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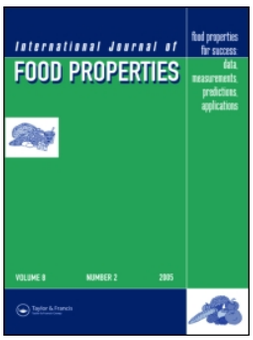

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Phytochemical characterization of ultrasound-processed sorghum sprouts for the use in functional foods

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ABSTRACT

In the recent years, application of ultrasound has been taken into consideration to enhance the nutritive value of processed food products, either by retaining or modulating their phytochemical components. Profile of phytochemicals in sorghum seeds was amended by optimizing ultrasound application for their use as sprouts. In this study, overall impact on various phytochemical constituents (alkaloids, phytates, saponins, and sterols), radical scavenging activity (2,2-diphenyl-1-picrylhydrazyl assay, ferric reducing antioxidant power assay, and oxygen radical absorbance capacity assay assay), phenolic profile (total phenolics content, total flavonoids content, ferulic acid, gallic acid, catechin, quercetin, and tannin) along with *in vitro* protein digestibility (IVPD %) influenced through germination was evaluated in ultrasonically processed sorghum sprouts. Among different treatment levels, sonication at 40% amplitude for 5 min showed the significant outcomes. After germination, the ultrasound-treated sorghum sprouts showed superior profile of phytochemicals that can serve as valuable raw material for producing high-protein functional/weaning foods with low cost.

KEYWORDS

Sorghum; sonication; germination; phenolic profile; protein digestibility

Introduction

Among cereal crops sorghum (*Sorghum bicolor* (L) Moench) is also a major dietary staple food in over 30 countries of the semiarid tropics having 500 million people.[1] Presence of phytochemical contents is an important attribute of sorghum. It contains various phytochemicals providing an excellent source of anthocyanins (particularly 3-deoxyanthocyanidins present in red sorghum), phenolic acids, policosanols, and sterols.[2] Among phytochemicals, phenolic compounds have potential biological activities such as antioxidant activity, anticancer property, antimicrobial effect, stimulation of the immune system and modulation of hormonal metabolism.[3] Different processing techniques have been introduced to improve the nutritional profile and functional characteristics in plant seeds.[4]

Like other processing techniques, germination seemed to be an effective technique to improve the nutritional composition of foods. During the germination process, variations in nutritional profile may occur due to breakage of complex substances into simpler ones and their further transformation into essential compounds.[5] For improving food values germinated grains have been consumed for centuries in Asian countries.[6] Through breakdown of nutritionally undesirable constituents, amount of the antinutritive materials such as phytates, tannin, etc. also decreased.[7] Several previous studies strengthen the argument of nutritional improvements in germinated grains like in sorghum increased total free amino acids content,[8] and carbohydrates content[9]; improved levels of polyphenol and

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antioxidant activities in rye and sorghum,[10] wheat,[11] and rice[12]; high contents of dietary fiber, free amino acid and phenolic compounds in germinated waxy wheat.[11]

Physical treatments has ability to enhance the seed activities by influencing their different physiological and biochemical functions occurring without having adverse effect on environment.[13] Recently application of ultrasound has attracted the attention of research groups as it does not require extensive technical training and sophisticated equipment.[14] Ultrasound treatment can change the structure and functions of biological molecules through interaction mechanisms causing acoustically induced cavitation activity. The process of cavitation contains series of phenomena including the creation, development and the collapse of microbubbles produced in a liquid when ultrasound waves travel through the medium. Stimulatory effect of ultrasound waves is linked with inducing high fluidity rate of cell wall and membrane.[15] Ultrasonic processing is an efficient technique among already used methods of pre-sowing treatment due to its simplicity, less cost,, multipurpose and environmentally safe features.[16] A big pool of literature reports positive results of sonication on the hydration process of seeds and grains, thus improves their germination performance.[15,16]

Unfortunately, still there is negligible extension in literature to investigate the phytochemicals profile of sprouts after ultrasound processing. Considering the importance of novel techniques for food processing as an innovative approach, this study was planned to assess the effects of ultrasonic stimulated sprouting on phytochemicals composition and on *in vitro* protein digestibility (IVPD %) of germinated sorghum grains.

Material and methods

Sorghum grains and reagents

Sorghum grains (commercial product; unknown variety) having white color, hearty texture, and mild, nutty flavor procured from a local grain market (Lincoln, Nebraska, USA) were put for soaking (5 min) in sodium hypochlorite solution (5%). Afterward, grains were properly washed in distilled water and again soaked overnight.[17] All the HPLC (High-Perfomrance Liquid Chromatography), analytical grade reagents, and standards were purchased from Duksan (Korea), Merck (KGaA, Darmstadt, Germany), Sigma-Aldrich (Tokyo, Japan), and Unichem, Avochem (UK).

Ultrasonic treatment

Grains were given ultrasound treatment by using two variables of amplitude (cavitation intensity) and time duration. Grains were placed into four groups 1 USD (amplitude: 40%, time: 5 min), 2 USD (amplitude: 60%, time: 5 min), 3 USD (amplitude: 40%, time: 10 min), and 4 USD (amplitude: 60%, time: 10 min). A 100 g of each sample dispersed in 500 mL of distilled water was exposed to ultrasound waves directly using a 25 mm probe system. Selection of conditions for ultrasound processing was according to preliminary trials and from different previous studies.[14,18] Pulsed on and pulsed off time was 5 and 10 s, respectively with constant temperature of 35°C (as temperature helps in better hydration of grains by improving their water absorption capacity so different preliminary trails were conducted at different temperatures, among which processing at 35°C showed better germination results). All ultrasonic treatments were applied through ultrasonic processor (SONICS & MATERIALS. INC, Model: VCX750) with frequency of 20 kHz, power 750 W, and volts 230 VAC~ 50/60 Hz NOM.

Germination test

Grains were germinated on moist paper towel placed in germination trays for 48 h at 25 ± 2°C and watered two to three times. Germination percentage of sprouted grains was calculated according to formula given by.[19]

$$G = \left[\text{Number of germinated grains } (N_g)/\text{Total number of grains } (N_t)\right] \times 100$$

Sample and extract preparation

Germinated grains were freeze dried and ground into a fine powder. Until further analysis the obtained powder samples were stored into air tight plastic containers (4°C). For analysis of radical scavenging activity and phenolic profile (excluding tannins), samples were processed following the procedure of.[20] Powder sample (1 g) was put to extraction for 2 h with methanol (70%). Supernatant was separated and remaining precipitates were again put for extraction. Supernatants from both extractions were mixed and then centrifuged. The finally obtained supernatant was stored in air tight tubes (4°C).

Quantitative analysis of phytochemical constituents

Alkaloids content analysis

Alkaloids content were analyzed according to Harborne method described by Eze, Ugwu.[21] Powder sample (5 g) was added in 10% solution (50 mL) of acetic acid in ethanol and kept at rest (4 h). Later the sample was filtered and put for evaporation. Alkaloids were precipitated by drop wise addition of concentrated NH_4OH, resultant precipitates were subjected to filtration. Sample was weighed and alkaloids content were calculated as mg/100 g of dry matter.

Phytates content analysis

Phytates content were determined according to method used by Tizazu, Urga.[22] Powder sample (0.15 g) was subjected to extraction for 1 h with 2.4 % HCl (10 mL). After extraction sample was centrifuged and 3 mL sample solution was mixed with Wade reagent (1 mL). Absorbance of samples was studied at 500 nm. Phytates content were given as mg sodium phytate equivalent per 100 g of dry matter.

Saponins content analysis

Saponins content were estimated by following the protocol of Okwu and Orji.[23] Powder sample (20 g) was put for extraction with 20% ethanol (200 mL), later filtered and concentrated. The concentrated sample was subjected to purification process with diethyl ether (20 mL) and n-butanol (30 mL). After that sample was washed by using aqueous NaCl (5%), properly dried and weighed. The saponins content were expressed as mg/100 g of dry matter.

Sterols content analysis

Sterols content were analyzed by using Liebermann-Burchard (LB) reagent according to method given by Nancy and Ashlesha.[24] Sample extract (1 mL) was mixed with 4 mL chloroform. Later LB reagent (2 mL) was added in it. Sample tubes were kept in dark area (15 min) to turn reaction mixture into green color. Absorbance was taken at 640 nm. Sterols content were calculated as mg cholesterol equivalent per gram of dry matter.

Radical scavenging activity

2,2-diphenyl-1-picrylhydrazyl assay (DPPH analysis)

DPPH analysis was conducted by following the protocol used by Zou, Chang.[25] Methanolic sample extract (100 µL) was mixed with methanolic solution of DPPH (3.9 mL). Mixture was kept for 60 min in dark and absorbance was taken at 515 nm. Percentage inhibition of DPPH for sample and trolox solution was estimated by using following formula:

$$\%\ \text{Inhibition} = 100^{*}(A_0 - A)/A_0$$

Where A_0: beginning absorbance of solvent; A: final absorbance of the test sample.

Ferric reducing antioxidant power assay (FRAP analysis)

FRAP analysis was conducted following the method described by Alka, Neelam.[26] FRAP reagent (1.3 mL) was added in sample extract (200 µL) and subjected for incubation at 37°C for 30 min. Absorption was taken at 595 nm and values expressed as mmol of Fe (II) equivalent per gram dry matter.

Oxygen radical absorbance capacity assay (ORAC analysis)

ORAC assay was determined according to method stated by Rhodes, Gadgil.[27] Fluorescein stock solution (4×10^{-3} mM) and AAPH solution (153 mM) was prepared by using trisodium phosphate buffer (75 mM, pH 7.4). Working solution of fluorescein solution was prepared by diluting (1: 1,000) it with trisodium phosphate buffer. Inner wells of microplate were filled with 150 µL of fluorescein working solution while exterior wells filled with 300 µL deionized water. About 25 µL of each diluted sample, diluted trolox (standard) and phosphate buffer (blank) were added in their particular wells. Further microplate was put at incubation for 30 min (37°C), after that 35 µL of AAPH solution was added. Fluorescence observations were started from 1 min intervals for 60 min by taking fluorescence at 485 nm (excitation) and 528 nm (emission). Values were calculated as micromoles of trolox equivalent per gram of dry matter.

Phenolic profile

Total phenolics content (TPC analysis)

TPC was analyzed through Folin–Ciocalteau method used by Hithamani and Srinivasan.[28] Sample extract (1 mL) was mixed with Folin–Ciocalteau reagent (4 mL). After 3 min sodium carbonate (5 mL) was added and subjected to incubation for 1 h at 40°C. Absorbance was examined at 760 nm and results expressed as mg gallic acid equivalent per gram of dry matter.

Total flavonoids content (TFC analysis)

Estimation of TFC was based on colorimetric method stated by Lallianrawna, Muthukumaran.[29] Sample extract (1.5 mL) was mixed with sodium nitrite solution (75 µL). Further $AlCl_3.6H_2O$ (150 µL) was added and kept at rest (6 min), after that NaOH (0.5 mL) was added and mixed. Volume of mixture was made up to 2.5 mL by using deionized water. Absorbance was studied at 510 nm and results described as mg quercetin equivalent per gram of dry matter.

Tannins content analysis

Vanillin-HCl assay was used for the determination of tannins as described by Dykes, Rooney.[30] In each of two culture tubes marked as sample and sample control, sample extract (1 mL) was taken. Tubes were subjected to incubation in water-bath for 5 min at 30°C. Vanillin reagent prepared from 1:1 of vanillin (1%) and HCl (8%). Later, 5 mL HCl (4%) was added to the sample control tube while vanillin reagent (5 mL) was added to the sample tube. Further tubes were incubated for 20 min at 30°C. Absorbance was measured at 500 nm and results were expressed as mg catechin equivalent per 100 g of dry matter).

Individual phenolic compounds (HPLC analysis)

Chromatographic analysis of methanolic samples extract was conducted by HPLC system for identification of individual phenolic compounds.[28] HPLC system (Agilent 1200 Series; Agilent Technologies Inc., Santa Clara, CA, USA) was composed of C18 analytical column (250 × 4.6 mm; 5 µm; Agilent Technologies Inc., USA) and equipped with a diode array detector. Methanol and trifluoroacetic acid (0.1%) were used as a mobile phase, and separation was conducted at flow with 1 mL/min. Total run time was 60 min with the gradient program including initial B concentration of 20%–40% in 40 min which was maintained for 10 min and then again to 20% B in next 5 min and

further 5 min of post run for reconditioning. Quantification was performed by using flavonoid and phenolic acid standards (270–370 nm).

In vitro protein digestibility (IVPD analysis)

IVPD analysis was carried out according to method stated by Afify, El-Beltagi.[31] Powder sample (1 g) was mixed with HCl, having pepsin (1.5 mg) and incubated at 37°C (3 h). Neutralization of suspension was conducted with sodium hydroxide and treated with pancreatin (4 mg) in 0.2 M phosphate buffer (7.5 mL). Later toluene (1 mL) was added in the mixture, slightly shaken and incubated (24 h). Sample was treated with 10% trichloroacetic acid (10 mL) and subjected to centrifugation. Kjeldahl method was used to analyze the protein of supernatant and calculated in percentage of protein digestibility.

Statistical analysis

The obtained data was statistically analyzed to determine the level significance for each observed response. All samples were prepared in triplicates, and data is given as mean values of each treatment for three replicates along with their standard deviation. Further data was assessed for statistical significance existing between the mean values at 5% significance level, using analysis of variance (ANOVA) (Minitab software version 17) and least significant difference (LSD) post hoc test (Statistical Software SPSS 21). Statistical significance is measured through pair wise comparison test (LSD test) and the number of alphabets (nomenclature) indicates the number of significant pairs that each treatment has with other treatments for each studied response. Moreover, principal component analysis (PCA) was applied to the mean values of the measured responses (Statistical software SPSS 21).

Results and discussion

Germination percentage

Regarding germination rate as compared to control, treatment 1 USD processed at 40% amplitude for 5 min showed the significantly ($p \leq 0.05$) higher percentage of germination (G: 94.00 ± 3.00%) among all processed treatments (Figure 1). Sorghum grains processed at different levels of amplitude and time resulted statistically significant ($p \leq 0.05$) variations in the germination trend. Germination performance was significantly decreased ($p \leq 0.05$) after long term exposure to ultrasound processing (10 min). For improving germination performance, the optimal ultrasonic amplitude was 40% for 5 min (US_1), while the ultrasonic amplitude of 60% for 10 min (US_4) resulted in the reduction of germination. It is

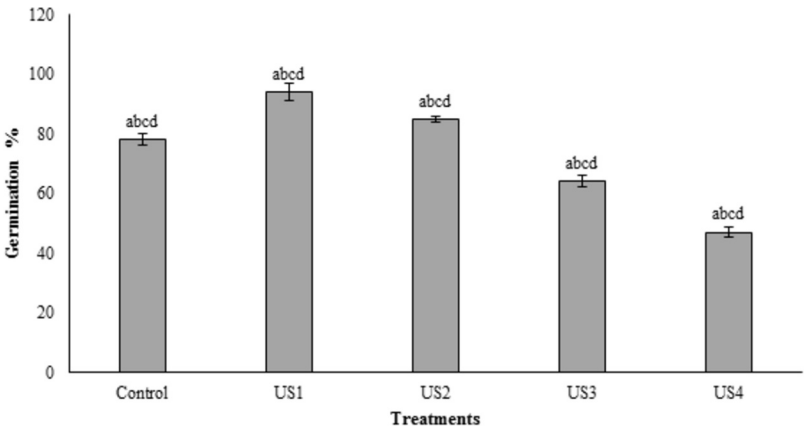

Figure 1. Germination performance of ultrasound-treated sorghum samples (values followed by different letters indicate statistically significant differences ($p \leq 0.05$); abcd: significant difference from other four treatments).

confirmed that ultrasound applies its major effects by causing mechanical changes (acoustic cavitation) and disturbance of cell walls. The ultrasonic application could fragment the seed shell causing larger porosity on the surface of grains by captivation of ultrasound.[32] Thus, increasing water uptake and water retention capacity results in better hydration which enhances the metabolic processes in the form of gibberellic acid release and activation of enzymes (diastase).[14] These phenomena may consequently lead to quicker germination and faster embryo growth. However, only ultrasound with proper amplitude and duration would increase the enzyme activities and stimulate physiological activities of cells, while at high amplitude there will be more damage to the cells or enzyme structure.[16]

Phytochemicals profile

All phytochemicals examined in ultrasound-treated germinated sorghum grains are given in Table 1. The ultrasonic processing caused marked changes in phytochemicals contents. In comparison to control sample, in sorghum treatment processed at 40% amplitude for 5 min (US_1) levels of alkaloids, saponins, tannins, and phytates were significantly ($p \leq 0.05$) decreased having amount of 0.035 ± 0.002 mg/100 g, 0.09 ± 0.02 mg/100 g, 0.131 ± 0.004 mg CE/100 g DM, and 143.25 ± 2.32 mg SPE/100 g DM, respectively. While the same treatment (US_1) contained the significantly ($p \leq 0.05$) increased level of sterols (0.697 ± 0.002 mg CHE/100 g DM). Results showed that with increasing rate of germination (US_1) the levels of anti-nutritional factors (alkaloids, saponins, and phytates) were significantly ($p \leq 0.05$) decreased or vice versa (US_3, 4 USD). In case of sterols content treatment 1 USD gave significantly ($p \leq 0.05$) increased level. Water stirring during soaking as presowing seed priming step leads to removal of large contents of alkaloids. Moreover, breaking of outer shell of grains during sprouting also assists in alkaloids removal.[33] Solubilization and resulting leaching of chemical compounds in soaking water is also linked with decreasing the saponins level.[34] The observed decreasing trend of alkaloids and saponins through germination are in accordance with previous works conducted on fenugreek F[35–38] and chickpea flour.[39] During germination major reason for decrease of tannins in leaching process might be the hydrophobic linkage of tannins with seed enzymes (phytase activity) and protein.[40] In study conducted by Nour et al.[41] on sorghum, also stated same decreasing trend of tannins reduction after germination. As compared to mature tissues of plant, the membrane biosynthesis becomes more rapid in new growing young plant tissues. Therefore, sterols production becomes more intensive during stages of seed development and germination.[42] In other work, germination of Australian sweet lupine showed the higher contents of sterols in flour sample after germination.[43]

Phenolic profile and radical scavenging activity

Results of phenolic profile and radical scavenging activity for all processed germinated sorghum samples are given in Table 2. As compared to control treatment 1 USD among processed germinated samples gave significant ($p \leq 0.05$) higher levels of TFC, TPC, DPPH, FRAP, and ORAC having concentration of 1.02 ± 0.06 mg QE/g DM, 1.26 ± 0.01 mg GAE/g DM, 89.11 ± 2.25%, 0.031 ± 0.004 mmol FE/g DM, and 27.06 ± 0.12 μmol TE/g DM, respectively. Other treatments 2

Table 1. Phytochemicals profile of ultrasound-treated sorghum samples.

Treatments	Alkaloids (mg/100 g)	Phytates (mg SPE/100 g DM)	Saponins (mg/100 g)	Sterols (mg CHE/g DM)	Tannins (mg CE/100 g DM)
Control	0.051 ± 0.004^{ab}	146.05 ± 2.25^{abcd}	0.15 ± 0.01^{a}	0.655 ± 0.025^{ab}	0.147 ± 0.001^{ns}
US_1	0.035 ± 0.002^{abcd}	143.25 ± 2.32^{abcd}	0.09 ± 0.02^{abc}	0.697 ± 0.002^{abcd}	0.131 ± 0.004^{a}
US_2	0.040 ± 0.003^{ab}	145.37 ± 2.51^{abcd}	0.12 ± 0.01^{a}	0.674 ± 0.015^{abc}	0.144 ± 0.005^{ns}
US_3	0.052 ± 0.004^{ab}	151.14 ± 2.03^{abcd}	0.15 ± 0.03^{a}	0.637 ± 0.001^{abc}	0.152 ± 0.001^{ns}
US_4	0.062 ± 0.001^{abcd}	154.46 ± 2.74^{abcd}	0.17 ± 0.05^{ab}	0.614 ± 0.003^{abcd}	0.162 ± 0.025^{a}

All data were presented as mean ± standard deviation. ns: non-significant; values followed by different letters indicate statistically significant differences ($p \leq 0.05$); a: significant difference from other one treatment; ab: significant difference from other two treatments; abc: significant difference from other three treatments; abcd: significant difference from other four treatments.

Table 2. Total phenols and radical scavenging activity of ultrasound-treated sorghum samples.

Treatments	TPC (mg GAE/g DM)	TFC (mg QE/g DM)	DPPH (%)	FRAP (mmol FE/g DM)	ORAC (μmol TE/g DM)
Control	1.18 ± 0.07^{a}	0.88 ± 0.04^{ab}	83.76 ± 1.50^{abcd}	0.029 ± 0.001^{ns}	25.38 ± 0.13^{abc}
US_1	1.26 ± 0.01^{abc}	1.02 ± 0.06^{abcd}	89.11 ± 2.25^{abcd}	0.031 ± 0.004^{ab}	27.06 ± 0.12^{abcd}
US_2	1.22 ± 0.02^{a}	0.91 ± 0.01^{abc}	86.35 ± 1.00^{abcd}	0.029 ± 0.005^{ns}	25.64 ± 0.11^{abc}
US_3	1.18 ± 0.03^{a}	0.82 ± 0.02^{ab}	81.24 ± 2.68^{abcd}	0.027 ± 0.002^{a}	23.11 ± 0.15^{abcd}
US_4	1.13 ± 0.04^{ab}	0.78 ± 0.03^{abc}	78.14 ± 1.75^{abcd}	0.026 ± 0.001^{a}	23.87 ± 0.13^{abcd}

All data were presented as mean ± standard deviation. ns: nonsignificant; values followed by different letters indicate statistically significant differences ($p \leq 0.05$); a: significant difference from other one treatment; ab: significant difference from other two treatments; abc: significant difference from other three treatments; abcd: significant difference from other four treatments.

USD, 3 USD and 4 USD gave significant ($p \leq 0.05$) lower levels of all these parameters due to their decreased germination rate. Results indicated that sorghum sprouts gave statistically significant ($p \leq 0.05$) variations in TPC, TFC, and radical scavenging activity through ultrasonic processing. Germination process can affect the composition of primary and secondary metabolites resulted from series of biochemical reactions, which in result cause changes in intrinsic phenolic profile, and radical scavenging activity.[44] In other study conducted by[45] stated an increase in levels of TFC and TPC of Hang rice after germination. Through germination contents of bioactive compounds and their activities intensely changed. Cell wall-degrading enzymes are active during germination, and they contribute to modification of the cell wall structure of the grain. The action of cell wall-degrading enzymes (mainly esterases) on these bonds contributes to the release of bound phenolic compounds. On the other hand, activation of phenylalanine ammonia lyase (key enzyme in phenolic biosynthesis) during germination of seeds also has been previously reported to contribute in the augmentation of phenolic compounds.[46] Similarly, higher values of DPPH and FRAP after germination were reported in yellow mustard, chickpea, cowpea,[46] and sorghum.[47] Besides germination an increase in phenolic contents can affect the values of ORAC assay. Similar trend of increased level of ORAC assay after germination was also analyzed in wheat grains.[44] Radical scavenging activity is directly associated with contents of polyphenolic compounds. During germination as enzyme synthesis enhance the inherent polyphenolic compounds. Sprouting alters the phenolic acids and other free phytochemicals might be present, leading to an improved radical scavenging activity in fully sprouted grain.[44]

Individual phenolic compounds

Major phenolic compounds (FA, GA, CA, and QC) examined in ultrasound processed sorghum samples are shown in Table 3. Among them FA gave the highest level of 120.33 ± 2.07 μg/g, followed by QC, GA and CA having concentrations of 21.86 ± 0.12 μg/g, 16.29 ± 0.09 μg/g and 5.89 ± 0.02 μg/g, respectively. In comparison to control, 1 USD treatment (40% amplitude for 5 min) showed significant ($p \leq 0.05$) higher contents for phenolic compounds because of giving maximum germination percentage. Whereas 4 USD treatment (60% amplitude for 10 min) showed significantly ($p \leq 0.05$) lower contents due to decreased germination rate. Statistically significant ($p \leq 0.05$) changes were observed in contents of all phenolic compounds after ultrasonic stimulated germination of sorghum grains.

Table 3. Phenolic profile and IVPD % of ultrasound-treated sorghum samples.

Treatments	Gallic acid (μg/g)	Ferulic acid (μg/g)	Catechin (μg/g)	Quercetin (μg/g)	IVPD (%)
Control	15.65 ± 0.10^{abcd}	118.45 ± 2.05^{abcd}	5.58 ± 0.04^{ab}	21.43 ± 0.14^{abcd}	71.30 ± 0.50^{abcd}
US_1	16.29 ± 0.09^{abcd}	120.33 ± 2.07^{abcd}	5.89 ± 0.02^{abcd}	21.86 ± 0.12^{abcd}	74.40 ± 0.20^{abcd}
US_2	15.86 ± 0.11^{abcd}	118.81 ± 2.11^{abcd}	5.71 ± 0.01^{abcd}	21.61 ± 0.13^{abcd}	72.80 ± 0.30^{abcd}
US_3	15.39 ± 0.12^{abcd}	117.79 ± 2.13^{abcd}	5.60 ± 0.06^{abc}	21.25 ± 0.15^{abcd}	65.20 ± 0.10^{abcd}
US_4	15.11 ± 0.13^{abcd}	117.25 ± 2.08^{abcd}	5.51 ± 0.05^{abc}	21.09 ± 0.11^{abcd}	64.70 ± 0.50^{abcd}

All data were presented as mean ± standard deviation. ns: nonsignificant; values followed by different letters indicate statistically significant differences ($p \leq 0.05$); a: significant difference from other one treatment; ab: significant difference from other two treatments; abc: significant difference from other three treatments; abcd: significant difference from other four treatments.

Generally, phenolic acids are bound to hydrolysable tannins, lignins, cellulose, and proteins, which are mainly structural components of bran, building a protective layer to inner parts of the caryopsis.[44] Synthesis of new hydroxinamic acids (cumaric acid) during germination was the main reason behind the increment of QC, CA, and GA.[48] In other study conducted by[49] on *Vitis californica* seeds, germination improved GA content from 42.40 to 204.00 µg/g of fresh weight sample. Similarly, wheat grains germinated for 24–48 h showed increased level of FA (932.4 mg/g).[50] Likewise, soybean grains after six days of germination gave improved levels of CA and QC ranged from 38.38 to 159.95 µmol/100 g and 523.82 to 955.29 µmol/100 g, respectively.[51]

In-vitro protein digestibility

According to data given in Table 3, treatment having maximum germination rate resulted in higher IVPD values. However, for all processed sorghum sprouts IVPD % was ranged from 64.70 ± 0.50 to 74.40 ± 0.20%. Treatment US_I (40% amplitude for 5 min) gave significantly ($p \leq 0.05$) higher value of IVPD (74.40 ± 0.20%) while treatment 4 USD (60% amplitude for 10 min) gave significantly ($p \leq 0.05$) lower value (64.70 ± 0.50%). In comparison to control (71.30 ± 0.50%), the ultrasound processed germination significantly ($p \leq 0.05$) improved the protein digestibility. Degradation of antinutrients is generally occurred after processing which ultimately improves the IVPD of sorghum. Germination process enhances the activity of endogenous protease enzyme responsible to hydrolyze the stored protein. Endogenous protease increases the protein digestibility by partially hydrolyzing the stored protein, it also increases the soluble protein which linked with increase in IVPD.[52] These results were in accordance with previous study conducted by[41] describing the change in IVPD % of raw sorghum (51.2%) up to 65.03% after germination. In another study[31] observed IVPD value in raw sorghum ranged 50.94–52.09 % which was increased up to 70–78% through germination.

Principal component analysis

PCA was used for conformation of relation existing among the examined responses analyzed from processed samples of sorghum. Two main PCs characterizing various analyzed variables of ultrasound-processed sorghum sprouts have cumulative total variance of 86.533% (Figure 2). The first PC (PC1) and second PC (PC2) accounted for 74.724% and 11.809% variability in the data having the maximum eigenvalue of 11.209 and 1.771, respectively. While the remaining other PCs progressively produced smaller eigenvalues. Figure 3 shows the loading variables in the first two PCs (PC1 and PC2), representing the correlation between analyzed responses and components. PC1 was positively correlated to germination, sterols, DPPH, FRAP, ORAC, TPC, TFC, CA, QC, FA, GA, and IVPD, on the other side PC2 was negatively correlated to alkaloids, phytates, saponins and tannins. PCA results indicated that ultrasonic processing caused a good correlation of high germination in sorghum grains with improved levels of DPPH, FRAP, ORAC, TPC, TFC, GA, FA, CA, and QC along with sterols and IVPD, while ultrasound processing negatively affected the antinutritional compounds (alkaloids, phytates, saponins, and tannins). Results of PCA showed similarity with findings reported in above section regarding the quantitative analysis and characterization of phytochemicals profile after ultrasound processing.

Conclusion

This study characterizes ultrasonic processed sorghum sprouts based on phytochemical constituents, phenolic profile, radical scavenging activity and in vitro protein digestibility. The sonication processing at 40% amplitude for 5 min showed the improved results regarding all analyzed responses. The treated sprouts were rich in radical scavenging activity and phenolic profile along with higher percentage of IVPD. The resulted findings were strengthened through statistical analysis. Therefore, the findings suggested that ultrasonic processing could be an important tool in terms of enhanced germination for the cereal, which in result improved the phytochemical composition and radical scavenging activity of

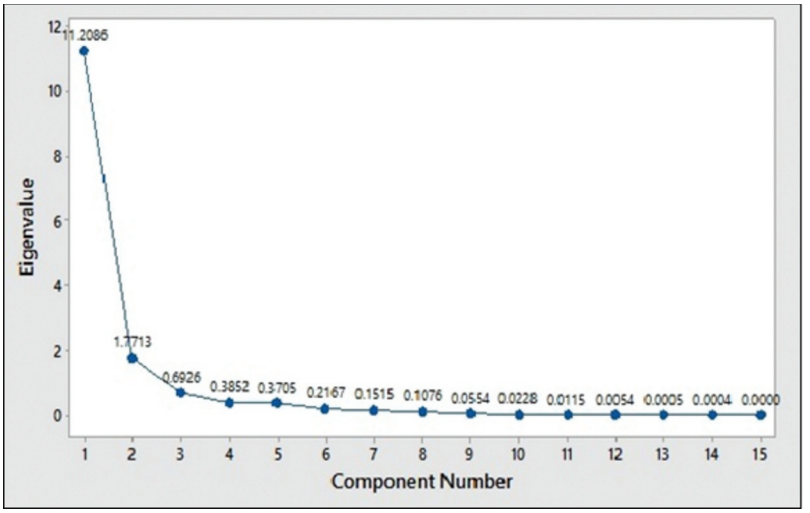

Figure 2. Eigenvalues for all principal components after PCA of ultrasound-treated sorghum samples.

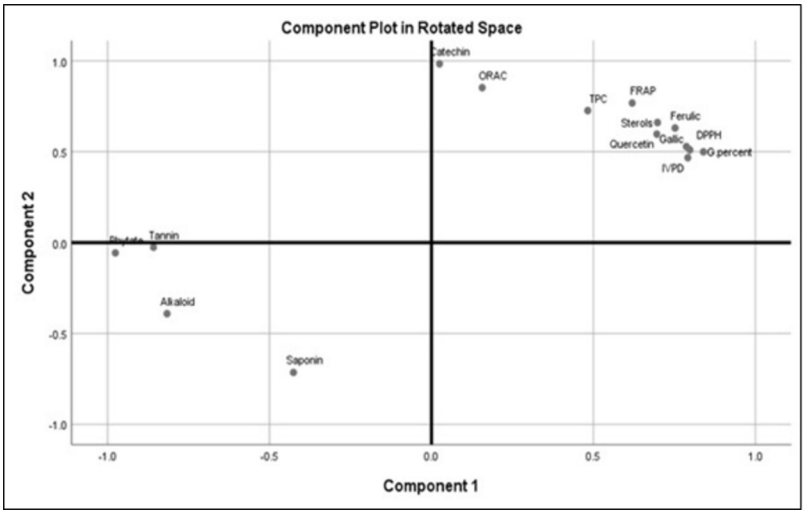

Figure 3. Loadings and biplot (PC1 × PC2) of scores after PCA of all studied responses of ultrasound-treated sorghum samples.

sorghum sprouts. Furthermore, these processed sprouts could be a significant source for improving nutritional value of different high-protein weaning/functional foods.

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