteristics before intervention

Subject characteristics	Mean±SD			*p*
	Supplement	Pudding	Control	
Age (years)**	21.70±1.90[a]	25.00±2.87[b]	23.70±2.83[ab]	0.027*
BMI (kg/m^2)	21.80±2.00	22.00±1.51	22.00±1.87	0.971
FBG (mg/dL)	87.60±7.30	84.50±5.06	85.30±7.35	0.567
BFP (%)	33.40±6.10	34.25±5.34	33.48±6.56	0.942

*Significant at $p<0.05$; **ANOVA followed by Duncan's multiple range test; BMI: Body Mass Index; FBG: Fasting Blood Glucose; BFP: Body Fat Percentage; SD: Standard Deviation

of life, which causes an imbalance in the body's oxidant and antioxidant status (Sharifi-Rad *et al.* 2020). Previous research showed different plasma MDA levels in adolescents, adults, and the elderly, with MDA values of 0.85 µM, 1.25 µM, and 2.54 µM, respectively (Mas-Bargues *et al.* 2021).

Food intake and physical activity

The data of subject's energy and nutrient intake were obtained through food intake data using a 2×24 hour food recall. Physical activity data were collected in 24 hours in the form of physical activity levels. Food intake and physical activity of the subjects are presented in Table 3.

The levels of carbohydrates, fibers, vitamin C, vitamin E, zinc adequacy, and cholesterol consumption were not significant among groups ($p>0.05$). The carbohydrate adequacy levels were categorized as moderate deficit for the supplement and pudding groups and severe deficit for the control group. All groups were in the same category for fiber, vitamin C, and vitamin E adequacy levels, namely severe deficit (Nurhidayati *et al.* 2017).

The level of energy adequacy of the pudding group was significantly higher than that of the control group but not of the supplement group. The categories of the energy adequacy level were moderate deficit for the supplement and pudding groups and severe deficit for the control group. The pudding group also demonstrated a higher protein adequacy level (mild deficit) compared to both the supplement and control groups (severe deficit). Although the levels of nutritional adequacy differed significantly, all was below 100% of the adequacy level.

Fat adequacy level of the control group was significantly lower than the pudding and supplement groups. The control group's category of fat adequacy level was normal, while the treatment groups were over the daily need (Nurhidayati *et al.* 2017). This could be due to the frequent consumption of fast food which is high in fat (Janssen *et al.* 2018). Based on the food recall analysis, the treatment groups consumed fast food 0–6 times per 8 days of food recall, while the control group only had a fast-food consumption frequency of 0–2 times. Adults prefer to consume fast food and sweetened beverages, and the trend is related to the availability and accessibility of fast food as well as hectic lifestyle (Abdullah *et al.* 2015). A study also demonstrated that 84% of university students consumed fast food (Habib *et al.* 2011). The difference in the fast food frequency consumption was likely caused by differences in the subjects' locations of residence. Groups were divided purposively to facilitate product distribution during the intervention period. The intervention groups were subjects

Table 3. Food intake and physical activity of the subject

Variable	Mean±SD			*p*
	Supplement	Pudding	Control	
Level of adequacy (%)				
Energy**	82.53±19.50^{ab}	90.74±11.98^{b}	69.82±13.94^{a}	0.019*
Carbohydrate**	71.54±20.45^{a}	70.99±10.07^{a}	61.23±14.84^{a}	0.273
Protein**	69.55±17.48^{a}	86.64±15.20^{b}	63.82±18.54^{a}	0.016*
Fat**	119.13±26.05^{b}	141.53±27.22^{b}	94.28±20.51^{a}	0.001*
Fiber**	21.20±9.73^{a}	20.24±7.23^{a}	21.88±9.31^{a}	0.917
Vitamin E**	15.26±4.24^{a}	15.63±4.98^{a}	17.74±6.01^{a}	0.514
Vitamin C**	29.24±31.17^{a}	30.26±36.48^{a}	31.84±29.85^{a}	0.984
Zinc**	45.68±11.52^{a}	43.71±15.90^{a}	51.97±16.60^{a}	0.440
Cholesterol (mg)**	244.22±118.55^{a}	298.41±90.73^{a}	196.62±61.66^{a}	0.068
Physical Activity (PAL)**	1.54±0.09^{b}	1.36±0.06^{a}	1.52±0.12^{b}	<0.001*

*Significant at $p<0.05$; **: ANOVA followed by Duncan's multiple range test; SD: Standard Deviation; mg: Milligram

who lived by renting a place nearby the campus with limited cooking facilities, while the control group lived at home with family members away from the campus. Thus, subjects in the control group tended to consume home-cooked food and had a lower frequency of fast food consumption than the treatment groups. High fat intake is associated with increased ROS production through the β-oxidation pathway in mitochondria (Kuchay *et al.* 2020). The accumulation of ROS production will induce lipid peroxidation, which results in MDA formation (Ayala *et al.* 2014).

The subjects' physical activity showed a significant difference in the pudding group ($p<0.001$), and all groups were in the same sedentary physical activity category (PAL 1.4–1.7) according to FAO (2001), with average scores of physical activity in the supplement group, pudding group, and control group being 1.54, 1.36, and 1.52, respectively.

Changes in superoxide dismutase levels

The administration of purple okra pudding and supplement for 28 days to adults with high body fat percentages had an effect on changes in the SOD levels. The SOD levels before and after intervention are shown in Table 4.

In this study, the intervention resulted in significantly different changes in SOD levels ($p=0.01$). The levels of quercetin as the major bioactive compound found in okra were different between supplement and pudding. The treatment group given the purple okra supplement with a quercetin content of 18.65 mg per day showed a decrease in SOD levels of 0.15±0.17 U/mL after the intervention. SOD activity was used as one of the parameters in determining the presence of antioxidant activity (Andrestian *et al.* 2019). The decrease in SOD levels of the subjects given the purple okra supplement can be explained by the role of the supplement in balancing the oxidative stress caused by free radicals. The antioxidants from the purple okra supplement are needed when endogenous antioxidant production is unable to compensate for the increase in free radicals (Moussa *et al.* 2019). The purple okra supplement has been identified as having a superior total phenol content, supported by apparent strong antioxidant activity which plays a role in changes the endogenous antioxidant levels. Flavonoids can easily donate hydrogen atoms to radical compounds, resulting in the reduction of highly oxidized radicals (Procházková *et al.* 2011; Kim *et al.* 2020).

On the other hand, there was an increase in SOD levels of 0.08±0.17 U/mL in the pudding group, which consumed 2.02 mg of quercetin per day after the intervention. This figure was significantly different from those of the supplement and control groups. The mechanism of bioactive components in functional food, such as quercetin, gives beneficial health effect by regulating the enzyme-mediated antioxidant system (Xu *et al.* 2019). The intervention of purple okra pudding, which had lower antioxidant activity (Table 1) compared to the intervention of purple okra supplement, contributed to the increase of SOD levels, which can be explained

Table 4. The changes in superoxide dismutase and fasting blood glucose levels

Group	Mean±SD			*p*
	Pre-intervention	Post-intervention	Δ	
Superoxide dismutase (U/mL)**				
Supplement	0.89±0.44	0.74±0.37	-0.15±0.17[a]	
Pudding	0.67±0.10	0.75±0.09	0.08±0.17[b]	0.01*
Control	0.73±0.08	0.65±0.12	-0.07±0.15[a]	
Fasting blood glucose (mg/dL)**				
Supplement	87.60±7.30	89.50±6.39	1.90±5.34	
Pudding	84.50±5.06	84.90±4.43	0.40±6.34	0.86
Control	85.30±7.35	86.50±5.58	1.20±6.53	

*Significant at $p<0.05$; **: ANOVA followed by Duncan's multiple range test; SD: Standard Deviation; Δ: Pre-Post Intervention
U/mL: Units per milliliter; mg/dL: milligrams per deciliter

by another endogenous antioxidant mechanism. It has been suggested that the mechanism promotes endogenous antioxidant defenses via upregulation (Kumar & Pandey 2013). The antioxidant activity and redox properties of flavonoids play a role in the activation of Antioxidant Responsive Element (ARE), which is a regulatory sequence of a group of genes encoding for phase II enzymes, including SOD (Banjarnahor & Artanti 2014). This is relevant to the fact that flavonoids such as quercetin glycosides, rutin, and isoquercitrin, which are also contained in okra, have distinct features in upregulating the production of intracellular antioxidant enzymes (Kozłowska & Szostak-Węgierek 2019). A preclinical study that examined the effects of okra pod methanol extract on lead acetate toxicity in mice kidneys concluded that the administration of okra methanol extract significantly increased SOD activity (Wahyuningsih *et al.* 2020). The two different mechanisms of purple okra supplement and pudding in changes of SOD levels are shown in Figure 1.

The control group showed a decrease in SOD levels of -0.07±0.15 U/mL, which was lower than the reduction in SOD levels of the supplement group. This indicate that the body's antioxidant defenses of the control group worked in maintaining the antioxidant status. The insignificant difference from the supplement group could be due to the variability of SOD levels, which could be influenced by other factors that were not observed in this study, such as exposure to pollutants, inflammation, physical and mental stress, radiation, etc. (Sanjay & Shukla 2021). The accumulation of free radicals from inside and outside the body that exceeds the limit of protective capacity has an impact on changes in SOD levels (Ayala *et al.* 2014).

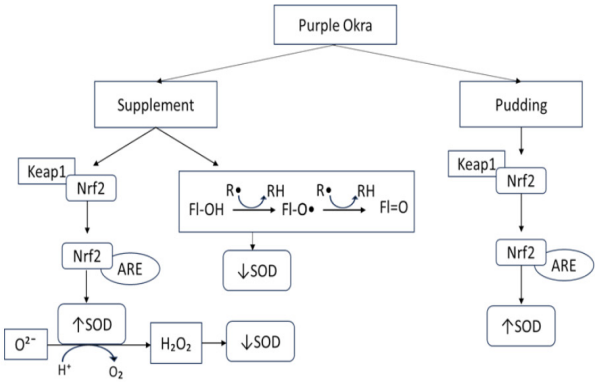

Figure 1. Hypothesized mechanism of antioxidant action from purple okra products (Modified from Xu *et al.* 2019; Kim *et al.* 2020; Liu *et al.* 2023)

The balance between oxidative and antioxidant processes appears to be sensitive to glucose levels, with early elevations of glucose affecting the oxidative status (Menon *et al.* 2004). Sujects' FBG levels were not significantly different (p=0.86), with each group having normal FBG levels. High blood glucose levels increase radical production, which affects levels of endogenous antioxidant SOD (Sachdeva *et al.* 2014). Hyperglycemia promotes collateral glucose metabolism through protein kinase C, polyol, and hexosamine routes. The mentioned processes undermine cellular structures, finally giving place to a progressively greater degree of oxidative stress with further hyperglycemia, metabolic alterations, and diabetes complications (González *et al.* 2023). FBG being at normal levels in all groups indicates that different antioxidant mechanisms play a role in maintaining normal physiological functions of the body.

CONCLUSION

Both purple okra pudding and supplement displayed potentials as sources of antioxidants based on their identified antioxidant capacity, IC_{50}, and total phenol. The intervention with both products resulted in different changes in SOD levels over the course of 28 days. This study has shown that okra intervention in different forms, pudding and supplements, possessed relatively high antioxidant activity. However, there were no significant differences between the control and treatment groups of healthy subjects. Both products can be used to maintain the balance of antioxidant status in adults. It is possible to further test the products in adults with certain health conditions to see the significant effect that they can impose.

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DECLARATION OF CONFLICT OF INTERESTS

The authors have no conflict of interest.

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