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Antioxidant properties, antidiabetic activity, and GC-MS phytochemical analysis of wheat-based bread fortified with *celosia argentea* seed flour

Lukuman Akande Azeez[1]
Kafayat Adebukola Babalola[2]
Oludolapo Akinyemi Osunrinade[3+]

[1,2,3]*Department of Food Science and Technology, The Oke-Ogun Polytechnic Saki, Oyo State, Nigeria.*
[1]*Email: azeezlukuman02@gmail.com*
[2]*Email: buki_kay_01@yahoo.com*
[3]*Email: dolaps2004@yahoo.com*

(+ Corresponding author)

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ABSTRACT

Pseudocereals like *Celosia argentea* seed have been indicated to have numerous therapeutic and prophylactic potentials that are yet to be incorporated into food materials. This study evaluated the antioxidant properties, antidiabetic potential, and phytochemicals of wheat-based bread fortified with Celosia argentea seed flour. Bread samples were produced using standard methods, fortifying refined and whole wheat with 5 and 10% *Celosia argentea* seed flour. The antioxidant properties and α-amylase inhibition of bread samples were determined using standard methods. The phytochemicals present in the methanolic extracts of bread samples were identified with Gas Chromatography-Mass Spectrometry (GCMS). Statistical significance was tested at $\alpha_{0.05}$. The result of the antioxidant properties for bread samples were Total flavonoid content (0.61-0.85 mg/g), Total Phenolic Content (1.1-1.31 mgGAE/g), total antioxidant capacity (1.19-3.34 mgGAE/g), Ferric reducing antioxidant power (0.13-0.54 mg/g) and DPPH (42.58-49.75%). The IC_{50} values obtained for *Celosia argentea* seed substituted wheat bread ranged between 58.20 and 171.05 µg/mL. The antioxidant properties and α-amylase inhibition showed a significant ($p<0.05$) increase as the percentage of inclusion of *Celosiae argentea* seed increased. Bioactive compounds detected in bread samples produced by the fortification of wheat flour (whole and refined) with *Celosia argentea* seed flour include 9,12-Octadecadienoic acid methyl ester; Docosanoic acid, methyl ester; E,E,Z-1,3,12-Nonadecatriene-5,14-diol; Linoleic acid ethyl ester; and Squalene. The predominant health benefits of phytochemicals detected in bread samples fortified with C. argentea seed were anti-inflammatory potentials, antioxidant protection, and reduction of lipid peroxidation. Including Celosia argentea seed impacted the antioxidant and antidiabetic potential of bread samples.

Contribution/Originality: This study utilised *Celosia argentea* seed flour to produce bread with functional attributes such as antidiabetics and antioxidant potential.

1. INTRODUCTION

Advances in functional food production is focused on the use of natural food additives and its incorporation into commonly consumed food products. The consumer's awareness of the need to eat healthy foods known as functional foods, that is, foods beyond the basic nutritional requirements, has increased [1]. Consumption of functional foods

does not only improve the nutritional status of the general population but also helps those suffering from degenerative diseases associated with today's changing lifestyles [2, 3].

Over the years, due to it ready to eat nature, specialty breads have been produced from whole-grain flour and other functional ingredients known as functional breads [4, 5]. The nutritional value of the bread depends on the type of flour used and on the variety of other ingredients added to the production.

Potent antioxidants and hypoglycemic properties have severally been reported to be a major components of plant families [6-9]. The discovery of new compounds from plants with therapeutic value against most common and very prevalent disease emphasized the importance of plants in human diet. Majority of the plants that have therapeutic application possess bioactive composites viz., alkaloids, glycosides, tannins, flavonoids, saponins, phenolics and vitamins [10-12].

Celosiae argentea L. is an annual herb that belongs to the Amaranthaceae family [13]. In folklore practice, C. argentea seeds' decoction has been reported to be helpful in diabetes mellitus [14, 15]. C. argentea seed has a theoblate, black, or reddish black seed. Findings have reported that semen Celosiae possesses miscellaneous pharmacological functions, which include antioxidant, hepatoprotection, antitumor, antidiarrhea, and antidiabetes [14, 16, 17].

Diabetes mellitus has become a global burden, affecting around 25% of the world population of both developed and developing countries [18, 19]. It has been projected that diabetes will be among the leading causes of death in 2030 [20, 21]. Diabetes cases are exponentially increasing due to societal influence and lifestyles, which can related to food products [22, 23]. In modern medicine, there is still no reasonably effective therapy or drug to cure diabetes [24]. One practical therapeutic approach to managing diabetes is by controlling postprandial hyperglycemia through daily meals [25, 26].

Functional bread-making aims to create bread fortified with physiologically functional ingredients [27]. Scholars [28-30] have highlighted concerns regarding the potential degradation or alteration of bioactive compounds in various flours during heat treatment and the baking process. The antioxidant capacity of bakery products is heavily influenced by factors such as manufacturing techniques, recipes, dough mixing, and kneading [27]. Additionally, research suggests that incorporating ground seeds may offer enhanced access to the bioactive compounds' benefits [31, 32]. Existing literature reveals investigations into the antioxidant and antidiabetic properties of Celosiae argentea L seed. However, there is a notable gap in understanding its utilization in fortifying wheat flour for bread production.

2. MATERIALS AND METHODS

2.1. Materials

Bakery facilities at the Department of Food Technology, The Oke-Polytechnic Saki, were used. *Celosia argentea* seed was obtained from local vegetable farmers in Saki, Oyo State, Nigeria. Bread-making raw materials such as yeast, sugar, refined wheat flour, salt, and margarine were obtained from local markets in Saki, Oyo State, Nigeria. All chemicals used were of analytical standard.

2.2. Sample preparation

2.2.1. Preparation of C. Argentea flour

Fresh whole plants of C. argentea were uprooted when the plants were in full bloom. The plants were identified and authenticated by a botanist at the Department of Crop Production, the Oke-Ogun Polytechnic Saki. The seeds were collected from the mature plants, shade-dried, and milled (80 mesh) using the laboratory manually operated attrition mill.

2.3. Bread Production

Bread samples were prepared following a standard formulation adapted from [33] using flour percentages, including *Celosiae argentea* seed flour of 5 and 10%, as presented in Table 1 The dough was prepared using an optimised straight-dough bread-making method [34]. The ingredients (Table 1) were mixed (for 10 min) in a stand mixer (Rohnson stand mixer, SC-623, Italy). The resulting dough was cut (200 g pieces), kneaded and shaped into a cylindrical shape prior to placement in baking pan (13 cm x 6 cm x 9.5 cm). Dough proofing was done in a proofing cabinet for 40 min at 38 °C. A static oven operated for 45 min at 220 °C was used for the baking operation. The resulting bread samples were carefully placed on the table in a clean cooling chamber until they reached room temperature. The cooled bread samples were packaged in a low-density polyethylene bag for further analysis.

Table 1. Bread formulation for Wheat based bread fortified with *C. argentea* seed.

Samples	HHT	LTF	TTS	FHC	WET	RWO
Whole wheat (g)	900	950	--	--	1000	--
Refined wheat (g)	---	---	900	950	--	1000
C. argentea flour (g)	100	50	100	50	--	--
Yeast (g)	30	30	30	30	30	30
Sugar (g)	40	40	40	40	40	40
Margarine (g)	50	50	50	50	50	50
Salt (g)	15	15	15	15	15	15
Water (ml)	600	600	600	600	600	600

Note: WETF – Whole Wheat Flour, RWOF – Refined Wheat Flour, CCSF – Celosia Argentea Seed Flour, WET – Whole Wheat Bread, RWO - Refined Wheat Bread, LTF – 95% Whole Wheat + 5 % Celiosia argentea seed, HHT – 90% Whole Wheat + 10 % Celiosia argentea seed, FHC – 95% Refine Wheat + 5 % Celiosia argentea seed, TTS - 90% Refine Wheat + 10 % Celiosia argentea seed.

2.4. Bread Sample Extraction for Analysis

The method outlined by Akinyemi, et al. [35] was employed to extract flour and bread samples for antioxidant analysis. In brief, bread samples were sliced into pieces with dimensions of 3 cm width and 1 cm thickness, then air-dried for a duration of 24 hours. Subsequently, the dried bread samples were finely ground using a hand-operated attrition mill. Flour and bread samples weighing 1g each were then subjected to extraction with 20 mL of 80% ethanol for a period of 48 hours, with intermittent agitation. The mixture was immediately filtered with Whatman No. 1 filter paper, and the resulting filtrate was kept at 4°C in a refrigerator for the analysis of samples' antioxidant properties.

2.5. Antioxidant Properties of Bread Samples

2.5.1. Determination of the Total Phenolic Content (TPC)

The TPC of bread samples was determined by the method of Akinyemi, et al. [35]. Briefly, the extract (0.2 mL) was allow to react with 1 mL of Folin–Ciocalteu's reagent previously diluted five times, prior to that addition of 7.5% Na_2CO_3 (0.8 mL). The reacting mixture was kept in the dark for 20 minutes at ambient temperature, and the absorbance was measured at 765 nm against a blank mixture. The TPC results were determine in mg of gallic acid equivalent (GAE) per gram of samples' dry weight.

2.5.2. Determination of Total Flavonoid Content (TFC)

The bread samples TFC determination was done according to Saikia, et al. [36]. Specifically, the bread sample extract (0.25 mL) and a standard catechin solution in triplicate were diluted with distilled water (1.25 mL). While 75 µL of a 5% $NaNO_3$ solution was added to each sample, before incubation for 6 minutes at ambient temperature. Then, 10% $AlCl_3$ (150 µL) was introduced to the reacting mixture prior to further incubation for 5 minutes. This as followed by the addition of 0.5 mL of 1 M NaOH solution to the reacting mixture which was immediately made up to 3 mL with distilled water. The resulting mixture absorbance was read at 510 nm using a spectrophotometer

(JENWAY, Model 7305). Standard catechin solution was used in place of samples to construct calibration curve that was used to determine the concentration of TFC in the bread samples in milligrams per gram.

2.5.3. Total Antioxidant Capacity of Milled Rice

The total antioxidant capacity of bread samples was determined using the method described by Akinyemi, et al. [35]. Briefly, the phosphomolybdenum reagent was prepared by thorough mixing of sulfuric acid (3.3 mL), sodium phosphate (335 mg), and ammonium molybdate (78.4 mg) in 100 mL of distilled water. The bread sample extract (0.5 mL) was mixed with the phosphomolybdenum reagent (7.5 mL), and the mixture was placed in water bath that operated at 95°C for 90 minutes. The resulting mixture was cooled and the absorbance read at 695 nm using a spectrophotometer. A standard curve was constructed using various concentrations of gallic acid as substitute for the bread extract. The equation derived from the standard curve was used to estimate the total antioxidant capacity of the bread extract milligrams of gallic acid equivalents per gram.

2.5.4. 1,1, Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Assay for Bread Samples

The DPPH radical scavenging assay reported by Akinyemi, et al. [35] was used to determine the radical scavenging potential of flour and bread samples. Breiefly, 4 mg of DPPH was thoroughly dissolved in 100 mL methanol. Sample extract (0.1 mL) was mixed with DPPH (0.3 mL) solution and the resulting mixture was placed in the dark for 30 minutes. The absorbance of the resulting mixture was measured at 516 nm. A control mixture was prepared using the DPPH reagent without the sample extract. The extract inhibition of DPPH reagent was determined in percentage using Equation 1.

$$Percentage\ inhibition = \frac{Ac - Ae}{Ac} \times 100\% \quad (1)$$

Where Ac = Absorbance of control.

Ae = Absorbance of extract.

2.5.5. Determination of Ferric Reducing Antioxidant Power (FRAP)

The method described by Sukrasno, et al. [37] was used to determine the FRAP of flour and bread samples. Briefly, the mixing of Acetate buffer, tripyridyltriazine TPTZ, and $FeCl_3.6H2O$ at a ratio of 10:1:1, respectively was used to prepare the FRAP reagent.

Sample extract (0.3 mL) diluted with distilled water (0.7 mL), was mixed with the FRAP reagent (2.85 mL). The resulting mixture was incubated for 20 minutes at 50°C before measuring its absorbance at 700 nm. A standard curve was constructed using ascorbic acid, to estimate the antioxidant power of the bread samples.

2.6. In-Vitro Alpha-Amylase Inhibitory Assay of Bread Samples

The method described by Shettar, et al. [38] was used to determine the Alpha-amylase inhibitory potential of methanolic extract of bread samples. In this method the sample extract (0.5 mL) mixed with 0.5 mL of α-amylase solution (0.5 mg/mL) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was incubated at ambient temperature for 10 minutes.

Then 0.5 mL of starch solution (1%) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added, and the resulting mixture was incubated for another 10 Minutes at ambient temperature. The reaction was terminated by the addition of dinitrosalicylic acid (1 ml) color reagent. Subsequently, the test tubes were placed in a water bath at 100°C for 5 minutes and then allowed to cool to room temperature. The mixture was diluted with 10 mL of deionized water, and the absorbance was measured at 540 nm. The absorbance of blank samples (buffer instead of extract and amylase solution) and control samples (buffer instead of extract) were also determined. Acarbose was used as a standard drug for comparison. The inhibition of α-amylase was calculated using Equation 2.

$$\% \text{ inhibition of } \alpha - \text{Amylase} = \frac{Abs_{Control} - Abs_{sample}}{Abs_{Control}} \times 100 \quad (2)$$

Where

$Abs_{control}$ = absorbance of the solution without bread extract (buffer instead of extract) and with α − amylase solution

Abs_{sample} = absorbance of solution with bread extract extract and α – amylase solution

2.7. GC-MS Profiling of Bread Samples Extracts

The analysis was performed using the Shimadzu Gas Chromatography-Mass Spectrometer (GC-MS) instrument, specifically the Pegasus 4D model from LECO Corporation, based in St. Joseph, MI, USA. A fused silica column was utilized, and helium served as the carrier gas at a constant flow rate of 1 mL/min. A 1 µL bread sample extract was injected into the instrument for analysis. The temperature parameters were set as follows: the initial temperature was maintained at 100°C, while the injector temperature was set to 250°C. Throughout the analysis, the temperature ramped up at a rate of 10°C/min. Separation of components occurred, and at the 24th minute, the final temperature was adjusted to 280°C, where it was held for 5 minutes [38]. Compounds were identified by comparing their mass spectra with library spectra databases, particularly those provided by the National Institute of Standards and Technology (NIST). The biological activity of all identified compounds was determined through an extensive literature survey.

2.8. Statistical Analysis

All experiments were performed in triplicates (n= 3), and the data are presented as the mean ± standard error. Differences between the means of the individual groups were analysed for variance using SPSS software version 20 (IBM). The significance of differences was defined at the $p < 0.05$ level.

3. RESULTS AND DISCUSSION

3.1. Antioxidant Properties of Flour and Bread Samples

To assess the antioxidant properties of bread produced from whole wheat flour fortified with Celosia argentea seed flour, various parameters, including total antioxidant capacity, total flavonoid content, ferric reducing antioxidant power (FRAP), total phenolic content, and DPPH were evaluated, and the results are presented in Table 2. Analysis of the flour samples revealed that Celosia argentea seed flour exhibited the highest antioxidant properties, followed by whole wheat flour. Conversely, refined wheat flour demonstrated the lowest values for all evaluated antioxidant properties in this study, except for total phenolic content. These findings align with previous research indicating that phenolic compounds are primarily concentrated in the outer layers of grains [39].

Borrelli, et al. [39] noted that 83% of the total phenolic compound in whole meal flour is located on the bran/germ fraction. The TPC (1.21 mgGAE/g) obtained for refined wheat flour in this work is lower but not significantly different from 1.26 mgGAE/g obtained by Ali, et al. [33] but higher than 0.64 mgGAE/g reported by Paucar-Menacho, et al. [40]. The total flavonoid content of refined wheat flour obtained by Ali, et al. [33] was lower than that obtained in this work. However, the result of TPC obtained for the whole (1.42 mgGAE/g) and refined wheat (1.21 mgGAE/g) flour in this study were within the range (0.896 – 1.61 mgGAE/g) reported by Borrelli, et al. [39] for different species and genotype of wheat flour. The TPC (1.63 mgGAE/g) of Celosia argentea seed flour obtained in this work is higher than that of amaranth (0.12 – 0.72 mgGAE/g), within the range of buckwheat products (1.46 – 6.78 mgGAE/g) and Quinoa (0.97 – 2.26 mgGAE/g) reported by Škrovánková, et al. [41] work on polyphenol and antioxidant activity in pseudocereals and their products. The TPC (1.45 mgGAE/g) of sprouted kiwicha (pseudocereal) flour reported by Paucar-Menacho, et al. [40] was lower than that obtained for Celosiae argentea seed flour.

The total antioxidant capacity of bread samples increased with the inclusion of Celosia argentea seed flour from 1.28 to 3.34 mgGAE/g for whole wheat-based bread and from 1.19 to 1.45 mgGAE/g for refined wheat-based bread. An Increase in Celosia Argentea seed flour percentage from 5 to 10% led to a significant increase in the TAC of bread samples. In contrast, there was no significant difference in TAC between bread samples produced from 5% and 10% inclusion of C. argentea seed flour in refined wheat flour. The result of TFC showed that the highest value (0.85mg/g) was obtained from bread produced from whole wheat flour supplemented with 10% Celosia argentea seed flour. At the same time, the lowest value (0.63mg/g) was from a bread sample baked with refined wheat flour. The increase in the percentage inclusion of C. argentea seed caused a significant increase in the TFC for whole and refined wheat flour bread. Celosia argentea seed addition to both whole and refined wheat flour increased the FRAP of whole wheat flour bread from 0.21 mg/g to 0.54 mg/g, while refined wheat flour bread from 0.13 to 0.37 mg/g. a marked increase in FRAP value was also reflected in the increment of inclusion of C. argentea seed from 5% to 10% for both whole and refined wheat flour. The TPC of the bread sample from whole wheat flour supplemented with 10% C. argentea seed flour had the highest value (1.31 mgGAE/g), while the lowest value of 1.10 mgGAE/g was from refined wheat flour. Generally, the addition of C. argentea seed flour to whole wheat and refined wheat flour significantly increased the TPC of bread samples produced. However, bread samples from whole wheat flour had significantly ($p<0.05$) higher values compared with C. argentea-supplemented refined wheat flour bread. The highest value of whole wheat bread is an offshoot of the high phenolic content of whole wheat flour. The result of this work agreed with the output of inclusion of pseudocereals like sprouted kiwicha and cañihua by Paucar-Menacho, et al. [40] who observed a significant increase in the TPC of bread samples up to 1.23 mgGAE/g and 2.51 mgGAE/g, respectively at 15% level of inclusion. The DPPH of the bread samples varied from 42.88 to 49.75%. Including C. argentea seed flour increased the DPPH of bread samples for whole wheat and refined wheat flour. There was a significant increase in DPPH as the percentage inclusion of C. argentea seed flour increased from 5% to 10% for whole wheat flour. In contrast, an increase in the percentage inclusion of C. argentea seed flour to refined wheat flour did not cause a significant increase in the percentage of DPPH value of the bread sample. The result of this work agrees with the work of Chlopicka, et al. [42] who recorded an increase in the TPC, TFC, FRAP, and DPPH as the percentage of pseudocereals (Buckwheat, amaranth, and Quinoa) inclusion in bread formulation increases. The findings of Keshani, et al. [43] also indicated higher antioxidant capacity and TPC of quinoa-wheat bread compared to wheat bread.

Table 2. Antioxidant properties of flour and bread samples.

Samples	Total antioxidant capacity mgGAE/g	Total flavonoid content mg/g	FRAP mg/g	Total phenolic content mgGAE/g	DPPH %
Flour samples					
WETF	$1.42^{c}\pm0.03$	$0.73^{d}\pm0.04$	$0.56^{b}\pm0.09$	$1.47^{a}\pm0.05$	$46.46^{cd}\pm1.00$
RWOF	$0.64^{b}\pm0.02$	$0.57^{h}\pm0.05$	$0.51^{b}\pm0.09$	$1.21^{c}\pm0.03$	$43.25^{e}\pm1.20$
CCSF	$1.63^{b}\pm0.13$	$1.14^{a}\pm0.04$	$0.70^{a}\pm0.05$	$1.35^{b}\pm0.02$	$62.30^{a}\pm2.32$
Bread samples					
WET	$1.28^{cd}\pm0.20$	$0.69^{de}\pm0.02$	$0.21^{a}\pm0.06$	$1.25^{c}\pm0.03$	$45.18^{de}\pm1.28$
RWO	$1.19^{d}\pm0.11$	$0.63^{fg}\pm0.01$	$0.13^{a}\pm0.02$	$1.10^{e}\pm0.04$	$42.88^{e}\pm1.59$
LTF	$1.35^{cd}\pm0.03$	$0.81^{c}\pm0.01$	$0.34^{c}\pm0.02$	$1.15^{d}\pm0.01$	$44.69^{de}\pm1.5$
HHT	$3.34^{a}\pm0.05$	$0.85^{b}\pm0.01$	$0.54^{b}\pm0.03$	$1.31^{b}\pm0.01$	$49.75^{b}\pm1.10$
FHC	$1.42^{c}\pm0.03$	$0.61^{g}\pm0.01$	$0.18^{a}\pm0.00$	$1.13^{de}\pm0.01$	$47.86^{bc}\pm0.50$
TTS	$1.45^{c}\pm0.03$	$0.67^{ef}\pm0.01$	$0.37^{c}\pm0.01$	$1.20^{c}\pm0.01$	$48.98^{b}\pm0.41$

Note: Means followed by the same letter down the column are not significantly different ($p<0.05$) from one another
WETF – Whole Wheat Flour, RWOF – Refined Wheat Flour, CCSF – Celosia Argentea Seed Flour, WET – Whole Wheat Bread, RWO - Refined Wheat Bread, LTF – 95% Whole Wheat + 5 % Celiosia argentea seed, HHT – 90% Whole Wheat + 10 % Celiosia argentea seed, FHC – 95% Refine Wheat + 5 % Celiosia argentea seed, TTS - 90% Refine Wheat + 10 % Celiosia argentea seed.

3.2. α-amylase Inhibition Activities of Celosia Argentea-Wheat Flour Bread Extracts

α-amylase is a digestive enzyme responsible for breaking down carbohydrate foods within the human body. The activity of these enzymes results in hyperglycemia, i.e., the amount of glucose within the bloodstream, resulting in a metabolic disorder known as diabetic mellitus. Prolonged digestion as a result of inhibition of α-amylase activity on carbohydrates results in low availability of glucose for absorption and ultimately low blood glucose level. As such, this could be a helpful technique in the management of *diabetic mellitus* [44]. However, this extension has been reported to be associated with health-related conditions such as flatulence resulting from carbohydrate fermentation within the system [45].

The $IC_{50,}$ i.e., the concentration of extract containing the enzyme inhibitor that inhibited the activity of α-amylase by 50% for all the bread sample extracts, is presented in Table 3. The IC_{50} values obtained for *Celosia argentea* seed substituted wheat bread ranged between 58.20 to 171.05 µg/mL for 10 and 5% inclusion in whole wheat bread, respectively. These values were lower than 198.4µg/mL reported for *Stevia rebaudiana* extract functional bread [46].

The refined wheat bread extract had the highest value of 312 µg/mL and the corresponding lowest percentage inhibition value (3.35%). This was followed by whole wheat bread extract with the values of 201.61µg/mL and 10.73% for IC_{50} and percentage inhibition, respectively.

Inclusion of *Celosia argentea* seeds in wheat bread results in a reduction of IC_{50} with a proportionate increment in percentage inhibition of α-amylase activity, 10% *Celosia argentea* seed substitution in refined wheat flour bread produced extract that requires double the concentration (132.06 µg/mL) of IC_{50} than the value for the standard acarbose (56.44 µg/mL). In contrast, approximately the same concentration of IC_{50} is necessary for the extract of 10% *Celosia argentea* seed flour inclusion in whole wheat flour bread (58.20 µg/mL) when compared with the standard acarbose; this value was higher than the value of 51.84 µg/mL documented for aqueous extract of *Ximenia Americana* [38].

The 28.15% and 30.79% inhibitions were obtained for 10% of Celosia argentea seed flour substituted in refined wheat and whole wheat bread extract, respectively. These values were compared favourably with the reference acarbose value (33.68%) α-amylase percentage inhibition value. The inclusion of *Celosia argentea* seeds reduces the activity of α-amylase, thereby slowing down the rate at which carbohydrate metabolism occurs; hence, this seed could be a raw material in the management of *diabetes mellitus.*

Table 3. α-amylase inhibition activities and IC_{50} of *Celosia argentea*-wheat flour bread extracts.

Samples	% inhibition	IC_{50} (µg/mL)
5% C. argentea-refined wheat	19.21 ± 0.61^{e}	171.05 ± 1.94^{c}
10% C. argentea-refined wheat	28.15 ± 0.24^{c}	132.06 ± 1.81^{d}
5% C. argentea- whole wheat	24.01 ± 0.33^{d}	121.83 ± 1.48^{e}
10% C. argentea- whole wheat	30.79 ± 0.47^{b}	58.20 ± 0.35^{f}
Whole wheat	10.73 ± 0.01^{f}	201.61 ± 0.52^{b}
Refined wheat	3.35 ± 0.32^{g}	312.00 ± 0.79^{a}
Acarbose	33.68 ± 1.48^{a}	56.44 ± 1.72^{f}

Note: Means followed by the same letter down the column are not significantly different ($p<0.05$) from one another
WETF – Whole Wheat Flour, RWOF – Refined Wheat Flour, CCSF – Celosia Argentea Seed Flour, WET – Whole Wheat Bread, RWO - Refined Wheat Bread, LTF – 95% Whole Wheat + 5 % Celiosia argentea seed, HHT – 90% Whole Wheat + 10 % Celiosia argentea seed, FHC – 95% Refine Wheat + 5 % Celiosia argentea seed, TTS - 90% Refine Wheat + 10 % Celiosia argentea seed

3.3. Phytochemicals Detected by GCMS Profiling

Bioactive compounds Table 4 detected in bread samples produced by the fortification of wheat flour (Whole and refined) with *Celosia argentea* seed flour include 9,12-Octadecadienoic acid methyl ester; Docosanoic acid, methyl ester; E,E,Z-1,3,12-Nonadecatriene-5,14-diol; Linoleic acid ethyl ester; and Squalene. However, decanoic acid,

methyl ester was detected in bread samples produced from whole wheat bread and bread samples produced by fortification of whole and refined wheat flours with *Celosia argentea* seed flour. The functionality of decanoic acid, methyl ester reported are improvement of blood lipids, antioxidant protection, reduction of lipid peroxidation, and enhancement of insulin oral absorption [47].

Table 4. Compounds identified in bread samples.

S/N	Compound	Retention time	% peak area	Similarity	Sample	Reported activities
1	9,12-Octadecadienoic acid, methyl ester	11.271	35.75	93	HHT	Essential fatty acids, cardiovascular health, anti-inflammatory
		11.806	3.16	99	TTS	
		11.243	14.6	96	FFC	
		15.553	0.33	91	TTS	
2	Decanoic acid, methyl ester	5.778	1.87	96	FFC	Improve blood lipids, antioxidant protection, reduce lipid peroxidation, oral absorption enhancer for insulin
		5.778	0.54	94	HHT	
		5.778	0.36	97	LTF	
		5.778	1.61	95	TTS	
		5.778	1.66	94	WET	
3	Docosanoic acid, methyl ester	15.243	0.23	91	FFC	Cardiovascular health, cognitive function, reduced inflammation, joint health, reduced cancer risk, type 2 diabetes prevention.
		15.215	1.13	95	HHT	
		15.271	0.17	90	LTF	
		15.243	0.1	91	TTS	
4	Dodecanoic acid, methyl ester	6.905	7.71	97	FFC	Anti-inflammatory and anti-fibrotic effects
		6.905	3.04	98	HHT	
		6.905	1.81	94	LTF	
		6.877	7.88	96	TTS	
		6.905	4.06	97	WET	
5	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	10.849	0.06	81	HHT	Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) inhibition, antibacterial and antifungal properties
		16.821	1.51	86	LTF	
6	Hexadecanoic acid, methyl ester	9.694	10.38	98	FFC	Anti-inflammatory and anti-fibrotic effects:
		9.778	5.6	86	LTF	
		9.694	4.45	97	RW	
		9.665	8.7	98	TTS	
		9.694	3.39	98	WET	
7	Linoleic acid ethyl ester	11.806	2.93	99	FHC	Reduce total and low-density lipoproteins (LDL) cholesterol levels
		11.863	4.01	90	LTF	
		14.905	0.31	96	TTS	
8	Methyl 5,9,12-octadecatrienoate	14.004	1.11	87	LTF	Hypertension prevention, anti-inflammatory properties calcium absorption and osteoporosis: antitumor potential
		13.187	0.26	92	WET	
9	Squalene	17.553	4.77	99	FHC	Anticancer effects, improve the immune response to vaccines.
		17.553	3.48	99	HHT	
		17.637	3.29	98	LTF	
		17.553	21.65	99	TTS	

Note: WET – Whole wheat bread, RWO - refined wheat bread, LTF – 95% Whole Wheat + 5 % *Celiosia Argentea Seed*, HHT – 90% whole wheat + 10 % *Celiosia Argentea Seed*, FHC – 95% Refine Wheat + 5 % *Celiosia Argentea Seed*, TTS - 90% Refine Wheat + 10 % *Celiosia Argentea* seed

The detection of 9,12-Octadecadienoic acid methyl ester in bread samples produced from 5% and 10% Celosia argentea seed flour supplemented with whole and refined wheat is an indication of its potential for several health benefits, which include cell membrane and prostaglandin synthesis, cardiovascular health and anti-inflammatory properties [48]. 9, 12-Octadecadienoic acid methyl ester was also reported as the predominant compound in *Helleborus bocconei* subsp. Intermedius [48] and *Jatropha curcas* [49]. The therapeutical and antioxidant potential

of Docosanoic acid, methyl ester found in bread samples fortified with C. argentea seed flour, has been reported to be present in *crinum defixum ker-gawler* leaves [50] and different extracts of *Haloxylon stocksii (Boiss.) Benth* [51].

Dodecanoic acid, methyl ester, which possesses the ability to cure diseases that are caused by oxidative stress [52] was found in whole wheat flour bread samples. The presence of Dodecanoic acid, methyl ester was also detected in all bread samples produced with flour fortification at 5 and 10% of C. argentea seed flour.

The addition of Celosia argentea seed flour to whole wheat flour at 5% (LTF) and 10% (HHT) levels caused the presence of E,E,Z-1,3,12-Nonadecatriene-5,14-diol to bread samples produced. This compound has been reported for its potential inhibitory effects against SARS-CoV-2, the virus responsible for COVID-19. The antibacterial and antifungal properties of E,E,Z-1,3,12-Nonadecatriene-5,14-diol has also been reported [53].

Linoleic acid methyl ester reported for its health benefit in reducing total and LDL cholesterol levels [54] was found in all bread samples supplemented with Celosia argentea seed. Squalene was found only in bread samples supplemented with Celosia argentea seed flours. Squalene is an intermediate for cholesterol biosynthesis, has been proposed to act similarly to statins via inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in the liver [55]. The anticancer effect and improvement of immune response to the vaccine have been reported as the significant benefit of squalene [49, 56].

4. CONCLUSION

The increase in the percentage inclusion of C. argentea seed caused a significant increase in the antioxidative content and activities of whole and refined wheat flour bread. Also, the inclusion of *Celosia argentea* seeds reduces the activity of α-amylase, thereby slowing down the rate at which carbohydrate metabolism occurs; hence C argentea seed could be a raw material in managing *diabetes mellitus*. The predominant health benefits of phytochemicals detected in bread samples fortified with C. argentea seed were anti-inflammatory potentials, antioxidant protection, and reduction of lipid peroxidation.

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