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Effects of gamma radiation on the stability and degradation kinetics of phenolic compounds and antioxidant activity during storage of (Oryza sativa L.) black rice flour

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HIGHLIGHTS

- Cyanidin-3-glucoside in black rice was effectively identified by HPLC-PDA.
- 1 kGy dose remained most stable for TAC and TPC.
- Kinetic data indicated a first-order reaction for the degradation of anthocyanins.

Abstract: The effects of gamma radiation (0, 1, 2 and 3 kGy) were used to evaluate the stability and thermal degradation kinetics of anthocyanins, as well as the stability of total phenolic compounds (TPC) and antioxidant activity at different temperatures (4, 25, 35 and 45 °C) during the storage (0, 30, 60, 90 and 120 days) of black rice flour. This flour can be used as ingredient for gluten-free cereal products with higher nutritional value. For this it is necessary to preserve the anthocyanin content during thermal processing and shelf-life periods. At time 0, the dose of 3 kGy provided all of the most available bioactive compounds, raising their antioxidant potential, except for TPC. During the storage at different temperatures up to 120 days, gradual losses occurred in all the analysed parameters. Regarding the total anthocyanin content and TPC, the sample irradiated with a 1 kGy dose remained most stable. The analysis of kinetic data indicated a first-order reaction for the degradation of anthocyanins. The combination of irradiation with different temperatures may improve the shelf-life of black rice flour.

Keywords: Anthocyanins; Bioactive compounds; Irradiation; Stability; Thermodynamic parameters; Pigmented rice.

INTRODUCTION

In recent times, pigmented rice varieties, such as black rice, have received increased attention from researchers and have become very attractive to consumers due to their health benefits. Black rice is a good source of anthocyanins, flavonoids, tocopherols and vitamins B and E [1,2]. These antioxidants act against possible harmful effects caused by free radicals and they are directly related in prevention of various diseases such as type II diabetes, obesity, cancer, cardiovascular disease, among others [1,3,4].

Anthocyanins are dark purple and red pigments that have been increasingly used by the food industry as natural replacements to synthetic dyes. Therefore, black rice flour can be used as ingredient for gluten-free cereal products with higher nutritional value [5]. In this context, there is a need to preserve the anthocyanin content during thermal processing, as well as prolonged storage and shelf-life periods. This bioactive compound is very reactive and can be simply degraded to colourless or brown-colour compounds. The stability of such substances in foods is influenced by a number of factors, including processing and storage conditions, physical and chemical properties of foods, the presence of copigments and metallic ions [6,7].

Temperature is the most important factor that affects the stability of anthocyanins and other phenolic compounds [8] in both food processing and storage [9]. The study of the degradation kinetics, with the reaction rate (k) as a function of storage temperatures, activation energy (E_a) and half-life ($t_{1/2}$) and thermodynamic parameters, with the free energy (ΔG), enthalpy (ΔH) and entropy (ΔS), could provide significant information concerning the thermal stability of anthocyanins as well as to be a very important factor in the prediction of food quality loss [7].

In terms of increasing the shelf-life of food products, radiation is a well-established non-thermal physical mode for food preservation and has been widely researched and its effects are known to act in reducing microbial load or sterilisation, increasing the shelf-life of products and mainly maintaining food quality [10]. Various papers have been published regarding the positive effect of gamma radiation on bioactive compounds with antioxidant potential in different raw materials such as coloured soybean [11], whole grain rice [12] and β-glucan extracted from button mushroom [13].

To date, there is a paucity of information concerning the effect of irradiation on the degradation kinetics of bioactive compounds during storage in pigmented rice. Thus, the objective of this study was to investigate the effects of gamma radiation on the stability and thermal degradation kinetics of anthocyanins, as well as the stability of total phenolic compounds and antioxidant activity at different temperatures during the storage of black rice flour.

MATERIAL AND METHODS

Sample preparation and radiation

All reagents were of the highest grade commercially available. The biodynamic black rice used in the experiments was cultivated according to Demeter biodynamic standards [14] and purchased in a local supermarket in the city of Curitiba, Paraná, Brazil. The black rice flour (BRF) was obtained according to the methodology used by Ito et al. [5]. This was separated and vacuum-packed in samples of about 200 g in small, non-toxic metallised polyester bags with hermetic sealing and protected from the light.

All the BRF samples were irradiated at doses of 0, 1, 2 and 3 kGy at a 0.221 kGy h^{-1} dose rate in ^{60}Co gamma irradiator (Gammacell Excell 220 - MDS Nordion, Ottawa, Canada). Harwell Amber 3042 dosimeters were used to measure the radiation dose and the uncertainty dose was less than 1%. The irradiation treatments were performed in the Centre for Nuclear Energy in Agriculture at the University of São Paulo, Brazil (CENA/USP).

Stability and storage conditions

In order to understand the effect of gamma radiation on the stability of the BRF the following were evaluated: four irradiation doses (0, 1, 2 and 3 kGy); four temperatures (4, 25, 35 and 45 °C); and five storage periods (0, 30, 60, 90 and 120 days).

After irradiation, all the samples were kept at 25 ± 2°C, analysed (first period - month 0) and then subjected to different storage temperatures in a B.O.D incubator (SP-500 SP Labor, Brazil): 4 ± 2°C, acting the refrigeration temperature (simulating at the use of the flour as an ingredient in low-temperature stored products); 25 ± 2°C, aiming room temperature; 35 ± 2°C, it is one of the temperatures studies for accelerated shelf-life[15]; and 45 ± 2°C, representing the start of degradation.

Extraction of anthocyanins

The anthocyanins were extracted in duplicate according to Shao et al. [16], with minor modifications. Briefly, 0.5 g of rice flour (BRF) was extracted twice with 15 mL of a solution of methanol and HCl (1 mol L^{-1}) (85:15, v v^{-1}) using a shaker for 45 min, under dark conditions. The samples were then centrifuged at 5000 x *g* (HIMAC CR-GII, Hitachi, Ibaraki, Japan) for 20 min at 20 °C. The mixture was vacuum filtered through a nylon syringe filter 0.22 µm (Waters, Milford, MA, USA) and the extract was corrected to a final volume of 50 mL with the extraction solvent.

The samples (in their respective treatments) were analysed for the total anthocyanin content (TAC), cyanidin-3-glucoside (C3G), total flavonoids (TF), total phenolic compounds (TPC), antioxidant activity measured by ABTS and FRAP assays. The analyses were carried out in triplicate.

Determination of total anthocyanin content (TAC)

The TAC was determined according to the pH differential spectrophotometric method adapted for microplate [17]. 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, aliquots of the extract (obtained as described in section 2.5) 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 using a microplate reader (Epoch microplate

spectrophotometer, Synergy-BioTek, Winooski, VT, USA). The TAC was expressed as cyanidin-3-glucoside equivalent, and was determined conform Shao et al. [16].

HPLC analysis of anthocyanins

The anthocyanin extracts were also submitted to HPLC analysis according to the method described by Pedro et al. [18], with minor changes. The analysis was conducted 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 using a 0.22 µm nylon membrane and then 10 µL of sample were 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 mobile phase consisted of A (0.1% formic acid) and B (acetonitrile). A linear gradient was applied as follows: 3-22% B (0-5 min), 22-35% B (5-15 min), followed by washing and reconditioning of the column. The anthocyanins were identified and quantified at 515 nm with 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). An example of the chromatogram that was obtained is shown in Figure 1.

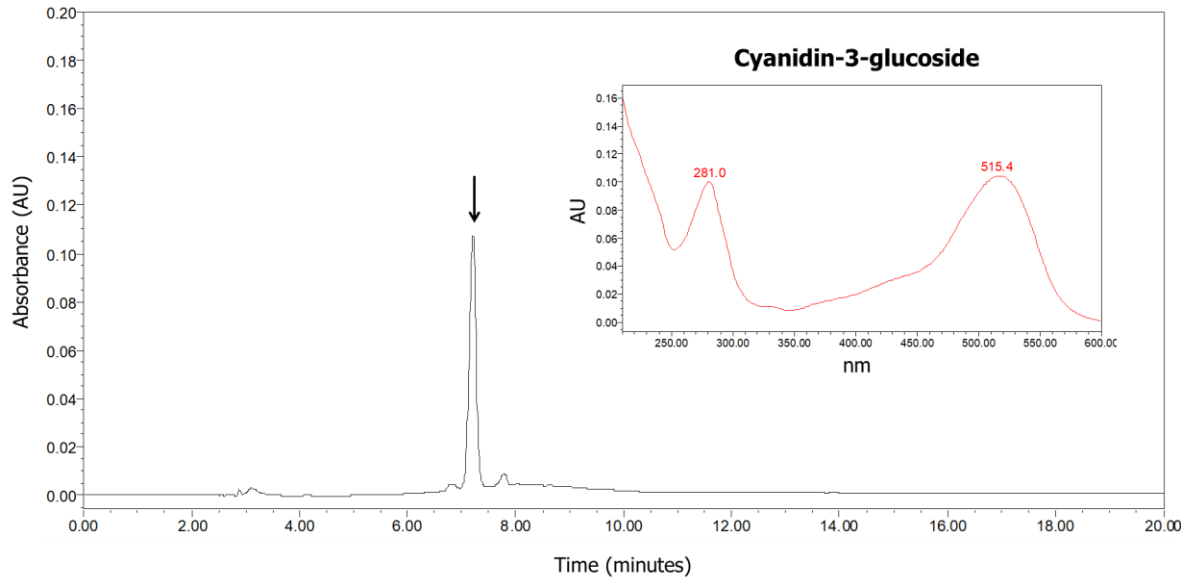

Figure 1. Chromatogram of anthocyanin extract of black rice flour at 515 nm.

Kinetic reaction and thermodynamic analysis

The thermal degradation of the anthocyanins from the black rice flour under various storage conditions (section 2.4) was evaluated by the following first-order equation according to Patras et al.[19].

$$C_t = C_0 \exp(-kt) \quad (1)$$

where C_t is the anthocyanin content at reaction time (t) (mgC3G.g^{-1}) and C_0 is the initial anthocyanin content (mgC3G.g^{-1}). The variation of k is the temperature-dependent rate constant (day^{-1}), and t is the heating time (day). The half-life ($t_{1/2}$) of the anthocyanins during heating was expressed as in Equation 2:

$$t_{1/2} = -\frac{\ln(0,5)}{k} \quad (2)$$

The effect of temperature on the kinetics of anthocyanin degradation was calculated follow the constants obtained from Equation (1), and were fitted to an Arrhenius Equation (3):

$$\ln k = \ln k_0 - \frac{E_a}{R.T} \quad (3)$$

where, E_a is the Arrhenius activation energy (kJ mol^{-1}); R is the universal gas constant (8.314 J mol^{-1} K); and T is absolute temperature (K). The activation energy was determinate by plotting ln (k) against 1/T conform to Equation (3).

The thermodynamic parameters as activation enthalpy ($\Delta H^{\#}$), free energy of inactivation ($\Delta G^{\#}$), and activation entropy ($\Delta S^{\#}$), were determined according to Equations 4-6, as described by Labuza [20].

$$\Delta H^{\#} = Ea - RT \quad (4)$$

$$\Delta G^{\#} = -R \cdot T \cdot ln\left(\frac{k \cdot h}{k_B \cdot T}\right) \quad (5)$$

$$\Delta S^{\#} = \frac{\left(\Delta H^{\#} - \Delta G^{\#}\right)}{T} \quad (6)$$

where h is Planck's constant (6.62 x 10^{-34} J s) and k_B is Boltzmann's constant (1.38 x 10^{-23} J K^{-1}).

Determination of total flavonoids (TF) and total phenolic compounds (TPC)

The TF from the black rice flour were quantified by UV–Vis spectrophotometry (Shimadzu UV-1800) at 374 nm. The total flavonoid content was expressed as mg quercetin equivalents (CE) per g of black rice flour and determined conform Pedro et al. [18].

The TPC was determined according to the Folin-Ciocalteu procedure described by Singleton & Rossi [21], with some modifications. Measurements were performed using a microplate reader, after 1 hour of reaction the absorbance was recorded at a wavelength of 720 nm. The sample absorbance values were compared against a calibration curve of gallic acid (GA) and the results were expressed as mg of gallic acid equivalents (GAE) per gram of black rice flour (mg GAE g^{-1}).

Measurement of the *in vitro* antioxidant activity

The total antioxidant potential of the BRF was determined using the ferric reducing antioxidant power (FRAP) assay, as describe by Benzie & Strain [22], with slight modifications. The absorbance was recorded at a wavelength of 593 nm after the solution had been allowed to stand in the dark for 2 hours. A standard curve (FRAP = 0.001 × absorbance; R^2 = 0.991; p < 0.001) was plotted using different concentrations of Trolox (0.1–1.0 mmol L^{-1}). The results were expressed in µmol Trolox equivalents per gram of sample (µmolTE g^{-1}).

The ABTS scavenging activity was determined using the method described by Re et al. [23], 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}).

Statistical analysis

The results were expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) was used to study the effect of gamma radiation for all the parameters. Duncan's tests were conducted to determine differences between the means at 95% confidence level ($p < 0.05$). Pearson's products (r) were used to evaluate the strength of correlation between the response variables [24]. The model parameters that needed to be fitted for all the equations were determined by non-linear squares regression using the Gauss–Newton algorithm or by linear squares regressions. The statistical significance of the equations was examined by ANOVA, and the goodness of fit was based on the regression coefficient (R^2). All the analyses were performed using STATISTICA v.13.3 software (TIBCO Software Inc., Palo Alto, CA, USA).

RESULTS

Effects of gamma radiation on the stability of anthocyanins

The effects of gamma radiation (0, 1, 2 and 3 kGy) were used to evaluate the stability of the anthocyanins at different temperatures (4, 25, 35 and 45 °C) during storage (0, 30, 60, 90 and 120 days) of the black rice flour. For total anthocyanin content (TAC), it was observed that the samples with the 3 kGy dose showed higher values compared with the samples treated with doses of 0, 1 and 2 kGy, as shown in Table 1. There was a significant reduction in the levels of anthocyanins for all the treatment samples ($p<0.05$) with increases in temperature and longer periods of time.

Table 1. Effects of gamma irradiation on the levels of total anthocyanins content (TAC) and cyanindin-3-glucoside (C3G) in BRF stored at 4, 25, 35 and 45 °C, at the beginning (T_0 - zero day) and end of storage (T_f).

		T_0 (0 days)	T_f (120 days)			
Parameters	Doses (kGy)	-	4(°C)	25(°C)	35(°C)	45(°C)
TAC (mgC3G.g^{-1})	0	1.81^{Ba}±0.01	1.57^{Cb}±0.02	1.52^{Cc}±0.01	1.43^{Bd}±0.01	1.43^{Cd}±0.02
	1	1.76^{Ca}±0.01	1.60^{ABb}±0.01	1.58^{Bc}±0.01	1.55^{Ad}±0.01	1.42^{Ce}±0.02
	2	1.81^{Ba}±0.01	1.58^{BCb}±0.01	1.58^{Bb}±0.02	1.54^{Ac}±0.02	1.46^{Bd}±0.02
	3	1.84^{Aa}±0.01	1.62^{Ab}±0.01	1.63^{Ab}±0.01	1.56^{Ac}±0.01	1.55^{Ac}±0.01
Cyanidin-3-glucoside (mg.g^{-1})	0	1.30^{Ca}±0.02	1.24^{Bb}±0.02	1.26^{Bb}±0.01	1.17^{Bc}±0.02	1.15^{Bc}±0.01
	1	1.37^{Ba}±0.01	1.29^{Ac}±0.01	1.32^{Ab}±0.01	1.30^{Abc}±0.02	1.30^{Abc}±0.02
	2	1.42^{Aa}±0.02	1.28^{Ab}±0.02	1.31^{Ab}±0.03	1.31^{Ab}±0.01	1.31^{Ab}±0.02
	3	1.43^{Aa}±0.01	1.29^{Ab}±0.01	1.33^{Ab}±0.02	1.31^{Ab}±0.03	1.31^{Ab}±0.01

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 difference between the temperatures and time. The significant differences at a level of 5% were performed by Duncan's test.

In addition, for the temperatures of 4 and 25 °C at the final time (120 days), the irradiated samples showed higher values when compared with the other temperatures (35 and 45°C). The control sample (0 kGy) had the highest reduction of anthocyanins (13.08 - 20.91%) for all temperatures. The sample irradiated with a 1 kGy dose remained most stable in relation to the storage temperatures (4, 25 and 35 °C), especially at 4 °C, and had a loss of only 9.33%. This decrease in anthocyanins may have been due to their polyphenolic structures, which are prone to oxidation and vulnerable to oxidative degradation during

storage. Furthermore, high temperatures can destabilise anthocyanin structures, contributing to degradation in the TAC [18]. Similar results were observed by Norkaew et al. [25] who reported that storage at 30 °C yielded an 18% reduction in the concentration of anthocyanins in black rice.

A study of black rice by Zhang et al. [3] found that the anthocyanins were mainly in free form, about 99.5 – 99.9% of total anthocyanins. The main anthocyanin in this cereal is cyanidin-3-glucoside (C3G), representing for 88% of the total anthocyanins [26]. Other anthocyanins were identified in previous studies; however, in lower levels, such as peonidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3,5-diglucoside [27], and malvidin-3-glucoside, petunidin-3-glucoside [28].The results obtained by HPLC for the cyanidin-3-glucoside (C3G) stored at 4, 25, 35 and 45 °C, at the start and the last storage time (0 and 120 days) are shown in Table 1.

The effect of irradiation and storage temperatures on cyanidin-3-glucoside (C3G) were also observed and showed the same behaviour observed for TAC. The samples with a 3 kGy dose at time 0 showed the highest values compared with the samples treated with doses of 0, 1 and 2 kGy. There was a decrease in the levels for all the samples ($p<0.05$) with increases in temperature and time. The control sample (0 kGy) had the highest reduction in C3G; 10% and 11.54% for the respective temperatures of 35 and 45 °C. The samples irradiated with 1, 2 and 3 kGy were stable between the temperatures of 35 and 45 °C, with losses of only 5.11, 7.75 and 8.39%, respectively.

Rodríguez-Pérez et al. [29] investigated the shelf-life of commercial cranberry syrup irradiated with gamma radiation at a rate of 5 kGy and stored for 6 months at 25 °C and 60% relative humidity. The authors reported a significant increase in the content of procyanidin after irradiation and concluded that the compounds were highly resistant to gamma-irradiation after one month of storage at room temperature. Tiwari et al. [30] reported that the effects of irradiation on anthocyanin depend upon the nature of the anthocyanin, and that diglycosides are stable in relation to doses of irradiation compared to monoglycosides.

Effects of gamma radiation on the thermal degradation kinetics of total anthocyanin content (TAC)

Kinetic models are often used for a quick and economic assessment of food safety [2]. In our study, the degradation of the TAC (Figure 2) was fitted according to the first-order kinetic model, showing a linear degradation in relation to time. These results are in agreement with previous studies which reported the use of the first-order kinetic model for fitting the thermal degradation of phytochemicals from black rice [2,27,31]. The first-order reaction rate constants (k) of the black rice flour are presented in Table 2.

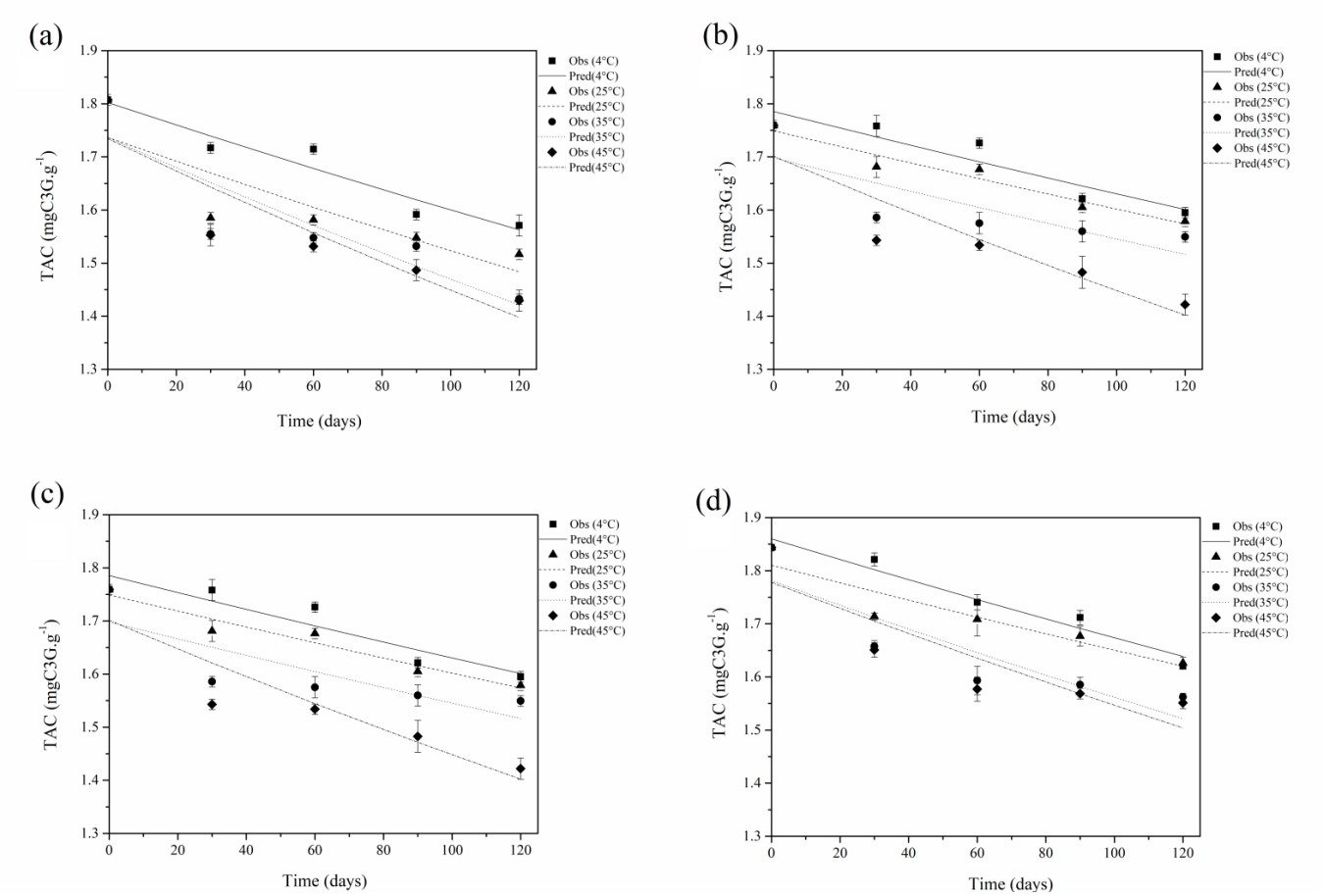

Figure 2. Total anthocyanins in black rice flour with: (a) 0 kGy; (b) 1 kGy; (c) 2 kGy; and (d) 3 kGy, storage at ■4, ▲25, ●35 and ♦45 °C, for 120 days. (Note: broken lines represent the behaviour predicted by the pseudo first-order kinetic model).

Table 2. Degradation kinetic parameters and activation energy (E_a) of anthocyanins in black rice flour stored at 4, 25, 35 and 45 °C for 120 days.

Doses	Temperature(°C)	k(days^{-1})	$t_{1/2}$ (days)	R^2	E_a (kJ mol^{-1})
0 kGy	4	0.0012	582.97	0.9299	7.6330
	25	0.0013	528.31	0.7391	
	35	0.0017	415.56	0.7870	
	45	0.0018	385.72	0.8186	
1 kGy	4	0.0009	761.70	0.8788	7.9725
	25	0.0009	787.67	0.9483	
	35	0.0009	734.27	0.6711	
	45	0.0016	430.53	0.8449	
2 kGy	4	0.0011	616.68	0.9157	6.2285
	25	0.0010	703.70	0.8474	
	35	0.0012	579.55	0.6785	
	45	0.0017	405.11	0.8224	
3 kGy	4	0.0011	656.39	0.9164	5.305
	25	0.0009	749.35	0.8649	
	35	0.0013	527.11	0.7839	
	45	0.0014	497.24	0.7805	

Note. Reaction rate (k), half-life ($t_{1/2}$) and activation energy (E_a).

The kinetic rate constant (*k*) is an indicator that predicts of the thermal degradation of anthocyanins in food products: a low value is better for the stability of anthocyanins [31]. In our results, the *k* values showed that the thermal stability of the TAC decreased with increasing temperature, especially from 35 to 45 °C for all the temperatures, and showed higher half-life time ($t_{1/2}$) values than the non-irradiated samples (Table 2), indicating that greater degradation can occur at higher processing temperatures. However, the use of gamma irradiation in black rice helped to preserve the anthocyanins.

The influence of temperature on the activation energy (E_a), fitted by Arrhenius equation, is commonly used to be the energy required to reach the transition state of a reaction [7]. As was observed for the *k* and half-life time values, the highest E_a value was found for the 1 kGy dose (7.9725 kJ mol^{-1}). According to Zhou et al. [32], a high E_a value indicates that the degradation reaction of anthocyanins is more difficult to activate and more susceptible to rises in temperature.

In order to verify if the developed kinetic models were thermodynamically possible, the estimation of the activation enthalpy ($\Delta H^{\#}$), Gibbs free energy ($\Delta G^{\#}$) and the activation entropy ($\Delta S^{\#}$) were performed (Table 3). $\Delta H^{\#}$ represents the energy difference between the reactant and activated complex [32]. In our study, the $\Delta H^{\#}$ values were positive and ranged from 2.66 to 5.67 kJ mol^{-1}, which revealed that the anthocyanin degradation showed an endothermic reaction [33]. The Gibbs free energy ($\Delta G^{\#}$) is the important parameter to measure the spontaneity of the chemical reaction [7]. Positive $\Delta G^{\#}$ values (from 83.23 to 95.48 kJ/mol) mean that anthocyanin degradation is a non-spontaneous reaction. The activation entropy ($\Delta S^{\#}$) measures the variation of disorder of the molecules in the system [7]. All the negative $\Delta S^{\#}$ values (from -281.91 to -293.51 J mol^{-1} K^{-1}) found in the present study showed a lower structural freedom than the reactants, which further confirmed that this is an irreversible process. The same behaviour for the evaluated thermodinamic parameter was reported by Turturicã et al.[34].

Table 3. Thermodynamic parameters of anthocyanins in black rice flour stored at 4, 25, 35 and 45 °C for 120 days.

		Temperatures			
Parameters	Doses (kGy)	4(°C)	25(°C)	35(°C)	45(°C)
$\Delta H^{\#}$ (kJ mol^{-1})	0	5.33	5.15	5.07	4.99
	1	5.67	5.49	5.41	5.33
	2	3.91	3.74	3.66	3.57
	3	3.00	2.83	2.74	2.66
$\Delta G^{\#}$ (kJ mol^{-1})	0	83.23	89.47	91.94	94.81
	1	83.84	90.46	93.40	95.10
	2	83.36	90.18	92.79	94.94
	3	83.50	90.34	92.55	95.48
$\Delta S^{\#}$ (J mol^{-1} K^{-1})	0	-281.07	-282.80	-281.91	-282.33
	1	-282.07	-284.98	-285.54	-282.18
	2	-286.64	-289.92	-289.26	-287.18
	3	-290.45	-293.51	-291.44	-291.76

Note. Enthalpy (ΔH), free energy (ΔG) and entropy (ΔS).

The stability of anthocyanin pigments is affected by several factors such as heat treatment, storage temperature, light, pH value, chemical structure, oxygen, solvents, and the presence of enzymes, proteins, flavonoids, and metallic ions [31]. The stability of anthocyanins is related to their structure and copigmentation capacity. Food sources with

high anthocyanin content contain mixtures of different compounds that act as copigments for intermolecular association with anthocyanins [27]. Furthermore, Hiemori et al. [35] suggested that anthocyanin stability increases with increasing number of methoxyl groups in the *B*-ring, and decreases as the number of free hydroxyl groups in the *B*-ring increases.

Effects of gamma radiation on the stability of phenolic compounds and antioxidant activity

Table 4 shows the total flavonoids (TF), total phenolics compounds (TPC), and *in vitro* antioxidant activity (ABTS and FRAP), which demonstrated different behaviours in relation to irradiation and storage temperature.

Table 4: Effects of gamma irradiation on the levels of total flavonoids (TF), total phenolic compounds (TPC) and antioxidant activity in BRF stored at 4, 25, 35 and 45 °C, at the beginning (T_0 zero day) and end of storage (T_f).

		T_0 (0 days)	T_f (120 days)			
Parameters	Doses (kGy)	-	4(°C)	25(°C)	35(°C)	45(°C)
TF (mgCE.g^{-1})	0	0.60^{Ba}±0.03	0.44^{Bb}±0.01	0.49^{Bb}±0.02	0.62^{Aa}±0.04	0.57^{Ca}±0.03
	1	0.62^{Ba}±0.02	0.48^{ABc}±0.02	0.52^{ABb}±0.03	0.62^{Aa}±0.01	0.61^{Ba}±0.01
	2	0.65^{Aa}±0.01	0.48^{ABc}±0.04	0.53^{ABb}±0.03	0.63^{Aa}±0.03	0.62^{ABa}±0.03
	3	0.65^{Aa}±0.02	0.49^{Ac}±0.02	0.56^{Ab}±0.03	0.65^{Aa}±0.02	0.63^{Aa}±0.02
TPC (mgGAE.g^{-1})	0	4.24^{Aa}±0.01	2.14^{Dc}±0.02	2.27^{Cb}±0.03	2.10^{Dc}±0.03	1.84^{Dd}±0.03
	1	4.24^{Aa}±0.02	2.89^{Ab}±0.02	2.80^{Ac}±0.02	2.41^{Bd}±0.02	2.09^{Ce}±0.02
	2	4.16^{Ba}±0.04	2.39^{Cc}±0.03	2.77^{Bb}±0.02	2.18^{Cd}±0.03	2.18^{Bd}±0.04
	3	4.24^{Aa}±0.02	2.75^{Bbc}±0.02	2.77^{Bb}±0.01	2.72^{Ac}±0.02	2.58^{Ad}±0.03
ABTS (µmolTE.g^{-1})	0	96.61^{Da}±0.06	73.03^{De}±0.02	74.58^{Dc}±0.03	75.39^{Cb}±0.03	73.59^{Cd}±0.02
	1	92.65^{Ca}±0.03	74.31^{Bc}±0.01	75.49^{Cb}±0.04	70.32^{De}±0.03	73.47^{Dd}±0.03
	2	95.38^{Ba}±0.03	78.82^{Ad}±0.02	81.55^{Ac}±0.01	84.32^{Ab}±0.02	76.95^{Be}±0.03
	3	99.59^{Aa}±0.04	74.21^{Ce}±0.03	77.33^{Bd}±0.03	79.68^{Bc}±0.03	85.06^{Ab}±0.02
FRAP (µmolTE.g^{-1})	0	89.68^{Da}±0.02	51.03^{Dc}±0.02	56.30^{Db}±0.03	49.18^{Dd}±0.01	48.78^{De}±0.02
	1	90.50^{Ca}±0.04	56.10^{Cc}±0.02	56.88^{Cb}±0.03	53.68^{Cd}±0.03	51.99^{Ce}±0.01
	2	98.91^{Ba}±0.02	63.17^{Bb}±0.03	63.13^{Bc}±0.03	59.30^{Bd}±0.03	56.18^{Be}±0.03
	3	110.32^{Aa}±0.03	71.92^{Ab}±0.03	71.93^{Ab}±0.01	67.89^{Ac}±0.02	66.62^{Ad}±0.03

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 difference between the temperatures and time. The significant differences at a level of 5% were performed by Duncan's test.

Regarding the levels of TF, it was observed that the irradiated samples showed higher values ($p < 0.05$) compared with the control sample (0 kGy). There was a decrease in the levels for all the samples for the temperatures of 4 and 25 °C at 120 days of storage. At temperatures of 35 and 45 °C, the TF showed a behaviour that was distinct from the other compounds because there was no significant difference in loss during storage.

In the case of the TPC, the results for the control sample (0 kGy - 0 day) were similar to those of studies using black rice [28]. Gamma radiation did not influence the level of TPC; only in the case of the storage period was there a significant decrease ($p < 0.05$) for all the samples. However, the control sample (0 kGy) showed the greatest reduction (56.7%) at the

temperature of 45 °C. The sample irradiated with a 1 kGy dose remained most stable during the storage period at 4 °C, with a reduction of about 31.8%.

Behgar, et al. [36] suggested that the alterations in the effect of gamma radiation on TPC may be due to the higher extractability of these compounds in irradiated products. Irradiation is able to break the chemical bonds of bioactive compounds, releasing soluble phenolic with low molecular weight and increasing these compounds with antioxidant potential [37].

The effect of irradiation and storage temperature were also observed regarding *in vitro* antioxidant activity (ABTS and FRAP), with a gradual loss during storage ($p < 0.05$) for all the samples. In terms of the ABTS assay, the 1 kGy dose showed the lowest level at time 0, as well as the highest degradation at 35 °C, about 24.11%. For the FRAP, the control sample (0 kGy) had the highest reduction, 45.6% for the temperature of 45 °C.

Previous findings have indicated that black rice varieties contain different contents of anthocyanins, as well as different ratios and types of polyphenols[27]; therefore exhibiting different antioxidant activities [1]. Zhang et al. [3], indicated that the total antioxidant activity of black rice bran was significantly correlated to the content of total phenolics ($r = 0.9810$, $p < 0.01$), total flavonoids ($r = 0.8281$, $p < 0.01$), and anthocyanins ($r = 0.5763$, $p < 0.05$).

In our results, the antioxidant activity measured by ABTS assay was positively correlated ($p < 0.05$) with TAC ($r = 0.99$). Yao et al. [38] also found a positive correlation between antioxidant activity and total anthocyanin content. The antioxidant activity measured by the FRAP assay was positively correlated ($p < 0.05$) with TAC ($r = 0.97$) and TPC (0.98). According to Shao et al. [12], the antioxidant activity tends to show a highly positive correlation with phenolic compounds. There were significant correlations ($p < 0.05$) between cyanidin-3-glucoside (C3G) content and total anthocyanins (TAC) ($r = 0.99$), total phenolic compounds (TPC) ($r = 0.97$) and total flavonoids (TF) ($r = 0.98$).

In view of the results regarding these parameters and their relations with irradiation, temperature and storage time, it is clear that the latter can be influenced by numerous aspects such as the choice of solvents used in the extraction, radiation dose, technological processes, as well as the specific nature of the product [39].

CONCLUSION

At time 0, a dose of 3 kGy provided all of the most available bioactive compounds, raising their antioxidant potential, except for total phenolic compounds, which maintained similar levels to the non-irradiated sample. In relation to the storage temperatures during 120 days, gradual losses occurred in all the analysed parameters. Thus, for the total anthocyanin content and total phenolic compounds, the sample irradiated with a 1 kGy dose remained most stable during the various storage temperatures; especially at 4 °C with losses of only 9.33, 31.79 and 10.98%, respectively.

The analysis of the kinetic data indicated a first-order reaction for the degradation of total anthocyanin content; the *k* values showed that the thermal stability of the anthocyanins decreased with increasing temperature, especially from 35 to 45 °C. At all the temperatures, the $t_{1/2}$ values were greater for the irradiated samples. In the case of cyanidin-3-glucoside, the irradiated samples were stable between 35 and 45 °C. The results showed that combination of irradiation with different temperatures may improve the shelf-life of black rice flour.

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