Bioactive Constituents and Bioactivities of Orange-Fleshed Sweet Potato Supplemented with Detarium microcarpum

Abstract

Orange-fleshed sweet potato (Ipomoea batatas) is a globally recognized functional food crop with significant nutraceutical components. This study investigated the bioactive constituents and bioactivities of orange-fleshed sweet potato flour supplemented with Detarium microcarpum at 5% and 10% proportions. The bioactive constituents (total phenolics, saponins, tannins, and flavonoids), antioxidant activities (DPPH, ABTS, and reducing power), and enzyme inhibitory activities (α-glucosidase, α-amylase, and angiotensin-converting enzyme) were determined. Results showed that supplementation with D. microcarpum significantly increased total phenolic, saponin, tannin, and flavonoid content compared to 100% orange-fleshed sweet potato. Samples supplemented with D. microcarpum demonstrated enhanced antioxidant capacity, with the 95% orange potato: 5% D. microcarpum blend showing the highest reducing power (4.55±0.01 mgGAE/g) and optimal DPPH scavenging activity (SC_{50} = 1.89±0.01 mg/mL). However, supplemented samples showed reduced enzyme inhibitory capacity compared to the control. The findings suggest that D. microcarpum serves as an important additive for enhancing the bioactive composition and antioxidant properties of orange-fleshed sweet potato, with potential applications in functional food development.

Keywords: Orange-fleshed sweet potato, Detarium microcarpum, bioactive compounds, antioxidant activity, enzyme inhibition, functional foods

1. Introduction

Orange-fleshed sweet potato (Ipomoea batatas) has emerged as a crucial food security crop and functional food due to its exceptional nutritional profile and bioactive compound content (Adebayo et al., 2021). This perennial crop is primarily cultivated for its tuberous roots, though the leaves are increasingly recognized for their nutritional value. The distinctive orange color of these varieties is attributed to high

carotenoid content, particularly β-carotene, which serves as a precursor to vitamin A (Musilova et al., 2021).

Figure 1 : Orange-fleshed Sweet Potato

Figure 2: Orange-fleshed sweet potato

The phytochemical makeup of orange-fleshed sweet potato encompasses diverse bioactive substances including phenolic acids, flavonoids, anthocyanins, and carotenoids, which provide antioxidant, anti-inflammatory, antimicrobial, and anti-carcinogenic benefits (Zhang et al., 2022). Studies have shown that orange-fleshed sweet potato foliage contains greater polyphenol levels than numerous commercial vegetables, such as spinach, cabbage, and lettuce (Okonkwo et al., 2023). These polyphenolic substances serve essential functions in preventing degenerative conditions, especially cancer and cardiovascular disorders, through their powerful antioxidant properties.

Detarium microcarpum, commonly called sweet detar or African oak, represents an indigenous African plant species from the Fabaceae family. This underutilized leguminous tree has been traditionally employed in African traditional medicine for treating diverse conditions (Abdulkadir et al., 2020). Recent phytochemical research has shown that D. microcarpum contains substantial quantities of bioactive substances, including phenolic compounds, flavonoids, tannins, and saponins, which contribute to its therapeutic properties (Omeje et al., 2021).

Figure 3: Detarium microcarpum (Ofor seed)

The approach of food fortification and enhancement has received significant attention in addressing malnutrition and improving the nutritional value of staple foods. The combination of orange-fleshed sweet potato with D. microcarpum represents an innovative method for developing nutrient-rich, functional food products. This enhancement strategy seeks to synergistically improve the bioactive compound composition and related health benefits of the final product (Nwosu et al., 2022).

Enzyme inhibition activities, especially those affecting α-amylase, α-glucosidase, and angiotensin-converting enzyme (ACE), represent important therapeutic targets for managing diabetes and hypertension. These enzymes serve crucial

functions in carbohydrate metabolism and blood pressure control, making their inhibition a valuable approach for preventing and managing metabolic conditions (Ademiluyi et al., 2024).

The purpose of this research was to comprehensively assess the bioactive constituents and biological activities of orange-fleshed sweet potato flour enhanced with D. microcarpum at various concentrations. Specifically, the study sought to examine the phytochemical profile, antioxidant properties, and enzyme inhibition characteristics of the enhanced products to evaluate their potential as functional food components.

2. Materials and Methods

2.1 Materials

Orange-fleshed sweet potato tubers and D. microcarpum seeds were obtained from local farmers in Ilorin, Kwara State, Nigeria. Commercial refined wheat flour was acquired from a commercial supplier and used as a control. All materials were manually sorted and cleaned to eliminate dirt and contaminants before processing.

Laboratory equipment consisted of test tubes, centrifuge tubes, transparent containers, electric blender, beakers, conical flasks, measuring cylinders, volumetric flasks, pipettes, funnels, analytical weighing balance, UV-Visible spectrophotometer, centrifuge, water bath, and refrigerator.

2.2 Chemicals and Reagents

All chemicals employed were of analytical grade and included: Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), hydrogen peroxide (H_2O_2), ethanol, methanol, ferric chloride ($FeCl_3$), concentrated sulfuric acid (H_2SO_4), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), p-nitrophenyl glucopyranoside (PNPG), aluminum chloride ($AlCl_3$), sodium phosphate buffer, trichloroacetic acid, sodium hydroxide (NaOH), potassium acetate, potassium ferricyanide, potassium persulfate ($K_2S_2O_8$), Folin-Denis reagent, vanillin reagent, diosgenin, soluble starch, 3,5-dinitrosalicylic acid (DNSA) color reagent, and distilled water.

2.3 Sample Preparation

2.3.1 Production of D. microcarpum Flour

D. microcarpum seeds were manually selected to eliminate impurities and foreign matter. The cleaned seeds were subsequently ground to a fine powder using an electric blender and stored in sealed containers until use.

2.3.2 Preparation of Orange-Fleshed Sweet Potato Flour

Fresh orange-fleshed sweet potato tubers were thoroughly washed, peeled using a sharp knife, and dried under direct sunlight until fully dehydrated. The dried tubers were ground to a fine powder using an electric blender and stored appropriately.

2.3.3 Preparation of Composite Samples

Five different sample formulations were prepared:

- Sample A: 100% D. microcarpum flour
- Sample B: 100% orange-fleshed sweet potato flour (Control A)
- Sample C: 95% orange-fleshed sweet potato flour + 5% D. microcarpum flour
- Sample D: 90% orange-fleshed sweet potato flour + 10% D. microcarpum flour
- Sample E: 100% wheat flour (Control B)

The composite samples were thoroughly mixed using an electric blender to ensure uniform distribution.

2.3.4 Preparation of Methanolic Extracts

Methanolic extracts were prepared following the method of Chan et al. (2007) with modifications. One gram of each flour sample was combined with 10 mL of methanol in a 50 mL centrifuge tube and shaken continuously for 1 hour at room temperature. The mixture was allowed to stand overnight, then filtered to obtain the supernatant. The methanolic extracts were stored at -4°C until analysis.

2.4 Phytochemical Analysis

2.4.1 Determination of Total Phenolic Content

Total phenolic content was assessed using the Folin-Ciocalteu method as described by Elemosho et al. (2021). Briefly, 300 µL of extract was combined with 7 mL of distilled water, followed by 1.5 mL of diluted Folin-Ciocalteu reagent (1:10 dilution) and 1.2 mL of 7.5% Na_2CO_3 solution. The mixture was incubated for 30 minutes at room temperature, and absorbance was measured at 765 nm. Results were expressed as gallic acid equivalents (GAE) in mg/g material.

2.4.2 Determination of Total Flavonoid Content

Total flavonoid content was assessed using the aluminum chloride method as reported by Kale et al. (2010). The reaction mixture contained 0.25 mL of extract, 1.75 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. After 30 minutes of incubation at room temperature, absorbance was measured at 514 nm. Results were expressed as quercetin equivalents (QE) in mg/g material.

2.4.3 Determination of Tannin Content

Tannin content was assessed following the method of Amorim et al. (2008). The assay mixture contained 0.1 mL of extract, 7.5 mL of distilled water, 0.5 mL of Folin-Denis reagent, and 1 mL of 35% sodium carbonate solution, diluted to 10 mL with distilled water. After 30 minutes of incubation, absorbance was measured at 760 nm. Results were expressed as tannic acid equivalents in mg/g material.

2.4.4 Determination of Total Saponin Content

Total saponin content was assessed using the method described by Makkar et al. (2007). The reaction mixture contained 0.25 mL of extract, 0.25 mL of 8% vanillin reagent in ethanol, and 2.5 mL of 72% aqueous H_2SO_4. The mixture was heated at 60°C for 10 minutes, cooled in ice for 4 minutes, then equilibrated to room temperature. Absorbance was measured at 544 nm using diosgenin as the standard.

2.5 Antioxidant Activity Assays

2.5.1 DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was assessed according to Cervato et al. (2000) with modifications. One milliliter of appropriately diluted extract was combined with 3 mL of 60 μM methanolic DPPH solution and incubated in darkness for 30 minutes. Absorbance was measured at 517 nm. The percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = [(A_{517}\text{control} - A_{517}\text{sample}) \div A_{517}\text{control}] \times 100$$

The SC_{50} value (concentration required for 50% scavenging) was calculated from the dose-inhibition curve.

2.5.2 ABTS Radical Scavenging Activity

ABTS radical scavenging activity was assessed according to Sellappan and Akoh (2002). The ABTS radical cation was generated by mixing equal volumes of 7 mM ABTS solution with 2.45 mM $K_2S_2O_8$ and incubating in darkness for 16 hours. The working solution was diluted with 95% ethanol to achieve an absorbance of 0.7 ± 0.02 at 734 nm. The reaction mixture contained 0.2 mL of extract and 2.0 mL of ABTS working solution. Absorbance was measured at 734 nm after 15 minutes, and results were expressed as Trolox equivalent antioxidant capacity (TEAC).

2.5.3 Reducing Power Assay

The reducing power was assessed by evaluating the ability of extracts to reduce $FeCl_3$ solution as described by Elemosho et al. (2021). The reaction mixture contained 2.5 mL of extract, 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6), and 2.5 mL of 1% potassium ferricyanide. After incubation at 50°C for 20 minutes, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 650 rpm for 10 minutes. Five milliliters of the supernatant was mixed with equal volume of water and 1 mL of 0.1% ferric chloride. Absorbance was measured at 700 nm.

2.6 Enzyme Inhibitory Activity Assays

2.6.1 α-Glucosidase Inhibition Assay

α-Glucosidase inhibitory activity was assessed using the method of Kareem et al. (2022). Five units of α-glucosidase enzyme was incubated with 20 µg/mL of extract for 15 minutes. Subsequently, 3 mM PNPG in 20 mM phosphate buffer (pH 6.9) was added as substrate. The reaction proceeded for 20 minutes at 37°C before termination with 2 mL of 0.1 M Na_2CO_3. Absorbance was measured at 400 nm, and percentage inhibition was calculated.

2.6.2 α-Amylase Inhibition Assay

α-Amylase inhibitory activity was conducted using porcine pancreas α-amylase and soluble starch as described by Kareem et al. (2022). The reaction mixture contained 500 µL of extract, 500 µL of enzyme solution (0.5 mg/mL in sodium phosphate buffer), and 500 µL of 1% starch solution. After incubation at 37°C for 15 minutes, 1.0 mL of DNSA reagent was added to terminate the reaction. The mixture was boiled for 5 minutes, cooled, and diluted with distilled water. Absorbance was measured at 540 nm.

2.6.3 Angiotensin-Converting Enzyme (ACE) Inhibition Assay

ACE inhibitory activity was assessed using the method of Cushman and Cheung (1971) with hippuryl-histidyl-leucine as substrate. The reaction mixture contained 50 µL of extract, 50 µL of ACE solution (4 mU/mL), and 150 µL of substrate solution. After incubation at 37°C for 30 minutes, the reaction was terminated with 1 M HCl. The hippuric acid produced was extracted with ethyl acetate, dried, and redissolved in water. Absorbance was measured at 228 nm.

2.7 Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation. Analysis of variance (ANOVA) was performed using SPSS 21 software, followed by Duncan's Multiple Range Test for mean comparison at 95% confidence level ($p < 0.05$).

3. Results

3.1 Phytochemical Composition

The phytochemical composition of orange-fleshed sweet potato enhanced with D. microcarpum is presented in Table 1. The results revealed significant variations in bioactive compound content among the different sample compositions.

Table 1: Phytochemical composition of orange-fleshed sweet potato enhanced with Detarium microcarpum

Sample	Total Phenolics Content (mg/g)	Total Tannins Content (mg/g)	Total Flavonoids Content (mg/g)	Total Saponin Content (mg/g)
Detarium microcarpum	3.59±0.05c	2.63±0.03d	0.17±0.02d	2.61±0.01c
Orange potato	16.68±0.08a	12.78±0.75c	0.34±0.03c	8.16±0.01a
95%OP-5%DM	14.35±0.54b	14.49±0.28b	0.43±0.03b	8.15±0.04a
90%OP-10%DM	16.57±1.46a	16.78±0.23a	0.37±0.01c	8.11±0.03a
Wheat	4.07±0.18c	12.37±0.29c	0.82±0.01a	6.32±0.14b

Data represent mean ± standard deviation of duplicate analysis. Values followed with different superscript letters along the same column vary significantly at $p < 0.05$. OP (Orange potato), DM (*Detarium microcarpum*).

Orange-fleshed sweet potato (100%) displayed the highest total phenolic content (16.68±0.08 mg/g) and saponin content (8.16±0.01 mg/g). The 90% orange potato: 10% D. microcarpum blend showed the highest tannin content (16.78±0.23 mg/g), while the 95% orange potato: 5% D. microcarpum blend demonstrated the highest flavonoid content (0.43±0.03 mg/g). Enhancement with D. microcarpum resulted in

significant increases in tannin and flavonoid content compared to the unenhanced orange potato control.

3.2 Antioxidant Activity

The antioxidant activities of the samples are presented in Table 2, showing significant variations in reducing power, DPPH scavenging ability, and ABTS radical scavenging capacity.

Table 2: Antioxidant activity of orange-fleshed sweet potato enhanced with Detarium microcarpum

Sample	**Reducing Power (mgGAE/g)**	**DPPH SC_{50} (mg/mL)**	**ABTS Scavenging ability (µM/gdw)**
Detarium microcarpum	1.99±0.01d	1.50±0.02d	9.29±0.69b
Orange potato	4.38±0.02b	1.63±0.06cd	10.66±0.13b
95%OP-5%DM	4.55±0.01a	1.89±0.01b	10.02±0.23b
90%OP-10%DM	4.16±0.00c	1.75±0.17bc	9.30±0.86b
Wheat	1.09±0.04e	2.68±0.01a	15.68±0.01a

Data represent mean ± standard deviation of duplicate analysis. Values followed with different superscript letters along the same column vary significantly at $p < 0.05$. OP (Orange potato), DM (*Detarium microcarpum*).

The 95% orange potato: 5% D. microcarpum blend exhibited the highest reducing power (4.55±0.01 mgGAE/g) and demonstrated superior DPPH scavenging activity with the lowest SC_{50} value (1.89±0.01 mg/mL) among the enhanced samples. For ABTS assay, wheat flour showed the highest scavenging ability (15.68±0.01 µM/gdw), while the enhanced samples showed moderate activities.

3.3 Enzyme Inhibitory Activity

The enzyme inhibitory activities of the samples are presented in Table 3, showing the IC_{50} values for α-amylase, α-glucosidase, and angiotensin-converting enzyme inhibition.

Table 3: Enzyme inhibitory activity of orange-fleshed sweet potato enhanced with Detarium microcarpum

Sample	Alpha-amylase IC_{50} (mg/mL)	Alpha-glucosidase IC_{50} (mg/mL)	Angiotensin I-Converting Enzyme IC_{50} (mg/mL)
Detarium microcarpum	12.13±0.49c	12.13±0.49c	13.38±0.08b
Orange potato	35.51±2.91a	13.98±0.05b	11.08±0.18c
95%OP-5%DM	32.78±0.19ab	14.77±0.21ab	15.29±0.39a
90%OP-10%DM	30.39±1.39b	15.04±0.50a	11.32±0.31c
Wheat	20.96±0.88c	11.68±0.05c	13.58±0.08b

Data represent mean ± standard deviation of duplicate analysis. Values followed with different superscript letters along the same column vary significantly at $p < 0.05$. OP (Orange potato), DM (*Detarium microcarpum*).

D. microcarpum (100%) demonstrated the strongest α-amylase and α-glucosidase inhibitory activities with the lowest IC_{50} values (12.13±0.49 mg/mL for both enzymes). Orange potato (100%) showed the strongest ACE inhibitory activity (IC_{50} = 11.08±0.18 mg/mL). The enhanced samples generally exhibited reduced enzyme inhibitory capacity compared to their individual components.

4. Discussion

4.1 Phytochemical Composition

The phytochemical analysis demonstrated that enhancement of orange-fleshed sweet potato with D. microcarpum significantly improved the bioactive compound profile of the composite samples. The elevated phenolic content observed in orange-fleshed sweet potato (16.68±0.08 mg/g) corresponds with previous reports emphasizing the rich phytochemical composition of this crop (Adebayo et al., 2021). The significant increase in tannin content upon enhancement with D. microcarpum demonstrates the synergistic effect of combining these two plant materials.

Tannins are recognized for their anti-diabetic and anti-hypertensive properties, making their enhanced presence in the supplemented samples particularly valuable for functional food applications (Irondi et al., 2021). The increase in flavonoid content in the 95% orange potato: 5% D. microcarpum blend (0.43±0.03 mg/g) suggests optimal bioactive compound interaction at this enhancement level.

The substantial saponin content maintained across the enhanced samples (>8.0 mg/g) is significant, as saponins exhibit various health benefits including anti-obesity, antioxidant, and anti-diabetic activities (Lu et al., 2020). The preservation of high saponin levels in the enhanced products indicates that D. microcarpum enhancement does not compromise this important bioactive component.

4.2 Antioxidant Activity

The antioxidant activity results demonstrate that enhancement with D. microcarpum can improve the free radical scavenging capacity of orange-fleshed sweet potato. The superior reducing power exhibited by the 95% orange potato: 5% D. microcarpum blend (4.55±0.01 mgGAE/g) suggests that this composition provides optimal antioxidant enhancement. This finding is consistent with the principle that moderate enhancement levels often yield synergistic effects in bioactive compound interactions (Nwosu et al., 2022).

The DPPH radical scavenging results indicate that while pure D. microcarpum showed the strongest individual activity (SC_{50} = 1.50±0.02 mg/mL), the enhanced blends maintained effective antioxidant capacity. The relationship between phenolic content and antioxidant activity observed in this study corroborates previous research demonstrating the positive correlation between these parameters (Zhang et al., 2022).

The ABTS assay results provide additional evidence of the antioxidant capacity of the enhanced samples. The moderate ABTS scavenging activities observed in the enhanced samples (9.30-10.66 μM/gdw) suggest that these products retain significant antioxidant potential suitable for functional food applications.

4.3 Enzyme Inhibitory Activity

The enzyme inhibitory activity results reveal complex interactions between the enhancement components. While D. microcarpum alone demonstrated strong α-amylase and α-glucosidase inhibitory activities, the enhanced samples showed reduced inhibitory capacity. This phenomenon may be attributed to potential antagonistic interactions between bioactive compounds from different plant sources or dilution effects (Ademiluyi et al., 2024).

The strong ACE inhibitory activity of orange-fleshed sweet potato (IC_{50} = 11.08±0.18 mg/mL) highlights its potential application in managing hypertension. The maintenance of reasonable ACE inhibitory activity in the enhanced samples suggests that the antihypertensive potential is preserved despite enhancement.

The differential enzyme inhibitory responses observed suggest that the mechanism of action may vary depending on the specific enzyme system and the bioactive compound profile of each sample. This complexity underscores the importance of comprehensive bioactivity evaluation in functional food development (Kareem et al., 2022).

4.4 Implications for Functional Food Development

The results of this study demonstrate that D. microcarpum enhancement can improve specific bioactive properties of orange-fleshed sweet potato while maintaining others. The optimal enhancement level appears to be 5% D. microcarpum, which provides improved antioxidant activity and enhanced phytochemical profile without significantly compromising enzyme inhibitory activities.

The enhanced tannin and flavonoid content in supplemented samples, combined with maintained saponin levels, suggests potential applications in developing functional foods targeting metabolic health. The preservation of antioxidant capacity in

enhanced products supports their potential use in addressing oxidative stress-related health conditions (Okonkwo et al., 2023).

5. Conclusions

This study successfully demonstrates that enhancement of orange-fleshed sweet potato with D. microcarpum improves the bioactive compound profile and antioxidant capacity of the composite products. The key findings include:

1. Enhancement with D. microcarpum significantly increased tannin and flavonoid content while maintaining high saponin levels in the composite samples.
2. The 95% orange potato: 5% D. microcarpum blend showed optimal antioxidant enhancement with the highest reducing power and effective DPPH scavenging activity.
3. While enzyme inhibitory activities were reduced in enhanced samples compared to individual components, the products retained significant bioactivity suitable for functional food applications.
4. D. microcarpum serves as an effective natural additive for improving the nutritional and functional properties of orange-fleshed sweet potato products.

The study contributes to the development of nutrient-dense, functional food products that could address malnutrition and support health promotion in regions where both crops are readily available. The enhanced bioactive composition of the supplemented products suggests potential applications in developing foods with targeted health benefits.

6. Recommendations

Based on the findings of this study, the following recommendations are proposed:

1. **In-vivo validation studies**: The bioactive properties and health benefits observed in this in-vitro study should be validated through animal and human intervention studies to confirm their physiological relevance.

2. **Optimization studies**: Further research should focus on optimizing the enhancement ratio and processing conditions to maximize bioactive compound retention and bioavailability.
3. **Product development**: The composite flour should be incorporated into various food products (bread, biscuits, porridge) to evaluate sensory acceptability and nutritional quality.
4. **Shelf-life studies**: Comprehensive storage stability studies should be conducted to determine the optimal storage conditions and shelf-life of the enhanced products.
5. **Scaling up**: Pilot-scale production studies should be undertaken to evaluate the commercial viability of producing D. microcarpum-enhanced orange-fleshed sweet potato products.
6. **Bioavailability studies**: Research should investigate the bioavailability of bioactive compounds in the enhanced products using appropriate in-vitro and in-vivo models.
7. **Sustainable sourcing**: Strategies for sustainable cultivation and harvesting of D. microcarpum should be developed to ensure consistent supply for commercial applications.

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