Removal of dark compounds from fruit juices by membrane separation

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Abstract

The objective of this work is to carry out de-coloration of browned apple and grape juice by physical separation of melanoidins with combined use of ultrafiltration (UF) and nanofiltration (NF) membranes. Proposed actions were focused to reduce de-coloration agents addition to juice, in accordance with current trends in food processing. Concentrated samples of apple and grape juices were stored at relatively high temperature (53 °C) until considerable browning level was obtained. De-coloration of browned juices by ultra (UF) and nanofiltration (NF) were performed with a cross-flow membrane filtration unit, using thin-film composite based in polyamide membranes, with cutoff in the range 150-450 Da; and cellulose acetate or polyvinylidene fluoride membranes, in the range 2-30 kDa. The following parameters were analyzed in permeates and compared with the non-browned clarified juice sample: Soluble sugars; pH; absorbance at 420 nm; soluble solids concentration (°Brix); and tristimulus color parameters, L, a, b and ΔE. Results indicated that after filtration with NF membranes (Cutoff < 2,000 Da), browned apple juice color was reduced to a level close to that of a fresh clarified juice. Although changes in pH of permeates were not observed, reduction in °Brix occurred after nanofiltration. Contrarily, color recovering by NF resulted unviable for highly colored grape juices and traditional active carbon discoloration should be used. Moreover, from a practical point of view, soluble solids concentration reduction after NF must be carefully taking into account, since additional concentration step may be required.

Keywords: apple, grape, fruit juice, membrane, discoloration

1. Introduction

Concentration of clarified fruit juice by evaporation ideally reduces costs and increases shelf-life by removing water without changing the solid composition. Multiple-effect evaporators were designed to concentrate juice at reduced temperatures but, in practice, temperatures become very high in the first effects and hydroxymethyl-furfural (HMF) formation can reach very high values (Lozano et al., 1995). HMF is an important intermediate in non-enzymatic browning (NEB) reactions and its production during processing may accelerate browning during storage. Fruit juice concentrates containing more than 65% total solids are normally stable from the standpoint of fermentation at any temperature but, when stored at relatively high temperatures, NEB reactions occur (Toribio and Lozano, 1984). Nonenzymatic browning via Maillard reactions between reducing sugars and amino acids is the major route of color formation in apple juice concentrate during processing and storage (Toribio and Lozano, 1986). These reactions involved the formation of reactive intermediates by a variety of pathways and these can yield both volatile flavor components and brown melanoidins of higher molecular weight (Mauron, 1983).

Fruit juice color can increase by browning until levels higher than those commercially acceptable. The influence of the chemical composition juice, the kinetics of color formation as a function of soluble solids concentration and temperature, and some inhibition and discoloration mechanisms of clarified apple juice have been studied (Toribio and Lozano, 1984; Giovanelli and Ravasini, 1993). Various pre- and post-treatments are available to avoid post-turbidity and browning of juices. Stabilization of beverages by gelatin, bentonite and silica gel are widespread conventional treatments (Lozano, 2006). The use of adsorbent resins for clear juice stabilization has been introduced as a final treatment after clarification. Príncipe and Lozano (1990) found good results applying adsorption process. At industrial scale, activated charcoal has been occasionally used to recover highly colored apple juice; however this method is troublesome at time of color adsorbent separation.

Successful development of membranes with increased service life, separation capacity and chemical resistance has been a reason for increasing use of membrane processes. Several studies have been published concerning the quality of ultrafiltered apple juice in comparison with conventional clarification techniques (Giovanelli and Ravasini, 1993; Constenla and Lozano, 1995; Gökmen et al., 1998; Girard and Fukumoto, 1999, Lozano et al., 2000). Ferrarini et al. (2001) presented an innovative application of nanofiltration in the concentration of grape juice. Warcsok (2004) studied the concentration of apple and pear juices by nanofiltration at low pressures. However, scarce works have been published concerning the application of membrane separation for remediation of browned juices (Borneman et al., 2001).

The objective of this work is to carry out discoloration of browned apple and grape juices by physical separation of melanoidins with combined use of ultrafiltration (UF) and nanofiltration (NF) membranes. Proposed actions are focused to reduce clarification and discoloration agents added to juice in according with current trends in food processing.

2. Materials and Methods

Necessary amount of apple fruits (Granny Smith variety) were sorted, washed, and crushed in a Model #6 Fitz Mill Comminutor (Fitzpatrick Co., Chicago, Ill, USA). Juice was produced by pressing in a hydraulic press, followed by screening through a 100 μm stainless steel mesh; steam heated to 95°C; and finally clarified by

ultrafiltration (batch mode) in a pilot scale UF unit (Amicon Model DC50P) with a single 50 kDa cutoff polysulphone hollow fiber cartridge. Temperature was kept at 25°C. Clarified juice was concentrated in a Büchi Rotovapor Model R-151 (Büchi; Switzerland) under vacuum at 60 °C to get a soluble solids concentration of 72 °Brix.
Manufacturing of grape juice (Merlot variety) included pressing the fruit in a hydraulic cloth and rack press (5 ton) through an 80-100 mesh filter (Bucher-Guyer Ag; Germany) and steam heating of the mash in the range 80-90ºC for 30 sec to inactivate native polyphenoloxidase (PPO). The enzymatic treatment for pectin colloid destabilization was done following the hot technique with pectinase (200 ppm Rohapect D5S; for 90 min at 30°C). Commercial sodium bentonite type I (La Helcha; Mendoza, Argentina; 900 mg/L) and gelatin (Cristagel Stauffer- 180 mg/L) were used as flocculants agents. Grape juice was finally filtered through a diatomaceous earth bed; concentrated to 72° Brix in a Rotavapor R-151, (BÜCHI A. G.; Switzerland) at 60 °C under vacuum (20.7 kPa).
This concentrated apple and grape juices were stored at relatively high temperature (53 °C) until considerable browning level was obtained. Samples with lower soluble solids concentration were obtained from the 72°Brix juices by dilution with distilled water.

2.1. Ultrafiltration/Nanofiltration (UF/NF) equipment

Ultra/nano-filtration system (Fig. 1) was assembled with: a (1) stainless steel cell body Sepa® CF Membrane Cell (Osmonics; Minnetonka; Mn; USA) which is a lab scale crossflow membrane filtration unit; (2) a hydraulic press to holds the cells tightly joined avoiding leaks; (3) a peristaltic pump, pressure gauge and tubing for retentate and permeate driving. The cell body accommodates any 19 cm x 14 cm flat sheet membrane for a full 155 cm^2 of effective membrane area. Characteristics of used membranes are described in Table 1.

2.2. Procedure

Restored browned juice was treated at 30 °C in the UF/NF equipment, in batch mode. UF experiences were done consecutively in diminishing order of membrane molecular weight cutoff (MWCO), using the obtained permeate in the previous experience like feed to the next separation. On the other hand, NF experiences were done using as feed the same ultrafiltered juice batch. All experiences were done at 0.05 L/min of retentate flow with a transmembrane pressure in the range of 41 to 90 kPa.

2.3. Physical and chemical analysis

The following parameters were analyzed in the obtained permeates and compared with the non-browned clarified juice sample: soluble sugars (by HPLC), pH (DigipHase Cole-Parker pHmeter); absorbance at 420 nm (Perkin-Elmer Lambda3 UV/VIS spectrophotometer); soluble solids concentration as °Brix (Reichert Abbe

Mark II digital refractometer); and tristimulus color parameters, L, a, b and ΔE in a Hunter Lab UltraScan XE colorimeter (Hunter Assoc. Laboratory; VA, USA).
Soluble sugars were quantified using a VARIAN VISTA 5500 (Varian, Assoc. Inc., Palo Alto, CA, USA) liquid chromatograph equipped with a differential refractometer VARIAN SERIES RI-3 and a Aminex® HPX-87C (Bio Rad; USA) column, under the following conditions: pressure < 90 atm; column temperature 85 °C; mobile phase: distilled and filtered water at 0.6 mL/min. Data was acquired and processed with a MILLENNIUM 2010 system. Glucose and fructose solutions in the range of 1,00 to 5,00 mg/mL were prepared with distilled water; filtered through Millipore (0.45 μm); injected in 20 μL aliquots and quantified by the external standard method. Juice samples (5.7°Brix) were further diluted (1:24) with distilled water; de-gassed in an ultrasonic bath; and filtered as indicated for the sugar standards.
Analyses were made in triplicate and mean values are reported.

3. Results and Discussion

Results presented in Table 2 show that pH value did not change significantly (α=0.05) after ultra- or nano-filtration. Moreover, the pH value of apple juice samples was not influenced by membrane composition. Similar results were obtained with grape juice.
However, as Table 2 also shows, soluble solids decrease with MWCO, particularly when apple juice was filtered. This effect was more significant when membrane porosity was reduced from 4 to 2 kDa, a value which may be assumed to be the boundary zone between UF and NF. Being the molecular weight of the involved sugars lower than the retention size of NF membranes, retention of sucrose was attributed to the molecular interactions between sugar and membrane. Fig. 2 shows changes in glucose, fructose, and sucrose content as a function of MWCO, in apple juice. Fructose content was continuously reduced in the MWCO range 20– 2 kDa. However, the concentration fructose remained practically constant after filtration through NF membranes of lower MWCO (< 2 kDa), independently of pore size and membrane composition. Giovanelli and Ravasini (1993) reported molecular interactions among components of apple juice and acetate cellulose membranes. Girard and Fukumoto (1999), studying micro- and ultrafiltration effect on depectinized apple juice; those authors found that 9 kDa polyethersulfone membrane retained sugars, flavanols and phenolics compounds. It was also found in the present work that fructose: glucose ratio remained practically constant throughout the ultra- and nano-filtration experiences.
Apple juice sample luminosity L increased with decreasing membrane pore size until a value similar to that of non-browned juice was obtained with NF membranes (Table 2). Fig. 3 shows that after filtering browned apple juice through NF membranes (treatments 7 and 8), color Hunter parameters *a* and *b* also approached the value of a non-browned juice. On the other hand, browned grape juice only reached Hunter values *a*= -5.5, *b*= 25 after treatment with the smaller membrane pore size. This values poorly compares with a no-browned grape juice, which after ultrafiltration with a 50 kDa membrane (treatment 1), shown Hunter coordinates *a*= -0.25, *b*=3.3.

Absorbance behavior at 420 nm of browned apple juice treated with different membrane MWCO (Fig. 4) shows an abrupt change in trends when passing from ultrafiltration to the nanofiltration zone. This result suggested that the bio-polymers, generically known as melanoidins, (responsible for the dark brown color in clarified apple juice), have molecular size in the range between 2,000 and 4,000 Da. Contrarily, after membrane treatment of browned grape juice, only a reduction of absorbance values between 0.9 and 1.0 where obtained.

4. Conclusions

After membrane treatment of highly browned apple juice with nanofiltration membranes whose size pores were below 2 kDa, permeates with color characteristics closer to a no-browned juice were obtained. From a practical point of view, soluble solids concentration reduction after NF must be carefully taking into account, since additional concentration step may be required. On the contrary, nanofiltration of highly browned grape juice did not help to recover its original characteristic color.

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TABLE 1. Characteristics of ultrafiltration and nanofiltration membranes used to reduce color of browned juices.

Membrane	Polymer*	Rejection size	Water permeability at 30ºC [g/min psi]	pH Tolerance
Nanofiltration				
DS-5-DL	TF	150-300 Da (96%$MgSO_4$)	0.144	2-11
BQ01	TF	350-450 Da	0.103	2-11
SP28	CA	2 kDa (92%Na_2SO_4)	0.023	2-9
Ultrafiltration				
GM(50)	TF	4 kDa	0.044	2-11
SN32	CA	20 kDa	1.150	2-9
AN09	PVDF	30 kDa	3.933	1-11

*: CA: cellulose acetate; TF: (TFC/TLC) thin-film composite based in polyamide; PVDF: polyvinylidene fluoride.

TABLE 2. Characteristic parameters of assayed apple (AJ) and grape (GJ) juices, at 23 °C.

N°	Membrane	°Brix		pH		L		ΔE	
		AJ	GJ	AJ	GJ	AJ	GJ	AJ	GJ
1	UF- 50,000 Da No-browned juice	9.4	12.2	3.61	3.52	94.86	89.81	0.02	0.0
2	UF-50,000 Da Browned juice	9.4	12.8	3.60	--	46.89	77.5	61.43	27.5
3	UF-30,000 Da	8.9	--	3.62	--	69.9	--	48.02	--
4	UF-20,000 Da	8.9	11.2	3.61	3.54	73.69	83.7	45.97	22.8
5	UF-4,000 Da	7.7	--	3.60	--	78.4	--	42.71	--
6	NF-2,000 Da	5.9	--	3.67	--	---	--	---	--
7	NF-450 Da	5.9	11.2	3.62	3.53	93.01	87.2	20.6	17.2
8	NF-300 Da	5.7	10.9	3.64	3.54	92.98	84.3	15.23	18.8

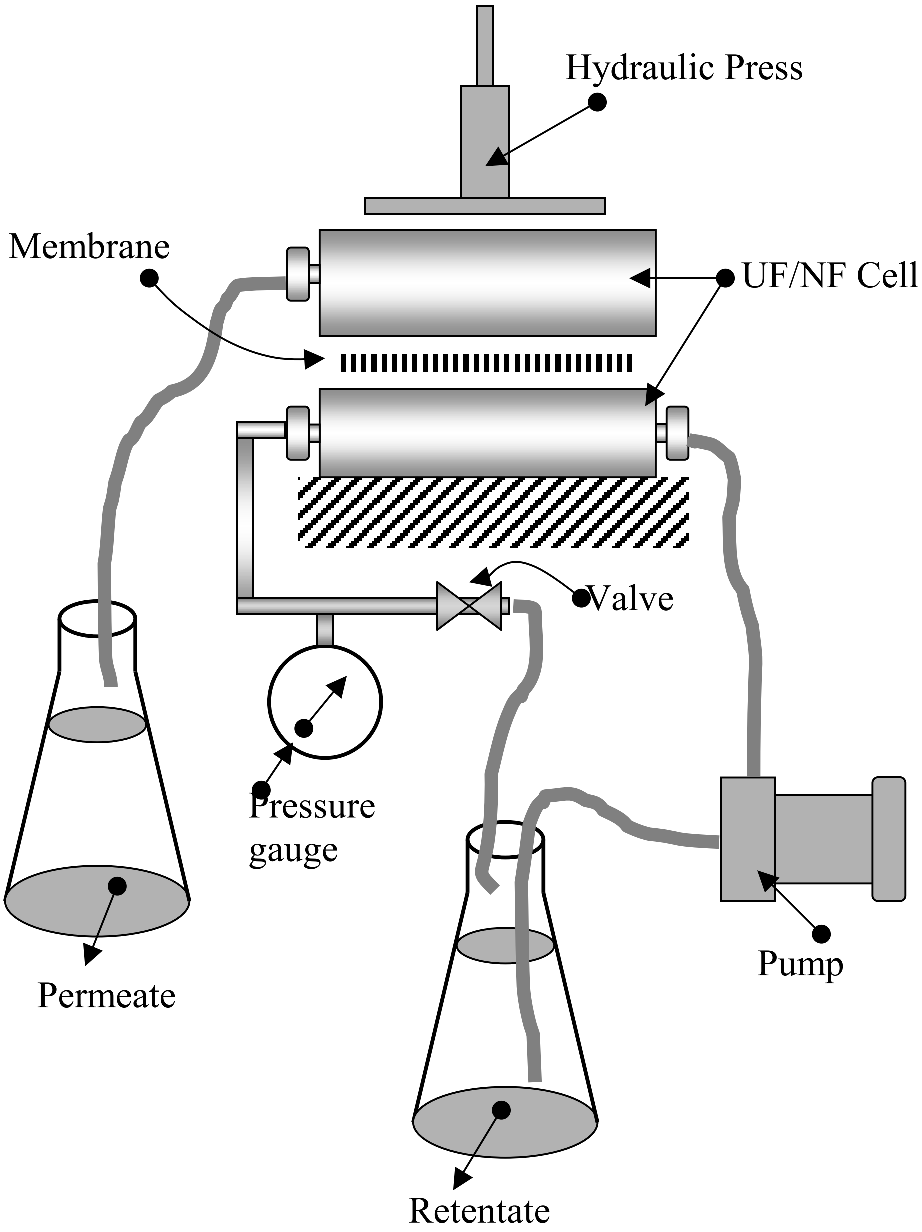

FIGURE 1. Scheme of the laboratory Ultrafiltration/Nanofiltration system.

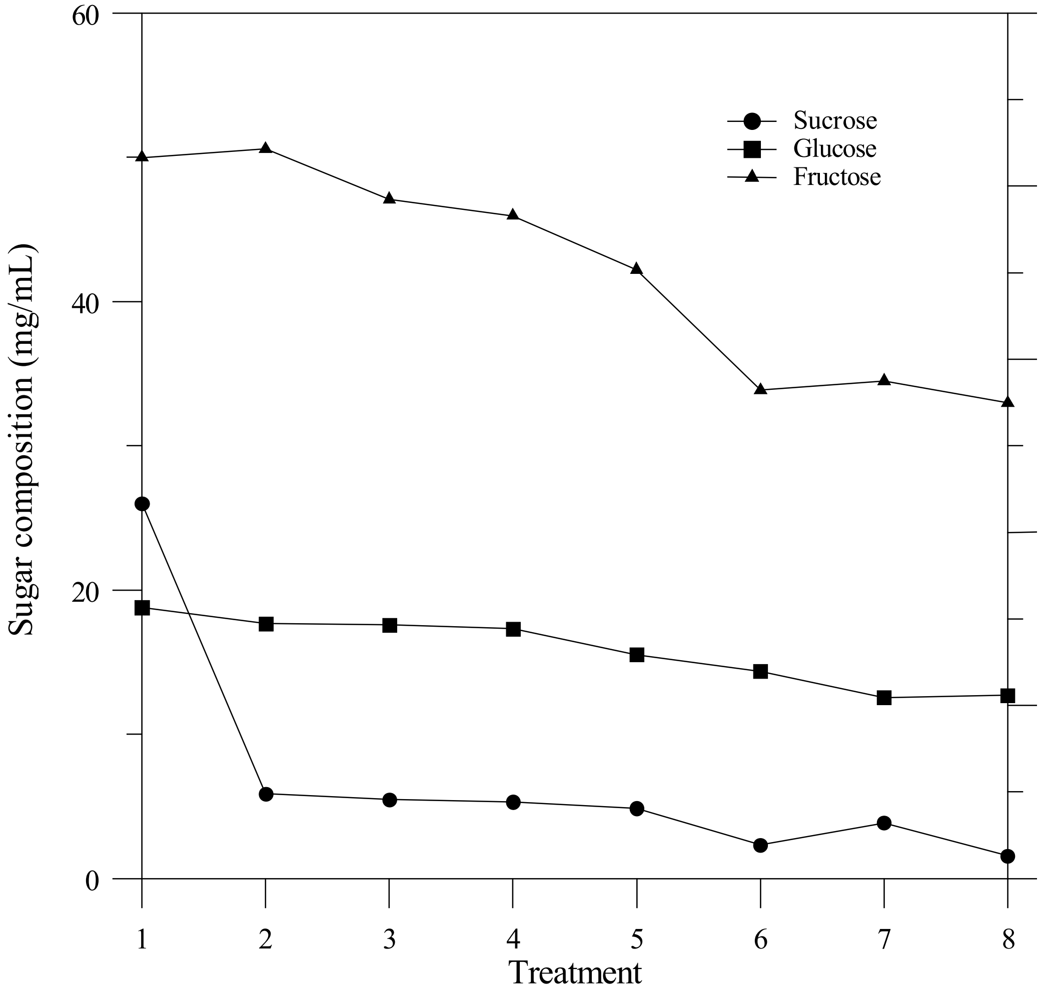

FIGURE 2. Effect of membrane molecular weight cutoff (MWCO) on clarified apple juice sugar content (see Table 2 for treatment description).

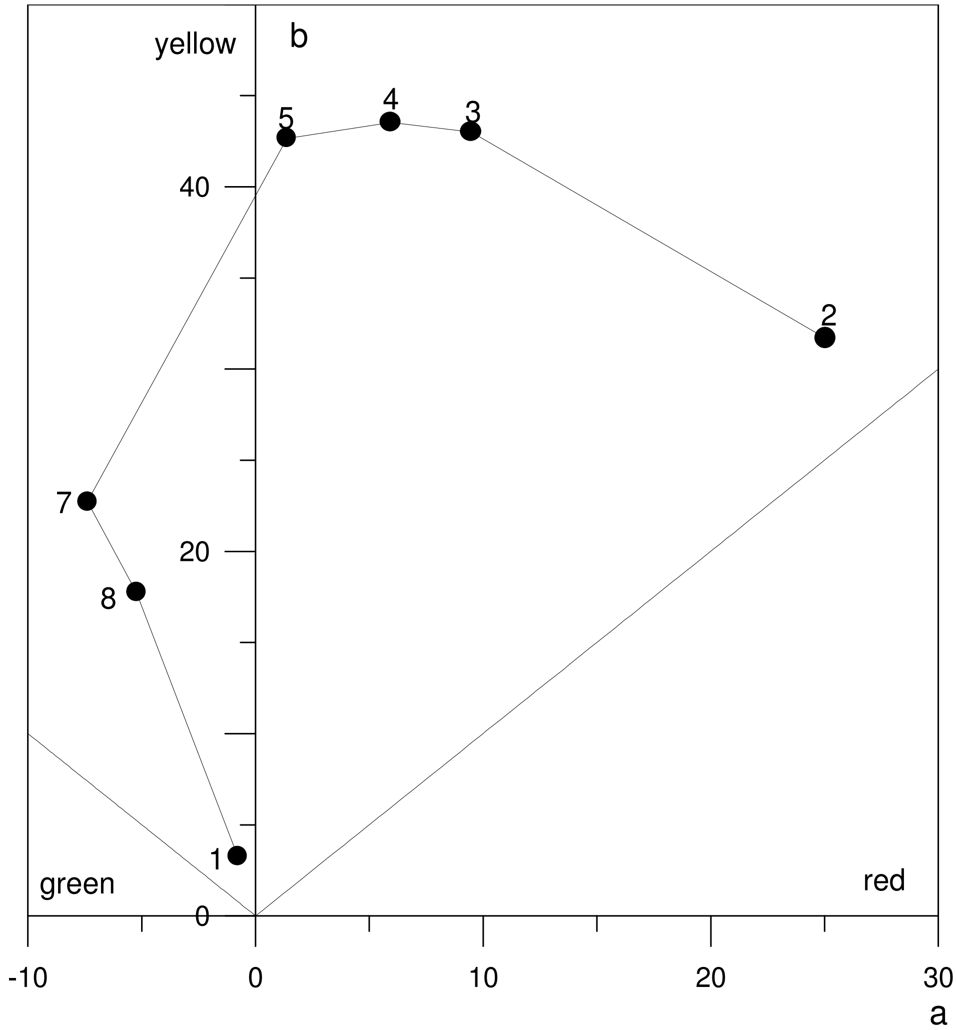

FIGURE 3. Color Hunter parameters of permeates obtained from browned apple juice with different membrane MWCO. (see Table 2 for sample characteristics)

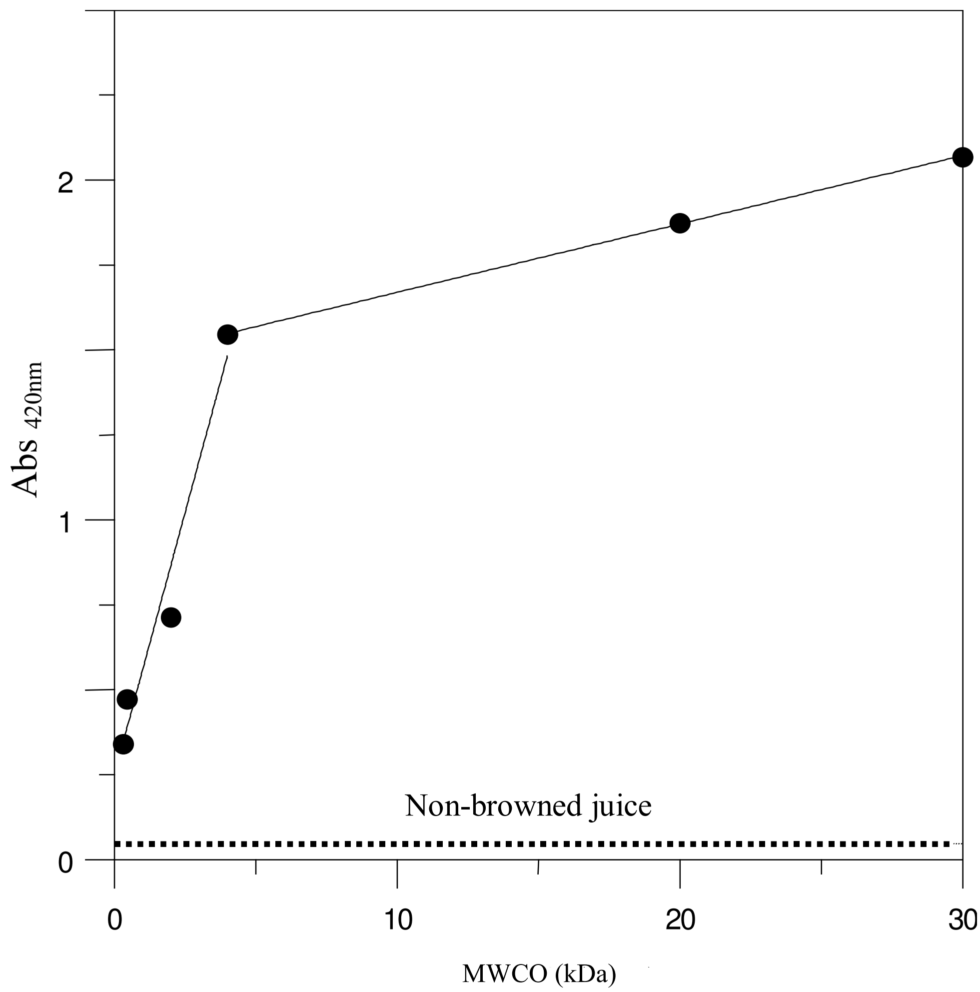

FIGURE 4. Absorbance at 420 nm, after filtration through different molecular weight cutoff (MWCO) membranes. (see Table 2 for sample characteristics)