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Total phenolic, flavonoid and antioxidant capacities of processed pearl millet and sorghum flours

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ABSTRACT
This study assessed the effects of sprouting and fermentation on total phenolic content, total flavonoid content and antioxidant property of pearl millet (*Pennisetum glaucum*) and sorghum (*Sorghum bicolor*). Pearl millet and sorghum were subjected to the treatment of sprouting and fermentation for 0 h, 24 h, 48 h, 72 h and 96 h. After each of the treatments, the samples were oven-dried at 60 °C for 24 h and milled into fine flour of 0.05 mm in size. The total phenolic content, flavonoid content and antioxidant capacities were quantified using standard methods. The results of the study revealed that sprouting significantly ($p<0.05$) increased the total phenolic, total flavonoid and antioxidant capacities of pearl millet and sorghum. However, sprouting time significantly ($p<0.05$) reduced the total phenolic content of pearl millet. A similar trend of increment was observed in fermentation where there was a significant ($p<0.05$) increase in the total phenolic, total flavonoid, reducing the power and antioxidant capacities of pearl millet and sorghum with an increase in fermentation time. Therefore, the results of this study revealed that the two bioprocesses (sprouting and fermentation) significantly increased the total phenolic, total flavonoid and antioxidant capacities of pearl millet and sorghum. However, pearl millet exhibited reduced TPC with an increase in sprouting time.

Introduction

Pearl millet grains (*Pennisetum glaucum*) are the traditional staple food of the dry land regions of the world. They contain nutrients such as proteins, essential fatty acids, dietary fibre, B-vitamins, and minerals such as calcium, iron, zinc, potassium and magnesium (FAO, 1995). Millet is rich in health-promoting phytochemicals like polyphenols, lignans, phytosterols, phytooestrogens, and phytocyanins. These compounds function as antioxidants, immune modulators, detoxifying agents, and hence, protect against age-related degenerative diseases like cardiovascular diseases (CVD), diabetes and cancer. Also, they help in the reduction of blood sugar levels and in the formulation of diet for people with celiac diseases, as well as regulate blood pressure (Rao et al., 2011). Despite these beneficial effects, the consumption of millet as food has significantly declined over the past three decades. The major reasons for a decrease in its consumption are attributed to a lack of awareness of nutritional merits, inconveniences in food preparation, lack of processing technologies and also the government policy of disincentives towards millet grain production (Rao et al., 2011).

Pearl millet is an indispensable food for millions of people inhabiting the semi-arid tropics. It constitutes a major carbohydrate source for millions of rural dwellers, which is attributed to the plant's ability to thrive in adverse weather conditions and on non-fertile soil. Different rural and urban communities that are familiar with the grains have evolved different home technologies in processing the grains into different

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millet-based products such as *fura, dambu, koko, kunun-zaki, pito* and *burukutu*. Efforts have been made to scale up and optimise the utilisation of millet and its products in an attempt to realise and derive the benefits thereof (Amadou et al., 2011). The bulk of the study on millet is geared towards its utilisation as an infant diet and adult breakfast gruel where the wet-milled paste is fermented into 'Ogi' in Nigeria, 'Uji' in Kenya and 'Kenkey' in Ghana (Ocheme and Chinma, 2008). Traditional processing technologies which include cooking, roasting, soaking, fermentation, and germination significantly affect the sensory attributes, nutritional composition, textural characteristics, bioactive constituents and wholesomeness/safety of the end products (James et al., 2020ab).

Sorghum (*Sorghum bicolor*) is a significant food source in Africa and Asia and is widely grown in the southern United States as cattle feed. It is a principal source of calories and proteins along with other cereals. The dry grain contains 10.15% moisture, 8 - 14% protein, 70 - 75% carbohydrates and 2 - 7% fat as well as a variety of minerals and vitamins. Among the cereals, sorghum has the highest content of phenolic compounds comprised of approximately 6% w/w and these include all the classes of phenolic compounds such as phenolic acids, flavonoids, carotenoids and condensed tannins (Cardoso et al., 2014; Chávez et al., 2017; Llopart and Drago, 2016; Stefoska-Needham et al., 2015). Local consumption of sorghum varies from one community to another; however, developed countries have a preference for its uses in industrial application. Industrially, sorghum is used in the production of biogas, starch-binders, brewing of alcoholic beverages and as a source of sweetener among others. The grains are domestically roasted, boiled, popped or milled into flour and used as a composite flour for making bread, and in making soft and in thick porridges (Beta et al., 2000; Dicko et al., 2005; Fall et al., 2006).

There is a dearth of information on the impact of some adopted local processing technologies on the availability of bioactive compounds. Brewer et al. (2014) examined the influence of flour size on the total phenolic content. The results revealed that flours with increased particle sizes ($p<0.05$) gave the highest yield of total phenolic compounds (both bound and soluble), and showed the highest antioxidant, as well as total anthocyanin and carotenoid capacity. In another vein, Luthria and Liu (2013) reported that phenols in sorghum are found in the outmost layers of the pericarp, which implies that sorghum bran could be used as a food additive with high antioxidant potential. Furthermore, Afify et al. (2012) evaluated the effects of soaking on phenolic compounds, flavonoids, tannins, vitamin E, β-carotene and antioxidant capacity of three varieties of sorghum. They reported that soaking time significantly ($p<0.05$) reduced total phenol, flavonoid and antioxidant capacity. Even thoughsorghum is a rich source of phenolic compounds, its bio availability may be affected by the technologies or processes applied before its intake, which may interfere with its biological potential (Brewer et al., 2014).

Sprouting allows the grains to be hydrated with appreciable amounts of moisture giving rise to biochemical and physiological changes. These include the activation of hydrolytic enzymes such as amylases, proteases, lipases, fibre-degrading enzymes and phytases which are inactive in the raw seeds. These enzyme pools contribute to the breakdown of food macromolecules thereby increasing their accessibility and digestibility (James et al., 2020a; Yan et al., 2010). The resultant products of decomposition of high-molecular-weight polymers during germination lead to the generation of biofunctional and novel substances. Hithamani and Srinivasan (2014) observed an increase in the content of total soluble phenols, tannins and phenolic acids after the germination. Furthermore, Dicko et al. (2005) examined the effect of germination on phenolic compounds and antioxidant activities in sorghum grain and the results showed that, on average, germination did not affect the content of total phenolic compounds but decreased the content of pro-anthocyanidins, 3-deoxyanthocyanidins, and flavan-4-ols. However, for other cereals and pseudocereals such as amaranth, quinoa, buckwheat and wheat, the total phenolic content in the free form was increased twofold after the germination process while it was reduced after baking (Alvarez-Jubete et al., 2010). Similar to the processing technologies, during germination, severalvariables such as time, temperature and seed variety are determining factors in the release of phenolic compounds (James et al., 2020c). The increase in phenolic compounds, the reduction of anti-nutritional compounds and the low processing cost are advantages in the production and diversification of functional foods from both millet and sorghum using local processing technologies (James et al., 2020abc).

Fermentation is a biological process that increases the digestibility of cereals. It is widely used by African people for preparing traditional porridge-like food (Dlamini et al., 2007; Zaroug et al., 2014; James et al., 2020a). Also, it has been reported that fermentation with Lactobacillus significantly ($p<0.05$) affected the content of polyphenols and the antimicrobial activity of red sorghum. This was mediated by the presence and activities of glucosidase, phenolic acid reductase and phenolic acid decarboxylase which can

metabolize phenolic acids, phenolic acid esters and flavonoid glucosides (Svensson et al., 2010). These bioprocesses lead through different mechanisms to both increase and loss in the bioactive profile of cereals. Therefore, this study was designed to evaluate the two most common local processing methods, sprouting and fermentation time on the availability of some bioactive compounds, as well as their antioxidant and reducing powers on millet and sorghum.

Materials and methods

Sample collection and preparation

Pearl millet and sorghum grains were purchased from Central Market, Minna and were identified at the Crop Production Department, Federal University of Technology, Minna, Nigeria. The grains were sorted manually to remove stones, damaged ones and other extraneous materials.

Sprouting

One thousand gramme of intact grain samples was sorted and cleaned. The millet was washed in tap water and sterilized in ethanol for about 3 h in the ratio of 1:3 (w/v) in clean water. This was done to prevent mould growth during sprouting. The soaked grains were drained off and spread on different four wet jute bags and were covered with the same jute bag material. The grains were allowed to sprout in the dark at room temperature (28 ± 0.2 ^{o}C) for 2, 3, 4 and 5 days. Water was sprayed on the grains at the 6 h interval to keep sprouting grain moist. After respective days, sprouted grains were oven-dried at 60 ^{o}C for 24 h to a constant weight and were milled into flour of 0.5 mm size, kept in plastic bags and then stored at 4 °C for further analysis (James et al., 2020a).

Fermentation

One thousand gramme of each sample was thoroughly washed and allowed to naturally ferment in tap water in a ratio of 1:3 (grains to water, w/v) contained in a beaker covered with aluminium foil. The samples have undergone the process of fermentation for 2, 3 and 4 days respectively at room temperature 28 ± 2 ^{o}C. After each fermentation time, the seeds were drained and oven-dried at 80 ^{o}C for 24 h in a laboratory air-convection oven (Gallenkamp, England) and milled in a disc attrition mill (7hp, China). The resultant flours were sieved to pass through a 0.5 mm sieve size, kept in plastic bags and stored at a refrigeration temperature of 4 °C for further analysis (Hui et al., 2004; James et al., 2020a).

Extract preparation for quantification of total phenolic, flavonoid and antioxidant power

Each sample (10 g) was transferred into dark-coloured flasks and mixed with 200 mL of solvents with different polarities (water, methanol, ethyl acetate, acetone, petroleum ether), and stored at room temperature. After 24 h the infusion was filtered through Whatman No. 1 filter paper and the residue was re-extracted with an equal volume of solvents. The process was repeated for 48 h. In the end, supernatants were combined and evaporated to dryness under vacuum at 40 ^{o}C using a rotary evaporator and were kept in a sterile sample tube and stored in a refrigerator at 4 ^{o}C (James et al., 2020a).

Determination of total phenolic content

The Folin-Ciocalteu method as described by Rajha et al. (2014) was used. An aliquot of 10 µL of the sample solution was mixed with a 100 µL of commercial Folin-Ciocalteu reagent and 1580 µL of water. After a brief incubation at room temperature (5 min), 300 µL of saturated sodium carbonate was added. The generated colour was read after 2 h at room temperature at 760 nm using a UV-Vis spectrophotometer (UV-9200, UK). Different concentrations of gallic acid solutions in methanol (25, 50, 75, and 100 µg/mL) were prepared from the standard solution. The correlation between the absorbance and gallic acid concentrations creates a calibration standard curve. The phenolic compound concentration of the samples was expressed as gallic acid equivalent in grams of GAE per 100 g of dry matter (g GAE/100 g DM).

Determination of total flavonoids

The total flavonoid content was determined by the aluminium trichloride method using catechin as a reference compound (Zhishen et al., 1999). A volume of 125 µL of the sample extract was added to 75 µL of a 5% $NaNO_2$ solution. The mixture was allowed to stand for about 6 min.150 µL of aluminium trichloride (10%) was then added and incubated for about 5 min, followed by the addition of 750 µL of NaOH (1M). The final volume of the solution was adjusted to 2500 µL with distilled water. After 15 min of incubation, the mixture turned pink and the absorbance was measured at 510 nm. The total flavonoid content was expressed as g of catechin per100 g of dry matter.

Ferric reducing antioxidant power

Ferric reducing antioxidant power (FRAP) assay was carried out using the method reported by Benzie and Strain (1996). When ferric chloride reacts with 2,4,6-tripyridyl-s-triazine (TPTZ) at low pH, ferric is converted into ferrous causing the formation of ferrous tripyridyl triazine complex. FRAP values were obtained by comparing the absorbance change at 593 nm in the reaction mixture with those containing ferrous ions in known concentrations. The FRAP assay also takes advantage of the electron transfer reactions, wherein a ferric salt, Fe (TPTZ)2 III, is used as an oxidant under acidic conditions, pH 3.6. Aqueous solutions of known ferrous ion concentration in the range of 100 – 1000 µl ($FeSO_4.7H_2O$) were employed for calibration. The working reagent was prepared freshly each day by mixing acetate buffer, pH 3.6, TPTZ in HCl and $FeCl_3$, in the ratio of 10:1:1 (v:v:v). FRAP reagent was mixed with a sample extract in a test tube and vortexed. Absorbance readings were recorded after 4 min of sample reagent mixing at a wavelength of 593 nm.

1,1'-Diphenyl-2-Picrylhydrazyl (DPHH●)

DPPH● (1,1′-diphenyl-2-picrylhydrazyl) assay was carried out using the method described by Bran-Williams et al. (1995). DPPH−free radical was obtained by dissolving DPPH in methanol and was stable when placed in the dark at −20 °C until usage. As DPPH● reacts with antioxidants present in the food sample, colour changes from violet to yellow and the absorbance of the obtained solution was measured spectrophotometrically at 515 nm.

Statistical analysis

The obtained data were in triplicates and the results were subjected to a one-way analysis of variance and expressed as mean with standard deviation. The differences between means were separated by Duncan's Multiple Range Test using IBM SPSS Statistics Programme, Version 19.0 (Illinois, USA). Significant differences were expressed at a 5% level.

Results

Effect of sprouting on total phenolic, flavonoid and antioxidant property of pearl millet

Table 1 showed the effect of sprouting on total phenolic and flavonoid content and antioxidant properties of pearl millet. The total phenolic content of the samples significantly ($p<0.05$) decreased with an increase in sprouting time. The values ranged from 16.25 to 20.14 mg/100 g. The control (unsprouted) sample had the highest total phenolic content (20.14 mg/100 g) while 96 h sprouting gave the lowest value (16.25 mg/100 g). The decrease in total phenolic content could be due to leaching during grain hydration prior to sprouting and biochemical breakdown through activities of favourable enzymes activated and generated on bioactive compounds which are predominant in the testa of the grains. The decreasing trend in TPC with an increase in sprouting time in this study agrees with the results obtained by Afiffy et al. (2012) who reported a similar finding in some selected cereals. However, the result does not correspond to the study conducted by James et al. (2020a) who reported that bound phenolic compounds get liberated by the action of cell wall-degrading enzymes, mainly esterases, which become active during sprouting leading to their availability. In the same vein, Duenas et al. (2009) also revealed that sprouting solubilises condensed tannins which leads to the migration of phenolic compounds to the outer layer of the sprouting seed which is indicated by the browning of the sprouted seeds. This leads to a rise in the total phenolic content of the sprouted seeds. However, during seed sprouting, the enzyme systems are mobilized and activated; seed colour concentration reduced especially the hydrophilic component with a resultant decrease in tannin, anthocyanin and total phenolic content (Sokrab et al., 2012). Furthermore, the production of phenolic compounds in some plant species during growth and development is a natural process. They help in protecting plants against biotic and abiotic factors such as diseases, insects and environmental stresses such as drought (Nderitu et al., 2013; Khang et al., 2016; James et al., 2020a). Therefore, structural change in phytochemicals during the germination process has been considered a natural phenomenon in plants. Unlike in TPC, the total flavonoid content significantly increased ($p<0.05$) with an increase in sprouting time. The values ranged from 6.36 to 7.87 mg/100 g with 98 h sprouting having the highest while the control had the lowestvalue. This result is in line with the finding of Duodu (2014) who reported a significant ($p<0.05$) increase in total flavonoid in sprouted cereals. In an attempt to explain the mechanism of increase in flavonoids during sprouting, Perales-Sánchez et al. (2014) revealed that free, bound and total flavonoids are released through hydrolytic activities of mobilised enzymes during the sprouting process. Also, James et al. (2020a) reported a significant ($p<0.05$) increase in the total flavonoid of sprouted African oil bean seeds at the end of the second, the third and the fourth day of sprouting.

Table 1. Effect of sprouting on total phenolic, flavonoid and antioxidant property of pearl millet

Sprouting time (h)	TPC (mg/100 g)	TFC (mg/100 g)	FRAP (mmol/100 g)	DPPH (mmol/100 g)
0	$20.14^{a} \pm 0.14$	$6.36^{e} \pm 0.07$	$29.63^{e} \pm 0.07$	$34.65^{e} \pm 0.21$
24	$19.43^{b} \pm 0.14$	$6.73^{d} \pm 0.14$	$31.25^{d} \pm 0.00$	$38.65^{d} \pm 0.07$
48	$17.9^{c} \pm 0.21$	$6.79^{c} \pm 0.14$	$31.65^{c} \pm 0.00$	$40.15^{c} \pm 0.00$
72	$17.45^{d} \pm 0.14$	$7.16^{b} \pm 0.07$	$32.00^{b} \pm 0.00$	$40.22^{b} \pm 0.07$
96	$16.25^{e} \pm 0.00$	$7.87^{a} \pm 0.14$	$32.15^{a} \pm 0.00$	$41.04^{a} \pm 0.14$

Values are means ± SD of duplicate determination, means with different subscripts along the column are significantly different ($p<0.05$). Keys: TPC: Total phenolic content (mg/100 g); TFC: Total flavonoid contents (mg/100 g) FRAP: Ferric reducing antioxidant power (mmol/100 g); DPPH 1,1-Diphenyl-2-picrylhydrazyl (radical scavenging activity mmol/100 g).

Also, James et al. (2020a) reported a significant ($p<0.05$) increase in the total flavonoid of sprouted African oil bean seeds at the end of the second, the third and the fourth day of sprouting. However, the results of this study strongly contrast the report of James et al. (2020a) who reported that sprouting significantly ($p<0.05$) reduced the total flavonoid content of cowpea, Bambara nut, red bean, pigeon pea, African breadfruit, African yam bean seed and groundnut with an increase in sprouting time. The mechanism of decrease is still unknown.

The ferric reducing antioxidant power (FRAP) exhibited a significant ($p<0.05$) increase with an increase in sprouting time. The values ranged from 29.63 to 32.15 mmol/100 g. Extended sprouting time (96 h) had the highest antioxidant property (32.15 mmol/100 g) when compared to the control (un-sprouted) sample (29.63 mmol/100 g). This result implies that sprouting has the capacity of improving the reducing power of cereal grains and hence, it can be applied in food systems requiring high physiological benefits. DPPH● radical scavenging activity showed a similar trend to the FRAB where there were significant ($p<0.05$) increases with an increase in sprouting time. The values ranged from 34.65 to 41.04 mmol/100 g. The sample with the extended sprouting time (96 h) had the highest radical scavenging activity (41.04 mmol/100 g) when compared with other sprouting times and the control (34.65 mm/100 g). The results of this study agree with those of Alvarez and Jubete et al. (2010) and James et al. (2020a) where they revealed that sprouting significantly ($p<0.05$) increased the antioxidant capacity of both cereals and pulses. However, the increase depends on the cereal type, crop variety as well as sprouting conditions. During sprouting, hydrolytic enzymes modify the endosperm across the matrix of the sample with a resultant increase in the antiradical activity of the flour. The observed increase in % DPPH inhibition upon sprouting implies that the active antioxidant components are better enhanced or activated during the sprouting process.

Effect of fermentation on total phenolic, flavonoid and antioxidant property of pearl millet

The result of the total phenolic, flavonoid and antioxidant capacity of fermented pearl millet is shown in Table 2. The TPC content of the samples showed a significant ($p<0.05$) increase with an increase in fermentation time. The TPC ranged from 20.14 to 25.62 mg/100 g. The highest value was observed at the end of 96 h fermentation (25.63 mg/100 g) while the lowest was in the control (unfermented) sample (20.14mg/100 g). The result of this finding implies that fermentation has the capacity to significantly ($p<0.05$) increase TPC with an increase in fermentation time. The increase in total phenolic capacity with an increase in fermentation time could be attributed to the favourable activities of microbial enzymes which in turn produce a more freely available form of plant secondary metabolites such as flavonoids, tannins, alkaloids and polyphenol propanoids (Messens et al., 2002; Nazarni et al., 2016; James et al., 2020a). A similar trend of increase in TPC with a rise in fermentation length was reported by Ng et al. (2011) in conventional legumes. The result of this finding agrees with Singh et al. (2013) who reported increased bioavailability of TPC in fermented cereal bran and fibre. However, the result of this finding is at variance with the study of (Alvarez et al., 2017) in fermented wild beans where they reported a significant loss in TPC with an increase in fermentation time. The loss is attributed to the activities of polyphenol oxidases which are responsible for catalyzing polyphenols to low molecular weight condensed polyphenols (Weisburger, 2001). Wollgast and Anklam (2000), James et al. (2020a) also revealed that the loss could be linked to the leaching of some of the components

of lipophilic polyphenols in the fermentation medium and their possible oxidation.

The total flavonoid content of the fermented pearl millet samples significantly ($p<0.05$) increased with an increase in fermentation time. The values were found to range from 6.36 to 8.02 mg/100 g. Four day (96 h) fermentation time had the highest flavonoid content (8.02 mg/100 g) while the unfermented sample (6.36 mg/100 g) had the lowest value. The finding of this result agrees with Singh et al. (2013). During fermentation, microbial enzymes such as glucosidase, amylase, cellulase, tannase, esterase, invertase and lipases are favourably activated while others are generated and their activities help in the hydrolysis of glycosides and breakdown of plant materials and starch. Activities of these enzymes play a role in the disintegration of plant matrix and consequently facilitate the extraction of flavonoids (Nazarni et al., 2016; Hur et al., 2014; James et al., 2020a). However, Duenas et al. (2009) reported that β-glucosidase of microbial origin could hydrolyze phenolics and flavonoids, especially the activities of L. Plantarum. Therefore, the activities of these microbial species can result in either increase or decrease in flavonoids.

The ferric reducing antioxidant power of the samples showed a significant ($p<0.05$) increase with an increase in fermentation time. The values ranged from 29.63 to 31.43 mmol/100 g. Fermentation time of four days (96 h) had the highest ferric reducing antioxidant power (31.43 mmol/100 g). The DPPH radical scavenging activity exhibited a similar trend as found in FRAB, where it was shown that DPPH radical scavenging activity significantly ($p\geq0.05$) increases with an increase in fermentation time. The values ranged from 34.65 to 38.83 mmol/100 g. Fermentation time of ninety six hours had the highest value (38.83 mmol/100 g). The results of this agree with the report of Prabhu et al. (2014) who reported a significant increase in DPPH in fermented cereals.

Table 2. Effect of fermentation on total phenolic, flavonoid and antioxidant property of pearl millet

Fermentation time (h)	TPC (mg/100 g)	TFC (mg/100 g)	FRAP (mmol/100 g)	DPPH (mmol/100 g)
0	$20.14^{e} \pm 0.14$	$6.36^{e} \pm 0.07$	$29.63^{e} \pm 0.07$	$34.65^{e} \pm 0.21$
24	$23.16^{d} \pm 0.07$	$7.52^{d} \pm 0.00$	$30.16^{d} \pm 0.07$	$36.63^{d} \pm 0.07$
48	$23.76^{c} \pm 0.14$	$7.85^{c} \pm 0.00$	$30.46^{c} \pm 0.07$	$37.16^{c} \pm 0.14$
72	$24.52^{b} \pm 0.14$	$8.00^{a} \pm 0.00$	$31.06^{b} \pm 0.07$	$38.16^{b} \pm 0.07$
96	$25.62^{a} \pm 0.21$	$8.02^{a} \pm 0.21$	$31.43^{a} \pm 0.14$	$38.83^{a} \pm 0.14$

Values are means ± SD of duplicate determination, means with different subscripts along the column are significantly different ($p<0.05$). Keys: TPC: Total phenolic content (mg/100 g); TFC: Total flavonoid contents (mg/100 g) FRAP: Ferric reducing antioxidant power (mmol/100 g); DPPH 1,1-Diphenyl-2-picrylhydrazyl (radical scavenging activity mmol/100 g).

Effect of sprouting on total phenolic, flavonoid content and antioxidant property of sorghum

The impact of sprouting on sorghum's total phenolic, flavonoid and antioxidant capacity is revealed in Table 3. The results showed that sprouting significantly ($p<0.05$) influenced the measured parameters. The phenolic content increased with an increase in the sprouting length and the values were found to range from 17.34 to 21.03 mg/100 g. Lopez-Amoros et al. (2006) reported that sprouting qualitatively and quantitatively modifies phenolic compounds and the changes depend on the type of legume and the employed sprouting conditions. These changes influence the functional properties as well the antioxidant capacity of the resultant flour (James et al. 2020a). The rise in the phenolic content of sprouted seeds has been explained by different researchers. James et al. (2020a), Sokrab et al. (2012) revealed that the increase in total phenolic compounds upon sprouting could be a result of the enzymatic release of free phenolics through the breakdown of complex chemical bonds between phenolic compounds and other food macromolecules. The result of this study implies that, as the sprouting time progressed, there was an increase in enzyme activity leading to a more recovery of phenolic compounds in the sprouted seeds. However, this is in contrast with the finding of Lopez-Amoros et al. (2006) who reported a decrease in some phenolic compounds, notably trans-ferulic acid and trans-p-coumaric acid as sprouting time increased. The reduction in phenolic contents could be due to the physical leaching of the phenolic compounds into the soaking water during the rehydration process prior to sprouting.

Flavonoids are widespread in most edible fruits, vegetables and pulses and are heat-sensitive phenolic compounds (Prasanna et al., 2018; James et al., 2020b). A similar trend of significant ($p<0.05$) increase was observed in the total flavonoid of

sprouted sorghum. The values ranged from 4.54 to 5.78 mg/100 g. The results imply that sprouting time positively affected the total flavonoid content of sorghum samples. Loss or gain in total flavonoids due to treatments could be attributed to a treatment type, nature of the food material and forms of the flavonoids present in the plant material (Prasanna et al., 2018; Hiemori et al., 2009). The increase in the total flavonoids with an increase in sprouting time might be related to its release as a result of enzyme activities from intracellular macromolecules such as carbohydrates and protein and altered cell wall structures (Prasanna et al., 2018).

Table 3. Effect of sprouting on total phenolic, flavonoid and antioxidant property of sorghum

Sprouting time (h)	TPC (mg/100 g)	TFC (mg/100 g)	FRAP (mmol/100 g)	DPPH (mmol/100 g)
0	17.34^{e} ±0.14	4.54^{e} ± 0.21	25.89^{e} ±0.07	22.76^{e} ± 0.14
24	18.14^{d} ±0.28	4.64^{d} ± 0.14	28.76^{d} ± 0.14	29.63^{d} ± 0.07
48	18.76^{c} ±0.14	5.00^{c} ± 0.00	30.15^{c} ±0.21	31.40^{c} ± 0.00
72	19.32^{b} ±0.07	5.63^{b} ±0.07	31.43^{b} ±0.14	31.57^{b} ± 0.07
96	21.03^{a} ±0.14	5.78^{a} ±0.07	32.63^{a} ±0.07	33.16^{a} ± 0.07

Values are means ± SD of duplicate determination, means with different subscripts along the column are significantly different ($p<0.05$). Keys: TPC: Total phenolic content (mg/100 g); TFC: Total flavonoid contents (mg/100 g) FRAP: Ferric reducing antioxidant power (mmol/100 g); DPPH 1,1-Diphenyl-2-picrylhydrazyl (radical scavenging activity mmol/100 g).

There was a significant ($p<0.05$) increase in the ferric reducing antioxidant power of sprouted sorghum at different lengths of time with an increase in sprouting time. The values ranged from 25.89 to 32.63 mmol/100 g. In the same vein, DPPH radical scavenging activity exhibited a similar trend of increment as found in FRAB, where there was a significant ($p<0.05$) increase with an increase in sprouting time. The values ranged from 22.76 to 33.16 mmol/100 g. The results of this study agree with the reports of Prabhu et al. (2014); James et al. (2020a) who reported a significant increase in DPPH in sprouted red bean, African oil bean seeds and some conventional cereals. Also, Fernandez-Orozco et al. (2008) reported increased total phenolics and the corresponding antioxidant activity in the sprouted cereals compared with the raw samples. These result imply that for optimal physiological benefits, sorghum should be sprouted for an extended time, preferably 72 h and 98 h.

Effect of fermentation on total phenolic, total flavonoid contents and antioxidant property of sorghum

Fermentation time significantly ($p<0.05$) influenced the total phenolic, flavonoid, FRAB and DPPH of sorghum with an increase in time (Table 4). The total phenolic content ranged from 17.34 to 22.32 mg/100 g. This trend implies that as fermentation time progressed, there was a corresponding increase in the total phenolic content of the fermenting samples. James et al. (2020a) reported that a continuous increase in the amount of phenolic content depends on the duration of fermentation, the adaptability of the generated enzymes which are responsible for the breakdown of phenolics bound to other macromolecules. However, at a point, the corresponding increase in TCP with an increase in fermentation time declines due to harmful microbial metabolites released in the fermenting medium.

Flavonoids are polyphenolic compounds known for their high antioxidant properties and free radical scavenging ability (Scherer and Godoy, 2009). The fermented sorghum seeds have significantly ($p> 0.05$) higher flavonoid content than the control or unfermented sorghum seeds. The total flavonoids at end of 0 h, 24 h, 48 h, 72 h and 96 h fermentation time were found to range from 4.56 to 6.43 mg/100 g. The increasing trend in total flavonoids is similar to total phenolic content. The result of this study is in agreement with the report of other researchers where they reported that fermentation caused an increase in the total flavonoid content of cereals (Moktan et al., 2008; Ademiluyi and Oboh, 2011; Yao et al., 2010). The increase in the total flavonoid content of the sorghum seeds as a result of increasing fermentation length might be due to an increase in the acidity of the fermentation medium; the acidic condition generated during fermentation helps in liberating bound flavonoid components, thereby making them more available. However, Ademiluyi and Oboh (2011) reported a remarkable increase in non-flavonoid phenolic compounds over the total flavonoid compounds in fermented sorghum grains. A similar trend has also been reported in fermented lesser-

known legumes in Nigeria (James et al., 2020ab). The results of this study showed that fermentation could enhance the release of several classes of phenolic phytochemicals.

Table 4. Effect of fermentation on total phenolic, flavonoid and antioxidant property of sorghum

Fermentation time (h)	TPC (mg/100 g)	TFC (mg/100 g)	FRAP (mmol/100 g)	DPPH (mmol/100 g)
0	$17.34^{e} \pm 0.14$	$4.56^{e} \pm 0.21$	$22.86^{e} \pm 0.07$	$22.76^{e} \pm 0.14$
24	$17.96^{d} \pm 0.07$	$5.25^{d} \pm 0.00$	$30.66^{d} \pm 0.07$	$30.46^{d} \pm 0.14$
48	$19.66^{c} \pm 0.07$	$5.83^{c} \pm 0.07$	$31.79^{c} \pm 0.07$	$32.63^{c} \pm 0.07$
72	$20.47^{b} \pm 0.07$	$6.15^{b} \pm 0.00$	$32.86^{b} \pm 0.07$	$33.76^{b} \pm 0.14$
96	$22.32^{a} \pm 0.14$	$6.43^{a} \pm 0.14$	$34.92^{a} \pm 0.14$	$35.83^{a} \pm 0.07$

Values are means ± SD of duplicate determination, means with different subscripts along the column are significantly different ($p<0.05$). Keys: TPC: Total phenolic content (mg/100 g); TFC: Total flavonoid contents (mg/100 g) FRAP: Ferric reducing antioxidant power (mmol/100 g); DPPH 1,1-Diphenyl-2-picrylhydrazyl (radical scavenging activity mmol/100 g).

The antioxidant activities of the raw, 24 h, 48 h, 72 h and 96 h fermented sorghum evaluated by the reducing power assay and the DPHH● methods exhibited a marked significant increase with a corresponding increase in fermentation time. The reducing power of the control and fermented samples was found to range from 25.86 to 34.92 mmol/100 g while the DPHH● values ranged from 22.76 to 35.83 mmol/100 g. This implies that fermented sorghum seeds are most suitable for dietary and physiological benefits since the seeds/flours are usually fermented in water before consumption either as dumplings or pap. The substantially high antioxidant activity and phytochemical content of fermented sorghum would contribute greatly to the health benefits of consumers familiar with it.

Conclusions

Bio-processing methods adopted in this study significantly influenced the total phenolic, total flavonoid content, reducing the power and antioxidant capacity of pearl millet and sorghum. Sprouting significantly increased the total flavonoid and antioxidant capacities of pearl millet and sorghum with an increase in sprouting time. Also, there were significant increases in the total phenolic, total flavonoid content, reducing the power and antioxidant capacities of pearl millet and sorghum with an increase in fermentation time. Both sprouting and fermentation significantly increased all quantified parameters in this study.

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