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Assessment of the stability of proanthocyanidins and other phenolic compounds in cranberry syrup after gamma-irradiation treatment and during storage

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ABSTRACT

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 (RH) and under accelerated stability conditions was investigated. High-performance liquid chromatography coupled to electrospray ionisation quadrupole-time-of-flight mass spectrometry (HPLC–ESI-QTOF-MS) was used to characterise cranberry syrup. Afterwards, these compounds were quantified by HPLC–ESI-QTOF-MS and 4-dimethylaminocinnamaldehyde (DMAC) assay. A significant increase in the content of procyanidin B isomer 1 (from 4.4 to 7.0 μg/ml) and procyanidin A2 (from 83 to 93 μg/ml) was observed after irradiation and compared with the non-irradiated syrup. Procyanidin B isomers and prodelphinidin were stable at 25 °C during the first month of storage, whereas quercetin and some derivatives remained constant for 3 months of storage at this temperature. In short, after gamma-irradiation in dose of 5 kGy, most compounds were highly stable for a month at 25 °C.

1. Introduction

American cranberry (*Vaccinium macrocarpon*) is a rich source of bioactive phenolic compounds with antiproliferative, antioxidant, anti-inflammatory, and antimicrobial activities (Es-Safi, Guyot, & Ducrot, 2006). This berry has been traditionally used to treat and prevent urinary-tract infections in women and digestive-tract complaints. Today its anticancer properties have also been studied (Neto, 2011). Previous research suggests that some of these activities may be associated with its rich composition in flavonoids such as quercetin and proanthocyanidins (PACs) and its wealth of phenolic acids (Formica, 1995; Iswaldi et al., 2012; Prior & Gu, 2005).

Proanthocyanidins (PACs) are oligomeric and polymeric end-products of the flavonoid-synthesis pathway, since they consist of sequences of flavanol monomeric units such as catechin, epicatechin, gallocatechin, epigallocatechin, afzelechin, and epiafzelechin, which are connected by C–C linkages (B-type) and sometimes also by C–O–C linkages (A-type), as classified by (He, Pan, Shi, & Duan, 2008). PACs are noteworthy for their antioxidant activity, since their aromatic rings can effectively scavenge free radicals. Besides their antioxidant properties, these key compounds may present some pharmacological and medicinal properties, such as anti-carcinogenic, anti-inflammatory, and vasodilatory properties (Neto, 2011; Reed, 2002). Thus, considerable research has examined the therapeutic applications of these compounds (Vadodkar, Suman, Lakshmanaswamy, & Damodaran, 2012), especially in regard to the prevention and reduction of urinary-tract infections.

To increase the shelf-life of food products, radiation is a well-established non-thermal physical mode for food preservation. This process is also known as cold-pasteurisation because foods are kept near or at ambient temperature during processing (Alothman, Bhat, & Karim, 2009). This technique has been introduced as an alternative to other preservation methods because it does not harm the environment, reduces the amount of weight loss during post-harvest, and leaves no residues on the fruits (Carocho et al., 2012). Currently, low-level irradiation of food and food-products has been approved by the U.S.A. Food and Drug Administration (FDA) to reduce the incidence of illness associated with food-borne pathogens.

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In this way, previous studies have evaluated the chemical changes of phenolic compounds in strawberries or its effect on the anthocyanins yield and shelf-life extension of grape pomace and pomegranate juices after gamma-irradiation (Alighourchi, Barzegar, & Abbasi, 2008; Ayed, Yu, & Lacroix, 1999; Breitfellner, Solar, & Sontag, 2003). However, there is a paucity of information concerning the effect of irradiation methods and storage on the phytochemical composition from cranberry-derived products, and especially PACs.

The complexity of PACs in terms of molecular weight and linkage type makes it difficult to use a single quantification method. The 4-dimethylaminocinnamaldehyde (DMAC) spectrophotometric assay has become increasingly popular as a rapid technique to quantify the total amount of proanthocyanidins present in foods and beverages. The DMAC method was used to substantiate French health claims for the bacterial anti-adhesion activity of cranberry juice concentrate and juice-concentrate extract powder, cranberry-juice cocktail, and fresh frozen and pureed cranberry by the French food-safety authority "Agence Française de Sécurité Sanitaire des Aliments", AFSSA (Prior et al., 2010). In this way, DMAC is considered the best analysis available for a global quantitation of flavanols (flavan-3-ols), both simple and oligomeric ones (proanthocyanidins), but it does not offer information about the specific compounds present in foods (Boudesocque, Dorat, Pothier, Gueiffier, & Enguehard-Gueiffier, 2013). For this reason it is necessary to combine this technique with other more selective ones. In this regard, high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) has become a powerful method due to its high resolution, the capacity to separate a wide range of polar compounds by means of polarity differences, as well as its specificity.

Thus, the aim of the present work is to evaluate the qualitative and quantitative changes in the phenolic composition of cranberry syrup after gamma-irradiation, as well as the stability of these compounds during 6 months of storage in the irradiated cranberry syrup under different conditions: 25 °C and 60% relative humidity (RH) and under 40 °C and 75% RH, the latter for accelerated stability testing.

2. Material and methods

2.1. Chemicals

Standards of proanthocyanidin A2, *p*-coumaric acid, quercitrin, quercetin-3-*O*-glucoside, myricetin, (+)-catechin, and luteolin were from Extrasynthese (Genay, France). Formic acid and acetonitrile used for preparing mobile phases were from Sigma–Aldrich (Steinheim, Germany) and Lab-Scan (Gliwice, Sowinskiego, Poland), respectively. Ultrapure water with a resistivity value of 18.2 MΩ was obtained from Milli-Q system (Millipore, Bedford, MA, USA). Solvents were filtered before use with a Solvent Filtration Apparatus 58061 (Supelco, Bellefonte, PA, USA). HPLC grade methanol (99.9%) and HPLC grade acetone were purchased from Fisher Scientific (Loughborough, Leics, UK). Acetic acid of analytical grade was acquired from Sigma–Aldrich. Hydrochloric acid (37%) was from Panreac (Barcelona, Spain). HPLC grade ethanol (99%) was from Analar Normapul (Fontenay-sous-Bois, France) and 4-dimethylaminocinnamaldehyde (DMAC) was from Sigma–Aldrich.

2.2. Syrup sample preparation

A total of 15 g of a commercial extract of American cranberry (*V. macrocarpon*) were mixed with 500 ml of water to prepare a syrup. The syrup was separated into 0.5 ml aliquots to facilitate storage and the irradiation process.

2.3. Preparation of standards solution

Procyanidin A2 (purity ⩾99%) was used as a standard in the DMAC assay and in HPLC–ESI-QTOF-MS quantification. For the plotting of the DMAC calibration curve, 1 mg was dissolved in ethanol to give a final concentration of 100 μg/ml and procyanidin A2 calibration curve was drawn ranging from 1 to 50 μg/ml. Also, for the HPLC–ESI-QTOF-MS quantification, standard calibration curves of procyanidin A2, *p*-coumaric acid, quercetin-3-*O*-glucoside, quercitrin, (+)-catechin and myricetin were prepared using different concentrations in methanol. In addition, luteolin was dissolved in methanol at 1 mg/ml and used as an internal standard (I.S.) to compensate for the potential variations in the instrumental analysis.

2.4. Irradiation treatment

A cobalt-60 irradiator was used for the irradiation treatment. The absorbed dosage was 5 kGy. Aliquots were separated to facilitate the storage process.

2.5. Storage conditions

The samples were stored for 6 months in two different chambers with different temperatures and humidities (chambers A and B). Chamber A had a temperature of 25 °C and 60% RH whereas chamber B was kept at 40 °C and 75% RH for the accelerated stability testing, according with the Committee for Human Medicinal Products (EMEA (European Medicines Agency), 2007).

2.6. Sample preparation

2.6.1. Syrup dilution for HPLC–ESI-QTOF-MS analysis

In 4 ml of methanol, 200 μl of syrup and 10 μl of the I.S. solution were dissolved, vortexed for 2 min in a G560E Vortex-Genie 2 (Scientific Industries, Bohemia, NY, USA), filtered with a polytetrafluoroethylene (PTFE) syringe filter (0.2 μm pore size), and injected directly into the HPLC system. Each sample was analysed in triplicate.

2.6.2. PAC extraction for the DMAC assay

The extraction process was performed according to (Prior et al., 2010), with some modifications. A total of 400 μl of syrup were dissolved in 5 ml of water. Bond Elut C18 cartridges (100 mg, 1 ml, Agilent Technologies, Palo Alto, CA, USA) were activated with 2 ml of methanol and washed with 2 ml of water. The cranberry syrup was added to each column and allowed to pass through the column by gravity. The column was washed twice with 1 ml of water and eluted twice with 1 ml of elution solution consisting of ethanol:water (80:20, v/v) with 0.5% of acetic acid. The eluted extract (2 ml) was collected, diluted again in a proportion of 1:25 in ethanol, and vortexed to mix well prior to the analysis by DMAC assay. Each sample was analysed in triplicate.

2.7. HPLC–ESI-QTOF-MS analysis

HPLC analyses were made with an Agilent 1200 series rapid-resolution LC system equipped with a binary pump, an autosampler, and a diode-array detector (DAD) following (Iswaldi et al., 2012), and a Zorbax Eclipse Plus C_{18} analytical column (150 × 4.6 mm, 1.8 μm particle size) was used for separation. The mobile phases consisted of water:acetonitrile (90:10, v/v) with 1% formic acid (phase A) and acetonitrile (phase B) at a constant flow rate of 0.5 ml/min using the following multi-step gradient: 0–20 min, from 5% B to 20% B; 20–25 min, from 20% B to 40% B;

25–30 min, from 40% B to 5% B; and 30–35 min, isocratic of 5% B. The injection volume was 10 μl.

The HPLC system was coupled to a microTOF-Q II mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) equipped with ESI operating in negative mode. The flow from HPLC delivered into the MS detector was split using a flow splitter at 1:2 for stable electrospray ionisation and reproducible results. The detection was made considering a mass-to-charge ratio of 50–1100 and using a capillary voltage of +4000 V, a dry gas temperature of 210 °C, a dry gas flow of 8.0 l/min, a nebulizer pressure of 2.0 bar, and spectra rate of 1 Hz. Moreover, automatic MS/MS experiments were performed using nitrogen as the collision gas and adjusting the collision-energy values as follows: *m*/*z* 100, 20 eV; *m*/*z* 500, 25 eV; *m*/*z* 1000, and 30 eV.

External instrument calibration was used to ensure the necessary mass accuracy to characterise compounds. The calibrant was sodium formate clusters consisting of 5 mM sodium hydroxide dissolved in water: 2-propanol, 1:1 (v/v), with 0.2% of formic acid. This calibrant was injected at the beginning of each run using a 74900-00-05 Cole Palmer syringe pump (Vernon Hills, IL, USA), which was equipped with a Hamilton syringe (Reno, NV, USA) and directly connected to the interface.

All procedures were controlled by DataAnalysis 4.0 software (Bruker Daltonik), which provided a list of possible elemental formulas by using the SmartFormula™ editor. The Editor uses a CHNO algorithm, which provides standard functionalities such as minimum/maximum elemental range, electron configuration, and ring-plus double-bond equivalents, as well as a sophisticated comparison of the theoretical with the measured isotope pattern (sigma value) for increased confidence in the suggested molecular formula.

2.8. Quantification by HPLC–ESI-QTOF-MS and method validation

Quantification of the phenolic compounds was carried out by HPLC–ESI-QTOF-MS, using internal calibration with luteolin as the internal standard (IS). The volume necessary to obtain a luteolin final concentration of 1 mg/ml was added to the standards and to the cranberry syrup samples. Extracted ion chromatograms of each compound were used to obtain the areas used in the quantification process.

The range of calibration of procyanidin A was from the limit of quantification (LOQ) to 100 ppm; *p*-coumaric acid was from LOQ to 250 ppm, and quercetin-3-*O*-glucoside, quercitrin, (+)-catechin and myricetin were from LOQ to 50 ppm, as showed in Table S1 (Supplementary Data).

Limits of detection (LOD) and quantification (LOQ) of the method were estimated to be 3 and 10 times the signal-to-noise ratio, respectively. Intra-day instrumental precision was determined by injecting a cranberry syrup sample 3 consecutive times in one day. Inter-day instrumental precision was determined by repeating the inter-assay procedure over three consecutive days. The linearity was individually verified for each compound, with analytical curves made from five calibration points in triplicate.

2.9. DMAC assay

DMAC is a colorimetric method that consists of the reaction between DMAC reagent and the C-8 position in the A-ring of the terminal unit of a proanthocyanidin (Wallace & Giusti, 2010). This reagent was found to be selective for flavanols, and also gives an intense reaction with monomers such as catechin or epicatechin. This assay was adapted from Prior et al. (2010). Briefly, procyanidin A2 standard (purity ⩾99%) was dissolved in ethanol to give a final concentration of 100 μg/ml. The incubation chamber was preheated to 25 °C, and 70 μl of ethanol (blanks), standard or sample solutions were dispensed into wells of a 96-well plate. Then, 210 μl of DMAC solution, consisting of 0.1% (w/v) DMAC reagent in acidized ethanol (ethanol:water:HCl, 75:12.5:12.5) was added. The absorbance was measured at 640 nm using a microplate reader Synergy Mx Monochromator-Based Multi-Mode Micro plate reader, by Bio-Tek Instruments Inc. (Winooski, VT, USA). Procyanidin A calibration curve was built ranging from 1 to 50 μg/ml. All samples were analysed in triplicate. The results were expressed as μg of procyanidin A2/ml of syrup.

2.10. Statistical analysis

Quantitative data are represented as mean ± standard deviation (SD). To evaluate the differences at a 95% confidence level ($p \leqslant 0.05$), a one-way analysis of variance (ANOVA) followed by

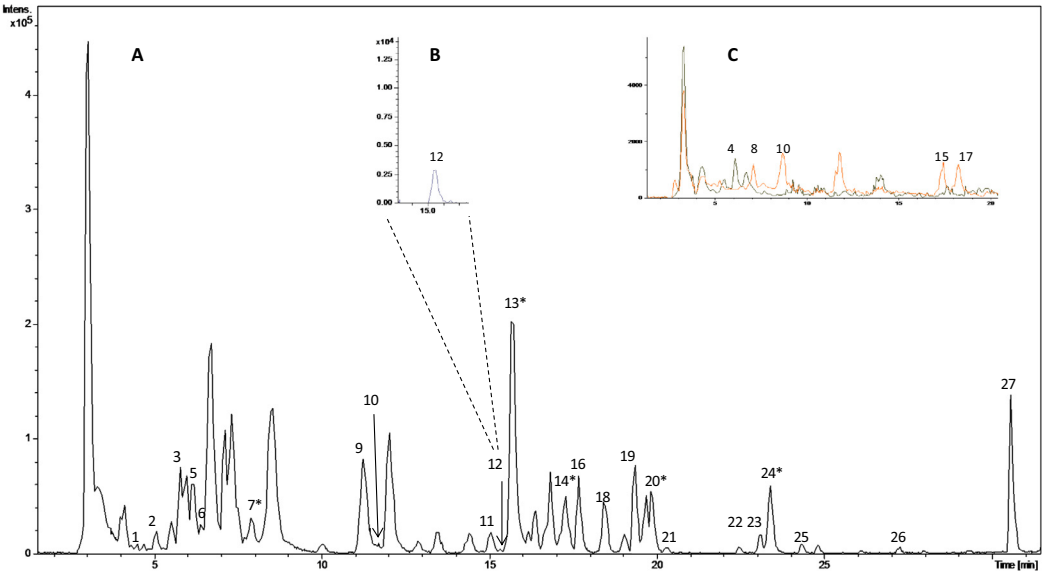

Fig. 1. Base-peak chromatogram of non-irradiated *cranberry syrup* (control) obtained by HPLC–ESI-TOF-MS analysis using negative ion mode (A) and extracted ion chromatogram of peak 12 (procyanidin B isomer) (B) and procyanidin A timers and pentamer (C). *Identification confirmed using commercial standards.

Tukey's test was performed using Origin (version Origin Pro 8 SR0, Northampton, MA, USA).

3. Results and discussion

3.1. Assessment of the HPLC–ESI-QTOF-MS method

Intraday and interday precision values were measured to evaluate the repeatability and reproducibility of the method and the relative standard deviations (RSDs) of the peaks area were determined. All the calibration curves presented good linearity, regression coefficients being higher than 0.99 (Table S1, Supplementary Data).

3.2. Compound characterisation and quantification of the cranberry syrup

3.2.1. Compound characterisation by HPLC–ESI-QTOF-MS and MS/MS

The base-peak chromatogram (BPC) of the non-irradiated cranberry syrup (control) obtained by HPLC–ESI-QTOF-MS analysis is shown in Fig. 1. The phenolic compounds characterised are indicated with numbers according to their elution order. The characterisation strategy was based on the accurate MS and MS/MS spectra of the compounds determined by QTOF mass analyzer and also taking account the data from literature. The identification of these compounds was also corroborated by comparing their retention times and MS/MS spectra with those of authentic standards whenever available. Table S2 (Supplementary Data) summarises the MS data of the compounds characterised, including experimental and calculated *m/z* for the molecular ions, their error, and the main fragments determined by MS/MS. Among these compounds, 27 phenolic compounds were identified, including simple flavonols, catechin, and oligomers such as 3 isomers of procyanidin B, procyanidin A2 and a procyanidin A isomer, prodelphinidin, four isomers of procyanidin A trimer (*m/z* 863) and one procyanidin A pentamer, identified on the basis of its doubly charged ion at *m/z* 719 (Foo, Lu, Howell, & Vorsa, 2000). Moreover, two isomers of myricetin and myricetin glycosylated derivatives, quercetin and quercetin glycosylated derivatives, and glycosylated derivatives of coumaric acid were detected as in Iswaldi et al. (2012).

3.2.2. Quantification by HPLC–ESI-QTOF-MS and DMAC methods

The main phenolic compounds were quantified by the aforementioned HPLC–ESI-QTOF-MS method. In this way, myricetin, catechin, quercetin-3-*O*-glucoside, quercitrin, and procyanidin A2 were quantified by the calibration curves determined from their

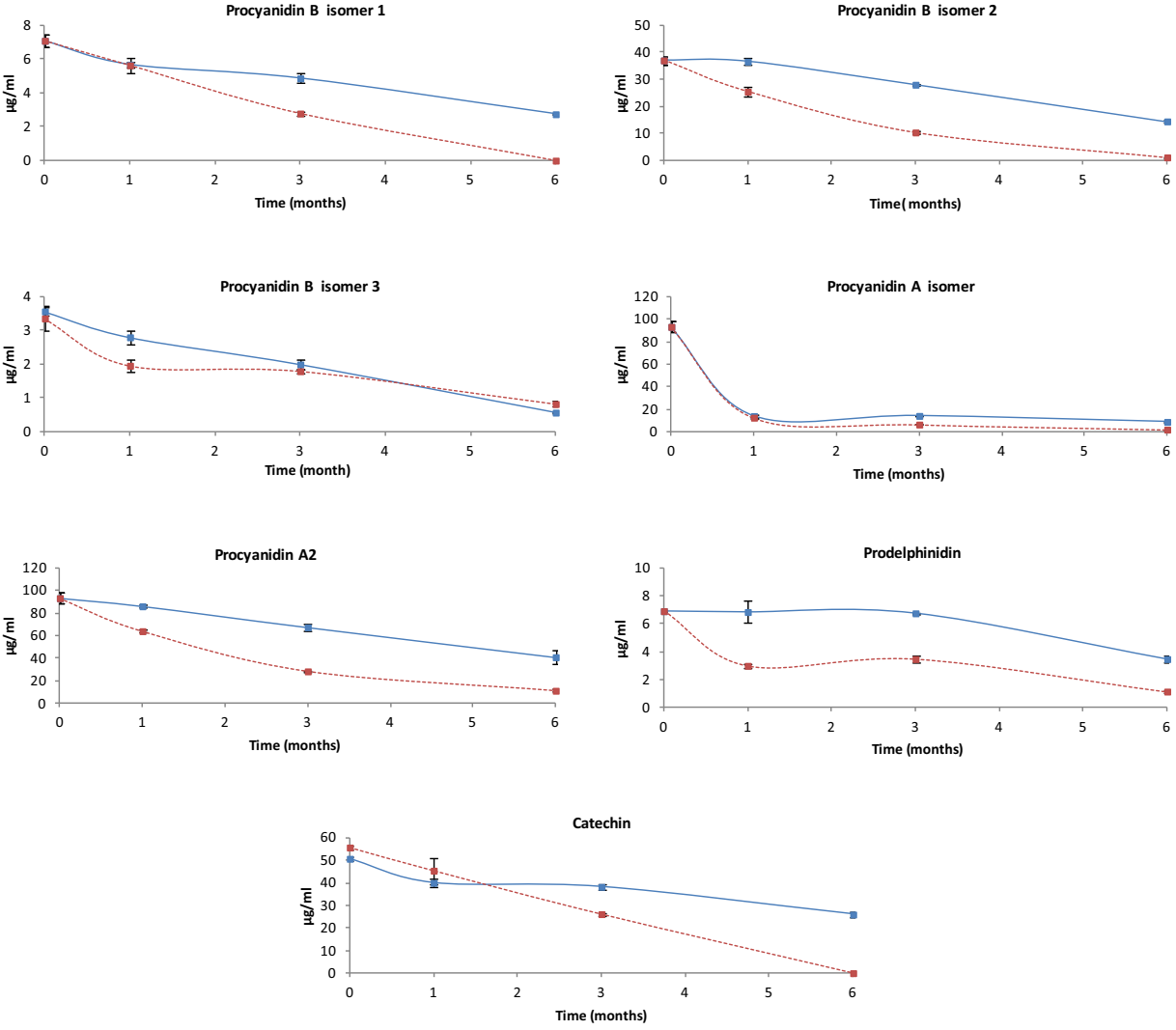

Fig. 2. Stability of PACs and catechin from gamma-irradiated cranberry syrup stored for 6 months at 25 °C/60% RH, chamber A (plain line), and at 40 °C/75% RH, chamber B (discontinuous line).

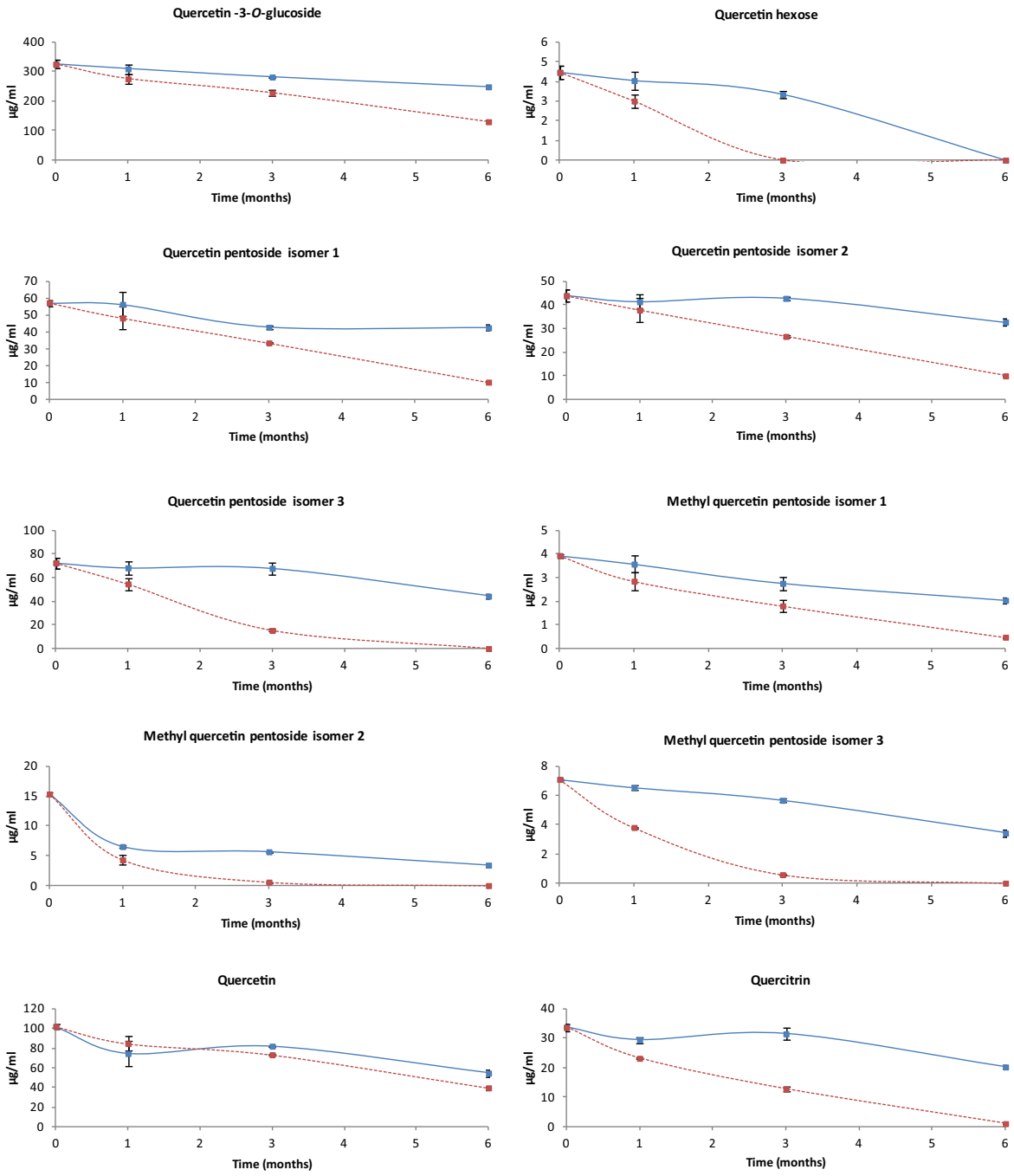

Fig. 3. Stability of quercetin and quercetin derivatives from gamma-irradiated cranberry syrup stored for 6 months at 25 °C/60% RH, chamber A (plain line), and at 40 °C/75% RH, chamber B (discontinuous line).

respective commercial standards. Since there are no available standards for all the characterised phenolic compounds, some compounds had their content estimated by using the analytical curves of compounds with similar chemical structures. In this way, proanthocyanidins were quantified using the procyanidin A2 calibration curve; myricetin derivatives were quantified using the myricetin calibration curve; quercetin and its derivatives were quantified by using the quercetin-3-*O*-glucoside calibration curve; and coumaroyl glycosides isomers were quantified using the coumaric acid calibration curve. The quantitative results of the main compounds are displayed in Figs. 2–4, and the whole data are presented in Tables S3 and S4 (Supplementary Data). The cranberry syrup used as control showed a rich composition in procyanidins, being procyanidin A2 the most abundant (83 µg/ml), which has been described as the most bioactive proanthocyanidin against urinary tract infections (Krueger, Reed, Feliciano, & Howell, 2013), followed by procyanidin B isomer 2 (35 µg/ml) and isomer of procyanidin A (20 µg/ml). The main flavonols were quercetin (80 µg/ml) and its glucoside (320 µg/ml).

Currently, the cranberry industry is using DMAC as a standard method to measure the PACs content of products (Krueger et al., 2013). Thus, to complement the latter quantitative results of each compound determined individually, DMAC assay was also performed to determine the overall PACs concentration, which include high mass oligomeric ones. These results are available in Table 1. The total PACs was 4130 µg/ml in the non-irradiated

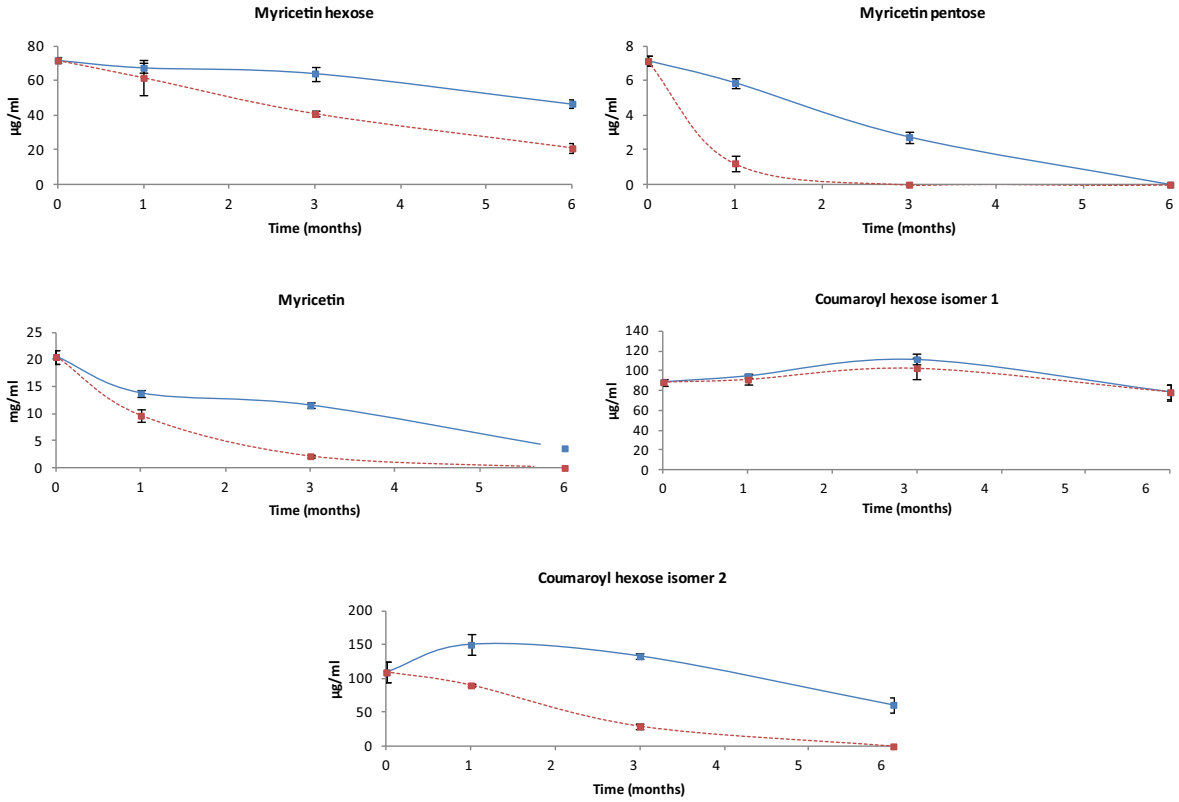

Fig. 4. Stability of myricetin, myricetin derivatives, and coumaroyl derivatives from gamma-irradiated cranberry syrup stored for 6 months at 25 °C/60% RH, chamber A (plain line), and at 40 °C/75% RH, chamber B (discontinuous line).

Table 1
Total PACs determined by DMAC assay.

Time (months)	Mean ± SD (µg/ml)
0 Control	4130 ± 50[a]
0 Irradiated	3460 ± 80[b]
Chamber A	
1	2720 ± 40[c]
3	2150 ± 160[d]
6	1420 ± 30[e]
Chamber B	
1	1910 ± 60[f]
3	1130 ± 40[g]
6	450 ± 10[h]

Data are expressed as mean ± standard deviation (SD). Mean values with different superscript letters are significantly different ($p < 0.05$).

syrup, which is higher than those results from the analysis by HPLC–QTOF-MS. It could be explained since one limitation of HPLC–MS methods is the difficulty to separate and detect higher polymeric proanthocyanidins, which may lead to underestimated quantitative results.

It is difficult to make a direct comparison between phenolic compounds found in this study and those reported by other authors because most articles refer to cranberry juices or fresh cranberries alone or in combination with other red fruits (Alighourchi et al., 2008; Boudesocque et al., 2013; Grace, Massey, Mbeunkui, Yousef, & Lila, 2012; Sánchez-Patán et al., 2012). In particular, PACs are usually characterised and quantified in previous studies by the degree of polymerisation, but not individually due to their structural heterogeneity and difficult analytical separation (Pappas & Schaich, 2009).

3.3. Resistance of phenolic compounds to gamma-irradiation and stability during storage

3.3.1. Assessment by HPLC–QTOF-MS

The main compounds were quantified by HPLC–ESI-QTOF-MS in syrup samples before gamma-irradiation, immediately after applying gamma irradiation and after 1, 3, and 6 months of storage of the irradiated syrup at 25 °C and 60% RH (chamber A) and 40 °C and 75% RH (chamber B). Interestingly, after the gamma-irradiation treatment, the qualitative composition of the syrup was similar to the non-irradiated one, showing poor quantitative differences. In any case, a significant increase ($p < 0.05$) in the content of procyanidin B isomer 1 (from 4.4 to 7.0 µg/ml) and procyanidin A2 (from 83 to 93 µg/ml) was observed after irradiation and compared with the non-irradiated syrup (control). The increase in procyanidin A2 could be associated with the degradation of procyanidin A trimers or pentamer, which were only observed in non-irradiated syrup. In this regard, other authors have reported greater anthocyanins content after irradiation at 5–6 kGy in strawberries (Ayed et al., 1999) and other vegetable foods (Dixit et al., 2012), but the formation pathway is not clear.

Fig. 2 displays the stability of the concentration of the flavanols, including PACs and the monomer catechin, after gamma-irradiation and during storage under the two conditions studied. In general, the most stable PACs were procyanidin B isomers 2 and 3, and prodelphinidin while the other isomer of procyanidin B and the isomer of procyanidin A showed higher susceptibility, e.g. isomers 2 and 3, remained stable in at 25 °C/60% RH (chamber A) until the 1st and the 3rd month of storage, respectively. Similarly, (Chang, Zuo, Chow, & Ho, 2006) also demonstrated the instability of B-type proanthocyanidins at room temperature after 6 months of storage, but remarkably our results show that this trend depends on the structure of procyanidin B. Not surprisingly, compared with the

initial time, the greatest losses were found when the syrup was stored under accelerated stability conditions, (40 °C/75% RH), in chamber B. In this case, in which all PACs showed significant changes ($p < 0.05$) in their concentration during storage except prodelphinidin, which remained constant under these conditions up to the first month.

In the case of the flavanol catechin, its content significantly decreased (from 65 to 50 μg/ml), around 23%, after gamma-irradiation and was less stable than other simple flavonoids. The same effect was observed by (Breitfellner et al., 2003) in strawberries in which the catechin concentration decreased as the irradiation dosage increased; specifically, at dosage of 5 kGy, the loss was over 25%. As found for PACs, the loss of this compound in chamber A was more gradual than in chamber B (Fig. 2), where it was more accused.

Flavonols were highly resistant to gamma-irradiation, and, as for flavanols, were less stable in chamber B conditions. As shown in Fig. 3, quercetin was the most stable compound over time under different storage conditions. The total loss in chamber A, at 6 months of storage, was approximately 35%, whereas in chamber B the loss was around 50%. Regarding myricetin, quercetin and its derivatives, there were no significant differences ($p < 0.05$) between irradiated samples and non-irradiated controls at the starting time. Quercetin-3-*O*-glucoside was the main flavonol in cranberry syrup control (320 μg/ml) and remained constant until 1 month of storage in both chambers A and B. Breitfellner et al., 2003 also reported that quercetin-3-*O*-glucoside in strawberry remained unchanged up to a dosage of 6 kGy (Breitfellner et al., 2003). The rest of the studied flavonols (myricetin hexose, quercitrin, quercetin xylopiranoside isomers, and methyl quercetin pentoside isomer 2) remained constant until the 3rd month of storage in chamber A, as showed in Figs. 3 and 4. In view of these results, most of the cranberry's phenolic compounds were quite stable for one month of storage, but the combination of gamma-irradiation with other treatments (refrigeration, freezing, etc.) may improve the shelf-life of this syrup.

3.3.2. Assessment by DMAC assay

The results from the DMAC assay showed that the total concentration of procyanidins decreased with the time in both chambers, PACs being present in less concentration over time in chamber B (Table 1). Thus, non-irradiated syrup (control) had a concentration of 4130 ± 50 μg/ml, which is significantly higher than that found in gamma-irradiated syrup (3460 ± 80 μg/ml). However, this only meant a loss of only about 16%.

On the other hand, the concentration of PACs in samples stored at 25 °C/60% RH (chamber A) significantly differed between the different months of storage, with a total loss over 60% after 6 months. The loss of PACs in samples stored at 40 °C/75% RH in chamber B was higher after 6 months, the total loss of PACs being around 80%. These results from the DMAC assay agreed with results from HPLC–ESI-QTOF-MS quantification. Overall, using the latter method and compared with the control samples, the total loss of PACs was over 65% in chamber A whereas in the chamber B the total loss was over 92%. Although the degradation pathway was not investigated in the present study, they could be related to their oxidation, hydrolysis or isomerization (Chang et al., 2006).

Although, DMAC assay is consider to be the best analysis available to perform an overall quantification of flavanols (Boudesocque et al., 2013), it is important to complement these results with the analysis of each individual compound, since the behaviour depend on the chemical structure, even stereochemistry, such as our results found for procyanidin B isomers.

4. Conclusion

The cranberry syrup studied was rich in flavonoids, mainly procyanidins and flavonols such as quercetin, myricetin, and their derivatives. The compounds were highly resistant to gamma-irradiation (dose of 5 kGy) and after one month of storage at room temperature. The combination of HPLC–ESI-QTOF-MS and DMAC assay demonstrated to be powerful techniques to quantify flavanols and other phenolic compounds. Our results showed that both methods should be used in a complementary way in order to gain more exhaustive information about the phenolic composition during processing and storage, particularly flavanols. However, further studies under different storage conditions are necessary to optimise preservation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.11.061.

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