Adsorption by Polyvinylpolypyrrolidone of Catechins and Proanthocyanidins from Beer

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The amounts of different phenolics adsorbed from beer and model solutions by various dosages of two commercial grades of polyvinylpolypyrrolidone (PVPP) were measured accurately by high-performance liquid chromatography, using electrochemical detection. Adsorption of simple phenolic acids and flavanols (catechins and proanthocyanidins) displayed classical Freundlich characteristics, and the isotherms obtained were compared to determine the extent of selectivity. Although PVPP preferentially adsorbed the more highly hydroxylated phenolic acids in model mixtures, a similar trend was not observed for the adsorption of dimeric flavanols in beer. The adsorptive capacity of commercial PVPP increased with decreasing particle size.

Keywords: *Adsorption; beer; flavanols; high-performance liquid chromatography; hydroxybenzoic acids; polyvinylpolypyrrolidone (PVPP)*

INTRODUCTION

Polyvinylpolypyrrolidone (PVPP) was introduced commercially as an adsorbent for beer phenolics as long ago as 1961; since then, it has been widely used as an agent for prolonging the stability of beers against haze formation (Dahlstrom and Sfat, 1972; Sfat, 1974; Sfat 1975; Dadic and Lavallee, 1983; McMurrough *et al.*, 1992a, 1993; O'Reilly, 1994). It is now suspected that treatment with PVPP may also have an impact on the flavor stability of beer because of proposals (Irwin *et al.*, 1991) that certain polyphenolic compounds might influence the development of stale, oxidized flavors in stored beer. On the basis of the results of model experiments it was suggested that 3′,4′,5′-trihydroxy flavans might decrease flavor stability by acting as prooxidants; in contrast, 3′,4′-dihydroxy flavans were supposed to act as antioxidants, by protecting certain alcohols and hydroxy fatty acids from oxidation. This implies that beer stabilization protocols might be made even more powerful by the selective removal of pyrogallol-constituted prooxidants in preference to catechol-constituted antioxidants. We have shown in detail (McMurrough *et al.*, 1992a, 1993) that increasing dosage of PVPP progressively extends haze stability. The objective of this study was to ascertain whether commercial PVPP exhibits structure-related selectivity for phenolic compounds that might be of relevance to the flavor stability of beer.

Haze stabilization of beer by treatment with PVPP is always accompanied by decreases in total polyphenols, total flavanols, simple phenolic acids, flavonol glycosides, catechins, proanthocyanidins, and complexes of polyphenols and proteins (Gramshaw, 1967a). Selectivity in the adsorption was noted, albeit cursorily (Weyh *et al.*, 1974), and there have been only a few systematic studies on the adsorption of specific phenolic compounds. A strong correlation was drawn (Vancraenenbroeck *et al.*, 1979) between the degree of PVPP-mediated stabilization and decreases in identified hydroxy flavans (proanthocyanidins). Gas chromatographic analysis of treated beers indicated a pronounced selectivity of adsorption of the proanthocyanidins rather than the catechins. The effect of variable PVPP dosage rate and the adsorption of polyphenols was complex; other workers (Singleton, 1967) proposed that the removal of polyphenols should be proportional to a geometric or Freundlich progression of increased dosage.

Systematic studies in model systems (Quarmby, 1968; Clifford, 1974) indicated that selective adsorption of phenolics depended on their degree of hydroxylation. Mennett and Nakayama (1969) concluded that the selectivity of adsorption of hydroxybenzoic acids by PVPP in very simple model systems was low. Recently, Doner *et al.* (1993) found that binding of flavonoids by PVPP, with a few exceptions, increased with the number of hydroxyl groups on the flavonoid nucleus.

To conduct this study of beer phenolics it was necessary to use a high-performance liquid chromatographic (HPLC) system with direct sample injection capability, so that the adsorption of each of the analytes of interest could be measured simultaneously. This was facilitated by highly selective and sensitive measurements with an electrochemical detector (Madigan *et al.*, 1994; McMurrough *et al.*, 1992a,b, 1993, 1994). Phenolic acids were used to confirm the general dependency of adsorption on hydroxyl group substitution, but selected simple hydroxy flavans were the main objects of attention. The simple flavanols (+)-catechin, procyanidin B3, and prodelphinidin B3 were studied because they are known precursors of beer haze (Gramshaw, 1967b, 1968, 1969; Gardner and McGuinness, 1977); their relevance to flavor stability is that the first two are potentially antioxidants, whereas the latter is potentially a prooxidant.

Our main finding was that two commercial grades of PVPP displayed very strong adsorption characteristics for catechins and proanthocyanidins which, when fitted to classical Freundlich isotherms, did not suggest any usable degree of selectivity between putative pro- and anti-oxidants.

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EXPERIMENTAL PROCEDURES

Reagents and Materials. Methanol was of HPLC grade. Ethanol, ethyl acetate, acetone, and phosphoric acid were of analytical reagent grade. (+)-Catechin and protocatechuic acid (3,4-dihydroxybenzoic acid) were obtained from Sigma Chemical Co., Dorset, U.K. Sodium benzoate, *p*-hydroxybenzoic acid, and gallic acid (3,4,5-trihydroxybenzoic acid) were obtained from BDH Chemical Co., Dorset, U.K. Prodelphinidin B3 and procyanidin B3 were isolated from barley (see procedure below). Deionized water was prepared using an Elga Prima/Maxima system (Elga Ltd., Bucks., England). PVPP (single-use Polyclar 10 and reusable Polyclar Super R) were obtained from International Speciality Products (ISP), Guildford, Surrey, U.K. (Polyclar 10 and Polyclar Super R are registered trademarks of ISP). Dosage rates for PVPP are expressed as grams per hectoliter in accordance with brewing industry practice. The minimum quality of all other chemicals used was reagent grade.

Instrumentation. Two HPLC systems were used as follows:

(1) For the measurement of hydroxybenzoic acids in model solutions, the system consisted of a Waters WISP Model 710B autosampler, a Waters Model 510 pump, and a Waters Model 481 UV spectrophotometer (Millipore U.K., Waters Chromatography Division, Hertfordshire, U.K.). The column was a 25 cm × 4 mm i.d. Nucleosil C_{18} 10 μm (Machery-Nagel, Duren, Germany) used with a Waters Guard-Pak guard column containing Nova-Pak C_{18} packing material. The mobile phase was $CH_3OH/H_2O/H_3PO_4$ (350:640:10 by volume) pumped at a flow rate of 1.0 mL/min. The injection volume was 20 μL and the detection wavelength 228 nm. The run time was 20 min. Calibration of the detector was by external standardization using solutions of hydroxybenzoic acids at concentrations in the range 1−100 mg/L.

(2) For the measurement of flavanols in beer, the system described previously (Madigan *et al.*, 1994) was used. This consisted of a Perkin-Elmer Integral 4000 liquid chromatograph (Perkin-Elmer, Bucks., U.K.). The diode array detector fitted to this instrument was by passed, and the column outlet was connected to a Coulochem II electrochemical detector equipped with a Model 5011 high-sensitivity analytical cell (ESA Analytical, Cambridgeshire, U.K.). This cell contains a porous graphite coulometric electrode connected upstream of a glassy carbon amperometric electrode. The column used was a 30 cm × 4 mm i.d. Nucleosil C_{18} 10 μm (Machery-Nagel) with a Waters Guard-Pak guard column containing Nova-Pak C_{18} packing material. The column was eluted at 1.0 mL/min with a mobile phase gradient as follows: (1) 2.5−10% v/v acetic acid over 60 min, (2) hold at 10% acetic acid for 10 min, (3) return from 10% to 2.5% acetic acid over 5 min, and (4) reequilibrate for 15 min.

The injection volume was 10 μL. The electrochemical detector settings were as follows: channel 1 (coulometric electrode), potential, +350 mV; output range, 5 μA; offset, +5%; polarity, positive; and filter, 2 s, channel 2 (amperometric electrode), potential, −650 mV; output range, 2 μA; offset, +5%; polarity, negative; and filter, 2 s. The detector was calibrated using (+)-catechin from Sigma and authentic prodelphinidin B3 and procyanidin B3 as described previously (Madigan *et al.*, 1994). Protocatechuic acid was used as an internal standard.

Electronic data acquisition and peak integration for both HPLC systems were performed using a Waters Maxima 820 chromatography workstation.

Preparation of Prodelphinidin B3 and Procyanidin B3 Mixture. The procedure described previously (McMurrough *et al.*, 1983) was modified to allow a quick extraction of prodelphinidin B3 and procyanidin B3 (Figure 1). Each of two samples (350 g) of Irish Blenheim variety barley (1993 crop) was ground in a blender for 2 min. The ground samples were both extracted with 1 L of 75% aqueous acetone at 0 °C for 1 h under N_2. The extracts were filtered through sintered glass, and the acetone was removed by rotary evaporation at 30 °C. The aqueous extracts were then extracted with 3 volumes of ethyl acetate. The ethyl acetate was evaporated to dryness, and the solids were redissolved in 10 mL of CH_3OH. This resulted in solutions containing approximately 2 mg/mL each of prodelphinidin B3 and procyanidin B3 and little other UV-absorbing material. The methanolic solution was stored under N_2 at −40 °C until use.

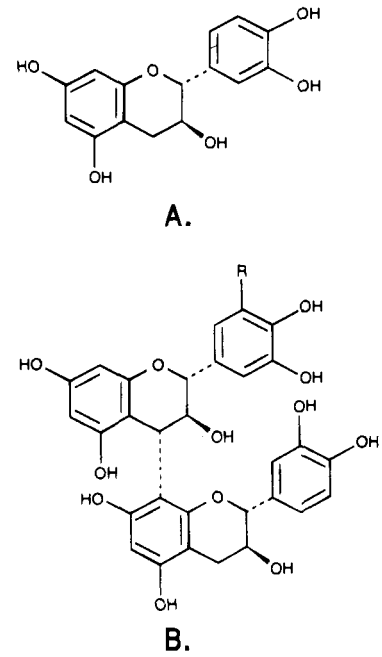

Figure 1. Structures of simple flavanols: (A) (+)-catechin; (B) dimers, prodelphinidin B3 (R = OH), procyanidin B3 (R = H).

Although barley usually contains more prodelphinidin B3 than procyanidin B3 (Madigan *et al.*, 1994), the procedure described above, which did not provide a quantitative extraction, yielded similar amounts of procyanidin B3 and prodelphinidin B3 due to the relatively low solubility of prodelphinidin B3 in ethyl acetate. The proanthocyanidins obtained as described were sufficiently pure for our purpose, as judged by the low level of interferents detected by HPLC.

Sampling of PVPP. For laboratory-scale treatments, representative subsamples of the PVPP powders were taken from bulk (25 kg drums). Because of the dust hazard and the waste associated with coning and quartering such a large amount, representative samples (ca. 100 g) from the top, bottom, center, and three points near the circumference were taken with a core sampler designed originally for the sampling of cereal grains. These samples were then combined and mixed well, and the combined sample (ca. 600 g) was termed "homogenized" PVPP.

Adsorption of Phenolic Acids from a Model System. A mixture of benzoic and hydroxybenzoic acids was prepared, with each acid present at 0.1 mM in 5% ethanol containing 0.1 M KH_2PO_4 adjusted to pH 4.0 with H_3PO_4. Samples (100−500 mL) of these solutions were treated with homogenized Polyclar 10 at 0, 5, 10, 15, 20, 25, 30, 40, 50, 75, 100, 150, 200, 500, and 1000 g/hL. The PVPP was allowed to react for 1 h at 0 °C with stirring. Samples (10 mL) were then centrifuged at 4500*g* for 15 min, and the supernatants were analyzed by HPLC using the method described above.

Adsorption of Flavanols from Beer. A sample (10 L) of unstabilized lager beer was taken from the storage vessel and centrifuged at 4000*g* for 30 min. A subsample (5 L) of this beer was then treated with an excess (approximately 5000 g/hL) of Polyclar 10 for 1 h at 0 °C to produce a "flavanol-free" beer. This beer was centrifuged at 3000*g* for 20 min to remove PVPP. The flavanol-free beer was then spiked with (+)-catechin (Sigma) and prodelphinidin B3 and procyanidin B3 (from barley) at 10 mg/L each. (+)-Catechin was weighed dry and added directly to the beer. Dimeric proanthocyanidins were added as concentrated methanolic solutions prepared as described above. The beer was agitated well to promote dispersal and dissolution of the flavanols. Samples (100−500 mL) of the spiked beer were then stirred with homogenized Polyclar 10 at 0, 5, 10, 15, 20, 25, 30, 40, 50, 75, 100, 150, 200,

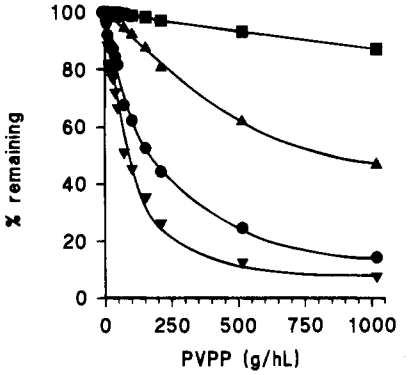

Figure 2. Adsorption of phenolic acids from a model system by Polyclar 10: (■) benzoic acid; (▲) p-hydroxybenzoic acid; (●) 3,4-dihydroxybenzoic acid; (▼) 3,4,5-trihydroxybenzoic acid. The phenolic acids (0.1 mM) were prepared in 5% ethanol containing 0.1 M KH_2PO_4, pH 4.0. Homogenized Polyclar 10 was added and allowed to react for 1 h at 0 °C. Centrifuged samples were analyzed for phenolic acids by HPLC.

500, and 1000 g/hL for 1 h at 0 °C. Samples (10 mL) of the treated beer were centrifuged at 4500*g* and 0 °C for 30 min to remove spent PVPP. The supernatants were analyzed by HPLC with electrochemical detection as described above. The procedure was then repeated with homogenized Polyclar Super R.

RESULTS AND DISCUSSION

Selective Adsorption by PVPP of Hydroxybenzoic Acids from a Model System. The isotherms obtained for the removal of hydroxybenzoic acids from a model mixture by Polyclar 10 are shown in Figure 2. This adsorbent demonstrated greatly increased affinity for compounds with higher degrees of hydroxylation. Benzoic acid was only slightly adsorbed, even at very high PVPP dosage levels; substitution of hydroxyl groups into the aromatic nucleus caused progressive increases in the extent of the adsorption. The affinity of the sorbent for the different acids was dependent on the equilibrium concentrations for each species, so that the complete removal of even the most strongly adsorbed acid was not possible. A similar exponential relationship for the adsorption of total polyphenols in beer has been reported by Narziss (1982).

Adsorption of Simple Flavanols from Beer by Commercial PVPP. To investigate the selective adsorption of specific beer polyphenols, it was necessary to diminish the background of phenolic interferents in the test beer matrix. This was done by treatment of the beer with a large excess of Polyclar 10 (5000 g/hL). The flavanol-free beer produced in this way gave the chromatogram shown in Figure 3A, and addition of pure (+)-catechin and the barley proanthocyanidin mixture gave the chromatogram shown in Figure 3B. The high sensitivity of the detection system toward phenolic compounds (Madigan *et al.*, 1994; McMurrough *et al.*, 1992a,b; McMurrough and Baert, 1994) confirmed that only small amounts of unidentified substances were added adventitiously to the beer in the barley proanthocyanidin mixture.

Treatment of spiked beer samples with various levels of Polyclar 10 and Polyclar Super R produced the isotherms shown in Figures 4 and 5. From these figures it can be seen that Polyclar 10 had a much higher adsorption capacity for flavanols than did Polyclar Super R. This was explicable by the greater particle size of Polyclar Super R, and consequently, lower specific surface area for adsorption (Polyclar Technical Data Sheets, 1994). The results indicated that neither

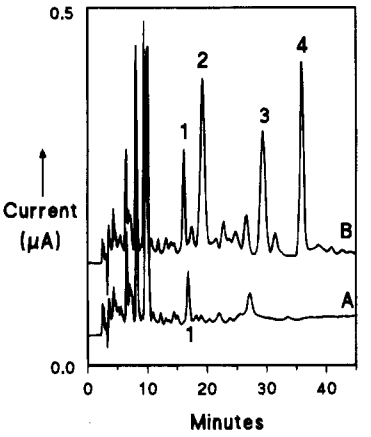

Figure 3. HPLC chromatograms of (A) flavanol-free beer after treatment with excess PVPP and addition of 1.0 mg/L protocatechuic acid (internal standard, peak 1) and (B) the same beer following addition of 2 mg/L protocatechuic acid (internal standard, peak 1) and 10 mg/L each of prodelphinidin B3 (peak 2), procyanidin B3 (peak 3), and (+)-catechin (peak 4). For chromatographic conditions, see text.

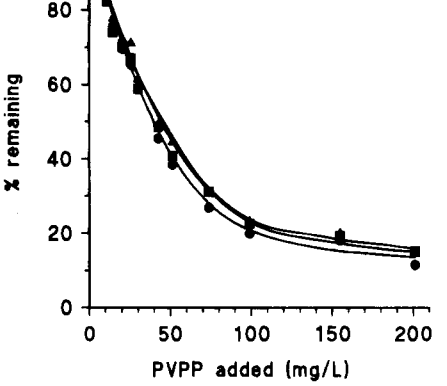

Figure 4. Adsorption of flavanols from flavanol-free beer, spiked with flavanols (10 mg/L), by Polyclar Super R: (●) prodelphinidin B3; (▲) procyanidin B3; (■) (+)-catechin.

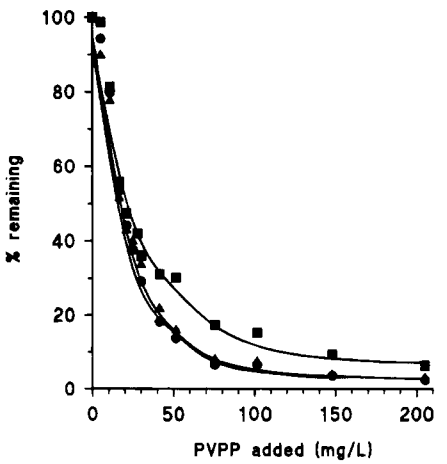

Figure 5. Adsorption of flavanols from flavanol-free beer, spiked with flavanols (10 mg/L), by Polyclar 10: (■) (+)-catechin; (▲) prodelphinidin B3; (●) procyanidin B3.

adsorbent showed significant differentiation between 3′,4′- and 3′,4′,5′-hydroxy-substituted flavanols, though the isotherms for Polyclar 10 were different from those obtained with Polyclar Super R. To quantify the differences displayed in adsorption behavior, the data were plotted in the form of Freundlich isotherms. The general parabolic Freundlich adsorption isotherm can be used as an empirical model of the adsorption of gases

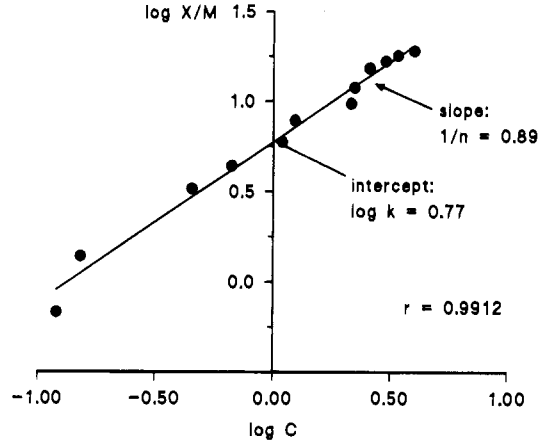

Figure 6. Freundlich isotherm for the adsorption of (+)-catechin from beer by Polyclar 10.

on solids or the adsorption of solutes at low concentrations by a solid sorbent. The Freundlich equation (Freundlich, 1926; Trapnell, 1955) is as follows:

$$x/m = kc^{1/n} \quad (1)$$

where x = mass of solute adsorbed (mg), m = mass of adsorbent (g), c = equilibrium concentration of solute after adsorption (mg/L), k = adsorption value, the amount of solute adsorbed at unit concentration, by unit mass of adsorbent (a constant dependent on temperature, surface area of the adsorbent, and the relative attraction of the solutes in a mixture for the solid surface), and $1/n$ = adsorption exponent (a temperature-dependent constant, characteristic of the particular system being studied and always less than unity when the adsorbent surface is partially covered).

Equation 1 may be written in a linear form as follows:

$$\log x/m = \log k + (1/n)(\log c) \quad (2)$$

From this equation it can be seen that the amount of solute adsorbed in dilute solution is determined by both a concentration-independent term ($\log k$) and a concentration-dependent term ($1/n \log c$).

Figure 6 shows, as one example, the Freundlich plot of $\log x/m$ vs $\log c$ for the adsorption of (+)-catechin on Polyclar 10, and from this were obtained values of 0.77 and 0.89 for $\log k$ and $1/n$, respectively. The values for the constants k and $1/n$ were calculated also for the adsorption of each of the hydroxybenzoic acids (Figure 2) and simple flavanols on Polyclar 10 (Figure 4) and Polyclar Super R (Figure 5) from the corresponding plots. Table 1 compares values of $1/n$ and k for the different hydroxybenzoic acids on Polyclar 10 and for flavanols on both adsorbents. The test for the applicability of the isotherm is the linearity of the double-log plot. It must be stated that few data points were obtained for benzoic acid and *p*-hydroxybenzoic acid, due to the low amounts of these compounds adsorbed, so high correlation coefficients do not necessarily imply better fits to the Freundlich isotherms.

The data reported herein are for solutes in competition for active sites on the sorbent, so solute displacement effects would be expected to have some influence on the isotherms obtained, even though the solutes were present individually at very low concentrations (Freundlich, 1926). This model was designed to mimic the anticipated solute behavior during production-scale beer stabilization.

There was a very obvious increase in the absorbance value, k (Table 1), obtained for Polyclar 10 with increasing hydroxylation of the benzoic acids. The adsorption value increased 10-fold, 34-fold, and 70-fold, respectively, with the introduction of one, two, and three hydroxyl groups per molecule. The adsorption value for (+)-catechin (Figure 1) was considerably greater than would have been predicted solely from its four aromatic hydroxyls per molecule, however, which suggested that structural dispositions were of importance also. The values of k obtained for the flavanoid dimers were significantly greater than that for (+)-catechin (Table 1). As judged by the correlations of data obtained for the dimers, however, their adsorption values were determined with much less accuracy than that for (+)-catechin.

Lower values for k for flavanol adsorption were observed with Polyclar Super R relative to Polyclar 10, and this is at least partially explained by the lower surface area per unit mass of the former sorbent. Freundlich has stated (Freundlich, 1926) that for two sorbents with identical surface characteristics, differing only in specific surface area, the adsorption isotherms should be affine, i.e. one curve should be the product of an ordinate transformation of the other. This would result in parallel isotherms, and hence the constant $1/n$ would be similar for both sorbents. A comparison of values for $1/n$ for Polyclar 10 ($1/n$ = 0.81−0.88) and Polyclar Super R ($1/n$ = 0.60−0.69) suggests a quantitative difference in adsorption behavior for flavanols in addition to that dictated by differences in effective surface area. The two sorbents also showed some differences in selectivity toward the flavanols. Values for k indicated a slight preferential adsorption of the dimers over (+)-catechin by Polyclar 10 but a much less obvious preference for dimers by Polyclar Super R. No consistent preference for the more highly hydroxylated dimer, prodelphinidin B3, over procyanidin B3 was obvious. Unlike the hydroxybenzoic acids, the complex molecular structures of the flavanols might sterically limit the number of hydroxyl groups that effectively participate in the adsorption process. Polyclar Super

Table 1. Determination by Linear Regression of Freundlich Constants (k, $1/n$) for the Adsorption of Phenolic Acids and Beer Flavanols by Commercial PVPP

compound	PVPP grade	k (mg/g)	$1/n$	r^a
benzoic acid	Polyclar 10	0.02	0.78	0.9999 (N^b = 4)
p-hydroxybenzoic acid	Polyclar 10	0.20	0.77	0.9834 (N = 4)
protocatechuic acid	Polyclar 10	0.67	0.91	0.9822 (N = 9)
gallic acid	Polyclar 10	1.4	0.91	0.9894 (N = 10)
prodelphinidin B3	Polyclar 10	9.2	0.85	0.9251 (N = 12)
procyanidin B3	Polyclar 10	10.0	0.81	0.9154 (N = 12)
(+)-catechin B3	Polyclar 10	6.0	0.89	0.9912 (N = 12)
prodelphinidin B3	Polyclar Super R	4.1	0.65	0.9842 (N = 11)
procyanidin B3	Polyclar Super R	3.5	0.60	0.9610 (N = 15)
(+)-catechin	Polyclar Super R	3.3	0.69	0.9830 (N = 11)

[a] r = correlation coefficient. [b] N = number of data points.

R is a regenerable sorbent and is manufactured to a high physical and mechanical stability specification, whereas Polyclar 10 is designed as a single-use disposable product. The observed differences in adsorptive behavior may, therefore, be due to differences in the extent of polymer cross-linking achieved during manufacture of the sorbents.

Conclusions. The adsorption of simple flavanols from beer was characterized by classical Freundlich isotherms over a much wider range of PVPP concentrations (0–1000 g/hL) than is used typically by the brewing industry (i.e. 0–50 g/hL). Polyclar 10 displayed almost twice the adsorptive capacity (k) for simple flavanols as did Polyclar Super R. Moreover, Polyclar 10 exhibited a slight preferential adsorption of dimeric proanthocyanidins in the presence of (+)-catechin, unlike Polyclar Super R. Neither of the adsorbents, however, showed any significant adsorption specificity for 3′,4′,5′-trihydroxy flavanols as compared with 3′,4′-dihydroxy flavanols. Presumably, the high affinity of PVPP for the flavanoid moiety masked any possible discrimination between the extent of aromatic B-ring hydroxylation. As a consequence, treatment of beer with commercial PVPP will effectively decrease the contents of all of these haze precursors and is unlikely to materially alter the concentration ratio of the putative prooxidants to the antioxidants. Further investigation will be required to determine separately the effects of PVPP treatment on flavor stability.

These findings explain, enhance, and expand on the results obtained by Vancraenenbroek *et al.* (1979), which were too few to interpret by classical adsorption rationale. Our results explain how the empirically determined relationship (Sfat, 1975) between haze stabilization and variable PVPP dosage rate—which is always nonlinear—is due to the effects on specific haze precursors. Such operational certainties support a logical approach to procedural optimization based on judicious correction of product composition. Whereas this study was undertaken with the requirements of the brewing industry in mind and was confined to the examination of lager beer, the emergent principles should be more generally applicable to the removal of polyphenols from aqueous extracts of plants.

LITERATURE CITED

Clifford, M. N. The use of poly-N-vinylpyrrolidone as the adsorbent for the chromatographic separation of chlorogenic acids and other phenolic compounds. *J. Chromatogr.* **1974**, *94*, 261–266.

Dadic, M.; Lavallee, J. G. The use of polyclar AT (PVPP) in brewing. *J. Am. Soc. Brew. Chem.* **1983**, *41*, 141–147.

Dahlstrom, R. V.; Sfat, M. The use of polyvinylpyrrolidone in brewing. *Brew. Dig.* **1972**, *47* (2), 75–80.

Doner, L. W.; Bécard, G.; Irwin, P. L. Binding of flavonoids by polyvinylpolypyrrolidone. *J. Agric. Food Chem.* **1993**, *41*, 753–757.

Freundlich, H. The physico-chemical foundations of colloid chemistry, A. Capillary chemistry. In *Colloid and Capillary Chemistry*; Methuen: London, 1926; Sections II–IV, pp 85–238.

Gardner, R. J.; McGuinness, J. D. Complex phenols in brewing—a critical survey. *Tech. Q. Master Brew. Assoc. Am.* **1977**, *14*, 250–261.

Gramshaw, J. W. Phenolic constituents of beer and brewing materials. I. Phenolic and nitrogenous components removed from beer by polyamide resins. *J. Inst. Brew.* **1967a**, *73*, 258–270.

Gramshaw, J. W. Phenolic constituents of beer and brewing materials. II. The role of polyphenols in the formation of non-biological haze. *J. Inst. Brew.* **1967b**, *73*, 455–472.

Gramshaw, J. W. Phenolic constituents of beer and brewing materials III. Simple anthocyanogens from beer. *J. Inst. Brew.* **1968**, *74*, 20–38.

Gramshaw, J. W. Phenolic constituents of beer and brewing materials IV. Further observations on anthocyanogens and catechins as haze precursors in beer. *J. Inst. Brew.* **1969**, *75*, 61–83.

Irwin, A. J.; Barker, R. L.; Pipasts, P. The role of copper, oxygen, and polyphenols in beer flavor instability. *J. Am. Soc. Brew. Chem.*, **1991**, *49*, 140–149.

Madigan, D.; McMurrough, I.; Smyth, M. R. Determination of proanthocyanidins and catechins in beer and barley by high performance liquid chromatography with dual electrode electrochemical detection. *Analyst* **1994**, *119*, 863–868.

McMurrough, I. Chromatographic procedures for measuring polyphenol haze precursors. In *European Brewery Convention, Proceedings of the 17th Congress, Berlin (West), 1979*; SDW: Dordrecht, The Netherlands, 1979; pp 321–324.

McMurrough, I.; Baert, T. Identification of proanthocyanidins in beer and their direct measurement with a dual electrode electrochemical detector. *J. Inst. Brew.* **1994**, *100*, 409–416.

McMurrough, I.; Loughrey, M. J.; Hennigan, G. P. Content of (+)-catechin and proanthocyanidins in barley and malt grain. *J. Sci. Food Agric.* **1983**, *34*, 62–72.

McMurrough, I.; Kelly, R.; Byrne, J.; O'Brien, M. Effect of the removal of sensitive proteins and proanthocyanidins on the colloidal stability of lager beer. *J. Am. Soc. Brew. Chem.* **1992a**, *50*, 67–76.

McMurrough, I.; Madigan, D.; Bogan, D. Measurement of proanthocyanidins and catechins in beer by high performance liquid chromatography using electrochemical detection. In *Proceedings of the 16th International Conference of Group Polyphenols, Lisbon, Portugal*; DTA: Narbonne, France, 1992b; pp 104–107.

McMurrough, I.; Kelly, R.; Madigan, D. Colloidal stabilization of lager beer. In *European Brewery Convention, Proceedings of the 24th Congress, Oslo, 1993*; Oxford University Press: Oxford, U.K., 1993; pp 663–672.

Mennett, R. H.; Nakayama, T. O. M. The adsorption of hydroxybenzoic acids by poly-n-vinyl pyrrolidone. *Am. J. Enol. Vitic.* **1969**, *20*, 169–175.

Narziss, L.; Reicheneder, E.; Freudenstein, L. Beer stabilization and flavor stability. *Monatsschr. Brau.* **1982**, *35*, 218.

O'Reilly, J. P. The use and function of PVPP in beer stabilisation. *Brew. Guardian* **1994**, *123* (9), 32–36.

Polyclar 10 and Polyclar Super R Technical Data Sheets. GAF Chemicals Corp., 1361 Alps Road, Wayne, NJ 07470, 1994.

Quarmby, C. The use of polyvinylpyrrolidone in the thin layer chromatographic separation of flavonoids and related compounds. *J. Chromatogr.* **1968**, *34*, 52.

Sfat, M. Evaluation of polyvinylpolypyrrolidone with American beers. *MBAA Tech. Q.* **1974**, *11* (3), 216–220.

Sfat, M. Recent experience with polyvinylpolypyrrolidone in the stabilization of U. S. beer. *MBAA Tech. Q.* **1975**, *12* (4), 243–248.

Singleton, V. L. Adsorption of natural phenols from beer and wine. *MBAA Tech. Q.* **1967**, *4* (4), 245–253.

Trapnell, B. M. W. *Chemisorption*; Butterworth Scientific Publications: London, 1955; Chapter 5, Adsorption Isotherms, p 109.

Vancraenenbroeck, R.; Kara-Zaitri, M.; Devreux, A. Influence of dimeric proanthocyanidin and catechin content in beer on colloidal stability. In *European Brewery Convention, Proceedings of the 17th Congress, Berlin (West), 1979*; SDW: Dordrecht, The Netherlands, 1979; pp 293–307.

Weyh, V. H.; Postel, W.; Drawert, F. Influence of polyvinylpolypyrrolidone (PVPP) on various properties and constituents of beer. II. Use of Polyclar AT (PVPP) in the brewery. *Brauwelt* **1974**, *16* (1), 295–300.

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