Waste Water from Citrus Processing as a Source of Hesperidin by Concentration on Styrene−Divinylbenzene Resin

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This paper describes a procedure for recovering hesperidin from the waste water of orange juice processing, namely, yellow water, by concentration of diluted extracts on styrene−divinylbenzene resin. Turbid raw material flowing out from centrifuges of essential oil separation contains considerable amount of hesperidin (∼1 g/L) mainly associated with solid particles. Yellow water was treated with calcium hydroxide until pH 12 to solubilize hesperidin, filtered, neutralized at pH 6, and loaded on resin up to saturation. Desorption with 10% ethanol aqueous solutions at different NaOH concentrations (0.23−0.92 M) assured high concentration of hesperidin in selected fractions (10−78 g/L), from which it precipitated in high yield and purity immediately after acidification at pH 5. Best results were obtained using 0.46 M NaOH as eluent: 71.5% of the adsorbed hesperidin was desorbed in 300 mL, with an overall 64% yield of isolated product at 95.4% purity (HPLC). These experiments can constitute a useful starting point for an industrial application.

Keywords: *Adsorption on resin; Citrus sinensis; hesperidin; styrene−divinylbenzene resin; waste water*

INTRODUCTION

Hesperidin is the predominant flavanone glycoside of sweet oranges. It is extracted from citrus peel (Lopez Sanchez, 1986) and required by the pharmaceutical industry for its therapeutic importance to many diseased capillary conditions (Struckmann and Nicolaides, 1994; Benavente-Garcia et al., 1997). Moreover, it was successfully tested as a chemopreventive of variously induced tumors in rats and mice (Tanaka et al., 1994, 1997a−c; Yang et al., 1997; Berkarda et al., 1998). We recently described a new procedure for recovering hesperidin from the residual peel of orange juice processing (Di Mauro et al., 1999), in which the classic alkaline treatment of peel was followed by an adsorption of the neutralized extract into a column filled with styrene−divinylbenzene resin (SDVB) and by a desorption using a much more reduced volume of alkaline solution as eluent, both in the absence and in the presence of ethanol (up to 10%). Hesperidin rapidly precipitates in high yield and purity after acidification of the more concentrated fractions. SDVB resin was previously used for recovering grapefruit oil from waste water (Ericson et al., 1990). The present work was aimed at application of this methodology using another raw material of orange processing as a source of hesperidin, that is, the turbid water flowing out from the centrifuges of essential oil separation, namely, yellow water (Figure 1). This effluent contains ∼1.0 g/L hesperidin together with other compounds, such as organic acids, phenolics, sugars, residual essential oil, and pectins, as already reported by Milnes and Agmon (1995). Each extractor

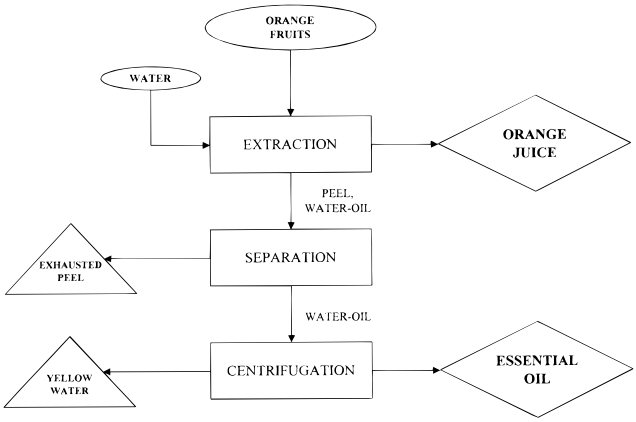

Figure 1. Simplified flow scheme of FMC technology for production of orange juice and essential oil.

in line utilizes ∼1.2 m^3/h of water to sprinkle the residual peel of 3−4 ton/h of orange fruits, discharging 1.0 m^3/h of yellow water from centrifuges. Therefore, a medium-sized factory working with 10 extractors for 10 h/day discharges ∼100 m^3/day of yellow water containing >100 kg/day of hesperidin. Exploitation of such waste material could be advantageous to balance at least the costs of the water purification plant.

MATERIALS AND METHODS

Samples of yellow water were drawn from the centrifuges of the FMC plant of Ruby International Co. (Catania, Italy) during the citrus season from January to April 1999. Each sample was constituted by mixing three aliquots drawn at different times of the same day. Values of pH, acidity, °Brix, and reducing and total sugars were determined according to standard methods. Limonene was quantified according to the procedure of Scott and Veldhuis (1966), and hesperidin was

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Table 1. Chemical Composition of Yellow Water from Citrus FMC Processing

sample	pH	acidity[a] (g/L)	°Brix	hesperidin (g/L)	reducing sugars (g/L)	total sugars (g/L)	limonene (g/L)
1	4.78	0.74	3.4	0.91			
2	4.60	1.38	4.5	1.11	25.5[b]	31.2[b]	3.84[b]
3	4.53	1.60	4.5	1.09			
4	4.57	1.41	4.5	1.10			
5	4.72	0.74	4.5	1.17	24.8[c]	29.9[c]	3.16[c]
6	5.18	0.48	3.5	0.72			
7	4.43	1.29	4.7	1.26	25.1	33.2	4.16
mean[d]	4.69 (0.25)	1.09 (0.43)	4.2 (0.50)	1.05 (0.18)	25.1 (0.40)	31.4 (1.70)	3.72 (0.50)

[a] As anhydrous citric acid. [b] Determined on the mixture of aliquots of samples 1−4. [c] Determined on the mixture of aliquots of samples 5 and 6. [d] Standard deviation in parentheses.

determined by HPLC as described later. Standard hesperidin (97% purity), *p*-coumaric acid, and ferulic acid were purchased from Aldrich (Milan, Italy), and narirutin was purchased from Extrasynthèse (Genay, France). The solvents used were of HPLC purity grade. Adsorbing material was a Kastel S-112 resin (Dow Italia, Milan, Italy), a nonpolar SDVB copolymer having a high degree of cross-linkage and the following physical properties: specific surface area, 450−600 m^2/g, dry; mean pore diameter, 40−60 Å; density, 1.10 g/cm^3; porosity, 40−70%. Before use, resin was treated with 95% ethanol/water (65:35, v/v) to remove soluble compounds and then washed with distilled water (pH 6).

Typical procedures of extraction, adsorption on resin, desorption and isolation of hesperidin, washing, and regeneration of resin were as follows. Finely powdered calcium hydroxide was added to the sample of yellow water under stirring until pH 12. The mixture was stirred for 2 h at room temperature and then filtered through glass wool. A second filtration on paper of medium porosity was necessary to eliminate the smallest particles. Aliquots of 500 mL were neutralized using 5 N HCl until pH 6 was reached and loaded in a glass column (length = 60 cm, i.d. = 3.7 cm) filled with 385 mL of hydrated resin (124 g of dry material). At intervals, the effluent (2.5 L/h) was analyzed by HPLC to check hesperidin concentration. *It* slowly increased to 100 mg/L, but after such a value it increased very rapidly, indicating saturation of the resin. The loading was then stopped when out-flow reached ∼100 mg/L. Hesperidin was desorbed using alkaline solutions containing 10% (v/v) ethanol (from 0.23 to 0.92 M NaOH). Different fractions were collected and analyzed, and those more concentrated were combined and acidified using concentrated HCl drop by drop under stirring. Hesperidin immediately precipitates when pH 5 is reached. It was filtered, washed with cold water, dried to constant weight (70 °C), and analyzed to determine purity. After desorption phase, the resin was washed with 4 bed volumes of hot water (70 °C) and 4 bed volumes of distilled water (pH 6) to make the resin ready for another adsorption−desorption cycle. After five cycles, the resin must be regenerated with 2 L of a 2 M NaOH solution and 95% ethanol (50:50, v/v) to eliminate the adsorbed limonene (Ericson et al., 1990) and other impurities, and then it is washed as above-described.

HPLC analysis of hesperidin in the yellow water (filtered after alkaline treatment) or in the eluted fractions was performed after neutralization of known volumes with 2 N HCl and dilution to a final known volume with an acetic acid/sodium acetate buffer solution (pH 4) (Di Mauro et al., 1999). Analyses were performed using a liquid chromatograph (Varian) equipped with a Star 9012 Q pump, a diode array detector (ProStar 330), and a 12.5 cm Lichrospher 100 RP-18 (5 μm) column (Merck, Milan, Italy). The mobile phase was isocratic $H_2O/CH_3CN/CH_3CO_2H$ (78:20:2, v/v/v) at a rate flow of 0.7 mL/min. Samples of 20 μL were injected and monitored at 283 nm. Each analysis was carried out in triplicate within 1 h from the sample preparation to avoid precipitation of hesperidin. Standard errors of the replicate HPLC measurements did not exceed ±5%. Quantitation of hesperidin was performed according to an external standard method using a calibration line based on standard hesperidin as previously reported (Di Mauro et al., 1999). Purity of the isolated hesperidin was obtained by HPLC comparison with a standard sample (Sigma Aldrich, Milan, purity = 97%), by dissolving a weighed quantity of isolated hesperidin in 0.3 mL of *N*,*N*-dimethylformamide and diluting to a final known volume with the HPLC mobile phase.

RESULTS AND DISCUSSION

The waste water flowing from centrifuges of essential oil separation appears to be yellow in color and turbid for the presence of suspended particles (∼3% dry weight). Values of pH, acidity, °Brix, reducing and total sugars, limonene, and hesperidin of the different samples of yellow water are reported in Table 1. The mean pH value is 4.69, ranging from 4.43 to 5.18; acidity (as anhydrous citric acid) changes from 0.48 to 1.60 g/L, with a mean value of 1.09 g/L; and the °Brix value is 4.2 (mean from 3.4 to 4.7). The distribution of reducing (25.1 g/L) and total sugars (31.4 g/L) appears to be different from that of prime orange juice, suggesting a large hydrolysis of sucrose to glucose and fructose. The mean concentration of limonene is 3.72 g/L, and that of hesperidin is 1.05 g/L with a minimum value of 0.72 g/L and a maximum of 1.26 g/L. Analyses were also performed in the samples of yellow water after filtration on 20−25 μm filter paper (Whatman No. 41). The corresponding values of pH, acidity, °Brix, and sugars remained unchanged, whereas limonene and hesperidin were significantly reduced, because these hydrophobic compounds remain associated with the suspended solids. The residual content of limonene in the clear yellow water was ∼3% of that present in the turbid yellow water, and the residual hesperidin was ∼19%. A lower amount of limonene in the filtered yellow water is useful for the subsequent adsorption on SVBD resin, because it could compete with hesperidin (Ericson et al., 1990); however, the loss of 81% hesperidin does not allow a convenient use of the clear yellow water as a starting material. Turbid yellow water was then directly treated with calcium hydroxide, and limonene was eliminated during the subsequent filtration of alkaline yellow water. Thus, the solution had the highest content of hesperidin and the lowest of limonene.

A simple stirring of alkaline yellow water (pH 12) at room temperature for 2 h was sufficient to ensure solubility of hesperidin and complete precipitation of pectins as calcium pectates. In fact, yellow water contains a lower amount of pectins (2−4 g/L) than peel wash (3−9 g/L) (Milnes and Agmon, 1995), and consequently a lower amount of calcium pectates should be formed. Subsequent filtration could be a critical operation if pectins were not completely transformed and precipitated. The deposit of solid particles on the resin during the adsorption phase is detrimental for its activity, and the presence of pectins or calcium pectates

Table 2. Adsorption of Yellow Water and Elution of Hesperidin with 10% Ethanol-Containing Solutions of NaOH at Different Concentrations

			eluted hesperidin			concn
	fraction[a]	vol (mL)	g	g/L	yield %	factor
expt 1	1	354	0.67	1.89	4.6	4.5
loading: 34.66 L	2	100	0.65	6.50	4.4	15.4
hesperidin: 14.66 g, 0.423 g/L	3*	100	2.20	22.00	15.0	52.0
0.23 MNaOH	4*	100	3.42	34.20	23.3	80.9
	5*	100	1.04	10.40	7.1	24.6
	6	500	0.10	0.20	0.7	0.5
	7	500	0.02	0.04	0.1	0.1
	total	1754	8.10		55.2	
expt 2	1	251	0.13	0.52	0.9	0.5
loading: 14.52 L	2	100	1.00	10.00	7.1	10.3
hesperidin: 14.11 g, 0.972 g/L	3*	100	4.05	40.50	28.7	41.7
0.46 M NaOH	4*	100	4.65	46.50	33.0	47.8
	5*	100	1.39	13.90	9.9	14.3
	6	500	0.59	1.18	4.2	1.2
	7	500	0.01	0.02	0.1	
	total	1651	11.82		83.9	
expt 3	1	307	0.55	1.79	3.8	1.6
loading: 13.00 L	2*	100	3.92	39.20	27.0	35.1
hesperidin: 14.52 g, 1.117 g/L	3*	100	5.48	54.80	37.7	49.1
0.55 M NaOH	4*	100	1.60	16.00	11.0	14.3
	5	100	0.52	5.20	3.6	4.7
	6	500	0.52	1.04	3.6	0.9
	7	500	0.03	0.06	0.2	0.1
	total	1707	12.62		86.9	
expt 4	1	302	0.98	3.24	6.6	2.6
loading: 12.00 L	2*	50	2.31	46.20	15.6	37.5
hesperidin: 14.78 g, 1.232 g/L	3*	100	7.82	78.20	52.9	63.5
0.69 M NaOH	4*	100	1.71	17.10	11.6	13.9
	5	100	0.43	4.30	2.9	3.5
	6	500	0.26	0.52	1.8	0.4
	7	500	0.02		0.1	
	total	1652	13.53		91.5	
expt 5	1	208	0.16	0.77	1.1	0.8
loading: 15.97 L	2*	100	1.81	18.10	12.5	20.0
hesperidin: 14.50 g, 0.907 g/L	3*	100	4.47	44.70	30.8	49.3
0.92 M NaOH	4*	100	1.84	18.40	12.7	20.3
	5	100	0.48	4.80	3.3	5.3
	6	500	0.48	0.96	3.3	1.1
	7	500	0.04	0.08	0.3	0.1
	total	1608	9.28		64.0	

[a] Fractions marked by asterisks were selected for recovery of hesperidin.

Table 3. Recovery of Hesperidin from Eluted Fractions

	expt 1[a]		expt 2		expt 3		expt 4		expt 5	
	eluted	precip[b]	eluted	precip	eluted	precip	eluted	precip	eluted	precip
loaded hesperidin (g)	14.66		14.11		14.52		14.78		14.50	
vol[c] (mL)	300		300		300		250		300	
g (% on adsorbed)	6.66 (45.4)	4.57 (31.2)	10.09 (71.5)	9.03 (64.0)	11.00 (75.8)	7.56 (52.1)	11.84 (80.1)	8.45 (57.2)	8.12 (56.0)	6.76 (46.6)
purity (%)	90.1		95.4		93.7		94.5		94.8	

[a] Key for numbers is that of Table 2. [b] Precipitated. [c] From selected fractions used to precipitate hesperidin (marked by asterisk in Table 2).

in the eluted fractions interferes with precipitation of hesperidin, lowering its yield and purity. Before loading of the adsorption column, the alkaline yellow water was acidified to the same pH value as the resin (pH 6). This pH value was suitable to give rise to complete protonation of the chalcone anion and cyclization (Di Mauro et al., 1999), avoiding precipitation of hesperidin on resin.

Table 2 reports the data of five cycles of adsorption−desorption using five different samples of yellow water. The loaded volume changed from 12 to 35 L, but the amounts of adsorbed hesperidin were almost the same for each experiment (14.1−14.8 g), indicating the constant efficiency of resin and the effectiveness of the washing after each cycle. The choice of 10% ethanol solutions as the more effective eluents came from the results of previous experiments using orange peel (Di Mauro et al., 1999). These alkaline eluents (0.23−0.92 M NaOH) assured desorption of most of the hesperidin

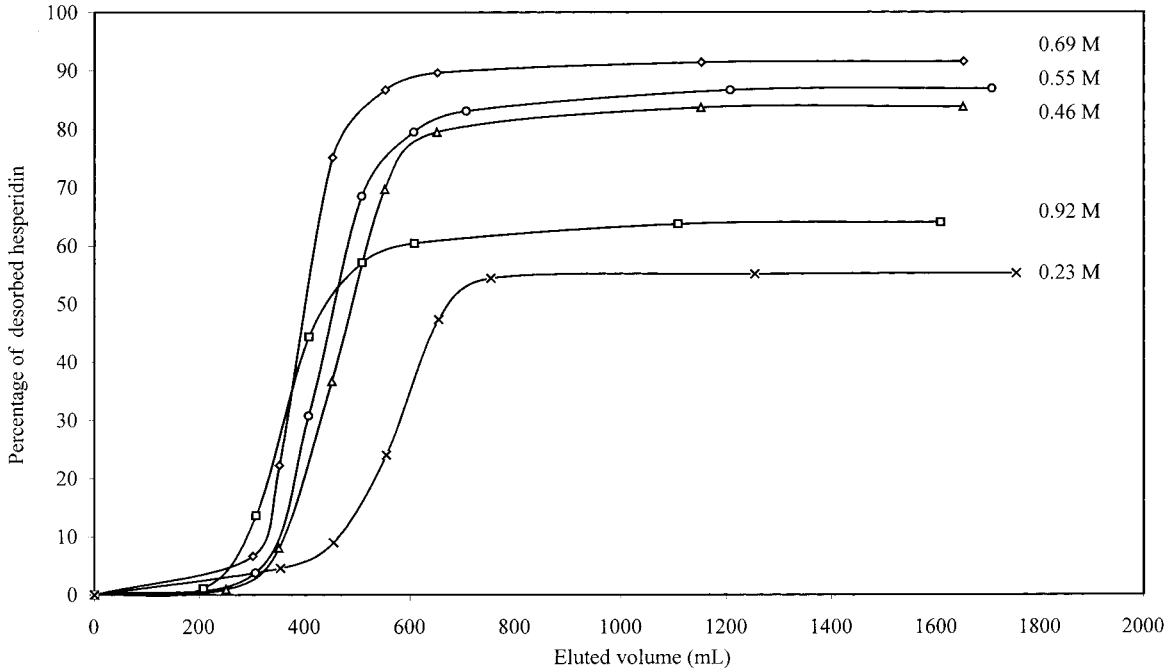

Figure 2. Cumulative percentage of desorbed hesperidin using eluents at different NaOH concentrations.

in 250−300 mL (total volume of fractions marked by asterisk in Table 2). The concentration of hesperidin in the selected fractions changed from 10.4 g/L (Table 2, experiment 1) to 78.2 g/L (Table 2, experiment 4). High concentration factors were also obtained using a 0.23 M NaOH solution, the weakest eluent. In this experiment ∼35 L of yellow water was loaded to saturate the resin, and the mixture of eluted fractions 3−5 was 52.5 times more concentrated than starting yellow water, but the yield of desorbed hesperidin was low (∼55%). Figure 2 reports the cumulative percentage of eluted hesperidin in the collected fractions of each experiment. In particular, desorption of >80% of the adsorbed hesperidin occurred with eluents at higher NaOH concentration. The elution yields were 83.9% using 0.46 M NaOH, 86.9% (0.55 M), and 91.5% (0.69 M). In the latter experiment fraction 3 contained 7.82 g of hesperidin in 100 mL, with a concentration factor 63.5 times with respect to the corresponding yellow water. A further increase of NaOH concentration (0.92 M) lowered the desorption yield, owing to swelling and a floating effect that hindered a correct diffusion of eluent through resin (Di Mauro et al., 1999). The most effective elution was obtained using an intermediate NaOH concentration in eluent (0.46−0.69 M).

Table 3 reports the data of isolation of hesperidin from those fractions where its concentration was maximum (marked by asterisks in Table 2). These fractions, containing 82.2−87.5% of the desorbed hesperidin, were combined and acidified at pH 5. A variable amount of product rapidly precipitated (4.57−9.03 g), depending on the combination of desorption and crystallization yields. The highest yields were obtained from the more concentrated fractions, as expected, and the best result was obtained in experiment 2 (0.46 M NaOH as eluent), where 9.03 g of hesperidin with 95.4% purity was isolated (64% of that adsorbed and 89.5% of that present in the mother liquor). Figure 3 reports HPLC chromatograms of different steps of hesperidin recovery. A typical chromatogram of yellow water (Figure 3A) shows the presence of large amounts of *p*-coumaric acid (peak I) and ferulic acid (peak II), together with hesperidin (peak

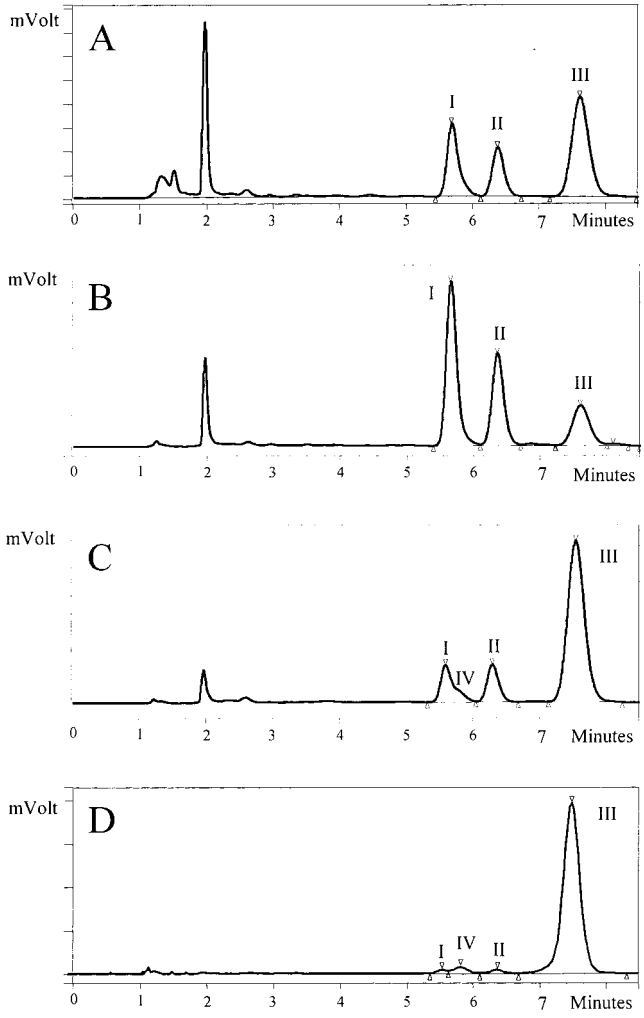

Figure 3. HPLC chromatograms of hesperidin-containing solutions: A, yellow water; B, first eluted fraction; C, hesperidin-rich fraction; D, isolated hesperidin. Peaks I, II, III, and IV correspond to *p*-coumaric acid, ferulic acid, hesperidin, and narirutin, respectively.

III). These *trans*-4-hydroxycinnamic acids are primarily present in orange peel and juice as esters (Peleg et al., 1991; Fallico et al., 1996; Rapisarda et al., 1998), but esters are hydrolyzed by the alkaline treatment to the free acids. The first eluted fraction (Figure 3B) contains less hesperidin than hydroxycinnamic acids, but successive fractions (Figure 3C) become richer in hesperidin and poorer in hydroxycinnamic acids, proving the selectivity of the SDVB eluent system in the desorption phase. Decrease of hydroxycinnamic peaks revealed the presence of a small amount of narirutin (peak IV). Figure 3D shows a typical analysis of the isolated hesperidin. Impurities of hydroxycinnamic acids and narirutin were identified by comparison of retention times and UV spectra (diode array) with those of standard compounds.

CONCLUSION

The procedure for recovering hesperidin by concentration of yellow water through SDVB resin appeared to be very effective in both adsorption and desorption phases. The concentration factors obtained using ethanol-containing alkaline solutions (0.46−0.69 M NaOH) as eluents assured, after acidification, immediate precipitation of hesperidin in high yield, thus overcoming disadvantages due to the high dilution. Recovery is highly selective as indicated by purity (93.5−95.4%). Best yield and purity were obtained using an NaOH concentration ∼0.5 M NaOH, according to previous results with orange peel (Di Mauro et al., 1999). The use of yellow water holds advantages over the use of solid peel as a source of hesperidin (Di Mauro et al., 1999) because of the simpler alkaline treatment and other operating facilities inherent in the liquid state. The results of the present study could be considered as a useful starting point for industrial application.

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