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Partial Purification of Anthocyanins from *Brassica oleracea* (Red Cabbage)

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ABSTRACT

Red cabbage has a high content of anthocyanin and it may become an important source of this pigment. Common extraction methods are not selective, and a great quantity of sugar is co-extracted with the dye. This work used adsorption-desorption process on Amberlite XAD7 and Sephadex LH20 resins to partially purify anthocyanins present in the whole juice of red cabbage. Both resins succeeded to purify anthocyanins, producing solutions with up to $0.5\,\mathrm{g\,L^{-1}}$ of sugars from an initial concentration of more than $25\,\mathrm{g\,L^{-1}}$. Using Amberlite XAD7 it was possible to recover 24 to 95% of the dye initially found in the whole juice while 11 to 56% was recovered using Sephadex LH20. The kinetics of adsorption of the dye on Amberlite XAD7 was described according to

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a pseudo-second-order model, and the sorption equilibrium was well described by the Freundlich isotherm. The adsorption enthalpy was -17.13 kcal mol^{-1}, indicating a possible chemical adsorption.

Key Words: Anthocyanin; Purification; Sorption; Red cabbage; Kinetics.

INTRODUCTION

Color is probably the most important factor in the acceptance of food products because it is used as an indicator of quality. Thus, it is extremely important to develop foods with an attractive appearance.

Artificial colorants are the most frequently used pigments due to their higher coloring power, stability, and number of colors, and also because they are less expensive to use. However, it has been shown that these compounds present some noxious effects to health.[1,2] For this reason, people increasingly demonstrate a preference for natural colorants.

Anthocyanins are pigments found in many vegetables; they are responsible for the most blue and reddish colors in the vegetable kingdom. In some fruits, such as some varieties of cherries, apples, and plums, anthocyanins are in the internal cells and in the skin but are not present in the whole pulp. Anthocyanins are also found in many other vegetables: grapes, strawberries, peaches, aubergines, red cabbage, radish, and so on. Some fruits have only one or two kinds of anthocyanins; others have a complex mixture of 20 or more types.[3] More than 250 kinds of anthocyanins have been identified.[4] The growing interest in this pigment is not only because it is a natural food colorant but also because it can be used in pharmaceutical products due to its various therapeutic effects. Anthocyanins are anticholesterics, antimutagenics, antidiabetics, and also antioxidants.[5–8]

Anthocyanins are, in general, obtained by extraction of the juice[9,10] or by extraction with polar solvents.[11–13] The extraction process is not selective, and a great number of components can be co-extracted.

Red cabbage (*Brassica oleracea*) is a rich and relatively cheap source of this pigment. Its extract has a powerful coloring and a superior stability compared with other anthocyanins, because of its chemical configuration.[14] Sulfurated substances are co-extracted with the pigment producing a disagreeable odor.[15] Red cabbage also has a high content of reducing sugars that accelerate the juice degradation or cause problems in subsequent processes such as spray drying.

Adsorption-desorption purification is a relatively simple method to discard undesirable components. This process was used by[9] to purify anthocyanin extract of Aronia melanocarpa var Nero. Sixteen adsorbent materials were tested as a solid phase on a laboratory scale. An extract, free from sugars, acids, and proteins, was obtained. The best results were given by Serdolit PAD IV and Amberlite XAD7.

The objective of our work was to separate sugars from colorant in the red cabbage juice, using two different adsorbents: Amberlite XAD7 and Sephadex LH20. These resins are commonly used in the separation and purification of colorants by chromatography.

METHODS

Experimental Procedure

Red cabbage juice was obtained through disintegration of the whole vegetable in a home juice extractor. The juice was filtered through a sinterized filter and centrifuged at 5000 rpm for 20 min to separate the insoluble solids. The colorant concentration (mg of colorant by 100 mL of solution) was obtained for partially purified and whole juices with the aid of a spectrophotometer at 550 nm, at pH 3.0. The concentration was referenced to a calibration curve prepared with red Congo synthetic colorant as recommended by.[16]

Batch tests were made using Sephadex LH20 (Pharmacia Biotech) and Amberlite XAD7 (Acros Organics) resins, at room temperature. Amberlite XAD7 is a non-ionic aliphatic acrylic polymer, which adsorptive properties are derived from the macroreticular structure. It also presents groups C═O that gives to the resin a moderate polarity with mean pore diameters around 400°A Sephadex is a dextrane gel where glicose units are α 1–6 bounded with ramifications on 1–2, 1–3, and 1–4.[17] The main characteristics of both resins are given in Table 1.

Table 1. Characteristics of resins used on partial purification of anthocyanins.

Properties	Amberlite XAD7HP	Sephadex LH20
Matrix	Macroreticular aliphatic intercruzade polymeric	Hidroxipropilated intercruzade dextrane gel
Physical form	Translucid white beads	Porous spheres
Specific mass	1.06 to 1.08	
Size particle	0.56 to 0.71 mm	18–111 μm
Superficial area	380 m^2/g	
Exclusion limit		4–5 kD depending on the solvent
Porosity	≥0.50 mL/mL	
Approximated bed volume (in water)		4.0 to 4.4 mL/g of dry powder

Kinetics study was performed by the contact of a defined mass of adsorbent under agitation at 80 rpm. At certain times, an aliquot was drained and the concentration in the liquid phase was measured. Equilibrium isotherms were made for different mass/volume ratios of resin and juice, at constant temperature, in the range of 30 to 50°C, for 2 h, when the concentration of dye in the liquid phase was constant.

The desorption was carried out using organic solvent (ethylic alcohol 70%, acidified) under the ambient conditions described above.

Evaluation of reducing sugar concentration was made by measuring the sugar concentration of the whole extract, of the supernatant after adsorption on the resin, and of the colorant solution desorbed from the resin. The determination of the reducing sugar was achieved by the DNS method.[18]

Mathematical Description of the Sorption Process

Sorption Isotherms

Sorption isotherms were described by the Freundlich model (Eq. 1)

$$q_e = K_F C_e^n \tag{1}$$

where q_e (mg g^{-1}) is the adsorbed mass in the solid phase, and C_e (mg L^{-1}) is the equilibrium concentration in the liquid phase, with K_F and n the Freundlich empirical parameters.

Sorption Kinetics

Three simplified models were considered to describe the sorption kinetics:

(a) Pseudo-first-order kinetics,[19, 20]

$$\frac{dq_t}{dt} = k_1(q_1 - q_t) \tag{2}$$

where k_1 is the pseudo-first-order sorption rate constant (min^{-1}), and q_1 and q_t are the equilibrium and instantaneous adsorbed mass (mg g^{-1}). Integrating Eq. (2) at the limits of 0 to t, and 0 to q_t, we have

$$\log(q_1 - q_t) = \log(q_1) - \frac{k_1}{2.303} \cdot t \tag{3}$$

where q_1 is the value provided by the kinetics when $t = \infty$.

(b) Peudo-second-order kinetics, based on the equilibrium adsorption capacity. It can be expressed by

$$\frac{dq_t}{dt} = k_2(q_2 - q_t)^2 \qquad (4)$$

where q_2 is the equilibrium adsorbed mass of the pigment (mg g^{-1}) and k_2 is the adsorption constant rate of a pseudo-second-order kinetics (mg g^{-1} min^{-1}).[19] By integration at the same limits as Eq. 2, we have

$$\frac{1}{(q_2 - q_t)} = \frac{1}{q_2} + k_2 t \qquad (5)$$

Equation 5 can be rearranged as follows:

$$\frac{t}{q_t} = \frac{1}{k_2 q_2^2} + \frac{1}{q_2} t \qquad (6)$$

The two presented models consider sorption as a chemical reaction depending on time.

(c) Homogeneous diffusion model

$$q_t = k_i t^{0,5} \qquad (7)$$

where k_i is the intraparticular diffusion rate constant (min$^{-0,5}$); k_i is a mass transfer coefficient that evolves effective diffusion, physical characteristics and dimensions of the particle.

Adsorption Enthalpy Calculation

Adsorption enthalpy ΔH (kcal mol^{-1}) on Amberlite was computed using the Henry constant, K (Table 4), where K (L g) is the inclination of each linear isotherm. These values were fitted to the Clausius-Clapeyron equation

$$K = A \exp\left(-\frac{\Delta H}{RT}\right) \qquad (8)$$

where R is the universal gas constant (kcal mol^{-1} K^{-1}) and T, the temperature (K).

RESULTS AND DISCUSSION

Choosing the Best Resin for the Mixture Colorant-Sugars Separation

Sorption and Desorption Kinetics

Figure 1 shows an adsorption kinetics of the red cabbage extract on Sephadex LH20 and Amberlite XAD7, at the initial concentration C_0 of 50 mg colorant 100 mL^{-1} solution. The rate of adsorption of dye is nearly the same and the equilibrium is achieved in about 30 min with Sephadex LH-20. With Amberlite XAD7, this equilibrium is achieved in 60 min.

Figure 2 shows the kinetics of desorption using different resins. Supernatant concentration rises quickly during the first 20 min, attaining a pseudoequilibrium in approximately 30 min. C_0, in this case, is the initial extract concentration used in the previous adsorption.

Sorption Isotherms

The selection of adsorbents for the separation process should also consider the evaluation of the equilibrium of adsorption. Adsorption isotherms on Amberlite XAD7 e Sephadex LH20 are shown in Fig. 3.

The parameters K_F and n were obtained by fitting experimental results to Eq. (1.) Table 1 shows that n, obtained for Amberlite, was very close to

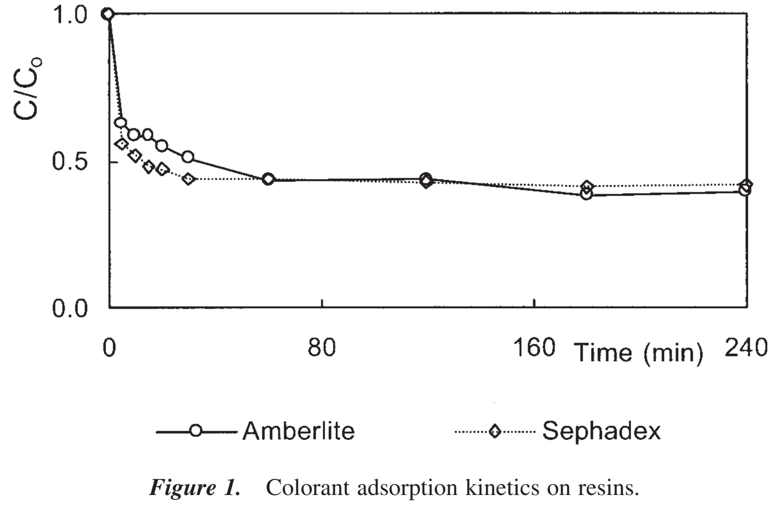

Figure 1. Colorant adsorption kinetics on resins.

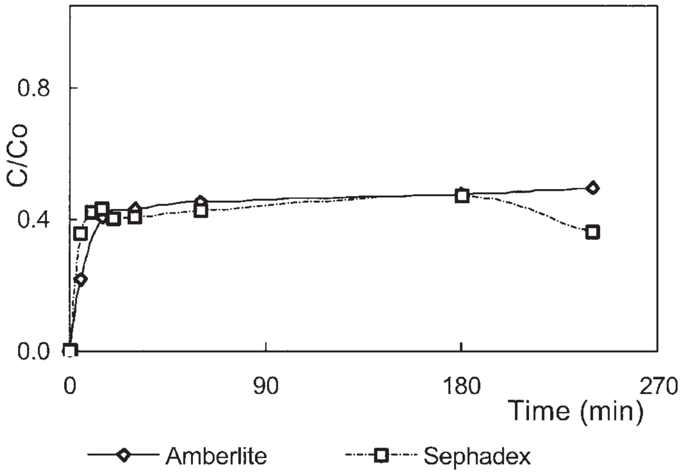

Figure 2. Colorant desorption kinetics using acidified alcohol.

1 (0.959), indicating a linear adsorption. Sephadex presents an n value of 1.245, which indicates that the colorant has a tendency to remain in the liquid phase.[21]

An additional linear fitting (n = 1) on experimental data was made. Determination coefficients found were 0.938 and 0.986 for Sephadex and Amberlite, respectively. K values found are also shown in Table 2. It can be seen that Amberlite has a k value twice that found for Sephadex; this shows its greater capacity to adsorb the pigment.

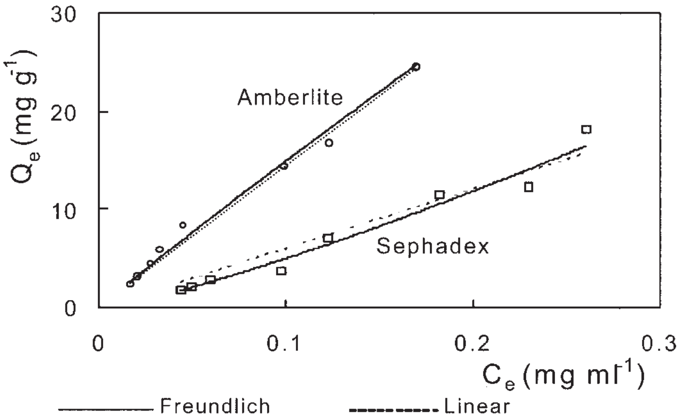

Figure 3. Colorant adsorption isotherms on Amberlite XAD7 and Sephadex LH20 resins.

Table 2. Linear and Freundlich isotherm parameters for adsorption and desorption with acidified ethanol.

Resin, isotherm	Amberlite		Sephadex	
	Adsorption	Desorption	Adsorption	Desorption
K (linear)	143.73	3.326	60.254	
R^2 linear	0.986	0.8317	0.938	
n	0.959	1.955	1.245	0.217
k_f	135.29	8.553	87.614	5.611
R^2 Freundlich	0.983	0.806	0.978	0.922

The resins have distinct compositions, and some considerations should be done about the differences of the adsorption-desorption process. Amberlite is a moderately polar adsorbent resin containing a macroreticular structure, properly constructed to adsorb phenols from water with the majority pores going from 350 to 400°A.[22] It makes access of the anthocyanins easy not only to the outer but also to the inner active sites of the polymer. On the other side, Sephadex LH20 has smaller pores, and it can provide chemical interactions between the matrix and the solid resulting in an irreversible bound.[17]

First, colorant desorption with water was carried out. As the desorbed mass (C_e) was very low and was also independent of the adsorbed quantity, 0.7% acidified alcohol was used for this operation.

Desorption isotherms with ethanol follow Freundlich behavior as shown in Fig. 4, and their parameters are presented in Table 2. It can be observed that $n = 1.955$ for Amberlite, and $n = 0.217$ for Sephadex. Similar to adsorption, anthocyanin dye has a tendency to remain in the liquid phase.

The amount of recuperated dye depends on the dosage of saturated adsorbent in the solution. For adsorbent dosage in the range 0.02 to 0.2 mg mL^{-1}, Amberlite recovers 24 to 95% of the dye initially found in whole juice while Sephadex gives back only 11 to 56%. Figure 5 shows the recuperated mass for partial purification.

Adsorbing a superior quantity of anthocyanin mass relating to Sephadex, a great degree of partial purification could be achieved by Amberlite XAD-7, resulting in a high recuperation rate of the pigment.

Sugar Separation Efficiency

Amberlite XAD7 and Sephadex LH20 efficiency in separating sugars was studied during kinetic essays. Figure 6 presents the values of the sugar concentrations (g L^{-1}) during adsorption and desorption processes.

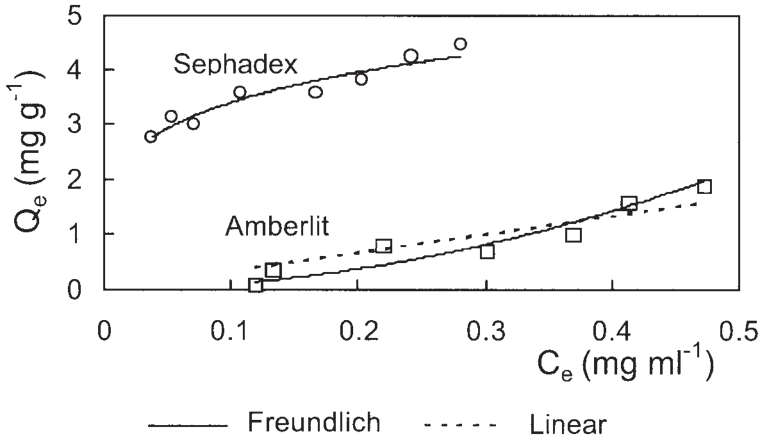

Figure 4. Desorption isotherms with ethanol on Amberlite XAD7 and Sephadex LH20.

During adsorption kinetics concentration, sugar concentration varied from 35 to 25 g L^{-1} for both resins. During desorption on Sephadex, the sugar concentration was from 0 to 0.5 g L^{-1}, and on Amberlite it was from 0 to 0.25 g L^{-1} (Fig. 6). After separation, the maximum sugar concentration obtained in our experiments was 0.5 g L^{-1} after desorption.

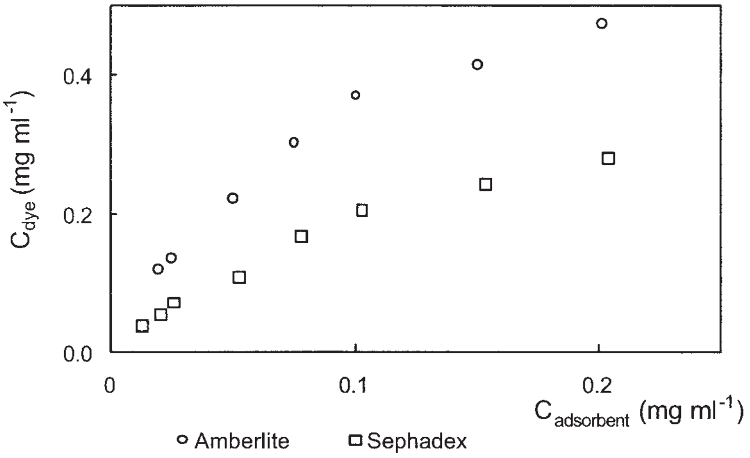

Figure 5. Recuperated dye concentration in solution, after partial purification.

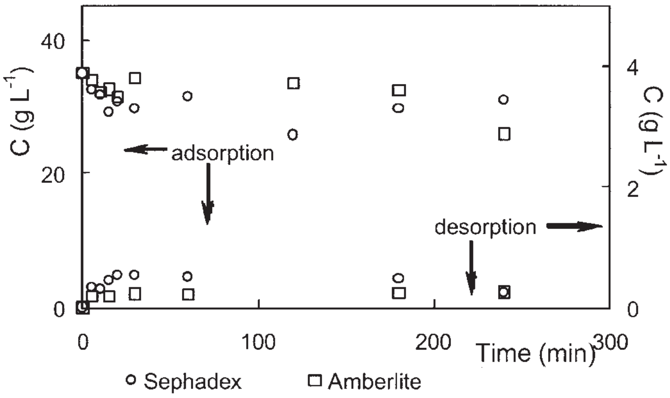

Figure 6. Sugar concentration in liquid phase.

Sorption Dynamics of the Colorant on Amberlite XAD7

Amberlite XAD7 gives the best results for colorant recuperation, so only its dynamics were studied. Three simplified models were tested.

Four kinetics experiments, at initial concentrations of 2.15, 4.81, 9.88, and 40.05 mg 100 ml^{-1}, were carried out (Fig. 7).

Two chemisorption models and one intraparticular model were tested. q_1, q_2 were obtained at $t_\infty = 1260$ min, denoted in Table 3 as $q_{\infty\ exp}$ and $t_{\infty\ exp}$. The description of the experimental results by the first-order and intraparticular diffusion models showed determination coefficient values, R, less than

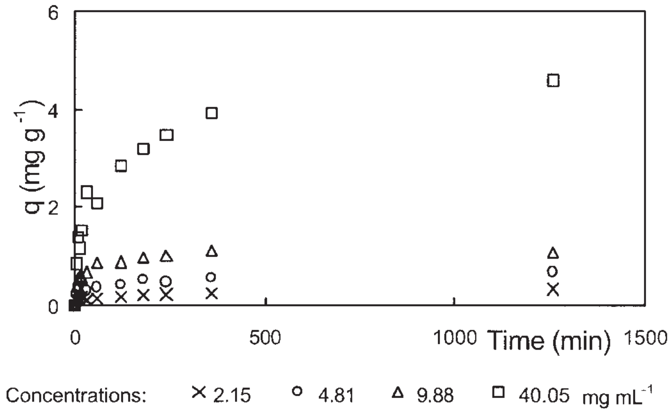

Figure 7. Colorant sorption kinetic for different initial concentrations.

Table 3. Parameters of the empirical models.

C (mg/ 100 mL)	$q_{\infty\ \mathrm{exp}}$	k_1 (min^{-1})	R_1	k_2 ($\mathrm{g\,mg}^{-1}\ \mathrm{min}^{-1}$)	R_2	k_i ($\mathrm{mg\,g}^{-1}\ \mathrm{min}^{-0,5}$)	R_i
2.15	0.326	−0.0016	—	0.0495	0.9918	0.0122	0.6874
4.81	0.6751	0.0033	0.8672	0.0303	0.9957	0.0258	0.5822
9.88	1.0843	0.014	0.7501	0.0697	0.9967	0.0502	—
40.05	4.6028	0.015	—	0.0045	0.9971	0.1791	0.7457

0.9. On the other hand, the second-order model showed R values greater than 0.99, the best model to describe the process. The second-order pseudo-kinetic term is applied because k is a function of the colorant concentration, is not a constant, and is independent of concentration, as would be expected for a true second order kinetic. Figure 8 shows linearized results.

Sorption Enthalpy

Adsorption isotherms were obtained at 39°C, 44°C, 47°C, and 50°C. Henry constant values (K) are shown in Table 4, for each initial concentration.

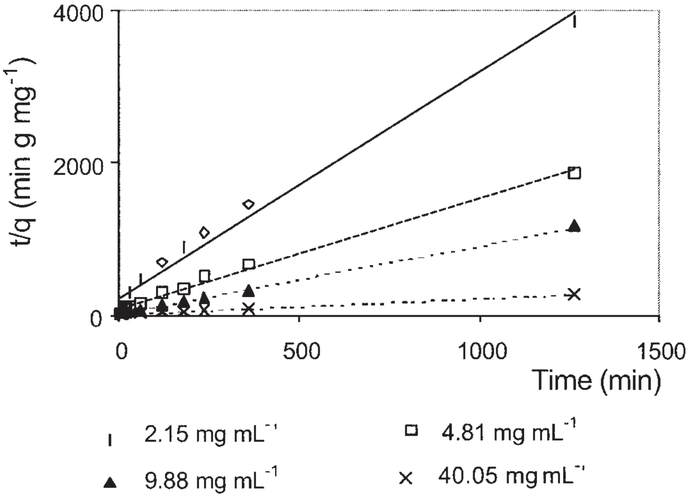

Figure 8. Pseudo-second-order linearized kinetic model for different initial concentrations.

Table 4. Linear isotherm constants at different temperatures.

Temperature	K
39	36.219
44	23.500
47	13.567
50	15.951

Adsorption enthalpy on Amberlite XAD7 was obtained using the Clausius-Clapeyron equation, and the value found was $-17.13\,\mathrm{Kcal\,mol^{-1}}$; it is possible that the colorant presents a chemical adsorption on Amberlite XAD7.

CONCLUSION

The adsorption–desorption method showed itself to be very efficient, and the resulting extract was practically free from reducing sugars ($0.50\,\mathrm{g\,L^{-1}}$).

Amberlite XAD7 resin has a higher adsorption capacity than Sephadex LH20, and acidified ethanol is an appropriate solvent to recuperate adsorbed colorant on the resin. Desorption from Amberlite XAD7 resin is more favorable than Sephadex LH20 resin, with the highest recuperated mass in the global process.

The adsorption kinetic on Amberlite follows a pseudo-second-order model, and the aparent adsorption enthalpy found is relatively high. The process is probably a chemical adsorption, which is reinforced by the necessity to change solvent in order to desorb colorant from the resin.

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