CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

GAMMA RADIATION EFFECTS ON PHYSICOCHEMICAL, MICROBIOLOGICAL AND ANTIOXIDANT PROPERTIES OF BLACK RICE (*Oryza Sativa* L.) FLOUR DURING STORAGE

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https://doi.org/10.34302/crpjfst/2019.11.3.14

Article history:
Received:
28 March 2019
Accepted:
29 August 2019

Keywords:
Bioactive compounds;
Irradiation;
Chemometric;
Pigmented rice.

ABSTRACT

Black rice has been categorised as a functional food because it contains high amounts of bioactive compounds. The effects of gamma radiation (at 0, 1, 2 and 3 kGy doses) on the free and bound total phenolics, antioxidant activity, and physicochemical and microbiological properties of black rice flour samples during storage were evaluated. The chemometric approach made it possible to observe the effects of irradiation and storage time on the samples. Regarding bioactive compounds, with the exception of the bound phenolic fractions the 3 kGy dose showed the highest values at time 0. At 120 days there was a decrease in these levels for all the samples, although the irradiated samples were most stable at the end of storage. Regarding the attribute of colour, the irradiation slightly modified all the parameters; in terms of microbiological analysis there was no growth of microorganisms at the end of storage.

1.Introduction

Rice (*Oryza sativa* L.) is one the most important staple foods worldwide; it is considered to be an important source of energy for populations from both developed and developing countries. In the last few years global rice production has been higher than consumption, with an annual production of almost 500 million metric tonnes (milled rice basis) and a harvested area of above 160 million hectares (USDA, 2018).

Even though white rice (non-pigmented) is the most cultivated variety, coloured (pigmented) rice has received increasing attention from researchers due to its antioxidant properties and bioactive compounds in the outer layer of the caryopsis (Finocchiaro et al., 2010; Ryu and Koh, 2017). Rice contains higher levels of phenolic acids (ferulic, caffeic and coumaric acid), than other cereal grains (Ti et al., 2014). Pigmented rice is a source of proanthocyanidins, anthocyanins and flavonoids, as well as tocopherols and vitamins (Hao et al., 2015; Rodríguez-Pérez et al., 2015).

In particular, black rice contains anthocyanins and phenolic acids, which is categorized as free phenolic acids or bound phenolic acids (Ito and Lacerda, 2019). Free phenolic acids are extractable mainly by using an 80% methanol solution. As the bound phenolic acids are present in insoluble form, a strong alkali is added to the residue obtained after the extraction of free phenolics (Alves et al., 2016). These bioactive compounds, which are present in different amounts in rice varieties, are associated with reducing the risks of developing several chronic diseases such as cancer, diabetes, cardiovascular disease (Hao et al., 2015).

It has known that irradiation is a powerful processing technology to inactivate microorganisms and insects to ensure hygienic quality, as well as extending the shelf life of foods. Gamma radiation is considered to be a physical and environmentally friendly technology that is widely acknowledged for its various applications in the food industry (Kumar et al, 2017).

Understanding the effects of radiation by using chemometrics could provide significant information concerning the bioactive compounds that are present in pigmented rice. This is fundamental to develop new food products and to improve human health. Therefore, the purpose of this research was to investigate the effects of gamma radiation on the free and bound total phenolics, antioxidant activity and physicochemical and microbiological properties of black rice flour during storage by using chemometrics.

2. Materials and methods

2.1. Sample preparation and radiation treatment

All reagents were of the highest grade commercially available. The biodynamic black rice used in the experiments was cultivated according to Demeter biodynamic standards (Demeter International, 2012) and purchased in a local supermarket in the city of Curitiba, Paraná, Brazil. The black rice flour was obtained according to the methodology used by Ito et al. (2018).

All samples were irradiated at doses of 0, 1, 2 and 3 kGy at a 0.221 kGy h^{-1} dose rate in a ^{60}Co gamma irradiator (Gammacell Excell 220 - MDS Nordion, Ottawa, Canada). The irradiation treatments were performed in the Centre for Nuclear Energy in Agriculture at the University of São Paulo, Brazil (CENA/USP).

2.2. Physicochemical analysis

The colour attributes lightness (L^*), redness (a^*), and yellowness (b^*) of the black rice flour samples were determined using a HunterLab MiniScan EZ colourimeter (Reston, VA, USA.), as described by Ito et al., 2016.

The moisture was determined gravimetrically in an air oven at 105 °C (AOAC 935.29). The water activity (A_w) was measured with a digital A_w meter (Aqualab®, USA).

2.3. Extraction and phenolic composition

2.3.1. Extraction of free and bound phenolics

The free phenolics were extracted using the method reported by Sumczynski et al. (2016) with minor modifications. Briefly, the sample (0.5 g) of black rice flour was treated twice with 8 mL of 80% aqueous methanol using an ultrasound device (47 kHz, 130 W, Ultrasonic Cleaners, Vernon Hills, USA) at 35 °C for 1 h. The supernatants were combined after centrifugation at 5000 *g* (HIMAC CR-GII, Hitachi, Ibaraki, Japan) for 30 min at room temperature and their pH was adjusted to 4.5−5.5.

To extract the bound phenolics the residues of black rice flour obtained above were re-washed using 20 mL of water. After removing the water the samples were blended twice with 20 mL of 4 M NaOH for 2 h in an ultrasonic device. The mixture was then adjusted. After centrifugation, the supernatant was used as the bound phenolic extract.

2.3.2. Phenolic composition

The free and bound phenolic fractions were determined by colorimetric analysis using Folin-Ciocalteu reagent, as described by Singleton, and Rossi (1965), with modifications. The absorbance was recorded at a wavelength of 720 nm after one hour of reaction and the measurements were performed using a microplate reader (Epoch microplate spectrophotometer, Synergy-BioTek, Winooski, VT, USA). The total phenolic content was calculated as the sum of the free and bound phenols. The results were expressed as mg of gallic acid equivalents (GAE) per gram of black rice flour (mg GAE g^{-1}).

The total anthocyanins content (TAC) was determined according to the pH differential spectrophotometric method adapted for microplate (Giusti & Wrolstad, 2001). Firstly, two solutions were prepared: one buffer at pH 1.0 (0.025 mol L^{-1} KCl water buffer, acidified with HCl) and another buffer at pH 4.5 (0.4 mol L^{-1} sodium acetate water buffer, acidified with HCl). Subsequently, using the method reported by Shao et al. (2014), aliquots of the extract were transferred to a 96-well microplate and 290 µL of corresponding buffer (pH 1.0 and 4.5) and allowed to equilibrate for 30 min. The absorbance was measured at 520 and 700 nm. The TAC was expressed as mg cyanidin-3-glucoside equivalent (C3G) per g of black rice flour.

The anthocyanin extracts were also analysed using HPLC, as previously described by Pedro et al. (2016), with minor changes. The analysis was performed in an Alliance 2695 separation module (Waters, Milford, MA, USA) coupled with photodiode detector (model PDA 2998, Waters, Milford, MA, USA), a quaternary pump and an auto sampler. Firstly, the extracts were filtered then 10 µL of sample was injected into the HPLC system. The separation was then performed using a XTerra® MS C18 column with dimensions of 4.6 × 250 mm, 5 µm (Waters, Milford, MA, USA) kept at 20 °C with a flow of 1.0 mL min^{-1}. The black rice flour anthocyanins were identified and quantified at 515 nm using a DAD detector by comparing the retention time with the standard of cyanidin-3-glucoside in the concentration range from 0.01 to 0.25 mg L^{-1} ($y = 25482x - 20152$; $R^2 = 0.999$).

The total flavonoids (TF) were quantified by UV–Vis spectrophotometry (Shimadzu UV-1800) at 374 nm as described by Pedro et al, (2016). The total flavonoid content was expressed as mg quercetin equivalents (QE) per g of black rice flour.

2.4. In *vitro* antioxidant activity

The total antioxidant potential of the black rice flour extracts was determined by assessing the ABTS scavenging activity of the extracts using the method described by Re et al. (1999), with modifications. The absorbance was recorded at a wavelength of 734 nm after the solution had been allowed to stand in the dark for 30 min. The results were compared with a standard curve (trolox 100–1000 µmol L^{-1}) and expressed in µmoL trolox equivalent per g of black rice flour (µmol TE g^{-1}).

The DPPH radical scavenging activity of the extracts was determined according to the method of Brand Williams et al. (1995), with minor modifications. The absorbance was recorded at a wavelength of 517 nm after the solution had been allowed to stand in the dark for 30 min. A standard curve (DPPH = 0.001 × absorbance; $R^2 = 0.995$; $p < 0.001$) was plotted using different concentrations of trolox (0.1 – 300 µmol L^{-1}). The results were expressed in µmol trolox equivalents per gram of sample (µmol TE g^{-1}).

2.5.Microbiological analysis

In order to evaluate the microbiological quality of the black rice flour, the following analyses were performed: *Bacillus cereus*, thermotolerant coliforms and *Salmonella* sp, according to the National Health Surveillance Agency, Collegiate Board Resolution No. 12 (ANVISA, 2001). In this study, the samples were included in the food group "flour, pasta and bakery products (industrialised and packaged) and similar" with a maximum tolerance for an indicative sample being $3x10^3$ NMP g^{-1} or CFU g^{-1} of *Bacillus cereus*; 10^2 NMP g^{-1} or CFU g^{-1} of thermotolerant coliform; and the absence of *Salmonella* sp in 25 grams. The analyses were performed in triplicate during the first and last stages of the research.

2.6. Statistical analysis

The experimental data were presented as the mean ± standard deviation. The analyses were performed using STATISTICA v.13.3 software (TIBCO Software Inc., Palo Alto, CA, USA). One-way analysis of variance (ANOVA) was used to study the effects of gamma radiation on bioactive compounds, antioxidant activity and colour parameters. Duncan's tests were conducted to determine differences between the means at 95% confidence level ($p < 0.05$). Pearson's correlation analysis (r) was applied between the different response variables to assess the strength of the correlation between the responses.

Principal component analysis (PCA) was used to analyse the interrelationships between the parameters. The hierarchical cluster analysis (HCA) was based on Euclidean distance and Ward's method. In order to compare the results between the groups proposed by HCA, homogeneity of variance (Levene's test) and analysis of variance (one-way ANOVA or Kruskal-Wallis ANOVA) were applied (Ito et al., 2016).

3. Results and discussions

3.1. Effects of gamma radiation on bioactive compounds and antioxidant activity during storage

The results obtained for phenolic compounds (PC) and antioxidant activity by the ABTS and DPPH assays regarding the free, bound and total phenolic fractions of the black rice flour are summarised in Table 1. Analysing the values for PC and antioxidant activity (ABTS and DPPH) of the free phenolic fraction of the black rice flour, it was observed that the sample with a 3 kGy dose showed the highest values compared with the samples treated with doses of 0, 1 and 2 kGy. At 120 days there was a decrease in the levels for all the samples ($p<0.05$).

Table 1..Effects of gamma radiation on the levels of phenolic compounds (PC) and antioxidant activity of free, bound and total phenolic fractions of black rice flour at the beginning (T_0 – zero days) and end of storage (T_f – 120 days).

Analysis	Doses (kGy)	T_0(0 days)	T_f (120 days)
Free PC (mgGA g)	0	$5.28^{Da}\pm0.03$	$3.25^{Cb}\pm0.04$
	1	$5.79^{Ba}\pm0.03$	$3.58^{ABb}\pm0.03$
	2	$5.67^{Ca}\pm0.01$	$3.54^{Bb}\pm0.03$
	3	$5.89^{Aa}\pm0.03$	$3.64^{Ab}\pm0.03$
Bound PC (mgGA g)	0	$1.84^{Aa}\pm0.02$	$0.95^{Db}\pm0.03$
	1	$1.67^{Ba}\pm0.02$	$0.99^{BCb}\pm0.04$
	2	$1.61^{Ca}\pm0.02$	$1.09^{Ab}\pm0.02$
	3	$1.59^{Ca}\pm0.01$	$1.01^{Bb}\pm0.02$
Total PC (mgGA g)	0	$7.12^{Ca}\pm0.02$	$4.19^{Bb}\pm0.06$
	1	$7.46^{Aa}\pm0.01$	$4.57^{Ab}\pm0.06$
	2	$7.28^{Ba}\pm0.03$	$4.63^{Ab}\pm0.01$
	3	$7.47^{Aa}\pm0.03$	$4.65^{Ab}\pm0.05$
Free ABTS (mgGA g)	0	$6.37^{Da}\pm0.03$	$5.19^{Db}\pm0.03$
	1	$7.35^{Ca}\pm0.03$	$6.05^{Bb}\pm0.02$
	2	$7.59^{Ba}\pm0.01$	$6.16^{Cb}\pm0.02$
	3	$8.13^{Aa}\pm0.02$	$6.77^{Ab}\pm0.03$
Bound ABTS (mgGA g)	0	$2.52^{Aa}\pm0.03$	$1.60^{Cb}\pm0.03$
	1	$2.48^{Ba}\pm0.03$	$1.61^{Bb}\pm0.03$
	2	$2.36^{Ca}\pm0.01$	$1.63^{Ab}\pm0.02$

	3	$2.35^{Da}\pm0.03$	$1.63^{Ab}\pm0.02$
Total ABTS (mgGA g)	0	$8.89^{Da}\pm0.01$	$6.80^{Db}\pm0.01$
	1	$9.83^{Ca}\pm0.06$	$7.66^{Cb}\pm0.04$
	2	$9.96^{Ba}\pm0.02$	$7.79^{Bb}\pm0.01$
	3	$10.48^{Aa}\pm0.01$	$8.40^{Ab}\pm0.05$
Free DPPH (mgGA g)	0	$6.19^{Da}\pm0.03$	$4.95^{Db}\pm0.03$
	1	$6.37^{Ca}\pm0.03$	$5.01^{Bb}\pm0.03$
	2	$6.60^{Ba}\pm0.02$	$5.13^{Bb}\pm0.02$
	3	$6.68^{Aa}\pm0.02$	$5.14^{Ab}\pm0.03$
Bound DPPH (mgGA g)	0	$2.14^{Aa}\pm0.03$	$1.31^{Cb}\pm0.02$
	1	$2.09^{Ba}\pm0.03$	$1.31^{Cb}\pm0.01$
	2	$2.05^{Ca}\pm0.01$	$1.33^{Bb}\pm0.03$
	3	$2.03^{Da}\pm0.02$	$1.34^{Ab}\pm0.02$
Total DPPH (mgGA g)	0	$8.33^{Da}\pm0.06$	$6.26^{Db}\pm0.01$
	1	$8.47^{Ca}\pm0.01$	$6.32^{Cb}\pm0.03$
	2	$8.65^{Ba}\pm0.03$	$6.46^{Bb}\pm0.05$
	3	$8.72^{Aa}\pm0.04$	$6.48^{Ab}\pm0.01$

Note - Results are expressed as mean ± standard deviation; Different capital letters in the same column indicate significant difference between the doses; Different small letters in the same line indicate significant differences during the time of storage. The significant differences at a level of 5% were performed by Duncan's test.

This increase in PC and antioxidant activity of the free phenolic fraction of gamma irradiated samples can be ascribed to the development of new double bonds due to of radiation degradation, which reduced the reactivity of the free radicals (Kumar et al., 2017). Another explanation is that, gamma irradiation may modify/activate some enzymes in rice and change the post-harvest physiology during storage (at room temperature), resulting in an improved synthesis of phenolic acids (Zhu et al., 2010).

The total phenolic content showed behaviour that was similar to that of the free fractions, where the irradiated samples showed the highest values compared with control sample ($p<0.05$); both at the beginning (T_0) and at the end (T_f). The free phenolic fractions accounted for about 78% of the total phenolic contents and 77% of the total antioxidant activity (ABTS and DPPH assays) in the irradiated samples. The main phenolic acids found in pigmented rice are protocatechuic, synaptic, vanillic, p-coumaric and ferulic acid (Zhang et al., 2015).

The values for PC and antioxidant activity of the bound phenolic fraction showed behaviour that was distinct from the free phenolic fraction of the black rice flour. The levels of the control sample were highest at time 0; however, the irradiated samples were most stable at the end of storage ($p<0.05$). The concentration of these phenolic fractions may be dependent on the radiation dosage, the time of storage, technological processes and also the specific nature of the product. Zhang et al., (2015) also found the TPC in soluble fractions were higher than insoluble bound fractions in the black rice; it could be due to some genes that control the linkage between phenolics and lignins which assorted resulting in different offsprings of rice.

Total flavonoids comprise a hydrophilic group of phenolic compounds, to which anthocyanins (highly coloured substances that are recognised for their antioxidant activity and are responsible for the red-purple colour of most vegetables) belong. Cyanidin-3-glucoside (88% of total anthocyanins) is a major anthocyanin in black rice (Abdel-Aal et al., 2006).

Evaluating the total anthocyanin content (TAC), cyanidin-3-glucoside (C3G) and total flavonoids (TF) at 120 days, the results were significantly different ($p < 0.05$) for all samples. The TAC values ranged from 1.84 - 1.52

mgC3G.g-[1]; cyanidin-3-glucoside ranged from 1.43 – 1.26 $mg.g^{-1}$ and TF ranged from 0.65 – 0.49 $mgCE.g^{-1}$. It is noteworthy that the sample with a 3 kGy dose at time 0 showed the highest values, and the control sample (0 kGy) at 120 days showed the lowest values. Sultan et al. (2018) also found a significant increase in TAC in pigmented brown rice flour using an irradiation dose of 2.5 and 5 kGy, as well as Zhu et al. (2010), who evaluated brown rice at an irradiation dose of 6 kGy.

3.2. Effects of gamma radiation on colour attributes during storage

With regard to the effects of gamma radiation on the colour attributes of the black rice flour during storage, the irradiation slightly modified all the parameters ($p < 0.05$), as shown in Figure 1.

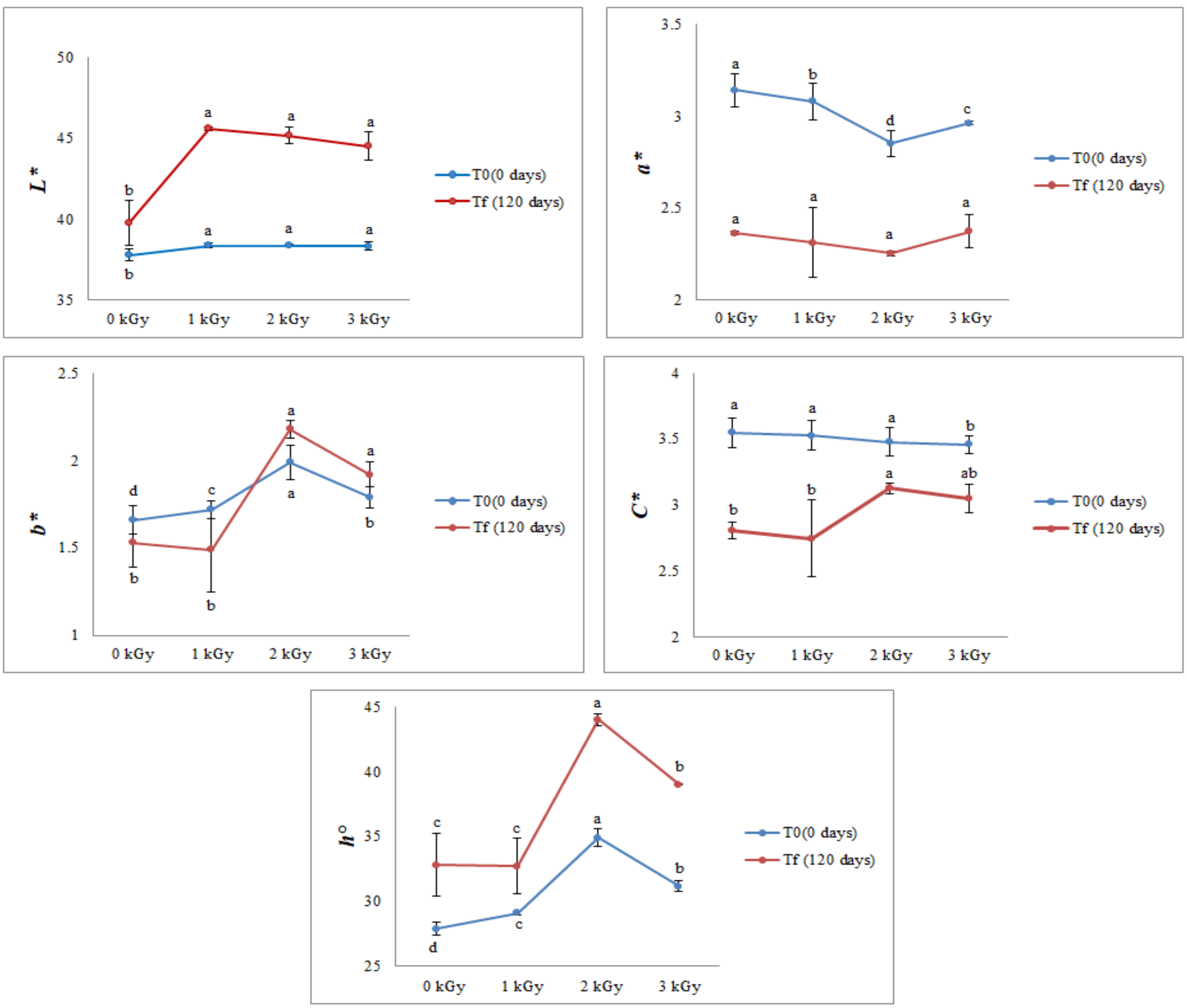

Figure 1. Effects of gamma radiation on the colour attributes (*L*, a*, b*, C** and *h*°) of black rice flour at the beginning (T_0 – zero days) and end (T_f) of storage.

In the irradiated samples, the values for Lightness (*L**) and hue angle (*h*°) were higher than the control sample. Moreover, these parameters increased for all the samples during storage, indicating a loss of colour intensity. *L** is negatively correlated to colour intensity

(Lago-Vanzela et al., 2014) and this behaviour may be associated with a decrease in phenolic content (Figueiredo-González et al., 2013). The *a** (red-green) values were lower in the irradiated samples and during storage this parameter decreased for all the samples.

The irradiated sample with a 2 kGy dose showed higher values for the *b** (yellow-blue) parameter and during storage the best stability for the chroma (*C**) parameter. The effects of gamma radiation on the colour parameters have been reported by other authors. For example, slight changes were found in hazelnuts and almond kernels treated with low doses (Mexis and Kontominas, 2009; Mexis et al., 2009). Therefore, these effects differed due to the individual characteristics of each product.

3.3. Multivariate analysis

The principal component analysis shown in Figure 2 relates to a two-dimensional graphical representation of the black rice flour samples. Principal Components 1 (eigenvalue 13.62) and 2 (eigenvalue 2.28) explained 93.5% of the variance of the data. The formation of two clusters based on the time of storage can be observed.

The left area of Principal Component 1 contained the samples with higher levels of free, bound and total PC, free bound and total DPPH, bound and total ABTS, TAC, TF and *C**. The right area of Principal Component 1 contained the samples with higher values for the *a** parameter. The upper area of Principal Component 2 contained a concentration of samples with higher levels of cianidin-3-glucoside, free ABTS, *L**, *b** and *h*°.

The samples analysed at day 0, were located in the second and third quadrants (left side) and the samples analysed at 120 days were located in the first and fourth quadrants. PCA was an appropriate approach to verify the differences between the gamma radiation doses and storage time in the samples of black rice flour.

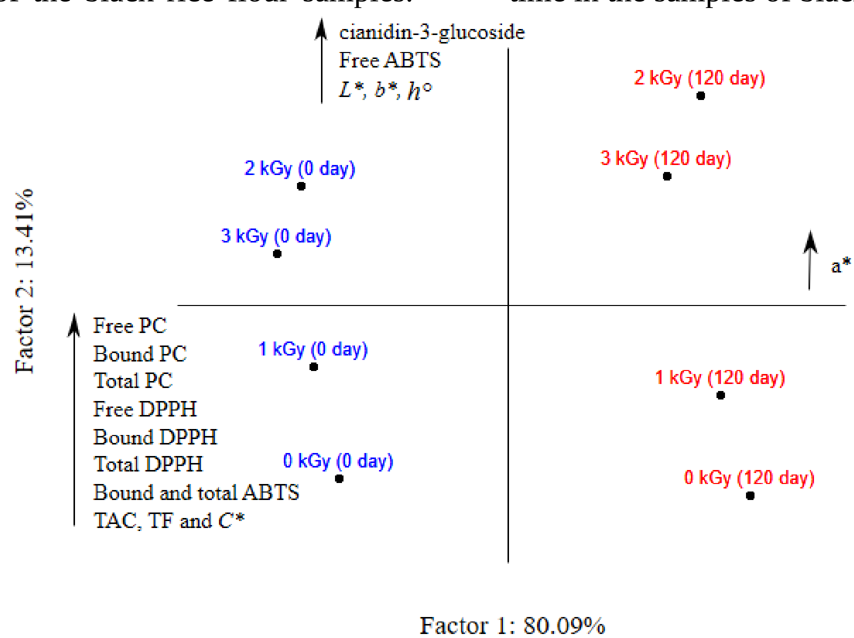

Figure 2. A scatter plot (Principal Component 1 × Principal Component 2) in relation to free, bound and total phenolic compounds, antioxidant activity and colour attributes in irradiated black rice flour during 120 days of storage.

The dendrogram in Figure 3 shows the association between the studied variables. The TAC showed a strong association and positive correlation with the free and total PC ($r = 0.96$; $p < 0.001$) and free and total antioxidant activity by DPPH ($r = 0.97$; $p < 0.001$). The TAC was also positively correlated with cianidin-3-glucoside (r = 0.76; $p < 0.05$), TF (r = 0.96; $p < 0.001$), free and total ABTS (r = 0.94; $p < 0.05$) and the *C** parameter (r = 0.93; $p < 0.05$). The bound PC, bound ABTS and bound DPPH also showed a strong association and positive correlation (r = 0.99; $p < 0.001$), as well as the cianidin-3-glucoside and free ABTS (r = 0.96; $p < 0.05$). Several studies have also demonstrated that phenolic content has a highly positive correlation with antioxidant activities (Shen et al., 2009; Shao et al., 2013).

The *L** and *a** colour parameters showed a strong association and positive correlation (r = 0.83; $p < 0.05$); in relation to the *h*° attribute there was an association with *b** (r = 0.74; $p < 0.05$).

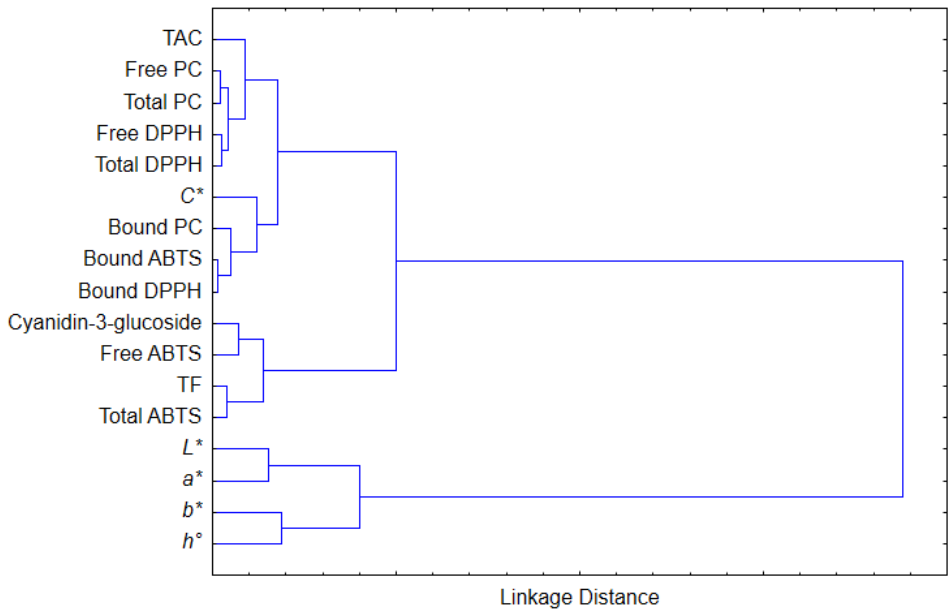

Figure 3. Dendrogram obtained from hierarchical cluster analysis for irradiated black rice flour during 120 days of storage applied to the variables in relation to free, bound and total phenolic compounds, antioxidant activity and colour attributes.

The similarity of the samples was evaluated by using hierarchical cluster analysis, where two clusters were suggested (Figure 4). Cluster 1 was characterised by samples analysed at 0 days and Cluster 2 was characterised by samples that were analysed at 120 days Table 2 shows that the means for each dependent variable were compared, and the ANOVA results for the clusters obtained by hierarchical cluster analysis were calculated.

Cluster 1 (0 days) had higher values for all the parameters, except ($p > 0.05$) for cianidin-3-glucoside, *b** and *h*°. Cluster 2 (120 days) showed lower values, which could have been due to the period of storage.

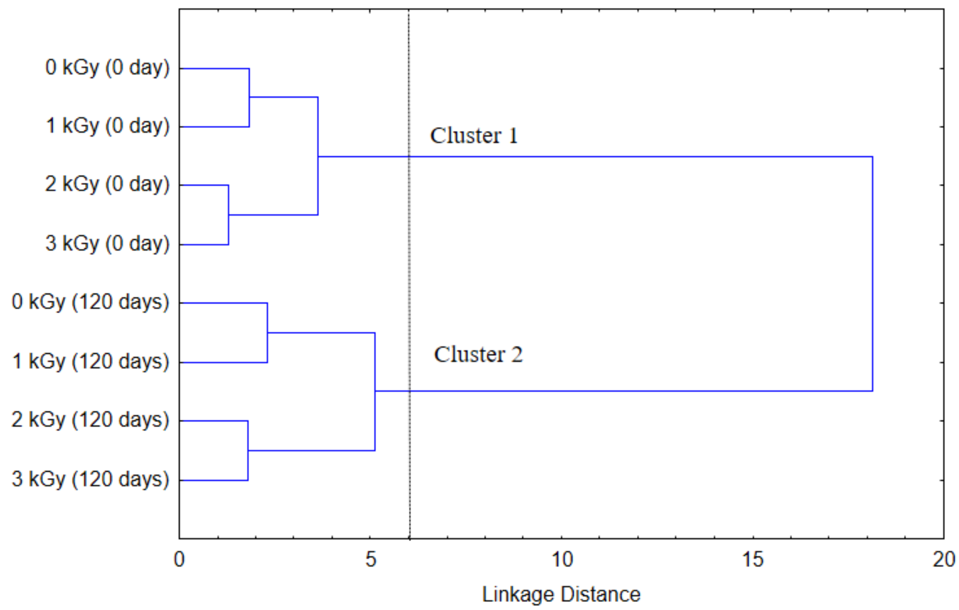

Figure 4. Dendrogram obtained from hierarchical cluster analysis for irradiated black rice flour during 120 days of storage applied to the samples in relation to free, bound and total phenolic compounds, antioxidant activity and colour attributes.

Table 2.Phenolic content – PC (mg GA g); antioxidant activity – ABTS and DPPH (μmol TE g), total anthocyanin content – TAC (mg C3G g^{-1}), cianidin-3-glucoside (mg g^{-1}), total flavonoids - TF (mg QE g^{-1}) and colour attributes of irradiated black rice flour using hierarchical cluster analysis (HCA).

Variables	Cluster 1 n= 4	Cluster 2 n=4	PSD	p-value*	p-value**
Free PC	5.66 ^{a}	3.50 ^{b}	1.53	0.52	<0.05*
Free ABTS	7.36 ^{a}	6.04 ^{b}	0.93	0.81	<0.05*
Free DPPH	6.46 ^{a}	5.06 ^{b}	0.99	0.05	<0.05*
Bound PC	1.68 ^{a}	1.01 ^{b}	0.47	0.30	<0.05*
Bound ABTS	2.43 ^{a}	1.61 ^{b}	0.58	<0.001	<0.05*
Bound DPPH	2.08 ^{a}	1.32 ^{b}	0.54	<0.05	<0.05*
TAC	1.81 ^{a}	1.58 ^{b}	0.16	0.67	<0.05*
Cianidin-3-glucoside	1.38	1.31	0.05	0.25	0.14
TF	0.63 ^{a}	0.53 ^{b}	0.07	0.93	<0.05*
L*	43.7 ^{a}	38.17 ^{b}	3.91	<0.05	<0.05*
a*	2.32 ^{a}	1.38 ^{b}	0.66	0.98	<0.05*
b*	1.79	1.78	0.01	<0.05	0.77
C*	3.5 ^{a}	2.93 ^{b}	0.40	<0.05	<0.05*
h°	37.15	30.77	4.51	0.16	0.08

Note: Results expressed as mean ± pooled standard deviation. PSD: pooled standard deviation; *Probability values obtained by Levene's test for homogeneity of variances; **Probability values obtained by one-way ANOVA or Kruskal–Wallis test. Different letters in the same line represent statistically different results ($p < 0.05$).

3.4. Effects of gamma radiation on physicochemical and microbiological properties during storage

There was an increase in all the samples during 120 days of storage regarding moisture and water activity (A_w). The moisture content ranged from 10.54 (3 kGy - 0 days) to 11.60 g.100g-1 (0 kGy – 120 days) and A_w values ranged from 0.41 (2 and 3 kGy - 0 days) to 0.48 (0 kGy – 120 days). The control sample (0 kGy) had the highest increase, 9.02% for moisture and 14.94% for A_w. This increase can be associated with the hygroscopic of flours and their tendency to respond to changes in ambient relative humidity, as well as the transfer properties of water vapour from the packaging.

Similar results were found by Silva et al. (2010), who evaluated the oxidative stability in irradiated wheat flour and cornmeal, and who observed a significant increase in moisture in the samples from the first 30 days onwards. Marathe et al. (2002) found that moisture levels in irradiated and non-irradiated wheat flour increased at 120 days of storage.

Regarding the microbiological analyses, the results were below those required by legislation (ANVISA, 2001). In the control sample (0 kGy), the colony-forming units per gram CFU g^{-1} were $<10^2$ for *Bacillus cereus*; the most probable number per gram NMP g^{-1} was < 3.0 for thermotolerant coliforms; and there was an absence of *Salmonella* sp in 25 grams. There was no growth of microorganisms during the storage period of 120 days. In the irradiated samples (1, 2 and 3 kGy) there were non-detectable values for these bacteria. Feliciano et al., (2017) demonstrated the effectiveness of low-dose gamma irradiation for the microbial decontamination of brown rice for a prolonged period of storage in ambient conditions.

4. Conclusions

Our study concluded that the effects of gamma - radioisotope ^{60}Co on black rice flour resulted in an increase in phenolic compounds and antioxidant activity of the free and total phenolic fraction, as well as total anthocyanins content, cyanidin-3-glucoside and total flavonoids. The dose of 3 kGy showed the highest values; at 120 days there was a decrease in these levels for all samples. However, the irradiated samples were most stable at the end of storage. With regard to the colour attributes, the irradiation slightly modified all the parameters. For the microbiological analysis, there was no growth of microorganisms in the samples during the storage period of 120 days.

The results demonstrated that the chemometric approach proved to be effective in facilitating the visualisation of the positive effects of irradiation, which may improve the shelf life of black rice flour. This pigmented rice can be a valuable ingredient in gluten-free, cereal products with higher nutritional value, as well as helping to partially reduce negative impacts on the environment.

5.References

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Acknowledgements

The authors are deeply grateful to the Coordination for the Improvement of Higher Education Personnel (CAPES) for scholarship.