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Changes in Phytochemical Compounds and Antioxidant Activity of Two Irradiated Sorghum (*Sorghum bicolor* (L.) Monech) Cultivars during the Fermentation and Cooking of Traditional Sudanese Asida

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Abstract: This study investigated the chemical changes of phytochemicals and antioxidant activity during the preparation of traditional Sudanese asida prepared from gamma-radiated (1.0 and 2.0 kGy) flour of two sorghum cultivars (Tabat and Wad Ahmed). For both cultivars, the irradiation process significantly ($p < 0.05$) increased the total phenolic content and antioxidant activity of the raw flour, while it caused a significant reduction in total flavonoid content and tannin content. Traditional asida (fermented food) prepared from irradiated sorghum flour caused a significant reduction in TPC, TFC, and tannin content in both sorghum cultivars, while the antioxidant activities (DPPH, reducing power, and H_2O_2 scavenging) were significantly increased in both the Tabat (85.0%, 3.8 mg AAE/g, and 84.6%, respectively) and the Wad Ahmed (89.6%, 3.9 mg AAE/g, and 83.1%, respectively) sorghum cultivar grains, particularly in those processed from 2.0 kGy-irradiated flour. A positive high correlation was observed between gamma radiation (2.0 kGy) and the antioxidant activity of asida prepared from both cultivars. In conclusion, traditional fermented asida obtained from irradiated flour showed high antioxidant activity in both sorghum cultivars.

Keywords: sorghum; radiation; processing; fermented food; antioxidant capacity

1. Introduction

Sorghum (*Sorghum bicolor* L. Monech) is the world's fifth-most important cereal after maize, rice, wheat, and barley. The annual world production of sorghum is about 57 million tons [1]. It is the most important cereal crop in Sudan due to its high yield and resistance to drought stress. In addition to dietary fiber, sorghum contains many health-promoting components such as vitamins, minerals, and phytochemicals, which include phenolic compounds, total flavonoids, and tannin. These phytochemicals, such as tannins, phenolic acids, anthocyanin, phytosterols and policosanols, play a significant role in human health; therefore, the consumption of the whole grain may help to minimalize the risk of many human diseases. The utilization of these phytochemicals as nutraceuticals and in functional foods has been broadly reported on by several scientists Awika & Rooney [2], Dykes & Rooney [3], Fardet [4].

Free radical reactions, especially with the participation of oxidative stress, are involved in many biological processes that cause damage to lipids, proteins, membranes, and nucleic acids, thus giving rise to a variety of diseases [5]. Reactive oxygen species (ROS) have been implicated in a huge range of human diseases, such as atherosclerosis, inflammatory injury, cancer, and cardiovascular disease [6]. Additionally, it has been recognized as playing an important role in the initiation, propagation, and termination stages of autoxidation, which occurs in various foods and renders them useless. On the other hand, it has been reported that the antioxidant effect of plant products is mainly due to phenolic compounds, such as flavonoids, phenolic acids, tannins, and diterpenes [7,8]. Like other types of grains, sorghum is stored in many ways and for different periods of time, and it is susceptible to infestation by different types of store pests during storage. Medium doses of gamma radiation are one of the important physical treatments that can prevent sorghum from insect infestation, and that might improve the storability and quality characteristics of sorghum grains. In addition to controlling insects and pests, gamma radiation also improves the nutritional and functional characteristics of grains, as stated by Mahmoud et al. [9]. Therefore, gamma radiation can be used as an effective alternative postharvest method for preserving and extending the shelf life of sorghum and its products [10].

Sorghum is traditionally processed into flour by removing the fibrous, and often colored, pericarp and teste layers. The flour is used to prepare a variety of traditional foods and beverages. The processing methods vary from one locality to another, depending on the local customs, traditions, and culture, as well as food habits. Traditional Asida is the most commonly consumed dish in Sudan, and it is prepared by cooking sorghum flour in boiling water before it is consumed. The preparation process may result in significant changes in the chemical composition and bioactive compounds, which might affect the bio-accessibility and concentration of nutrients. Therefore, this study aims to investigate the effect of irradiation and the preparation process on the phytochemical properties of sorghum.

2. Materials and Methods

2.1. Sample Collection

Two cultivars of sorghum, Tabat (low-tannin) and Wad Ahmed (high-tannin), were obtained from the Agriculture Research Commission—Sudan. The grains were cleaned manually, freed from broken seeds and impurities, and then stored in a plastic bottle during the study.

2.2. Radiation Process

Sorghum grains were irradiated at the Kaila Irradiation Processing Unit, Sudanese Atomic Energy Corporation (SAEC, Khartoum, Sudan), using an experimental cobalt-60 gamma source (Nordion Gammacell 220 Excel) with doses of 1.0 and 2.0 kGy. About 250 g of sorghum grain was exposed to gamma rays with a dose rate of 1.30838 kGy/h at 25 °C and normal relative humidity. During the radiation process, three dosimeters (Gafchromic HD-810 film, International Specialty Products, NJ, USA, FAO/IAEA/USDA 2003) were included to measure the dose received in each batch. Furthermore, the radiation treatments were performed three times. Non-irradiated seeds served as a control. Irradiated and nonirradiated grain was cleaned and milled to pass through a 0.4 mm screen (Retsch, Haan, Germany) and kept in glass bottles at room temperature for analysis.

2.3. Fermentation

The fermentation of the sorghum flour was achieved following the traditional method after adding of starter from a previously fermented dough of the same nature, as described by Mahgoub, Ahmed, Ahmed & El Nazeer [11]. Sorghum flour was mixed with water in a ratio of 1:3 (*w*/*v*). The fermentation process was carried out at 37 °C for 24 h.

2.4. Cooking of the Traditional ASIDA

The Sudanese traditional asida was prepared traditionally from raw and fermented sorghum dough. The fermented dough was mixed with water and cooked in the water bath at 80 °C for 15 min until a thick paste formed after gelatinization. The dough was dried by an oven (Nabertherm, Lilienthal, Germany) at 60 °C, then ground, kept in plastic containers, and stored at −18 °C.

2.5. Extraction

The extraction of the phytochemical compound, as well as the antioxidants, was performed as described by Talhaoui et al. [12]. The samples were suspended in methanol (1:25, w/v) and shaken at 25 °C for 24 h. The extracts were then dried using a vacuum rotary evaporator (Shimadzu, Kyoto, Japan) and kept for further analysis.

2.6. Determination of Total Phenolic Content

The Folin-Ciocalteu's reagent method of Waterhouse [13] was used to measure the total phenolic content of the untreated and treated samples. An aliquot solution (20 µL) of dried methanolic extract (1 mg/mL) solution (1:10 w/v) was mixed with 1.58 mL of H_2O and 100 µL of the Folin-Ciocalteu reagent. Then, about 300 µL of Na_2CO_3 was added to the solution and then kept in the dark at 20 °C for 2 h. The absorbance was detected at 765 nm using an ultraviolet–visible (UV–VIS PD-303 UV, Jenway, Staffordshire, UK) spectrophotometer in contradiction to the blank solution. A calibration curve was performed using different concentrations of gallic acid (R_2 = 0.9672), and the results were expressed as mg gallic acid equivalents GAE/g sample dry weight (DW).

2.7. Determination of Total Flavonoid Content

The total flavonoid content of the extracts (1 mg/mL) was estimated as described by Kim, Jeong & Lee's [14] method. An amount of 1 mL of the extract was mixed with a 5% $NaNO_2$ solution (300 µL) and 10% aluminium chloride (300 µL) and incubated at 25 °C for 5 min. After incubation, 2 mL of 1 NaOH (1 M) was added. Directly, the volume was then completed to 10 mL with H_2O, and the absorbance was detected at 510 nm using an ultraviolet–visible (UV–VIS PD-303 UV) spectrophotometer. A calibration curve was performed using different concentrations of catechin (R_2 = 0.974). Total flavonoid content was stated as mg catechin equivalents (CE)/g sample (DW).

2.8. Tannin Determination

The tannin content of the sorghum samples was measured using the spectrophotometric vanillin-HCl method described by Price, Van Scoyoc & Butler [15]. Different concentrations of catechin were prepared to the standard curve, and tannin results were expressed as catechin equivalents (mg CE/100 g).

2.9. Antioxidant Activity Assays

2.9.1. DPPH Scavenging Assay

The scavenging activity of the diphenyl- 2-picrylhydrazyl (DPPH) radicals of the extracts was used to measure the antioxidant activity in the samples' extracts (1 mg/mL). About 0.9 mL of 50 mM Tris-HCl buffer (pH 7.4) and 0.1 mL of the extracts or deionized H_2O (as a control) were incubated at room temperature for 30 min, and then the absorbance was determined at 517 nm using an ultraviolet–visible (UV–VIS PD-303 UV) spectrophotometer [16]. The DPPH scavenging was calculated according to the following formula:

$$\text{DPPH scavenging } (\%) = \frac{(\text{Absorbance control} - \text{Absorbance sample})}{\text{Absorbance control}} \times 100 \qquad (1)$$

2.9.2. Reducing Power Assay

The total reducing power (RP) of the samples was determined following the method of Gülçin, Oktay, Küfrevioğlu & Aslan [17]. Briefly, 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1%) were added to the prepared concentrations of methanolic extract (1 mg/mL). The mixture was incubated at 50 °C for 20 min, and then 2.5 mL of trichloroacetic acid (10%) was added to the mixture and centrifuged at 1038 g for

10 min. About 2.5 mL of the supernatant was then mixed with 2.5 mL of H_2O and 0.1% ferric chloride (0.5 mL). The absorbance was detected at 700 nm using an ultraviolet–visible (UV–VIS PD-303 UV) spectrophotometer. The total reducing power inhibition is expressed as mg ascorbic acid equivalents (AAE)/g sample (DW).

2.9.3. Hydrogen Peroxide Scavenging Assay

The hydrogen peroxide scavenging assay (H_2O_2) was determined according to Jayaprakasha, Rao & Sakariah [18]. A solution of H_2O_2 (40 mM) was prepared in a phosphate buffer (pH 7.4). About 1 mg/mL extract was prepared and was mixed with 3 mL of phosphate buffer, and then 1 mL H_2O_2 (40 Mm) was added. After 10 min incubation, the absorbance was noted at 230 nm using an ultraviolet–visible (UV–VIS PD-303 UV) spectrophotometer. The H_2O_2 scavenging ability was calculated as follows:

$$\text{H}_2\text{O}_2 \text{ scavenging ability of samples } (\%) = \frac{(\text{Absorbance control } - \text{Absorbance sample})}{\text{Absorbance control}} \times 100 \quad (2)$$

2.10. Statistical Analysis

For the analytical data, mean values and standard deviation were reported. The data obtained were subjected to analysis of variance (ANOVA) using completely randomized factorial design (CFRD), and differences at the 5% level of significance were compared using the least significant difference test (LSD). Multivariate analysis was conducted using HJ-Biplot PCA algorithms as described in the XSTAT software package [19].

3. Results and Discussion

3.1. Total Phenolic Content

Table 1 shows the changes in the total phenolic content (TPC) during the preparation of traditional Sudanese asida prepared from raw and irradiated sorghum flour of the Tabat and Wad Ahmed cultivars. The TPC was found to be higher in the raw flour of the Wad Ahmed cultivar compared to that of the Tabat cultivar. Gamma radiation treatment at 1.0 and 2.0 kGy caused a significant ($p < 0.05$) increase in the TPC of both cultivars compared to raw, untreated flour. There was a progressive increase as the radiation dose was increased ($p < 0.05$). Gamma irradiation at a high dose (2.0 kGy) significantly increased the TPC compared to nonirradiated flour and flour treated at a 1.0 kGy gamma-ray dose. Similar to raw flour, in cooked samples, gamma irradiation significantly increased the TPC in both cultivars with an increase of the gamma-ray dose reaching the maximum values at a 2.0 kGy dose. The TPC of fermented and asida samples of both sorghum cultivars were reduced as the gamma-ray dose increased, reaching the minimum values at a 2.0 kGy dose. In the nonirradiated samples of both cultivars, asida had the highest levels of TPC, whereas the cooked samples showed the lowest level.

Table 1. The effect of gamma radiation and/or domestic processing on the total phenolic content (TPC mg GAE/g) of sorghum cultivars.

Cultivar	Processing	Radiation Dose		
		0 kGy	1.0 kGy	2.0 kGy
Tabat	Raw flour	8.9 ± 0.42 Bb	9.0 ± 0.24 Bb	9.4 ± 0.36 Aa
	Cooked	7.6 ± 0.23 Cc	8.6 ± 0.24 Bc	9.6 ± 0.32 Aa
	Fermented	8.7 ± 0.82 Ab	8.5 ± 0.24 Ac	7.7 ± 0.12 Bc
	Asida	9.8 ± 0.19 Aa	9.5 ± 0.39 Ba	8.8 ± 0.40 Cb
Wad Ahmed	Raw flour	9.7 ± 0.80 Bb	9.8 ± 0.83 Bc	10.2 ± 0.94 Ab
	Cooked	7.1 ± 0.01 Cc	9.8 ± 0.30 Bc	12.1 ± 0.01 Aa
	Fermented	9.5 ± 0.69 Bb	10.2 ± 0.54 Ab	9.2 ± 0.57 Bd
	Asida	11.2 ± 0.42 Aa	11.1 ± 0.17 Aa	9.8 ± 0.19 Bc

Values are means (±SD) of triplicate samples. Values followed by the same letter are not significantly different ($p < 0.05$) as assessed by LSD. Upper-case letters indicate significant differences among radiation doses, whereas lower-case letters indicate significant differences in processed samples.

Treatment Wad Ahmed cultivar samples with 1 kGy gamma irradiation showed the highest level of TPC in asida, followed by the fermented and cooked and the raw samples, while Tabat asida showed the highest TPC content, followed by raw flour, and the cooked and fermented sample. Increasing the radiation dose to 2.0 kGy for both cultivars revealed that that the cooked samples had the highest TPC levels, followed by raw flour, while the fermented sample showed the lowest level in both cultivars. Variyar et al. [20] stated that gamma radiation at doses up to 5 kGy increased the free phenolic content of soybeans. Also, Zaroug, Orhan, Senolv & Yagi [21] found that fermentation, cooking, and fermentation followed by cooking enhanced the TPC of sorghum grain. Increasing the TPC due to gamma irradiation treatment could be associated with the release of phenolic compounds from glycosidic components and the degradation of larger phenolic compounds into smaller ones [22]. Moreover, Ademo et al. [23] stated that gamma radiation is able to break the chemical bonds of polyphenols, resulting from the destructive oxidation reaction, which may lead to the releasing of the soluble polyphenols, particularly those with low molecular weights.

3.2. Total Flavonoid Content

The changes in the total flavonoid content (TFC) during the preparation of traditional Sudanese asida prepared from raw and irradiated sorghum flour of the Tabat and Wad Ahmed cultivars are described in Table 2. The statistical analysis showed significant differences ($p < 0.05$) in the TFC between irradiation doses and processing treatments (Table 2). The Tabat cultivar has a higher TFC compared to the Wad Ahmed cultivar in untreated samples. In the raw flour and processed samples of both cultivars, increasing the gamma radiation dose significantly reduced the TFC, with the maximum reduction being observed in samples subjected to a 2.0 kGy dose. The domestically processed (cooking, fermentation, and asida preparation) samples of both cultivars showed significantly lower TFC compared to raw samples, indicating that these processes negatively affected the TFC of the sorghum flour. Overall, both gamma irradiation and domestic processing treatments greatly reduced the TFC of both cultivars, with the highest reduction being found in the Tabat cultivar. The variable effects of the treatments on TFC among cultivars could be attributed to genetic and environmental factors.

Table 2. The effect of gamma radiation and/or domestic processing on the total flavonoid content (TFC mg CE/g) of sorghum.

Cultivar	Processing	Radiation Dose		
		0 kGy	**1.0 kGy**	**2.0 kGy**
Tabat	Raw flour	60.4 ± 1.52 Aa	52.4 ± 2.78 Ba	50.5 ± 2.53 Ca
	Cooked	45.4 ± 0.84 Ac	34.7 ± 1.10 Bc	31.8 ± 0.51 Cc
	Fermented	49.6 ± 2.42 Ab	38.4 ± 0.42 Bb	36.2 ± 0.23 Cb
	Asida	34.1 ± 3.79 Ad	31.8 ± 0.51 Bd	25.7 ± 1.82 Cd
Wad Ahmed	Raw flour	52.5 ± 0.05 Aa	50.2 ± 0.51 Ba	49.0 ± 0.51 Ba
	Cooked	41.7 ± 1.33 Ab	37.5 ± 1.51 Bb	34.0 ± 1.91 Cb
	Fermented	37.3 ± 1.33 Ac	37.0 ± 0.75 Ab	31.9 ± 1.23 Bc
	Asida	33.8 ± 1.01 Ad	29.8 ± 3.03 Bc	28.3 ± 0.51 Bd

Values are means (±SD) of triplicate samples. Values followed by the same letter are not significantly different ($p < 0.05$) as assessed by LSD. Upper-case letters indicate significant differences among radiation doses, whereas lower-case letters indicate significant differences in processed samples.

Decreases in TFC due to gamma radiation treatment were also described in several reports. Mun′im, Ramadhani, Chaerani, Amelia & Arrahman [24] found that radiation of *Peperomia pellucida* (L.) Kunth significantly reduced its TFC. Moreover, gamma irradiation of up to 5 kGy lowered the TFC of spices compared with control samples [25]. The reduction of TFC in irradiated sorghum grains might be due to the conformational change of the molecules and rearrangement in the matrix structure, which leads to more bound and less-extractable compounds [26]. Similar observations were also reported by [27].

3.3. Tannin Content

Table 3 shows the changes in the tannin content (TC) during the preparation of traditional Sudanese asida prepared from raw and irradiated sorghum flour of the Tabat and Wad Ahmed cultivars. The tannin content of sorghum flour was adversely affected by both gamma irradiation and domestic processing treatments (Table 3). Regardless of the treatment, the tannin content (2.3–5.0 mg/g) of the Wad Ahmed cultivar was significantly higher than that of the Tabat cultivar (0.4–1.4 mg/g). The tannin content of both cultivars was concomitantly reduced as the gamma radiation dose increased, reaching the minimum values at a 2.0 kGy dose. Similar findings were also stated by Hassan, Osman, Rushdi, Eltayeb & Diab [28] and Mahmoud et al. [9].

Table 3. The effect of gamma radiation and/or domestic processing on the tannin content (mg/g) of sorghum cultivars.

Cultivar	Processing	Radiation Dose		
		0 kGy	1.0 kGy	2.0 kGy
Tabat	Raw flour	1.4 ± 0.00 ^{Aa}	1.2 ± 0.03 ^{Aa}	0.8 ± 0.07 ^{Ba}
	Cooked	1.0 ± 0.07 ^{Ab}	0.5 ± 0.09 ^{Bb}	0.5 ± 0.00 ^{Bb}
	Fermented	0.6 ± 0.02 ^{Ac}	0.5 ± 0.09 ^{Abb}	0.4 ± 0.00 ^{Bb}
	Asida	0.9 ± 0.09 ^{Ab}	0.4 ± 0.07 ^{Bb}	0.4 ± 0.02 ^{Bb}
Wad Ahmed	Raw flour	5.0 ± 0.07 ^{Aa}	4.8 ± 0.07 ^{Aa}	4.3 ± 0.14 ^{Ba}
	Cooked	3.3 ± 0.51 ^{Ac}	3.2 ± 0.06 ^{ABc}	3.1 ± 0.07 ^{Bb}
	Fermented	3.8 ± 0.43 ^{Ab}	3.5 ± 0.27 ^{Bb}	3.1 ± 0.00 ^{Cb}
	Asida	2.7 ± 0.07 ^{Ad}	2.4 ± 0.17 ^{Bd}	2.3 ± 0.14 ^{Bc}

Values are means (±SD) of triplicate samples. Values followed by the same letter are not significantly different ($p < 0.05$) as assessed by LSD. Upper-case letters indicate significant differences among radiation doses, whereas lower-case letters indicate significant differences in processed samples.

The reduction of tannin content as affected by gamma radiation might be due to chemical degradation by the action of free radicals formed by the radiation. In addition, all domestic processing treatments significantly reduced the tannin content of both cultivars, with the highest reduction observed in Asida samples. On the other hand, it was noted that the reduction of tannin content is associated with the increase in the total phenolic content since the increase of total phenolic content is mainly caused by the degradation of tannins into a simple phenol compound [29].

3.4. Antioxidant Activity

The changes in antioxidant activity during the preparation of Sudanese traditional asida prepared from raw and irradiated sorghum flour of the Tabat and Wad Ahmed cultivars were determined using several assays of DPPH scavenging activity, and reducing power (RP) and hydrogen peroxide scavenging activity (H_2O_2) assays (Table 4). The DPPH radical scavenging activity of raw sorghum flour differed between the cultivars, with Tabat (56.0%) showing higher values than the Wad Ahmed (45.5%). The DPPH radical scavenging activity was increased concurrently with increases in the gamma-ray dose, reaching the highest values at 2.0 kGy. Interestingly, the highest increment was observed in the Wad Ahmed cultivar, which showed the maximum values of DPPH radical scavenging activity at a 2.0 kGy dose. Domestic processing methods also increased the DPPH radical scavenging activity of both cultivars, with the highest increment being found in the asida samples.

The reducing power (RP) scavenging activity assay also indicated that both gamma irradiation treatment and domestic processing increased the RP with different magnitudes. In raw samples, the RP values of the Wad Ahmed sorghum were higher than those of the Tabat ones; however, the irradiation of the samples at 2.0 kGy greatly increased the RP to the maximum values in the Tabat flour rather than the Wad Ahmed. Among the domestic processing treatments, the asida showed the highest values of RP in both the nonirradiated and irradiated samples of the Tabat and Wad Ahmed cultivars, followed by cooking, and then fermentation treatments. Overall, gamma irradiation at low doses

(1.0 or 2.0 kGy) followed by cooking or asida preparation could lead to increasing the RP scavenging activity of the products.

Table 4. The effect of gamma radiation and/or domestic processing on the antioxidant activity (DPPH scavenging, reducing power (RP), H_2O_2 scavenging) of sorghum cultivars.

Antioxidant Method	Cultivar	Processing	Radiation Dose		
			0 kGy	1.0 kGy	2.0 kGy
DPPH scavenging (%)	Tabat	Raw flour	56.0 ± 1.00 ^{Cc}	70.0 ± 2.61 ^{Bc}	74.0 ± 1.01 ^{Ad}
		Cooked	69.9 ± 1.47 ^{Cb}	75.9 ± 1.50 ^{Bb}	78.9 ± 3.35 ^{Ac}
		Fermented	71.1 ± 1.48 ^{Cb}	80.4 ± 1.50 ^{Ba}	82.9 ± 1.09 ^{Ab}
		Asida	77.5 ± 0.52 ^{Ca}	81.9 ± 3.48 ^{Ba}	85.0 ± 2.43 ^{Aa}
	Wad Ahmed	Raw flour	45.5 ± 0.98 ^{Cd}	67.9 ± 2.54 ^{Bc}	81.1 ± 0.06 ^{Ac}
		Cooked	67.9 ± 2.22 ^{Cb}	73.3 ± 2.54 ^{Bb}	82.2 ± 2.31 ^{Abc}
		Fermented	61.1 ± 1.39 ^{Cc}	73.3 ± 1.67 ^{Bb}	83.5 ± 0.75 ^{Ab}
		Asida	76.4 ± 2.08 ^{Ca}	81.7 ± 1.50 ^{Ba}	89.6 ± 0.46 ^{Aa}
RP (mg AAE/g)	Tabat	Raw flour	1.0 ± 0.03 ^{Bb}	2.7 ± 0.19 ^{Aa}	3.2 ± 0.08 ^{Ab}
		Cooked	2.4 ± 0.03 ^{Aa}	2.7 ± 0.01 ^{Aa}	3.2 ± 0.06 ^{Ab}
		Fermented	1.1 ± 0.02 ^{Ab}	1.4 ± 0.02 ^{Ab}	1.5 ± 0.03 ^{Ac}
		Asida	2.5 ± 0.01 ^{Ba}	2.8 ± 0.04 ^{ABa}	3.8 ± 0.02 ^{Aa}
	Wad Ahmed	Raw flour	1.5 ± 0.00 ^{Bc}	2.5 ± 0.03 ^{ABb}	2.9 ± 0.05 ^{Ac}
		Cooked	3.3 ± 0.03 ^{Aa}	3.4 ± 0.02 ^{Aa}	3.4 ± 0.02 ^{Ab}
		Fermented	2.1 ± 0.02 ^{Ab}	2.2 ± 0.00 ^{Ab}	2.9 ± 0.02 ^{Ac}
		Asida	3.5 ± 0.03 ^{Aa}	3.6 ± 0.04 ^{Aa}	3.9 ± 0.02 ^{Aa}
H_2O_2 scavenging (%)	Tabat	Raw flour	79.1 ± 1.09 ^{Ca}	81.5 ± 0.29 ^{Bab}	84.4 ± 0.65 ^{Aa}
		Cooked	79.3 ± 0.49 ^{Ca}	80.6 ± 1.09 ^{Bb}	83.8 ± 0.31 ^{Aab}
		Fermented	78.4 ± 0.78 ^{Ca}	81.7 ± 0.00 ^{Bab}	82.8 ± 0.00 ^{Ab}
		Asida	78.9 ± 0.78 ^{Ca}	82.1 ± 1.68 ^{Ba}	84.6 ± 0.00 ^{Aa}
	Wad Ahmed	Raw flour	76.9 ± 1.65 ^{Cb}	81.8 ± 0.49 ^{Ba}	82.8 ± 0. 43 ^{Aa}
		Cooked	79.0 ± 1.23 ^{Ca}	82.7 ± 0.78 ^{Ba}	83.6 ± 0.93 ^{Aa}
		Fermented	79.2 ± 1.82 ^{Ca}	82.4 ± 1.17 ^{Ba}	83.8 ± 0.74 ^{Aa}
		Asida	78.9 ± 2.14 ^{Ca}	81.6 ± 0.22 ^{Ba}	83.1 ± 0.36 ^{Aa}

Values are means (±SD) of triplicate samples. Values followed by the same letter are not significantly different ($p < 0.05$) as assessed by LSD. Upper-case letters indicate significant differences among radiation doses, whereas lower-case letters indicate significant differences in processed samples.

The percentage of H_2O_2 scavenging activity of both sorghum cultivars was concomitantly increased with increasing the gamma-ray dose to the highest values at 2.0 kGy, suggesting the positive effects of gamma rays on the H_2O_2 scavenging activity of sorghum flour of both cultivars. However, no effect of processing treatments on the H_2O_2 scavenging activity was seen.

Overall, the combined effect of gamma irradiation and domestic processing treatments indicated that both treatments enhanced the DPPH, RP, and H_2O_2 scavenging activity of both sorghum types. Also, the highest antioxidant activity values of asida from both cultivars were obtained from irradiated flour with 2.0 kGy. The increase in antioxidant activity of irradiated samples may be attributed to the free radicals generated by gamma rays, which may cause oxidative damage to the biomolecules present in the samples. It was noted that the increase in the antioxidant activity in the irradiated samples depended on the dose level of gamma rays. The application of high doses may cause a reduction in the antioxidant activity as reported by Variyar, Limaye & Sharma [30]; Moosavi, Hosseini, Dehghan & Jahanban-Esfahlan [31]; and Bhat, Sridhar & Tomita-Yokotani [32]. Furthermore, increases in the antioxidant activity of irradiated grains might be associated with increasing in the total phenolic content. It has been stated that the enhanced antioxidant activity could be attributed to the increase in phenolic compounds due to the hydrolysis of phenolic glycosides and the release of aglycone, which contributes to increased antioxidant activity [33].

3.5. Principle Component Analysis (PCA)

Figure 1a,b describe the HJ-biplot based on principle component analysis of the interactive changes on the bioactive compounds (TPC, TFC, and tannin) and antioxidant activities (DPPH, RP, and H_2O_2) during preparation of traditional Sudanese asida prepared from gamma-irradiated (1.0 and 2.0 kGy) sorghum grain cultivars Tabat (Figure 1a) and Wad Ahmed (Figure 1b) flour. In the figure, the contribution of the axes of the principal components, PC1 and PC2, was (62.96% and 19.17%) and (63.26% and 19.43%), which resulted in a high variability, 82.13% and 82.69%, of the plotted components for the Tabat and Wad Ahmed cultivars, respectively.

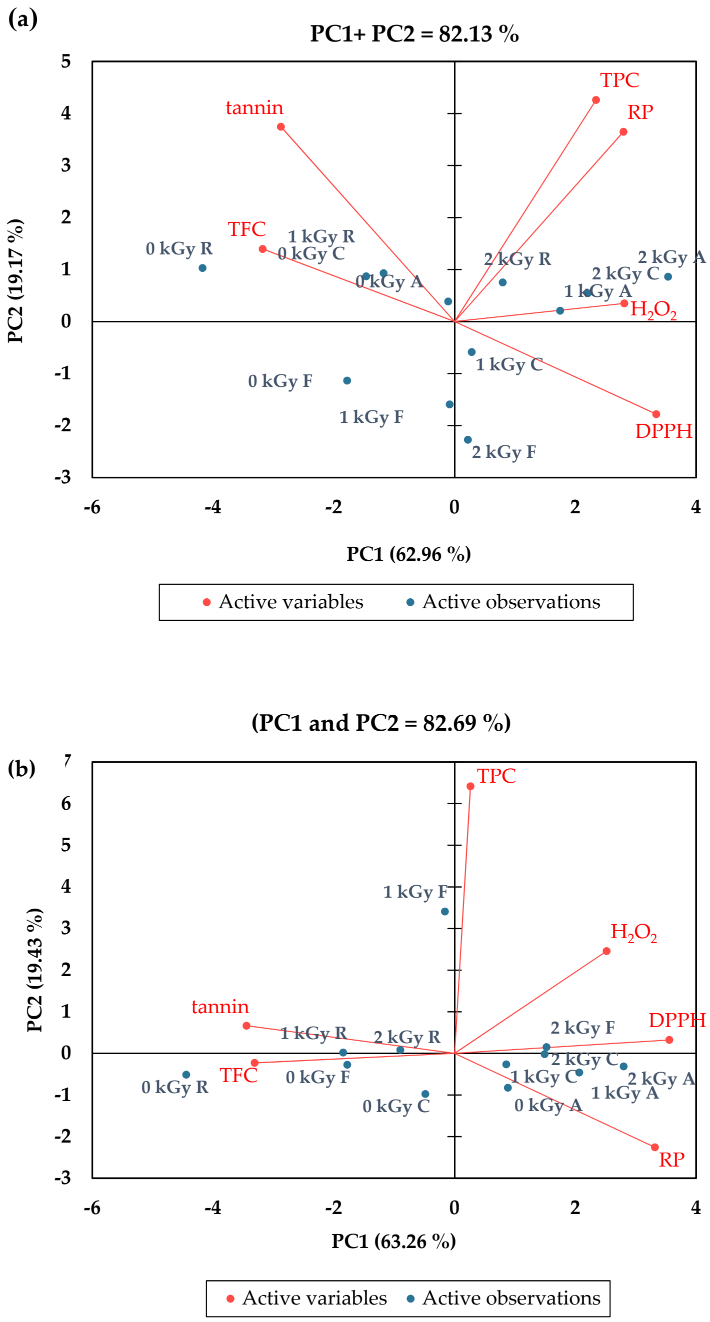

Figure 1. The HJ biplot based on principle component analysis of the interactive changes on the TPC, TFC, tannin, and DPPH, RP, and H_2O_2 during preparation of traditional Sudanese Asida prepared from gamma-irradiated (1.0 and 2.0 kGy), R: raw; C: cooking; F: fermentation; A: Asida, for sorghum grain cultivars Tabat (**a**) and Wad Ahmed (**b**).

In the biplot, the short distance between the treatment points indicated the similarity, whereas the cosine of the angle between the vector of the traits indicated the correlation between them, in which acute, obtuse, and right angles indicate positive, negative, and no correlation, respectively [34]. Accordingly, a strong positive correlation was evident between radiation treatment alone or radiation followed by fermentation or cooking, and the antioxidant capacity of the sorghum flour obtained from both the Tabat and Wad Ahmed cultivars. Consequently, nonradiated samples (0 kGy) for both cultivars (Figure 1a,b) showed greater values for tannin content and TFC, whereas the irradiated sorghum, particularly with 2.0 kGy, showed the highest correlation between treatments, content of phytochemical compounds, and antioxidant activity.

Overall, changes that occurred in the phytochemical compounds and antioxidant activity during the preparation of traditional Sudanese asida revealed that these properties could be improved by gamma radiation treatment at a dose of 2.0 kGy alone or followed by domestic processing treatments (fermentation or cooking).

4. Conclusions

In this study, there was considerable variation among the gamma radiation process and the domestic treatments. Generally, the obtained results revealed that the irradiation process with gamma radiation followed by asida could increase the bioavailability of phenolic, which subsequently might improve the antioxidant activity of sorghum grain of the Wad Ahmed and Tabat cultivars, respectively. Gamma radiation up to 2.0 kGy alone or followed by domestic processing (fermentation and cooking) has the potential to improve the antioxidant capacity of traditional Sudanese asida prepared from the Tabat and Wad Ahmed sorghum cultivars and thus contribute significantly to the health benefits associated with sorghum-based food consumption. However, further research is recommended in order to involve most of the traditional fermented food in food manufacturing.

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