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Effects of gamma radiation on the phenolic compounds and *in vitro* antioxidant activity of apple pomace flour during storage using multivariate statistical techniques

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ABSTRACT

The aim of this study was to evaluate the effects of gamma radiation on the phenolic compounds and antioxidant activity of apple pomace flour (0, 1 and 2 kGy dose at times of 0, 3, 6 and 9 months) during storage using multivariate techniques. The a^* and $h°$ colour attributes at a dose of 1 kGy, and the phenolic compound phloridzin showed constant levels during the experiment. The dose of 1 kGy at time 0 provides better results for antioxidant activity, total phenolic compounds and some individual compounds. Chlorogenic acid and its class of hydroxycinnamic acids increased proportionally with time in the irradiated samples. The chemometric approach made it possible to observe the influence of irradiation and storage time on the samples of apple pomace flour. The effect of irradiation on the phenolic compounds was related to the intensity and the characteristics of each individual compound. *Industrial relevance:* Irradiation has been shown to be an economically viable alternative for the treatment of foods; it has several advantages compared to traditional processing methods and can be applied independently or combined with existing techniques such as dehydration. Thus, this combination of treatments, as well as increasing the shelf life of apple pomace also increases the concentrations of nutrients and can be applied in the pharmaceutical, cosmetics and food industries. This advantage can translate into economic advantages, with added values in various applications and help to partially reduce negative impacts on the environment.

1. Introduction

The sustainable production of food and the recovery of waste are important issues in the food industry. The steady increase in the production and sale of apples worldwide has resulted in a huge amount of residue, apple pomace, which generates about 12 million tons per year (USDA, 2014), representing around 18% of production.

With added economic value and appropriate treatments, there are several alternatives to add value to this by-product. It can serve as a promising raw material for the direct extraction of bioactive compounds, the bioproduction of high value-added products, as well as being incorporated into foods, in ethanol production, as flavour constituents, in natural gas, as citric acid in solid state fermentation, in the formation of culture media for the production of edible mushrooms, and in the extraction of enzymes and pectin, among others (Coelho & Wosiacki, 2010, Dhillon, Kaur, & Brar, 2013, Dhillon, Kaur, Brar, & Verma, 2012, Paganini, Nogueira, Silva, & Wosiacki, 2005 and Rabetafika, Bchir, Blecker, & Richel, 2014).

Apple pomace is a good source of phenolic compounds (Schieber, Stintzing, & Carle, 2001), with phenolic acids and flavonoids being the most commonly found (Suárez et al., 2010). These antioxidants are

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recognised as bioactive compounds that act against possible harmful effects caused by free radicals. They work in the prevention and control of cardiovascular and pulmonary diseases, type 2 diabetes, allergies, obesity, hypercholesterolemia and they have anticarcinogenic activity, among others (Neto, Loving, & Liberty, 2008, Pierini, Gee, Belshaw, & Johnson, 2008 and Shahidi, 2012). Thus, the consumption of products that are the sources of these phytochemicals, allied to a healthy lifestyle, can provide beneficial health effects. The use of chemometrics was recently applied in studies related to apples and fruit juices to classify the antioxidant capacity and phenolic compounds (Braga et al., 2013, Zielinski et al., 2014a), with positive results.

Food irradiation is a processing technology that aims to improve food security. In recent decades it has been widely researched and its effects are known to act in preservation, reducing microbial load or sterilisation, increasing the shelf life of products and especially to maintain the quality of food. Its efficacy and safety are proven (FAO, IAEA, WHO). With regard to the effects of radiation treatment on the levels of antioxidants and phytochemicals, this will depend on the dose that is applied, on the product specificity, and the sensibility of each phytochemical (Alothman, Bhat, & Karim 2009). Although the radiation dose administered to a product plays an important role in the concentration of antioxidants, studies have mentioned that the choice of extraction solvent also plays a significant role (Tsai, Tsai, & Mau, 2007).

Several papers have been published on the positive impact of radiation on the preservation and antioxidant potential plant products (Alighourchi, Barzegar, & Abbasi, 2008; Khan et al., 2015; Pereira et al., 2014; Song et al., 2006). To date, there have been no studies in the literature on the effect of irradiation on the phenolic compounds and antioxidant activity of apple pomace stored for long periods. Thus, the aim of this study was to evaluate the effects of gamma radiation on the phenolic compounds and antioxidant activity of apple pomace flour during storage by using multivariate techniques.

2. Materials and methods

2.1. Materials and chemicals

Apples ('Fuji' cultivar) used in the experiments were purchased in the local supermarket at commercial maturity in the city of Ponta Grossa (25° 05′ 42″ S 50° 09′ 43″ O), Paraná, Brazil, from the 2013 harvest.

Acetone, acetonitrile, methanol, and acetic acid were purchased from J. T. Baker (Phillipsburg, NJ, USA). The reagents Folin–Ciocalteau, DPPH (2,2-diphenyl-2-picrylhydrazyl)TPTZ (2,4,6-Tri (2-pyridyl)-s-triazine), Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid), phloridzin, phloretin, (+)-catechin, (−)-epicatechin, procyanidin B1, procyanidin B2, quercetin, quercetin-3-D-galactoside, quercetin-3-β-D-glucoside, quercetin-3-*O*-rhamnoside, quercetin-3-*O*-rutinoside, chlorogenic acid, *p*-coumaric acid, caffeic acid and gallic acid were purchased from Sigma–Aldrich (St. Louis, MO, USA). The aqueous solutions were prepared using ultra-pure water (Milli-Q, Millipore, São Paulo, SP, Brazil).

2.2. Processing of apple pomace

To obtain the pomace, the apples were selected, washed in 1% chlorinated water, cut and ground in a Wiley mill (Metvisa) (Coelho & Wosiacki, 2010). The residue was washed three times with room temperature water at a ratio of 2:1 to remove the soluble solid surface, according to the methodology proposed by Paganini, Nogueira, Silva, & Wosiacki (2005). After this procedure, the pomace was subject to dehydration in a circulation oven and hot air renewal (MA 035 model, Marconi, Piracicaba, São Paulo Brazil) at a temperature of 90 °C to dry. It was then ground and sieved using 60 mesh to obtain the apple pomace flour (APF). This flour was separated and packed in samples of about 250 g in small non-toxic polyethylene bags with hermetic closure. Then, the samples were irradiated with 3 doses (0, 1 and 2 kGy) and 4 storage periods (0, 3, 6 and 9 months). After irradiation, the sample with 0 kGy dose (control), 1 and 2 kGy were subjected to the analysis (time 0). The other samples were stored at room temperature until the end of each storage time.

2.3. Sample Irradiation

All the samples of the apple pomace flour were subjected to gamma radiation at doses of 0, 1 and 2 kGy at 0.87 kGy/h dose rate. Harwell Amber 3042 dosimeters were used to measure the radiation dose and the uncertainty dose was less than 1%. The irradiation source was with Cobalt 60 (Gammacell Excell 220—MDS Nordion) located in the Centre for Nuclear Energy in Agriculture (CENA/USP).

2.4. Colour and A_w measurement

The colour properties L^* (lightness), a^* (redness), and b^* (yellowness) of the APF samples were determined using a HunterLab MiniScan EZ colorimeter (Reston, Va., U.S.A.). The samples were placed in cells of 1 cm and the L^*, a^*, and b^* values were performed 6 times using Iluminant D65 with observation angle of 10°. The Chroma (C^*) parameter was calculated by $C^* = (a^{*2} + b^{*2})^{1/2}$. The hue angle ($h°$) parameter was calculated by $h° = \tan^{-1}(b^*/a^*) + 180°$ when $a^* < 0$ and $h° = \tan^{-1}(b^*/a^*) + 180°$, where $a^* < 0$ and $h° = \tan^{-1}(b^*/a^*)$, where $a^* > 0$ (Zielinski et al., 2014b). The water activity (A_w) was measured

Table 1
Chromatographic parameters of phenolic compounds analysed by HPLC-DAD.

Phenolic compounds	Retention time (min)	UV bands (nm)	Regression equation	R^2	LOD (μg/mL)	LOQ (μg/mL)
Caffeic acid	19.76	323.9	y = 4E + 07x − 237524	0.999	0.13	0.44
Catechin	8.91	278.7	y = 6E + 06x − 114983	0.999	0.08	0.28
Chlorogenic acid	9.00	326.9	y = 2E + 07x − 695106	0.998	0.19	0.62
Coumaric acid	15.33	309.6	y = 5E + 07x + 1E + 06	0.995	0.03	0.09
Epicatechin	12.56	278.4	y = 6E + 06x − 123503	0.999	0.07	0.23
Gallic acid	3.25	271.5	y = 2E + 07x − 371284	0.999	0.15	0.50
Kaempferol	28.44	364.4	y = 1E + 08x − 501059	0.997	0.77	2.56
Myricetin	32.86	364.4	y = 6E + 06x − 106416	0.997	0.15	0.50
Phloretin	32.14	285.5	y = 3E + 07x − 209182	0.998	0.03	0.10
Phloridzin	25.43	284.3	y = 2E + 07x − 217901	0.999	0.09	0.30
Procyanindin B1	6.91	278.7	y = 4E + 06 − 3175	0.997	0.54	1.81
Procyanindin B2	9.85	279.8	y = 5E + 06 − 2351	0.997	0.17	0.56
Quercetin	23.50	376.2	y = 1E + 07x − 163002	0.994	0.98	3.26
Quercetin-3-D-galactoside	18.73	355	y = 7E + 07x − 69383	0.998	0.06	0.19
Quercetin-3-D-glucoside	19.27	353.8	y = 2E + 07x − 90936	0.998	0.26	0.87
Quercetin-3-*O*-rhamnoside	21.47	349	y = 2E + 07x − 4352.3	0.998	0.27	0.89
Quercetin-3-*O*-rutinoside	18.49	355	y = 1E + 07x − 356302	0.998	0.07	0.23

Note: LOD: limit of detection; LOQ: limit of quantification.

with a digital a_w meter (Aqualab®, USA). All determinations were performed in triplicate.

2.5. Extraction of phenolic compounds

Phenolic compounds were extracted from APF according to the methodology described by Alberti et al., (2014). 1 g of the sample was extracted (2 times) with 15 mL of 84.5% methanol for 15 min at 28 °C and 65% acetone for 20 min at 10 °C. After the mixture was centrifuged at 8160 rpm for 20 min at 4 °C (HIMAC CR-GII, Hitachi, Ibaraki, Japan) and lyophilized. Before the analysis the samples were reconstituted with 2 mL of 2.5% acetic acid and methanol (3:1) and filtered through a nylon syringe filter 0.22 μm (Waters, Milford, MA, USA).

2.6. Measurement of in vitro antioxidant activity

The total antioxidant potential of the extracts was determined using the ferric reducing antioxidant power (FRAP) assay according to the method proposed by Benzie and Strain (1996), with minor changes. The assay is based on the reducing power of antioxidants present in extracts, in which a potential antioxidant reduces the ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ). Measurements were performed using a microplate reader (Epoch microplate spectrophotometer, Synergy-BioTek, Winooski, VT, USA), and the absorbance was recorded at a wavelength of 593 nm after 6 min. A standard curve (FRAP = 805.80 × absorbance; $R^2 = 0.999$; $p < 0.001$) was plotted using different concentrations of Trolox (0.1–1.0 mmol/L). The results were expressed in mmol Trolox equivalents per gram of sample (mmoL TE/g). All determinations were performed in triplicate.

The DPPH radical scavenging activity of the extracts was determined in triplicate according to the method of (Brand-Williams, Cuvelier, & Berset, 1995) with minor modifications. This method determines the hydrogen donating capacity of molecules and does not produce oxidative chain reactions or react with free radical intermediates. Diluted samples (100 μL) were mixed with 3.9 mL of 60 μmol/L methanolic DPPH. The absorbance was measured at 517 nm using a spectrophotometer (model Mini UV 1240, Shimadzu) after the solution had been allowed to stand in the dark for 30 min. Methanol was used as a negative control (blank). The free-radical scavenging activity of each sample towards DPPH radical was calculated using Eq. 1. All determinations were performed in triplicate.

$$\text{Antioxidant activity\%inhibition} = [1-(\text{Abs517 sample}/\text{Abs517 blank})] \times 100 \quad (1)$$

2.7. Total phenolic content

The total phenolic content was determined by colorimetric analysis using Folin–Ciocalteau reagent as described by Singleton & Rossi (1965). In a test tube, 8.4 mL of distilled water, 100 μL of sample or (+)-catechin (standard, 10–400 mg/L), and 500 μL of Folin–Ciocalteau reagent were added and the mixture was vortexed for 10 s. After 3 min, 1.0 mL of saturated sodium carbonate was added into each tube and the tube was agitated immediately in a vortex. After 1 h, the absorbance was measured using a spectrophotometer (model mini UV 1240, Shimadzu, Tokyo, Japan) at 720 nm. The total phenolic content was expressed as catechin equivalents (mg catechin/100 g of APF) of the sample. All determinations were performed in triplicate.

2.8. HPLC analysis of phenolic compounds and method validation

Chromatographic analysis was performed in HPLC apparatus (2695 Alliance, Waters, Milford, MA, USA), with a photodiode array detector PDA 2998 (Waters, Milford, MA, USA) and Symmetry C_{18} (4.6 × 150 mm, 3.5 μm) column (Waters, Milford, MA, USA) at

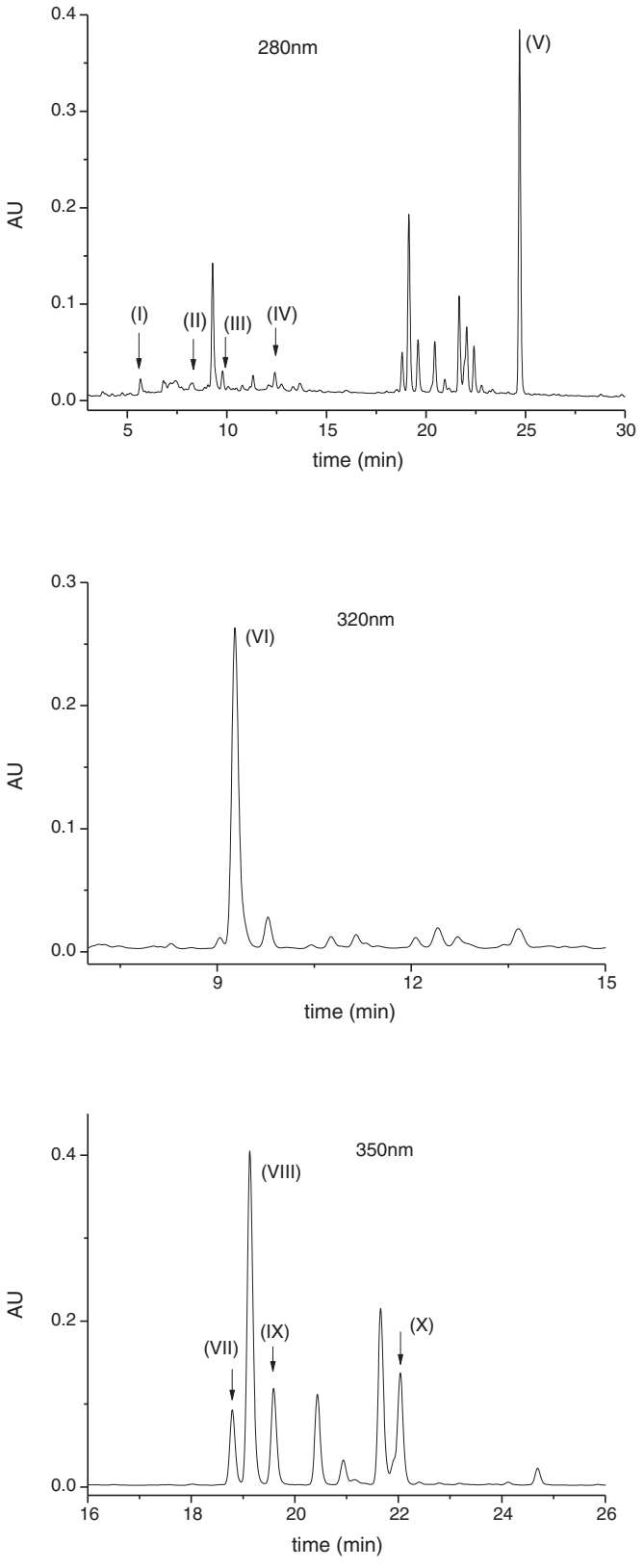

Fig. 1. Chromatograms obtained of phenolic extract of APF (1 kGy dose at time 3 month) at 280 ηm, 320 ηm and 350 ηm. (I) procyanindin B1, (II) catechin, (III) procyanindin B2, (IV) epicatechin, (V) phloridzin, (VI) chlorogenic acid, (VII) quercetin-3-O-rutinoside, (VIII) quercetin-3-O-galactoside, (IX) quercetin-3-O-glucoside and (X) quercetin-3-O-rhamnoside.

20 °C. The mobile phase was composed of solvent A (2.5% acetic acid, *v*/*v*) and solvent B (acetonitrile) with the following gradient: 3–9% B (0–5 min), 9–16% B (5–15 min), 16–36.4% B (15–33 min), followed by an isocratic run at 100% of B (5 min) and reconditioning of the column (3% of B, 10 min). The flow rate was 1.0 mL/min. Identification of phenolic compounds was performed by comparing their retention time and spectra with those of standards. The runs were monitored at 280 nm (flavan-3-ols and dihydrochalcones), 320 nm (hydroxycinnamic acids) and 350 nm (flavonols) (Alberti et al., 2014). Quantification was performed using calibration curves of standards (at least seven concentrations were used to build the curves). All determinations were performed in triplicate.

The regression equations of pure chemical standards, limits of detection, quantification limits, retention time and wavelength used to quantify the phenolic compounds present in apple pomace flour samples are shown in Table 1. The limits of detection (LOD) and the limit of quantitation (LOQ) were determined by a method based on analytic curve parameters, according Eq. 2 (ANVISA, 2003).

$$LOD = \frac{\mathrm{dp} \times 3,3}{\mathrm{S}} \quad (2)$$

$$LOQ = \frac{\mathrm{dp} \times 10}{\mathrm{S}} \quad (3)$$

where: dp: standard deviation of the response; S: slope of the analytical curve.

Examples of the obtained chromatograms are shown in Fig. 1.

2.9. Statistical analysis

The data was presented as mean ± standard deviation and the means was compared by Duncan's test ($p < 0.05$). A chemometric approach consisting of principal component analysis (PCA) and hierarchical cluster analysis (HCA) applied on the colour attributes, compounds phenolic and antioxidant activity analysis. All the results were previously autoscaled to standardise the statistical significance for all the variables. Then, a matrix of samples (n = 36) and response variables (n = 21) was constructed; the samples were adopted as lines and the variables as columns, making a total of 756 data points. Pearson products (r) were used to evaluate the strength of correlation among the response variables.

PCA was applied to separate the samples according to the colour attributes, phenolic compounds and antioxidant activity analysis. The results obtained for each of the parameters were adjusted as columns and the samples were used as lines. Eigenvalues greater than 1.0 were adopted to explain the projection of the samples on the graph and two-dimensional analysis was based on linear correlations (Granato, Branco, Faria, & Cruz, 2011).

For the HCA, the similarities of the samples and the variables were calculated based on the Euclidean distance, and the hierarchical agglomerative Ward method was used to cluster the samples (Granato, Oliveira, Caruso, Nagato, & Alaburda, 2013). In order to compare the results between the clusters that were formed, Levene's test was performed to verify the homogeneity of variance, while one-way Anova and Fisher's LSD test were used to identify the differences between the clusters. The Kruskal–Wallis test was used to compare the variables with non-parametric data ($p < 0.05$). Statistical analysis was performed using STATISTICA 7.0 software (Stat-Soft Inc., Tulsa, OK, USA).

3. Results and discussion

The effects of gamma radiation on the colour parameters of the APF during storage are shown in Table 2. Yan & Kerr (2013) evaluated the colours of dried apple pomace, and the L^* values ranged from 71.50 to 75.70; for a^* they ranged between 6.19 and 6.28; and for b^* they ranged between 21.30 and 26.70, at temperatures from 80 to 110 °C. Canteri, Nogueira, Wosiacki, & Petkowicz (2012) found values of $L^* = 51.8$, $a^* = 5.4$ and $b^* = 18.2$.

Brightness values range from light to dark; the value 0 (zero) corresponds to black and one hundred (100) to white. Evaluating the L^* colour attribute, the values ranged from 70.48 (2 kGy dose at times of 6 and 9 months) to 72.60 (1 kGy dose at time 0), indicating a colouration with a tendency to white (Table 2). Gamma radiation did not influence the brightness of the apple pomace flour; however, during the storage period this parameter was significantly decreased ($p < 0.05$) at a dose of 1 kGy. Camargo, Canniatti-Brazaca, Mansi, Domingues & Arthur (2011) obtained similar results in a study of irradiated peanuts, where there was a decrease in L^* during storage.

The values of a^* and b^* represent the colours, ranging from red to green (a^*) and from yellow to blue (b^*). The values for the a^* parameter, in the irradiated samples at time 0, were lower when compared ($p < 0.05$) to the control sample, however, during the storage period the values of a^* decreased with a 0 kGy dose and increased in the samples at a dose of 2 kGy. The b^* parameter in the samples irradiated with a dose of 2 kGy was stable during the storage period, in contrast, at a dose of 0 and 1 kGy these levels decreased (Table 2). Lee, Sung, Lee, and Kim (2004), did not detect changes in the colour of red pepper powder irradiated with doses between 0 and 7 kGy.

Table 2
Effects of gamma irradiation on the colour attributes in APF during storage.

Color attributes	Doses (kGy)	Time (months)			
		0	3	6	9
L^*	0	71.50 ± 1.24	70.74 ± 0.62	71.83 ± 1.52	71.46 ± 1.18
	1	72.60 ± 1.16^{a}	71.72 ± 0.98^{ab}	71.21 ± 0.28^{ab}	70.69 ± 0.36^{b}
	2	71.88 ± 0.79	71.57 ± 1.27	70.48 ± 0.43	70.48 ± 0.68
a^*	0	9.72 ± 0.14^{Aa}	8.27 ± 0.25^{b}	8.28 ± 0.25^{Ab}	8.24 ± 0.19^{Ab}
	1	7.52 ± 0.78^{B}	7.45 ± 0.60	6.83 ± 0.67^{B}	6.95 ± 0.53^{B}
	2	7.42 ± 0.51^{Bb}	7.42 ± 0.27^{b}	8.90 ± 0.53^{Aa}	8.91 ± 0.52^{Aa}
b^*	0	22.70 ± 1.06^{a}	23.36 ± 0.43^{a}	20.84 ± 0.23^{Ab}	20.25 ± 0.70^{b}
	1	22.07 ± 1.32^{a}	21.67 ± 1.70^{a}	18.41 ± 1.55^{Bb}	18.26 ± 1.09^{b}
	2	20.87 ± 0.96	20.25 ± 1.52	20.45 ± 1.26^{AB}	20.00 ± 1.00
C^*	0	24.70 ± 0.97^{a}	24.78 ± 0.46^{a}	22.42 ± 0.30^{Ab}	21.87 ± 0.72^{b}
	1	23.32 ± 1.37^{a}	22.92 ± 1.71^{a}	19.63 ± 1.69^{Bb}	19.54 ± 1.20^{b}
	2	22.15 ± 0.92	21.57 ± 1.47	22.31 ± 1.23^{A}	21.89 ± 1.07
$h°$	0	66.80 ± 1.03^{Bc}	70.50 ± 0.44^{a}	68.34 ± 0.40^{ABb}	67.85 ± 0.24^{Abc}
	1	71.19 ± 1.71^{A}	71.00 ± 1.43	69.65 ± 0.32^{A}	69.18 ± 0.49^{A}
	2	70.41 ± 1.49^{Aa}	69.82 ± 1.22^{a}	66.45 ± 1.54^{Bb}	66.01 ± 0.94^{Bb}

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 storage. The significant differences at a level of 5% were performed by Duncan's test.

C^* values close to zero are indicative of more neutral colours (white/gray) and those colours close to 60 are indicative of greater intensity. The samples irradiated with a 2 kGy dose remained more stable during the storage period, the opposite occurred with the 0 and 1 kGy, which decreased the C^* values over time (Table 2). The results for the hue angle indicated red colour at 0 $h°$, yellow at 90 $h°$, green at 180 $h°$ and blue at 270 $h°$. The irradiated samples at time 0, presented a colouration that was closer to yellow when compared to the control sample, and the sample irradiated with a 1 kGy dose remained stable during the storage period (Table 2).

The results obtained for the individual phenolic compounds (IPC) and their classes determined by HPLC, total phenolics compounds (TPC), and antioxidant capacity by FRAP and DPPH assays are shown in Table 3.

By analysing the values for antioxidant activity (FRAP) and phenolic compounds it was observed that the samples with the 1 kGy dose on day 0 showed greater values ($p < 0.05$) compared with the samples treated with doses of 0 and 2 kGy. Over time there was a decrease in the levels for all the samples. However, at a dose of 1 kGy, there was no significant difference in loss, for the TPC, during storage. The results showed that the inhibition of DPPH ranged from 30.68 (0 kGy dose at time 9) to 31.66% (1 kGy dose at time 0) of reduction. The levels of DPPH remained stable during storage ($p \geq 0.05$) and the dose of 1 kGy showed a higher inhibition at time 0.

Koike et al., (2015) studied edible, lyophilised and irradiated flowers, and they found that all the phenolic compounds and antioxidant activity were detected in higher amounts in the irradiated samples (especially with a 1 kGy dose). This occurrence might be due to differences in the atmospheric composition inside the polyethylene bags, where samples were kept, or to alterations in the molecular oxygen availability induced by the ionizing effect of the irradiation.

Table 3
Individual phenolic compounds (IPC) and their classes, total phenolic compounds (TPC) and antioxidant capacity in APF during storage.

Analysis (mg/100 g)	Time (months)				
	Doses (kGy)	0	3	6	9
Hydroxycinnamic acids	0	19.88 ± 0.21^{Ca}	18.03 ± 0.35^{Cb}	17.32 ± 0.26^{Cc}	15.74 ± 0.50^{Cd}
	1	30.43 ± 0.44^{Ad}	33.12 ± 0.17^{Ab}	35.14 ± 0.50^{Ac}	39.07 ± 0.61^{Ad}
	2	24.18 ± 0.35^{Bd}	25.75 ± 0.44^{Bc}	27.82 ± 0.25^{Bb}	28.55 ± 0.47^{Ba}
Chlorogenic acid	0	15.33 ± 0.56^{Ca}	13.50 ± 0.10^{Cb}	12.72 ± 0.62^{Cc}	11.03 ± 0.23^{Cd}
	1	27.55 ± 0.55^{Ad}	28.43 ± 0.39^{Ac}	30.07 ± 0.59^{Ab}	34.69 ± 0.99^{Aa}
	2	19.07 ± 0.65^{Bd}	20.99 ± 0.24^{Bc}	23.21 ± 0.28^{Bb}	24.41 ± 0.28^{Ba}
Flavanols	0	nd	4.63 ± 0.66^{Cc}	7.77 ± 0.81^{Cb}	10.53 ± 0.50^{Ca}
	1	56.82 ± 1.03^{Ab}	58.13 ± 1.35^{Aa}	50.61 ± 1.44^{Ac}	38.09 ± 0.87^{Ad}
	2	46.79 ± 1.15^{Ba}	40.61 ± 1.70^{Bb}	34.85 ± 1.46^{Bc}	30.50 ± 2.34^{Bd}
Catechin	0	nd	nd	nd	2.01 ± 0.25^{Ca}
	1	nd	6.07 ± 0.65^{Ba}	5.70 ± 0.18^{Bb}	2.13 ± 0.53^{Bc}
	2	14.62 ± 0.46^{Aa}	11.12 ± 0.25^{Ab}	9.09 ± 0.84^{Ac}	7.82 ± 0.49^{Ad}
Epicatechin	0	nd	nd	nd	nd
	1	21.96 ± 1.15^{Aa}	21.45 ± 0.63^{Ab}	14.93 ± 0.60^{Ac}	7.68 ± 1.03^{Ad}
	2	13.63 ± 1.14^{Ba}	13.22 ± 1.15^{Bb}	10.04 ± 0.50^{Bc}	7.06 ± 1.07^{Bd}
Procyanindin B1	0	nd	nd	3.12 ± 0.36^{C}	4.46 ± 0.62^{C}
	1	17.70 ± 0.35^{Aa}	13.47 ± 0.68^{Ab}	13.08 ± 0.78^{Ac}	11.53 ± 0.65^{Ad}
	2	7.43 ± 0.25^{Ba}	5.06 ± 0.53^{Bb}	4.63 ± 0.61^{Bc}	4.61 ± 0.19^{Bc}
Procyanindin B2	0	nd	4.63 ± 0.66^{Ca}	4.66 ± 0.57^{Ca}	4.06 ± 0.55^{Cb}
	1	17.16 ± 0.53^{Aa}	17.14 ± 0.36^{Aa}	16.90 ± 0.05^{Ab}	16.76 ± 0.57^{Ac}
	2	11.12 ± 0.65^{Bab}	11.22 ± 0.60^{Ba}	11.09 ± 0.89^{Bb}	11.01 ± 0.63^{Bb}
Dihydrochalcones	0	38.53 ± 0.49^{C}	38.63 ± 0.49^{C}	38.60 ± 0.11^{C}	38.63 ± 0.51^{C}
	1	61.77 ± 0.65^{A}	61.84 ± 0.39^{A}	61.90 ± 0.26^{A}	61.70 ± 0.26^{A}
	2	41.97 ± 0.65^{B}	42.04 ± 0.39^{B}	42.14 ± 0.39^{B}	41.94 ± 0.39^{B}
Phloridzin	0	34.53 ± 0.49^{C}	34.40 ± 0.25^{C}	34.23 ± 0.67^{C}	34.43 ± 0.59^{C}
	1	57.77 ± 0.65^{A}	57.57 ± 0.95^{A}	57.60 ± 1.17^{A}	57.70 ± 1.17^{A}
	2	37.97 ± 0.65^{B}	37.65 ± 0.25^{B}	37.74 ± 1.21^{B}	37.77 ± 2.29^{B}
Flavonols	0	50.35 ± 4.15^{Ba}	36.56 ± 0.37^{Cb}	35.46 ± 0.79^{Cc}	30.57 ± 0.86^{Cd}
	1	72.60 ± 1.16^{Aa}	63.90 ± 1.30^{Ab}	61.03 ± 0.44^{Ac}	55.94 ± 1.05^{Ad}
	2	49.88 ± 0.65^{Ba}	43.48 ± 0.90^{Bb}	42.20 ± 0.93^{Bc}	35.62 ± 1.24^{Bd}
Quercetin-3-*O*-rutinoside	0	8.11 ± 0.13^{Ca}	6.23 ± 0.57^{Cb}	5.27 ± 0.14^{Cc}	3.74 ± 0.34^{Cd}
	1	25.89 ± 0.89^{Aa}	21.78 ± 0.40^{Ab}	21.20 ± 0.08^{Ac}	20.79 ± 0.89^{Ad}
	2	20.15 ± 0.57^{Ba}	18.21 ± 0.68^{Bb}	17.83 ± 0.56^{Bc}	15.68 ± 0.75^{Bd}
Quercetin-3-D-galactoside	0	19.02 ± 0.66^{Ba}	11.46 ± 0.46^{Bd}	12.58 ± 0.51^{Bb}	12.04 ± 0.63^{Bc}
	1	20.40 ± 0.14^{Aa}	16.73 ± 0.63^{Ab}	16.12 ± 0.41^{Ac}	15.60 ± 0.12^{Ad}
	2	13.82 ± 0.56^{Ca}	11.32 ± 0.17^{Cb}	11.04 ± 0.36^{Cc}	8.25 ± 0.09^{Cd}
Quercetin-3-β-D-glucoside	0	11.47 ± 4.47^{Aa}	7.31 ± 0.20^{Bb}	7.13 ± 0.30^{Bb}	4.80 ± 0.04^{Bc}
	1	10.49 ± 0.15^{Ba}	10.46 ± 0.29^{Ab}	10.25 ± 0.35^{Ac}	6.96 ± 0.08^{Ad}
	2	6.69 ± 0.06^{Cb}	6.73 ± 0.21^{Ca}	4.61 ± 0.2^{Cc}	3.22 ± 0.11^{Cd}
Quercetin-3-*O*-rhamnoside	0	11.75 ± 0.26^{Ba}	11.56 ± 0.06^{Bb}	10.48 ± 0.25^{Bc}	10.00 ± 0.06^{Bd}
	1	15.82 ± 0.31^{Aa}	14.94 ± 0.37^{Ab}	13.46 ± 0.47^{Ac}	12.60 ± 0.06^{Ad}
	2	9.23 ± 0.36^{Ca}	7.22 ± 0.17^{Cb}	8.72 ± 0.20^{Cc}	8.47 ± 0.43^{Cd}
TPC	0	562.66 ± 49.79^{Ba}	542.99 ± 25.69^{Bab}	482.98 ± 27.6^{b}	477.75 ± 29.74^{Cb}
	1	660.58 ± 33.15^{A}	640.58 ± 7.51^{A}	622.84 ± 69.50	582.67 ± 12.98^{A}
	2	645.64 ± 25.55^{ABa}	622.31 ± 4.90^{Aab}	540.70 ± 108.67^{ab}	533.96 ± 28.25^{Bb}
FRAP (mmol TE/g)	0	36.19 ± 1.36^{Ba}	32.43 ± 0.35^{Bb}	28.34 ± 1.20^{c}	24.44 ± 0.10^{Cd}
	1	57.30 ± 1.15^{Aa}	46.80 ± 2.50^{Ab}	32.22 ± 3.53^{c}	27.16 ± 0.03^{Ad}
	2	37.39 ± 1.18^{Ba}	34.56 ± 0.14^{Bb}	29.17 ± 0.79^{c}	26.70 ± 0.29^{Bd}
DPPH (%reduction)	0	30.99 ± 0.05^{B}	30.89 ± 0.59	30.81 ± 1.16	30.68 ± 0.62
	1	31.66 ± 0.05^{A}	31.17 ± 0.32	30.97 ± 1.02	30.90 ± 1.90
	2	31.09 ± 0.05^{B}	30.86 ± 0.91	30.82 ± 0.78	30.70 ± 0.20

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 storage. The significant differences at a level of 5% were performed by Duncan's test; nd = not detected or values below LOD.

Table 3 shows the classes and individual phenolic compounds, which resulted in different behaviours in relation to irradiation and storage. This study evaluated the classes of hydroxycinnamic acids (chlorogenic acid), flavanols (catechin, epicatechin, procyanidin B1 and B2), dihydrochalcones (phloridzin), and flavonols (quercetin-3-D-galactoside, quercetin-3-β-D-glucoside, quercetin-3-*O*-rhamnoside, quercetin-3-*O*-rutinoside). Gallic acid, coumaric acid, caffeic acid, phloretin, myricetin and kampferol were also evaluated, but were not detected in the samples of apple pomace flour, or the values were below the detection limit.

Flavonols were the class that had the highest levels of phenolic compounds, followed by dihydrochalcones. Hydroxycinnamic acids and flavanols were the classes that had the lowest values, respectively (Table 3). Lavelli & Corti (2011) studied the behaviour of phenolic compounds in dehydrated apple pomace stored for 9 months at different levels of A_w. They reported that the flavonols and dihydrochalcones resulted in higher concentrations compared with the other classes.

Phloridzin (dihydrochalcone) was the individual phenolic compound with the greatest concentration. It ranged from 34.23 to 57.77 mg/100 g and during the storage period remained constant at all doses. The 1 kGy dose showed the highest ($p < 0.05$) content of this compound (Table 3). This stability, which was derived from phloridzin, was also reported by Lavelli & Corti (2011). This result is in agreement with a previous evaluation of the ranking of phenolic reactivity. Phloridzin contains a monophenolic β ring, unlike other phenolic compounds which are present in apple rind, which contains a diphenolic β ring (René, Abasq, Hauchard, & Hapiot, 2010).

Chlorogenic acid was the second highest compound found in concentrations ranging from 11.03 to 34.69 mg/100 g (Table 3). The doses of 1 and 2 kGy were greater ($p < 0.05$), respectively, compared to the non-irradiated sample. This compound, and its class of hydroxycinnamic acids, showed a behaviour that was distinct from the others, the levels of the irradiated dose increased over time, whereas for the samples with 0 kGy, the values decreased. According to Alothman et al. (2009), the concentration of these phenolic compounds may be dependent on the time of evaluation, the dose administered, technological criteria, as well as the specific nature of the product.

The effect of irradiation and shelf life were also observed for the class of flavanols. Catechin content was only detected in the control sample after 9 months of storage; however, it was at a low concentration when compared to the 2 kGy dose, which showed catechin levels in all the periods, with a gradual loss during storage ($p < 0.05$). Epicatechin was not detected in the control sample; however, it was found in all periods in the doses of 1 and 2 kGy, with losses that were proportional to time; the sample irradiated with 1 kGy showed higher concentrations. The levels of procyanidin B1 in the control sample were detected from the sixth month, and for the irradiated samples, the behaviour was similar to that of epicatechin. Procyanidin B2 was the phenolic compound from this group that was most stable during the time, although it was not detected in the control sample at time 0; the dose of 1 kGy showed higher levels ($p < 0.05$) of phenolic compounds (Table 3).

This behavior of flavanols may be due to the destructive processes of oxidation and gamma radiation, which are able to break the chemical bonds of polyphenols, releasing soluble phenols with low molecular weight and increasing these compounds with antioxidant action (Adamo et al., 2004). Behgar, Ghasemi, Naserian, Borzoie, & Fatollahi (2011) reported that the differences in the effect of ionizing radiation on phenolic compounds may be partly due to the higher extractability of these compounds in irradiated samples. Rodríguez-Pérez et al., (2015) evaluated the stability of phenolic compounds in syrup cranberry irradiated and stored, and they observed that the procyanidin B1 significantly increased in samples with gamma radiation and catechin showed less stability.

All the samples of the flavonols class proved to be unstable during the period of analysis, and the dose of 1 kGy showed higher levels of phenolic compounds (Table 3). A study by Lavelli & Corti (2011) demonstrated that the instability of phenolic compounds present in apple pomace is related to the level of A_w, and they determined that a value above 0.32 is indicative of a reduction in those levels during storage. They also reported that the compounds in the classes of flavanols and flavonols were detected with levels above 0.54. In our study, the levels of A_w were also investigated, and the average A_w of the samples was 0.25 (0 months), 0.33 (3 months), 0.39 (6 months) and 0.43 (9 months). These results are in agreement with the study by Lavelli & Corti (2011) and some compounds, with or without irradiation, showed a reduction after the third month.

Regarding the behavior of these bioactive compounds and their interaction with irradiation and storage time, many are the factors that can influence, such as the choice of radiation, the dose administered, solvents used in the extraction, treatment and technological processes, level of Aw during storage and mainly the characteristics of each phenolic and each product. In this study the dose of 1 kGy at time 0 showed better results for antioxidant activity, total phenolic compounds and individual (epicatechin, procyanidin B1 and B2, quercetin-3-*O*-rutinoside, quercetin-3-D-galactoside, quercetin-3-*O*-rhamnoside) (Table 3).

In order to understand the data set, inter-relationships and the differences between the samples of the apple pomace flour during the storage period, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied to the following variables: colour parameters; individual phenolic compounds and their classes determined by HPLC, total phenolic compounds; and antioxidant capacity (FRAP).

The PCA shown in Fig. 2 relates to a two-dimensional graphical representation of the apple pomace flour samples. Principal component 1 (PC1, eigenvalue 11.68) and 2 (PC2, eigenvalue 4.83) explained 79.69% of the variance of the data.

The formation of three clusters based on the irradiated doses can be observed in Fig. 2. In the left area of PC1 were the samples with higher levels of flavanols, epicatechin, procyanidin B1 and B2, dihydrochalcones, phloridzin, flavonols, quercetin-3-*O*-rutinoside and TPC. With respect to PC2, the upper area concentrated samples with higher levels of brightness (L^*), chroma (C^*), b^*, FRAP, quercetin-3-D-galactoside and quercetin-3-β-D-glucoside. In the lower area were located the samples with higher chlorogenic acid content. The samples irradiated with a 1 kGy dose at all times, (coded as 2, 5, 8 and 11) were located in the second and third quadrants (left side). The samples irradiated with a 2 kGy

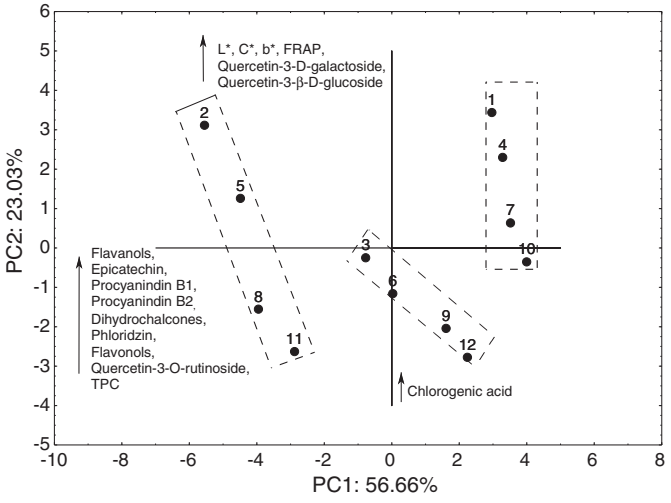

Fig. 2. A scatter plot PC1 × PC2 in relation to colour attributes, individual phenolic compounds (IPC) and their classes, total phenolic compounds (TPC) and antioxidant capacity (FRAP) in APF during storage. Samples: 1 (0 kGy – 0 months), 2 (1 kGy – 0 months), 3 (2 kGy – 0 months), 4 (0 kGy – 3 months), 5 (1 kGy – 3 months), 6 (2kGy – 3 months), 7 (0 kGy – 6 months), 8 (1 kGy – 6 months), 9 (2 kGy – 6 months), 10 (0 kGy – 9 months), 11 (1 kGy – 9 months), and 12 (2 kGy – 9 months).

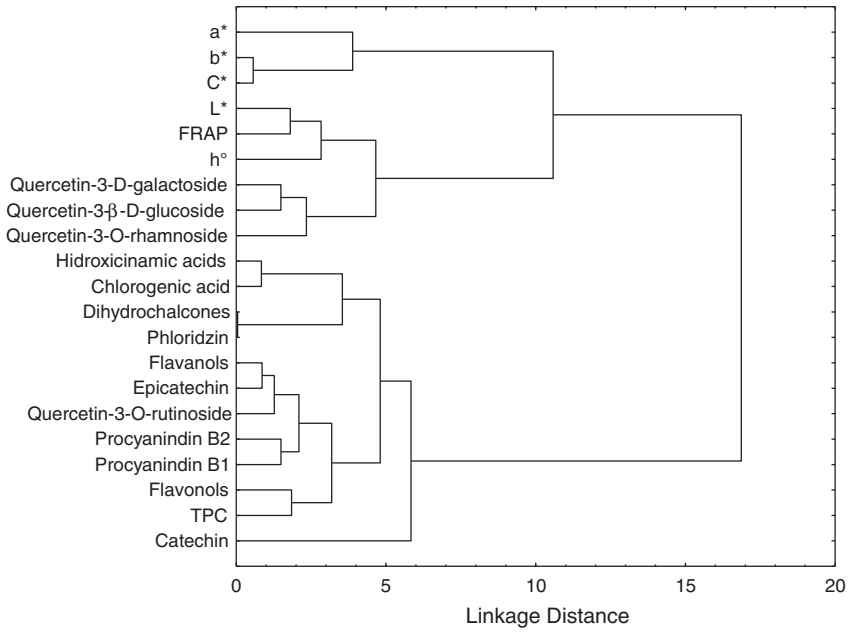

Fig. 3. Dendrogram obtained from hierarchical cluster analysis for APF samples during storage applied to the variables in relation colour attributes, individual phenolic compounds (IPC) and their classes, total phenolic compounds (TPC) and antioxidant capacity (FRAP).

dose (3, 6, 9 and 12) were located in the third and fourth quadrants, and the samples that were not irradiated (1, 4, 7 and 10) were located in the first and fourth quadrants (Fig. 2). PCA was an appropriate approach to verify the differences between the gamma radiation doses and storage time in the samples of apple pomace flour.

The dendrogram in Fig. 3 shows the association between the variables that were studied. The colour parameters b^* and C^* showed a strong association and positive correlation ($r = 0.98$; $p < 0.001$), and that the C^* parameter correlated with a^* ($r = 0.63$; $p < 0.001$). The brightness (L^*) was associated with FRAP ($r = 0.49$; $p < 0.05$), and in the h° attribute there was also an association with FRAP ($r = 0.65$; $p < 0.05$).

All the individual phenolic compounds showed a significant correlation ($p < 0.001$) with their respective classes. The antioxidant activity (FRAP) correlated significantly with epicatechin ($r = 0.73$; $p < 0.05$), procyanidin B1 ($r = 0.61$; $p < 0.05$) and with the class of flavonols ($r = 0.79$; $p < 0.05$); quercetin-3-D-galactoside ($r = 0.70$; $p < 0.05$); quercetin-3-β-D-glucoside ($r = 0.67$; $p < 0.05$); and quercetin-3-*O*-rhamnoside ($r = 0, 65$, $p < 0.05$). Among the IPC and their classes analysed, only chlorogenic acid (hydroxycinnamic acids), catechin and procyanindin B1 (flavanols) did not show significant ($p \geqslant 0.05$) correlation with antioxidant capacity by DPPH assay.

The total phenolic compounds (TPC) were positively correlated ($p < 0.05$) with all the individual phenolic compounds and classes, as

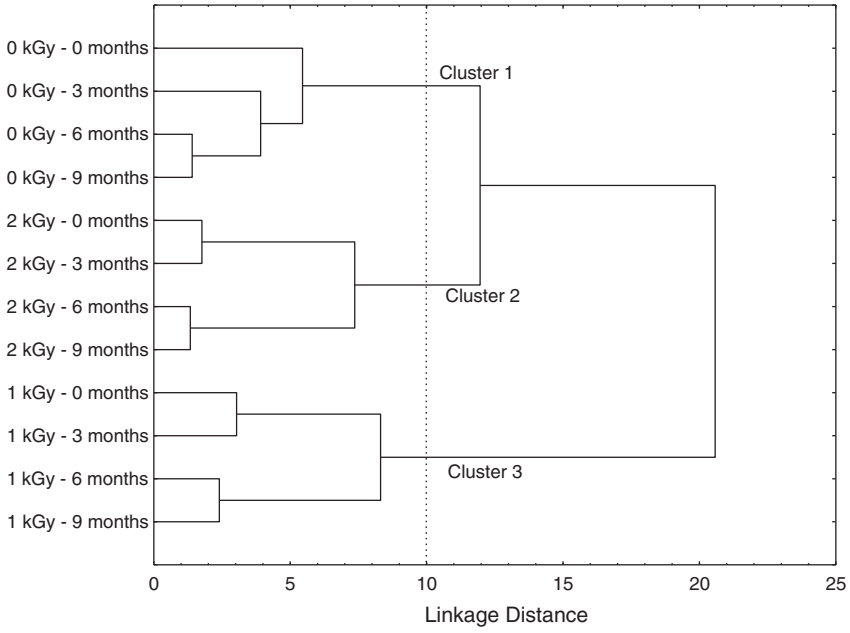

Fig. 4. Dendrogram obtained from hierarchical cluster analysis for APF samples during storage applied to the samples in relation to colour attributes, individual phenolic compounds (IPC) and their classes, total phenolic compounds (TPC) and antioxidant capacity (FRAP).

Table 4
Colour attributes, individual phenolic compounds—IPC (mg/100 g), total phenolic compounds—TPC (mg/100 g) and antioxidant capacity—FRAP (mmol TE/g) of APF using hierarchical cluster analysis (HCA).

Variables	Cluster 1 (0 kGy) n = 4	Cluster 2 (2 kGy) n = 4	Cluster 3 (1 kGy) n = 4	PSD	p-Value*	p-Value**
L^*	71.34	71.10	71.56	0.61	<0.05	0.50
a^*	8.63[a]	8.16[a]	7.19[b]	0.88	0.27	<0.001*
b^*	21.79[a]	20.39[b]	20.10[b]	1.54	0.48	<0.05*
C^*	23.44[a]	21.98[b]	21.35[b]	1.62	0.41	<0.05*
$h°$	68.38[b]	68.17[b]	70.25[a]	1.81	0.06	<0.05*
Hydroxycinnamic acids	17.74[c]	26.58[b]	34.44[a]	7.50	0.49	<0.001*
Chlorogenic acid	13.14[c]	21.92[b]	30.19[a]	7.61	0.06	<0.001*
Flavanols	5.74[c]	38.19[b]	50.92[a]	20.90	<0.05	<0.001*
Catechin	0.50[c]	10.66[a]	3.48[b]	4.98	<0.05	<0.001*
Epicatechin	nd	10.99[b]	16.51[a]	8.13	<0.001	<0.001*
Procyanindin B1	1.90[c]	5.43[b]	13.94[a]	5.96	<0.05	<0.001*
Procyanindin B2	3.34[c]	11.11[b]	16.99[a]	5.63	<0.05	<0.001*
Dihydrochalcones	38.60[c]	42.02[b]	61.80[a]	10.68	0.45	<0.001*
Phloridzin	34.40[c]	37.78[b]	57.66[a]	10.72	<0.05	<0.001*
Flavonols	38.24[c]	42.80[b]	63.37[a]	13.14	<0.001	<0.001*
Quercetin-3-O-rutinoside	5.84[c]	17.97[b]	22.41[c]	7.54	0.16	<0.001*
Quercetin-3-D-galactoside	10.95[b]	8.41[c]	14.20[a]	2.67	<0.05	<0.001*
Quercetin-3-β-D-glucoside	13.78[b]	11.11[c]	17.21[a]	3.59	<0.05	<0.001*
Quercetin-3-O-rhamnoside	7.68[b]	5.32[c]	9.54[a]	2.64	<0.001	<0.001*
TPC	516.59[b]	585.68[a]	626.67[a]	62.62	<0.05	<0.001*
FRAP	30.35[b]	31.96[b]	40.87[a]	9.41	<0.001	<0.05*

Note: Results expressed as mean ± pooled standard deviation. PSD: pooled standard deviation; nd: not detected or values below LOD. Different letters in the same line represent statistically different results ($p < 0.05$).
* Probability values obtained by Levene's test for homogeneity of variances.
** Probability values obtained by one-way ANOVA or Kruskal–Wallis test.

well as with the antioxidant activity (FRAP) ($r = 0.65$; $p < 0.001$) and DPPH ($r = 0.65$; $p < 0.05$). According to Picinelli, García, Sánchez, Madrera, & Valles (2009), the antioxidant method of ferric reducing power (FRAP) tends to show a highly significant correlation with phenolic compounds. Zielinski et al., (2014a), analysed apple juice and found significant association and correlation ($p < 0.01$) between antioxidant activity (FRAP) and total phenolic compounds ($r = 0.92$) and with chlorogenic acid ($r = 0.66$). The antioxidant capacity of hydroxycinnamic acids (chlorogenic acid) is related to the presence of catechol, which generates a semiquinone that is stabilised by hydrogen bonds when it is captured from the chemical structure (Amorati, Pedulli, Cabrini Zambonin, & Landi, 2006).

The similarity of the samples was evaluated by using HCA, where three clusters were suggested (Fig. 4). Cluster 1 was characterised by doses of 0 kGy, cluster 2 by doses of 2 kGy and cluster 3 by doses of 1 kGy.

In Table 4 the averages of each response variable were compared and the Anova results for the groups obtained using HCA were calculated.

Cluster 1 (0 kGy) had higher values for the colour parameters, such as a^* and b^*, and C^*, and showed no levels of epicatechin. Cluster 2 (2 kGy) showed lower values for the colour parameters of L^* and $h°$, quercetin-3-D-galactoside, quercetin-3-β-D-glucoside, quercetin-3-*O*-rhamnoside, as well as higher levels of catechin. Cluster 3 (1 kGy) had higher levels of all the classes and the IPC, except for catechin. The TPC and FRAP values were also classified in this cluster, confirming the positive correlations that were cited (Table 4).

4. Conclusion

Using a chemometric approach it was possible to observe the influence of irradiation and storage time on the samples of apple pomace flour, in which the effect of gamma radiation, using a ^{60}Co radioisotope, showed positive results. The irradiated samples remained stable during the storage period for all parameters colour, values of a^* and $h°$ were constant in the doses of 1 kGy and L^*, b^* and C^* in the doses of 2 kGy.

A dose of 1 kGy provided the most available individual phenolic compounds and total phenolic compounds, raising their antioxidant potential. During the storage period of 9 months, it was concluded that, regardless of radiation, gradual losses occurred in almost all the analysed parameters, except for phloridzin, which maintained constant levels during the research. Chlorogenic acid, as well as its class of hydroxycinnamic acids, proportionally increased with time in the irradiated samples. The effects of irradiation on the phenolic compounds will depend on the dose applied. It can increase, decrease or have insignificant effects, and it is necessary to take into consideration the specificity of the product and the sensitivity of each photochemical.

Irradiated apple pomace is a rich source of phytonutrients and it is stable for some individual phenolic compounds. It can be applied in products and can result in high added value and economy, as well as reducing the environmental impact.

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