PHYSICOCHEMICAL AND ANTIOXIDANT PROPERTIES OF OKRA (*Abelmoschus esculentus*) GUM EXTRACT AND OKRA SEEDLESS POD FLOUR

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Abstract

The health consciousness of consumers and increasing demand for functional ingredient has stimulated research interest on novel plant-based hydrocolloids. There is limited information on the physicochemical and antioxidant activity of okra gum extract and okra seedless pod. Physicochemical and antioxidant properties of okra gum extract and okra seedless pod flour were investigated. Okra gum extract (OGE) and okra seedless pod flour (OSPF) were prepared using standard procedures. Physicochemical, phytochemical and antioxidant properties of the samples were assayed following standard methods. It was found that okra seedless pod flour contained significantly higher yield, ash, protein (16.48 g/ 100 g), insoluble, soluble and total dietary fiber (15.38 g/ 100 g), micronutrients (minerals, vitamins A and C), total phenolic content (26.74 mg GAE /g), total flavonoid and bioactivity (DPPH radical inhibition activity and ferric reducing antioxidant power) than okra gum extract. The study established that OSPF is a rich source of protein, total dietary fiber, micronutrients, phytochemicals and antioxidant activity. The incorporation of OSPF and OGE in wheat flour as a food additive is advocated but more studies are required to elucidate their functionality.

Keywords: Okra products, physicochemical characteristics, bioactivity

Introduction

The high cost of importation of food hydrocolloids and increasing consumer demand for functional ingredients have encouraged the inclusion of novel plant-based hydrocolloids in the production of confectionaries such as bread. Hydrocolloids are water soluble polysaccharides used in bakery products to improve dough performance, bread and cake properties, sensory quality, and shelf life (Salehi, 2020). Several works have reported the potential use of natural additives, and their positive impact on products such as bread, biscuits, cakes, and pasta formulation (Salehi, 2020).

Okra (*Abelmoschus esculentus*) is one of the most heat- and drought-tolerant vegetable species cultivated throughout the tropical and warm regions of the world (Padayachee *et al.*, 2012). Okra pods (the immature pods of the *Abelmoschus esculentus* plant), okra seedless pod flour (obtained from okra seedless pods) and okra gum (extracted from the pods, often through water extraction) are used as thickeners and flour improver, among others, have food, pharmaceutical and industrial applications. *Okra* is a rich source of high-quality protein, phenolic compounds, vitamins A and C, and dietary fiber while its mucilage could form viscous gels and impact viscosity required in food product development (Padayachee *et al.*, 2012). *Okra* is highly perishable and wasted during seasonal glut due to poor preservation technology in Nigeria. There is the need thus convert *okra* to valuable raw materials for application in food systems in order to minimize post-harvest losses experienced during the peak harvest season.

There is relatively scarce information on the physicochemical and antioxidant properties of okra gum extract and okra seedless pod flour. Such information is important in determining the potential of okra gum extract and okra seedless pod flour as a functional ingredient. It is, therefore, hypothesized in this study that differences may exist between the physicochemical and antioxidant properties of okra gum extract compared to okra seedless pod flour. The objective of this study was to determine the physicochemical and antioxidant properties of okra gum extract and okra seedless pod flour.

Materials and Methods

Source of materials

Fresh okra fruits planted in 2024 farming season were obtained from the Teaching and Research Farm of the Federal University of Technology Minna, Nigeria. All the chemicals used for the study were purchased from a reputable Agrochemical company based in Minna, Nigeria.

Extraction of Okra Gum Extract (OGE)

Okra gum extract (OGE) was prepared following a standard procedure (Amari *et al.*, 2013) with minor modification in terms of the extraction liquid used. Fresh okra pods were cleaned and manually cut into halves with a kitchen knife and the seeds removed to obtain okra seedless pod. A 100 g of the okra seedless pods were blended in distilled water (500 mL) for 5 minutes using a blender. The supernatant was separated after centrifugation at 12,000 × g, and the extraction was repeated to obtain the okra gum. The combined supernatant was adjusted to pH 7, dried in an air-draft oven at 40 °C until < 10% moisture content was achieved. The dried okra gum extract was blended and sieved (100 μm mesh size) to obtain okra gum extract (OGE) flour. The OSPF was stored in an airtight container at 4°C for further analysis. The percentage OGE yield was calculated as:

$$\text{Yield (\%)} = (\text{OGE weight}/\text{okra weight used}) \times 100\%. \qquad (1)$$

Preparation of Okra Seedless Pod Flour (OSPF)

Cleaned fresh okra pods were manually cut into halves with a kitchen knife and the seeds removed to obtain okra seedless pod. The seedless pods were blended without water to obtain a slurry. The okra slurry was dried in an air-draft oven at 40 °C until a moisture content of < 10% was achieved. The dried okra meal was milled and sieved (100 μm mesh size) to obtain okra seedless pod flour (OSPF). The OSPF was stored in a plastic container with lids at 4 °C for further analysis.

Determination of Physicochemical properties of OGE and OSPF

The pH of the slurry was obtained using a standardized digital pH meter. The proximate composition of the samples was analyzed according to the method of AOAC (2012). Moisture content was determined in a hot air circulating oven. Total ash was determined by incineration. Fat content was determined using petroleum ether. Protein content (N x 6.25) was assayed by the micro-Kjeldahl method. Soluble dietary fiber and insoluble dietary fiber were determined using a Dietary Fiber Analyzer. The proximate composition of the samples was analyzed according to the method of AOAC (2012). Moisture content was determined in a hot air circulating oven. Total ash was determined by incineration. Fat was assayed using petroleum ether. Protein content (N x 6.25) was determined by the micro-Kjeldahl method. Soluble dietary fiber and insoluble dietary fiber was analyzed using a total dietary fiber kit (Megazyme International Ltd., Wicklow, Ireland). Mineral composition of the OGE and OSPF samples were profiled using inductively coupled plasma optical emission spectrometry (ICP-OES) (Chinma *et al.*, 2023). Vitamins A and C content (dry basis) were analyzed using a standard procedure (AOAC 2012).

Determination of phytochemical and antioxidant properties of OGE and OSPF

Preparation of extract

A methanolic extract for the determination of total phenolic content, total flavonoid and antioxidant activities of samples were employed following a standard method (Chinma *et al.*, 2020). A 0.2 g of each sample was extracted twice with 4 mL methanol (80 %) and kept in a shaking water bath at 40°C for 2 hours and centrifuged at 2000 g for 100 minutes. The TPC, TFC, DPPH and ferric reducing antioxidant power were measured using extracts stored at 4 °C.

Determination of total phenolic content

Total phenolic content was determined on the methanolic extract (ME) of the samples. A 10 µL of the extract was placed in a test tube containing distilled water (500 µL) and reacted with 50 µL of Folin–Ciocalteu phenol reagent. This was kept in the dark for 3 minutes, followed by the addition of 200 µL of 20 g/L Na_2CO_3 (g/v), and finally distilled water (245 µL). The absorbance of 300 µL of the mixture was measured on a UV–Visible spectrophotometer. The results were reported in mg GAE /g (dry basis).

Determination of total flavonoid content (TFC)

A 2 mL extract was mixed with potassium acetate (0.1 mL 1 M), and 0.01 mL aluminum chloride solution (10%) before being incubated for 30 minutes at 25 °C (Yakubu *et al.*, 2022). The absorbance was measured at 415 nm and results reported as mg quercetin equivalent (QE) per g dry sample.

Determination of DPPH (1,1-diphenyl-2-picryl-hydrazil) radical scavenging activity

A standard procedure (Polat *et al.*, 2020) was used for measuring DPPH. The DPPH solution (2.8 mL) was added to 0.2 mL sample extract/standard, and the resulting mixture was maintained for 30 minutes at room temperature in the dark. Thereafter, a UV/VIS spectrophotometer (Jenway 6850, Cole-Parmer, Staffordshire, UK) was used to measure the absorbance at 516 nm. Trolox was used as standard, and DPPH inhibition value was reported in %.

Determination of Ferric reducing antioxidant power (FRAP)

The FRAP was assayed using a standard procedure (Beta *et al.*, 2005). Sample (0.2 g) sample was mixed with methanol (4 mL of 80%), sonicated at 25 °C at 12 minutes, and centrifuged for 10 minutes at 1935 x g. A 300 µL of the supernatant was mixed with 2.85 mL of freshly made stock solution (10:1:1), which contained 300 mM acetate buffer pH 3.6, 10 mM TPTZ [2, 4, 6 Tri (2-pyridyl) s-triazine] and 20 mM ferric chloride. Thereafter, the mixture was incubated in the dark for 30 minutes at 37 degrees Celsius. The absorbance was measured using UV–Visible spectrophotometer at 593 nm. The results were reported in mmol TE (Trolox equivalent)/g (dry sample).

Statistical analysis

All analyses were performed in triplicates. The resulting data were subjected to analysis of variance (ANOVA) using SPSS version 23 (IBM Corp., Armonk, NY, USA). Differences between means were determined using Tukey's test at a 5% significance level ($p < 0.05$).

Results and Discussion

Physicochemical properties of okra gum extract and okra seedless pod flour

Table 1 shows the physicochemical properties of okra gum extract and okra seedless pod flour. OGE showed no statistical difference in pH and moisture content compared to OSPF. The pH values of OGE (6.16) and OSPF (6.29) suggest that the samples are slightly acidic to neutral which is generally suitable for most food applications. Higher yield ($p \leq 0.05$) was obtained for OSPF than OGE. The low moisture value of OGE and OSPF suggest that the products are shelf stable. The ash content of OSPF increased significantly ($p \leq 0.05$) by 85.49% compared to OGE; an indication of higher mineral content of the samples compared to OGE. The variation in ash content could be due to high ash content of okra pod than okra gum extract (Padayachee *et al.*, 2012). Total dietary fiber content of OSPF was significantly higher (p 0.05) than OGE. The soluble and insoluble fiber content of OGE significantly ($p \leq 0.05$) decreased by 312.41% and 154.71%, respectively, compared to the OSPF. The variation in fiber constituents may be attributed to low total dietary fiber in OGE compared to OSPF. OSPF is a rich source of dietary fiber compared to okra gum extract (Padayachee *et al.*, 2012). Consumption of foods rich in dietary fiber are known to promote health and wellness. In terms of mineral composition, okra seedless pod flour contained significantly ($p \leq 0.05$) higher mineral (calcium, iron, magnesium, potassium, phosphorus and zinc) compared to okra gum extract. Similarly, vitamins A and C content of okra gum extract were observed to be significantly lower than okra seedless pod flour. This implies that OSPF is a rich source of micronutrients than OGE and could be incorporated into food materials such as wheat flour which are known to be relatively low in micronutrients.

Antioxidant properties of okra gum extract and okra seedless pod flour

Antioxidant activity of food materials is important in determining their potential health properties. Total phenolic content and flavonoid content of OSPF significantly ($p \leq 0.05$) increased by 161.39% and 192.24% respectively, compared to OGE (Table 1), suggesting higher phytochemical constituents in OSPF than OGE. Similarly, the DPPH radical scavenging ability and ferric reducing antioxidant power of OSPF (62.88 % and 0.75 mmol/TE/g, respectively) were significantly ($p \leq 0.05$) higher than OGE (54.09% and 0.41 mmol/TE/g, respectively) (Table 1). Higher antioxidant activity of OSPF than OGE could be related to its higher phytochemical constituents. Antioxidant activity from natural dietary sources has been reported to supplement the human resistance to numerous modern-lifestyle diseases arising from oxidative stress (DJordjevic *et al*, 2010). The inclusion of OSPF in food formulation is advocated due to its higher bioactivity.

Table 1. Physicochemical and antioxidant properties of okra gum extract and okra seedless pod flour

Parameter	Okra gum extract	Okra seedless pod flour
Yield (%)	14.82 ± 0.13^{b}	17.11 ± 0.20^{a}
pH	6.16 ± 0.05^{a}	6.29 ± 0.07^{a}
Moisture (g/100g)	8.14 ± 0.12^{a}	8.05 ± 0.10^{a}
Protein (g/100g)	2.30 ± 0.05^{b}	16.48 ± 0.21^{a}
Ash (g/100g)	1.41 ± 0.07^{b}	9.72 ± 0.15^{a}
Fat (g/100 g)	0.92 ± 0.03^{b}	1.06 ± 0.06^{a}
Insoluble dietary fiber (g/100g)	3.82 ± 0.36^{b}	9.73 ± 0.45^{a}
Soluble dietary fiber (g/100g)	1.37 ± 0.24^{b}	5.65 ± 0.31^{a}
Total dietary fiber (g/100g)	5.19 ± 0.13^{b}	15.38 ± 0.49^{a}
Calcium (mg/100 g)	45.42 ± 1.28^{b}	84.66 ± 1.73^{a}
Iron (g/100g)	0.36 ± 0.03^{b}	1.83 ± 0.05^{a}
Magnesium (mg/100g)	53.40 ± 0.84^{b}	126.75 ± 1.17^{a}
Potassium (mg/100g)	106.81 ± 2.19^{b}	332.92 ± 1.83^{a}
Phosphorus (mg/100g)	28.92 ± 0.53^{b}	63.47 ± 1.83^{a}
Zinc(mg/100g)	0.20 ± 0.08^{b}	1.05 ± 0.16^{a}
Vitamin A (µg/ 100 g)	10.33 ± 0.16^{b}	28.79 ± 0.48^{a}
Vitamin C(mg/100g)	14.73 ± 0.25^{b}	23.56 ± 0.33^{a}
Antioxidant properties		
Total phenolic content (mg GAE /g)	10.23 ± 0.38^{b}	26.74 ± 0.52^{a}
Total flavonoid (mg GAE /g)	1.16 ± 0.07^{b}	3.39 ± 0.11^{a}
DPPH radical inhibition (%)	54.09 ± 0.28^{b}	62.88 ± 0.35^{a}
Ferric reducing ability power (mmol/TE/g)	0.41 ± 0.05^{b}	0.75 ± 0.14^{a}

Each value is mean of triplicates ± standard deviation of triplicates. Means with no common letters within a row significantly differ ($p \leq 0.05$).

Conclusions

The study established variations in physiochemical and antioxidant properties between okra gum extract and okra seedless pod flour. OSPF contained significantly ($p \leq 0.05$) higher yield, ash, protein, total dietary fiber, minerals, phytochemicals and antioxidant activity than OGE. This demonstrated its potential as a functional food ingredient which could be explored as a composite in cereal flour such as wheat flour to improve its protein quality, micronutrients and bioactivity. Research is ongoing in our laboratory on the rheological, functional, thermal characteristics and quality attributes of bread containing okra gum extract/okra seedless pod flour.

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