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PII: S2213-4530(18)30110-1
DOI: https://doi.org/10.1016/j.fshw.2019.03.012
Reference: FSHW 172

To appear in:

Received date: 6 October 2018
Revised date: 14 February 2019
Accepted date: 29 March 2019

Please cite this article as: Irondi EA, Adegoke BM, Effion ES, Oyewo SO, Alamu EO, Boligon AA, Enzymes inhibitory property, antioxidant activity and phenolics profile of raw and roasted red sorghum grains *in vitro*, *Food Science and Human Wellness* (2019), https://doi.org/10.1016/j.fshw.2019.03.012

# Enzymes inhibitory property, antioxidant activity and phenolics profile of raw and roasted red sorghum grains *in vitro*

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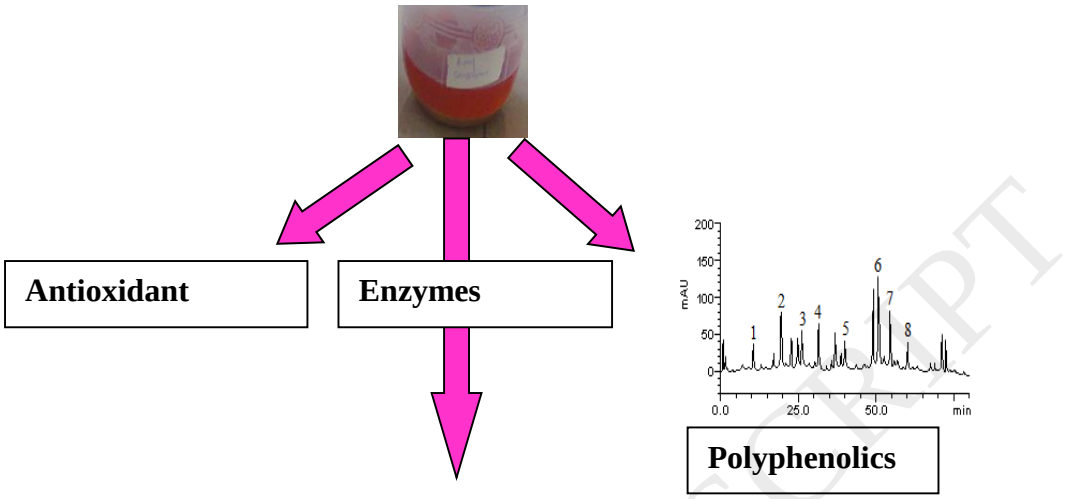
**Graphical Abstract**




## Highlights

- Raw sorghum grains extract was rich in phenolic acids and flavonoids.
- The extract exhibited strong enzymes inhibitory and antioxidant activities.
- Levels of phenolic compounds decreased with increasing roasting temperature.
- Enzymes inhibitory activity decreased with increasing roasting temperature.
- Antioxidant activity increased as the roasting temperature increased.


## Abstract

Whole grain cereals are important dietary sources for management of metabolic diseases due to the bioactive components they contain. Hence, this study investigated enzymes (pancreatic lipase, α-amylase, α-glucosidase, xanthine oxidase and angiotensin 1-converting enzyme) inhibitory property, antioxidant activity and phenolics profile of raw and roasted red sorghum (*Sorghum bicolor*) grains *in vitro*. Extracts of flours of raw and roasted (150°C and 180°C, for 20 min) grains were assayed for enzymes inhibitory and antioxidant activities using spectrophotometric methods; while their phenolic constituents were characterized using HPLC-DAD. The raw grains exhibited

strong enzymes inhibitory and antioxidant activities, and contained phenolic acids (gallic, chlorogenic, caffeic, ellagic and *p*-coumaric acids) and flavonoids (quercetin, luteolin and apigenin). However, whereas the enzymes inhibitory activity and levels of the phenolic compounds in the grains decreased significantly ($p < 0.05$) with increasing roasting temperature, the antioxidant activity increased. Hence, roasting at high temperature may not be recommended for the optimum retention of the enzymes inhibitory property and phenolic compounds of red sorghum grains.

**Keywords**: Antioxidant activity; Enzymes inhibition; Metabolic diseases; Phenolic compounds; Sorghum grains.

## 1. Introduction

Metabolic diseases including obesity, type 2 diabetes (T2D), hyperuricemia and hypertension are major health challenges of this twenty-first century, with high prevalence. These diseases form a constellation of cardiovascular risks factors referred to as metabolic syndrome [1-3]. Clinically, there are pharmacotherapies for treating these metabolic diseases. Examples include orlistat for obesity, acarbose for T2D, allopurinol for hyperuricemia and captopril for hypertension. These drugs elicit their therapeutic action mainly by inhibiting certain enzymes whose over-activities promote excessive production and accumulation of metabolites that predispose to these metabolic diseases. Thus, orlistat inhibits pancreatic lipase [4], which catalyzes the hydrolysis of dietary fats to produce fatty acid [5]; acarbose inhibits α-amylase and α-glucosidase, which catalyze the hydrolysis of starch to release glucose [6]. Similarly, allopurinol inhibits xanthine oxidase (XO) [7], which catalyzes the oxidation of hypoxanthine first to xanthine, and ultimately

to uric acid [8]; while captopril inhibits angiotensin 1-converting enzyme (ACE) [9], which catalyzes the proteolytic cleavage of angiotensin 1 to form angiotensin II [10]. However, these drugs have some side effects that interfere with their clinical uses. Such side effects include hepatic and gastrointestinal tract dysfunction for orlistat [11], flatulence for acarbose [12], renal and hepatic dysfunction for allopurinol [13], and hypotension for captopril [14].

The high prevalence of the aforementioned metabolic diseases and the side effects of the available drugs for their management have aroused research efforts targeted at finding affordable and effective novel strategies for preventing the onset and decelerating the progression of these diseases [15]. In this context, it is gratifying to know that plant-based bioactive components such as phenolic compounds in functional foods have been evidently shown to be beneficial [16]. Some studies have demonstrated the efficacy of phenolics-rich plant extracts to inhibit relevant enzymes implicated in metabolic diseases [17, 18].

Sorghum, a grain cereal that originated in sub-Saharan Africa, serves mainly as food in many parts of Africa and Asia, and as animal feed in the western region [19]. It is prominent for its rich polyphenols content, relative to other grain cereals such as barley, wheat, rye and millet [20]. Previous studies have reported some bioactivities of different varieties of sorghum grains extract including anti-inflammatory [21], antioxidant [22] and anti-proliferative [23] activities. In addition to these, a recent study by Tayo et al. [24] concluded that supplementation of routine hematinics with sorghum extract ameliorated preoperative anemia in human subjects better than the routine hematinics alone. However, most grain cereals are subjected to different processing methods, which may influence their bioactive constituents and potential health benefits [25]. Therefore, this study investigated enzymes (pancreatic lipase, α-amylase, α-glucosidase, xanthine

oxidase and angiotensin 1-converting enzyme) inhibitory property, antioxidant activity and phenolics profile of raw and roasted red sorghum grains *in vitro*.

## 2. Materials and method

### *2.1. Chemicals and reagents*

Porcine pancreatic lipase, α-amylase, *Bacillus stearothermophillus* α-glucosidase, rabbit lung ACE, xanthine oxidase, acarbose, allopurinol, xanthine, orlistat, captopril, hippuryl-histidyl-leucine, Trolox, luteolin, apigenin and quercetin, L-ascorbic acid, 2,2´-azino-bis-3-ethylbenzthiazoline-6-sulphonic (ABTS) and 2,2-diphenylpicrylhydrazyl (DPPH) were products of Sigma (St. Louis, USA). Gallic, chlorogenic, *p*-coumaric, ellagic and caffeic acids, methanol, and phosphoric acid were products of Merck (Darmstadt, Germany).

### *2.2. Sample collection and preparation*

Sample (1.5 Kg) of dry sorghum grains (red variety) was purchased from Malete market in Ilorin, Kwara State, Nigeria. The sample was later authenticated at the Department of Plant and Environmental Biology, Kwara State University, Malete, Ilorin, Nigeria. Subsequently, the sample was sorted and divided into three portions of 0.5 kg each. Out of the three portions, a portion (raw) was not roasted; another portion was roasted at 150 ± 2 $^{o}$C for 20 min; while the last portion was roasted at 180 ± 2 $^{o}$C for 20 min in a hot-air oven with thermostatic regulation. After roasting, the samples were allowed to cool to room temperature for 30 min, and each portion was later milled into flour (0.5 mm). The flours were hermetically packed in opaque plastic containers, and kept at 4 $^{o}$C during analysis.

### *2.3. Flours extract preparation*

Each flour sample was extracted by soaking in methanol in a ratio of 1:8 (w/v) for 24 h with intermittent shaking. Afterwards, the mixture was filtered through Whatman (No. 1) filter paper, and the filtrate was collected. The filtrate was concentrated at 45 °C using a rotary evaporator, and the residue was used for the various analyses.

*2.4. In vitro enzymes inhibition assays*

*2.4.1. Pancreatic lipase inhibition assay*

Pancreatic lipase inhibition was assayed as per the spectrophotometric method described by Eom et al. [26]. P-nitrophenyl butyrate served as substrate, while orlistat served as a reference inhibitor. Enzyme solution was prepared by mixing 30 µL of pancreatic lipase (10 units) in 10 mmol/L morpholinepropane sulphonic acid and 1 mmol/L EDTA (pH 6.8), and 850 µL of Tris buffer containing (100 mmol/L Tris-HC1 and 5 mmol/L $CaCl_2$, pH 7.0). Next, 100 µL of varied concentrations (5, 10, 15 and 20 µg/mL) of the extract (or orlistat) and 880 µL of the enzyme solution were incubated at 37 ºC for 10 min. Following this, 20 µL of 10 mM p-nitrophenyl butyrate solution in dimethyl formamide was added to initiate hydrolytic reaction at 37 ºC for 20 min. Absorbance of the p-nitrophenol produced from the hydrolytic reaction was measure at 405 nm, and percentage pancreatic lipase inhibition by the extract was calculated.

*2.4.2. Alpha-amylase inhibition assay*

This was conducted following the protocol described by Kwon et al. [27], using soluble starch as substrate and acarbose as a reference inhibitor. Briefly, 500 µL of different concentrations (10, 20, 30 and 40 µg/mL) of extract and 500 µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 0.5 mg/mL α-amylase solution were incubated at 37°C for 10 min. Thereafter, 500 µL of 1% starch solution in 0.02 M sodium phosphate buffer was added, and the reaction mixture was incubated at 37°C for 15 min. To terminate the hydrolytic reaction, 1.0 mL

of DNSA reagent (1% 3, 5-dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 M NaOH) was added. Next, the reaction mixture was incubated for 5 min in a boiling water bath, cooled to room temperature, and diluted with 10 mL distilled water. The absorbance was measured at 540 nm, and percentage α-amylase inhibition by the extract was calculated.

*2.4.3. Alpha-glucosidase inhibition assay*

This was conducted following the method reported by Kim et al. [28], using *p*-nitrophenylglucopyranoside (PNPG) as substrate and acarbose as a reference inhibitor. Briefly, 5 units aliquot of α-glucosidase was incubated with different concentrations (5, 10, 15 and 20 µg/mL) of extract for 15 min. Next, 3 mM PNPG dissolved in 20 mM phosphate buffer, pH 6.9 was added as a substrate to initiate the hydrolytic reaction. The hydrolytic reaction was allowed to proceed for 20 min at 37°C, after which it was terminated by adding 0.1 M $Na_2CO_3$ (2 mL). The absorbance of the p-nitrophenol released from PNPG hydrolysis was measured at 400 nm, and percentage α-glucosidase inhibition by the extract was calculated.

*2.4.4. Xanthine oxidase (XO) inhibition assay*

This assay was performed as per the spectrophotometric method reported by Osada et al. [29]. Xanthine was used as substrate, while allopurinol was used as a reference inhibitor. Xanthine (15 mM) and XO (0.1 mU/µL) solutions were freshly prepared with Tris–HCl buffer (50 mM, pH 7.4). Afterwards, 40 µL of xanthine solution, 10 µL of xanthine oxidase solution and 1950 µL of varied concentrations (10, 20, 30 and 40 µg/mL) of extract were mixed and incubated at 37 $^{o}$C for 10 min. Next, 50 µL of perchloric acid solution in the same Tris–HCl buffer (3.2% (v/v) was added to terminate the XO-catalyzed hydrolysis of xanthine. Absorbance of the uric acid formed was then measured at 292 nm, and the percentage xanthine oxidase inhibition by extract was calculated.

*2.4.5. Angiotensin 1-converting enzyme (ACE) inhibition assay*

This was performed as per the method reported by Cushman and Cheung [30], using hippuryl-histidyl-leucine as substrate and captopril as a reference inhibitor. In brief, 50 μL of different concentrations (10, 20, 30 and 40 μg/mL) of the extract (or captopril, 64 nmol/L) and 50 μL of ACE solution (4 mU/mL) were incubated at 37 $^{o}$C for 15 min. Afterwards, 150 μL of 8.33 mM of hippuryl-histidyl-leucine in 125 mM Tris–HCl buffer (pH 8.3) was added to the mixture, and this was incubated for 30 min at 37 $^{o}$C. Next, 250 μL of 1 M HCl was added to terminate the hydrolytic reaction, and the hippuric acid produced was extracted with ethyl acetate (1.5 mL), and separated by centrifugation. Then, 1.0 mL of the ethyl acetate layer was dispensed into a clean test tube and evaporated to dryness in an oven. The hippuric acid residue was redissolved with 1.0 mL of deionized water; following which its absorbance was measured at 228 nm, and the percentage ACE inhibition by the extract was calculated.

*2.4.6. Antioxidant activity assays*

DPPH$^{*}$ scavenging ability of extracts was determined according the method described by Cervato et al. [31], using ascorbic acid as a reference antioxidant. ABTS$^{*+}$ scavenging ability was carried as per the method described by Re et al. [32], and expressed as trolox equivalent antioxidant capacity (TEAC). Iron (II) ($Fe^{2+}$) chelation assay was performed using the method reported by Puntel et al. [33], using ascorbic acid as a reference antioxidant. Reducing power assay was carried out as per the method reported by Oyaizu [34], and expressed as gallic acid equivalent (GAE).

*2.4.7. Analysis of phenolic compounds using HPLC-DAD*

Phenolic compounds in the extracts were characterized at ambient temperature in triplicates using a reverse-phase high-performance liquid chromatography with diode-array detection (HPLC-DAD) (Shimadzu, Kyoto, Japan). The extracts were injected at 12 mg/mL and separation of the phenolic compounds was achieved using reversed phase Phenomenex $C_{18}$ column (4.6 mm

x 250 mm) with particles of 5 μm diameter. The mobile phase was HPLC water with 1% phosphoric acid (v/v) (solvent A) and HPLC grade methanol (solvent B) at a flow rate of 0.6 mL/min and injection volume 50 μL. The gradient system used was as previously described by Bitencourt et al. [35]. Quantification of phenolic compounds in the extracts was performed by integrating the chromatography peaks using the external standard method. The chromatography peaks were confirmed by matching their retention time with those of reference standards and by DAD spectra (200 to 600 nm).

### 2.5. Statistical analysis

One-way analysis of variance (ANOVA) was performed on mean values of triplicate determinations, followed by least significant difference (LSD) test, using SPSS statistical software (version 17), at $p < 0.05$. Concentration of extracts that scavenged DPPH$^{*}$ by 50% ($SC_{50}$) and concentration of the extracts that inhibited enzymes activity by 50% ($IC_{50}$) were calculated using version 4.0 of Graphpad Prism® (Sandiego, CA).

## 3. Results and discussion

### *3.1. Enzymes inhibitory activity*

Enzymes (pancreatic lipase, α-amylase, α-glucosidase, XO and ACE) inhibitory activity of extracts of raw and roasted sorghum grains, presented as concentration of extract that caused 50% enzyme inhibition ($IC_{50}$), varied significantly ($p < 0.05$) (Table 1). Generally, raw grain had the lowest $IC_{50}$ values for all the enzymes, followed by grain roasted at 150 $^{o}$C and grains roasted at 180 $^{o}$C; indicating a decreasing order of inhibitory strength with increasing roasting temperature.

#### *3.1.1. Pancreatic lipase inhibitory activity*

Pancreatic lipase inhibitory activity of raw sorghum extract ($IC_{50}$: 12.72 ± 1.13 μg/mL) is much stronger than those of some other grains that have been reported to inhibit pancreatic lipase,

including twenty Canadian lentil cultivars ($IC_{50}$ range: 6.26 to 9.26 mg/mL) [36] and two *Vigna* Species namely, mung bean ($IC_{50}$: 17.74 ± 2.00 mg/mL) and moth bean ($IC_{50}$: 7.32 ± 1.29 mg/mL) [37]. The strong pancreatic lipase inhibitory activity of the raw sorghum extract suggests that it may be useful for decelerating the rate of formation, absorption and accumulation of fatty acids from dietary fats digestion, which is an important strategy for controlling obesity [36]. However, orlistat displayed a much stronger pancreatic lipase inhibitory activity ($IC_{50}$: 0.36 ± 0.02 μg/mL) than the raw sorghum extract.

The $IC_{50}$ values of the sorghum on pancreatic lipase increased from 12.72 ± 1.13 μg/mL in the raw grain to 14.13 ± 1.42 and 17.09 ± 1.75 μg/mL in grain roasted at 150 and 180 $^{o}$C, respectively; indicating that the pancreatic lipase inhibitory activity of the grain decreased with increasing roasting temperature. This observation partially agrees with the findings of a recent study which reported a decrease, no effect and an increase in the pancreatic lipase inhibitory activity of cocoa beans roasted at 100, 150 and 170 °C, respectively [38].

*3.1.2. Alpha-amylase and α-glucosidase inhibitory activity*

The catalytic role α-amylase and α-glucosidase play in the digestion and absorption of dietary carbohydrates has made their inhibition clinical targets in the control of postprandial hyperglycemia associated with T2D. The $IC_{50}$ values of raw sorghum extract on α-amylase and α-glucosidase (16.93 ± 1.08 and 10.78 ± 0.63 μg/mL, respectively) (Table 2) indicate that the extract had stronger inhibitory effects on these two enzymes than barnyard millet ($IC_{50}$: 32.59 ± 1.04 and 18.60 ± 0.83 μg/mL on α-amylase and α-glucosidase, respectively [39].

Unlike, acarbose (a reference inhibitor) that had a stronger inhibitory effect on α-amylase ($IC_{50}$: 10.13 ± 0.82 μg/mL) than α-glucosidase ($IC_{50}$:18.04 ± 1.25 μg/mL), raw sorghum extract had a more potent inhibitory effect on α-glucosidase than α-amylase. This inhibition pattern has

therapeutic importance, and is in agreement with some earlier reports that indicated similar pattern [40, 41]. Since the adverse effects associated with the clinical use of acarbose are due to its stronger inhibition of α-amylase than α-glucosidase [12], the observed pattern of inhibition implies that such adverse effects may not be present when the extract of sorghumis used to manage T2D.

Similar to the impact of roasting on PL inhibition, both the α-amylase and α-glucosidase inhibitory activity of the grain decreased as the roasting temperature increased, as indicated by the higher $IC_{50}$ values of the roasted grain on these enzymes (Table 2). Whereas this is in concordance with the findings of Vadivel et al. [25], who reported a decrease in the α-amylase and α-glucosidase inhibitory effects of some under-utilized legume grains due to roasting, it contradicts the report of Kunyanga et al. [42] that indicated an increase in α-amylase and α-glucosidase inhibitory activities of some indigenous grain cereal and oil seeds, including pearl millet and pigeon pea, due to roasting.

*3.1.3. XO inhibitory activity*

Raw sorghum extract exhibited a strong inhibitory effect on XO, with $IC_{50}$ of 28.35 ± 1.86 μg/mL. Extracts of other grain cereals such as kodo millet [43] have been previously been reported as potent inhibitors of XO. Whereas the raw sorghum extract had a stronger XO inhibitory effect than raw *Brachystegia eurycoma* seed extract ($IC_{50}$: 45.17 ± 2.14 μg/mL) as recently reported by Irondi et al. [44]; allopurinol, a reference XO inhibitor with $IC_{50}$ of 7.04 ± 0.44 μg/mL in this study, had a more potent inhibitory effect than it. The $IC_{50}$ values of the extracts increased significantly ($p < 0.05$) from 28.35 ± 1.86 μg/mL in raw grain to 30.75 ± 1.90 μg/mL and 33.06 ± 1.97 μg/mL in grain roasted at 150 and 180 °C, respectively. This indicates a decreasing order of XO inhibitory activity with increasing roasting temperature. A similar finding in which the XO inhibitory effect of *Brachystegia eurycoma* seed decreased after roasting was recently reported [44]. Through the

inhibition of XO, and consequently, deceleration of uric acid formation, raw sorghum extract may be beneficial for mitigating ROS generation, hyperuricemia and inflammation, which result from excessive XO activity [43].

*3.1.4. ACE inhibitory activity*

As the enzyme that catalyzes the formation of angiotensin II (a physiologically potent vasoconstrictor) by cleavage of angiotensin 1, inhibition of ACE has become a therapeutic target for regulating the blood pressure [45]. Raw sorghum extract inhibited ACE ($IC_{50}$: 19.64 ± 1.06 μg/mL), but not as much as captopril, a standard ACE inhibitor with $IC_{50}$ of 6.34 ± 0.72 μg/mL. The result further shows that the $IC_{50}$ values increased from 19.64 ± 1.06 μg/mL in the raw grain to 20.99 ± 1.17 and 22.81 ± 1.52 μg/mL in grain roasted at 150 and 180 °C, respectively. Thus, the ACE inhibitory activity of sorghum grains decreased as the roasting temperature increased. This is in agreement with the findings of Hyun et al. [46], who also reported a decrease in ACE inhibitory activity of *Cassia tora* seed due to roasting.

*3.2. Antioxidant activity*

Potentiating cellular antioxidant ability has been noted as being crucial for a successful treatment of the various metabolic diseases [47]. Hence, the antioxidant activity of raw and roasted sorghum grains was tested and the results are presented in Table 2. Raw sorghum extract effectively scavenged $DPPH^{*}$ and $ABTS^{*+}$, chelated $Fe^{2+}$ and reduced $Fe^{3+}$. However, in contrast to the impact of roasting on the enzymes inhibitory activity of the grain, the antioxidant activity increased significantly ($p < 0.05$) as the roasting temperature increased. This increment notwithstanding, ascorbic acid, a standard antioxidant, had stronger $DPPH^{*}$ scavenging and $Fe^{2+}$ chelating ability than both raw and roasted grain. There have been conflicting reports by some previous studies on the effect of roasting on antioxidant activity of grains. Whereas Vadivel et al.

[48] reported a drastic decrease in antioxidant activity of ten under-utilized legume grains; Kunyanga et al. [49] reported a significant increase in antioxidant activity of finger millet and sunflower seed due to roasting.

### *3.3. Phenolics composition*

Phenolics composition of raw and roasted sorghum grains (Table 3) shows that both phenolic acids (gallic, chlorogenic, caffeic, ellagic and *p*-coumaric acids) and flavonoids (quercetin, luteolin and apigenin) were present in the raw grain. Representative chromatograms of raw and roasted grain are shown in Figure 1A - C. Chlorogenic acid was the most abundant phenolic acid, followed by ellagic and caffeic acids; while quercetin was the most abundant flavonoid followed by luteolin. Generally, the levels of all the phenolic acids (except caffeic acid) and flavonoids reduced with increasing roasting temperature. Chlorogenic acid and apigenin were not detected in grain roasted at 180 $^{o}$C, while luteolin was not detected in grain roasted at 150 $^{o}$C and 180 $^{o}$C; suggesting absolute degradation of these compounds at the respective roasting temperatures.

Loss of phenolic compounds during thermal processing have been reported by some previous studies [44, 50]. This loss is attributed to thermal decomposition and heat-induced oxidation of the phenolic compounds [51, 52]. For instance, phenolic acids were reported to easily volatilize during heat treatment, especially during extended heating; while the highly hydroxylated structure of flavonoids, which makes them vulnerable to redox reactions, promotes their degradation during thermal treatments [50]. Regarding heat-induced oxidation of phenolic compounds, roasting promotes the formation of Maillard reaction products at the expense of phenolics; the extent of which is dependent on the roasting temperature and time [53].

The parallel decrease in enzymes inhibitory activity and phenolics composition of the grain with increasing roasting temperature suggests that phenolic acids and the flavonoids might be the major enzymes inhibitors in the grain. Some earlier studies had demonstrated the ability of extracts of some plants such as *Ziziphus mistol* [54] and leaf of different *Momordica* species [55] rich in phenolic compounds to inhibit some important enzymes linked to metabolic diseases. Phenolic compounds of plant origin have affinity for peptides; a property made possible through hydrogen and hydrophobic bonding. This affinity enables phenolics-rich extracts to denature peptide enzymes, thereby inhibiting their catalytic activity [18]. Consequently, as the phenolic acids and flavonoids levels of the grain decreased, the enzymes inhibitory activity also decreased due to increasing roasting temperature.

In contrast, the increase in antioxidant activity of the grains as the levels of phenolic compounds decreased with increasing roasting temperature suggests that the antioxidant activity of the grains may not be exclusively attributed to the phenolics. Instead, Maillard reaction products that may have been formed during roasting at 150 and 180 $^{o}$C possibly contributed to the antioxidant activity. Roasting process is known to result in the formation of Maillard reaction products, which are mainly responsible for some noticeable changes in the organoleptic properties of roasted foods, such as alteration in colour and flavor [56]. These Maillard reaction products possess antioxidant activity [57]. Thus, as Maillard reaction and its products may have increased with increasing roasting temperature, the antioxidant activity of the grain also increased.

## 4. Conclusions

Raw sorghum grains extract displayed strong enzymes (pancreatic lipase, α-amylase and α-glucosidase, XO and ACE) inhibitory and antioxidant activities. The extract contained phenolic acids (gallic, chlorogenic, caffeic, ellagic and *p*-coumaric acids) and flavonoids (quercetin, luteolin

and apigenin). However, whereas the enzymes inhibitory activity and phenolic compounds levels of the extract decreased with increasing roasting temperature of the grains; the antioxidant activity increased. Hence, roasting at high temperatures may not be recommended for the optimum retention of the enzymes inhibitory property and phenolic compounds of red sorghum grains, and the associated health benefits.

**Acknowledgement**

The authors acknowledge the laboratory of the Department of Biochemistry, Kwara State University, Malete, Ilorin, Nigeria, where the wet analyses in this study were conducted.

**Conflict of interest statement**

There is no conflict of interest to declare.

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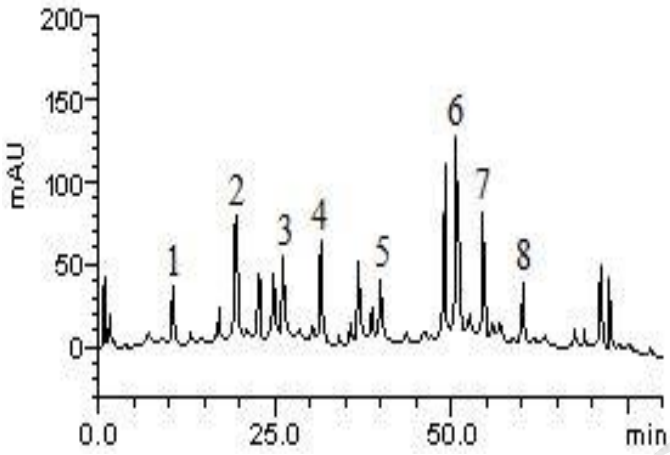
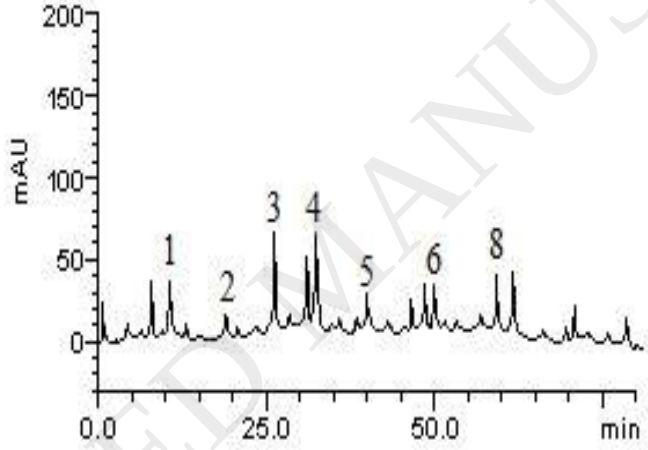
**Figure caption**

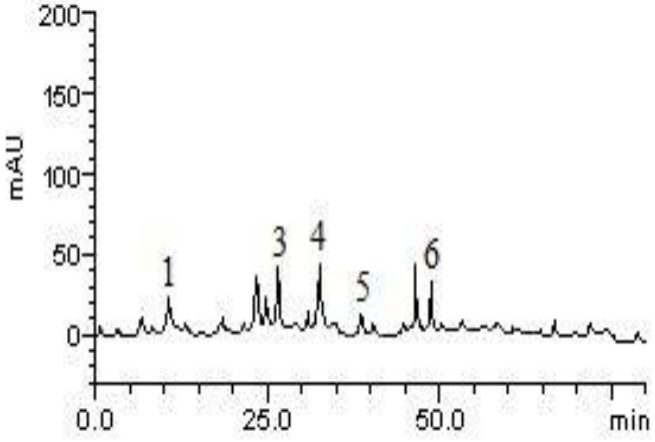
Figure 1. HPLC-DAD chromatograms depicting the polyphenolic constituents of sorghum grains: 1A - raw grains; 1B - grains roasted at 150 ºC for 20 min; 1C - grains roasted at 180 ºC for 20 min; peak 1 - gallic acid; peak 2 - chlorogenic acid; peak 3 - caffeic acid; peak 4 - ellagic acid; peak 5 - *p*-coumaric acid; peak 6 - quercetin; peak 7 - luteolin; peak 8 - apigenin.




1A. Raw sorghum




1B. Roasted sorghum, 150 ºC



1C. Roasted sorghum, 180 ºC

**Tables**

Table 1. $IC_{50}$ values of raw and roasted sorghum grains extracts on pancreatic lipase (PL), α-amylase, α-glucosidase, angiotensin I-converting enzyme (ACE) and xanthine oxidase (XO) activities

| Enzyme | Raw (μg/mL) | Roasted, 150 ºC (μg/mL) | Roasted, 180 ºC (μg/mL) | Orlistat (μg/mL) | Acarbose (μg/mL) | Allopurinol (μg/mL) | Captopril (μg/mL) |
|---|---|---|---|---|---|---|---|
| PL | 12.72 ± 1.13$^{c}$ | 14.13 ± 1.42$^{b}$ | 17.09 ± 1.75$^{a}$ | 0.36 ± 0.02$^{d}$ | - | - | - |
| α-amylase | 16.93 ± 1.08$^{c}$ | 19.15 ± 1.24$^{b}$ | 21.82 ± 1.51$^{a}$ | - | 10.13 ± 0.82$^{d}$ | - | - |
| α-glucosidase | 10.78 ± 0.63$^{d}$ | 12.29 ± 0.96$^{c}$ | 14.69 ± 1.02$^{b}$ | - | 18.04 ± 1.25$^{a}$ | - | - |
| XO | 28.35 ± 1.86$^{c}$ | 30.75 ± 1.90$^{b}$ | 33.06 ± 1.97$^{a}$ | - | - | 7.04 ± 0.44$^{d}$ | - |
| ACE | 19.64 ± 1.06$^{c}$ | 20.99 ± 1.17$^{b}$ | 22.81 ± 1.52$^{a}$ | - | - | - | 6.34 ± 0.72$^{d}$ |

Results are means ± standard deviations (SD) of triplicate determinations. Along the same row, values having different superscript letters vary significantly ($p < 0.05$). $IC_{50}$: concentration of extract that inhibited enzyme activity by 50%.

Table 2. $DPPH^{*}$ and $ABTS^{*+}$ scavenging ability, $Fe^{2+}$ chelation and reducing power of raw and roasted sorghum grains extracts

| Antioxidant activity | Raw | Roasted, 150 °C | Roasted, 180 °C | Ascorbic acid |
|---|---|---|---|---|
| $ABTS^{*+}$ scavenging ability (mmol TEAC/g) | $5.47 \pm 0.29^{c}$ | $6.32 \pm 0.34^{b}$ | $8.09 \pm 0.51^{a}$ | - |
| $DPPH^{*}$ $SC_{50}$ (μg/mL) | $12.04 \pm 0.85^{a}$ | $10.81 \pm 0.63^{b}$ | $8.46 \pm 0.61^{c}$ | $5.89 \pm 0.64^{d}$ |
| $Fe^{2+}$ chelation $CC_{50}$ (μg/mL) | $19.83 \pm 1.02^{a}$ | $17.68 \pm 0.94^{b}$ | $14.64 \pm 0.87^{c}$ | $9.75 \pm 0.63^{d}$ |
| Reducing power (mg GAE/g) | $72.41 \pm 1.42^{c}$ | $75.18 \pm 1.80^{b}$ | $77.92 \pm 1.96^{a}$ | - |

Results are means ± standard deviations (SD) of triplicate determinations. Along the same row, values having different superscript letters vary significantly ($p < 0.05$). TEAC: Trolox equivalent antioxidant capacity; $SC_{50}$: extract concentration that scavenged 50% of $DPPH^{*}$; $CC_{50}$: extract concentration that chelated 50% of $Fe^{2+}$; GAE: Gallic acid equivalent.

Table 3. Phenolic constituents of raw and roasted sorghum grains extracts

| Compounds (mg/g) | Raw | Roasted, 150 °C | Roasted, 180 °C | $t_R$ (min) | LOD (μg/mL) | LOQ (μg/mL) |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| Gallic acid | 1.78 ± 0.02[a] | 1.64 ± 0.03[b] | 0.96 ± 0.02[c] | 10.09 | 0.015 | 0.049 |
| Chlorogenic acid | 4.15 ± 0.03[a] | 0.49 ± 0.01[b] | ND | 19.45 | 0.028 | 0.093 |
| Caffeic acid | 2.63 ± 0.01[b] | 3.87 ± 0.01[a] | 1.82 ± 0.05[c] | 25.11 | 0.009 | 0.030 |
| Ellagic acid | 4.09 ± 0.01[a] | 3.92 ± 0.02[a] | 1.93 ± 0.01[b] | 31.62 | 0.023 | 0.075 |
| *p*-Coumaric acid | 1.81 ± 0.05[a] | 0.85 ± 0.04[b] | 0.51 ± 0.01[c] | 39.76 | 0.011 | 0.036 |
| Quercetin | 7.93 ± 0.02[a] | 1.76 ± 0.04[b] | 0.91 ± 0.01[c] | 50.97 | 0.024 | 0.078 |
| Luteolin | 4.20 ± 0.01 | ND | ND | 54.87 | 0.018 | 0.064 |
| Apigenin | 1.75 ± 0.01[a] | 1.67 ± 0.05[a] | ND | 59.74 | 0.013 | 0.042 |

Results are expressed as mean ± standard deviations (SD) of three independent determinations. Mean values followed by different letters along the same row differ significantly at $p < 0.05$. $t_R$: retention time; LOD: limit of detection; LOQ: limit of quantification; ND: not detected.