

Impact of Gamma Irradiation on Physicochemical, Technological, Antioxidant and Microbiology Properties of Whole Sorghum Flours

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This study aimed to evaluate the effect of gamma irradiation (GI) on the physicochemical, technological, antioxidant and microbiological characteristics of different whole sorghum flours (WSF), as well as to characterize the profile of chemical constituents by paper spray mass spectrometry (PS-MS). The doses applied interfered in the staining characteristics of the evaluated flours, providing the darkening of the same. For the other physicochemical, technological, and antioxidant parameters, no significant influence of the process was observed. The fingerprint obtained in both ionization modes had not been influenced by irradiation, being flavonoids, phenylpropanoids, amino acids, benzoic acid derivatives, carboxylic acids, and sugars tentatively identified. In microbiological terms, there was a reduction of molds, yeasts, and *Bacillus cereus* in irradiated WSF compared to control (non-irradiated). Therefore, the application of low doses of gamma irradiation represents an advantageous alternative for the conservation of WSF and maintenance of bioactive compounds identified by the PS-MS technique.

Keywords: cereal technology, food preservation, paper spray mass spectrometry, PS-MS

Introduction

Originally from Central Africa, the sorghum (*Sorghum bicolor* L.) is a graminaceous cereal that, in terms of production, it is in the position of fifth most produced in the world, overcoming only by rice, wheat, corn, and barley. Sorghum has a lower production cost when compared to most other cereals, for this reason, it has aroused the interest of researchers in different areas of activity, mainly aiming at its introduction into human food.¹

Some specific genotypes are source of minerals as iron, phosphorus, magnesium, and zinc^{2,3} and others present significant concentrations of bioactive compounds, such as anthocyanins and tannins, which are components of

secondary metabolism of plants with antioxidant action, capable of kidnapping free radicals and promoting the improvement of human health.^{4,5}

Due to the benefits that sorghum can provide to human health, it is interesting to develop new products added from the flour of this cereal. However, due to its planting conditions and storage forms, sorghum becomes susceptible to microbial contamination and insect attack, requiring non-thermal conservation methods with a proposal to preserve its functional and nutritional composition, and gamma irradiation could be an alternative treatment.

Gamma irradiation (GI) is applied to improve food safety and stability. It is mainly used to reduce pathogenic and deteriorating microorganisms, besides providing disinfestation and inhibition of the proliferation of insects and agricultural pests in stored products.⁶

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There is little knowledge about the effects of gamma irradiation treatment on the antioxidant properties and phytochemical levels in flours compounds. Furthermore, it is known that this condition is dependent on the doses applied in each product and its sensitivity to exposure. The use of non-recommended irradiating doses can cause deformation in the physical structure of starch, interfering with the physicochemical and technological characteristics of these foods.⁷

Therefore, the objective of this study was to evaluate the influence of gamma irradiation on the physicochemical, antioxidant, technological, and microbiological characteristics of whole sorghum flours (WSF) of different genotypes, as well as to characterize the profile of chemical constituents by paper spray mass spectrometry (PS-MS).

Experimental

Reagents

The reagents of analytical grade acetone, ethanol, sulfuric acid, boric acid, and sodium hydroxide were acquired from Vetec (São Paulo, SP, Brazil). Folin-Ciocalteu, catechin, vanillin, ethanolamine, 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 6-hydroxy 2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were acquired from Sigma-Aldrich (St. Louis, MO, USA). Gallic acid and methanol were acquired from Neon (São Paulo, SP, Brazil). The chromatographic paper used was acquired from Whatman (Little Chalfont, Buckinghamshire, United Kingdom).

Sorghum sample

Sorghum grains of BRS 305 and SC 319 genotypes (brown pericarp and with tannin) and BR 501 (white pericarp and without tannin), belonging to the Embrapa Milho e Sorgo (Brazil) breeding program were used. Planting was carried out at the experimental field, in Sete Lagoas, Minas Gerais, Brazil, located at 19°27'54" south latitude and 44°14'79" west longitude. Approximately 3 kg of the grains of each sorghum cultivar were manually selected and milled three times in a stone mill model HM-1 (Hawos, Bad Homburg vor der Höhe, Germany) to obtain the WSF. The flours were stored in polyethylene plastic bags, protected from light, and stored under refrigeration (4 ± 1 °C).

Flours irradiation

The WSF samples were divided into three lots, which two were irradiated with GI at 3 kGy (IR3) and one at

5 kGy (IR5) doses at Nuclear Technology Development Center/National Nuclear Energy Commission (CDTN/CNEN), Belo Horizonte, Minas Gerais, Brazil. The samples were irradiated in cobalt-60 source irradiator multipurpose panoramic and secondary standard dosimeter PTW LS01 with a dose rate of 1212.57 Gy h⁻¹, at room temperature (25 ± 2 °C), at 29 cm from target material for 247 s.⁸ After irradiation, all samples were refrigerated (4 ± 1 °C). The irradiation doses were defined by a compilation of studies which used irradiation in different flours.

Physicochemical parameters

The L* (lightness), a* (-a* = greenness and +a* = redness), and b* (-b* = blueness and +b* = yellowness) values of the flour samples were determined using colorimeter model CM-2600D (Konica Minolta, Osaka, Japan). Titratable acidity (TA) was determined by titrating using NaOH 0.01 mol L⁻¹, with phenolphthalein as an indicator until it reaches pH 8.1 ± 0.2 , and the results were expressed as g of citric acid 100 g⁻¹ of flour, according to the Association of Official Analytical Chemists (AOAC).⁹ The pH was determined using a benchtop pH meter (Bante Instruments, 920, China), and the water activity was detected by direct measurement in a water activity analyzer (Aqualab Series 3-TE) applying the dew point sensor methodology (978.18).⁹

The determination of moisture, lipids, proteins, and ash was determined according to the AOAC methods.⁹ The carbohydrate content was obtained by difference, which protein, fat, ash, and moisture contents were reduced by 100%. The energy values (VE) of the flours were obtained by equation [VE = (protein × 4) + (fat × 9) + (carbohydrate × 4)].

Technological properties

Water, milk, and oil absorption capacities (WAC, MAC and OAC) and water solubility index (WSI) were determined according to the methods described by Becker *et al.*,¹⁰ with minor modifications. For the evaluation of the water, oil, and milk absorption capacities, samples (1 g) were mixed with water, oil, or milk separately (20 mL), posteriorly were agitated in an agitator water-bath and centrifuged (1372 × g). The supernatants with oil and milk were discarded, the supernatant with water was reserved, the final mass was measured and divided by the mass of the samples (g g⁻¹). The reserved supernatant was dehydrated for 12 h at 105 °C, and WSI was determined as the percent mass ratio between the dehydrated and wet samples.

Antioxidant properties

The analyses were performed in triplicate with three lab replicates for each one, on dry basis. The total anthocyanins were extracted with 1% HCl methanol solution, separated and quantified by ultra-high-performance liquid chromatography (Acquity UPLC[®] Class, Waters, Milford, MA, USA), in a C18 reverse-phase column (mobile phases: water with 4% formic acid and acetonitrile, with a flow of 1.0 mL min⁻¹ in gradient) and the results expressed in total anthocyanins µg g⁻¹ of the sample.¹¹

The total phenolics (TP) were determined using the Folin-Ciocalteu reagent method, according to Kaluza *et al.*¹² The absorbance of samples was read at 600 nm in a spectrophotometer (Hitachi U-1100 UV-Visible, Tokyo, Japan) and results were calculated and expressed as mg gallic acid equivalent (GAE) g⁻¹ sample. Condensed tannins (CT) were determined by the vanillin/HCl method, described by Price *et al.*,¹³ with absorbance read at 500 nm and results expressed as mg catechin equivalent (CE) g⁻¹ sample.

Antioxidant capacity (AC) was performed according to Awika *et al.*¹⁴ by the ABTS 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonate) radical cation, and the absorbance of samples was read at 734 nm in a spectrophotometer. The results were expressed as µmol Trolox g⁻¹.

Obtaining flours extract for paper spray mass spectrometry

The flours extracts (2.5 g of the sample, previously homogenized) were obtained according to the methodology described by Campelo *et al.*¹⁵ Initially, the WSF was mixed with 10 mL of methanol/water (50:50 v/v) and incubated for 1 h at room temperature (25 ± 2 °C). Then, the samples were centrifuged at 3493 × g for 22 min (Excelsa II, FANEM, 206BL, Brazil) and the supernatant was recovered. Subsequently, 10 mL of acetone/water (70:30, v/v) were added to the residue, and new centrifugation was performed using the same conditions. In the end, both supernatants were mixed, and distilled water was added until 25 mL volume.

Chemical profile by paper spray mass spectrometry

The chemical profile was evaluated using LCQ Fleet mass spectrometer (Thermo Scientific, San Jose, CA, USA) equipped with a paper spray ionization source. The analysis was performed in positive and negative ionization modes. An amount of 2.0 µL of the extract and 40 µL of methanol were applied on chromatographic paper, cut in triangular shape (equilateral-1.5 cm) and positioned in front of the equipment entrance.

The instrumental conditions of analysis were voltage of the PS-MS source equal to -3.0 kV (negative mode) and +4.0 kV (positive mode), a capillary voltage of 40 V, the voltage of tube lenses of 120 V, transfer tube temperature of 275 °C and mass range of 100 to 1000. To identify the compounds, a comparison was made between the mass/load ratios obtained in the study with the literature, through fragmentation by sequential mass spectrometry. The collision energy used to fragment the compounds ranged from 15 to 40 V and the mass spectra obtained were processed in the Xcalibur software.¹⁵

Microbiological analysis

To evaluate the hygienic-sanitary quality of the control and irradiated WSF, analyses of total coliforms at 35 °C and thermotolerant at 45 °C, *Salmonella* sp., *Bacillus cereus*, and molds and yeasts were performed according to the methodologies of the Manual of Microbiological Analysis Methods of Food and Water.¹⁶ The results obtained were compared with the maximum limits for the indicative flour sample, determined by the Technical Regulation of Microbiological Standards for Food, Resolution No. 12 of January 2001.¹⁷

Statistical design

The experiment was conducted according to a completely randomized design with three repetitions, with the treatments arranged in a 3 × 3 factorial scheme, factor 1-three levels of the flour (BRS 305, SC 319, and BR 501) and factor 2-three levels of the irradiation (control, 3 and 5 kGy). The data were collected in triplicate and submitted to variance analysis with two factors (ANOVA two-way), after verifying of the premises of normality and homogeneity by the Shapiro-Wilk and Levene tests. Subsequently, the Tukey's test for the comparison of means was applied, using the free software R version 3.6.3 and SISVAR (UFLA, Lavras, MG),¹⁸ with 5% probability ($p \leq 0.05$). The mass spectra were analyzed by the software Xcalibur (Thermo Fisher Scientific Inc.), and the PS-MS spectra in modes positive and negative were tabulated using a Microsoft Excel 2016 spreadsheet.¹⁹

Results and Discussion

Physicochemical parameters

There was no significant interaction between the two factors, thus, they were analyzed separately. The results of the colorimetric characteristics, pH, titratable acidity (TA)

and centesimal composition of WSF BRS 305, BR 501, and SC 319 control and irradiated are presented in Table 1.

To verify the influence of irradiation doses on color, samples were compared with the control. A significant effect of this process was observed in relation to the luminosity attribute for the three WSF IR5 evaluated ($p \leq 0.05$). There was a decrease in the mean values with darkening of the flours. In terms of chromaticity a^* and b^* , the effect of gamma irradiation was observed in WSF SC 319 for both attributes and WSF BR 501 for chroma b^*

($p \leq 0.05$). The treatments irradiated with 5 kGy showed the highest values for both a^* and b^* .

According to Youssef *et al.*,²⁰ this occurrence can be explained by the increase in the activity of polyphenol oxidases as a response to the stress that occurred during the irradiation process. These enzymes present in sorghum flour in contact with oxygen promote the oxidation of phenolic compounds and formation of quinones, responsible for the dark tint in products, causing decreased in the luminosity and interference in other color attributes.

Table 1. Physicochemical characteristics of whole sorghum flour (WSF) control and irradiated at 3 kGy (IR3) and irradiated at 5 kGy (IR5)

	Treatment	WSF BRS 305	WSF SC 319	WSF BR 501
Luminosity	control	70.39 ± 0.5 ^{ab}	63.14 ± 0.7 ^{ac}	77.24 ± 0.5 ^{aA}
	IR3	68.75 ± 0.8 ^{bb}	62.98 ± 0.6 ^{ac}	76.09 ± 0.6 ^{bA}
	IR5	68.30 ± 0.4 ^{bb}	61.66 ± 0.6 ^{bc}	76.23 ± 0.5 ^{bA}
a^*	control	6.13 ± 0.3 ^{ab}	8.89 ± 0.3 ^{bA}	4.01 ± 0.2 ^{ac}
	IR3	6.34 ± 0.2 ^{ab}	8.85 ± 0.3 ^{bA}	4.17 ± 0.3 ^{ac}
	IR5	6.36 ± 0.4 ^{ab}	9.32 ± 0.3 ^{aA}	4.19 ± 0.3 ^{ac}
b^*	control	13.06 ± 0.4 ^{ac}	13.95 ± 0.4 ^{bb}	17.48 ± 0.3 ^{bA}
	IR3	13.14 ± 0.4 ^{ac}	13.85 ± 0.5 ^{bb}	17.89 ± 0.4 ^{abA}
	IR5	13.18 ± 0.5 ^{ac}	14.72 ± 0.5 ^{ab}	18.17 ± 0.6 ^{aA}
pH	control	6.25 ± 0.1 ^{ab}	6.27 ± 0.0 ^{ab}	6.66 ± 0.1 ^{aA}
	IR3	6.24 ± 0.1 ^{ab}	6.23 ± 0.1 ^{ab}	6.67 ± 0.1 ^{aA}
	IR5	6.24 ± 0.0 ^{ab}	6.23 ± 0.1 ^{ab}	6.66 ± 0.1 ^{aA}
Titratable acidity / (g 100 g ⁻¹)	control	0.94 ± 0.0 ^{aA}	0.90 ± 0.1 ^{aA}	0.48 ± 0.1 ^{ab}
	IR3	0.92 ± 0.1 ^{aA}	0.89 ± 0.0 ^{aA}	0.49 ± 0.0 ^{ab}
	IR5	0.93 ± 0.0 ^{aA}	0.88 ± 0.1 ^{aA}	0.50 ± 0.1 ^{ab}
Moisture / %	control	11.96 ± 0.5 ^{ac}	13.55 ± 0.1 ^{aA}	12.85 ± 0.3 ^{ab}
	IR3	11.49 ± 0.4 ^{ab}	13.37 ± 0.4 ^{aA}	12.79 ± 0.1 ^{aA}
	IR5	11.85 ± 0.4 ^{ab}	12.92 ± 0.3 ^{aA}	12.79 ± 0.1 ^{aA}
Protein / %	control	12.16 ± 0.3 ^{aA}	12.05 ± 0.3 ^{aA}	12.23 ± 0.1 ^{aA}
	IR3	11.81 ± 0.3 ^{aA}	12.01 ± 0.6 ^{aA}	11.88 ± 0.0 ^{aA}
	IR5	11.75 ± 0.2 ^{aA}	11.78 ± 0.2 ^{aA}	11.81 ± 0.2 ^{aA}
Lipids / %	control	3.41 ± 0.3 ^{aA}	3.33 ± 0.3 ^{aA}	3.83 ± 0.1 ^{aA}
	IR3	3.25 ± 0.6 ^{aA}	2.29 ± 1.7 ^{aA}	4.06 ± 0.3 ^{aA}
	IR5	3.17 ± 0.2 ^{aA}	2.99 ± 0.6 ^{aA}	3.50 ± 0.4 ^{aA}
Ash / %	control	1.24 ± 0.0 ^{ab}	1.81 ± 0.1 ^{aA}	1.77 ± 0.0 ^{abA}
	IR3	1.18 ± 0.1 ^{ac}	1.64 ± 0.2 ^{ab}	1.83 ± 0.1 ^{aA}
	IR5	1.21 ± 0.1 ^{ac}	1.76 ± 0.1 ^{aA}	1.54 ± 0.0 ^{bb}
Carbohydrates / %	control	71.23 ± 0.5 ^{aA}	69.26 ± 0.6 ^{ab}	69.33 ± 0.1 ^{ab}
	IR3	72.27 ± 0.5 ^{aA}	70.67 ± 1.1 ^{ab}	69.44 ± 0.4 ^{ab}
	IR5	72.02 ± 0.6 ^{aA}	70.54 ± 0.3 ^{ab}	70.36 ± 0.3 ^{ab}
Water activity	control	0.64 ± 0.00 ^{ac}	0.71 ± 0.00 ^{abA}	0.68 ± 0.00 ^{ab}
	IR3	0.65 ± 0.01 ^{ab}	0.70 ± 0.01 ^{aA}	0.68 ± 0.01 ^{aA}
	IR5	0.63 ± 0.02 ^{ac}	0.71 ± 0.00 ^{abA}	0.66 ± 0.02 ^{ab}

Mean ± standard deviation. Averages followed by different lowercase letters in the column for each evaluated attribute showed a significant difference between radiation doses ($p < 0.05$). Averages followed by different capital letters in the line present a significant difference between the WSF evaluated for the same intensity ($p < 0.05$).

Bashir *et al.*²¹ showed decrease in the luminosity and increase in the parameter b^* in wheat flours irradiated with 5 and 10 kGy, compared to the control sample. According to the authors, the change in these parameters may be justified by the caramelization of the monosaccharides present in the cereal, which were affected during the irradiation process.

When comparing whole sorghum flours, each other, the lowest luminosity was verified for the WSF BRS 305 and SC 319 samples compared to the BR 501 sample, showing higher values for parameter a^* (redness) and lower values for parameter b^* (yellowing). These significant differences ($p \leq 0.05$) can be explained by the chemical and nutritional composition of the sorghum grains of the different genotypes with and without the presence of pigmented 'testa' that were evaluated in this study. These results corroborate the fact that sorghum grains BRS 305 and SC 319 present high concentrations of anthocyanins, condensed tannins, and other flavonoids, responsible for the dark and reddish coloration of the evaluated flours.⁸

There was no significant effect of gamma irradiation in the pH ($p > 0.05$). Regarding the TA, in this study there was no significant difference concerning the applied dosages ($p > 0.05$). WSF BR 501 sample showed higher pH and lower acidity values when compared to WSF BRS 305 and SC319 sample ($p \leq 0.05$), but all of them can be considered flours of low acidity because they have a pH higher than 4.5.

No significant changes were observed in all centesimal parameters ($p > 0.05$) regarding the irradiation dosages applied to WSF BR 305 and SC 319 samples. However, it was observed reduction ($p \leq 0.05$) in the ash content of the WSF BR 501 sample submitted to 5 kGy of GI.

In general, plants and their grains when exposed to stress conditions may present mechanisms involving physiological, biochemical, and morphological alterations. The mineral content is significantly affected due to the sensitivity of specific genotypes and the effect caused by stress on their centesimal composition.³

Although the irradiation process did not influence the moisture parameter, significant differences were observed between of the WSF samples, with higher values for the SC 319 sample (12.92 to 13.55%) compared to the BRS 305 and BR 501 samples. Lipid contents showed a variation from 3.17 to 3.41% for WSF BRS 305 sample, 2.29 to 3.33% for SC 319 sample and 3.50 to 4.06% for BR 501 sample ($p > 0.05$).

The quality and integrity of proteins in cereals significantly influence their nutritional value. According to Silva *et al.*²² high doses of radiation (30 kGy) do not affect protein content, however, increase the free amino acids, isoleucine, tyrosine, valine, and alanine contents.

The water activity represents the free water content present in the food, influencing microbial growth and therefore in the shelf life, and as changes in moisture, texture, aroma, and color parameters. The samples analyzed showed low water activity, ranging from 0.63 to 0.71; WSF BRS 305 presented lower (W_a) indices compared to the SC 319 and BR 501 samples ($p \leq 0.05$). It is known that foods with lower water activity, especially dry, sugary, and frozen foods, have the indirect effects of radiolytic products minimized.⁷

The calculated average energy contents (BRS 305 = 364.48 kcal 100 g⁻¹, SC 319 = 354.24 kcal 100 g⁻¹, BR 501 = 360.88 kcal 100 g⁻¹) were relatively similar to other sorghum flours, such as genotype BRS 309 (325 kcal 100 g⁻¹) and BR 007B (320 kcal 100 g⁻¹).²

Technological properties

Table 2 shows the results of the technological properties for the evaluated WSF samples. The absorption and solubility indices in water did not undergo significant changes ($p > 0.05$) in the WSF studied samples, regardless of the applied irradiation dosage.

The WAC in products of plant origin may be mainly related to the high fiber content present in these foods, especially the whole ones. Specific sorghum genotypes are considered rich in dietary fibers, as in the case of BRS 305² which was evaluated in this study. Mean values of WAC between 1.49-4.72 g g⁻¹ are considered fundamental for raw materials used in food production, especially viscous ones, such as soups and gravies.²³ Given the above, sorghum flour has potential for insertion and enrichment of these products.

WSI is a parameter that measures the degree of total degradation of the starch granule, and this indicator ranged from 3.59 to 4.61% for the WSF. There was no significant difference between the results found for the different sorghum genotypes evaluated ($p > 0.05$). Flours with high WSI values can be used in products that require low temperatures to be prepared (instantaneous) or as a component in the formulation of desserts and sauces, which require ingredients with greater solubility in water.²⁴

All evaluated flours presented values close to 1.8 g g⁻¹, indicating low OAC. This property is of great importance in food formulations, such as cake doughs since oils improve the taste and sensation of food in the mouth.²⁵

The mean MAC of the WSF were from 3.15 to 3.18 g g⁻¹ for BRS 305 sorghum; 2.97 to 3.00 g g⁻¹ for genotype SC 319 and 2.68 to 2.71 g g⁻¹ for genotype BR 501. For the addition of flours in viscous products such as bases for instant puddings, and children's foods, MAC is an important parameter related to the homogenization of the

Table 2. Technological properties: water absorption capacity (WAC), water solubility index (WSI), oil absorption capacity (OAC), and milk absorption capacity (MAC) of whole sorghum flour (WSF) control and irradiated

	Treatment	WSF BRS 305	WSF SC 319	WSF BR 501
WAC / (g g ⁻¹)	control	2.69 ± 0.09 ^{abA}	2.63 ± 0.09 ^{abA}	2.06 ± 0.12 ^{abB}
	IR3	2.72 ± 0.06 ^{abA}	2.62 ± 0.12 ^{abA}	2.06 ± 0.16 ^{abB}
	IR5	2.73 ± 0.14 ^{abA}	2.71 ± 0.10 ^{abA}	2.07 ± 0.10 ^{abB}
WSI / %	control	3.81 ± 1.56 ^{abA}	4.55 ± 1.41 ^{abA}	3.96 ± 0.99 ^{abA}
	IR3	3.59 ± 0.79 ^{abA}	4.61 ± 0.82 ^{abA}	3.79 ± 1.14 ^{abA}
	IR5	3.95 ± 0.76 ^{abA}	4.15 ± 1.14 ^{abA}	3.89 ± 0.57 ^{abA}
OAC / (g g ⁻¹)	control	1.81 ± 0.08 ^{abA}	1.88 ± 0.13 ^{abA}	1.78 ± 0.13 ^{abA}
	IR3	1.83 ± 0.08 ^{abA}	1.80 ± 0.11 ^{abA}	1.75 ± 0.05 ^{abA}
	IR5	1.79 ± 0.03 ^{abA}	1.85 ± 0.05 ^{abA}	1.71 ± 0.08 ^{abA}
MAC / (g g ⁻¹)	control	3.18 ± 0.11 ^{abA}	3.00 ± 0.30 ^{abA}	2.71 ± 0.14 ^{abA}
	IR3	3.17 ± 0.09 ^{abA}	2.99 ± 0.24 ^{abA}	2.68 ± 0.31 ^{abA}
	IR5	3.15 ± 0.12 ^{abA}	2.97 ± 0.27 ^{abA}	2.69 ± 0.23 ^{abA}

Mean ± standard deviation. Averages followed by different lowercase letters in the column for each evaluated attribute showed a significant difference between radiation doses ($p < 0.05$). Averages followed by different capital letters in the line present a significant difference between the WSF evaluated for the same intensity ($p < 0.05$). IR3: samples irradiated at 3 kGy; IR5: samples irradiated at 5 kGy.

product, avoiding, for example, technological problems such as syneresis.¹⁰ There was no significant difference between the WSF for the attribute's absorption capacities in oil and milk and neither influence of gamma radiation on these parameters ($p > 0.05$).

Antioxidant properties

The results regarding the characterization of antioxidants of WSF samples are presented in Table 3.

Regarding antioxidant compounds, significant

differences were not observed between irradiated flours and control sample for none of the evaluated parameters ($p > 0.05$). Results that are interesting from a nutritional point of view due to the importance of these compounds in food, since they can kidnap free radicals and contribute to the prevention of chronic non-communicable diseases, such as certain types of cancers, diabetes mellitus, and kidney diseases.^{5,11}

These results corroborate the data previously published by Mustapha *et al.*,²⁶ which studied Tunisian millet flours submitted to gamma irradiation (1, 2, 3, and 5 kGy) and

Table 3. Antioxidant properties: total anthocyanins (ANT), condensates tannins (CT), total phenolic (TP), and antioxidant capacity by the ABTS method (AC) of whole sorghum flour (WSF) control and irradiated

	Treatment	WSF BRS 305	WSF SC 319	WSF BR 501
ANT	control	27.34 ± 5.9 ^{abB}	55.41 ± 5.0 ^{abA}	6.53 ± 1.4 ^{acC}
	IR3	28.17 ± 1.4 ^{abB}	51.14 ± 5.5 ^{abA}	8.03 ± 1.7 ^{acC}
	IR5	26.04 ± 2.4 ^{abB}	46.99 ± 3.7 ^{abA}	6.15 ± 1.7 ^{acC}
CT	control	71.39 ± 5.0 ^{abA}	45.16 ± 5.8 ^{abB}	ND
	IR3	66.22 ± 5.0 ^{abA}	43.81 ± 3.6 ^{abB}	ND
	IR5	70.66 ± 5.5 ^{abA}	42.60 ± 4.2 ^{abB}	ND
TP	control	21.29 ± 2.4 ^{abA}	18.85 ± 0.9 ^{abA}	0.90 ± 0.1 ^{abB}
	IR3	21.07 ± 2.1 ^{abA}	18.47 ± 0.2 ^{abA}	0.89 ± 0.1 ^{abB}
	IR5	21.08 ± 2.5 ^{abA}	18.42 ± 0.5 ^{abA}	0.95 ± 0.0 ^{abB}
AC	control	325.39 ± 10.9 ^{abA}	304.83 ± 18.8 ^{abB}	12.55 ± 4.9 ^{acC}
	IR3	323.08 ± 13.4 ^{abA}	286.68 ± 5.8 ^{abB}	13.69 ± 5.9 ^{acC}
	IR5	326.41 ± 4.9 ^{abA}	286.19 ± 8.8 ^{abB}	11.97 ± 3.6 ^{acC}

Mean ± standard deviation. Averages followed by different lowercase letters in the column for each evaluated attribute showed a significant difference between radiation doses ($p < 0.05$). Averages followed by different capital letters in the line present a significant difference between the WSF evaluated for the same intensity ($p < 0.05$). IR3: samples irradiated at 3 kGy; IR5: samples irradiated at 5 kGy; ND: not detected.

did not identify a significant effect of ionizing radiation on the total phenolic content and antioxidant capacity by the ABTS method. The influence of radiation on TP content may be related to geographic and environmental conditions, plant type, and physical state of the sample, in addition to extraction conditions, temperatures applied during evaluation, and radiation doses.²⁶

Proanthocyanidins or CT vary between dimers and oligomers, and this difference in the structure of molecules may be related to their degradation in the application of gamma radiation. In the case of large structures, such as oligomers and insoluble phenolic compounds, these are connected to the cell wall, a process that hinders intrastructural breakdown of components, enabling their maintenance in food, even after low doses applied.²⁷

Comparatively, when analyzing the WSF for all treatments, as expected, the flours from genotypes BRS 305 and SC 319 presented concentrations of AC, ANT and TP higher than those found in BR 501 flour. BRS 305 and SC 319 have pigmented 'testa' and high levels of tannins which is the component that most raises the values of total phenolic and antioxidant capacity in the samples. BR 501 does not present pigmented 'testa' and condensed tannins content were below to the limit of detection of the methodology employed ($p \leq 0.05$).

PS-MS chemical profile

Examples of spectra (PS-MS) of chemical profiles of sorghum flours, control and irradiated in negative mode are shown in Figure 1.

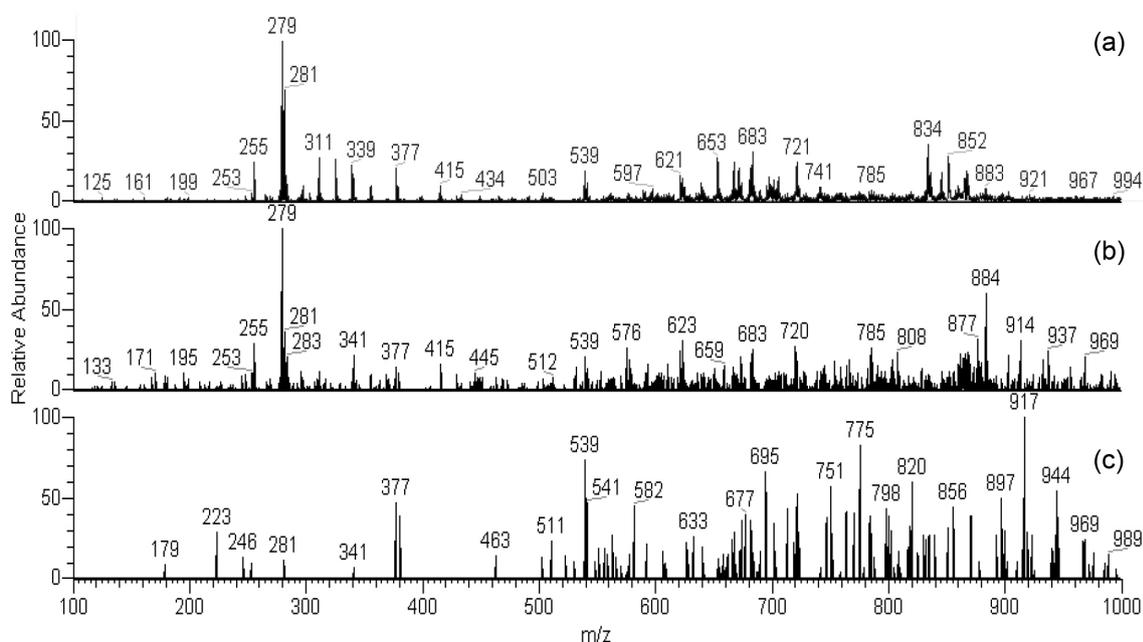


Figure 1. Representation of (a) (–) PS-MS of a sample BRS 305, (b) (–) PS-MS of a sample SC 319, (c) (–) PS-MS of a sample BR 501.

The irradiation process did not influence the detection of WSF chemical compounds in the BR 501, BRS 305, and SC 319 genotypes. In positive and negative ionization modes, 38 compounds were provisionally identified, shown in Table 4. Positive ionization mode was able to tentatively identify 13 compounds, whereas negative ionization provided insight into 25 compounds in the extracts. The chemical profile is composed mainly of flavonoids (58.0%), benzoic acid derivatives and phenylpropanoids (21.0%) and other compounds (21.0%) including sugars, amino acids, and organic acids.

Flavonoids

The flavonoid group contains the highest number of substances identified. Flavonoids are secondary metabolites and the largest group of natural phenolics present in plants. The ingestion of these compounds is related to the preventive action against certain non-communicable chronic diseases, with anti-inflammatory, antioxidant, anticarcinogenic potential, and cellular protection capacity.⁵

In WSF, the ion of m/z 289 in negative mode was possibly identified as catechin present in WSF BRS 305 and SC 319 samples. Most phenolic compounds are present in the outer layers of the grain, with pigmented 'testa'. This condition (Table 5) is verified in the SC 319 and BRS 305 genotypes, which are associated with the presence of condensed tannins.¹⁵

Other flavonoids with important bioactive action were also possibly identified by ions: (m/z 301) diosmetin, (m/z 317) 3-*O*-methylquercetin, (m/z 463) apigenin mono-*C*-glycoside and *C*-hexosyl-chrysoeriol, (m/z 595)

Table 4. Compounds determined by paper spray ionization mass spectrometry (PS-MS) positive and negative modes in extracts of whole sorghum flour (WSF) control and irradiated

Tentative identification	<i>m/z</i>	ID	MS/MS	Reference
Glycine betaine	118	[M – H] ⁺	59	Campelo <i>et al.</i> ¹⁵
Histidine	156	[M – H] ⁺	110	Campelo <i>et al.</i> ¹⁵
L-Arginine	175	[M + H] ⁺	129	Loyola <i>et al.</i> ²⁸
Diosmetin	301	[M – H] ⁺	258, 286	Rodrigues <i>et al.</i> ²⁹
3- <i>O</i> -Methylquercetin	317	[M – H] ⁺	301; 274; 273	Campelo <i>et al.</i> ¹⁵
Sucrose	381	[M – H] ⁺	201; 219	Campelo <i>et al.</i> ¹⁵
β-Sitosterol	397	[M + H – H ₂ O] ⁺	243	Loyola <i>et al.</i> ²⁸
Apigenin mono- <i>C</i> -glycoside	463	[M – H] ⁺	361, 349, 337	Campelo <i>et al.</i> ¹⁵
<i>C</i> -Hexosyl- chrysoberyl	463	[M – H] ⁺	445, 427, 409	Campelo <i>et al.</i> ¹⁵
Kaempferol rutinoside	595	[M – H] ⁺	287	Rodrigues <i>et al.</i> ²⁹
Myricetin-hexose	597	[M – H] ⁺	319	Campelo <i>et al.</i> ¹⁵
Rutin	611	[M – H] ⁺	618, 606, 602	Nascimento <i>et al.</i> ³⁰
<i>O,C</i> -Ramosyl-glycosyl-apigenin <i>O,O</i> dihexoside	903	[M – H] ⁺	739, 271	Campelo <i>et al.</i> ¹⁵
Malic acid	133	[M – H] ⁻	115	Loyola <i>et al.</i> ²⁸
Protocatechuic acid	153	[M – H] ⁻	109	Campelo <i>et al.</i> ¹⁵
ρ-Coumaric acid	165	[M – H] ⁻	119, 147	Campelo <i>et al.</i> ¹⁵
Gallic acid	169	[M – H] ⁻	125	Loyola <i>et al.</i> ²⁸
Caffeic acid	179	[M – H] ⁻	135	Campelo <i>et al.</i> ¹⁵
Citric acid	191	[M – H] ⁻	173, 111, 87	Loyola <i>et al.</i> ²⁸
Ferulic acid	193	[M – H] ⁻	134, 149, 178	Campelo <i>et al.</i> ¹⁵
Hexose	215	[M – H] ⁻	71, 89, 179	Loyola <i>et al.</i> ²⁸
Apigeninidin	253	[M – H] ⁻	179, 209, 225	Campelo <i>et al.</i> ¹⁵
Luteolin	285	[M – H] ⁻	165, 167, 175	Campelo <i>et al.</i> ¹⁵
Catechin	289	[M – H] ⁻	125, 179, 205	Campelo <i>et al.</i> ¹⁵
Quercetin	301	[M – H] ⁻	179, 151	Campelo <i>et al.</i> ¹⁵
Ellagic acid	301	[M – H] ⁻	257, 229	Loyola <i>et al.</i> ²⁸
Epigallocatechin	305	[M – H] ⁻	125, 179, 219	Campelo <i>et al.</i> ¹⁵
Isorhamnetin	315	[M – H] ⁻	300	Campelo <i>et al.</i> ¹⁵
Coumaryl-hexoside	325	[M – H] ⁻	163	Loyola <i>et al.</i> ²⁸
Chlorogenic acid	353	[M – H] ⁻	191, 179	Loyola <i>et al.</i> ²⁸
6- <i>C</i> -Pentosyl luteolin	417	[M – H] ⁻	399, 290, 269	Nascimento <i>et al.</i> ³⁰
Apigenin-6- <i>C</i> -hexoside	431	[M – H] ⁻	370, 361, 318	Nascimento <i>et al.</i> ³⁰
Naringenin hexoside II	433	[M – H] ⁻	271, 313, 415	Campelo <i>et al.</i> ¹⁵
Swertisin	445	[M – H] ⁻	207	Nascimento <i>et al.</i> ³⁰
Isoorientin	447	[M – H] ⁻	429, 419, 330	Nascimento <i>et al.</i> ³⁰
Isoscoparin	461	[M – H] ⁻	425, 407, 363	Nascimento <i>et al.</i> ³⁰
Procyanidin B3	577	[M – H] ⁻	451, 425, 407	Loyola <i>et al.</i> ²⁸
Quercetin di-deoxyhexose	755	[M – H] ⁻	609	Campelo <i>et al.</i> ¹⁵

kaempferol rutinoside, (*m/z* 597) myricetin-hexose, (*m/z* 611) rutin and (*m/z* 903) *O,C*-ramnosyl-glycosyl-apigenin *O,O* dihexoside in mode positive.

Additionally, in mode negative the compounds were (*m/z* 253) apigeninidin, (*m/z* 285) luteolin, (*m/z* 301) quercetin, (*m/z* 305) epigallocatechin, (*m/z* 315) isorhamnetin, (*m/z* 417) 6-*C*-pentosyl luteolin, (*m/z* 445)

swertisin, (*m/z* 447) isoorientin, (*m/z* 461) isoscoparin, (*m/z* 577) procyanidin B3 and (*m/z* 755) quercetin di-deoxyhexose. The flavanone naringenin and flavone apigenin was possibly identified by ions (*m/z* 433) and (*m/z* 431), respectively. These compounds were also found in sorghum with brown and red pericarps in the study of Campelo *et al.*¹⁵ when evaluating extruded sorghum flours

Table 5. Compounds identified by paper spray ionization mass spectrometry (PS-MS) positive and negative modes in extracts of whole sorghum flour (WSF) control and irradiated

Compound	[+][-]	BR 501			BRS 305			SC 319		
		CTL	IR3	IR5	CTL	IR3	IR5	CTL	IR3	IR5
Flavonoids										
Apigeninidin	-				x	x	x	x	x	x
Luteolin	-				x	x	x	x	x	x
Catechin	-				x	x	x	x	x	x
Quercetin	-				x	x	x	x	x	x
Epigallocatechin	-				x	x	x	x	x	x
Isorhamnetin	-	x	x	x	x	x	x	x	x	x
6-C-Pentosyl luteolin	-				x	x	x	x	x	x
Apigenin-6-C-hexoside	-				x	x	x	x	x	x
Naringenin hexoside II	-				x	x	x	x	x	x
Swertisin	-				x	x	x	x	x	x
Isoorientin	-				x	x	x	x	x	x
Isoscoparin	-	x	x	x	x	x	x	x	x	x
Procyanidin B3	-				x	x	x	x	x	x
Quercetin di-deoxyhexose	-				x	x	x	x	x	x
Diosmetin	+	x	x	x	x	x	x	x	x	x
3-O-Methylquercetin	+				x	x	x	x	x	x
Apigenin mono-C-glycoside	+				x	x	x	x	x	x
Kaempferol rutinoside	+	x	x	x	x	x	x	x	x	x
C-Hexosyl- chrysoberyl	+	x	x	x	x	x	x	x	x	x
Myricetin-hexose	+				x	x	x	x	x	x
Rutin	+				x	x	x	x	x	x
O,C-Ramnosyl-glycosyl-apigenin O,O dihexoside	+				x	x	x	x	x	x
Benzoic acid derivatives										
Protocatechuic acid	-				x	x	x	x	x	x
Ellagic acid	-				x	x	x	x	x	x
Gallic acid	-				x	x	x	x	x	x
Phenylpropanoids										
Caffeic acid	-	x	x	x	x	x	x	x	x	x
Ferulic acid	-				x	x	x	x	x	x
p-Coumaric acid	-				x	x	x	x	x	x
Chlorogenic acid	-	x	x	x	x	x	x	x	x	x
Coumaryl-hexoside	-	x	x	x	x	x	x	x	x	x
Other compounds										
Malic acid	-				x	x	x	x	x	x
Citric acid	-	x	x	x	x	x	x	x	x	x
Hexose	-	x	x	x	x	x	x	x	x	x
Glycine betaine	+	x	x	x	x	x	x	x	x	x
Histidine	+	x	x	x	x	x	x	x	x	x
L-Arginine	+	x	x	x	x	x	x	x	x	x
Sucrose	+	x	x	x	x	x	x	x	x	x
β-Sitosterol	+	x	x	x	x	x	x	x	x	x

CTL: sample control; IR3: samples irradiated at 3 kGy IR5: samples irradiated at 5 kGy.

samples by PS-MS and in the study by Pinheiro *et al.*³¹ when evaluating six sorghum genotypes with and without tannin by high-performance liquid chromatography with diode array detection.

According to Nascimento *et al.*,³⁰ plants of Poaceae family are very interesting economically, as they represent some of the largest culture for human and animal consumption. Therefore, the presence of flavonoids in sorghum flours represents an important healthy potential because compounds such as rutin and quercetin, for example, present pharmacological properties and act against free radicals responsible for degenerative diseases.²⁹

Benzoic acid derivatives and phenylpropanoids

All phenylpropanoids were possibly identified in the negative ionization mode, being represented by ρ -coumaric acid (m/z 165), ferulic acid (m/z 193), coumaryl-hexoside (m/z 325) and chlorogenic acid (m/z 353). Caffeic acid was identified in this study by the ion (m/z 179), this compound was also found by Rodrigues *et al.*²⁹ in malt bagasse flours. The benzoic acid derivatives exhibit high antioxidant capacity and may provide benefits to human health, such as protocatechuic acid (m/z 153), ellagic acid (m/z 301) and gallic acid. Campelo *et al.*¹⁵ observed the presence of protocatechuic, ρ -coumaric, caffeic and ferulic acids in extruded sorghum flours.

Other compounds

According to the fragmentation profile, the signals of m/z 381 (positive mode) and m/z 215 (negative mode) were recognized as sugars. Loyola *et al.*²⁸ using the same method also tentatively identified sugars in peel banana flour with the same transition MS/MS. Regarding the amino acids and phytosterols contents, β -sitosterol (m/z 397), histidine (m/z 156), L-arginine (m/z 175), and a quaternary ammonium compound (m/z 118, glycine-betaine) were possibly identified. Regarding organic acids, malic (m/z 133) and citric (m/z 191) acids prevailed, that also found in work by Rodrigues *et al.*²⁹ when analyzing cassava flours.

Microbiological analysis

The Resolution No. 12¹⁷ establishes for starches, flours, and powdered or flaked cornmeal, a most probable number (MPN) maximum limit of 5×10^2 MPN g^{-1} for coliforms at 45 °C; 3×10^3 CFU (colony forming unit) g^{-1} for *B. cereus* and absence of *Salmonella* sp. 25 g of product.¹⁷ Although the Brazilian legislation does not establish a limit for fungi and yeasts in flours, it is relevant to evaluate the presence of these microorganisms because they are frequent contaminants in these types of foods.

Molds and yeasts were identified with values of 3.43 log CFU g^{-1} in WFS BR 501 (control sample), 3.35 log CFU g^{-1} in WFS BRS 305 (control sample), and 3.39 log CFU g^{-1} in WFS SC 319 (control sample). After irradiation (3 and 5 kGy), there was a reduction of 3 log¹⁰ CFU g^{-1} in both genotypes evaluated for molds and yeasts.

Gamma irradiation proved to be an efficient technology in reducing the initial load of these microorganisms. The presence of typical and atypical colonies of *B. cereus* was verified in non-irradiated flours (control). These results can be explained because these microorganisms are commonly found in soil, air, plantations and are usually present in cereal grains. After irradiation (3 and 5 kGy), there was a reduction of 2 log¹⁰ CFU g^{-1} for *B. cereus*.

The application of GI at low dosages (≤ 3 kGy) in food products has been instigated to reduce the contamination by bacteria and extend the life of products. However, many microorganisms can adapt to the applied radiation, allowing them to survive ordinarily lethal exposures, which require the higher dosages. Spore-producing bacteria such as *B. cereus* can quickly repair modified deoxyribonucleic acid (DNA), generally becoming more resistant than vegetative cells or non-spore-producing bacteria.³²

According to Henriksen and Maillie³³ irradiation causes a direct effect on microorganisms since, during this process, there is the formation of ions and free radicals that can interact with proteins and DNA molecules, causing the mutation of microbial cells or even cell death. With the damage of the structural and functional units of the organism, they become unable to adapt to the environment.

The effect of gamma irradiation on microbial inactivation in sorghum flours was also evaluated by Mukisa *et al.*³⁴ The authors found that a dose of 10 kGy had a significant effect, reducing three logs on the count of total aerobic microorganisms in malted flours. Total coliforms, thermotolerant coliforms (45 °C), and *Salmonella* sp. were not detected in the WSF samples evaluated.

As well as chemical disinfection and heat treatment, the contaminant microbiota present in food also significantly influences the efficiency of gamma irradiation treatment that is, the larger the number of cells, the lower the process efficiency. However, it is emphasized that non-thermal technologies, such as irradiation, can inactivate microorganisms at room temperature, thus avoiding undesirable effects that heat can cause in the nutritional value, flavor, and color of products.³⁵

Conclusions

The application of irradiation at doses of 3 and

5 kGy showed to be an effectively method to reduce microorganisms in tannin and non-tannin sorghum flours, without altering functional, technological, and physicochemical constitution of the sample, interfering only in instrumental color parameters of samples. The fingerprint obtained in both ionization modes had not been influenced by radiation process. Therefore, the application of low doses of gamma irradiation represents an advantageous alternative for conserving the analyzed flours and allows its safe application in products intended for human consumption.

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Author Contributions

V.T.V.C. was responsible for conceptualization, formal analysis, investigation, data curation, writing original draft; D.F.D. for conceptualization, formal analysis, investigation, data curation, writing original draft; V.A.V.Q. for writing - review and editing, supervision, resources; A.L.C.C.R. for formal analysis and writing - review; M.C.C.M. for formal analysis; J.O.F.M. for formal analysis, writing - review and editing; R.A. for formal analysis; A.A.F. for conceptualization, writing - review and editing, resources, supervision; I.C.A.L. for conceptualization, writing - review and editing, resources, supervision; C.A.F. for conceptualization, writing - review and editing, resources, supervision.

References

- Rumler, R.; Bender, D.; Schönlechner, R.; *J. Cereal Sci.* **2022**, *104*, 103425. [Crossref]
- Martino, H. S. D.; Tomaz, P. A.; Aguiar Moraes, E.; da Conceição, L. L.; Oliveira, D. S.; Queiroz, V. A. V.; Rodrigues, J. A. S.; Pirozi, M. R.; Pinheiro-Sant'ana, H. M.; Ribeiro, S. M. R.; *Rev. Inst. Adolfo Lutz* **2012**, *71*, 337.
- Paiva, C. L.; Queiroz, V. A. V.; Simeone, M. L. F.; Schaffert, R. E.; de Oliveira, A. C.; da Silva, C. S.; *Food Chem.* **2017**, *214*, 400. [Crossref]
- Taleon, V.; Dykes, L.; Rooney, W.; Rooney, L.; *J. Food Compos. Anal.* **2014**, *34*, 178. [Crossref]
- Queiroz, V. A. V.; Oliveira, K. G.; Paiva, C. L.; Carlos, L. A.; Menezes, C. B.; Barros, F. A. R.; Pinheiro-Sant'ana, H. M.; Anunciação, P. C.; *Rev. Bras. Milho e Sorgo* **2018**, *17*, 522. [Crossref]
- Gowthamraj, G.; Jubeena, C.; Sangeetha, N.; *Radiat. Phys. Chem.* **2021**, *183*, 109403. [Crossref]
- Tezotto-Uliana, J. V.; Silva, P. P. M.; Kluge, R. A.; Spoto, M. H. F.; *Rev. Virtual Quím.* **2015**, *7*, 267. [Crossref]
- Correia, V. T. V.; D'Angelis, D. F.; dos Santos, A. N.; Ronchetti, E. F. S.; Queiroz, V. A. V.; Figueiredo, J. E. F.; Silva, W. A.; Ferreira, A. A.; Fante, C. A.; *LWT* **2022**, *154*, 112672. [Crossref]
- Association of Official Analytical Chemistry (AOAC); *Official Methods of Analysis*, 19th ed.; AOAC: Washington D.C., USA, 2012.
- Becker, F. S.; Eifert, E. C.; Soares Jr., M. S.; Tavares, J.-A. S.; Carvalho, A. V.; *Cienc. Agrotec.* **2014**, *38*, 367. [Crossref]
- Awika, J. M.; Rooney, L. W.; Waniska, R. D.; *Food Chem.* **2004**, *90*, 293. [Crossref]
- Kaluza, W. Z.; Mcgrath, R. M.; Roberts, T. C.; Schroder, H. H.; *J. Agric. Food Chem.* **1980**, *28*, 1191.
- Price, M. L.; van Scoyoc, S.; Butler, L. G.; *J. Agric. Food Chem.* **1978**, *26*, 1214. [Crossref]
- Awika, J. M.; Rooney, L. W.; Wu, X.; Prior, R. L.; Cisneros-Zevallos, L.; *J. Agric. Food Chem.* **2003**, *51*, 6657. [Crossref]
- Campelo, F. A.; Henriques, G. S.; Simeone, M. L. F.; Queiroz, V. A. V.; Silva, M. R.; Augusti, R.; Melo, J. O. F.; Lacerda, I. C. A.; Araújo, R. L. B.; *J. Braz. Chem. Soc.* **2020**, *31*, 788. [Crossref]
- Silva, N.; Junqueira, V. C. A.; Silveira, N. F. A.; Taniwaki, M. H.; dos Santos, R. F. S.; Gomes, R. A. R.; *Manual de Métodos de Análise Microbiológica de Alimentos*; Varela: São Paulo, BR, 2010.
- Agência Nacional de Vigilância Sanitária (ANVISA); Resolução RDC No. 12: *Regulamento Técnico sobre os Padrões Microbiológicos para Alimentos*; Ministério da Saúde: Brasília, DF, 2001. [Link] accessed in July 2022
- Ferreira, D. F.; *Cienc. Agrotec.* **2011**, *35*, 1039. [Crossref]
- Redmond, W. E.; *Excel 2016*; Microsoft, USA, 2016.
- Youssef, B. M.; Asker, A. A.; El-Samahy, S. K.; Swailam, H. M.; *Food Res. Int.* **2002**, *35*, 1. [Crossref]
- Bashir, K.; Swer, T. L.; Prakash, K. S.; Aggarwal, M.; *LWT - Food Sci. Technol.* **2017**, *76*, 131. [Crossref]
- da Silva, R. C.; Pino, L. M.; Spoto, M. H. F.; D'Arce, M. A. B. R.; *Food Sci. Technol. (Campinas)* **2010**, *30*, 406. [Crossref]
- Aletor, O.; Oshodi, A. A.; Ipinmoroti, K.; *Food Chem.* **2002**, *78*, 63. [Crossref]
- Leonel, M.; Freitas, T. S.; Mischan, M. M.; *Sci. Agric.* **2009**, *66*, 486. [Crossref]
- Ocloo, F. C. K.; Okyere, A. A.; Asare, I. K.; *Radiat. Phys. Chem.* **2014**, *103*, 9. [Crossref]
- Mustapha, M. B.; Bousselmi, M.; Jerbi, T.; Bettaieb, N. B.; Fattouch, S.; *Food Chem.* **2014**, *154*, 230. [Crossref]

27. de Camargo, A. C.; Regitano-d'Arce, M. A. B.; Gallo, C. R.; Shahidi, F.; *J. Funct. Foods* **2015**, *12*, 129. [Crossref]
28. e Loyola, A. C. F.; Silva, V. M. D.; Silva, M. R.; Rodrigues, C. G.; dos Santos, A. N.; Melo, J. O. F.; Augusti, R.; Fante, C. A.; *J. Braz. Chem. Soc.* **2021**, *32*, 953. [Crossref]
29. Rodrigues, C. G.; Silva, V. D. M.; e Loyola, A. C. F.; Silva, M. R.; Augusti, R.; Melo, J. O. F. M.; Carlos, L. A.; Fante, C. A.; *Quim. Nova* **2022**, *45*, 403. [Crossref]
30. do Nascimento, C. D.; de Paula, A. C. C. F. F.; de Oliveira Jr., A. H.; Mendonça, H. O. P.; Reina, L. C. B.; Augusti, R.; Figueiredo-Ribeiro, R. C. L.; Melo, J. O. F.; *Plants* **2021**, *10*, 1617. [Crossref]
31. Pinheiro, S. S.; Cardoso, L. M.; Anunciação, P. C.; Menezes, C. B.; Queiroz, V. A. V.; Martino, H. S. D.; Della Lucia, C. M.; Sant'ana, H. M. P.; *J. Food Compos. Anal.* **2021**, *100*, 103892. [Crossref]
32. Lima Filho, T.; Teixeira, L. J. Q.; da Rocha, C. T.; Ferreira, G. A. M.; de Souza, M. C.; *Bol. Centro Pesqui. Process. Aliment.* **2012**, *30*, 243. [Crossref]
33. Henriksen, T.; Maillie, H. D.; *Radiation & Health*; CRC Press/ Taylor & Francis: UK, 2002.
34. Mukisa, I. M.; Muyanja, C. M. B. K.; Byaruhanga, Y. B.; Schüller, R. B.; Langsrud, T.; Narvhus, J. A.; *Radiat. Phys. Chem.* **2012**, *81*, 345. [Crossref]
35. Cabo Verde, S.; Trigo, M. J.; Sousa, M. B.; Ferreira, A.; Ramos, A. C.; Nunes, I.; Junqueira, C.; Melo, R.; Santos, P. M. P.; Botelho, M. L.; *J. Toxicol. Environ. Health* **2013**, *76*, 291. [Crossref]

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