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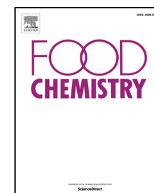

Kinetics of polyphenol content of dry flowers and floral bio-residues of saffron at different temperatures and relative humidity conditions

Natalia Moratalla-López[a], Cándida Lorenzo[a], Soukaina Chaouqi[b,c], Ana M. Sánchez[d], Gonzalo L. Alonso[a,*]

[a] Cátedra de Química Agrícola, ETSI Agrónomos y Montes, Universidad de Castilla-La Mancha, Campus Universitario, 02071 Albacete, Spain
[b] Laboratory of Materials, Environment and Electrochemistry, Faculty of Science, Ibn Tofaïl University, PO Box 242, Kénitra, Morocco
[c] Research Unit of Aromatic and Medicinal Plants and Soil Products, Institut National de la Recherche Agronomique (INRA), PO Box 6356-Instituts Avenue Mohamed Belarbi Alaoui, 10101 Rabat, Morocco
[d] Unidad de Hortofruticultura, Centro de Investigación y Tecnología Agroalimentaria de Aragón, CITA. Instituto Agroalimentario de Aragón IA2 (CITA-Universidad de Zaragoza), Avda. Montañana 930, 50059 Zaragoza, Spain

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ABSTRACT

The cultivation of *Crocus sativus* L. is valued for its dried stigmas, but the rest of the parts of its flowers are increasingly important. Saffron flowers (SF) are natural sources of antioxidant compounds. Kaempferols and anthocyanins are the main compounds of the high-phenolic content of SF. This work studies the evolution of flavonols and anthocyanins of dry SF and floral bio-residues of saffron (FBR) and their kinetics at different temperatures and relative humidity (RH) conditions. There was a degradation process of anthocyanins that fitted a second-order kinetic model and kaempferols showed better fit in a first-order kinetics model. The best storage conditions for anthocyanins studied in SF and FBR was 25 °C and 23% RH. The main kaempferol (Kaempferol 3-*O*-sophoroside) was no deteriorated in FBR. These results could contribute to the using SF and FBR as food and active ingredients in cosmetic industry, as well as development of new food products.

1. Introduction

The cultivated saffron (*Crocus sativus* L.) stands out for the highly valued stigmas of its flowers used as spice, mainly, but also as food additive and medicinal drug (Licón, Carmona, Llorens, Berruga, & Alonso, 2010). Apart from the use of *Crocus sativus* L. as spice, its flower with all the parts (tepals, stamens, style and stigma) and these parts independently has been studied (Serrano-Díaz, Sánchez, Alvarruiz, & Alonso, 2013; Serrano-Díaz et al., 2012; Serrano-Díaz, Sánchez, Martínez-Tomé, Winterhalter, & Alonso, 2013). The phenolic content of the saffron tepals and their antioxidant activity have been reported (Goupy, Vian, Chemat, & Caris-Veyrat, 2013; Sánchez-Vioque et al., 2012). Saffron flowers (SF) possess high-phenolic content and excellent antioxidant properties. The perianth (tepals) is the part of SF, which has the major content of anthocyanins and flavonols (Licón et al., 2010). Anthocyanins are responsible of the attractive colour of their tepals. In addition to anthocyanins (delphinidin, petunidin and malvidin glycosides), kaempferol glycosides are main flavonols in the flowers constituting between 70 and 90% of the total content of flavonols in the perianth (Moratalla-López, Lorenzo, Alonso, & Sánchez, 2016; Nørbæk, Brandt, Nielsen, Ørgaard, & Jacobsen, 2002).

Currently, there is an increasing demand for natural food consumption, instead of other products made with synthetic additives. The characterization of SF regarding to its proximate composition, minerals, dietary fiber, sugars, anions and organic acids has been studied previously (Serrano-Díaz, Sánchez, Martínez-Tomé et al., 2013). Thus, SF provide low energy and lipids, possess high carbohydrate content, have high content of some minerals and are rich in dietary fiber. Furthermore, the food safety of SF extracts has also been reported by Serrano-Díaz, Estevan et al. (2014). SF are valuable natural sources of antioxidants and phenolic compound that could to contribute to the development of new food products (Serrano-Díaz et al., 2012). Moreover, SF are used as active ingredients in high-end cosmetic products (ES2646415B1, 2018).

Traditionally, saffron spice production requires the separation of stigmas from other parts of SF and this generates a large amount of floral bio-residues (FBR) made up of tepals, stamens and styles. Nowadays, the mechanization of saffron crop is being introduced, which will increase the generation of FBR in a short period of time.

In the production of saffron spice, about 93 g per 100 g of SF are

* Corresponding author.
E-mail address: Gonzalo.Alonso@uclm.es (G.L. Alonso).

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FBR and about 63 kg of bio-residues are generated to produce 1 kg of saffron spice (Serrano-Díaz et al., 2012; Serrano-Díaz, Sánchez, Martínez-Tomé et al., 2013). This large amount of FBR needs to be stabilized because of their quickly deterioration. Serrano-Díaz et al. (2013) proposed to dehydrate them with hot air convection at 90 °C and 2–6 m/s. It is known that temperature and relative humidity are relevant parameters in the conservation of the saffron spice. Studies about the storage conditions of this spice have been done, being a low temperature and a low relative humidity the best storage conditions (Alonso, Varón, Salinas, & Navarro, 1990, 1993). Besides, kinetics of its main compounds have also been studied (Sánchez, Carmona, Jarén-Galán, Mínguez Mosquera, & Alonso, 2011; Sánchez, Carmona, Ordoudi, Tsimidou, & Alonso, 2008). In a previous work, the evolution of total polyphenol content of FBR was studied (Moratalla-López, Sánchez, Campayo, Salinas, & Alonso, 2017). To our knowledge, there are not assays regarding the stability of detailed compounds in SF and in FBR during storage. The aim of this work was to study the evolution of flavonols and anthocyanins of dry SF and dry FBR and their kinetics at different temperatures and relative humidity (RH) conditions. The results may establish the best storage conditions, regarding to temperature and relative humidity, of these phenolic compounds that could contribute to the using SF and FBR as food and active ingredients in cosmetic industry, as well as the development of new food products.

2. Materials and methods

2.1. Plant material and reagents

SF were obtained from the company "Molineta de Minaya" (Minaya, Spain). SF are made up of tepals, stamens, styles and stigma. According to the traditional procedures of "Azafrán de La Mancha" Protected Designation of Origin (OJEC, 2000), the stigma was separated from the rest of the flower, obtaining FBR composed by tepals, stamens and styles. SF and FBR were dehydrated in a hot air drier at 90 °C and constant airflow of 4 m/s. Then, they were ground in a pulverizing mill and sieved through 500 m mesh size.

2.2. Storage conditions

Storage conditions were: 25 °C and 40 °C, and three RH: 23%, 57% and 75%. Laboratory oven was set at 25 °C and another one at 40 °C. Each laboratory oven contained three hermetically sealed boxes having different saturated solutions: potassium acetate, sodium bromide and sodium chloride to produce 23%, 57% and 75% RH, respectively. Twelve circular plastic boxes with diameters of 8.5 cm and thickness of 1.5 cm with 15 g of SF and other twelve circular plastic boxes with 15 g of FBR were used. The plant material reached a thickness between 0.5 and 0.7 cm. The hermetically sealed boxes had dimensions of 29 × 20 × 10 cm (length × width × height). Two circular plastic boxes of SF and other two circular plastic boxes of FBR were placed in each box separated from the corresponding saturated solution by a grid.

2.3. Preparation of extracts of flowers and floral bio-residues of saffron

Aliquots of approximately 150 mg were withdrawn, at the beginning, every seven days from each box of laboratory oven set at 25 °C and every four days from each box of laboratory oven set at 40 °C. Then, they were withdrawn periodically based on their stability. They were analysed for a period of 248 days.

Extracts of SF (125 mg) and FBR (125 mg) were prepared with 10 mL water:HCL (100:1 v/v), and were stirred for 1 h at 500 rpm. Then, they were centrifuged at 3500 rpm for 10 min (Selecta, Barcelona, Spain).

Halogen lamp moisture balance model XM-120T (Cobos, Barcelona, Spain) at 105 °C was used to determine the moisture content of the samples. When moisture loss was less than 0.1% in 180 s was considered that samples had reached constant mass.

2.4. Anthocyanin content

Anthocyanin content, for the study of kinetic parameters, was measured according to Ribéreau-Gayon, Peynaud, Sudraud, and Ribéreau-Gayon (1982). For determination of total anthocyanin content (TA), 1 mL of extract prepared before was dissolved in 25 mL of 0.1 M HCL solution in duplicate and their absorbance at 520 nm was measured after 30 min at room temperature. Anthocyanins were quantified based on calibration curves of 0.022–0.200 mg/mL delphinidin 3,5-di-*O*-glucoside; $C = A{\cdot}103.92{\cdot}10^{-3} - 9.77{\cdot}10^{-3}$, where C is the concentration (mg/L) and A is the absorbance at 520 nm. Results were expressed as weight of delphinidin 3,5-di-*O*-glucoside equivalents (mg) per weight of plant material (g) on dry basis.

The results are average of four measurements. Of each circular plastic box was withdrawn an aliquot sample of plant material and two measurements of each sample were carried out in duplicate. In total, it was obtained four measurements of SF and four of FBR, for each storage condition studied.

2.5. HPLC-DAD analysis

Solvents with HPLC purity or analytical grade were employed. Ultrahigh-purity water was produced using a Milli-Q system (Millipore, Bedford, MA). Acetonitrile and trifluoroacetic acid were supplied from Scharlau (Barcelona, Spain). Aliquots of 30 μL of each sample were filtered through a PTFE filter (0.45 μm). Kaempferols glycosides, quercetin 3-*O*-sophoroside, isorhamnetin 3–4́-di-*O*-glucoside, kaempferol aglycone and anthocyanins of SF and FBR were analysed and identified by RP-HPLC-DAD according to Serrano-Díaz, Sánchez, Martínez-Tomé, Winterhalter, and Alonso (2014) using an Agilent 1200 HPLC chromatograph (Palo Alto, CA, USA) equipped with a 250 × 4.6 mm, 5 μm, Develosil ODS-HG-5 chromatographic column (Phenomenex, Le Pecq Cedex, France) at 40 °C. The DAD detector was set at 266 nm for flavonols detection, and at 520 nm for anthocyanins detection. Quantification of compounds was based on the six-point calibration curves: $C_i = A_i{\cdot}0.50{\cdot}10^{-3} - 78.34{\cdot}10^{-3}$; (0.01–12.00 mg/L) for kaempferol 3-*O*-sophoroside-7-*O*-glucoside (K 3-*O*-s-7-*O*-g), kaempferol 3,7-di-*O*-glucoside (K 3,7-di-*O*-g), kaempferol 3,7,4́-tri-*O*-glucoside (K 3,7,4́-tri-*O*-g), quercetin 3-*O*-sophoroside (Q 3-*O*-s), isorhamnetin 3,4́-tri-*O*-glucoside (I 3,4́-tri-*O*-g), kaempferol 3-*O*-rutinoside (K 3-*O*-r), kaempferol 3-*O*-glucoside (K 3-*O*-g) and kaempferol 7-*O*-glucoside (K 7-*O*-g). $C_i = A_i{\cdot}25.83{\cdot}10^{-3} - 11.87$; (0.01–700.00 mg/L) for kaempferol 3-*O*-sophoroside (K 3-*O*-s). $C_i = A_i{\cdot}10.03{\cdot}10^{-3} - 488.82{\cdot}10^{-3}$; (0.04–90.00 mg/L) for kaempferol aglycone (K). $C_i = A_i{\cdot}27.19{\cdot}10^{-3} - 6.96$; (15.00–300.00 mg/L) for delphinidin 3,5-di-*O*-glucoside (D 3,5-di-*O*-g), delphinidin 3-*O*-glucoside (D 3-*O*-g), malvidin 3,5-di-*O*-g (M 3,5-di-*O*-g) and $C_i = A_i{\cdot}31.80{\cdot}10^{-3} - 118.60{\cdot}10^{-3}$; (1.00–85.00 mg/L) for petunidin 3,5-di-*O*-g (P 3,5-di-*O*-g). Being C_i the concentration (mg/L) and A_i the HPLC peak area of the corresponding compound *i*. Duplicate measurements for every aliquot were taken, obtaining four measurements of each plant material (SF and FBR).

2.6. Kinetics studies

An integral method (Fogler, 1992) was used to obtain the kinetic parameters. Of each reaction were obtained: reaction order, rate constants (k) and half-life periods ($t_{1/2}$). This method uses a trial-and-error procedure to find reaction order. If the order assumed is correct, the appropriate plot of the concentration–time data [concentration against time (zero-order), ln concentration against time (first-order), and 1/concentration against time (second-order)] should be linear. To perform these calculations the spreadsheet Excel (Office, Microsoft; 2013) was used. The result showing the best correlation coefficient (R^2) was selected.

Table 1
Initial concentration (mg/g plant material in dry weight) of flavonols and anthocyanins of saffron flowers and floral bio-residues of saffron.

	Flavonols				
	K 3-*O*-s-7-*O*-g	K 3,7-di-*O*-g	K 3,7,4́-tri-*O*-g	Q 3-*O*-s	I 3,4́-tri-*O*-g
Flowers[a]	0.15	0.01	0.01	0.08	0.03
Floral bio-residues[a]	0.17	0.01	0.01	0.10	0.03
	K 3-*O*-s	K 3-*O*-r	K 3-*O*-g	K 7-*O*-g	K
Flowers[a]	51.89	0.01	0.04	0.08	0.39
Floral bio-residues[a]	55.43	0.03	0.03	0.08	0.19
	Anthocyanins				
	D 3,5-di-*O*-g	P 3,5-di-*O*-g	D 3-*O*-g	M 3,5-di-*O*-g	
Flowers[a]	6.88	1.90	0.74	0.75	
Floral bio-residues[a]	10.23	1.97	0.77	0.75	

K 3-*O*-s-7-*O*-g, kaempferol 3-*O*-sophoroside-7-*O*-glucoside; K 3,7-di-*O*-g, kaempferol 3,7-di-*O*-glucoside; K 3,7,4́-tri-*O*-g, kaempferol 3,7,4́-tri-*O*-glucoside; Q 3-*O*-s, quercetin 3-*O*-sophoroside; I 3,4́-tri-*O*-g, isorhamnetin 3,4́-tri-*O*-glucoside; K 3-*O*-s, kaempferol 3-*O*-sophoroside; K 3-*O*-r, kaempferol 3-*O*-rutinoside; K 3-*O*-g, kaempferol 3-*O*-glucoside; K 7-*O*-g, kaempferol 7-*O*-glucoside; K, aglycone kaempferol; D 3,5-di-*O*-g, delphinidin 3,5-di-*O*-glucoside; P 3,5-di-*O*-g, petunidin 3,5-di-*O*-g; D 3-*O*-g, delphinidin 3-*O*-glucoside; M 3,5-di-*O*-g, malvidin 3,5-di-*O*-g.

[a] Values are the mean of two extracts conducted in duplicate (2 × 2n).

2.7. Statistical analysis

One-way analysis of variance (ANOVA) was performed on each determination. Mean values were compared by Duncańs test at $p < 0.05$ using SPSS 23 for Windows (SPSS INC., Chicago, IL, USA).

3. Results and discussion

3.1. Polyphenol content

Table 1 shows the initial concentration (t = 0 min) of flavonols (kaempferols glycosides, Q 3-*O*-sophoroside, I 3–4́-di-*O*-glucoside and K) and anthocyanins in SF and FBR before being subjected to storage. The values obtained are closed to those reported by other authors. Regarding to K 3-*O*-s-7-*O*-g, the content in dried stigmas ranges from 1.47 to 2.58 of equivalent mg of rutin/g according to Carmona et al. (2007). In tepals, contents of 1.90 equivalent mg of K 3-*O*-g/g on dry weight (dw) was reported (Goupy et al., 2013). And in FBR the content of this kaempferol glycoside was 0.11 and 0.03 equivalent mg of K 3-*O*-g/g dw, after calculations from data reported by Serrano-Díaz et al. (2014) and Tuberoso, Rosa, Montoro, Fenu, and Pizza (2016), respectively. K 3,7-di-*O*-g content in saffron tepals was 0.42 equivalent mg of K 3-*O*-g/g dw according to (Goupy et al., 2013). Other studies showed, after calculations from data reported, in FBR 0.01 and 0.02 equivalent mg of K 3-*O*-g/g dw (Serrano-Díaz, Sánchez et al., 2014; Tuberoso et al., 2016). The content of K 3,7,4́-tri-*O*-g ranging from 0.59 to 1.09 of equivalent mg of rutin/g were reported in dried stigmas of *Crocus sativus* L. by Carmona et al. (2007). In FBR 0.01 equivalent mg of K 3-*O*-g/g dw of this kaempferol glycoside was obtained (Serrano-Díaz, Sánchez et al., 2014). Regarding to Q 3-*O*-s and I 3-4́-di-*O*-g, 0.07 and 0.01 equivalent mg of K 3-*O*-g/g dw were reported, respectively by Serrano-Díaz et al. (2014) after calculations from their data obtained. K 3-*O*-s content in dried stigmas ranging from 0.61 to 3.12 equivalent mg of rutin/g according to Carmona et al. (2007). From FBR an extraction yield of 2.3 mg/g dry weight was reported by Sánchez, Jerz, Serrano-Díaz, Alonso, and Winterhalter (2011). This kaempferol represents about 82.42% of the total of kaempferols and anthocyanins studied in SF and about 79.42% of FBR. Other work showed that this kaempferol glycosides represented about 55% of the total flavonoids in saffron tepals (Goupy et al., 2013), with contents of 33.6 equivalent mg of K 3-*O*-s/g dw, after calculations from data according to (Serrano-Díaz, Sánchez et al., 2014). K 3-*O*-r was detected in saffron tepals with contents of 0.26 equivalent mg of K 3-*O*-g/g dw (Goupy et al., 2013) and 0.01 equivalent mg of K 3-*O*-glucoside/g dw in FBR (Serrano-Díaz, Sánchez et al., 2014). K 3-*O*-g was reported as a major flavonol in tepals (Nørbæk et al., 2002). According to Goupy et al. (2013) 1.90 mg/g dw the content of K 3-*O*-g were reported. Other studies showed, after calculations, that in FBR, the content of this kaempferol glycoside was less than 0.01 mg/g dry weight (Serrano-Díaz, Sánchez et al., 2014; Tuberoso et al., 2016). K 7-*O*-g content in FBR was also reported by Serrano-Díaz et al. (2014) and Tuberoso et al. (2016) with 0.01 and 0.22 equivalent mg of K 3-*O*-g/g dw, respectively, after calculations from their data obtained. And 0.46 mg of kaempferol aglycone/g dw was obtained in FBR by Serrano-Díaz et al. (2014), after calculations from their data reported.

Regarding to anthocyanins, the initial concentration obtained (Table 1) was similar to the concentration showed after calculations from data reported by Serrano-Díaz et al. (2014) in FBR: 9.68 mg of D 3,5-di-*O*-g/g dw, 2.7 mg of P 3,5-di-*O*-g/g dw, D 3-*O*-g expressed as 1.47 equivalent mg of D 3,5-di-*O*-g/g dw and M 3,5-di-*O*-g expressed as 0.62 equivalent mg of D 3,5-di-*O*-g/g dw.

Fig. 1 presents the evolution of the main kaempferol glycoside (K 3-*O*-s) and anthocyanin (D 3,5-di-*O*-g) with the general parameters: Folin-Ciocalteu (FC) and absorbance at 520 nm, both in SF and in FBR on dry basis during the first 99 days of the storage at 25 °C. From day 99 to day 248 only were registered data of K 3-*O*-s and D 3,5-di-*O*-g at 25 °C with 23% RH, both in SF and in FBR, and at 25 °C with 57% and 75% RH, K 3-*O*-s was the only parameter which obtained values that remained constant. The values shown of FC and absorbance at 520 nm in Figs. 1 and 2 are the results reported in a before work (Moratalla-López et al., 2017). Thereby, the evolution of the most important compound regarding to kaempferols and anthocyanins can be compared with the evolutions of the general parameters.

SF (Fig. 1A) and FBR (Fig. 1B) at 25 °C and 23% RH showed values of K 3-*O*-s, FC and D 3,5-di-*O*-g, which did not vary significantly during the first 99 days. The absorbance at 520 nm values decreased slightly in SF, but in FBR from initial day to day 15 this value increased and then, decreased slightly until the day 45 where remained practically constant until the day 99.

At 25 °C with 57% and 75% RH (Fig. 1C–F), both in SF and in FBR, all the parameters was remained practically constant, except the D 3,5-di-*O*-g that decreased slightly and a sharp decrease in the absorbance at 520 nm values was observed.

Fig. 2 presents the evolution of K 3-*O*-s, FC, D 3,5-di-*O*-g and absorbance at 520 nm, both in SF and in FBR on dry basis during the first 92 days of the storage at 40 °C. At 40 °C and 23% RH, both in SF and in FBR, was observed that K 3-*O*-s decreased slightly (Fig. 2A, B). The absorbance at 520 nm decreased sharply, being the 49th the last day that this parameter could be measured. FC values increased slightly and D 3,5-di-*O*-g decreased, being the 92nd the last day that this parameter could be measured. At 40 °C and 57% RH (Fig. 2C, D), different behaviour of K 3-*O*-s was observed in SF and in FBR. K 3-*O*-s decreased sharply from the day 17 in SF (Fig. 2C), while its concentration remained constant in FBR during storage period (Fig. 2D). FC values increased slightly. A sharp decrease in the absorbance at 520 nm values was observed in 10 days of storage, both in SF and in FBR on dry basis. D 3,5-di-*O*-g decreased sharply in 17 days of storage in SF (Fig. 2C) and its concentration in FBR decreased slightly until the day 92 (Fig. 2D). The same trend showed the results of the parameter studied at 40 °C and 75% RH (Fig. 2E, F), but in FBR, for FC, the day 36 was the last day that

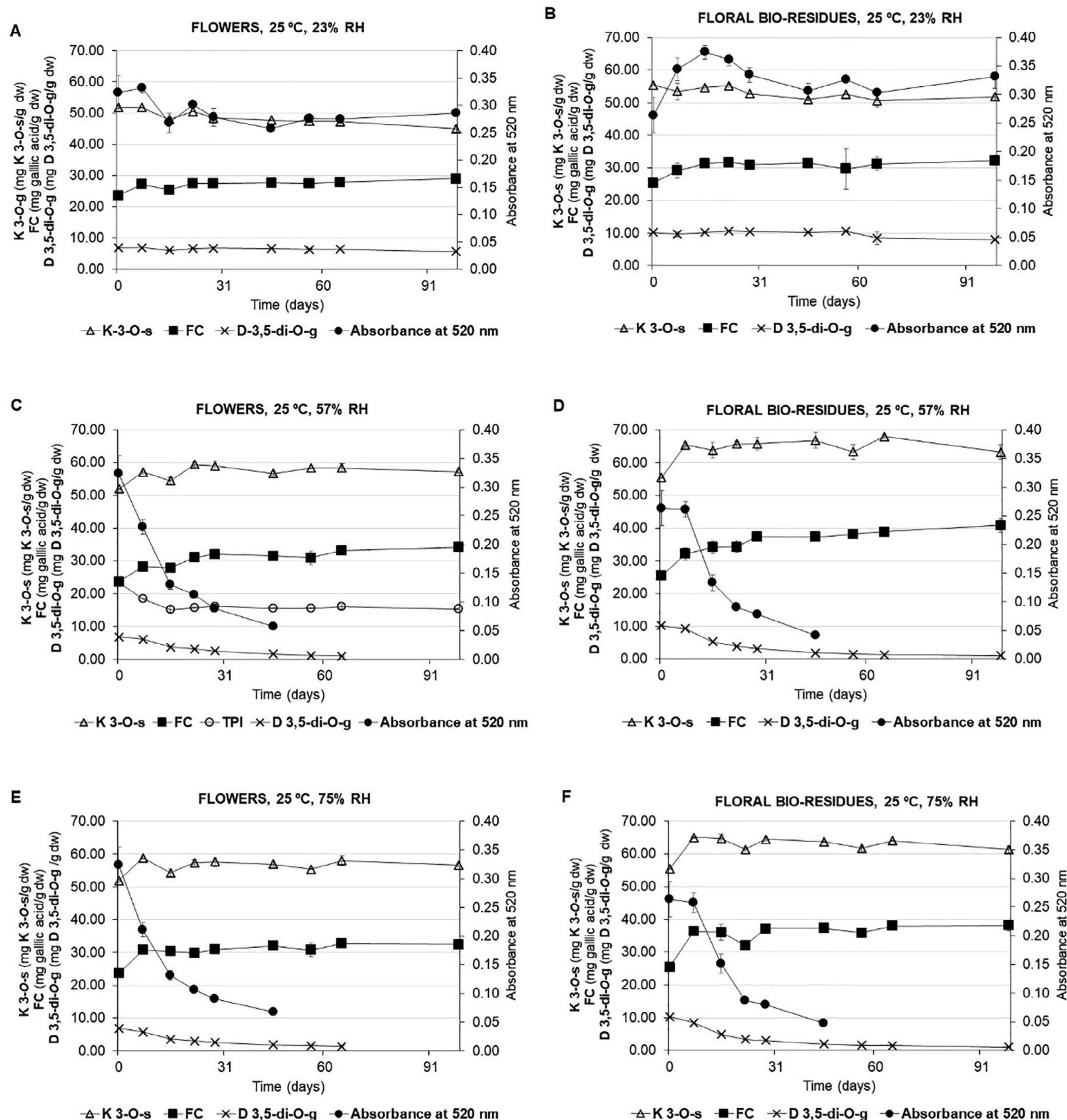

Fig. 1. Mean values of two extracts conducted in duplicate (2 × 2n) and its standard deviation of: kaempferol 3-*O*-sophoroside (mg K 3-*O*-s/g vegetal material on dry weight, dw), FC (Folin-Ciocalteu assay; mg gallic acid/g of vegetal material on dry weight, dw), delphinidin 3,5-di-*O*-glucoside (mg D 3,5-di-*O*-g/g vegetal material on dry weight, dw) and absorbance at 520 nm versus time (days) for saffron flowers and floral bio-residues of saffron at 25 °C with 23%, 57% and 75% relative humidity (RH) conditions.

could be measured. At 40 °C with the two highest RH studied, it is remarkable that the behaviour of the concentration evolution of K 3-*O*-s and D 3,5-di-*O*-g was different in SF and in FBR, being more stable these compounds in FBR. The difference between SF and FBR is the presence of stigma in SF. So, the stigma could cause the deterioration of K 3-*O*-s and D 3,5-di-*O*-g at 40 °C with 57% and 75% RH. In this way, the aromatization process of olive oils with aquoes extract rich in safranal was studied by Sena-Moreno et al. (2018). Safranal is one of the main metabolites of saffron. In that study, at room temperature, was observed a lower oxidative stability of the flavoured oils at production, against control olive oils without safranal. Besides, after the first month of storage, a sharp decreased was showed with an oxidative stability loss of approximately 51%, while the control oils remained more stable. It seems that in these cases, both in olive oil and in SF, safranal acts as a pro-oxidant and not as an antioxidant, as other authors have shown in other conditions (Madan & Nanda, 2018; Samarghandian, Samini, Azimi-Nezhad, & Farkhondeh, 2017).

By comparing the polyphenol content, FC values, both in SF and in

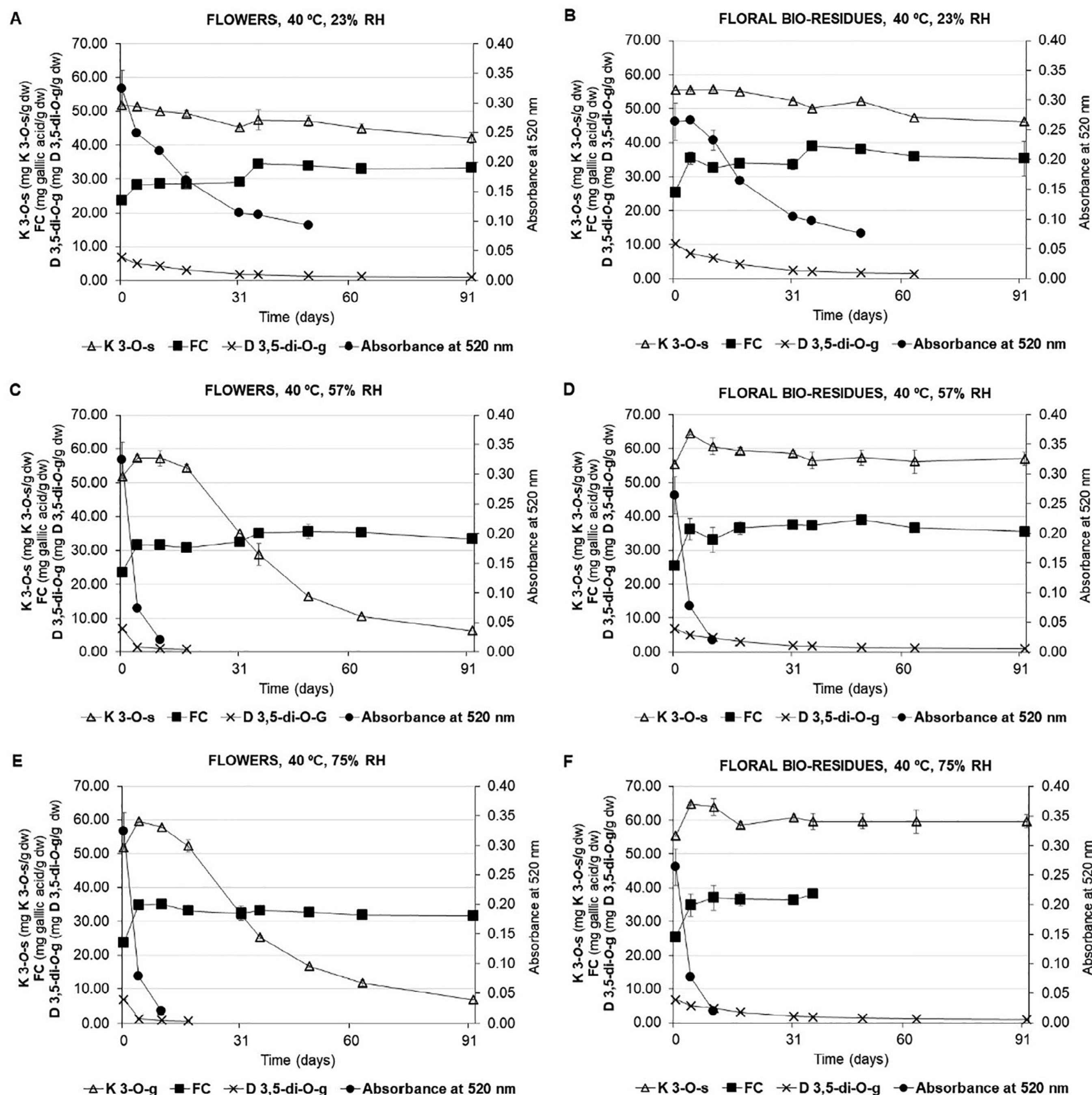

Fig. 2. Mean values of two extracts conducted in duplicate (2 × 2n) and its standard deviation of: kaempferol 3-*O*-sophoroside (mg K 3-*O*-s/g vegetal material on dry weight, dw), FC (Folin-Ciocalteu assay; mg gallic acid/g of vegetal material on dry weight, dw), delphinidin 3,5-di-*O*-glucoside (mg D 3,5-di-*O*-g/g vegetal material on dry weight, dw) and absorbance at 520 nm versus time (days) for saffron flowers and floral bio-residues of saffron at 40 °C with 23%, 57% and 75% relative humidity (RH) conditions.

FBR, with the evolution of the main kaempferol and anthocyanin, it is remarkable that FC values at 25 °C and 23% RH increased slightly, showing a greater increase at 25 °C with 57% and 75% RH, while K 3-*O*-s remained constant and D 3,5-di-*O*-g decreased under these storage conditions. At 40 °C, FC values increased during the storage period, while K 3-*O*-s and D 3,5-di-*O*-g decreased, being this decrease more pronounced in SF. Thereby, the general parameter of polyphenol content (FC) at 25 °C with 57% and 75% RH and at 40 °C with the three RH studied, increased its values during storage, while the main compounds responsible of the polyphenol content decreased (K 3-*O*-s and D 3,5-di-*O*-g). This could be due to the compounds of SF with the passage of time are deteriorated and news substances appear, which interfere in the measurement of this general parameter. So, it is unwise to measure polyphenol content of SF and FBR by FC. There are various studies where the results obtained by HPLC-DAD and C for the same saffron extract were compared; being more appropriate to use HPLC-DAD method (García-Rodríguez et al., 2017; García-Rodríguez et al., 2014). The results of the present study indicate again that it is preferable to carry out measurements to determine the saffron composition by HPLC-DAD that by UV–vis spectrophotometry.

3.2. Kinetic parameters

Data corresponding to the loss of anthocyanin content showed

Table 2
Rate constants (k), determination coefficients (R^2), and half-life periods ($t_{1/2}$) of anthocyanins content loss in an extract of saffron flowers and floral bio-residues at different temperatures (T) and relative humidity (RH) conditions.

T (°C)	RH (%)	Compounds	FLOWERS			FLORAL BIO-RESIDUES		
			(k ± SD) [a] × 10^{-3} (d^{-1})	R^2 (m)[b]	$t_{1/2}$ (d)	(k ± SD)[a] × 10^{-3} (d^{-1})	R^2 (m)[b]	$t_{1/2}$ (d)
25	23	D 3,5-di-*O*-g	0.20 ± 0.02 a	0.625 (10)	740	0.11 ± 0.02 a	0.773 (9)	873
		P 3,5-di-*O*-g	0.88 ± 0.06f	0.815 (9)	600	0.66 ± 0.17 f	0.872 (9)	824
		D 3-*O*-g	5.91 ± 0.00	0.934 (4)	232	3.92 ± 0.00	0.707 (4)	449
		M 3,5-di-*O*-g	3.60 ± 0.520	0.775 (4)	379	2.75 ± 0.54	0.727 (4)	500
	57	TA	7.76 ± 1.61 i, j	0.895 (4)	3	4.62 ± 1.90 i	0.649 (4)	1
		D 3,5-di-*O*-g	10.45 ± 0.25 b	0.979 (6)	15	10.21 ± 1.17 b	0.981 (7)	9
		P 3,5-di-*O*-g	44.76 ± 1.83 g	0.965 (5)	12	57.06 ± 0.99 g	0.891 (5)	8
	75	TA	13.15 ± 5.67 j	0.786 (4)	2	2.65 ± 1.14 i	0.697 (4)	12
		D 3,5-di-*O*-g	9.05 ± 0.21 b	0.994 (7)	16	9.74 ± 0.60 b	0.990 (8)	10
		P 3,5-di-*O*-g	52.06 ± 0.56 h	0.967 (6)	10	57.12 ± 1.74 g	0.943 (5)	9
40	23	TA	7.04 ± 0.53 i	0.855 (5)	4	27.65 ± 9.32 j	0.764 (5)	1
		D 3,5-di-*O*-g	12.20 ± 0.63 c	0.992 (7)	12	10.34 ± 0.11 b	0.993 (6)	10
		P 3,5-di-*O*-g	54.69 ± 3.51 h	0.990 (6)	10	67.10 ± 16.92 g	0.986 (4)	10
	57	D 3,5-di-*O*-g	57.48 ± 0.79 d	0.881 (4)	3	65.81 ± 1.43 c	0.973 (4)	2
	75	D 3,5-di-*O*-g	60.44 ± 2.62 e	0.877 (4)	3	68.09 ± 0.96 d	0.943 (4)	2

D 3,5-di-O-g, delphinidin 3,5-di-O-glucoside; P 3,5-di-O-g, petunidin 3,5-di-O-glucoside; D 3-di-O-g, delphinidin 3-O-glucoside; M 3,5-di-O-g, malvidin 3,5-di-O-glucoside and TA, total anthocyanin content.

[a] Values are the mean of two extracts conducted in duplicate (2 × 2n), SD = standard deviation.

[b] Minimum number of experimental data points. At each compound, different letters between rows indicate significant differences at the 0.05% level: a, b, c, d, e for D 3,5-di-O-g; f, g, h for P 3,5-di-O-g; i, j for TA.

better fits in a second-order kinetic model than in zero-order, or first-order one, while kaempferols content obtained the best fit for the first-order kinetic model. It was selected the kinetic model according to the best correlation coefficient reported. Table 2 shows rate constants (k) and half-life periods ($t_{1/2}$) of anthocyanins content loss and total anthocyanin content (TA) loss according to a second-order kinetic model, in SF and FBR at various temperatures (25 °C and 40 °C) and relative humidity conditions (23%, 57% and 75%). Within the group of anthocyanins, D 3,5-di-O-g, P 3,5-di-O-g, D 3-O-g and M 3,5-di-*O*-g have been studied. In SF, at 25 °C and 23% RH, the half-life period of D 3,5-di-*O*-g was approximately 2 years. Rate constant value of D 3,5-di-*O*-g it was multiplied by 53 (at 25 °C and 57% RH) and 46 (at 25 °C and 75% RH); showing significant differences for rate constant of D 3,5-di-*O*-g when the RH increased. At 40 °C and 23% RH the half-life period decreased substantially for D 3,5-di-*O*-g and k value was multiplied by 4.7 and 5 at 57% RH and 75% RH, respectively, against 23% RH. At 40 °C, D 3,5-di-*O*-g showed significant differences of rate constant for 23%, 57% and 75% RH so, an increase in the RH was relevant to accelerate the deterioration of the main anthocyanin. The best storage conditions for D 3,5-di-*O*-g in SF were 25 °C and 23% RH. In the same way, it was observed significant differences for the rate constant of D 3,5-di-O-g in FBR; being equally relevant for the deterioration of this anthocyanin the increased of RH at 25 °C from 23% to 57% and 75% RH that at 40 °C with 23% RH. An increased of RH at 40 °C showed less stability of this anthocyanin, being at 40 °C with 75% RH the worst storage conditions.

In SF, the half-life period of P 3,5-di-*O*-g was approximately 1.6 years at 25 °C and 23% RH. The same as D 3,5-di-O-g, the half-life period of P 3,5-di-*O*-g decreased substantially at 40 °C and 23% RH. P 3,5-di-*O*-g showed significant differences for k values at 25 °C between 23%, 57% and 75% RH. In this case, rate constant value it was multiplied by 51 and 59, at 25 °C with 57% and 75%, respectively. At 40 °C with 23% RH was observed the same instability of this anthocyanin that at 25 °C with 75% RH, and at 40 °C with 57% and 75% RH, P 3,5-di-O-g was completely deteriorated in less than 4 days. In FBR, this anthocyanin showed the same behavior as in SF under the different storage conditions studied. In this case, it has not been contemplated kinetics parameter at 40 °C with 57% and 75% RH due to this anthocyanin was completely deteriorated in 17 days under these storage conditions. Therefore, it was repeated that an increased in the RH conditions causes a less stability of the anthocyanin studied.

D 3-*O*-g and M 3,5-di-*O*-g were minor anthocyanins in SF and in FBR. At 25 °C with 23% RH the k values of D 3-*O*-g and M 3,5-di-*O*-g were an order of magnitude greater than for D 3,5-di-*O*-g and P 3,5-di-O-g, which represented lower values of half-life period, around 0.6 and 1 year, respectively. Both in SF and in FBR a good kinetic fit could not be obtained at 25 °C with 57% and 75% and at a temperature of 40 °C, because these anthocyanins were completely deteriorated in SF 4 days after storage and in FBR in less time.

Regarding to the kinetics of the TA at 25 °C with 23% RH there was no deterioration, both in SF and FBR. At this temperature, an increased in the RH showed a second-kinetic model with very low half-life periods. At 40 °C with 23% RH in SF, the k value was similar to the k at 25 °C and 57% RH and an increase of RH at 40 °C generated a fast deterioration of the TA in 4 days, both in SF and FBR. In FBR, an increase in the RH at 25 °C did not show significant differences, while an increased in the temperature obtained significant differences (40 °C with 23% RH). The different behavior between TA and the anthocyanins studied showed that it is unwise to measure anthocyanin content by TA.

Table 3 shows the kinetic parameters of flavonols content loss according to a first-order kinetic model in SF and in FBR at different temperatures (25 °C and 40 °C) and relative humidity conditions (23%, 57% and 75%). K 7-*O*-g, Q 3-*O*-s and I 3,4́-tri-*O*-g remained stable at different storage conditions studied. The same behaviour was obtained for K 3,7,4́-tri-*O*-g in SF at 25 and 40 °C. A strange behaviour was observed in FBR at 25 °C for this kaempferol, showing a slight deterioration from the first day until the 45th day, then until the 168th day increased slightly. At 40 °C was observed a slight deterioration from the beginning of storage period.

K 3-*O*-s, main kaempferol in SF and in FBR, was no deteriorated in FBR under the all different storage conditions studied. In SF, at 25 °C with 57 and 75% RH remained constant. At both temperatures studied with 23% RH was slightly deteriorated, influencing significantly at 40 °C the increase from 23 to 57% RH in its deterioration, while an increased from 57 to 75% RH did not show significant differences. This kaempferol represented in SF and in FBR about 98.93% and 98.49%, respectively, of the total content of kaempferol glycosides plus its aglycone. The best storage conditions in SF were at 25 °C with 57% and 75% RH and any of the studied conditions in FBR. It is clear that for preserving the main kaempferol glycoside it is necessary to remove the stigma of the saffron flower before its storage period.

Table 3
Rate constants (*k*), determination coefficients (R^2), and half-life periods ($t_{1/2}$) of kaempferols content loss in an extract of saffron flowers and floral bio-residues at different temperatures (T) and relative humidity (RH) conditions.

T (°C)	RH (%)	Compounds	FLOWERS			FLORAL BIO-RESIDUES		
			(k ± SD)[a] × 10^{-3} (d^{-1})	R^2 (m)[b]	$t_{1/2}$ (d)	(k ± SD)[a] × 10^{-3} (d^{-1})	R^2 (m)[b]	$t_{1/2}$ (d)
25	23	K 3-*O*-s	1.79 ± 0.11 a	0.928 (6)	389	–	–	–
		K 3,7-di-*O*-g	23.67 ± 3.23 c, d	0.800 (7)	30	29.12 ± 5.69 c	0.819 (6)	25
		K 3-*O*-r	11.51 ± 1.04 e	0.817 (7)	61	22.67 ± 2.73 f	0.810 (9)	31
		K 3-*O*-g	27.30 ± 0.81 i	0.827 (5)	25	26.00 ± 9.45 i	0.855 (5)	29
	57	K 3-*O*-s-7-*O*-g	11.95 ± 1.04 n	0.940 (8)	76	14.37 ± 0.43 d	0.922 (10)	48
		K 3,7-di-*O*-g	33.37 ± 9.82 d	0.743 (8)	22	28.92 ± 4.61 c	0.834 (6)	24
		K 3-*O*-r	15.47 ± 3.61 e	0.762 (6)	46	18.57 ± 1.69 e	0.825 (9)	37
		K 3-*O*-g	133.25 ± 20.17 l	0.820 (4)	6	49.50 ± 10.54 j	0.892 (4)	14
	75	K 3-*O*-s-7-*O*-g	5.86 ± 0.20 m	0.909 (10)	118	8.967 ± 0.11 b	0.925 (10)	77
		K 3,7-di-*O*-g	19.62 ± 3.94 c	0.749 (7)	36	22.37 ± 4.06 c	0.816 (7)	32
		K 3-*O*-r	10.88 ± 3.07 e	0.709 (7)	67	18.45 ± 1.18 e	0.857 (9)	38
		K 3-*O*-g	65.32 ± 11.84 k	0.829 (4)	11	40.20 ± 3.88 j	0.920 (6)	17
40	23	K 3-*O*-s	2.18 ± 0.45 a	0.901 (7)	328	–	–	–
		K 3,7-di-*O*-g	19.20 ± 2.36 c	0.851(6)	36	27.07 ± 5.14 c	0.807 (4)	26
		K 3-*O*-r	23.37 ± 1.31 f	0.862 (8)	30	17.12 ± 0.78 e	0.791 (4)	40
		K 3-*O*-g	44.30 ± 0.96 j	0.931 (4)	16	20.37 ± 5.74 i	0.836 (4)	36
	57	K 3-*O*-s	21.77 ± 5.88 b	0.810 (6)	35	–	–	–
		K 3-*O*-s-7-*O*-g	76.75 ± 0.87 p	0.890 (6)	9	10.70 ± 1.08 c	0.938 (8)	65
		K 3,7-di-*O*-g	22.50 ± 5.97 c, d	0.990 (6)	33	32.50 ± 5.77 c	0.895 (9)	22
		K 3-*O*-r	41.67 ± 5.62 h	0.800 (6)	17	24.77 ± 4.14 f	0.898 (9)	28
		K 3-*O*-g	46.45 ± 2.11 j	0.853 (5)	15	19.65 ± 5.89 i	0.949 (8)	37
	75	K 3-*O*-s	25.37 ± 0.41 b	0.966 (4)	32	–	–	–
		K 3-*O*-s-7-*O*-g	58.10 ± 7.45 o	0.952 (4)	13	6.28 ± 0.46 a	0.802 (5)	111
		K 3,7-di-*O*-g	27.65 ± 11.72 c, d	0.842 (4)	29	56.40 ± 29.03 d	0.870 (4)	15
		K 3-*O*-r	34.05 ± 0.42 g	0.839 (6)	20	18.52 ± 3.43 e	0.903 (5)	39
		K 3-*O*-g	45.85 ± 12.83 j	0.881 (6)	16	46.60 ± 3.73 j	0.955 (5)	15

K 3-*O*-s, kaempferol 3-*O*-sophoroside; K 3,7-di-*O*-g, kaempferol 3,7-di-*O*-glucoside; K 3-*O*-r, kaempferol 3-*O*-rutinoside; K 3-*O*-g, kaempferol 3-*O*-glucoside; K 3-*O*-s-7-*O*-g, kaempferol 3-*O*-sophoroside-7-*O*-glucoside.

[a] Values are the mean of two extracts conducted in duplicate (2 × 2n), SD = standard deviation.

[b] Minimum number of experimental data points. At each compound, different letters between rows indicate significant differences at the 0.05% level: a, b for K 3-*O*-s; c, d for K 3,7-di-*O*-g; e, f, g, h for K 3-*O*-r; i, j, k, l for K 3-*O*-g; m, n, o, p for K 3-*O*-s-7-*O*-g.

In case of K 3-*O*-s-7-*O*-g, good fits to a zero-order kinetic model was also found. This kaempferol, both in SF and in FBR at 25 °C and 40 °C with 23% RH was stable. It is noticed that in both plant material was shown greater *k* values at 57% RH than at 75% RH.

K 3,7-di-*O*-g at 25 °C in SF showed lower *k* values when RH increased from 57% to 75% RH, the same trend that it was observed for K 3-*O*-s-7-*O*-g. At 40 °C the behaviour was different that at 25 °C, an increase of RH at 40 °C obtained similar *k* values. In FBR, at 25 °C an increase of RH did no show a greater deterioration of this compound. At 40 °C did not find significant differences between 23% and 57% RH, being the most unfavourable storage conditions at 40 °C with 75% RH.

K 3-*O*-r at 25 °C showed the same stability under three different RH studied in SF. At 40 °C in SF, it was observed the same behaviour that for K 3-*O*-s-7-*O*-g and K 3,7-di-*O*-g; at 57% RH was showed greater *k* values than at 75% RH. In FBR this behaviour was also observed at 40 °C and at 25 °C with the rise between 23% and 57% RH, being the worst storage conditions at 25 °C with 23% RH and 40 °C with 57% RH.

An increased in SF of RH at 25 °C from 57% to 75% RH for K 3-*O*-g showed again lower *k* values. The worst storage conditions for this kaempferol in SF was 25 °C with 57% RH. In FBR an increase of RH at 25 °C from 23% to 57% RH and at 40 °C from 57% to 75% RH evinced greater *k* values.

Regarding to kaempferol aglycone, in both plant material studied under all different storage conditions, showed an increase of its concentration during the first days followed by a deterioration period. This could be due to in the deteriorate process of kaempferols glycosides, by hydrolysis, could be generate kaempferol aglycone at first, and with the passage of time, it would occur the deterioration of kaempferol glycosides and the kaempferol aglycone.

The results revealed that all kaempferols studied did no show the same trend under different storage conditions and their behaviour was also different in several cases according to the plant material studied. In contrast with that happened with the anthocyanins studied, an increase of RH did no always show greater *k* values for kaempferols. An increase of RH from 57% to 75% did no always obtain lower *k* values for K 3-*O*-s-7-O-g, K 3,7-di-*O*-g, K 3-*O*-r and K 3-*O*-g. The same behavior showed K 3-*O*-s in SF at 25 °C and 23% RH, being stable this kaempferol if the RH increased from 23% to 57% and 75% RH. It seems that humidity could have a protective effect on the deterioration of these kaempferols. In this way, results about other spice (paprika) revealed that the humidity had a clearly protective effect on its color stability when samples were stored during 160 days at different conditions (Ladrón de Guevara, Bernabeu, Picazo, Gonzalez, & Varón, 2005). A moderate increase in the relative humidity could generate a protection against the deterioration process of the main metabolites by the oxygen, as it happens with the carotenoids of the paprika.

4. Conclusions

K 3-*O*-s and D 3,5-di-*O*-g were the main phenolic compounds in SF and FBR. K 3-*O*-s represented more than 79% of polyphenolic content obtained in both plant material studied. The comparison of the general parameters (FC and TA) with the evolution of the main kaempferol and anthocyanin indicates that is more appropriate to measure polyphenol content in SF and FBR by HPLC-DAD than by UV–vis spectrophotometry.

Temperature and RH conditions affect the stability of dry SF and FBR. The degradation process of anthocyanins showed better fits in a second-order kinetic than in zero-order, or first-order one. The loss of kaempferols glycosides content presented better fits in a first-order kinetic model.

Among the studied storage conditions in both plant material, the

most convenient ones to avoid a loss of anthocyanins were 25 °C and 23% RH. On the contrary, 40 °C and 75% RH were the storage conditions which produced the fastest degradation of anthocyanins in SF and FBR. The degradation process of kaempferols content did not show the same trend for all of them. A different behavior was found in SF and FBR for some kaempferol glycosides. K 3-O-s was no deteriorated in FBR under the different storage conditions studied.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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