International Journal of Food Science +Technology

Institute of Food Science +Technology ifst

Original article

Effects of different souring methods on phenolic constituents and antioxidant properties of non-alcoholic gruels from sorghum and amaranth

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(Received 7 October 2022; Accepted in revised form 4 December 2022)

Summary Functional foods have developed in modern food markets as a result of consumers' rising choice for healthier food options in recent years. This study looked at how non-alcoholic gruels made from sorghum and amaranth were affected by lactic acid fermentation and exogenous acidification with lactic acid in terms of their phenolic content and antioxidant capacities. The phenolic compounds were identified and quantified by liquid chromatography–mass spectrometry (LC-MS). Total phenolic content was determined by Folin–Ciocalteu method, and antioxidant properties by 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonicacid) (ABTS), 2,2′-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO) assays as well as inhibition of oxidative damage of vector DNA. In comparison to cooked gruels without fermentation or acidification, both fermented and lactic acid acidified gruels (cooked) had considerably greater total phenolic acid (4%–32%), total flavonoid content (20%–30%), and radical scavenging capacities (114%–195%, 166%–208%, and 10%–28% for ABTS, DPPH and NO radical scavenging capacities respectively). Overall, fermentation and exogenous acidification enhanced the extractability of phenolic compounds and radical scavenging capacities. Therefore, souring of cereal-based foods either by fermentation or exogenous acidification has the potential to shield the body from illnesses linked to oxidative stress.

Keywords ABTS, acidification, amaranth, DPPH, fermentation, gruels, LC-MS, nitric oxide, sorghum, total phenolic content, vector DNA.

Introduction

Consumers' growing preference for healthier food options in recent times has fuelled the development of functional foods in modern food markets. Fermentation, for example, which was once primarily employed to preserve food, is now gaining popularity due to the realisation that it produces nutrient-dense foods that provide other benefits beyond basic nutrition and sensory appeal. Various clinical investigations on people involving the consumption of fermented foods have recently been conducted, with the results indicating a positive correlation to improving health status (Saleh *et al.*, 2017). Beneficial bacteria in naturally fermented foods alter the chemical composition of plant-based raw materials during food fermentation, improving the food's sensory, nutritional, and health-promoting qualities (Laaksonen *et al.*, 2020). These microbes specifically aid in increasing bioavailability of essential micronutrients (iron and zinc) (Adeyanju *et al.*, 2019), enriching food with bioactive compounds (Svensson *et al.*, 2010), conferring probiotic capabilities, breaking down harmful and anti-nutritive components (Adeyanju *et al.*, 2019), and providing bio-preservative benefits by producing antioxidant and antibacterial substances. As a result, nutritionists and food scientists are paying increased attention to the relationship between food and human health. Despite the development of many fermented food products from other sources, cereal grains and their processed foods remain the primary sources of human nutrition, particularly in developing countries.

In sub-Saharan African nations, cereals are frequently soured through lactic acid fermentation to make a variety of foods, including alcoholic and non-alcoholic gruels and porridges (Gabaza *et al.*, 2017). However, a much simpler process of souring (exogenous acidification) such as addition of acidic plant

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doi:10.1111/ijfs.16245
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extracts (e.g. lemon juice extract, tamarind pulp) and food grade acids (lactic acid, citric acid) is practised in a number of rural communities in Africa. Various ready-to-eat soured porridge products made using exogenous acidification are also readily available commercially for urban dwellers (Onyango *et al*., 2005; Onyango & Wanjala, 2018). The rise in popularity of exogenously acidified products is primarily due to convenience, but it is also due to issues like inconsistent flavour and long processing times that come with traditional lactic acid fermentation.

Sorghum is a crucial food grain, especially for many populations in Africa's semi-arid tropical regions (Nedumaran *et al*., 2012; Awika, 2017). In recent years, the pseudocereal amaranth has also been advocated as an alternative crop to help ensure food security, especially in dry and semi-arid regions of East Africa (Alemayehu *et al*., 2015). This grain (amaranth) has received a lot of attention in part because of its higher protein content and overall quality compared to cereal grains (D'Amico & Schoenlechner, 2017). In comparison to sorghum-only gruels, our prior research has demonstrated that adding grain amaranth to sorghum improves the gruels' protein content, protein digestibility, reactive lysine, and bioaccessible iron (Adeyanju *et al*., 2019). Sorghum and amaranth grains are becoming more widely recognised as important sources of bioactive phenolic compounds, and given the rising prevalence of and mortality from diet-related non-communicable diseases, which is a growing concern, particularly in developing countries in sub-Saharan Africa, the potential health-promoting properties of sorghum- and amaranth-based foods are of interest.

While there has been a lot of research on the effects of lactic acid fermentation on the profile of phenolic compounds in sorghum (Towo *et al*., 2006; Dlamini *et al*., 2007; Svensson *et al*., 2010), there is still a lot of information missing for amaranth. Furthermore, the impact of exogenous acidification on phenolic compounds and antioxidant properties in sorghum and amaranth has not been thoroughly explored. This is despite the fact that, as previously noted, there is a rising trend in some African countries to produce sour food products through exogenous acidification. This important information is now even more important because the potential health benefits that grain-based products may offer due to their phytochemical components heavily depend on the processing technique used (Taylor & Duodu, 2015). Therefore, the current study sets out to examine the effects of souring using lactic acid fermentation (with *Lactobacillus plantarum* starter culture and by back-slopping) and exogenous acidification with lactic acid on phenolic compounds and radical-scavenging capacity of non-alcoholic gruels made from sorghum and amaranth.

Materials and method

Materials

Amaranth grains (*Amaranthus caudatus*) supplied by Advance Seed, Krugersdorp, South Africa, and a red non-tannin sorghum (variety MMSH 625), obtained from the Golden Valley Research Station in Chisamba, Zambia, were used. Phenolic acids and flavonoids standard were purchased from Sigma-Aldrich. Similarly, catechin, trolox, 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH), 2,2′-diphenyl-1-picrylhydrazyl (DPPH), and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) were purchased from Sigma-Aldrich. pBR 322 vector DNA was purchased from Promega (Madison, WI, USA). All other chemicals, unless stated otherwise, were purchased from Merck Chemical Co. (South Africa).

Sample preparation

The flour samples were prepared from whole-grain sorghum and amaranth as previously described by Adeyanju *et al*. (2019). The flours were packaged separately into airtight plastic containers and stored at 4 °C prior to further processing and/or analysis.

Preparation of *L. plantarum* starter culture and back-slopped inoculum

L. plantarum starter culture and back-slopped inoculum were prepared based on the preparation procedure described by Adeyanju *et al*. (2019). *L. plantarum* strain (FS2) was revived in MRS broth at 37 °C for 18 h for three successive generations. The resultant cell suspension was centrifuged at 1095× ***g*** for 15 min at 4 °C, and the supernatant was then decanted off. The bacteria cells were centrifuged after being washed twice in 0.1% sterile buffered peptone water (BPW). Bacterial cells were suspended in 0.1% sterile BPW after the supernatant was decanted off. Using a McFarland densitometer (DEN-1 Grant Instruments, Royston, UK), which had already been calibrated with McFarland standard, the bacteria suspension was standardised (bacterial concentration was determined). A back-slopped inoculum was made by first creating a slurry in a plastic container using 30 g of each flour and 500 mL of distilled water. The slurry was covered and left to ferment at 30 °C to pH 3.5. The fermented slurry was utilised as a starter culture (10% v/v, i.e. 10 mL of the fermented slurry + 90 mL of fresh slurry).

Gruel preparation

According to the preparation method outlined by Adeyanju *et al*. (2019), lactic acid fermented, lactic

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acid acidified, and unsoured gruels were made from sorghum flour and amaranth flours. Each flour sample was made into a slurry (60 g flour in 1 Litre of distilled water), which was then heated on an electric hot plate while being continuously stirred and allowed to boil for 10 min at a temperature of around 96 °C. The finished gruels were labelled as fermented/acidified/unsoured and frozen at −20 °C.

Extraction of phenolic compounds, both free and bound

Free phenolic extracts for LC-MS analysis were prepared from raw sorghum flour, raw amaranth flour, and freeze-dried fermented as well as acidified gruels using a modification of the extraction procedure previously described by Apea-Bah *et al.* (2014). Approximately 2.5 g of each flour sample was extracted in duplicate using 10 mL acidified methanol (1% (v/v) conc. HCl in methanol) by magnetic stirring for 2 h. The suspension was centrifuged at 1500× ***g*** for 15 min at 4 °C, and the supernatant was decanted into a 50 mL centrifuge tube. The residue was similarly re-extracted twice each with 5 mL acidified methanol for 30 min. The supernatants were then pooled together and stored at −20 °C in the dark prior to analysis.

Extraction of bound phenolics was carried out using the residues from the acidified methanol extractions above. The residue from each of the flour samples was placed separately into 100 mL beakers and saponified with 20 mL 2 N NaOH for 4 h by magnetic stirring at room temperature. Concentrated HCl was added to the saponified residues in order to adjust the pH to 2.0 ± 0.2. The reaction mixture was centrifuged at 3500× ***g*** for 20 min, and the resulting supernatant was transferred into a separating funnel and extracted with diethyl ether: ethyl acetate (1:1) four times each with 20 mL. The organic phase was pooled and dried with anhydrous sodium sulphate, filtered through Whatman no 4 filter paper into a collecting beaker and evaporated to dryness under vacuum at 40 °C with a rotary evaporator. The sample was re-dissolved in 10 mL 1% (v/v) conc. HCl–methanol and stored at −20 °C in the dark prior to analysis.

Liquid chromatography–mass spectrometry (LC-MS) analysis for the identification and quantification of phenolic compounds (LC-MS)

Acrodisc PSF 0.45 μm syringe filters (Pall Life Sciences, Ann Arbor, MI, USA) were used to filter the free and bound phenolic extracts. Following the procedure described by Apea-Bah *et al.* (2014), phenolic compound characterisation by LC-MS was carried out.

Preparation of phenolic extracts for total phenolic content and radical scavenging assays

Phenolic extracts were prepared from sorghum flour, amaranth flour, and freeze-dried fermented/acidified gruels using the extraction procedure previously described by Awika *et al.* (2003).

Determination of total phenolic content

The total phenolic content of the extracts was determined using the Folin–Ciocalteu method as described by Apea-Bah *et al.* (2014).

Determination of $ABTS^{\cdot+}$ radical scavenging capacity

The method outlined by Apea-Bah *et al.* (2016) was used to test the acidified-methanolic extracts' ability to scavenge ABTS radicals. Using the trolox standard as a standard, the sample's ability to scavenge ABTS radicals was determined and represented as micromoles of trolox equivalent per gram of sample (μmol TE/g) on a dry weight basis.

Determination of DPPH radical scavenging capacity

The ability of the sample extracts to scavenge DPPH radical was assessed using a modified version of the Apea-Bah *et al.* (2014) method. Absolute methanol was used to dilute the extracts 10 times. Also, in absolute methanol, a 0.609 mM DPPH stock solution and a 0.102 mM working solution were prepared.

Determination of nitric oxide radical scavenging capacity

Using the method described by Jagetia & Baliga (2004), the extracts' ability to scavenge nitric oxide (NO) radical was assessed. To calculate the amount of NO that the extracts were able to scavenge, a standard curve of $NaNO_2$ (0.02–0.1 mM) was plotted. The results were presented as μmol $NaNO_2$ equivalents per gram of flour or freeze-dried gruel on a dry weight basis.

Inhibition of oxidative DNA damage assay

Inhibition of 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH)-induced oxidative DNA damage was determined according to a modification of the method described by Nderitu *et al.* (2013). The phenolic extracts were diluted 10 times with 0.2 M sterile phosphate buffered saline (PBS) pH (7.4). To test the inhibitory effect of the sample extracts against AAPH-induced oxidative DNA damage, 2.5 μL of the 10 times diluted sample extracts was reacted with 2.5 μL of 20 times diluted 1 mg/mL pBR 322 vector DNA

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and 2.5 μL of 3 mM AAPH in Eppendorf tubes. The positive control consisted of 2.5 μL PBS, 2.5 μL AAPH, and 2.5 μL DNA, while the reaction mixture comprising 2.5 μL distilled water, 2.5 μL PBS, and 2.5 μL DNA served as negative control. The reaction mixtures were incubated for 90 min at 37 °C. After incubation, gel red (2 μL) was added to the reaction mixtures and 8 μL of the final reaction mixtures was loaded into a 1% (w/v) agarose gel. The gel was subjected to electrophoresis (60 V, 500 mA, 150 W) for 60 min and the DNA bands were then imaged (Gel Doc EZ Imager, Bio-Rad Labs, Hercules, CA, USA) and the bands analysed (Image Lab 5.1, Bio-Rad Labs, Hercules, CA, USA).

Statistical analysis

The LC-MS analysis was repeated twice for each sample, while the radical scavenging activities of the extracts were carried out in three replicates. The data obtained were analysed by one-way analysis of variance (ANOVA) at $P < 0.05$ using IBM SPSS statistical for windows version 20.0 (IBM, Armonk, NY, USA). Turkey's HSD post hoc test was applied to determine differences between specific means.

Results and discussion

Figure 1 shows the base peak ion (BPI) chromatograms of the extracts from sorghum and amaranth. Table 1 shows the retention times, maximum UV-visible absorption wavelengths, and mass spectral (MS) data for the phenolic compounds which were identified in sample extracts of the flours and gruels. The phenolic compounds were identified by comparing their retention times, UV-visible absorption maxima, and mass spectral data with authentic standards (where available) and data from literature. Phenolic acids identified in the samples included four hydroxybenzoic acid derivatives, three hydroxycinnamic acid derivatives, and five phenolic acid glycerol esters. Flavonoids identified in the samples included six flavones and six flavanones. Additionally, one phenolamide was found.

Phenolic acids and their glycerol esters

The compounds labelled as peaks 1, 2, 9, and 10 were identified as protocatechuic acid, p-hydroxybenzoic acid, gallic acid, and vanillic acid, respectively, while compounds labelled as peaks 5, 8, and 18 were identified as caffeic acid, p-coumaric acid, and ferulic acid respectively (Table 1). These compounds were identified by comparing their chromatographic retention times and mass of their molecular ion with those of authentic standards. Vanillic acid produced a fragment at m/z 135 [(M-H)-32] due to the loss of a $–OCH_3$ group. For caffeic acid, the fragment at m/z 135 was due to the loss of one carbon dioxide molecule (−44 amu) from caffeic acid (Wu *et al*., 2016). Ferulic acid produced two fragments at m/z 133 and 161 (Table 1). The fragment at m/z 133 could be due to simultaneous loss of a methyl (CH_3; −15 amu) from the methoxy group, loss of CO_2 (−44 amu) from the carboxylate moiety on the ethylene short chain of the ferulic acid molecule and the loss of a hydride ion (H; 1 amu). The fragment at m/z 161 could be due to either loss of OCH_3 or the loss of O_2 (−32 amu) from the ferulate anion (Svensson *et al*., 2010).

Peak 4 (t_R = 11.17 min, λ_{max} = 325 nm) and **peak 6** (t_R = 12.54 min, λ_{max} = 325 nm) had the same $[M-H]^-$ at m/z 253.The Ms/Ms fragmentation pattern of these two compounds was identical as both produced fragment ions at m/z 179 [(M-H)-74] due to loss of a glycerol unit, m/z 161 [(M-H)-92] due to loss of a glycerol unit and oxygen atom, and m/z 135 [(M-H)-118] due to loss of a glycerol unit together with the COOH of caffeic acid. These fragments matched the fragmentation pattern of caffeoyl glycerol as previously reported by Svensson *et al*. (2010) and Kang *et al*. (2016). According to Ma *et al*. (2007), the caffeoyl glycerol compounds with substitution of the caffeoyl moiety at the 2-*O*-position of glycerol elute earlier than the one at the 1-*O*-position. Therefore, **peak 4** was identified as 2-*O*-caffeeoyl-glycerol and peak 8 as 1-*O*-caffeoyl-glycerol.

Peak 11 (t_R = 14.70 min, λ_{max} = 280 nm) had a $[M-H]^-$ at m/z 237. The Ms/Ms fragmentation produced ions at m/z 175 (loss of $C_2H_5O_2$ from the glycerol unit), m/z 145 (loss of glycerol residue), and m/z 119 (loss of glycerol unit together with the COOH of coumaroyl unit). Thus, **peak 11** was identified as 1-*O*-coumaroyl-glycerol as reported earlier (Svensson *et al*., 2010; Kang *et al*., 2016).

Peak 21 (t_R = 22.80 min, λ_{max} = 243 and 295 nm) had a $[M-H]^-$ at m/z 415 and was identified as 1,3-*O*-dicaffeoyl-glycerol as previously reported by Apea-Bah *et al*. (2014) and Kang *et al*. (2016). The Ms/Ms fragmentation yielded ions at m/z 253 (due to loss of a caffeoyl unit) [(M-H)-162], m/z 179 (due to loss of a caffeoyl and a $–C_3H_6O_2$ unit from the glycerol) [(M-H)-162–74], m/z161 (due to loss of a caffeoyl and glycerol unit) [(M-H)-254], and m/z 135 (due to loss of a caffeoyl, glycerol, and –COOH group) [(M-H)-162-74-44].

Peak 23 (t_R = 23.80 min, λ_{max} = 232 nm) had a $[M-H]^-$ at m/z 399.The Ms/Ms fragmentation pattern was largely similar to that of 1,3-*O*-dicaffeoyl-glycerol (described above) and produced ions at m/z 253, 179, 163, 135, and 119 (Table 1). The fragment m/z 253 was due to loss of a coumaroyl unit [(M-H)-146], m/z 179 was due to loss of a coumaroyl and a $–C_3H_6O_2$ unit from the glycerol, m/z 163 was due to loss of a coumaroyl and glycerol unit, m/z 135 was due to loss

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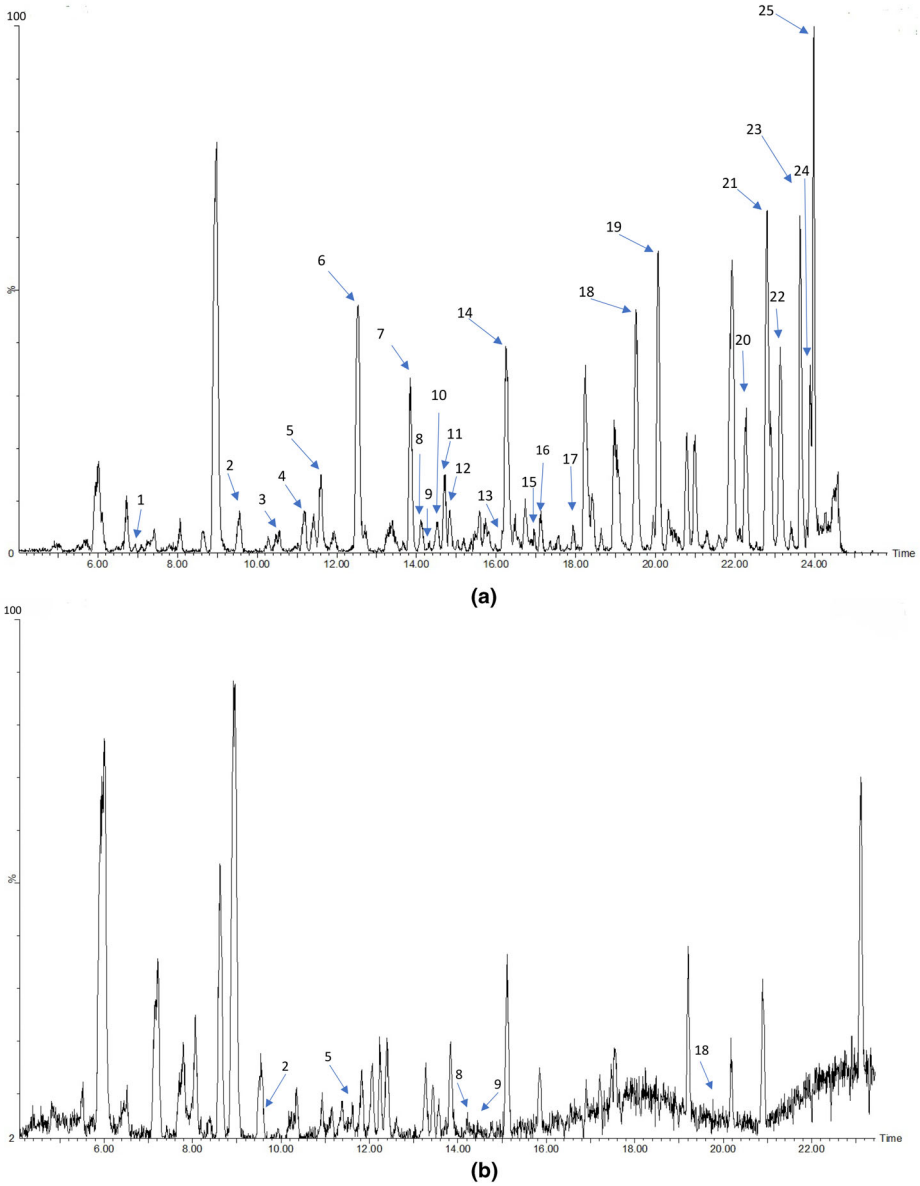

Figure 1 UPLC-ESI-MS base peak ion chromatograms (BPI) of the acidified-methanolic extract of (A) whole-grain red non-tannin sorghum and (B) whole-grain amaranth.

of a coumaroyl, glycerol, and –COOH group, and m/z 119 was due to loss of a caffeoyl, glycerol, and –COOH group. Thus, **peak 23** was identified as 1,3-*O*-coumaroyl-caffeoyl-glycerol (Svensson *et al.*, 2010; Kang *et al.*, 2016).

Phenolamide

Peak 7 (t_R = 13.84 min, λ_{max} = 295 and 319 nm) had a $[M\text{-}H]^-$ at m/z 468. The Ms/Ms fragmentation pattern of this compound yielded ions at m/z 332 [(M-H)-136], m/z 306 [(M-H)-162], m/z 161 [(M-H)-307], and m/z 135 [(M-H)-333] (Table 1). These fragmentations indicated the presence of caffeoyl unit and amide moiety. The fragmentation at m/z 332 was due to loss of fragment of caffeoyl unit without the carboxylic bond. The ion at m/z 306 was due to loss of fragment of caffeoyl unit, while ions at m/z 161 and m/z 135 are characteristic fragments of caffeoyl. This peak was identified as N^1, N^4-dicaffeoyl-spermidine based on its

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Table 1 Retention time, UV-visible absorption maxima, and mass spectral characteristics of phenolic compounds identified in extracts from raw sorghum and amaranth flour, lactic acid fermented, and lactic acid acidified gruels

Rt (min)	λ_{max} (nm)	[M-H]$^-$	Ms/Ms fragments	Proposed compound	Peak no
Benzoic acid derivatives					
7.03	239	153	153 (100)	Protocatechuic acid	1
9.66	247	137	137 (100)	p-Hydroxybenzoic acid	2
14.51	280	169	169 (100)	Gallic acid	9
14.6	280	167	167 (100), 135 (35)	Vanillic acid	10
Cinnamic acid derivatives					
11.60	295, 325	179	179 (74), 135 (100)	Caffeic acid	5
14.23	280	163	163 (100)	p-Coumaric acid	8
19.52	243, 289	193	193 (79), 161 (43), 133 (100)	Ferulic acid	18
Glycerol esters of phenolic acid					
11.17	325	253	253 (100), 179 (19), 161 (36), 135 (22)	2-*O*-Caffeoyl-glycerol	4
12.54	325	253	253 (100), 179 (15), 161 (58), 135 (27)	1-*O*-Caffeoyl-glycerol	6
14.70	280	237	237 (56), 175 (16), 145 (100), 119 (59)	1-*O*-Coumaroyl-glycerol	11
22.80	243, 295	415	415 (100), 253 (73), 179 (34) 161 (71), 135 (84)	1,3-*O*-Dicaffeoyl-glycerol	21
23.80	232	399	399 (100), 253 (21), 179 (7), 163 (21), 135 (16), 119 (15)	1,3-*O*-Coumaroylcaffeoyl-glycerol	23
Phenolamide					
13.84	295, 319	468	468 (100), 332 (41), 306 (43), 161 (17), 135 (54)	N^1,N^4-Dicaffeoyl-spermidine	7
Flavones					
10.56	280	315	315 (28), 271 (35), 206 (10), 179 (10), 161 (11), 153 (100), 109 (8)	6-Methoxyluteolin	3
14.85	280	269	269 (100), 161 (6), 133 (9), 107 (4)	7,3′,4′-Trihydroxyflavone	12
16.24	276, 475	253	253 (100), 135 (4), 117 (8)	7,4′-Dihydroxyflavone	14
17.13	244, 264	447	447 (100), 285 (64), 255 (33)	Luteolin-7-*O*-β-D-glucoside	16
23.14	244	285	285 (100), 175 (2), 151 (5), 133 (18)	Luteolin	22
23.98	280	269	269 (100), 151 (21), 119 (19)	Apigenin	25
Flavanones					
16.18	280	433	433 (79), 271 (82), 253 (100), 151 (25)	Naringenin-7-*O*-glucoside	13
16.89	244, 280	449	449 (82), 287 (100), 151 (70), 135 (61)	Eriodictyol-7-*O*-β-D-glucoside	15
18.96	239, 285	721	721 (83), 559 (14), 433 (100), 287 (37), 151 (36), 135 (19)	5,7,3′,4′,-Tetrahydroxy-flavan-5-*O*-β-galactosyl-4-8-eriodictyol	17
20.09	238, 285	721	721 (71), 559 (64), 433 (100), 287 (28), 151 (31), 135 (8)	5,7,3′,4′,-Tetrahydroxy-flavan-5-*O*-β-glucosyl-4-8-eriodictyol	19
22.27	286, 289	287	287 (62), 179 (9), 151 (100), 135 (99), 107 (13)	Eriodictyol	20
23.94	244	271	271 (100), 151 (21), 119 (12)	Naringenin	24

fragmentation pattern and comparison with published literature (Gancel *et al.*, 2008; Kang *et al.*, 2016).

Flavones and derivatives

Peak 3 (t_R = 10.56 min, λ_{max} = 280 nm) had a [M-H]$^-$ at m/z 315. The Ms/Ms fragmentation pattern produced ions at m/z 271, 206, 179, 161, 153, and 109. This compound was tentatively identified as 6-methoxyluteolin and its proposed fragmentation pattern is shown in Fig. 2. The ion at m/z 271 corresponds to loss of CO from the C ring (−28 amu) and CH_3 (−15 amu) from the methoxy group to produce a derivative with a 5-membered C ring as shown in Fig. 2. The ion at m/z 206 corresponds to loss of B-ring (−109 amu). Further loss of CO from the C ring of the m/z 206 ion produces the ion at m/z 179. The ions at m/z 161 and m/z 153 are produced from cleavage of the heterocyclic C-ring as shown in Fig. 2.

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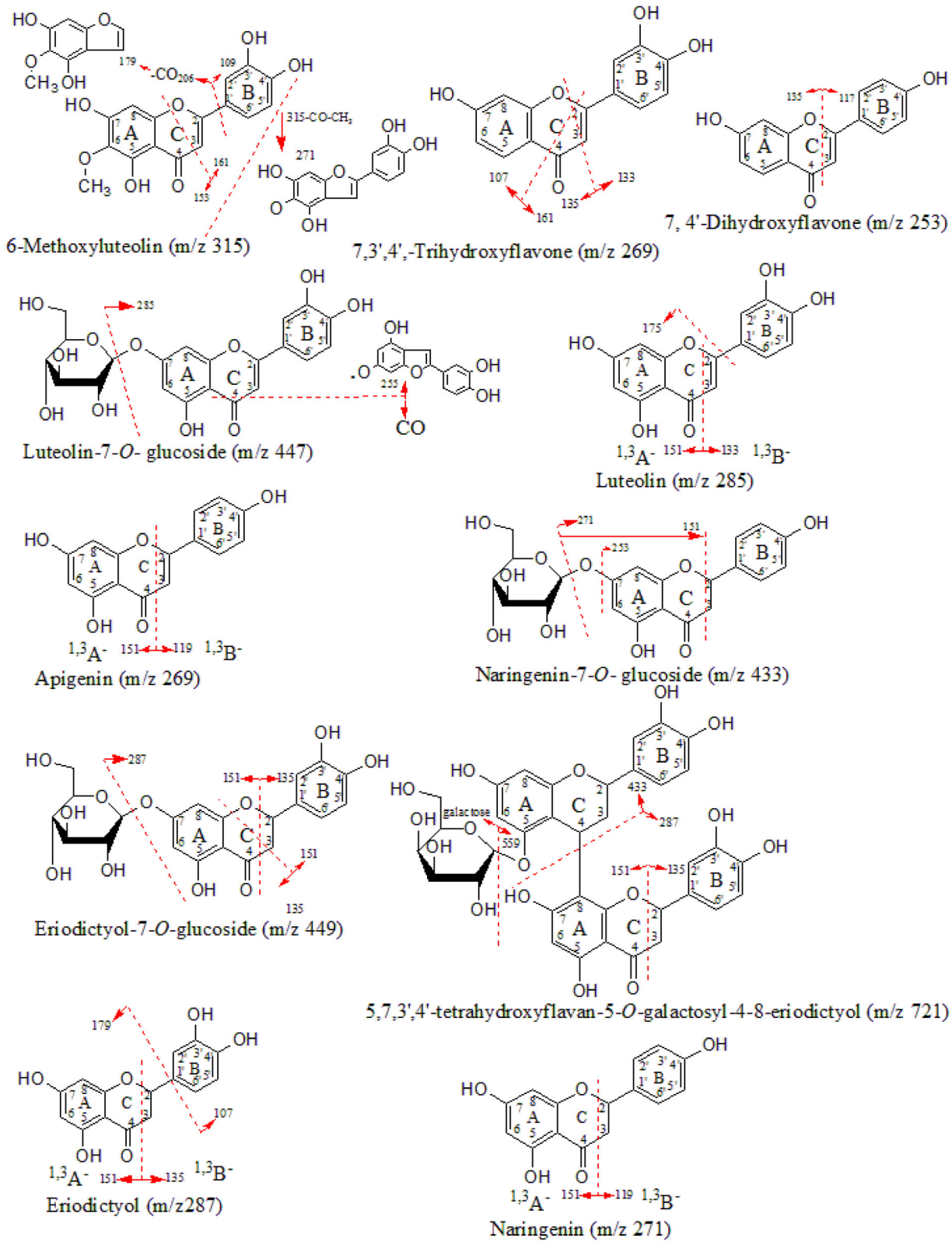

Figure 2 Proposed fragmentation patterns of flavones, flavanones, and their derivatives.

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Peak 12 (t_R = 14.85 min, λ_{max} = 280 nm) had a [M-H]$^-$ at m/z 269 and was identified as 7,3′,4′-trihydroxyflavone based on its fragmentation pattern (Kang *et al*., 2016). The Ms/Ms fragmentation pattern produced ions at m/z 161, 133, and 107. Fragmentation of the C ring at positions 1 and 4 produces a $^{1,4}B^-$ fragment (m/z 161) and a $^{1,4}A^-$ fragment (m/z 107) (Fig. 2). Fragmentation of the C ring at positions 1 and 3 produces a $^{1,3}B^-$ fragment (m/z 133).

Peak 14 (t_R = 16.24 min, λ_{max} = 276 and 475 nm) had a [M-H]$^-$ at m/z 253 and was tentatively identified as 7,4′-dihydroxyflavone (Pereira *et al*., 2012; Kang *et al*., 2016). The Ms/Ms fragmentation pattern produced ions at m/z 135 and 117 (Table 1). These ions are produced by fragmentation of the C ring at positions 1 and 3 to produce a $^{1,3}A^-$ fragment (m/z 135) and a $^{1,3}B^-$ fragment (m/z 117) (Fig. 2).

Peak 16 (t_R = 17.13 min, λ_{max} = 244, 264 nm) had a [M-H]$^-$ at m/z 447 (Table 1). The Ms/Ms fragmentation produced a major fragment ion at m/z 285 (loss of hexose unit) and another ion at m/z 255 (loss of hexose unit and CO from the C ring) (Fig. 2). This compound was identified as luteolin-7-*O*-β-D-glucoside based on comparison of its chromatographic retention time and Ms/Ms fragmentation pattern with an authentic standard and reported literature (Apea-Bah *et al*., 2014; Jaiswal *et al*., 2014; Kang *et al*., 2016).

Peak 22 (t_R = 23.14 min, λ_{max} = 244 nm) had a [M-H]$^-$ at m/z 285. The Ms/Ms fragmentation pattern produced ions at m/z 175, 151, and 133 (Table 1). This compound was identified as luteolin by comparing its chromatographic retention time and fragmentation pattern with that of an authentic standard and literature data. The fragment ion at m/z 175 corresponds to loss of the B-ring, while cleavage of the heterocyclic C-ring at positions 1 and 3 produced fragment ions at m/z 151 ($^{1,3}A^-$) and m/z133 ($^{1,3}B^-$) (Fig. 2).

Peak 25 (t_R = 23.98 min, λ_{max} = 280 nm) had a [M-H]$^-$ at m/z 269.The Ms/Ms fragmentation pattern indicated cleavage of the C ring at positions 1 and 3 to produce ions at m/z 151 ($^{1,3}A^-$) and m/z119 ($^{1,3}B^-$) (Fig. 2). This compound was identified as apigenin by comparing its chromatographic retention time and fragmentation pattern with apigenin standard and literature data (Kang *et al*., 2016).

Flavanones and derivatives

Peak 13 (t_R = 16.18 min, λ_{max} = 280 nm) had a [M-H]$^-$ at m/z 433.The Ms/Ms fragmentation pattern produced ions at m/z 271, 253, and 151 (Table 1). The fragment ion at m/z 271 indicates loss of hexose [(M-H)-162], while the ion at m/z 253 indicates loss of hexose and oxygen atom [(M-H)-180]. The ion at m/z 151 results from cleavage of the heterocyclic C-ring at positions 1 and 3 to produce a $^{1,3}A^-$ fragment (Fig. 2). This compound was identified as naringenin-7-*O*-glucoside by comparing its fragmentation pattern with the literature (Svensson *et al*., 2010).

Peak 15 (t_R = 16.89 min, λ_{max} = 244 and 280 nm) had a [M-H]$^-$ at m/z 449 and was identified as eriodictyol-7-*O*-β-D-glucoside. The Ms/Ms fragmentation pattern yielded ions at m/z 287, 151, and 135 (Table 1). The ion at m/z 287 corresponds to eriodictyol aglycone after loss of a glucose unit (−162 amu). Two possible fragmentation patterns of the C-ring may produce the ions at m/z 151 and 135. After loss of the glucose unit, cleavage of the C-ring at positions 1 and 3 produces a $^{1,3}A^-$ fragment (m/z 151) and a $^{1,3}B^-$ fragment (m/z 135) (Fig. 2). Cleavage of the C-ring at the heterocyclic oxygen and position 3 produces a $^{0,3}A^-$ fragment (m/z 135) and a $^{0,3}B^-$ fragment (m/z 151).

Peak 17 (t_R = 18.96 min, λ_{max} = 239, 285 nm) and **peak 19** (t_R = 20.09 min, λ_{max} = 239, 285 nm) had the same [M-H]$^-$ at m/z 721. They produced similar fragments at m/z 599, 433, 287, 151, and 135 (Table 1). The fragment ion at m/z 559 indicates loss of a hexose unit [(M-H)-162] (Fig. 2). The fragmentation ion at m/z 433 corresponds to loss of an eriodictyol aglycone unit [(M-H)-288] (Fig. 2). The remaining two fragments are products of cleavage of the C-ring of eriodictyol aglycone at positions 1 and 3 to produce a $^{1,3}A^-$ fragment (m/z 151) and a $^{1,3}B^-$ fragment (m/z 135). Based on the fragmentation pattern and reported literature (Gujer *et al*., 1986; Apea-Bah *et al*., 2014), the compounds represented by **peak 17** and **peak 19** were identified as 5,7,3′,4′,-tetrahydroxy-flavan-5-*O*-β-glucosyl-4-8-eriodictyol and 5,7,3′,4′-tetrahydroxy-flavan-5-*O*-β-galactosyl-4-8-eriodictyol respectively. According to Prior *et al*. (2001), the sequence of elution of glucosides in reverse phase HPLC is galactoside followed by glucosides. Thus, **peak 17** was identified as the galactoside derivative and **peak 19** as the glucoside derivative.

Peak 20 (t_R = 22.27 min, λ_{max} = 286 and 289 nm) had a [M-H]$^-$ at m/z 287. The Ms/Ms fragmentation pattern produced ions at m/z 179, 151, 135, and 107 (Table 1), which are characteristic fragments of eriodictyol (Svensson *et al*., 2010; Apea-Bah *et al*., 2014; Kang *et al*., 2016). Fragmentation ions at m/z 151 ($^{1,3}A^-$) and m/z 135 ($^{1,3}B^-$) are produced from the cleavage of heterocyclic C-ring at positions 1 and 3 (Fig. 2), while cleavage of the bond linking the B-ring to the C-ring produces the ions at m/z 179 and 107. The retention time of **peak 20** matched that of eriodictyol standard and therefore was identified as such.

Peak 24 (t_R = 23.94 min, λ_{max} = 244 nm) had [M-H]$^-$ at m/z 271 and was identified as naringenin by comparing its chromatographic retention time and fragmentation pattern with an authentic naringenin standard and literature data (Svensson *et al*., 2010;

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Apea-Bah *et al*., 2014). Its fragmentation ions at m/z 151 and 119 are produced from cleavage of the C-ring at positions 1 and 3 to produce $^{1,3}A^{-}$ (m/z 151) and $^{1,3}B^{-}$ (m/z 119) fragments (Fig. 2).

Effects of fermentation and acidification on the phenolic acid concentration

Seven phenolic acids were quantified (against standards) as free phenolic acids in the acidified methanolic extract of sorghum flour and gruels, namely, protocatechuic acid, p-hydroxybenzoic acid, caffeic acid, p-coumaric acid, gallic acid, vanillic acid, and ferulic acid (Table 2). Caffeic acid, p-hydroxybenzoic acid, and protocatechuic acid were the most abundant phenolic acids as previously reported in sorghum grains (Svensson *et al*., 2010; Afify *et al*., 2012; Apea-Bah *et al*., 2014). In addition, p-hydroxybenzoic acid, caffeic acid, gallic acid, vanillic acid, and ferulic acid were quantified in the saponified residue as bound phenolic acids, with the major ones being p-hydroxybenzoic acid, caffeic acid, and ferulic acid. Similarly, five phenolic acids (namely, p-hydroxybenzoic acid, caffeic acid, p-coumaric acid, gallic acid, and ferulic acid) were quantified as free phenolic acids in the acidified methanolic extract of amaranth gruels with the major ones being gallic acid, caffeic acid, and p-coumaric acid (Table 2). The saponified residue of amaranth gruels (bound phenolic acids) also comprised of p-hydroxybenzoic acid and ferulic acid.

Fermentation and exogenous acidification with lactic acid had different effects on individual phenolic acids, with some appearing to decrease while others appeared to increase in concentration. Generally, total phenolic acids decreased significantly (at $P < 0.05$) in the fermented sorghum and amaranth gruels compared to their corresponding raw flours. On the other hand, total phenolic acid concentration was higher in the exogenously acidified sorghum and amaranth gruels compared to their corresponding raw flours, although the increase was not significant in the case of sorghum. When the raw, fermented, and acidified gruels were all cooked, there was no significant difference in total phenolic acids between the fermented and cooked sorghum gruels and cooked sorghum gruels without fermentation or acidification. However, fermented and cooked amaranth gruels still had significantly lower total phenolic acids compared to cooked amaranth gruels without fermentation or acidification. For both sorghum and amaranth, exogenously acidified and cooked gruels were significantly higher in total phenolic acids compared to their corresponding cooked gruels without fermentation or acidification.

The observed reductions in the levels of some phenolic acids after fermentation may be attributed to degradation by phenolic acid decarboxylases and phenolic acid reductases as previously reported (Svensson *et al*., 2010). Such reductions have also been linked to the activity of polyphenol oxidase enzymes (either from the grain or the fermenting microflora), which act on phenolics as a substrate and in the process convert them to quinones (Taylor & Duodu, 2015). Moreover, the acidic environment created during fermentation has been reported to trigger hydride ion abstraction from phenolic compounds, thereby leading to the rearrangement of the phenolic structure (Taylor & Duodu, 2015).

Furthermore, reduction in the extractable phenolic compounds due to self-polymerisation or interaction with proteins has also been linked to the fermentation process (Beta *et al*., 2000). However, from the results obtained in this study, it seems that the reduction in phenolic acids after fermentation was more likely to be due to the degradation by the fermenting microflora and possibly polyphenol oxidase activity rather than hydride ion abstraction from the phenolic compounds as a result of the acidic environment created by the fermentation process; since such reductions were not observed in the lactic acid acidified treatments, where acidic environment was also created.

The reduced level of total phenolic acids in cooked gruels without fermentation or acidification compared to their corresponding raw flours (Table 2) may be due to reduction in extractability of these phenolic acids as a result of possible complexation with protein and other cell wall components during cooking. According to Dykes & Rooney (2006), thermally denatured storage proteins in cereals can interact during cooking with free extractable phenolics acids present in the pericarp, testa, and aleurone layer of the kernel. Also, a possible covalent interaction between phenolic acids and protein at high temperature has been reported (Prigent *et al*., 2003). Furthermore, oxidative degradation of the phenolic compounds during thermal processing is another possibility (reviewed by Taylor & Duodu, 2015). On the other hand, an increase in the concentration of total phenolic acids observed in the fermented and lactic acid acidified gruels after cooking compared to their fermented and acidified counterparts without cooking implied that pretreatments such as fermentation and acidification may increase the extractability of these phenolic acids during subsequent cooking. The low pH and cooking temperature may have possibly facilitated the disruption of cell wall components, thereby leading to the release of cell wall-bound or esterified phenolics (Guo & Beta, 2013). Notable is the fact that matter can neither be created nor be destroyed. Therefore, in line with this, apparent decreases in phenolic acids after cooking may presumably be due to the phenolic acids becoming less extractable, while apparent increases in

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Table 2 Effects of fermentation and acidification on the phenolic concentration (μg/g) of extracts from sorghum and amaranth gruels

Sample	Raw flour	Cooked flour	Fermented*	Fermented* and cooked	Fermented**	Fermented** and cooked	Acidified	Acidified and cooked
Sorghum								
Acidified methanol extract								
Protocatechuic acid	82.85^{a} ± 1.61	78.88^{a} ± 1.09	95.28^{b} ± 0.44	108.18^{c} ± 5.51	98.10^{b} ± 0.28	100.85^{bc} ± 1.24	83.58^{a} ± 1.38	102.14^{bc} ± 1.94
p-Hydroxy benzoic acid	121.91^{b} ± 5.00	100.43^{a} ± 2.63	139.32^{de} ± 1.28	165.56^{f} ± 4.71	125.96^{bcd} ± 3.56	136.32^{cde} ± 3.43	122.60^{bc} ± 2.67	139.96^{e} ± 2.97
Caffeic acid	236.80^{f} ± 1.08	215.36^{e} ± 0.69	70.12^{c} ± 0.90	80.44^{d} ± 0.45	41.82^{a} ± 0.47	50.14^{b} ± 0.45	239.97^{f} ± 4.46	274.65^{g} ± 3.13
p-Coumaric acid	57.92^{bc} ± 1.53	54.01^{bc} ± 0.46	38.99^{a} ± 0.76	51.88^{b} ± 3.78	61.21^{c} ± 1.57	72.80^{d} ± 1.51	60.55^{c} ± 2.36	74.36^{d} ± 0.60
Gallic acid	29.93^{a} ± 1.98	25.52^{a} ± 0.25	44.16^{cd} ± 0.50	49.14^{de} ± 0.25	52.68^{ef} ± 1.42	54.63^{f} ± 1.49	37.28^{b} ± 1.28	41.71^{bc} ± 1.23
Vanillic acid	21.93^{c} ± 0.09	21.50^{bc} ± 0.16	21.67^{c} ± 0.15	24.79^{d} ± 0.15	21.95^{c} ± 0.14	22.00^{c} ± 0.20	19.53^{a} ± 0.05	20.92^{b} ± 0.17
Ferulic acid	47.25^{d} ± 0.05	55.54^{f} ± 0.17	14.93^{c} ± 0.13	9.08^{b} ± 0.03	5.41^{a} ± 0.09	4.49^{a} ± 0.06	52.64^{e} ± 0.09	59.13^{g} ± 0.82
Saponified residue								
p-Hydroxy benzoic acid	48.25^{ab} ± 1.52	41.95^{a} ± 0.29	73.14^{c} ± 3.13	101.30^{d} ± 1.71	80.40^{c} ± 1.48	96.74^{d} ± 3.14	46.32^{ab} ± 2.67	54.15^{b} ± 1.98
Caffeic acid	34.74^{ab} ± 1.44	27.59^{a} ± 0.46	39.82^{be} ± 0.68	49.62^{cd} ± 3.61	44.65^{ce} ± 3.29	57.02^{d} ± 2.26	35.78^{ab} ± 0.70	50.40^{cd} ± 1.79
Gallic acid	4.17^{a} ± 0.26	3.12^{a} ± 0.17	6.60^{b} ± 0.41	15.39^{d} ± 0.58	n.d.	n.d.	6.99^{b} ± 0.26	12.11^{c} ± 0.41
Vanillic acid	2.86^{bc} ± 0.28	2.57^{b} ± 0.09	4.17^{e} ± 0.09	1.56^{d} ± 0.11	0.85^{a} ± 0.07	0.60^{a} ± 0.07	5.33^{f} ± 0.14	3.35^{c} ± 0.11
Ferulic acid	9.75^{d} ± 0.25	7.30^{c} ± 0.06	3.90^{b} ± 0.01	2.48^{a} ± 0.04	4.19^{b} ± 0.07	3.08^{a} ± 0.06	11.62^{e} ± 0.33	7.53^{c} ± 0.13
Total phenolic acids	698.36^{d} ± 9.19	633.77^{bc} ± 0.81	552.10^{a} ± 5.92	659.42^{c} ± 20.94	537.22^{a} ± 6.18	598.67^{b} ± 0.65	722.19^{d} ± 7.88	840.41^{e} ± 3.60
Amaranth								
Acidified methanol extract								
p-Hydroxy benzoic acid	17.16^{b} ± 1.56	13.50^{a} ± 0.66	19.75^{bcd} ± 0.43	23.33^{e} ± 1.18	20.68^{cde} ± 0.61	23.07^{de} ± 0.30	19.11^{bc} ± 0.64	21.21^{cde} ± 0.61
Caffeic acid	58.09^{b} ± 0.84	45.87^{a} ± 1.62	n.d.	n.d.	n.d.	n.d.	59.44^{b} ± 1.92	74.95^{c} ± 0.56
p-Coumaric acid	48.15^{b} ± 0.96	44.46^{a} ± 0.56	n.d.	n.d.	n.d.	n.d.	49.65^{b} ± 0.96	58.44^{c} ± 0.18
Gallic acid	126.66^{e} ± 1.05	96.98^{d} ± 1.27	86.31^{b} ± 0.62	91.33^{c} ± 1.74	75.70^{a} ± 1.59	87.74^{bc} ± 0.76	131.96^{f} ± 0.92	140.13^{g} ± 0.86
Ferulic acid	0.46^{c} ± 0.04	0.29^{b} ± 0.01	0.16^{a} ± 0.01	0.10^{a} ± 0.00	n.d.	n.d.	0.51^{c} ± 0.01	0.24^{b} ± 0.01
Saponified residue								
p-Hydroxy benzoic acid	3.59^{a} ± 0.05	8.39^{d} ± 0.03	5.80^{c} ± 0.12	14.61^{g} ± 0.11	8.94^{e} ± 0.06	12.18^{f} ± 0.10	4.38^{b} ± 0.06	8.92^{e} ± 0.10
Ferulic acid	0.54^{d} ± 0.01	1.54^{e} ± 0.02	0.36^{c} ± 0.01	2.17^{f} ± 0.04	0.12^{a} ± 0.01	0.24^{b} ± 0.02	0.57^{d} ± 0.01	0.62^{d} ± 0.00
Total phenolic acids	254.65^{d} ± 4.43	211.03^{c} ± 0.47	112.38^{a} ± 1.15	131.54^{b} ± 3.06	105.44^{a} ± 0.94	123.23^{b} ± 0.98	265.62^{e} ± 2.58	304.51^{f} ± 0.53
Sorghum								
Acidified methanol extract								
Naringenin-7-*O*-glucoside (naringenin equivalents)	170.25^{b} ± 1.86	88.02^{a} ± 2.90	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Eriodictyol-7-*O*-β-D-glucoside	53.77^{c} ± 1.70	29.06^{b} ± 1.09	n.d.	n.d.	n.d.	n.d.	23.48^{a} ± 0.74	25.18^{a} ± 0.79
Luteolin-7-*O*-β-D-glucoside	72.76^{b} ± 0.19	66.24^{a} ± 0.18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Eriodictyol	657.25^{b} ± 3.10	577.23^{a} ± 1.12	887.37^{f} ± 1.64	860.37^{e} ± 1.27	943.17^{h} ± 0.76	897.17^{g} ± 1.28	748.25^{d} ± 3.40	710.44^{c} ± 3.43
Luteolin	137.79^{a} ± 1.41	151.71^{b} ± 2.43	176.75^{c} ± 1.85	199.58^{ef} ± 0.66	187.41^{d} ± 1.38	206.91^{f} ± 1.50	172.37^{c} ± 0.68	197.11^{e} ± 3.98
Naringenin	777.49^{a} ± 4.39	817.97^{b} ± 4.28	1017.58^{c} ± 2.38	1041.90^{d} ± 2.89	1086.43^{f} ± 5.05	1160.62^{g} ± 3.30	1032.69^{d} ± 1.14	1063.06^{e} ± 3.80
Saponified residue								
Luteolin-7-*O*-β-D-glucoside	8.92^{a} ± 0.59	9.90^{a} ± 0.65	9.51^{a} ± 0.39	n.d.	n.d.	n.d.	9.37^{a} ± 0.46	n.d.
Luteolin	18.01^{a} ± 0.81	22.93^{b} ± 0.15	22.02^{b} ± 0.32	27.76^{c} ± 0.66	23.80^{b} ± 0.36	29.01^{c} ± 0.50	23.55^{b} ± 0.33	27.59^{c} ± 0.71
Naringenin	6.21^{a} ± 0.77	10.85^{b} ± 0.86	6.28^{a} ± 0.35	5.82^{a} ± 0.37	4.84^{a} ± 0.63	4.51^{a} ± 0.14	5.62^{a} ± 0.14	4.72^{a} ± 0.08
Total flavonoids	1902.45^{b} ± 8.62	1773.91^{a} ± 2.00	2119.51^{d} ± 1.39	2135.43^{d} ± 2.49	2245.65^{e} ± 8.18	2298.22^{f} ± 4.16	2015.33^{c} ± 4.75	2028.10^{c} ± 9.64

Note: Values are means ± standard deviation. Means in a row with different superscripts are significantly different ($P < 0.05$) from each other. Fermented* = fermentation with *Lactobacillus plantarum* starter culture; Fermented** = fermentation by back-slopping; n.d. = below limit of quantification.

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phenolic acid content may be due to the phenolic acids becoming more extractable as a result of cooking.

Effects of fermentation and acidification on the flavonoid composition

Flavonoids were quantified only in sorghum flour and gruel but not in amaranth. The absence of quantifiable amounts of flavonoids in amaranth flour and gruel suggests low levels of flavonoids in the amaranth used. Previous studies have reported the presence of flavonoids, primarily flavonols like rutin (quercetin-3-*O*-rutinoside) (Kalinova & Dadakova, 2009; Vollmannova *et al*., 2013) and nicotiflorin (kaempferol-3-*O*-rutinoside) (Barba de la Rosa *et al*., 2009; Steffensen *et al*., 2011) in amaranth grains. However, low levels (4.0–10.2 μg/g) have been reported by some of these authors (Barba de la Rosa *et al*., 2009). Other authors (Repo-Carrasco-Valencia *et al*., 2010), however, have observed that there were no quantifiable amounts of flavonoids in amaranth, which is consistent with the current findings.

The flavonoids quantified in the acidified methanolic extracts (sorghum) included naringenin-7-*O*-glucoside, eriodictyol-7-*O*-β-D-glucoside, luteolin-7-*O*-β-D-glucoside, eriodictyol, luteolin, and naringenin. Luteolin-7-*O*-β-D-glucoside, luteolin, and naringenin were quantified in the saponified residue (Table 2). Both fermentation and exogenous acidification with lactic acid seemed to have the same effect on the total flavonoid content. In both cases, the total flavonoid content was significantly higher compared to the raw flour with most of the glycosides apparently not detected. In addition, the flavonoids aglycones (such as eriodictyol, luteolin, and naringenin) were substantially higher in the fermented and lactic acid acidified treatments compared to the raw flour. Evidently, cooking had different effects on individual flavonoids in the raw, fermented, and acidified sorghum flours, with some appearing to decrease while others appeared to increase in concentration (Table 2). However, the total flavonoid content was significantly higher in the fermented/acidified and cooked gruels compared to the cooked gruels without fermentation or acidification.

The fact that flavonoid glycosides (such as naringenin-7-*O*-glucoside, eriodictyol-7-*O*-β-D-glucoside, and luteolin-7-*O*-β-D-glucoside) were not detected in the fermented treatments indicated a possible hydrolysis by fermenting microflora. It has been reported that lactobacilli possess glycosidase activities, which make them release flavonoid aglycones from the corresponding glucosides (Ávila *et al*., 2009; Marazza *et al*., 2009). Svensson *et al*. (2010) also reported hydrolysis of naringenin-7-*O*-glucoside by *Lactobacillus fermentum* during its growth as binary strain combination in sorghum dough. Similar observation or reduced levels of these glycosides in the acidified treatments can also be attributed to a possible hydrolysis by the natural grain microflora since the samples were firstly acidified to pH 5 and held at 50 °C for 2 h; otherwise, the hydrolysis might just be due to the low pH.

Hydrolysis of flavonoid glycosides leading to the release of aglycones during lactic acid fermentation or exogenous acidification could be responsible for the higher levels of the aglycones (eriodictyol, luteolin, and naringenin) in the fermented and acidified gruels compared to the gruels without fermentation or acidification. Apart from the hydrolysis of flavonoid glycosides, fermentation-induced or acidification-induced structural breakdown/modification of the cell wall leading to the release of phenolic compounds bound to the insoluble cell wall material could have also contributed to higher levels of eriodictyol, luteolin, and naringenin in the fermented and acidified gruels compared to the gruels without fermentation or acidification. With regard to the effect of cooking on flavonoid content, the observed reduction in the levels of some flavonoids after cooking can be attributed to a possible interaction between such flavonoids and macromolecules such as protein thereby making them less extractable (Guo & Beta, 2013).

Effects of fermentation and lactic acid acidification on the total phenolic content and radical scavenging capacity

The total phenolic content (TPC) of extracts from the flours and gruels ranged between 4.09 and 5.88 mg CE/g and between 2.06 and 3.78 mg CE/g sample (dry basis) for 100% sorghum and 100% amaranth respectively (Table 3). The 100% sorghum flour treatments had significantly ($P < 0.05$) higher TPC (average, 5.33 ± 0.01 mg CE/g) compared to 100% amaranth which had 2.95 ± 0.01 mg CE/g. In comparison to the raw flour, the souring of 100% sorghum flour using fermentation by *Lactobaccillus plantarum* starter culture, fermentation by back-slopping, and lactic acid acidification resulted in a significant ($p < 0.05$) increase in total phenolic content by 5.6%, 8.4%, and 10.8% respectively (Table 3). Similarly, the souring of 100% amaranth resulted in a significant increase in TPC by 14.6%, 9.5%, and 7.1% for fermentation using *L. plantarum*, fermentation by back-slopping, and lactic acid acidification, respectively, in comparison to the raw flour (Table 3).

The effect of fermentation and acidification on total phenolic content was more pronounced when the raw, fermented, and acidified treatments were cooked. The TPC was significantly higher by 39.6%–43.8% in the fermented and acidified treatments from 100% sorghum after cooking compared to the cooked flour without fermentation or acidification. Concerning 100% amaranth, the TPC was significantly higher by

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Table 3 Effects of fermentation and lactic acid acidification on the total phenolic content (TPC), ABTS, DPPH, and NO radical scavenging activities of gruels from sorghum, amaranth, and their composites

Flour type	Treatment	TPC (mg CE/g)	ABTS (μmol TE/g)	DPPH (μmol TE/g)	NO (μmol $NaNO_2$/g)
Sorghum	Raw flour	$4.98^{c} \pm 0.05$	$30.87^{d} \pm 0.81$	$9.15^{d} \pm 0.28$	$1.94^{c} \pm 0.05$
	Cooked flour (unfermented)	$4.09^{a} \pm 0.09$	$24.49^{a} \pm 0.02$	$6.46^{a} \pm 0.11$	$1.78^{b} \pm 0.01$
	Fermented (*L. plantarum*) (uncooked)	$5.26^{d} \pm 0.05$	$31.34^{de} \pm 0.06$	$10.07^{e} \pm 0.05$	$2.01^{cd} \pm 0.00$
	Fermented (*L. plantarum*) and cooked	$5.71^{f} \pm 0.03$	$32.11^{fg} \pm 0.06$	$10.32^{ef} \pm 0.16$	$2.14^{ef} \pm 0.01$
	Fermented by back-slopping (uncooked)	$5.40^{de} \pm 0.05$	$31.77^{ef} \pm 0.10$	$10.60^{fg} \pm 0.14$	$2.08^{de} \pm 0.01$
	Fermented by back-slopping and cooked	$5.83^{fg} \pm 0.05$	$32.14^{fg} \pm 0.06$	$10.88^{gh} \pm 0.06$	$2.25^{g} \pm 0.00$
	Acidified (lactic acid) (uncooked)	$5.52^{e} \pm 0.09$	$32.97^{h} \pm 0.06$	$10.83^{g} \pm 0.05$	$2.14^{ef} \pm 0.04$
	Acidified (lactic acid) and cooked	$5.88^{g} \pm 0.04$	$32.79^{gh} \pm 0.05$	$11.22^{h} \pm 0.10$	$2.16^{f} \pm 0.05$
	Average	$5.33^{b} \pm 0.01$	$31.06^{b} \pm 0.08$	$9.94^{b} \pm 0.03$	$2.06^{b} \pm 0.01$
Amaranth	Raw flour	$2.53^{bc} \pm 0.07$	$12.58^{c} \pm 0.04$	$3.64^{b} \pm 0.05$	$1.18^{b} \pm 0.01$
	Cooked flour (unfermented)	$2.06^{a} \pm 0.09$	$8.33^{a} \pm 0.12$	$2.06^{a} \pm 0.09$	$1.11^{a} \pm 0.01$
	Fermented (*L. plantarum*) (uncooked)	$2.90^{de} \pm 0.13$	$19.33^{f} \pm 0.51$	$5.69^{d} \pm 0.40$	$1.31^{e} \pm 0.01$
	Fermented (*L. plantarum*) and cooked	$3.78^{f} \pm 0.11$	$24.56^{g} \pm 0.14$	$6.27^{e} \pm 0.31$	$1.42^{f} \pm 0.01$
	Fermented by back-slopping (uncooked)	$2.77^{bd} \pm 0.08$	$19.90^{f} \pm 0.09$	$5.31^{d} \pm 0.06$	$1.21^{bc} \pm 0.01$
	Fermented by back-slopping and cooked	$3.76^{f} \pm 0.03$	$24.26^{g} \pm 0.02$	$6.34^{e} \pm 0.05$	$1.30^{e} \pm 0.00$
	Acidified (lactic acid) (uncooked)	$2.71^{bcd} \pm 0.11$	$12.82^{c} \pm 0.08$	$4.19^{c} \pm 0.02$	$1.22^{bc} \pm 0.04$
	Acidified (lactic acid) and cooked	$3.05^{e} \pm 0.09$	$17.85^{e} \pm 0.14$	$5.48^{d} \pm 0.06$	$1.22^{bc} \pm 0.00$
	Average	$2.95^{a} \pm 0.01$	$17.45^{a} \pm 0.11$	$4.87^{a} \pm 0.06$	$1.25^{a} \pm 0.00$

Note: Means in a column with different superscripts are significantly different ($p < 0.05$) for each sample type.
Abbreviations: ABTS, trolox equivalent antioxidant capacity (μmol TE/g sample dry basis); DPPH, 2,2′-diphenyl-1-picrylhydrazyl (μmol TE/g sample dry basis); NO, values are means ± standard deviation; TPC, total phenolic content (mg CE/g sample dry basis).

48.1%–83.8% in the fermented and acidified treatments after cooking compared to the cooked flour without fermentation or acidification.

Regarding the radical scavenging capacity of extracts from the flours and gruels, 100% sorghum treatments had significantly ($P < 0.05$) higher ABTS, DPPH, and NO radical scavenging capacity (29.86 μmol TE/g, 9.53 μmol TE/g, and 1.97 μmol $NaNO_2$ /g for ABTS, DPPH, and NO respectively) compared to 100% amaranth (16.46 μmol TE/g, 4.79 μmol TE/g, and 1.24 μmol $NaNO_2$/g for ABTS, DPPH, and NO respectively) (Table 3).

The souring of 100% sorghum flour using fermentation by *Lactobacillus plantarum*, fermentation by backslopping, and lactic acid acidification resulted in a significant ($P < 0.05$) increase in ABTS radical scavenging capacity by 1.5%–6.8%, DPPH by 10.1%–18.4%, and NO by 3.6%–10.3% compared to the raw flour. When the raw, fermented, and acidified flours were cooked, fermented and lactic acid acidified treatments had a significantly higher ABTS by 31.0%–34.0%, DPPH by 59.8%–73.7%, and NO by 20.2%–26.4% compared to the cooked flour without fermentation or acidification. Similarly, fermentation and lactic acid acidification of 100% amaranth resulted in a significant increase in ABTS radical scavenging capacity by 41.9%–58.2%, DPPH by 15.1%–56.3%, and NO by 2.5%–11.0% in comparison to the raw flour. After cooking, the ABTS radical scavenging capacity significantly increased by 114.3%–194.8%, DPPH by 166.0%–207.8%, and NO by 9.9%–28.0% in the fermented and lactic acid acidified treatments compared to the cooked flour without fermentation or acidification. The results further revealed that treatments with the higher total phenolic content correspondingly exhibited the higher radical scavenging capacities, which imply that radical scavenging capacity may be a result of their phenolic contents.

The observed increases in TPC and radical scavenging capacity in all the fermented treatments compared to the raw flour may be attributed to the structural breakdown of cell walls of the grain by cell wall degrading enzymes released by the fermenting microflora, leading to the release of bound phenolics, which makes the phenolic compounds more extractable (Shahidi & Yeo, 2016). This proposed release of bound phenolics may also account for the observed increase in TPC and radical scavenging capacity in the acidified treatments compared to the raw flour since the samples were firstly acidified to pH 5 and held at 50 °C for 2 h. During this period, the endogenous microflora in the grains could have possibly had sufficient time to release cell wall hydrolysing enzymes that could breakdown the cell wall leading to the release of bound phenolics. Further increase in TPC and radical scavenging capacity after cooking the fermented and acidified treatments suggested that pre-treatments like fermentation and acidification may enhance the extractability of phenolics during subsequent cooking. In addition, hydrolysis of glycosidic bonds and ester/ether bonds

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between phenolic compounds, lignins, and hemicelluloses/cellulose increases during cooking, leading to the release of bound phenolic acids (Gong *et al*., 2012).

On the other hand, the reduction in TPC observed after cooking those treatments without fermentation or acidification may be attributed to the reduction in the extractability of phenolic compounds due to possible complexation with protein and other macromolecules during cooking (Dykes & Rooney, 2006). It may also be due to possible heat-induced structural degradation of phenolic compounds (Zadernowskl *et al*., 1999; Taylor & Duodu, 2015).

Principal component analysis (PCA) was applied to determine relationships among the treatments in relation to the total phenolic content and radical scavenging capacity. The sample principal component plot (Fig. 3a) separated treatments such as acidified, acidified and cooked, fermented (*L. plantarum*), fermented (*L. plantarum*) and cooked, fermented (by back-slopping), fermented (by back-slopping) and cooked on the right side of the plot from treatments such as raw samples, and cooked samples without fermentation or acidification on the left side of the plot. Fig. 3b shows the relationship between TPC and radical scavenging activity. On the right side of the plot, treatments such as acidified, acidified and cooked, fermented (*L. plantarum*), fermented (*L. plantarum*) and cooked, fermented (by back-slopping), fermented (by back-slopping) and cooked were strongly associated with TPC and radical scavenging capacity, suggesting that such treatments could enhance the TPC and radical scavenging capacity of the gruel. This supports the earlier statement that fermentation and lactic acid acidification may improve the extractability of phenolic compounds, leading to the increase in TPC and radical scavenging capacity.

The Pearson correlation coefficients between total phenolic content (TPC) and radical scavenging activity (table not shown) clearly showed that a strong and significant ($p < 0.05$) positive correlation existed between total phenolic content and antioxidant activity as measured by ABTS, DPPH, and NO ($r = 0.944$, $r = 0.862$, $r = 0.959$ for TPC and ABTS, TPC and DPPH, TPC and NO respectively). The strong and significant positive correlation between total phenolic content and radical scavenging capacity agrees with earlier reports (Kayitesi *et al*., 2012; Adarkwah-Yiadom & Duodu, 2017). This is due to the fact that those treatments with higher TPC also exhibited higher radical scavenging capacity and *vice versa*, thus, suggesting that radical scavenging activity of the extracts may depend largely on their constituent phenolic compounds as earlier stated.

Inhibition of AAPH-induced DNA damage by extracts from sorghum, amaranth, and their composite gruels

The scission of supercoiled plasmid DNA to an open-circular form (single-strand breaks) or to a linear form (double-strand breaks) in the presence of radicals has been used as an indicator of DNA damage (Madhujith *et al*., 2004; Wei *et al*., 2006). The agarose gel

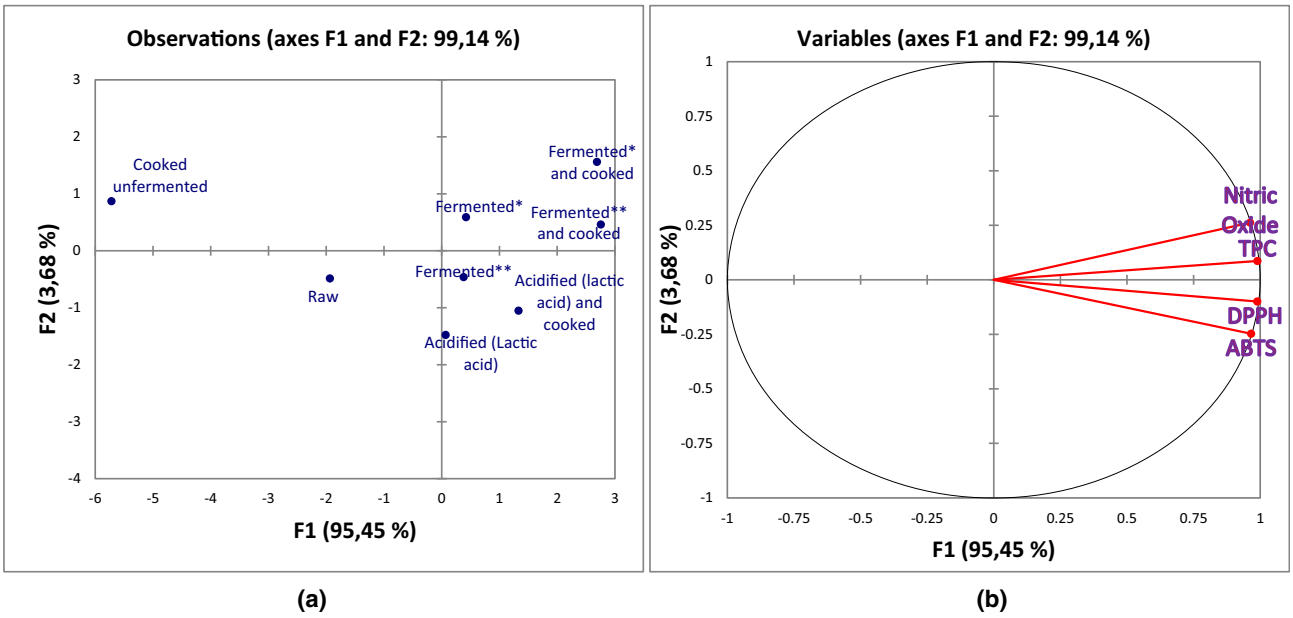

Figure 3 Principal component analysis between the treatments (a) and the parameters determined (b). ABTS, trolox equivalent antioxidant capacity; DPPHS, 2,2′-diphenyl-1-picrylhydrazyl; TPC, total phenolic content

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electrophoretograms showing the effect of the extracts from unfermented, fermented, and lactic acid acidified gruels from sorghum, amaranth, and their composite flour on pBR 322 plasmid DNA treated with AAPH are shown in Fig. 4. For the negative control (DNA + distilled water) in lane 1 of Fig. 4, there was clear migration of the plasmid DNA through the agarose gel to position F, signifying that its original supercoiled form was retained. However, in the presence of AAPH radical (positive control) (Lane 2), the pBR 322 plasmid DNA failed to migrate effectively through the gel (which is an indication of damage) and therefore could not move beyond position E, suggesting that it now existed mainly in the damaged open circular form, which has low electrophoretic mobility. This may be due to the cleavage of hydrogen bonds in the DNA molecule by alkyl peroxyl radicals generated by AAPH, leading to the unfolding to open circular from its original supercoiled form (Wei *et al*., 2006).

The ability of the extracts (unfermented, fermented, and lactic acid acidified gruels from sorghum and amaranth) to protect the DNA against oxidative damage was revealed when the extract was added to the plasmid DNA in the presence of AAPH. The results clearly indicated that in spite of the presence of AAPH, the DNA maintained its predominantly supercoiled form in all the relevant lanes as shown by its effective migration to position F as was observed for the negative control. This may be attributed to scavenging of the AAPH-generated radicals by phenolic compounds in these extracts.

Various workers have reported a similar protective effect of extracts from different plant sources against oxidative DNA damage. Such reports include those by Nderitu *et al*. (2013) (*in vitro* digests of cooked cowpeas), Apea-Bah *et al*. (2016) (*in vitro* digests of sorghum porridge), and Adarkwah-Yiadom & Duodu (2017) (*in vitro* digests of extruded tannin sorghum porridges). It is worth noting that all the lanes containing plasmid DNA treated with AAPH in the presence of the extracts showed faint bands attributable to open circular forms of the DNA. According to Adarkwah-Yiadom & Duodu (2017), this could be attributed to the inability of the sample extracts to completely protect against oxidative DNA damage. There could also have been some open circular DNA forms in the plasmid DNA as obtained from the manufacturer as can be observed in lane 1 (negative control, DNA + distilled water), where faint bands due to open circular DNA forms were present. According to Wei *et al*. (2006), there seems to be a proportion of open circular DNA forms present with supercoiled DNA in variable amounts depending on the batch.

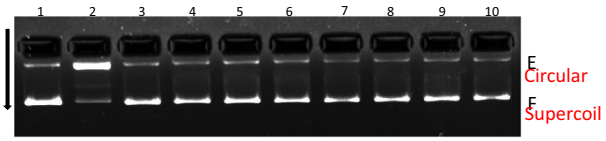

Figure 4 Effects of fermented and lactic acidified sorghum, amaranth, and their composite gruels extracts on AAPH-induced DNA damage. Lane 1 negative control (DNA + H_2O); lane 2 positive control (DNA + AAPH); lane 3 (DNA + AAPH + cooked 100% sorghum gruel); lane 4 (DNA + AAPH + fermented (*L. plantarum*) and cooked 100% sorghum gruel); lane 5 (DNA + AAPH + fermented (by back-slopping) and cooked 100% sorghum gruel); lane 6 (DNA + AAPH + acidified and cooked 100% sorghum gruel); lane 7 (DNA + AAPH + cooked 100% amaranth gruel); lane 8 (DNA + AAPH + fermented (*L. plantarum* and cooked 100% amaranth gruel); lane 9 (DNA + AAPH + fermented (by back-slopping) and cooked 100% amaranth gruel); lane 10 (DNA + AAPH + acidified and cooked 100% amaranth gruel).

Conclusions

This study demonstrates that extractable phenolic compounds in sorghum and amaranth gruels are affected by souring caused by both lactic acid fermentation and exogenous acidification with lactic. Fermented and acidified gruels have higher levels of flavonoid aglycones such as eriodictyol, luteolin, and naringenin compared to gruels without fermentation or acidification, with most of the glycosides apparently not detected in both cases. Overall, lactic acid-fermented and lactic acid-acidified gruels have higher levels of extractable phenolics and antioxidant properties (as determined by ABTS, DPPH, and NO radical scavenging assays) than their corresponding unsoured gruels. Therefore, based on the observed effect of exogenous acidification with lactic acid on phenolic composition of sorghum and amaranth gruels, it may be inferred that ready-to-eat soured porridge products made using exogenous acidification as a simple process of souring have the potential to shield the body from illnesses linked to oxidative stress.

Acknowledgments

This publication was made possible through support provided by the Office of Agricultural Research and Policy, Bureau for Food Security, U.S. Agency for International Development, under the terms of Cooperative Agreement No. AID-OAA-L-14-00003. The opinions expressed herein are those of the author(s) and do not necessarily reflect the views of the U.S. Agency for International Development.

Author contributions

Adeyemi Ayotunde Adeyanju: Conceptualization (supporting); data curation (lead); investigation (lead);

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methodology (lead); project administration (lead); writing – original draft (lead). **Kwaku Gyebi Duodu:** Conceptualization (lead); funding acquisition (lead); supervision (lead); writing – review and editing (supporting).

Conflict of interest

The authors report no potential conflicts of interest.

Ethical approval

Ethics approval was not required for this research.

Peer review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.16245.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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This reference was used in the current work because it was part of earlier research done by our research group. In particular, it was used to discuss the defence mechanism of the phenolic molecule in sorghum against oxidative DNA damage. The authors' most recent work, however, focused on red non-tannin sorghum and amaranth, while the earlier work focused on *in vitro* digests of extruded tannin sorghum porridges.

Adeyanju, A.A., Kruger, J., Taylor, J.R.N. & Duodu, K.G. (2019). Effects of different souring methods on the protein quality and iron and zinc bioaccessibilities of non-alcoholic beverages from sorghum and amaranth. *International Journal of Food Science and Technology*, **54**, 798–809.

This reference was used because the current work builds on our previous research that was published in this journal. The current investigation focuses on the phenolic constituent and antioxidant activities, whereas the previous work (Adeyanju *et al.*, 2019) concentrated on protein quality and iron and zinc bioaccessibilities. The method used in the previous work to prepare the fermented and acidified gruels was used in the current investigation.

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