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RESEARCH ARTICLE

Antioxidant Potential of Simulated in Vitro Gastrointestinal Digestion and Mineral Composition of Wheat and Wheat Products (Triticum spp.)

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ABSTRACT

A wide range of bioactive phytochemicals has been recognized to support overall health through their antioxidant potential. The current investigation seeks to evaluate the phytochemical and mineral composition of wheat and wheat products and their potential antioxidant activities after being simulated in vitro gastrointestinal digestion. Mineral analysis and phytochemical screening were carried out on the aqueous-methanolic extract and powdered samples, respectively, followed by sequential in vitro enzyme digestion. The presence of antioxidant molecules was determined based on the measurement of Total Phenol Content and Total Flavonoid Content. 1,1-diphenyl-2-picrylhydrazyl and ABTS radical scavenging ability assay, Ferric reducing antioxidant power assay, Nitric oxide and hydroxyl radical scavenging assay were carried out for antioxidant activity determination. The lipid peroxidation inhibitory activity was assessed in the brain and liver homogenates from rats induced with Iron (II) sulphate. The results indicate that raw wheat and spaghetti products are rich in important minerals like phosphorus, sodium, and potassium. Similarly, both wheat samples possess important phytochemicals, including phenol and flavonoid, both of which are decreased upon in-vitro digestion. Interestingly, our findings reveal increase in antioxidative activity upon in-vitro simulation. Finally, the results revealed that all wheat and spaghetti fractions inhibit Malondialdehyde production, suggesting the inhibition of lipid oxidation in the liver sample. However, the aqueous methanolic extracts of wheat and spaghetti, compared to their simulated in vitro digested samples, demonstrated higher inhibitory activity. The finding suggests that wheat and its processed product spaghetti offer huge benefits to individual health through its antioxidative activity.

Keywords: *Wheat, flavonoids, antioxidant, in-vitro, gastrointestinal*

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INTRODUCTION

For a very long time, cereal grains have remained a major source of staple foods worldwide. Whole grain cereals are good sources of phenolic compounds, which include derivatives of benzoic and cinnamic acids, anthocyanidins, quinines, flavonols, chalcones, flavones, flavanones, and amino phenolic compounds (Bangar & Kaushik, 2022). Grains contain tocotrienols and tocopherols (Lachman et al., 2018), and oryzanols (Ranathunga & Suwannaporn, 2022). This wide range of phytochemicals has been recognized to support overall health through their antioxidative potential (R. H. Liu, 2007). The protective function of antioxidants in the body is through balanced oxidative stress-free radicals (Zhang & Tsao, 2016). Cereal grain antioxidants are thought to act as direct free radical scavengers, cofactors of antioxidant enzymes or as indirect antioxidants (Fardet et al., 2008; Rudrapal et al., 2022). Free radical compounds result from normal metabolic activities as well as from the diet and environment (Law et al., 2017). Excess amounts of these reactive substances can cause oxidative damage to biomolecules such as lipids, protein and DNA (Halliwell & Gutteridge, 2015) resulting in an increased risk of chronic disease and contributing to general inflammatory response and tissue damage. Reactive oxygen species (ROS) including superoxide anion, hydrogen peroxide, peroxyl radical, hydroxyl radical, singlet oxygen and peroxynitrite are produced during cellular metabolic processes (Halliwell & Gutteridge, 2015) and they are key players in a variety of physiological and pathological processes, including cell signaling transduction, cell proliferation, differentiation, apoptosis, as well as ischemia-reperfusion, inflammation and many neurodegenerative disorders (Ames et al., 1993; Averill-Bates, 2024; Checa & Aran, 2020; S. Liu et al., 2025).

ROS is generated due to imbalance between pro-oxidants and antioxidants leading to oxidative stress (Ames et al., 1993). The process of oxidation in cells is usually regulated by antioxidants, which delay or prevent cellular damage by maintaining a healthy balance between pro-oxidants and endogenous and/or dietary antioxidants (Ames et al., 1993). The dietary antioxidants (minerals, trace elements, vitamins, carotenoids, polyphenols, alkylresorcinols, betaine, choline, sulfur amino acids, phytic acids, lignans and avenanthramides) of whole grain cereals are primarily located in the bran and germ fractions (Fardet, 2010; Fardet et al., 2008). Whole cereal grain antioxidants are water and fat-soluble, providing protection through the entire digestive tract (Nirmala & Joye, 2020). Wheat is an example of a cereal grain that belongs to the family of Triticum spp. (Singh et al., 2012). Botanically, it is a type of fruit called caryopsis, originally from the Levant region of the Near East but now cultivated worldwide. Globally, wheat is the leading source of vegetable protein in human food, having higher protein content than other major cereals like maize (corn), or rice (Poutanen et al., 2022; Sharma & Kumar Sharma, 2025). Growing evidence indicates that intake of whole wheat foods may be associated with potential health benefits including the reduced risk of coronary heart diseases and certain types of cancer (Tighe et al., 2010; Wei et al., 2022). These beneficial effects are attributed to non-digestible carbohydrates and a group of small molecular weight phytochemicals with antioxidative properties present in wheat grain (J. Liu et al., 2020).

The antioxidant compounds present in cereals, and other plant foods have been extracted and measured by different methods (Djordjevic et al., 2011; Singh et al., 2012). While the plant products investigated clearly demonstrate a range of antioxidant contents, they do not necessarily relate to the available antioxidants when the cereals are degraded by the enzymes and juices of the gastrointestinal tract, which can be released during passage through the gastrointestinal tract. Upon ingestion, several proteolytic enzymes break proteins into peptides of various sizes and free amino acids, the pancreatic amylase degrade starches, simple sugars and other digestible carbohydrates into disaccharides and trisaccharide, while fats are hydrolyzed by pancreatic lipase, cholesterol esterase and phospholipase, before absorption by the body (Dhillon et al., 2017; Lass et al., 2011; Williams & Goldfine, 1985). All the digested nutrients are absorbed through the intestinal walls, and the waste products are propelled into the colon for excretion. This process potentially modifies the biological activities of digested food, playing a tremendous role in the final benefits of the ingested food. Therefore, the present investigation sought to evaluate the mineral and phytochemical composition of wheat and wheat products and the potential changes in their antioxidant activities after in-vitro enzyme digestion, with a view to provide information on the effect of gastrointestinal tract digestive enzymes on the antioxidant activities of the selected cereals.

METHODS

Sample Preparation

Raw wheat used in the production of Spaghetti and the Spaghetti finished product were obtained from BUA Pasta Limited, Apapa, Lagos State, Nigeria.

The samples were milled into powdered form using electric blender and then stored in a container and kept at a temperature of 4-10°C. Aqueous-methanolic extraction was carried out by dissolving 5g of powder sample in 25ml acidified water -ethanol containing 80% methanol, 1% concentrated Hydrochloric acid and 19% distilled water and allowed for 24hr and the process repeated by resuspending in 25ml acidified water -ethanol. The mixture was then filtered through Whatman filter paper No. 42. The filtrate was collected inside amber bottle and stored at -4°C prior to analysis. The in vitro digestion using sequential α-amylase, pepsin and pancreatin enzymatic steps is based on a slightly modified method reported by (Delgado-Andrade et al., 2010). In brief, 2g of the milled samples [raw wheat and spaghetti] was weighed and dissolved in 40ml of distilled water. 300µl of α- amylase [32.5mg of α-amylase was dissolved in 25ml of 1mM calcium chloride at pH 7] was added to the tubes. The tubes were incubated in a shaking water bath set at 37°C for 10 minutes and at 80 strokes/min. After 10 minutes, the pH was adjusted to 2 using concentrated HCl. After 30 minutes incubation in a shaking water bath set at 37°C, 2mg pepsin which was dissolved in 1ml of 0.05M HCl was added to the tube. The tubes were then incubated in a shaking water bath set at 37°C for 10 minutes at 80 strokes/minute. After 20 minutes of shaking the tubes, the pH was adjusted to 6 using NaOH. Then 10ml of pancreatin [3gram of pancreatin was dissolved in 20ml distilled water] was added and the tubes were incubated in a shaking water bath set at 37°C for 20 minutes. The pH was adjusted finally to pH 7.5 using NaOH [simulating pH conditions in the small intestine]. Then the tubes were incubated for 10 minutes in a shaking water bath set at 37°C. The digested samples were incubated at 100°C for 4 minutes to inactivate the enzymes, and the digested sample was then centrifuged for 60 minutes at 3200 revolutions/minute, and the soluble fraction was kept in the refrigerator for antioxidant, and lipid peroxidation analysis. The insoluble portion was discarded. An undigested control was done with the same procedure used for the in vitro digestion without the enzymes.

Animals Care

Healthy male Albino rats weighing 200-250g were selected and used for the study. They were housed at 253^{0}C according to standard animal handling procedures and care of animals (Ethical Approval Number- FUTA/BCH/020). They were acclimatized for a week, fed with standard feed pellets and water.

Phytochemical Screening

Phytochemical screening test was carried out on the aqueous-methanolic extract to identify the presence of alkaloids, saponins, tannins, phylobatannins, anthraquinones, terpenoids, flavonoids and cardiac glycosides using standard procedures as described by (Trease & Evans, 1989), (Edeoga et al., 2005) and (Sofowora, 1993) with slight modifications.

Antioxidant and Activity Assays

The total phenolic content of the aqueous-methanolic extract of wheat and the wheat product (Spaghetti) samples was determined by the Folin-Ciocalteu assay as described by (Waterman & Mole, 1994). The total flavonoid content of the aqueous-methanolic extract of wheat and the wheat product (Spaghetti) samples was determined using the method reported by (Meda et al., 2005).

The Ferric reducing power of the extracts was determined by assessing the ability of each extract to reduce FeCl3 solution as described by (Oyaizu, 1986) . In vitro DPPH radical scavenging activity was done according to the method of (Brand-Williams et al., 1995), with a little modification. ABTS radical scavenging activity of the aqueous-methanolic extract of wheat and the wheat product (Spaghetti) samples was determined using ABTS antiradical assay as described by Awika et al. (Awika et al., 2003) . The ferric reducing antioxidant power (FRAP) of the samples were carried out using the method of (Halliwell & Gutteridge, 1981). In addition, the nitric oxide radical scavenging capacity of the aqueous-methanolic samples was measured by Griess reaction (Sangameswaran et al., 1970).

In Vitro Lipid Peroxidation Assay

The rats were euthanized using cervical dislocation and the cerebral and hepatic tissue (whole-brain and liver, respectively) were rapidly dissected and placed on ice and weighed. The tissues were subsequently homogenized in cold Tris HCl buffer (pH 7.4) (1:10 w/v) with about 10-up-and-down strokes at approximately 1,200 rev/minute in a Teflon-glass homogenizer. The homogenate was centrifuged for 10 minutes at 3000g to yield a pellet that was discarded, and the low-speed supernatant (S1) was kept for lipid peroxidation assay.

The ability of the aqueous-methanolic extract of wheat and the wheat product (Spaghetti) sample to inhibit lipid peroxidation was tested using a modified method of (Ohkawa et al., 1979). Briefly, S1 fraction (liver and brain homogenate) was mixed with a reaction mixture containing 100mM Tris HCl buffer (pH 7.4), aqueous-methanolic samples freshly prepared pro-oxidant solution (0.071mM FeSO4 and 7mM Sodium Nitroprusside). The colour reaction was carried out by addition of 8.1% sodium dodecyl sulphate, acetic acid/HCl solution (pH 3.4) and 0.6% thiobarbituric acid, respectively. The absorbance was read at a wavelength of 532nm. As control, the homogenate was peroxidized with the pro-oxidant solutions without the sample. The reaction without the pro-oxidant solutions, the homogenates and the sample was used as a blank.

Mineral Analysis

Five grams (5g) of the sample was tri-ashed in an electric furnace at 550°C for 24 hours. The resulting ash was cooled in desiccators, weighed and dissolved with 2ml of concentrated HCl and few drops of concentrated HNO3 were added. The solution was then evaporated almost to dryness and the content transferred to 100ml volumetric flask and diluted to volume with deionized water. Following appropriate dilution, each element analysis was carried out using Buck Atomic absorption spectrophotometer model 210A, as described in the official method of the (Association of Official Analytical Chemists., 1990).

Data Analysis

All the analyses were run in triplicate. Statistical analyses were conducted using Prism software, version 4 (Graph Pad) and Microsoft Excel software (Microsoft Corporation, Redmond, WA). P values < 0.05 were significant.

RESULTS

Phytochemical Screening

The result of the phytochemical screening of wheat and wheat products is as presented in Table 1.

Table 1: *Phytochemical Constituents of Wheat and Wheat Product (Spaghetti)*

TEST	RW	RS
Alkaloid	+	+
Saponin	+	+
Tannin	-	-
Phlobatannin	-	-
Terpenoid	+	-
Flavonoid	+	+
Steriod	-	-
Anthraquinones	-	-
Salkwoski	-	+
Cardiac Glycosides	+	+

+ = Present,- = Absent, RW = Raw Wheat, RS = Raw Spaghetti

The phytochemical screening revealed the presence of alkaloids, saponins, flavonoids and cardiac glycosides in both the raw wheat and its processed product (spaghetti). In addition, terpenoids were present in raw wheat (RW) while Salkowski is present in the raw spaghetti (RS). Both samples were lacking tannins, phlobatannins, anthraquinones and steroids.

Phenolic and Flavonoid Content

Figure 1 presents the total phenolic and mean flavonoid value of the aqueous methanolic extracts and simulated in vitro enzyme digest of wheat and spaghetti. The total phenolic content (mg/100g) of the samples expressed in tannic acid equivalent is shown in Figure 1A. Generally, the digested samples demonstrated a higher phenolic content than that of the aqueous methanolic extract in both samples, with the digested raw wheat sample significantly higher in phenolic content (57.71) than the digested spaghetti (38.88). The mean values of total flavonoid content of the samples are presented in Figure 1B. Findings revealed a higher mean total flavonoid content in digested spaghetti 91.989 mg/g while the aqueous methanolic extract of raw wheat contains the least, with a total flavonoid content of 81.77 mg/g. While the digested and processed products indicate a higher concentration of total flavonoid, there is, however, no significant difference between the samples.

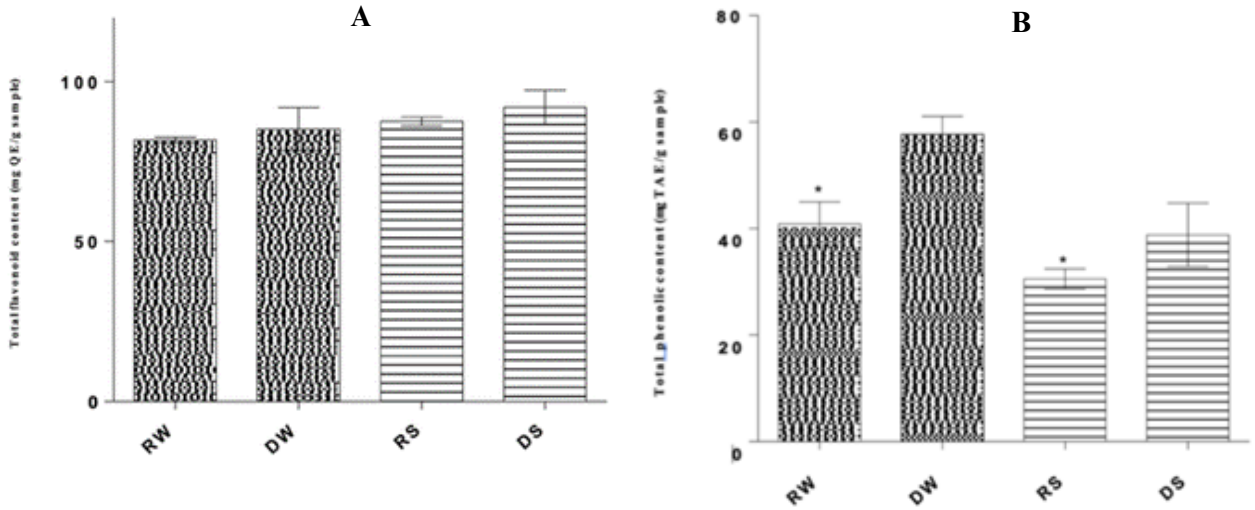
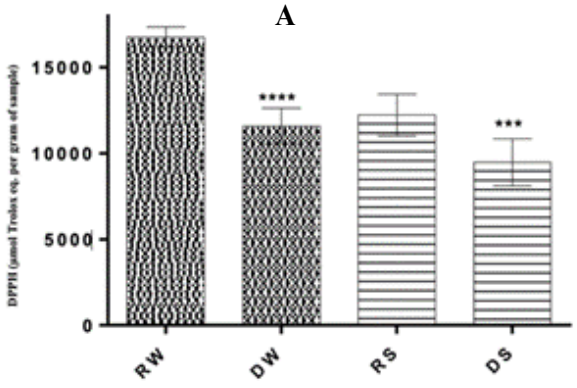


Figure 1: presents the total phenolic and mean flavonoid value of the aqueous methanolic extracts and simulated in vitro enzyme digest of wheat and spaghetti. (A) Total Flavonoid content of raw wheat and spaghetti samples in Quercetin Equivalent; The results are expressed as milligrams of quercetin equivalent (mg QE) per 100g. (B) Total phenolic content of raw wheat and spaghetti; Result is expressed as mg of the tannic acid equivalents (TAE) per 100 g of dry weight of the dry sample. RW = aqueous methanolic extract of raw wheat; RS = aqueous methanolic extract of spaghetti; DW= simulated in vitro digested wheat; DS = simulated in vitro digested spaghetti.

Antioxidant Activity

The antioxidative effects of the aqueous methanolic extract and simulated in vitro enzyme digest of wheat and spaghetti were evaluated by estimating the Ferric reducing antioxidant power, DPPH scavenging ability, ABTS scavenging activity, Nitric Oxide scavenging activity of the samples and hydroxyl ion scavenging activity. The result is presented in Figure 2. Assessment of DPPH scavenging ability of the samples expressed in µmol Trolox eq. per gram of sample (Figure 2A) reveals that Aqueous methanolic extract of raw wheat demonstrated the highest scavenging ability (16769.2) followed by aqueous methanolic extract of raw spaghetti (12230.8). Similarly, the result of ABTS scavenging activity test expressed in mg of Trolox equivalents is presented in Figure 2B. Findings revealed a high oxidant scavenging activity of the aqueous methanolic extract of both samples with ABTS scavenging abilityin aqueous methanolic extract of raw wheat estimated at (60833.33 mg/g) while that of the aqueous methanolic fraction of raw spaghetti (approximately 72000 mg/g). The least oxidant scavenging activity observed was for simulated in vitro digested spaghetti. On the other hand, evaluation of the ferric reducing antioxidant power expressed in mg ascorbic acid (Figure 2C) revealed that simulated in vitro digested samples possessed a significantly high ferric reducing antioxidant power compared to the aqueous methanolic fraction. However, the raw wheat product demonstrates better activity in each category compared to the processed wheat product. Specifically, the in vitro digested raw wheat sample had the best activity (15.577 mg/g) while the aqueous methanolic fraction of processed wheat had the least activity (2.80mg/g). Theresult shows that the simulated in vitro digested samples have more ferric reducing power than the aqueous methanolic extract of the samples. The result of the nitric oxide scavenging activity for the samples expressed as per cent scavenging ability is provided in Figure 2D. Nitric oxide scavenging activity in simulated in vitro digested wheat was 67.17%, and in simulated in vitro digested spaghetti, 94.49%, while the least value was observed in aqueous methanolic extract of raw wheat (21.36%). These findings revealed that simulated in vitro digested samples offer a higher scavenging activity compared to the aqueous methanolic fractions of both raw wheat and spaghetti. However, compared to the raw wheat products, Spaghetti has better Nitric oxide scavenging activity. Finally, the OH radical inhibitory capacity of the samples was estimated, and the result is shown in Figure 2E. The rate of hydroxyl radical generation decreases with increasing concentration of both samples, with the in vitro simulated digested sample having the most significant inhibitory activity



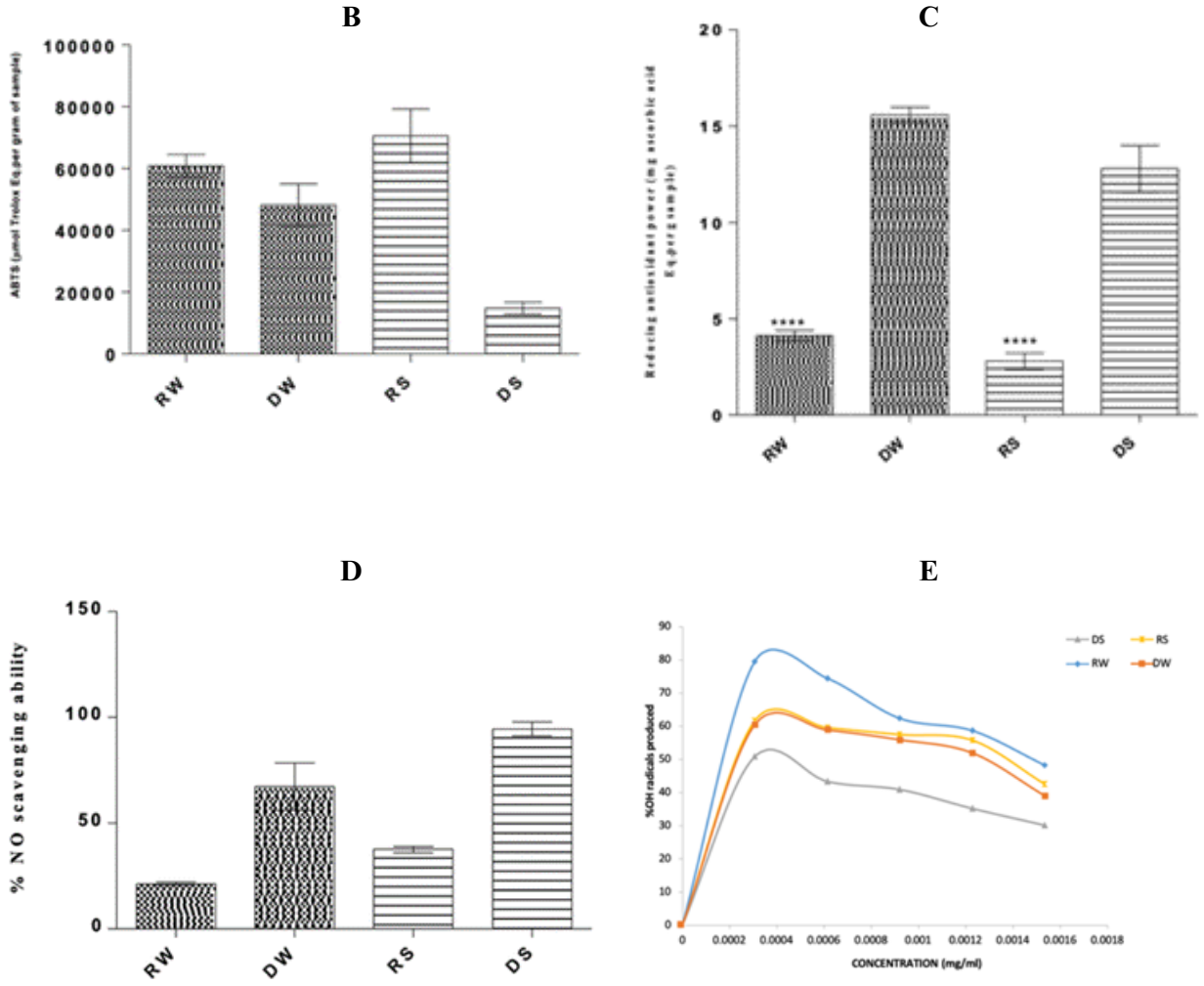
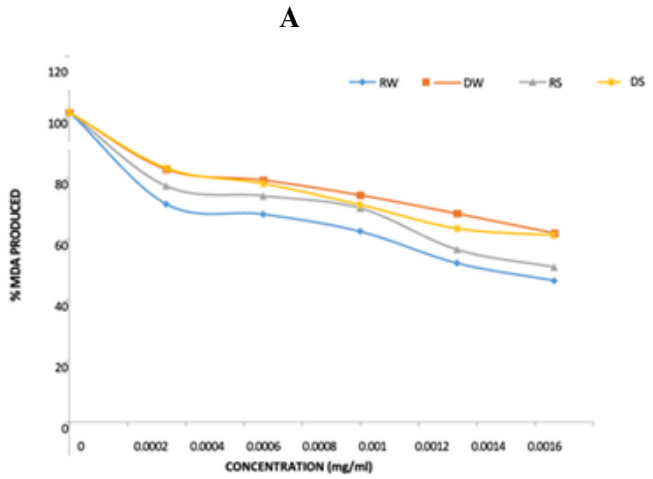


Figure 2: Antioxidative properties of wheat and spaghetti. (A) DPPH scavenging activity of the raw wheat and spaghetti samples expressed in mg of Trolox equivalents per sample. (B) ABTS radical scavenging activity test expressed in mg of Trolox equivalents per sample (C) Ferric Reducing Power of the samples extracts and Ascorbic acid; Result is expressed in mg ascorbic acid equivalent (AAE) per 100g of dry weight of sample. (D) Percentage Nitric oxide Scavenging ability of raw and digested wheat and spaghetti. (E) Hydroxyl radical inhibition capacity of raw and digested wheat and spaghetti. RW = aqueous methanolic extract of raw wheat. RS = aqueous methanolic extract of spaghetti; DW = simulated in vitro digested wheat; DS = simulated in vitro digested spaghetti.

In Vivo Activity Assay

The in vivo protective effect of the studied phenolic compounds on Iron (II) sulphate induced lipid peroxidation was investigated using rat's liver and rat's brain homogenate as lipid rich source and the result is presented in Figure 3.The result revealed an increase in the inhibitory action of the phenolic fraction of wheat and spaghetti against lipid oxidation in rat's liver (Figure 3A) and brain (Figure 3B) homogenate in a concentration-dependent manner. Findings revealed decrease inhibitory activity of simulated in vitro digested fractions in the liver sample compared to the methanolic extract of both samples in the same organ (Figure 3A). On the other hand, inhibitory action against lipid oxidation on the brain homogenate is most prominent in the in vitro digested fraction of spaghetti (Figure 3B).



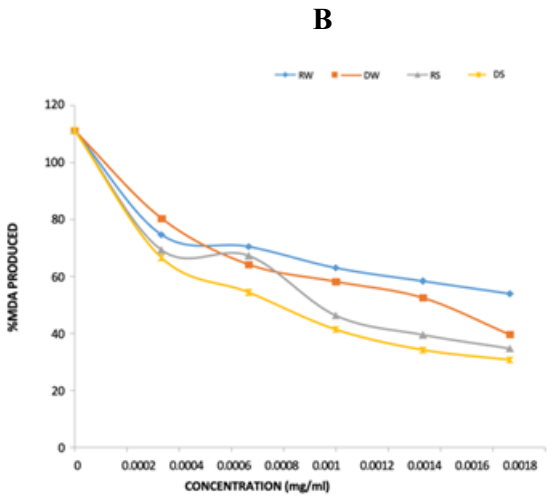


Figure 3: The inhibitory effects of methanolic extract and digested phenolic compound present in raw wheat and spaghetti on Iron (II) sulphate induced lipid peroxidation. (A) Effect on rat's liver homogenate (B) Effect on rat's brain homogenate

Mineral Analysis

Mineral analysis carried out on raw wheat and spaghetti powder reveals the quantity of both macro element and microelements found in the sample. The value of each mineral analyzed is presented in Table 2. Findings revealed that wheat and spaghetti samples contain important minerals such as iron, magnesium, zinc, calcium, cobalt, sodium, potassium and phosphorus with the absence of lead in both. The level of iron, magnesium, zinc, calcium, is significantly higher in raw wheat samples compared to Spaghetti, while there is no significant difference in the proportion of sodium potassium and phosphorus. However, while manganese is also present in raw wheat, it is absent in spaghetti powder used in the study (Table 2).

Table 2: *Mineral Composition (mg/100g) of Raw Wheat and Spaghetti Flour Blend*

Sample	RW	RS
Fe2+	34.21±5.44b	13.11±0.99a
Mg2+	244.67±13c	224.65±11a
Zn2+	17.13±1.76b	12.02±1.33a
Ca2+	221.19±14.02a	188.09±3.22d
Mn2+	39.22±3.24a	-
Pb	-	-
Co	3.07±0.11c	3.00±0.00a
Na	16.88±2.11a	25.56±8.03d
K	500.04±47.2c	600.00±56.70d
P	541.20±65.4c	742.11±22.11a

DISCUSSION

The findings of this study show the phytochemical screening of aqueous methanolic extract of raw wheat and spaghetti in Table 1, revealing the presence of alkaloids, saponins, flavonoids and cardiac glycosides in both the raw wheat and its processed product (spaghetti). These suggest a potential biological activity and health benefits of wheat and wheat products.

For instance, saponins are thought to boost the immune system, reduce cancer risk, as well as lower serum cholesterol by binding to intestinal cholesterol (Elekofehinti et al., 2021; Navarro del Hierro et al., 2018; Timilsena et al., 2023). Similarly, flavonoids are widely reported to possess antioxidant, anticancer, anti-allergic, anti-inflammatory, and anticarcinogenic and gastro protective properties (Jomova et al., 2025; Ullah et al., 2020).

Animal studies have shown that dietary phytochemical antioxidants can remove free radicals and thereby exhibiting potent antioxidant activities (Sahin, Orhan, Akdemir, et al., 2012; Sahin, Orhan, Tuzcu, et al., 2012). Wheat contains a diverse array of bioactive compounds that may contribute to its antioxidant capacity. The result of the total flavonoid content (TFC) for aqueous methanolic extracts of wheat and spaghetti presented in (Figure 1A) revealed that the aqueous methanolic extract of spaghetti contains higher flavonoid content than the aqueous methanolic extract of raw wheat. Interestingly, the result also indicates that in vitro digested samples possess a very high total flavonoid content (TFC) compared to the respective aqueous methanolic extract of the sample. This finding corroborates the work of Nayak et al. (2022), who concluded that in vitro digestion enhances both TFC and TPC of roselle and mustard leaves. Similarly, Balakrishnan and Schneider (2020) demonstrated that antioxidant activities are enhanced after in vitro enzyme digestion of quinoa flavonoids. Polyphenols in plant foods are important because plant phenolics represent one of the major groups of compounds acting as primary antioxidants or free radical terminators (Upasana & Jyotirekha, 2022). The Total Phenolic content (mg tannic acid equivalent/g of the sample) presented in Figure 1B revealed that wheat, especially in its raw form, possesses a very high phenolic content, which increased upon enzyme digestion. This result agrees with the report observed by (Nayak et al., 2022) that enzymic digestion enhances TPC release from plant products. According to Grace et al. (2009), polyphenols are compartmentalized in plant tissues and need to be mechanically released before they can be absorbed by the body. These bound phenolics might be released during the heating process, thus increasing the total phenolic content of the sample. On the other hand, increasing formation of Maillard reaction products may significantly alter the TPC content (Maillard & Berset, 1995). However, contrary to our findings, some authors observed a decrease in TPC after digestion of plants products (Maghsoudlou et al., 2019; Palermo et al., 2014). This difference is likely due to the nature and extent of heat treatment. In the current work, the heating temperature occurs during the sequential enzyme digestion process which is relatively low compared to the temperature of 180°C used by (Maghsoudlou et al., 2019). At such a high temperature, phytochemicals in plants can be reduced by the heat application.

To evaluate the antioxidative effects of the cereals, five distinct methods of DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging ability, ABTS scavenging activity, the Ferric reducing antioxidant power, Nitric Oxide scavenging activity and the hydroxy radical inhibitory ability of the samples were adopted. The DPPH radical scavenging method, compared to other methods, requires a relatively short time for activity determination. The result in Figure 2A shows that the cereals possess DPPH radical scavenging properties, which decrease with enzyme digestion. This finding agrees with Abozed et al (Abozed et al., 2014) which reported increasing TPC from different fractions of whole wheat and Bran. In a separate experiment, Wong et al. also found out significant decrease in DPPH scavenging activity after in vitro digestion of Hibiscus cannabinus L. (Wong et al., 2014). According to Awika et al. (2003), there is a positive link between the determination of phenolic antioxidants using the ABTS+ cation radical and DPPH radical assays (Awika et al., 2003). Thus, monitoring the antioxidant activity of phenolic compounds by their ability to scavenge ABTS+ radical was demonstrated to give a good prediction as seen in Figure 2B. The findings again reveal that wheat possesses ABTS+ cation radical scavenging properties, which decrease with enzyme digestion. The characteristic decline in the radical scavenging properties has been reported by other scholars (Li et al., 2024; Mercatante et al., 2022) and could be attributed to loss of polyphenolic antioxidant molecules through processing (Mercatante et al., 2022; Minaxi et al., 2013). A reduction in antioxidant activity estimated using DPPH radical assays and FRAP (Ferric reducing antioxidant power) was also reported by Wanyo et al, following in vitro enzyme simulation of rice bran (Wanyo et al., 2024). However, in our study, the FRAP of the cereals expressed in mg ascorbic acid (Figure 2C) supports a significantly high ferric reducing antioxidant power of the in vitro enzyme-digested wheat and wheat products. This discrepancy might be due to the differences in the reaction mechanism governing each reaction as was rightly noted by the author cited (Rumpf et al., 2023). Similarly, the in-vitro digested cereals, especially spaghetti, exhibited good NO scavenging activity, leading to the significant reduction of the nitrite concentration in the assay medium. The toxicity of NO increases when it reacts with superoxide anion radical to form the peroxynitrite anion ($ONOO^-$), which is a potential strong oxidant that can decompose to produce OH radical, and NO_2 (Ford & Miranda, 2020). The present study shows that enzyme digestion of wheat and its product (spaghetti) significantly increase the nitric oxide scavenging activity (Figure 2D). Moreover, our findings suggest a dose-dependent inhibition of hydroxyl radical generation, thus supporting the findings that polyphenols and flavonoids elicit antioxidative properties by preventing free radical accumulation (Rudrapal et al., 2022; Upasana & Jyotirekha, 2022; Zhang & Tsao, 2016).

The in vitro simulated digested sample having the most significant hydroxyl radical inhibitory activity highlights the potentiating effect of enzymatic processing on antioxidative molecules. Taken together, FRAP, NO assay and hydroxyl radical inhibitory assays demonstrated that in vitro enzyme-digested samples showed an increase in antioxidant capacity compared to aqueous methanolic extract of the samples.

Dietary intake of minerals such as sodium plays an important role in the absorption of other nutrients, like glucose, amino acids, and water. Hence, ten important mineral compositions of the wheat powder were determined and summarized in Table 2. Findings revealed that wheat and spaghetti samples contain important minerals, including iron, magnesium, zinc, calcium, cobalt, sodium, potassium and phosphorus, with the absence of lead in both. Although the level of iron, magnesium, zinc, and calcium is significantly higher in raw wheat samples compared to the Spaghetti, there is no significant difference in the proportion of sodium, potassium and phosphorus. Moreover, while manganese is also present in raw wheat, it is absent in spaghetti powder according to this study. This is in correlation with an earlier report, which implies that refined cereals contain significantly less minerals and trace elements (Garutti et al., 2022). Processing conditions such as heat treatment and extrusion may have led to the reduced level of mineral content in the spaghetti (Garutti et al., 2022).

The inhibitory effect of the aqueous methanolic extract and the in vitro enzyme-digested samples on Iron II sulphate-induced lipid peroxidation (Lotfipour Nasudivar et al., 2024) was evaluated using liver and brain homogenate as shown in Figure 3. The cereals demonstrated a inhibitory activity at sample concentration in a dose dependent manner, evidenced by reduced MDA generation. Furthermore, the results revealed that all wheat and spaghetti fractions inhibit Malondialdehyde (MDA) production starting at the smallest tested concentration 3.33 x 10^{-4}mg/m, suggesting the inhibition of lipid oxidation in the liver sample. Although the aqueous methanolic extracts of wheat and spaghetti compared to their simulated in vitro digested samples demonstrated higher inhibitory action in the liver homogenates, while in the brain homogenates, the simulated in vitro digested samples demonstrated higher inhibitory action. Elsewhere, it has also been shown that Cyprinus carpio haematopterus simulated gastrointestinal digestion products effectively inhibit lipid peroxidation (Xiao et al., 2019). This observation again highlights the understanding that in vitro enzyme digestion enhances antioxidative activity and consequently increases the protective ability of phytochemicals (Grace et al., 2009; Maghsoudlou et al., 2019), which, according to Grace et al. (2009), could be a result of the disruption of the complex polyphenol structure by heat, thus increasing antioxidative phenolics obtained after heating.

CONCLUSION

Wheat contains bioactive compounds such as phytochemicals, including flavonoids and polyphenol that possess antioxidant function in addition to other biological activity. These compounds contribute immensely to the health benefits associated with wheat consumption but elucidating this relationship requires research that characterizes their content as well as the impact of agricultural practices and food processing on their health benefits. This research shows that the enzyme digestion of wheat and wheat products increases their antioxidant ability, and while the refining process lowers the mineral composition of the cereals, many important minerals are however retained in spaghetti according to this study. Taken together, our finding suggests that consumption of wheat and its processed product spaghetti offer huge benefit to individual via its antioxidative effects

LIMITATIONS

This study demonstrates the antioxidative effect of enzyme digested wheat and wheat by product in an in-vitro study. Further experiment is needed to characterize how the antioxidative activity of wheat and wheat products are modified in-vivo.

RECOMMENDATION

Future studies should validate these in vitro findings with in vivo animal studies (e.g., rodent models) to confirm that the increased antioxidant capacity after enzyme digestion translates into measurable biological benefits. Moreover, researchers should characterize the specific phenolic and flavonoid profiles, and their bio accessibility, across wheat varieties and growing conditions. Based on this study, consumers and dietary guidelines may emphasize whole-grain and minimally processed wheat products, including spaghetti, as part of a balanced diet.

Availability of Data and Materials

The data used and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare no conflicts of interest.

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Authors' Contributions Statement

O.S.O.: Investigation, Data curation, Project administration, Writing of the original draft. O.S.: Supervision, Conceptualization, Methodology. M.A.O.: Formal analysis, Data interpretation, Writing, review and editing. M.A.A.: Formal analysis, Methodology, Software, Visualization. A.B.A.: Validation, Resources, Data curation. A.O.: Validation, Quality control, Resources. E.E.A.: Literature review, Data curation, Writing of the original draft. P.T.A.: Formal analysis, Visualization, Writing, review and editing. T.F.A.: Validation, Quality assurance, Writing, review and editing. O.A.O.: Data curation, Resources, Writing, review and editing.

All authors have read and approved the final manuscript.

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