Changes in the antioxidant properties of *Ocimum africanum* leaves aqueous extract from the southeastern region of Nigeria after in vitro gastrointestinal digestion

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**ABSTRACT**

The potential for antioxidant compounds to be released from the original food matrix is greatly determined by bioaccessibility. Digestive enzymes have a substantial impact on the antioxidant properties of dietary items after ingestion. The present study aimed to determine the antioxidant properties of *Ocimum africanum* leaves aqueous extract (OALAE) before and after *in vitro* digestion. *In vitro* gastro-pancreatic digestion of extracted samples (steamed, air dried and fried) was done using artificial gastric fluid, and 4 mL of artificial intestinal fluid. Antioxidant analyses were carried out on the OALAE samples (group 1: steamed, group 2: fried, group 3: air dried and group 4: garlic acid standard), before digestion (undigested), post-intestinal digestion with enzymes and post-intestinal digestion without enzymes. The trends of antioxidant properties of the OALAE (*in-vitro*) were as follows: air dried > steamed > fried. A significant \((p < 0.05)\) decrease in 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), ferric reducing antioxidant power (FRAP), total antioxidant capacity (TAC), reduced glutathione (GSH), vitamin E, vitamin C, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and non-heme iron content of OALAE in post-intestinal digestion with enzyme and post-intestinal digestion without enzyme was found compared to the undigested extract in group 1, 2, 3, and 4. Significant decrease was observed in antioxidant activity (illustrated using DPPH, ABTS, FRAP, and TAC) of post-intestinal digestion with enzyme compared to post-intestinal without enzymes in group 1, 2, 3 and 4. The results suggested that antioxidant properties of *O. africanum* leaves are unstable and may show limited activity after stimulated gastrointestinal digestion.

**Introduction**

Antioxidants phytochemicals are an integral part of the human diet because they are found in almost all plant-derived foods, such as vegetables and fruits, spices and herbs, and related processed food items.\(^{[1,2]}\) According to studies, using synthetic antioxidants in excess of a specific concentration can cause ailments in people.\(^{[3,4]}\) Enzymatic and non-enzymatic antioxidants serve as an imperative natural defense against contaminants. Superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX) are the most effective enzymatic antioxidants of the defense framework by which free radicals that are produced through metabolic responses are evacuated.\(^{[5]}\) Antioxidant activity is one of the most studied bioactive potential of phenolic compounds.\(^{[4]}\) Examples of common non-enzymatic
Antioxidant compounds are greatly determined by the bioaccessibility and bioavailability of bioactive molecules (the fraction of an ingested compound that, through the systemic circulation, reaches specific sites). However, in *vitro* digestion models based on human functioning are widely used for studying digestibility, structural changes, and release of the components that are present in food under simulated gastrointestinal conditions. A simulated digestion model has been used in a few research to examine how the bioavailability of polyphenols and the antioxidant qualities of spicy plants progress during digestion. In discern, phenolic compounds in green tea plant, predominantly catechins, show up to be profoundly steady beneath gastric conditions. However, they are to some degree touchier to near-neutral conditions (pH 6.0–7.5) within the small intestine due to the epimerization and auto-oxidation of catechins. Nevertheless, antioxidant activity of plants is mainly attributed to phenolic acids.

*Ocimum americanum* L., synonym *Ocimum africanum* is commonly known as American basil, lemon basil, hoary basil, and/or curry leaves. The plant contains antioxidants/phenolic compounds that avert impairment due to the action of reactive oxygen species (ROS). Leaf paste of the plant is used as a cure for parasitical skin diseases. *O. africanum* plant belongs to the Rutaceae family and it is also called curry leaf in Nigeria. Although it is indigenous to India, it is grown in Africa’s tropical and subtropical regions. It bears white flowers from early March to early April and can reach heights of 20–40 cm. Since ancient times, lemon basil leaf has been consumed in many culinary preparations, including foods, sauces, condiments, soups, stews, and stir-fried dishes. It mixes well with other herbs, such as rosemary, mustard, parsley, and pepper. Also, the phytochemical composition of the leaves of the plant showed the presence of alkaloids, saponins, flavonoids, tannins, and steroids. In Africa, especially the coastal regions of Nigeria, the plant is used to cure epilepsy, high fever, and diarrhea, and mental disease in the Savannah areas. While the Igbos of South Eastern Nigeria use it to control the neonatal umbilicus and keep wound surfaces sterile. In our previous study, the bioaccessibility of polyphenols and flavonoids in the leaves extract of *O. africanum* has been examined. Bioaccessibility is the term used to refer to the portion of bioactive substances that are liberated from the food matrix during digestion and made available for intestinal absorption. In this instance, the antioxidants wouldn’t be voluntarily open for absorption until after efficient digestion. Based on the uses of *O. africanum* leaves in culinary of soups, this study was aimed to determine the antioxidant properties of *O. africanum* leaves aqueous extract after *in vitro* simulated gastro-pancreatic digestion.

**Materials and methods**

**Chemicals/reagents and collection of sample**

All the chemicals used for this work are of analytical grade. Curry leaves (*Ocimum africanum*) was purchased from Anambra State, Nigeria. The plant was identified and authenticated at forestry research institute of Nigeria Ibadan with voucher number FHI 734512 deposited in their herbarium.

**Samples preparation and extraction**

Group 1 (Sample 1): Fresh sliced curry leaves (200 g) was steamed with little water (100 mL) in a pot with continuous stirring for 10 minutes. The parboiled leaves were dried open air at room temperature. Group 2 (Sample 2): Vegetable oil (100 mL) was added to a cooking pot set on heat. The oil was
allowed to heat before adding 200 g of fresh sliced curry leaves. This was fried for the next 10 minutes, with occasional stirring to avoid burning. The fried leaves were allowed to cool and dried open air at room temperature. Group 3 (Sample 3): Exactly 200 g of fresh sliced curry leaves were dried open air at room temperature. Group 4 (Sample 4): Garlic acid standard. Sample 1, 2 and 3 were ground to powder form. One hundred grams of the powder samples (1, 2, and 3) were dissolved in water (400 mL, 45°C) for 48 hours and then filtered using muslin cloth. The water extract was evaporated under reduced temperature of 37°C using water bath until no moisture was visible and dry sample was used for the in vitro gastro-pancreatic digestion.

**Simulation of gastrointestinal digestion (in-vitro)**

The digestion was performed as described by Helal et al.$^{12}$ Briefly, 1 g of *O. africanum* extract was adjusted to pH 2.5 with 40 mM HCl. After addition of 5 mL of artificial gastric fluid (300 U/mL pepsin and 2 g/L NaCl), the mixture was incubated in water bath for 2 h at 37°C. Thereafter, the pH was adjusted to 7.5 with 0.1 M NaHCO$_3$ and 4 mL of artificial intestinal fluid (0.8 g/L pancreatin and 5 g/L bile extract) was added. The mixture was incubated again in bath for another 2 h at 37°C. The ending of the enzymatic reaction in each phase occurred by cooling the sample for 10 min in ice bath. After the intestinal phase, 1 mL aliquot was withdrawn from the mixture and 100 mL of methanol was added, and then centrifuged at 10,000 xg for 10 min at 4°C. The supernatant was filtered and then acidified to pH 2.5 to ensure the stability of the phenolic compounds current in the mixture. Digestion without enzymes was carried out to differentiate the result of the chemical environment from that of the digestive enzymes. This process was also performed using Gallic acid as standard for comparison. In vitro assessment of antioxidant activity was carried out on the samples. Sample treatments (in-vitro) are shown Table 1.

**Biochemical analyses**

**Reduced glutathione** assay: The reduced glutathione concentration was estimated using the method of Ellman.$^{13}$ Sample (0.5 mL) was added to 2 mL 10% trichloroacetic acid and centrifuged. One milliliter (1 mL) of supernatant was added to Ellman’s reagent (0.5 mL) and 3 mL of phosphate buffer. A series of standard were done in similar manner along with a blank containing 3.5 mL of buffer. Samples were read at 412 nm.

**DPPH free radical scavenging assay:** The free radical scavenging ability of the sample against DPPH free radical was estimated using the method described by Ursini et al.$^{14}$ The extracts 50 μg was diluted with 3 mL ethanol and mixed with 3 mL DPPH solution. The reaction mixture was shaken, and then incubated in dark for 30 minutes. The absorbance of the solution was measured against a blank at 517 nm.

| Table 1. Simulation of gastrointestinal digestion (in-vitro) sample treatment. |
|---|
| **Samples** | **Treatment** |
| Group 1 (Steamed *O. africanum* leaves) | Before digestion (undigested) |
| | Post-intestinal digestion with enzymes |
| | Post-intestinal without enzymes |
| Group 2 (Fried *O. africanum* leaves) | Before digestion |
| | Post-Intestinal digestion with enzymes |
| | Post-intestinal without enzymes |
| Group 3 (Air dried *O. africanum* leaves) | Before digestion |
| | Post-intestinal digestion with enzymes |
| | Post-intestinal without enzymes |
| Group 4 (Garlic acid standard) | Before digestion |
| | Post-intestinal digestion with enzymes |
| | Post-intestinal without enzymes |
ABTS radical scavenging activity: ABTS radical-scavenging activity was determined according to method of Re et al. [15]. The ABTS⁺⁺ radical cation was stimulated by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate (K₂S₂O₈) and incubating for 16 hours in the dark at room temperature. Then, 4.5 mL of ABTS⁺⁺ was mixed with 0.5 mL of sample and the absorbance was read at 734 nm after 6 min.

FRAP assay: The method described by Benzie and Strain[16] was used for FRAP assay. Samples (0.5 mL) were added to 3 mL of FRAP reagent which is a mixture of 300 mM sodium acetate buffer at pH 3.6, 10.0 mM of tripyridyl triazine (TPTZ) solution, and 20 mM FeCl₃·6H₂O solution in a ratio of 10:1:1 (v/v). The reaction mixture was allowed to incubate for 30 min at 37°C. Absorbance was measured at 593 nm.

Ascorbic acid assay: Ascorbic acid content was determined by Sadasivam and Manickam[17] method. Sample (40 μL) and standard ascorbic acid (20–100 μL) was place in test tubes. A drop of thiourea solution and 1 mL of 2,4-dinitrophenyl hydrazine reagent was added to each tube. Thereafter, 100 μL of 4% oxalic acid was added and incubated at 37°C for 3 hours. The tubes were allowed to cool on ice water, then 5 mL of 85% sulfuric acid was added to the tubes. The orange color formed was read against a reagent blank at 540 nm.

α-Tocopherol assay: α-Tocopherol content was determined using the method described by Kivcak and Mert.[18] The volume of 20 μL-100 μL of standard α-tocopherol solution and 40 μL of the sample was used for the estimation. Volume was made up to 3 mL using chloroform, 1 mL of 2, 2-dipyridyl, and 1 mL of FeCl₃ solution, incubated at 37°C for 15 min, and the absorbance of the reaction mixture was read at 520 nm.

TAC estimation

The method described by Prior et al.[19] was used for the estimation of TAC. Sample (0.1 mL) was added to 1 mL of reagent solution (28 mmol/L Na₂PO₄, 4 mmol/L ammonium molybdate, and 0.6 mol/L H₂SO₄) in test tubes. The tubes were incubated in a thermal block at 95°C for 90 min. After cooling the mixture at room temperature, the absorbance was taken at 695 nm against blank.

Superoxide dismutase assay

The activity of superoxide dismutase was determined by following the auto-oxidation of epinephrine as described by Misra and Fridovich.[20] The assay was carried out by adding 0.2 mL of sample to 2.5 mL of 0.05 M carbonate buffer, pH 10.2. The reaction was on-going by addition of 0.3 mL freshly prepared epinephrine. The reference cuvette contained 2.5 mL of the buffer; 0.3 mL of the substrate and 0.2 mL of distilled water. The increase in absorbance at 480 nm due to the adrenochrome formed was monitored every 30 seconds for 120 seconds.

Catalase assay

The method of Kaplan et al.[21] was adopted for the assay of catalase activity. Catalase breaks down H₂O₂ directly into water and oxygen. The decrease in H₂O₂ concentration may be monitored spectrophotometrically. Two milliliters (2 mL) of H₂O₂ was added to 1 mL of sample in the reaction cuvette. Absorbance was read at 360 nm for 70 seconds. The reference cuvette contained 2 mL H₂O₂ and 1 mL of water.

GPx assay

The activity of GPx was measured by the method described by Ellman.[13] The reaction mixture contained 0.2 mL phosphate buffer, 0.1 mL sodium azide, 0.5 mL of sample, 0.2 mL glutathione, and
0.1 mL of H₂O₂ was added. The contents were incubated at 37°C for 10 mins, then 2 mL of 10% TCA was added. Thereafter, the reaction mixture was centrifuged. One milliliter of the supernatant was added to 0.5 mL of Ellman’s reagent and 3 mL of phosphate buffer. Standards and blank were treated similarly containing 3.5 mL of buffer. The absorbance of all samples was read at 412 nm.

Statistical analysis

All the results were expressed in means ±SD and all data were analyzed using analysis of variance (ANOVA). Significant difference between means were determined at p < 0.05 confidence level using Least Significant Difference (LSD).

Results and discussion

The results in Table 2 depicted DPPH, ABTS, FRAP, and TAC of steamed, fried, and air dried OALAE. Significant decrease was observed in DPPH, ABTS, FRAP, and TAC of OALAE (Group 1, 2, 3, and 4) in post-intestinal digestion with and without enzyme compared to samples that was not digested (before digestion). It can be assumed that the action of digestive enzymes, as well as changes in pH, caused degradation, alteration, or transformation of the chemical structure of antioxidant compounds. The significant decrease observed in DPPH, ABTS, FRAP, and TAC of post-intestinal with enzymes in comparison with post-intestinal without enzymes in the test samples may likely be as a results of the digestive enzymes inhibiting the antioxidant activities. The digestive enzymes may have impaired the integrity and stability of the antioxidant compounds. Bioactive molecules form two chiral enantiomers called racemization in the digestion environment, may have led to various reactivities in the respective digested fractions. However, other studies have shown that simulated digestion decreases the antioxidant activity of green tea infusions, while the phenol in green tea infusions is comparatively stable through digestion. This study provides convincing

Table 2. DPPH, ABTS, FRAP, and total antioxidant capacity of O. africana leaves aqueous extract.

| Group          | Before digestion | Post-intestinal digestion with enzymes | Post-intestinal digestion without enzymes | Before digestion | Post-intestinal digestion with enzymes | Post-intestinal digestion without enzymes | Before digestion |
|----------------|------------------|----------------------------------------|------------------------------------------|------------------|----------------------------------------|------------------------------------------|------------------|
| Group 1 (Steamed) | 27.84 ± 0.42a | 11.32 ± 1.89b | 16.27 ± 3.09c | 25.18 ± 3.01a | 11.40 ± 1.14b | 18.26 ± 1.09c | 40.18 ± 0.92d |
| Group 2 (Fried)   | 42.18 ± 2.10a | 21.22 ± 1.04b | 30.53 ± 9.38c | 38.26 ± 3.20a | 22.38 ± 4.01b | 25.93 ± 4.89b | 40.23 ± 0.23a |
| Group 3 (Air dried) | 58.23 ± 5.80a | 40.29 ± 4.07b | 37.47 ± 2.33b | 52.47 ± 2.23c | 42.55 ± 1.19b | 46.32 ± 5.97b | 69.50 ± 5.66d |
| Group 4 (Garlic acid) | 280.15 ± 6.05a | 130.34 ± 8.06b | 160.55 ± 4.33c | 271.44 ± 3.80d | 100.29 ± 20.07e | 117.37 ± 23.44f | 300.20 ± 50.07g |

Triplicate values are given in mean ± SD. Mean values in the same column with different letter differ at p < 0.05.
Table 3. SOD, CAT, and GPx of *O. africanum* leaves.

| Group                  | Before digestion | Post-intestinal digestion with enzymes | Post-intestinal digestion without enzymes |
|------------------------|------------------|----------------------------------------|------------------------------------------|
| Group 1 (Steamed)      | 30.02 ± 7.14 b   | 24.31 ± 4.34 a                         | 15.26 ± 1.92 a                          |
| Group 2 (Fried)        | 29.12 ± 3.57 a   | 22.18 ± 1.94 a                         | 14.40 ± 3.91 a                          |
| Group 3 (Air dried)    | 35.42 ± 0.75 b   | 10.12 ± 0.25 b                         | 9.23 ± 2.35 b                           |

Triplicate values are given in mean ± SD. Mean values in the same column with different letter differ at *p* < 0.05.

evidence that *in vitro* gastrointestinal digestion of air dried, steamed, and fried OALAE with or without digestive enzymes reduces residual antioxidant activity.

In addition, the permanence of DPPH, ABTS, FRAP, and TAC under post-intestinal conditions without enzymes was found to be better than that of the post-intestinal digestion with enzymes. An antioxidant assay based on electron transfer, the DPPH free radical technique yields a violet solution in ethanol. The most popular antioxidant assay for plant extract is the DPPH and ABTS' assay. In the DPPH and ABTS' tests, this investigation showed that the OALAE might lower or scavenge free radicals during a simulated digestion. In general, the pH of the environment induces changes in the concentration and structural transformation of antioxidant compounds, which affect antioxidant properties. Under pH elevation, structural transfiguration of antioxidant compounds occurred and caused various forms with wide-ranging chemical properties. In this study, acidifying the samples to pH 2.5 may ensure the stability of the antioxidant compounds present in the mixture. This may confirm the high antioxidant property observed in garlic acid digestion and air dried OALAE when compared to the steamed and fried OALAE. One of the key elements influencing antioxidant activity is temperature. Heating typically accelerates the initiation processes, which results in less antioxidant activity from the existing or newly added antioxidants. Therefore, the occurrence of different individual phenolic compounds structures may reflect residual antioxidant activity.

Table 3 shows SOD, CAT, and GPx of *O. africanum* leaves. There were significant decreases in SOD, CAT, and GPx activity of OALAE of post-intestinal and post-intestinal without enzyme compare to OALAE before digestion in Group 1, 2, and 3. Decrease was observed in SOD, CAT, and GPx activity of OALAE in post-intestinal digestion with enzymes when compared to post-intestinal digestion without enzymes, however this was not significant. The reduction in phenolic component concentration and/or transformation into new structural forms with altered chemical characteristics brought about by the digestive process has an impact on the antioxidant properties. Additionally, elements including pH variations and the interaction of antioxidant compounds with other sample components may result in changes in of SOD, CAT, and GPX activities.

Table 4 showed GSH, vitamin C, E, and non-heme iron content of *O. africanum* leaves. Significant decrease were observed in GSH, Vitamin E, C, and non-heme iron content of OALAE in post-intestinal using enzymes and post-intestinal without enzymes compared to OALAE before digestion in Group 1, 2, and 3. In Group 1, 2 and 3 decrease were observed in SOD, CAT, and GPx activity of OALAE in post-intestinals with enzymes when compared to post-intestinal without enzymes. Conversely, this effect was not significant. It may be suggested that, the action of digestive enzymes could have resulted to changes in the chemical structures of antioxidant compounds, especially in the functional groups of these compounds. Consequently, their solubility becomes decreased in the intestinal lumen, making them unavailable for absorption. Vegetables, herbs, and spices contain phenolic compounds and have been shown to restrain the assimilation of non-heme iron. However, antioxidant properties of spices are beneficial as a consequence of lowering oxidative
Table 4. GSH, vitamin C, E, and non-heme iron content of O. africanum leaves.

| Groups          | GSH (Units/g DW) | Vit C (Units/g DW) | Vit E (Units/g DW) | Non-heme iron content (Units/g DW) |
|-----------------|------------------|--------------------|--------------------|-----------------------------------|
| Group 1 (Steamed) | Before digestion | 6.20 ± 3.25 a      | 3.23 ± 1.16 a      | 15.13 ± 1.81 a                    | 7.36 ± 2.10 a                     |
|                 | Post-intestinal digestion with enzymes | 2.30 ± 0.10 b      | 0.50 ± 0.20 b      | 9.23 ± 1.02 b                     | 6.27 ± 2.21 a                     |
| Group 2 (Fried) | Before digestion | 4.30 ± 0.12 ab     | 1.03 ± 1.52 bc     | 10.20 ± 4.25 b                    | 5.37 ± 1.95 a                     |
|                 | Post-intestinal digestion with enzymes | 4.23 ± 1.23 ab     | 2.00 ± 0.50 ab     | 14.00 ± 2.00 a                    | 9.46 ± 0.66 a                     |
| Group 3 (Air dried) | Before digestion | 2.12 ± 0.31 bc     | 0.70 ± 0.41 bc     | 10.40 ± 3.15 c                    | 7.33 ± 3.60 a                     |
|                 | Post-intestinal digestion with enzymes | 2.53 ± 0.63 bc     | 1.50 ± 0.50 bc     | 12.13 ± 2.20 bc                   | 6.21 ± 1.82 a                     |
|                 | Post-intestinal digestion without enzymes | 8.16 ± 0.57 c     | 1.16 ± 0.57 bc     | 20.33 ± 2.20 d                    | 10.33 ± 4.01 ab                    |
|                 | Post-intestinal digestion with enzymes | 3.00 ± 2.00 b      | 0.26 ± 0.15 b      | 16.13 ± 6.10 a                    | 4.33 ± 2.32 ac                     |
|                 | Post-intestinal digestion without enzymes | 4.17 ± 1.74 ab      | 0.70 ± 0.20 b      | 18.00 ± 3.00 ad                   | 5.17 ± 2.02 a                     |

Triplicate values are given in mean ± SD. Mean values in the same column with different letter differ at p < 0.05.

stress[32] and the inhibition of non-heme iron absorption by air dried, fried, and steamed OALAE may institute a potential of reducing adverse effect of excess iron.

Conclusion

This study showed that fried, steamed, and air dried O. africanum leaves possess antioxidant properties and it suggest that enzymes play significant roles in the digestion of OALAE. The study established significant decrease in antioxidant properties of air dried, fried, and steamed OALAE in post-intestinal (with enzyme) in comparison to post-intestinal without enzymes. Meanwhile, in the enzymatic post-intestinal digestion, the assayed antioxidant parameters of the fried and steamed O. africanum leaves were lower compared to air dried O. africanum leaves extract. Comparisons of the antioxidant properties of O. africanum leaves revealed that digestion appeared to be an important factor in maintaining and increasing this property. Digestive enzymes hasten the process by which large food molecules are broken down into smaller ones that the body can use. Animals would not be able to break down food molecules rapidly enough to give them the energy and nutrients they need to survive without digestive enzymes. Interestingly, larger particles that include more intact cells likely to exhibit higher antioxidant properties in their digested fluid when lacking digestive enzymes. In other words, even if the plant was processed before consumption, the intracellular components may still be shielded from oxidative reactions when there is lacking digestive enzymes. This work could also be regarded as an appreciated knowledge of the functional properties of O. africanum leaves. This may contribute to the development of new types of functional foods/ingredients.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Authors’ contributions

B.O.G., conceived, designed, planned, and supervised the study. G.O.O. and E.O.U. performed the experiments and wrote initial draft. J.O analysed and interpreted results. B.O.G and J.O critically revised and edited the manuscript. All authors read the final manuscript and approved submission.
Ethical approval

Experimental protocols and procedures used in this study were approved by the Delta State University, Faculty of Science (Abraka) (approval no. REL/FOS/21/05).

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