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Effect of Centrifugal Ultrafiltration on the Composition of Aqueous Extracts of Saffron Spice (*Crocus sativus* L.)

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The purpose of this research was to study the effect of centrifugal ultrafiltration (UF) on the composition of aqueous extracts of saffron spice. The contents of seven crocetin esters, picrocrocin, and two kaempferol glycosides were analyzed by UV−vis and HPLC in the filtrate and retentate fractions from 16 centrifugal filter devices with regenerated cellulose (RC) and polyethersulfone (PES) membranes ranging from 1−100 kDa nominal molecular weight cutoff (MWCO). The separation of crocetin esters from picrocrocin and their concentration with centrifugal UF have been demonstrated. A great heterogeneity of results regarding devices with equal MWCO was found that could not be related to the membrane material or manufacturer. Four devices of 5 and 10 kDa MWCO, three of which had RC membranes, showed the best results. The device having the lowest MWCO also showed a potential to obtain picrocrocin without crocetin esters and could be considered in successive UF steps. The less polar crocetin esters were rejected better than the others.

KEYWORDS: Saffron (*Crocus sativus* L.); carotenoids; crocetin esters; picrocrocin; kaempferol glycosides; centrifugal ultrafiltration; regenerated cellulose; polyethersulfone

INTRODUCTION

Mainly, three groups of compounds stand out in the composition of aqueous extracts of saffron (*Crocus sativus* L.) spice (**Figure 1**): (1) a group of water-soluble carotenoids responsible for saffron color and coloring capacity that consists of various esters of crocetin ($C_{20}H_{24}O_4$, 8,8′-diapo-Ψ,Ψ′-carotenedioic acid), where glucose, gentiobiose, neapolitanose, or triglucose are the sugar moieties (*1*) and where trans- or cis-configuration is found; (2) picrocrocin (4-(β-D-glucopyranosyl)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde) and its related compounds; (3) flavonoids such as kaempferol glycosides, which are thought to contribute to the bitter taste of saffron together with the previous group (*1–4*). Saffron spice also contains safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde), the major compound of its volatile fraction, although its solubility in water is very low (*5*). Until now, separation and purification procedures for water-soluble saffron components consisted of column chromatography (*6, 7*), preparative or analytical HPLC (*8, 9*), multilayer coil countercurrent chromatography (*3*), and TLC (*10, 11*). An attractive alternative to these methods is ultrafiltration (UF) because of its mild operating conditions and relatively high selectivity. However, there are no studies on its application to saffron extracts, even though there are enough differences in molecular weight (Mw) among the principal compounds responsible for saffron's color and taste to consider their partial or total separation with UF technologies.

UF is widely used in the agro-food industry for recovering peptides, proteins, polysaccharides, and other biopolymers of animal or vegetal origin (*12–15*). It is particularly suitable for the separation of suspended solids in liquid foods and as a preliminary step to other processes, such as concentration by reverse osmosis or the deacidification and debittering of fruit juices (*16*). This technique offers the food industry the advantages of a chemical-free separation treatment, the possible diafiltration of the retentate, and maximum protection of the sample against external factors.

New applications for separation, concentration, or purification in saffron analysis and the saffron industry could arise from careful research into the behavior of saffron components when they are subjected to UF processes. The relevance of UF in saffron chemistry lies in its potential for component separation and concentration together with a better handling of labile and easily oxidized components such as crocetin esters. From the technological point of view, this field of research might contribute to the introduction of modifications in the color, taste, or functional properties of saffron extracts by changing the proportion of its components.

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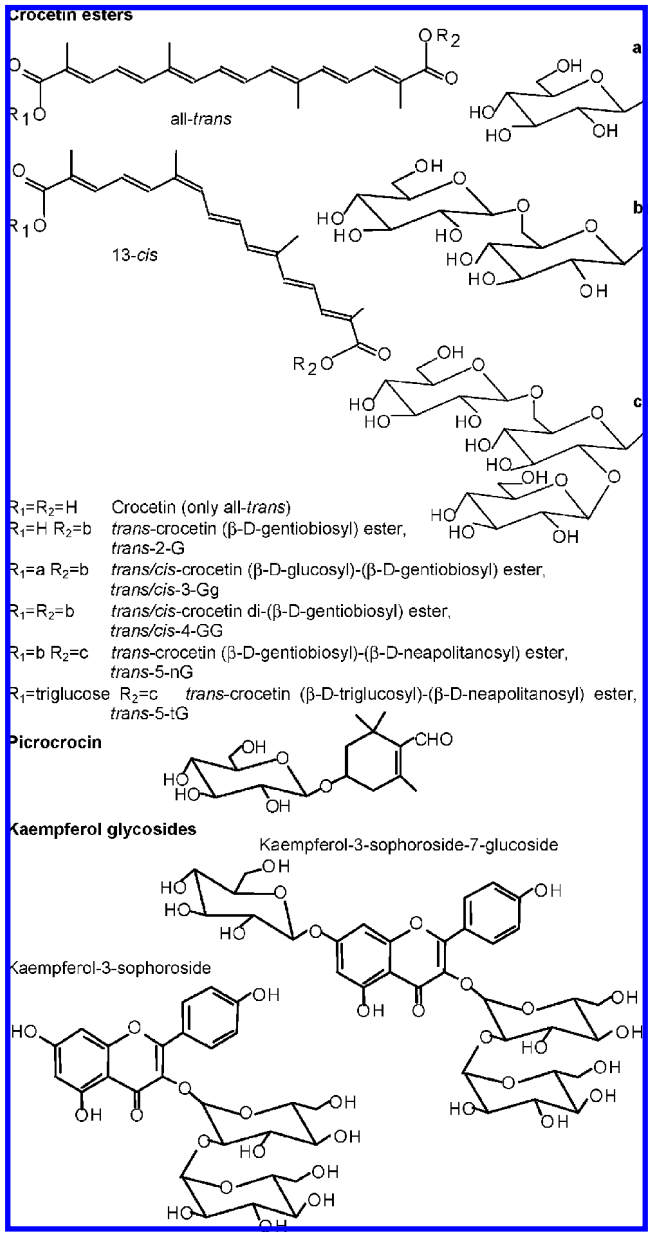

Figure 1. Structures of saffron compounds under discussion. In the case of crocetin esters with *cis*-configuration, the position of the substitutes R_1 and R_2 could not be exactly determined in relation to the C_{13-14} bond.

The performance of a given UF membrane depends on several factors including transmembrane pressure, cross-flow velocity, concentration of dissolved solids, fouling characteristics, and nominal molecular weight cutoff (MWCO) (*15*). The MWCO is a term associated with pore size and is used for describing the separating capabilities of a UF membrane. It refers to the Mw of a solute, such as a globular protein, which is 90% rejected by the membrane under standard conditions (*13*). But MWCO may not be valid for all solutes since it depends on molecular dimensions and behavior, making a test of each membrane necessary for the solutes of interest.

In the application of UF to saffron, the vast supply of membranes available on the market and their high cost, together with the high price of the spice, make small scale procedures necessary to select the most appropriate membranes using small volumes of saffron extract. This can be carried out by using centrifugal filter devices. These filter devices are widely used for the concentration, purification and desalting of protein and nucleic acid solutions, but they have never been applied to the study of saffron extracts.

The purpose of this work was to study the effect of centrifugal UF on the composition of crocetin esters, picrocrocin, and kaempferol glycosides in aqueous extracts of saffron spice, in order to determine the possibility of concentration or purification of these components and to select the most appropriate membranes to attain it. Special attention was paid to comparing the performance of regenerated cellulose and polyethersulfone membranes ranging from 1 kDa to 100 kDa MWCO.

MATERIALS AND METHODS

Samples and Standards. Saffron spice (*Crocus sativus* L.) was used from the 2006 harvest of the Protected Designation of Origin Azafrán de La Mancha. Rutin hydrate (95%) was purchased from Sigma-Aldrich (Steinheim, Germany).

Centrifugal Filter Devices. The centrifugal filter devices under study and their technical specifications are listed in **Table 1**. A total of 16 different filter devices were used, designated from 1 to 16. They came from three manufacturers (Millipore, Bedford, MA; Pall, Ann Arbor, MI; and Sartorius, Goettingen, Germany) and had five different membranes (Omega, Amicon, Ultracel, Vivaspin and Biomax) made of polyethersulfone (PES) or regenerated cellulose (RC) with an MWCO ranging from 1 to 100 kDa.

Saffron Extract Preparation. To reduce the coextraction of nonpolar compounds, 1 g of powdered saffron was extracted twice with 20 mL of cyclohexane. Each extraction was carried out at room temperature in the dark for 5 h with sporadic agitation. Then the organic solvent was discarded, and the solid residue was dried under reduced pressure. Five-hundred milligrams of the thus treated saffron powder was extracted with 1 L of ultra high purity water by stirring the suspension in the dark at room temperature for 1 h. Next, to obtain the initial extract for the centrifugal UF treatment, the extract was clarified by centrifugation at 4280 × *g* (7000 rpm) for 20 min and successive dead-end microfiltration through 0.8, then 0.45 and then 0.2 μm pore size cellulose acetate membrane filters from Albet (Barcelona, Spain). This aqueous saffron extract preparation was carried out just before each treatment in order to avoid storage and degradation of crocetin esters.

Centrifugal UF Treatment. First, in order to set centrifugation time, the filtration profile, that is to say filtrate volume versus centrifugation time, was studied with 10 mL feed volume of aqueous saffron extract and the centrifuge's maximum rcf: 3220 × *g* (4000 rpm), in a swinging bucket at 20 °C for 10, 15, 20, 30, 40, 50, and 60 min. Once the centrifugal conditions were selected (3220 × *g*, 20 min), experiments were conducted in triplicate for each device. The only exceptions were devices 4 and 5 (**Table 1**), which were centrifuged in a fixed rotor at 2687 × *g* (4300 rpm) for 20 min, due to their dimensions and maximum rcf.

Spectrophotometric Analysis. Spectroscopic characteristics of aqueous saffron extracts, filtrate and retentate fractions were monitored by scanning from 190 to 700 nm using a Perkin-Elmer Lambda 25 spectrophotometer (Norwalk, CT, USA) with UV WinLab 2.85.04 software (Perkin-Elmer). Saffron quality characteristics, moisture and volatile matter content, coloring strength ($E^{1\%}_{1cm}$ 440 nm), $E^{1\%}_{1cm}$ 257 nm, and $E^{1\%}_{1cm}$ 330 nm were determined according to ISO 3632/TS (*17*). The picrocrocin molar absorption coefficient in aqueous solution was determined by following the Beer−Lambert law. All analyses were done in triplicate.

RP-HPLC-DAD Analysis. Forty microliters of each sample were injected into an Agilent 1100 HPLC chromatograph (Palo Alto, CA) operating with a 150 mm × 4.6 mm i.d., 5 μm Phenomenex (Le Pecq Cedex, France) Luna C_{18} column, thermostated at 30 °C. Eluents were water (A) and acetonitrile (B), with the following gradient: 20% B, 0−5 min; 20−80% B, 5−15 min and 80% B, 15−20 min. The flow rate was 0.8 mL/min. The DAD detector (Hewlett-Packard, Waldbronn, Germany) was set at 250, 330, and 440 nm for picrocrocin, kaempferol glycoside and crocetin ester detection, respectively. All analyses were carried out in triplicate. The measurements of the extract used as feed

Table 1. Technical Specifications of the Centrifugal Membrane Filter Devices under Study

no.	centrifugal filter device trade name	membrane trade name	membrane material[a]	MWCO[b] (kDa)	active membrane area (cm^2)	maximum rcf (g)	manufacturer[c]
1	Macrosep	Omega	PES	1	10.00	5000	Pall
2	Macrosep	Omega	PES	3	10.00	5000	Pall
3	Vivaspin−20	Vivaspin	PES	3	6.00	5000	Sartorius
4	Centriplus	Amicon YMT	RC	3	2.34	3000	Millipore
5	Centriprep	Ultracel YM-3	RC	3	2.84	3000	Millipore
6	Vivaspin−20	Vivaspin	PES	5	6.00	5000	Sartorius
7	Amicon Ultra-15	Ultracel	RC	5	7.60	4000	Millipore
8	Centricon Plus-20	Biomax	PES	5	9.50	4000	Millipore
9	Amicon Ultra-15	Ultracel	RC	10	7.60	4000	Millipore
10	Centricon Plus-20	Ultracel PL	RC	10	9.50	4000	Millipore
11	Macrosep	Omega	PES	10	10.00	5000	Pall
12	Vivaspin−20	Vivaspin	PES	10	6.00	5000	Sartorius
13	Vivaspin−20	Vivaspin	PES	30	6.00	5000	Sartorius
14	Vivaspin−20	Vivaspin	PES	50	6.00	5000	Sartorius
15	Amicon Ultra-15	Ultracel	RC	50	7.60	4000	Millipore
16	Centricon Plus-20	Ultracel PL	RC	100	9.50	4000	Millipore

[a] PES, polyethersulfone; RC, regenerated cellulose. [b] MWCO: nominal molecular weight cutoff. [c] Pall, (Ann Arbor, MI), Sartorius (Goettingen, Germany), Millipore (Bedford, MA).

Table 2. Molecular Weight (Mw), Composition (Mean and Standard Deviation: SD, $n = 3$) in Kaempferol Glycosides, Picrocrocin, and Crocetin Esters, UV−Vis Maxima and Retention Times (RT) of the Initial Saffron Extract

compound	Mw	mean content ± SD (mg/L)	mean content ± SD (% on dry basis)	UV−vis λmax (nm)	RT (min)
kaempferol-3-sophoroside-7-glucoside[a]	772	7.78 ± 0.37	1.66 ± 0.08	265, 321sh, 345	2.9
kaempferol-3-sophoroside[a]	610	8.00 ± 0.82	1.71 ± 0.18	266, 295sh, 350	5.6
total kaempferol glycosides		15.78 ± 0.45	3.37 ± 0.11		
picrocrocin	330	86.21 ± 7.81	18.43 ± 1.54	250	5.9
trans-5-tG	1139	1.72 ± 0.11	0.34 ± 0.02	263, 443, 467	9.6
trans-5-nG	1139	1.69 ± 0.60	0.33 ± 0.12	263, 422sh, 440, 467sh	10.0
trans-4-GG	977	66.35 ± 3.12	13.04 ± 0.70	262, 442, 465	10.3
trans-3-Gg	815	40.76 ± 2.91	8.01 ± 0.54	262, 441, 465	10.9
trans-2-G	653	5.57 ± 0.72	1.10 ± 0.15	259, 434, 459	11.5
cis-4-GG	977	6.71 ± 0.27	1.32 ± 0.06	262, 327, 435, 458	12.0
cis-3-Gg	815	2.67 ± 1.05	0.53 ± 0.21	262, 325, 434, 458	12.7
total crocetin glycosides		125.48 ± 2.56	24.66 ± 0.68		

[a] Kaempferol glycoside content expressed as equivalent mass of rutin.

for centrifugal filter devices were made at the same time as the measurements of the filtrate and retentate fractions in order to minimize variations in the results due to crocetin ester degradation.

Identification and Quantification of Saffron Components. Identification of crocetin esters, picrocrocin, and kaempferol glycosides by HPLC-DAD-MS was carried out as previously described (*1, 4*). Total crocetin esters were first determined by UV−vis spectrophotometry, using their absorbance at 440 nm. The results were expressed as the percent of *trans*-4-GG, as reported by Basker et al. (*18*) but using the molar absorption coefficient determined by Speranza (*8*). Because of the lack of pure standards of each crocetin ester, their quantification was based on the following equation (*19, 20*):

$$\%\ \text{of crocetin ester } i \text{ on dry basis} = \frac{\text{Mw}_i(E^{1\%}_{1\ \text{cm}}\ 440\ \text{nm})A_i}{10\varepsilon_{\text{t,c}}} \qquad (1)$$

where Mw_i stands for the molecular weight of the crocetin ester i, $E^{1\%}_{1\text{cm}}$ 440 nm is the coloring strength, A_i is the percent peak area of the crocetin ester i at 440 nm and $\varepsilon_{\text{t,c}}$ is the molar absorption coefficient value (89000 for *trans*-crocetin esters and 63350 for *cis*-crocetin esters) (*8*). For comparative purposes, besides the results for each crocetin ester, the total crocetin ester content was assessed with HPLC data.

Quantification with UV−vis data was based on the determined picrocrocin molar absorption coefficient (10515 L cm^{-1} mol^{-1}). Quantification with HPLC data was based on calibration curves of the picrocrocin concentration, c, (mg/L) as a function of its peak area, a, in the range of 2−315 mg/L: $c = 0.0354\ a + 0.0018$, $r^2 = 0.999$, for a total of six data points. Picrocrocin was purified according to the procedure described below.

Quantification of kaempferol glycosides referred to a rutin standard, whose concentration, d (mg/L), as a function of its HPLC peak area, b, also exhibited good linear regression in the 5−100 mg/L range ($d = 0.0882b + 0.0021$, $r^2 = 0.996$, for 10 data points). The content of kaempferol glycosides was expressed as equivalent mg of rutin/100 mg of dry saffron.

Picrocrocin Isolation. Picrocrocin was extracted from saffron and isolated by column chromatography by using a C_{18} adsorbent (125×10^{-8} cm pore size, 55−105 μm particle size) from Waters (Milford, MA). For extraction, 30 mL of cyclohexane (HPLC-grade from Scharlau, Barcelona, Spain) were added to 5 g of powdered saffron, and the suspension was left in the dark at room temperature for 24 h with sporadic agitation. Then the organic solvent was discarded, and the solid residue was dried under reduced pressure. Sixty milliliters of nitrogen-saturated water were added to the thus treated saffron, and the resulting suspension was stirred for 1 h in the dark at room temperature. Then the extract was centrifuged at 4000 rpm for 10 min, and the supernatant was collected and loaded on the previously conditioned C_{18} column (8 cm high × 2.7 cm i.d.). Picrocrocin was eluted with 90 mL of 10% acetonitrile/water (v/v) after the elution of flavonoids with 20 mL of 2% acetonitrile/water (v/v). Finally, the solvent was eliminated by evaporation to dryness under reduced pressure, and the purified picrocrocin was kept at −20 °C until its utilization. The chromatographic purity of the obtained picrocrocin was 96%, calculated as the percent of the total peak area at 250 nm.

Centrifugal Filter Membrane Performance. Performance of the centrifugal filter membranes was expressed in terms of the parameters described below. Volume concentration ratio (*VCR*) is defined as:

$$VCR = \frac{V_O}{V_R} \quad (2)$$

where V_0 is the volume of the feed extract, and V_R is the volume of the retentate fraction. Rejection (R) of any solute is defined as the variation (<0 = an increase, >0 = a decrease) in the solute concentration, expressed as a percentage of its concentration in the feed extract, which is observed in the filtrate fraction:

$$R\,(\%) = \left(1 - \frac{C_F}{C_O}\right) \times 100 \quad (3)$$

where C_F is the concentration of the solute in the filtrate fraction, and C_0 is the concentration of the solute in the feed extract. Filtrate, retentate, and total recoveries (%) were calculated by a direct weighing procedure, considering the density of the aqueous solutions as equal to 1 g/mL and using the measured concentrations as follows:

$$\text{Filtrate recovery}\,(\%) = \left(\frac{V_F \times C_F}{V_O \times C_O}\right) \times 100 \quad (4)$$

$$\text{Retentate recovery}\,(\%) = \left(\frac{V_R \times C_R}{V_O \times C_O}\right) \times 100 \quad (5)$$

$$\text{Total recovery}\,(\%) = \text{Retentate recovery}\,(\%) + \text{Filtrate recovery}\,(\%) \quad (6)$$

$$\text{Losses}\,(\%) = 100 - \text{Total recovery}\,(\%) \quad (7)$$

where V_F is the filtrate volume, V_0 is the initial extract volume (feed volume), V_R is the retentate fraction volume, C_F is the concentration of the solute in the filtrate fraction, C_0 is the concentration of the solute in the initial extract, and C_R is the concentration of the solute in the retentate fraction.

Nomenclature for Crocetin Esters. Abbreviations were adopted from Carmona et al. (*1*): *trans*-5-tG, *trans*-crocetin (β-D-triglucosyl)-(β-D-gentiobiosyl) ester; *trans*-5-nG, *trans*-crocetin (β-D-neapolitanosyl)-(β-D-gentiobiosyl) ester; *trans*-4-GG, *trans*-crocetin di-(β-D-gentiobiosyl) ester; *trans*-3-Gg, *trans*-crocetin (β-D-glucosyl)-(β-D-gentiobiosyl) ester; *trans*-2-G, *trans*-crocetin (β-D-gentiobiosyl) ester; *cis*-4-GG, *cis*-crocetin di-(β-D-gentiobiosyl) ester; and *cis*-3-Gg, *cis*-crocetin (β-D-glucosyl)-(β-D-gentiobiosyl) ester.

Statistics. Data were subjected to an analysis of variance (ANOVA) using the SPSS 15.0 statistical program for Windows (SPSS Inc.).

RESULTS AND DISCUSSION

Saffron Quality Characteristics and Chemical Composition of the Initial Saffron Extract. Results indicated that the commercial saffron sample used belonged to ISO category I (*17*): 8.1% moisture and volatile matter content; $E^{1\%}_{1cm}$ 440 nm = 239.2 ± 3.1 (coloring strength); $E^{1\%}_{1cm}$ 257 nm = 91.3 ± 1.2; $E^{1\%}_{1cm}$ 330 nm = 25.8 ± 0.5.

It was observed that the cyclohexane removed from the sample preparation was yellow; therefore, after this process the $E^{1\%}_{1cm}$ 440 nm, $E^{1\%}_{1cm}$ 257 nm, and $E^{1\%}_{1cm}$ 330 nm aqueous extracts were measured. The results indicated that after cyclohexane extraction, the $E^{1\%}_{1cm}$ 440 nm of the aqueous extract remained the same (239.1 ± 5.6), while $E^{1\%}_{1cm}$ 257 nm and $E^{1\%}_{1cm}$ 330 nm were slightly higher (95.5 ± 1.0 and 27.8 ± 0.4, respectively). The similarities in $E^{1\%}_{1cm}$ 440 nm indicated an extraction of nonwater-soluble pigments such as α and β-carotene, lycopene, or zeaxanthin, whose presence in saffron has already been described (*21, 22*), rather than a removal of crocetin esters.

The composition of the initial saffron extract in kaempferol glycosides, picrocrocin and crocetin esters is shown in **Table 2**. It was expressed as mg/L and as a percentage on a dry basis so that the comparison to previously reported data could be made. In addition, this table shows Mw, UV−vis maxima and retention times (RT) for the above-mentioned compounds. The initial concentration of kaempferol glycosides in the extract

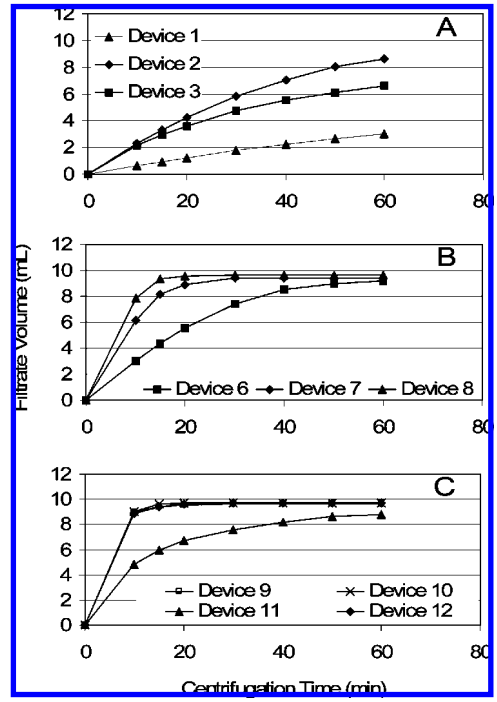

Figure 2. Filtration profile for saffron extracts from ≤10 kDa MWCO devices centrifuged at 3220 × *g*. (**A**) 3 kDa MWCO; (**B**) 5 kDa MWCO; (**C**) 10 kDa MWCO.

determined by HPLC was ~16 mg/L, while the picrocrocin concentration was ~86 mg/L, and the one for crocetin esters was ~125 mg/L. The main kaempferol glycosides found were kaempferol-3-sophoroside-7-glucoside and kaempferol-3-sophoroside, which showed fairly similar contents, ~1.7 mg of rutin/100 mg of dry saffron each. These results were higher than the data reported in previous research: 0.258 and 0.312 mg of rutin/100 mg of dry saffron, respectively (*4*). The picrocrocin content of the sample used here (18.4%) was also higher than what Alonso et al. reported (0.79−12.94%) (*23*), and than results by Iborra et al. (*10*) (13.9%). Crocetin esters were the major compounds, comprising ~25%. Among them, *trans*-4-GG was the most abundant (13.04%), followed by *trans*-3-Gg (8.01%), *cis*-4-GG (1.32%), *trans*-2-G (1.10%), *cis*-3-Gg (0.53%), *trans*-5-tG (0.34%), and *trans*-5-nG (0.33%). Alonso et al. (*23*) also reported lower values for *trans*-4-GG (0.46−12.12%) in Spanish saffron, but results from the present study for *trans*-3-Gg, *cis*-4-GG and *cis*-3-Gg remained within the ranges previously given in ref *23* for the above-mentioned crocetin esters (0.01−9.44%; 0.04−8.53%; 0.01−2.26%, respectively).

Filtration Profile for Aqueous Saffron Extracts. Filtration profiles from the centrifugal filter devices have been established by manufacturers for several well-known materials (bovine serum albumin, cytochrome *c*, etc.). However, actual performance depends on the nature of the specific solute under study, making a study with saffron extracts indispensable. The filtration profile for saffron extracts consisted of an increasing filtrate volume as centrifugation time increased until a plateau was reached. **Figure 2** shows the filtration profile from ≤10 kDa MWCO devices centrifuged at 3220 × *g*. When using centrifugal devices from 1 to 3 kDa MWCO (**Figure 2A**), filtrate volume did not reach the plateau in 60 min and was on the increase as MWCO increased. Also, differences were found between devices 2 and 3 (both of 3 kDa MWCO). Device 2 showed higher filtrate volumes, especially after 20 min of centrifugation, probably due to its higher active membrane area or the kind of membrane. In 5 kDa MWCO devices (**Figure**

Table 3. Volume Concentration Ratio (*VCR*) and Mean ± Standard Deviation, $n = 3$

centrifugal filter device	*VCR*[a]	centrifugal filter device	*VCR*
1	1.2 a ± 0.1	9	43.4 f ± 8.7
2	1.7 a ± 0.1	10	76.0 g ± 1.3
3	1.8 a ± 0.1	11	2.6 a ± 0.1
4	1.6 a ± 0.3	12	37.4 e ± 1.9
5	1.4 a ± 0.2	13	31.8 d ± 1.6
6	2.8 a ± 0.3	14	91.6 h ± 4.6
7	14.3 b ± 4.4	15	88.7 h ± 15.4
8	20.6 c ± 4.6	16	15.1 b ± 0.8

[a] The same letter in the *VCR* columns indicates nonsignificant differences according to Duncan's test at the 0.05% level.

2B), it was found that the filtrate volumes were ordered from lower to higher active membrane areas, and all but device 6 had reached a stable filtrate volume at about 20 min. Comparing their characteristics, the highest similarities were found in devices from the same manufacturer, rather than in devices with the same membrane material. In spite of having different membrane materials and active membrane areas, the filtration profiles of all 10 kDa MWCO devices (**Figure 2C**) were the same, except for device 11, which showed lower filtrate volumes. For the same type of device and therefore, the same active membrane area, it was observed that device 8 (5 kDa MWCO, PES membrane) and device 10 (10 kDa MWCO, RC membrane) had equal filtration profiles for saffron extracts. It seemed that PES was more permeable than RC in this type of device. In light of these results and so that all devices would have the same experimental conditions, 20 min was selected as the centrifugation time. Regarding the filtration profiles that are not presented in **Figure 2**, devices 4 and 5 showed filtration profiles similar to device 2, and device 13 was very similar to device 12. Filtration profiles of devices 14 and 15 reached maxima filtrate volumes in the early min of centrifugation, while devices 16 and 7 had similar filtration profiles.

Volume Concentration Ratio (*VCR*). A vast range of *VCR* was reached with the membranes studied, from ~1 to ~92 (**Table 3**). The lower limit of this range corresponded mainly to devices with MWCO membranes under 5 kDa. These devices (from device 1 to 5) and devices 11 and 6 did not present significant differences in *VCR*. However, the highest *VCR* value was found for devices 14 and 15, which had 50 kDa MWCO. There was a great heterogeneity in the *VCR* of devices sharing the same MWCO membrane, even when the membrane material was the same but the manufacturer was different. This could be seen when comparing devices 6 and 8, both with a 5 kDa MWCO and a PES membrane, or devices 11 and 12, both having a 10 kDa MWCO and a PES membrane. As for the membrane material (= MWCO, = manufacturer), PES gave slightly higher *VCR* results than RC (comparing devices 7 and 8). However, the low magnitude of the difference could be due rather to its higher active membrane area. In general, from the comparison of devices 1, 2 and 11 or 3, 6, 12, 13 and 14 or 7, 9 and 15, it was found that *VCR* increased with increasing MWCO (= membrane material, = manufacturer and type of device). Sometimes the differences were not significant, however, and in devices 12 and 13, the tendency was inverted.

Performances of Each Membrane Determined by UV−Vis Spectrophotometry versus Those Determined by HPLC. Results indicated that total crocetin ester *R* and recoveries were very similar for both means of quantification, and therefore, they could be used equally. These similarities were in consonance with the good correlation found between UV−vis and HPLC results (*20*). However, higher values of picrocrocin *R* and lower or equal picrocrocin recoveries were found in most cases with UV−vis data than with HPLC data. This may be due to the fact that not only picrocrocin, but also other compounds, such as crocetin esters or flavonoids, absorb at 250 nm. Consequently, HPLC results will be shown and used throughout this discussion, but not UV−vis results, which can be seen in the Supporting Information.

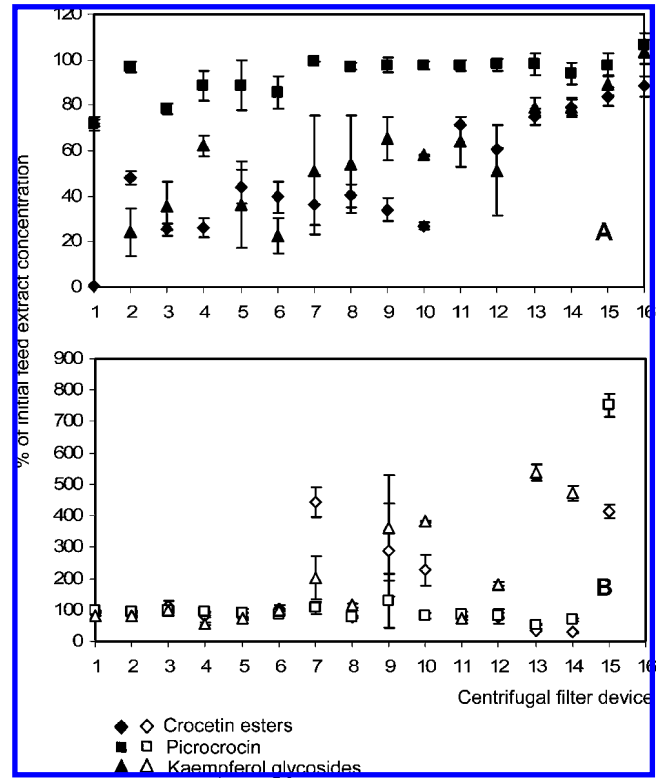

Figure 3. Concentration of crocetin esters, picrocrocin, and kaempferol glycosides compared to their concentrations in the saffron feed extract, in filtrate (**A**) and retentate (**B**) fractions.

Crocetin Ester Composition in the Filtrate and Retentate Fractions. The concentration of the main components in filtrate and retentate fractions compared to their concentrations in the saffron feed extract is presented in **Figure 3**. Crocetin ester concentration in the filtrate fractions (**Figure 3A**) decreased to a greater or lesser extent depending on the centrifugal filter device, especially in those with ≤10 kDa MWCO. The filtrate fractions from devices 13−16 underwent the lowest concentration changes. The magnitude of these decreases of the crocetin ester *R* (**Table 4**) varied from ~12% for the membrane with the highest MWCO, corresponding to device 16, to ~99% for device 1, which contained the membrane with the lowest MWCO. However, *R* did not always follow a decreasing order with increasing MWCO. Moreover, significant differences were found in *R* from devices having the same MWCO, as happened in the 3 kDa MWCO and the 10 kDa groups of devices. In the former group, two subgroups of *R* values were distinguished that were not connected either with their membrane material or manufacturer. The first subgroup consisted of devices 2 and 5; the second one, of devices 3 and 4. In the latter group, three subgroups of *R* values were found corresponding to devices 9−10, 11, and 12. They depended on the manufacturer but not on the membrane material since both devices with PES membranes showed different *R* values. Within the same type of devices, there was a general trend of decreasing crocetin ester *R* as MWCO rose, especially when dealing with the same

Table 4. Rejection (*R*), Filtrate Recovery (%), and Losses of Crocetin Esters, Mean ± Standard Deviation, $n = 3$

centrifugal filter device	crocetin esters[a]		
	R (%)[b]	filtrate recovery (%)[b]	losses (%)[b]
1	99.4 j ± 0.1	0.1 a ± 0.1	21.1 a,b ± 2.9
2	52.4 e ± 3.4	18.3 c ± 0.9	32.8 c,d ± 3.0
3	74.7 i ± 2.5	10.3 b ± 1.3	26.9 b,c ± 9.3
4	73.9 h,i ± 4.1	4.5 a,b ± 1.1	29.3 b,c ± 1.7
5	55.8 e,f ± 7.4	17.8 c ± 2.8	32.4 c,d ± 0.2
6	60.9 e,f,g ± 7.3	24.5 c,d ± 5.6	38.6 d ± 8.3
7	63.9 f,g ± 13.0	32.9 e,f ± 12.9	35.1 c,d ± 7.4
8	59.9 e,f,g ± 4.9	38.3 f,g ± 5.9	59.1 e ± 6.5
9	66.0 g,h ± 5.0	32.8 e,f ± 5.1	62.3 e ± 5.2
10	73.1 h,i ± 1.3	26.1 d,e ± 1.3	70.9 f ± 0.7
11	28.6 c ± 0.1	42.4 g ± 0.7	28.1 b,c ± 0.1
12	39.3 d ± 0.2	58.1 h ± 0.1	40.4 d ± 0.2
13	25.1 c ± 0.1	71.5 i ± 0.1	27.4 b,c ± 0.1
14	21.2 b,c ± 0.2	76.7 i,j ± 0.1	23.0 a,b ± 0.1
15	16.2 a,b ± 0.1	82.9 j ± 0.1	16.6 a ± 0.1
16	11.7 a ± 0.1	81.1 j ± 0.1	n.a.[c]

[a] Sum of individual crocetin esters determined by HPLC. [b] The same letter in a column indicates nonsignificant differences according to Duncan's test at the 0.05% level. [c] Data not available.

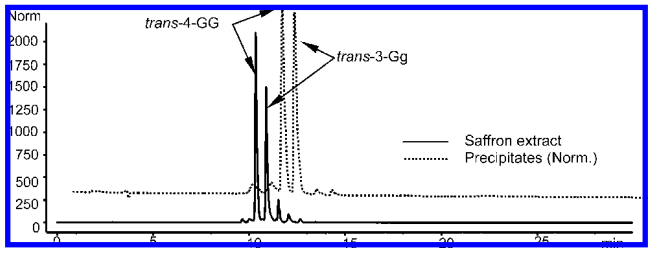

Figure 4. Chromatograms at 440 nm of the saffron extract and the precipitates of crocetin esters.

membrane. Device 8 (5 kDa, PES membrane) was the exception, having lower crocetin ester *R* than device 10 (10 kDa, RC membrane). Besides device 1, which had the only filtrate fraction nearly free from crocetin esters, devices 3, 4, and 10 presented the highest reductions in crocetin ester concentrations from filtrate fractions (*R* ~74%), followed by devices 6, 7, 8, and 9, which showed reductions of ~60%. They came from three different manufacturers; devices 1, 3, 6, and 8 were made of PES, while the others were made of RC. As previously stated, devices with MWCO higher than 10 kDa showed the lowest crocetin ester *R*, which was too low for consideration in the separation of crocetin esters from picrocrocin and kaempferol glycosides. Regarding mass balance, the highlighted devices had crocetin ester filtrate recoveries lower than 40%; this parameter was just 0.1% in device 1 (**Table 4**). Retentate results (**Figure 3B**) showed that higher concentrations of crocetin esters were found than in the initial extract only in devices 7, 9, 10, and 15. Because of the low retentate volume and some problems with its recuperation, it was not possible to analyze the retentate fraction of device 16. Devices 7, 9, 10, and 15 showed concentration factors for crocetin esters of 4.4, 2.9, 2.3, and 4.1, respectively. However, a phenomenon of crocetin ester precipitation that was not included in the results shown in **Figure 3B** was observed in the retentate fractions of devices 2−4 and 6−11. The precipitates were washed with water, redissolved in 50% acetonitrile/water v/v, and analyzed under chromatographic conditions (see Materials and Methods) (**Figure 4**). A mixture of crocetin esters was found, which had almost the same proportion of *trans*-4-GG and *trans*-3-Gg. The magnitude of precipitated crocetin esters and/or their adsorption on membranes were considered losses and were estimated through the difference between 100% and total recovery. Loss values of the initial crocetin ester mass ranged from nearly 17 to 71%, and the highest values were found in device 10 followed by devices 9 and 8 (**Table 4**). These results indicated the possibility of purifying the main crocetin esters through concentration and precipitation, which should be further studied.

With regard to each crocetin ester, it was found that the less polar ones (see RT in **Table 2**) and therefore cis-isomers were better rejected by the majority of membranes. The proportion of these esters was changed in the retentate and filtrate fractions as reported, for example, by Kalbasi et al. (*24*), for monomeric and polymeric anthocyanin fractions. The device 1 membrane totally rejected all crocetin esters, and only small quantities of *trans*-4-GG were detected in its filtrate fraction; therefore, they are not shown in **Figure 5**. **Figure 5A** shows the *R* results of the two main *trans*- and *cis*-crocetin esters. Devices 6, 7, and 8, all having 5 kDa MWCO and previously emphasized for their high crocetin ester *R*, showed a tendency to reject the less polar crocetin esters better than devices 3, 4, 9, and 10. In this way, -3-Gg forms were better rejected than -4-GG forms, although sometimes the differences were not significant. Apart from device 1, the *R* for *trans*-5-tG, *trans*-5-nG, and *trans*-4-GG in the various devices ranged from 0 to ~70%, whereas the *R* for *trans*-3-Gg, *trans*-2-G, *cis*-4-GG, and *cis*-3-Gg were ≥20, ≥50, ≥54, and ≥66%, respectively. Furthermore, *trans*-5-tG, *trans*-5-nG, and *trans*-4-GG were recovered in the filtrate at a higher proportion than the rest of the crocetin esters for all the devices under study. **Figure 5B**, shows the recovery results of the two main *trans*- and *cis*-crocetin esters. The greatest losses were found in *trans*-2-G, *cis*-4-GG, and *cis*-3-Gg for devices 8−10 and 12. **Figure 5C** shows the losses found in the two main *trans*- and *cis*-crocetin esters.

Picrocrocin Composition in the Filtrate and Retentate Fractions. The picrocrocin concentration in the filtrate fractions was almost equal to its concentration in the feed extract (**Figure 3A**). A narrow range of picrocrocin *R* values were found, from 0.5% (device 16) to 28% (device 1) (**Table 5**). Heterogeneous results were observed, the same as for crocetin esters, with significant differences among devices with the same MWCO membrane. These differences could not be related to the membrane material or manufacturer, making membrane tests necessary. For example, picrocrocin *R* from device 2 was lower than the other values found for devices with 3 kDa membranes, while the value from device 6 was higher than the other values from devices with 5 kDa membranes. Within the same type of device, a general trend of picrocrocin *R* to decrease or remain stable was observed as the MWCO rose. When attention is focused on picrocrocin filtrate recoveries from devices 7 through 16, except for device 11, there were values of ≥90%, picrocrocin being the best recovered component, having the highest filtrate and total recovery. This trend for filtrate recovery within the same type of device as the MWCO increased was the same as for picrocrocin *R*. Picrocrocin losses reached as high as 16% (devices 5 and 6), which is a much lower result than that for crocetin esters. The only picrocrocin concentration in the retentate fractions (**Figure 3B**) that was clearly higher than in the initial extract was from device 15. However, the retentate fraction from device 13 had only 53% of the initial picrocrocin concentration.

Kaempferol Glycoside Composition in the Filtrate and Retentate Fractions. Like in crocetin esters, the total kaempferol glycoside concentration in the filtrate fractions (**Figure 3A**) decreased depending on the centrifugal filter device, with the lowest concentration changes corresponding to the filtrate

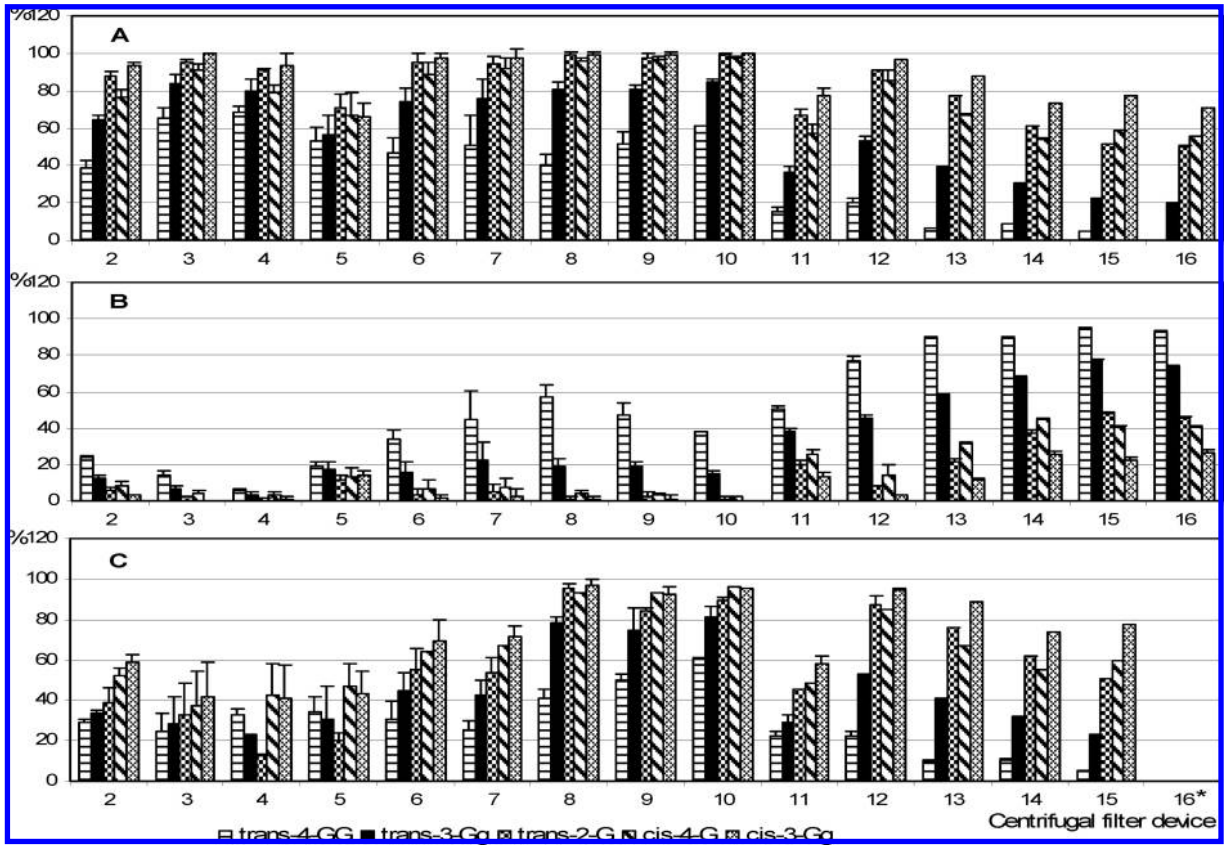

Figure 5. (**A**) Rejection, *R* (%); (**B**) filtrate recovery (%), and (**C**) losses (%) of the two main *trans*-crocetin esters and *cis*-crocetin esters individually. *Data not available.

Table 5. Rejection (*R*), Filtrate Recovery (%), and Losses of Picrocrocin, Mean ± Standard Deviation, $n = 3$

centrifugal filter device	picrocrocin[a]		
	R (%)	filtrate recovery(%)	losses (%)
1	28.1 d ± 1.6	8.4 a ± 0.5	8.0 b ± 1.6
2	3.3 a,b ± 1.4	36.7 c ± 1.2	7.2 a,b ± 3.3
3	13.5 c ± 8.0	35.1 c ± 6.4	8.1 b ± 5.4
4	11.5 b,c ± 3.7	15.2 b ± 8.9	8.7 b ± 1.5
5	11.4 b,c ± 6.4	35.7 c ± 4.1	15.5 c ± 0.2
6	14.6 c ± 4.8	52.7 d ± 7.3	16.0 c ± 6.7
7	0.8 a ± 0.1	89.8 e ± 2.3	2.7 a ± 1.5
8	3.0 a,b ± 1.0	92.3 e,f ± 2.4	5.6 a,b ± 1.2
9	2.3 a,b ± 1.8	94.2 e,f ± 2.2	4.1 a,b ± 2.0
10	2.7 a,b ± 1.0	94.2 e,f ± 2.0	4.7 a,b ± 2.1
11	2.7 a,b ± 1.4	57.7 d ± 2.4	8.9 b ± 0.5
12	2.2 a,b ± 1.6	93.6 e,f ± 3.2	4.7 a,b ± 2.9
13	2.1 a,b ± 0.1	93.3 e,f ± 0.1	5.5 a,b ± 0.5
14	6.3 a,b,c ± 0.1	91.3 e,f ± 0.1	7.9 b ± 0.2
15	2.4 a,b ± 0.1	96.5 f ± 0.2	2.5 a ± 0.1
16	0.5 a ± 0.4	97.8 f ± 0.1	n.a.[b]

[a] The same letter in a column indicates nonsignificant differences according to Duncan's test at the 0.05% level. [b] Data not available.

Table 6. Rejection (*R*), Filtrate Recovery (%), and Losses of Kaempferol Glycosides, Mean ± Standard Deviation, $n = 3$

centrifugal filter device	kaempferol glycosides[a]		
	R (%)[b]	filtrate recovery (%)[b]	losses (%)[b]
1	27.2 d ± 1.1	8.5 a ± 0.1	21.4 b,c ± 1.9
2	65.4 i ± 1.1	13.2 a,b ± 0.1	40.0 f ± 1.9
3	58.4 h ± 3.3	16.7 b,c ± 0.7	27.5 c,d ± 3.0
4	37.8 e,f ± 4.4	10.5 a ± 0.2	43.4 f ± 4.2
5	44.6 g ± 2.2	22.2 c ± 1.1	30.8 d,e ± 3.5
6	69.8 i ± 3.5	17.5 b,c ± 0.9	41.1 f ± 2.9
7	35.8 e ± 8.6	58.8 e ± 9.0	26.7 c,d ± 9.1
8	36.1 e ± 1.3	58.1 e ± 2.1	36.5 e,f ± 2.2
9	29.8 d ± 3.9	67.9 f ± 4.3	26.3 c,d ± 4.2
10	42.0 f,g ± 0.4	56.2 e ± 0.2	38.8 f ± 0.3
11	25.0 c,d ± 1.3	45.2 d ± 2.3	25.8 c,d ± 3.7
12	28.7 d ± 1.4	68.5 f ± 3.4	28.7 c,d ± 3.6
13	20.8 c ± 1.0	75.5 g ± 3.8	7.6 a ± 4.6
14	20.9 c ± 1.0	77.0 g ± 3.9	17.7 b ± 4.1
15	10.9 b ± 0.5	88.1 h ± 4.4	6.4 a ± 4.7
16	3.0 a ± 0.2	94.9 i ± 4.7	n.a.[c]

[a] Kaempferol glycoside content expressed as equivalent mass of rutin. [b] The same letter in a column indicates nonsignificant differences according to Duncan's test at the 0.05% level. [c] Data not available.

fractions from devices 13−16. The results for total kaempferol glycoside *R* (**Table 6**) reached values up to ∼70% (device 6) and did not decrease with increasing MWCO. Within the same type of devices, a decreasing kaempferol glycoside *R* was observed as the MWCO rose only in the group of devices 7, 9, and 15. The highest kaempferol glycoside filtrate recoveries were found from device 16 (∼95%) and the lowest, from devices 1, 2, and 4 (∼10%). Losses of kaempferol glycosides ranged from ∼6% (device 15) to ∼43% (device 4). Once more, the heterogeneity of results is noteworthy in relation to devices with the same MWCO membrane that could not be related to the membrane material or manufacturer. In the retentate fractions (**Figure 3B**), half of the devices were able to concentrate kaempferol glycosides. These devices, in an increasing order of concentration factors, were 8, 12, 7, 9, 1, 14, 13, and 15. Kaempferol glycoside concentration from the latter device was 4334 ± 217% of the initial concentration in the feed extract (data not shown in **Figure 3B** due to the scale).

Regarding each kaempferol glycoside (**Figure 6**), the highest kaempferol-3-sophoroside-7-glucoside *R* came from devices 2 and 6 (∼70%) and the lowest from devices 11−16. In addition,

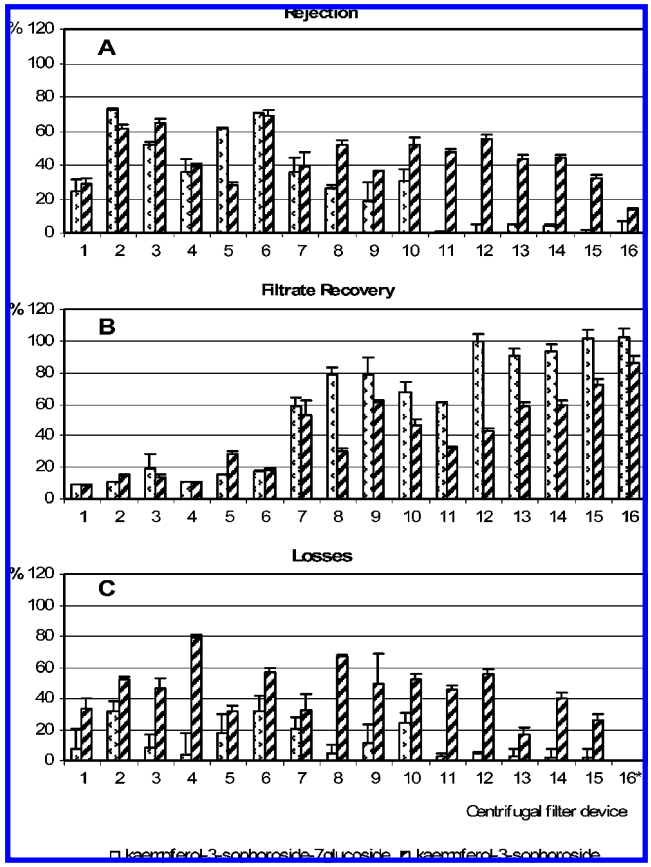

Figure 6. (**A**) Kaempferol glycoside rejection, *R* (%); (**B**) kaempferol glycoside filtrate recovery (%), and (**C**) kaempferol glycoside losses (%).*Data not available.

the highest kaempferol-3-sophoroside *R* were from devices 2, 3, and 6 (between 61 and 69%) and the lowest, from device 16 (~14%). No significant differences were found in the *R* of either quantified kaempferol glycoside with devices 1, 4, 6, and 7, but kaempferol-3-sophoroside-7-glucoside showed higher *R* than kaempferol-3-sophoroside with devices 2 and 5. On the contrary, lower *R* values from the former kaempferol glycoside were found with the remaining devices. Nearly total filtrate recoveries of kaempferol-3-sophoroside-7-glucoside were found for devices 12, 15, and 16, whereas the lowest values were found for devices 1, 2, and 4 (~10%). However, for kaempferol-3-sophoroside, filtrate recoveries extended from 8% for device 1 to 86% for device 16. In comparing both kaempferol glycosides, kaempferol-3-sophoroside-7-glucoside had similar (devices 1−4 and 6−7) or higher (devices 8−16) filtrate recoveries than kaempferol-3-sophoroside, with the sole exception of device 5. The highest losses of kaempferol-3-sophoroside-7-glucoside came from devices 2, 6, and 10 (~30%) and the lowest losses, from devices 11−15 (~5%). The highest loss of kaempferol-3-sophoroside came from device 4 (~79%) and the lowest, from device 13 (~17%). Kaempferol-3-sophoroside-7-glucoside had similar (devices 5 and 7) or lower losses (the rest of the devices) than kaempferol-3-sophoroside.

Membrane Selection for Concentration or Purification of Saffron Components. First, UF can be used for the separation of crocetin esters from picrocrocin. Different degrees of purification were obtained depending on the membrane employed. The best results corresponded to devices 1, 3, 4, 7, 8, 9, and 10, which had the highest coefficient between picrocrocin and crocetin ester filtrate recoveries since they let picrocrocin pass through the membrane while keeping a great deal of crocetin esters in their retentate fractions. The four latter devices were in the group of devices with the highest picrocrocin filtrate recoveries and the highest crocetin ester *R* as well. Therefore, they are a good choice for this separation. All of them had RC membranes except for device 8, which had a PES membrane. Besides, their higher MWCO in relation to the membranes in devices 1, 3, and 4 makes them more suitable for further studies in a pilot plant UF unit. However, device 1 can especially be considered for a second UF step, once the extract has been partially purified of crocetin esters, in order to obtain picrocrocin nearly free from crocetin esters.

Filtrate recoveries of kaempferol glycosides in devices 1, 4, and 16 were equal to or, in the remaining devices, lower than filtrate recoveries of picrocrocin; thus, a partial purification of picrocrocin was produced in most filtrate fractions.

Devices 7, 9, and 10 were also the most suitable ones for crocetin ester concentration in their retentate fractions. Devices 9 and 10 showed high losses of these compounds due to precipitation, making them also suitable for the purification of crocetin esters. Picrocrocin concentration was observed only in the retentate fractions from devices 9 and 15, but their picrocrocin filtrate recoveries were ~95% with a very low mass of picrocrocin remaining in the retentate fractions. Kaempferol glycoside concentration was attained in the retentate fractions of devices 8, 12, 7, 9, 10, 14, 13, and 15, in this order according to the increase in their concentration factors. Of these devices, the lowest kaempferol glycoside filtrate recoveries were found in devices 7−9, which consequently showed the most interesting results from this viewpoint.

In summary, this is the first time that the application of centrifugal UF to saffron spice is reported. Filtration profiles showed good results for most devices in short centrifugation times, which is very interesting from an analytical point of view. Results show that centrifugal UF modified the proportion of main saffron components in a vast range, making it possible to tailor crocetin ester, picrocrocin, and kaempferol glycoside proportions or even purify them by selecting the appropriate membrane. The possibility of using successive UF steps has also been inferred. Results from this research establish the first basis of knowledge for the application of UF to aqueous saffron extracts, and, although further studies will be conducted in a pilot plant UF unit, these promising results from commercially available membranes prompted the consideration of new UF applications in the analysis and modification of the principal saffron components with the subsequent repercussion on their associated properties.

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Supporting Information Available: Rejection (*R*), filtrate recovery (%) and losses of crocetin esters and picrocrocin determined from UV−vis data, mean ± standard deviation, $n = 3$. This material is available free of charge via the Internet at http://pubs.acs.org.

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