

Deodorization of a Colorant Prepared from Red Cabbage

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ABSTRACT

A process for preparing odor-free colorant from red cabbage was developed. Anthocyanins in an acidified aqueous cabbage extract were adsorbed on Amberlite XAD-7, water-washed to remove residual cabbage odor, eluted with ethanol containing 0.1% HCl, and concentrated by evaporation. Optimal conditions for single stage batch contact and column deodorization were determined. Pigment recovery generally exceeded 90%. The stability, spectral, and colorant properties of the recovered pigments were not altered significantly by the process. Spent adsorbent could be regenerated by water washing without adversely affecting process efficiency or colorant performance.

INTRODUCTION

FOOD COLORANTS derived from red cabbage (*Brassica oleracea*) have been proposed as potential substitutes for FD&C synthetic red food dyes (Shewfelt and Ahmed, 1977, 1978). In a previous study, we showed that the pigments of red cabbage have superior colorant and stability properties (Sapers et al., 1980). However, we also found that compounds with an objectionable cabbage-like odor were co-extracted with pigments in aqueous or alcoholic preparations.

In an earlier investigation, we observed that Amberlite XAD-4, an hydrophobic polymeric adsorbent (Rohm and Haas Company, Philadelphia, PA) could be used to recover cherry anthocyanins from cherry processing waste streams (Sapers, 1975). In the present study, we investigated the use of a related product, Amberlite XAD-7, to produce an odor-free colorant from an aqueous red cabbage extract.

EXPERIMENTAL

Comparison of polymeric adsorbents

In a preliminary study we compared four polymeric adsorbents: Amberlite XAD-2, XAD-4, XAD-7, and XAD-8, the latter two being more hydrophilic than the former products (Anon., 1972). We determined their affinity for red cabbage anthocyanins by adding 0.6g of each adsorbent to 100-ml aliquots of a 1:10 dilution of cabbage extract, prepared by homogenizing equal parts of cabbage and 1% aq HCl for 2 min at high speed with a Waring Blendor and then filtering the slurry through Whatman No. 2 paper. We stirred the mixtures for 5 min before removing the adsorbents by filtration and examining them visually for differences in color intensity.

Preparation of standard cabbage extracts

In subsequent studies we prepared red cabbage extract from pigmented cabbage leaves that had been steam blanched for 3 min at 100°C in an autoclave operated at atmospheric pressure, cooled to room temperature, combined in 300g batches with 300 ml of 0.1M HCl and blended at high speed in a Waring Blendor for 2 min. The resulting slurry was clarified with an Acme Supreme Juicerator (Acme Juicer Mfg. Co., Lemoyne, PA), lined with Whatman No. 1 filter paper. Residual pigments in the filter cake were recovered by twice redispersing the cabbage solids in 300 ml portions of 0.1M

HCl and passing the resulting slurry through the juicer. The three extracts were combined and refrigerated until used.

Single stage batch contact deodorization

With the exception of the preliminary comparison of adsorbents, we used Amberlite XAD-7 for all deodorization experiments. The adsorbent was preconditioned by a modification of the method recommended by Rohm and Haas (Anon., 1972b) which consisted of hydrating one volume of adsorbent in five volumes of distilled H₂O for 30 min with stirring; removing the hydrated adsorbent with a coarse fritted disc filter funnel; washing the adsorbent with four successive one-volume portions of distilled H₂O, each time resuspending the adsorbent in H₂O, stirring for 5 min and then removing the adsorbent by filtration as before; washing the adsorbent with five successive one-volume portions of absolute methanol by the same procedure used to wash with water; removing the alcohol with four successive water washes as before; and finally, washing the adsorbent with one volume of 0.1M HCl. One hundred grams of new adsorbent yielded approximately 125g of preconditioned adsorbent having a volume of approximately 171 ml.

To determine the effect of contact time on pigment recovery, we added 15, 30, and 60g of preconditioned adsorbent to 200 ml portions of red cabbage extract and stirred the suspensions with a magnetic stirrer (Fisher Thermix set to a speed of 3) for as long as 90 min, removing 11-12 ml aliquots after 2, 5, 10, 15, 30, and 90 min. Following adsorption, we immediately separated the spent cabbage extract from the adsorbent by filtration through Whatman No. 2 filter paper with suction and retained the filtrates for spectrophotometric analysis.

To determine pigment recovery at different adsorbent usage levels, we added 5, 10, 15, 20, 25, 30, 35, and 40g portions of preconditioned adsorbent to 100 ml portions of red cabbage extract and stirred the mixtures for 15 min. The spent extract was separated from the adsorbent by filtration and retained for spectrophotometric analysis. This procedure was repeated with cabbage extract diluted with 0.1M HCl to concentrations between 5 and 75% of full strength to determine the effect of pigment concentration on recovery.

To evaluate the efficacy of a single stage batch contact deodorization process, we determined the pigment loss for each unit operation of the process by adding 15 or 30g of preconditioned Amberlite XAD-7 to 100 ml portions of red cabbage extract and stirring the suspensions for 15 min. Adsorbent was separated from spent extract by filtration through Whatman No. 2 paper with suction. The pigment-bearing adsorbent was washed by mixing with 100 ml of distilled H₂O for 5 min, the wash water being removed by filtration as described above. Pigments from the washed adsorbent were recovered by extraction with two successive 100 ml portions of 0.1% HCl in 95% ethanol, mixing the adsorbent and extractant for 5 min each time. The alcoholic solutions of extracted pigments were separated from spent adsorbent by filtration as before, combined, and concentrated to a volume of approximately 10 ml with a rotary evaporator (Rotavapor R110, Brinkmann Instruments, Inc., Westbury, NY), operated at a vacuum of 1-2 mm Hg and a water bath temperature of 40°C. Samples of fresh and spent cabbage extracts, washings, and concentrates were retained for analysis by spectrophotometric and tristimulus colorimetric procedures.

To regenerate adsorbent following recovery of the deodorized cabbage pigments, we employed the preconditioning treatments described previously. The performance of the regenerated adsorbent was evaluated by substituting it for fresh adsorbent in the process described above.

Column deodorization

We developed and tested a prototype column procedure for deodorizing red cabbage pigments with Amberlite XAD-7. A chro-

matography column (400 × 30 mm), plugged with glass wool, was charged with a suspension in distilled H₂O of hydrated adsorbent (corresponding to 100g of dry adsorbent) which on settling occupied a bed volume (B.V.) of 150 ml. The adsorbent was backwashed with sufficient water upflow to increase the B.V. by 50% as recommended by the manufacturer (Anon., 1972b), washed with four B.V. of distilled water followed by five B.V. of absolute methanol, four B.V. of distilled H₂O, and one B.V. of 0.1M HCl, all at a flow rate of approximately four B.V. per hour.

Red cabbage extract to be passed through the column was clarified by adding 5% Celite Analytical Filter Aid (Johns-Manville) and filtering the slurry through Whatman No. 2 paper with suction. Each batch of extract was analyzed by spectrophotometric and tristimulus procedures. We added clarified extract to the column in 200 ml increments at a flow rate of approximately four B.V. per hour and collected 200 ml portions of corresponding effluent for spectrophotometric analysis to determine the unadsorbed pigment. Leakage of pigments through the column was expressed in terms of the quantity of unadsorbed pigment in the effluent, calculated as a percentage of the colorant contained in the same volume of influent. When the extent of leakage reached 1%, extract was added to the column in 100-ml increments. The capacity of the column was defined as the colorant load which would result in a leakage of 5%. When this quantity of cabbage extract had been added, we washed the column with four 250-ml portions of deionized H₂O at a rate of four B.V. per hr and collected the washings for analysis. After washing, we eluted adsorbed pigments from the column with 500 ml of 95% ethanol containing 0.1% HCl at a rate of four B.V. per hr. The eluate was concentrated, diluted, and analyzed by the same procedures employed with the batch method described previously.

To permit the reuse of XAD-7 adsorbent after pigment elution, we passed four B.V. of deionized water through the column followed by two B.V. of 0.1M HCl. The column was then ready for another adsorption cycle. The performance of XAD-7 columns was evaluated during the course of as many as five successive cycles of use.

Measurement of spectral and colorant properties

Spectrophotometric analyses were performed with a Perkin-Elmer Model 552 UV-VIS Double Beam Spectrophotometer (Perkin-Elmer Corp., Norwalk, CT) and 10 mm light path rectangular silica cells. Solutions of red cabbage pigments were diluted with 0.1M HCl and adjusted to pH 1.0 with HCl to attain maximum absorbance values (Fuleki and Francis, 1968). Turbid samples were clarified by adding 1% Celite Analytical Filter Aid (2% for column effluents), and filtering through Whatman No. 5 paper. Any residual haze was removed by further filtration through a Falcon 0.22 micron disposable membrane filter unit. We obtained visible and UV absorption spectra between 600 and 250 nm and measured sample absorbance at the maxima. Colorant strength was expressed as the visible absorption maximum ($A_{\lambda_{\max\text{-vis}}}$), multiplied by the appropriate dilution factor rather than in terms of total anthocyanin concentration as defined by Fuleki and Francis (1968), since the proportions of individual cabbage anthocyanins and their extinction coefficients were not known. The quantity of cabbage anthocyanins added to an adsorbent column or mixed with the adsorbent in a batch contact treatment was expressed in colorant units, defined as the product of $A_{\lambda_{\max\text{-vis}}}$, the dilution factor required for analysis, and the volume of undiluted cabbage extract added to the adsorbent.

For the measurement of tristimulus coordinates, we diluted samples with 0.1M HCl so that the visible absorption maximum would be 1.0. Measurements were performed with a Gardner XL-23 Tristimulus Colorimeter (Gardner Laboratory, Inc., Bethesda, MD) operated in the transmission mode. Pigment solutions were analyzed in optical cells, 57.1 mm in diameter (ID), filled to a depth of 20 mm (50-ml sample).

Determination of storage stability

We compared the storage stability of red cabbage pigments in solutions prepared from a red cabbage extract (Ruby Ball Hybrid CV) and from the corresponding deodorized concentrate, obtained by the XAD-7 column procedure, following methods described previously (Sapers and Hornstein, 1979; Sapers et al., 1980). The cabbage extract was not boiled to inactivate enzymes since it was prepared from steam blanched cabbage. Dilutions of red cabbage extract and the deodorized concentrate were prepared with pH 3.0, 3.5 and 4.0 McIlvaine's buffer or with a simulated beverage formulation containing 10% sucrose and 0.3% citric acid monohydrate in distilled water, adjusted with 10% aqueous NaOH to pH 3.0 or 3.5. The dilutions were clarified by the addition of 5% Celite followed

by filtration through Whatman No. 5 filter paper with suction and then were sterilized by membrane filtration. Tubes of sterilized pigment solutions were stored under fluorescent light (80 foot-candles) or in darkness. After storage, spectrophotometric and tristimulus measurements were made, as described previously. Anthocyanin retention was calculated from values of the difference between ($A_{\lambda_{\max\text{-vis}}} \times \text{dilution factor}$) at pH 1.0 and at pH 4.5, at the beginning of the study and after storage.

Evaluation of odor

The odor of fresh and spent red cabbage extract, spent wash water, and aqueous pigment concentrates, prepared following treatment with Amberlite XAD-7, was evaluated by an informal panel comprising the author and two technicians. Samples were examined at room temperature and after warming to approximately 60°C on a steam bath.

RESULTS & DISCUSSION

Batch contact deodorization with Amberlite XAD-7

A preliminary evaluation of the ability of Amberlite polymeric adsorbents to remove anthocyanins from red cabbage extract, based on visual comparison of the adsorbents following treatment, demonstrated that XAD-7 had the highest affinity for the cabbage pigments followed by XAD-8, XAD-4, and XAD-2 in order of decreasing adsorbed pigment. Consequently, we used XAD-7 in all subsequent pigment recovery studies.

The single stage batch contact deodorization process consisted of five steps: extraction of pigment from red cabbage with 0.1M HCl, removal of pigments from odiferous cabbage extract, recovery of pigments from washed adsorbent by extraction with acidic ethanol, and concentration of the alcoholic pigment solution. Cabbage-like aroma notes were found in the spent extract and washings; however, aqueous dilutions of pigment concentrates prepared by this process were completely free of the cabbage odor.

The pigment extraction procedure yielded an intensely colored solution having a pH of 1.5–1.7. With three successive extractions, we were able to obtain 95–97% of the pigment that could be recovered with five extractions, and the extracted solids appeared to contain little residual color. When we lowered the concentration of HCl in the extractant from 0.1 to 0.01M, the extract pH increased to 4.7, and we recovered approximately 5–8% less pigment. The lower pigment yield may have been due to oxidation during blending and juice extraction; anthocyanin stability in air is greater at lower pH values (Markakis, 1974).

XAD-7 to be used for pigment recovery was preconditioned in order to remove fines, preservatives, and residual monomeric compounds. Preliminary studies demonstrated that without preconditioning, the adsorbent imparted an objectionable aroma of its own to red cabbage pigments. We used methanol as the alcoholic solvent in the preconditioning treatments described herein; however, we obtained equivalent results with isopropyl alcohol. Consequently, the choice of alcohol can be based on considerations of cost, safety, and FDA approval.

When we mixed preconditioned adsorbent and red cabbage extract in batch contact deodorization trials, pigment adsorption depended on the adsorbent-extract ratio as well as on the contact time (Fig. 1). Increasing the proportion of XAD-7 to extract resulted in more rapid equilibration and decreased the quantity of unadsorbed pigment (expressed as a percentage of the original colorant strength) in equilibrium with the adsorbent. In subsequent studies with the batch contact system, we standardized the contact time at 15 min.

When we treated diluted red cabbage extracts containing different pigment concentrations with XAD-7, we obtained a linear relationship between the pigment adsorbed per gram of XAD-7 and the pigment concentration in the

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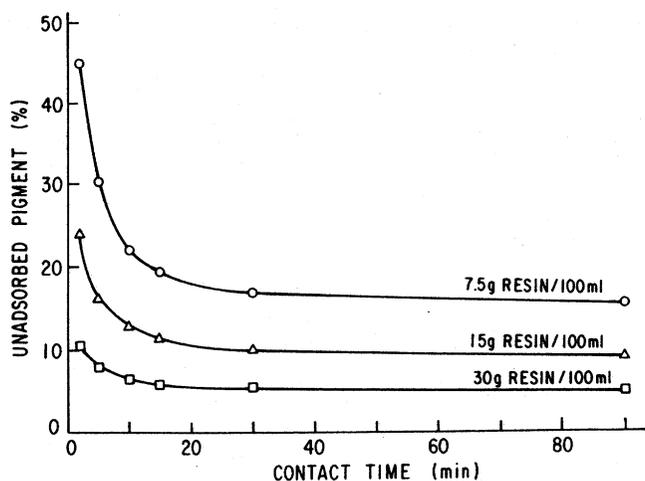


Fig. 1—Effect of contact time on pigment recovery with Amberlite XAD-7 polymeric adsorbent.

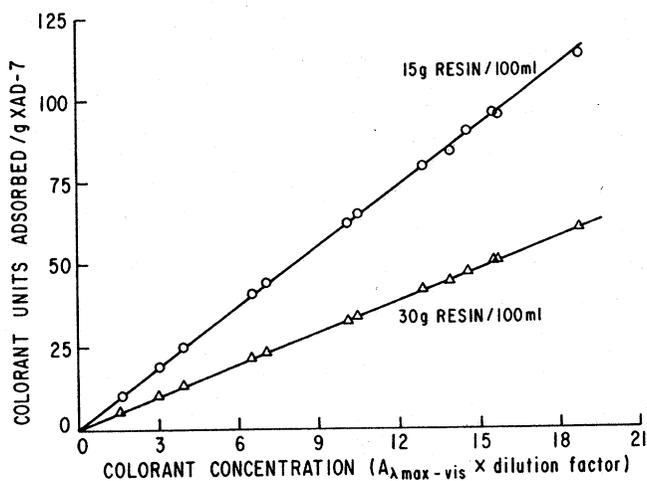


Fig. 2—Effect of colorant concentration ($A_{\lambda_{\max-\text{vis}}} \times \text{dilution factor}$) on pigment adsorption (colorant units adsorbed per g XAD-7) for different proportions of Amberlite XAD-7 to red cabbage extract.

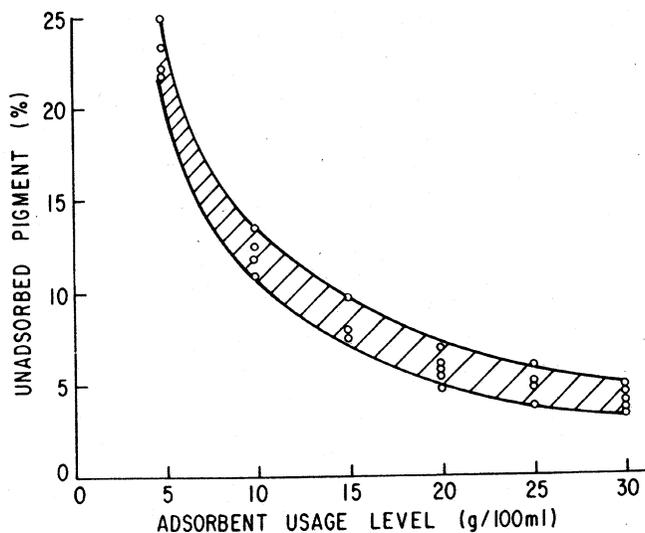


Fig. 3—Effect of Amberlite XAD-7 usage level on pigment loss during adsorption.

extract (Fig. 2). Increasing the proportion of resin to extract decreased the slope of this line. Simpson (1972) reported a linear relationship between adsorption on XAD-7 and solute concentration for aqueous solutions of acetic acid.

To show the relationship between pigment loss and adsorbent usage, we plotted the percentage of unadsorbed pigment against the amount of XAD-7 (Fig. 3). These data indicate that the pigment loss decreased to approximately 3–5% when 25–30g of XAD-7 were used to treat 100 ml of red cabbage extract, which is equivalent to 1g XAD-7 per gram red cabbage.

In addition to pigment losses resulting from incomplete adsorption of red cabbage anthocyanins on XAD-7, further losses occurred during washing, colorant recovery, and concentration steps. Data showing the magnitude of these losses with different amounts of new or regenerated XAD-7 are given in Table 1. The loss of pigment in adsorbent washings was less than the loss from incomplete adsorption since washing removed the residual adhering red cabbage extract from the adsorbent. Pigment recovery generally exceeded 90% but was somewhat variable. No differences in the distribution of pigment losses were seen between trials with new XAD-7 and trials in which regenerated XAD-7 was used.

Spectral and colorant properties of red cabbage extract and corresponding colorants prepared by XAD-7 treatment are compared in Table 2. Colorants recovered from new or regenerated adsorbent and the original cabbage extracts were similar with respect to absorption maxima, the ratios of UV to visible adsorption bands, and the tristimulus parameters. It would appear that cabbage pigments are not greatly changed by adsorption on XAD-7, recovery or concentration. Unadsorbed pigments contained in red cabbage extract following XAD-7 treatment (and also in the aqueous washings) were different from the original and recovered colorants, however. The visible absorption maximum decreased from 520 nm to 513 nm, while the UV absorption bands appeared as indistinct shoulders superimposed on a broader band. Ratios of the absorption band maxima were different with the unadsorbed pigment fraction, reflecting the change in the appearance of the UV spectrum. Tristimulus measurements indicated that the unadsorbed pigments imparted a more orange shade of red than that produced by the original and recovered colorants. Whether the unadsorbed pigments represent a minor constituent of red cabbage or an artifact formed during extract preparation or XAD-7 treatment is not known.

Deodorization with Amberlite XAD-7 column

When Amberlite XAD-7 columns are used to deodorize red cabbage pigments, the adsorbent may be loaded with extract until significant leakage, i.e., 5% of pigments occurs (Fig. 4). Quantitative studies of colorant recovery by the column procedure indicate that this endpoint corresponds to a column capacity of approximately 250 colorant units per gram of XAD-7, more than five times the capacity obtained by single stage batch contact deodorization (Table 3). At the 5% leakage level, the actual pigment loss in the effluent was less than 2% of the column load. Additional losses during washing represented 3–5% of the column load. Generally, over 90% of the pigments applied to the column were recovered in odor-free concentrates of the column eluate. Spectral and colorant properties of the cabbage pigments were relatively unchanged by the column deodorization process; a small decrease in the ratios of UV to visible absorption maxima reflects the higher purity of the deodorized product.

Amberlite XAD-7 columns may be reused following a simple water washing step to remove residual alcohol and pigments. We carried out as many as five successive

Table 1—Pigment loss during single stage batch contact deodorization of red cabbage extract with new and regenerated Amberlite XAD-7

Source of pigment	Pigment distribution (%) ^{a,b}			
	Level of XAD-7 addition (g/100 ml)			
	15		30	
	New	Regen.	New	Regen.
Red cabbage extract	100	100	100	100
Extract after adsorption	7.4–8.2	7.8–9.6	4.2–4.4	3.6–5.8
Washings	3.2–3.7	1.9–5.3	1.9–2.3	2.2–4.0
Recovered pigment	92.3–108.7	90.9–102.7	91.8–99.8	92.0–102.0

^a Calculated from values of $A\lambda_{\max\text{-vis}}$ x dilution factor x volume.
^b Range for three trials

Table 2—Effect of treatment with Amberlite XAD-7 on spectral and colorant properties of red cabbage pigments

Property	Red cabbage extract	Unadsorbed pigments	Recovered pigment ^a	
			New XAD-7	Regenerated XAD-7
Spectral measurements				
$\lambda_{\max\text{-vis}}$ (nm)	520	513	522	522
$\lambda_{\max\text{-uv}}$ (nm)	325, 290, 280	Sh ^c	325, 290, 280	325, 290, 280
$A_{325}/A\lambda_{\max\text{-vis}}$	1.24	0.54	1.16	1.16
$A_{290}/A\lambda_{\max\text{-vis}}$	1.17	1.33	1.08	1.08
$A_{280}/A\lambda_{\max\text{-vis}}$	1.20	1.81	1.09	1.08
Tristimulus measurements ^b				
L	59.2	66.4	58.2	58.1
a_L	75.2	62.2	76.5	76.6
b_L	10.3	21.2	10.4	10.2
\ominus	7.8	18.8	7.7	7.6
Saturation index	75.9	65.7	77.2	77.3

^a Usage level of 15g XAD-7 per 100 ml extract.
^b Obtained with samples diluted so that $A\lambda_{\max\text{-vis}} = 1.0$.
^c UV absorption spectrum displays shoulders corresponding to 325, 290, and 280 nm.

deodorization/washing cycles with one column and observed no changes in the column capacity, in pigment recovery or in the odor, colorant, and spectral properties of the recovered pigments (Table 3).

Column performance was adversely affected by increasing the influent flow rate (Table 4). When red cabbage extract was added at a rate of 8 or 12 B.V. per hour instead of four B.V. per hour, the column capacity decreased substantially, and colorant recovery decreased slightly. In addition, the purity of the product, as judged by the ratios of UV to visible absorption maxima, decreased slightly. However, no change occurred in the colorant hue angle.

Effect of deodorization on pigment stability

Spectrophotometric and tristimulus data plotted in Fig. 5 show that the deodorization process did not adversely affect pigment stability during storage at 25°C in the absence of light. Anthocyanin retention was greater with deodorized red cabbage extract than with untreated extract, both compared in buffer and in a simulated beverage at pH 3–4. In the presence of light, solutions containing treated and untreated cabbage extracts were similar in stability but less stable than equivalent samples stored in darkness. The hue angle of solutions containing deodorized cabbage pigments did not change during storage even with samples stored in light. Solutions of untreated cabbage pigment increased in hue angle during storage, the change being greater with samples stored in light.

The stabilizing effect of the deodorization treatment may be due to the separation of cabbage anthocyanins from such components as ascorbic acid and/or copper ions that can promote anthocyanin degradation in the presence of

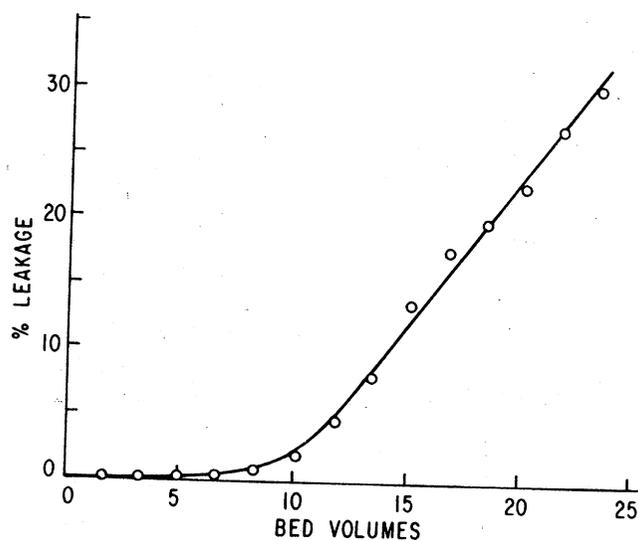


Fig. 4—Adsorption of red cabbage colorant on Amberlite XAD-7 column at 25°C and flow rate of 4 bed volumes per hour.

oxygen (Starr and Francis, 1968; Shrikhande and Francis, 1974).

CONCLUSIONS

AMBERLITE XAD-7, a polymeric adsorbent, may be used to prepare an odor-free colorant from red cabbage without adversely affecting the functional properties or stability of

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Table 3—Performance of Amberlite XAD-7 column in deodorization process for red cabbage colorant with multiple cycling

	Cycle number					
	0	1	2	3	4	5
Column capacity, colorant units/g ^a	—	274	246	274	259	245
Loss from leakage (%) ^b	—	1.9	1.8	1.5	1.7	1.7
Loss in washings (%) ^b	—	2.7	2.7	3.4	3.8	5.2
Recovered (%) ^b	—	98.2	99.6	88.6	98.9	93.2
Hue angle	10.9	10.2	10.2	10.0	10.2	10.2
A ₃₂₅ /A _{λmax-vis}	1.10	1.07	1.03	1.05	1.07	1.08
A ₂₈₀ /A _{λmax-vis}	1.26	1.11	1.08	1.03	1.08	1.12

^a For 5% leakage in effluent

^b Colorant units (A_{λmax-vis} × dilution factor × extract volume) per g Amberlite XAD-7 in column.

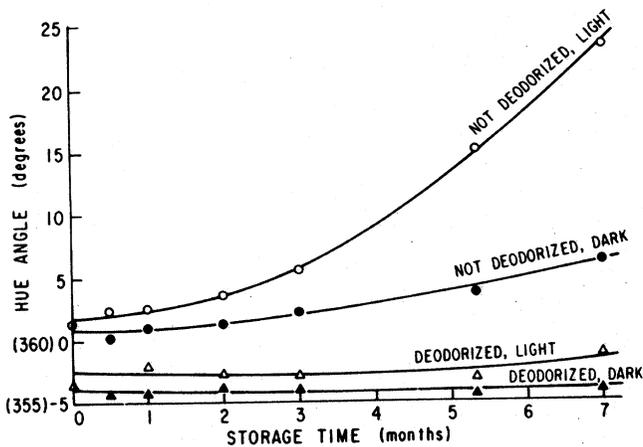
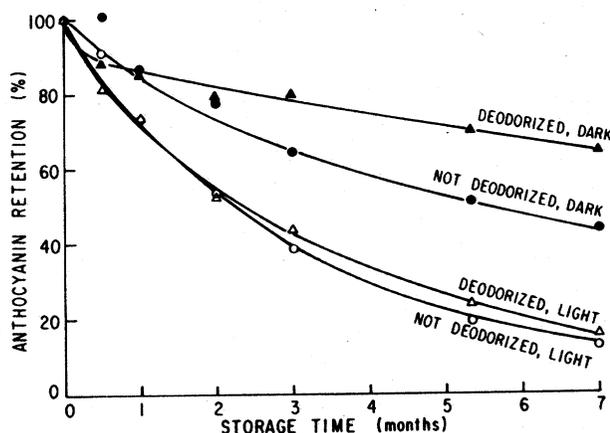


Fig. 5—Effect of deodorization treatment on stability of red cabbage pigment in simulated beverage (pH 3) stored at 25°C in darkness or exposed to fluorescent light (80 ft-c).

the product. The adsorbent may be regenerated by water washing

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Table 4—Effect of flow rate on the performance of Amberlite XAD-7 column in deodorization process for red cabbage colorant

	Flow rate (bed volumes per hr)			
	0	4	8	12
Column capacity, colorant units/g ^a	—	263	222	185
Loss from leakage (%) ^b	—	2.0	2.3	2.6
Loss in washings (%) ^b	—	2.1	3.2	3.7
Recovered (%) ^b	—	97.5	95.4	93.6
Hue angle	11.2	10.0	10.0	10.1
A ₃₂₅ /A _{λmax-vis}	1.13	1.05	1.09	1.11
A ₂₈₀ /A _{λmax-vis}	1.28	1.07	1.11	1.13

^a For 5% leakage in effluent

^b Colorant units (A_{λmax-vis} × dilution factor × extract volume) per g Amberlite XAD-7 in column.

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