A Comparison of Polyvinylpolypyrrolidone (PVPP), Silica Xerogel and a Polyvinylpyrrolidone (PVP)–Silica Co-Product for Their Ability to Remove Polyphenols from Beer

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ABSTRACT

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Proanthocyanidins have been characterized and levels compared in an ale and a lager using normal-phase liquid chromatography–mass spectroscopy (LC/MS) after a pre-concentration step employing Sephadex LH-20 chromatography. Twenty proanthocyanidins were identified including both non-galloylated and galloylated forms of the monomers, dimers and trimers. Oligomers greater than trimer were not detected. A proportion of all classes of proanthocyanidin adsorbed non-specifically to a silica xerogel product, but polyvinylpolypyrrolidone (PVPP) and a product that comprises polyvinylpyrrolidone (PVP) on a surface of amorphous silica displayed greater capability (i.e. specificity) for binding these materials. Of the two specific adsorbents, it was the PVPP that had the greater binding capability compared to the PVP-silica co-product.

Key words: Beer, high-performance liquid chromatography, mass spectrometry, polyphenolics, polyvinylpolypyrrolidone, proanthocyanidins, PVP-silica composite, silica.

INTRODUCTION

Beer is inherently colloidally unstable and, unless precautions are taken, it will develop hazes, bits, sediments or precipitates[2]. A diversity of chemical species can enter into such insoluble complexes, however the most extensively studied haze systems are those involving hordein-derived polypeptides[1] and polymeric polyphenols[10]. Siebert[14] has developed models to explain the mechanism by which such components interact. Strategies for avoiding the development of this type of haze may either involve a removal of protein, of polyphenol or a proportion of both. Those advocating the removal of protein (which may either be by adsorption on silica hydrogels or xerogels[8], by precipitation with tannic acid[12] or by hydrolysis with papain[4]) claim that this is preferable to eliminating polyphenols, as the latter are important in imparting body to beer[7] and as antioxidants[6]. Those championing the removal of polyphenols, using polyvinylpolypyrrolidone (PVPP[13]), stress that the removal of protein is to jeopardise foam stability.

Recently products have been introduced that are claimed to have enhanced ability to remove polyphenols. These agents comprise PVP dispersed on a surface of amorphous silica[15] and are marketed for polyphenol removal alone – the silica representing a backbone carrier for the PVP moieties rather than as an adsorbing surface for protein.

The primary polyphenolics in beer affecting colloidal stability are the proanthocyanidins or condensed tannins. Proanthocyanidins are polymers of flavan-3-ols or flavanols (Fig. 1). Beer contains primarily procyanidins (polymers of [epi] catechin) and prodelphinidins (derived from [epi] gallocatechin). The most common flavanol–flavanol linkages are C–C bonds [B-type, 4→6 or 4→8], however mixed double linkages can occur [A type, 4→8, 2→7][16]. It has been suggested that these compounds contribute to the astringency, mouthfeel and after-bitterness of beer, however it has also been reported that the level of these molecules in beers, even those not treated with PVPP, is too low to have a material contribution to these properties[9]. Bushnell et al.[3] showed that the removal of polyphenol was without impact on the flavour stability of beer.

On the assumption that the removal of polyphenol from beer should be maximised in the interests of prolonged haze stability, the question is begged of which is the more effective treatment in removing polyphenols: is it the "traditional" PVPP or is it the newer silica bound PVP (hitherto referred to as PVP-silica)? This paper addresses that question.

MATERIALS AND METHODS

Chemicals

Protocatechuic acid was obtained from Sigma (St. Louis, MO, USA). HPLC-grade acetone, methylene chloride, methanol and acetic acid were obtained from Fisher Scientific (Houston, TX, USA). Reagent-grade, bacteria-free water was generated by a Barnstead E-pure 4-module de-

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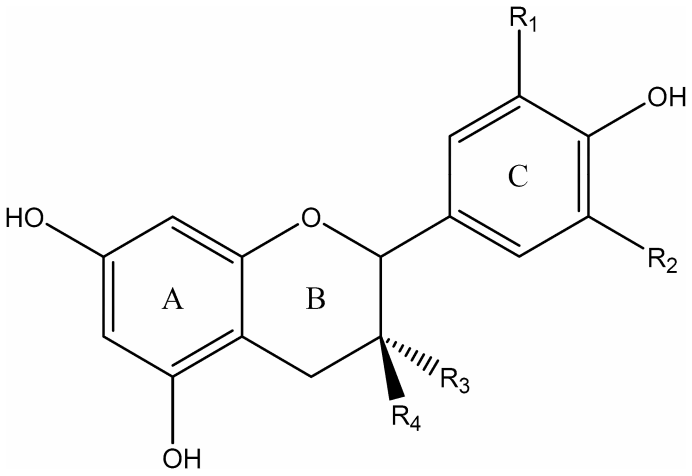

Proanthocyanidin subclass	Flavan-3-ol monomer	R_1	R_2	R_3	R_4
Procyanidin	Catechin	OH	H	H	OH
	Epicatechin	OH	H	OH	H
Prodelphinidin	Gallocatechin	OH	OH	H	OH
	Epigallocatechin	OH	OH	OH	H

Fig. 1. Structure of flavan-3-ol and substitution patterns of proanthocyanidins found in beer.

ionisation system (Dubuque, IA). PVPP (Polyclar 10) was from International Specialty Products, Wayne, New Jersey. PVP-silica (Lucilite TR) was from Brewers Wholesale Supply Inc. (http://www.brewerswholesale.com/brewerswholesale) and manufactured by Ineos. Synthetic silicon dioxide (Crosfield XLC) was from Ineos, Joliet, IL.

Beer treatment

Commercial samples of a lager and ale were obtained fresh from the breweries at the cold conditioning stage and prior to stabilisation and filtration. Dosage rates were selected on the basis of discussion with suppliers and perusal of product information sheets. The beers were routinely treated at 20 ± 1°C with either PVPP, PVP-silica or silica xerogel at three different concentrations (5, 10 and 15 g/hL for the lager; 10, 15 and 20 g/hL for the ale) and for three different contact times (5 min, 10 min and 30 min) prior to filtration through Whatman No. 1 filter paper (cat. no. 1001 110). As stabilisation treatments in breweries are customarily performed at or near 0°C, a comparison of the adsorption performance of PVPP and PVP-silica at 2°C and 20°C was also made. In all experiments treated beer samples were held in sealed containers at 4°C prior to analysis, which was within 7 days of the adsorption treatment. Although special precautions were not taken to rigorously exclude oxygen from the samples, conditions were identical for all samples in respect of time of storage before analysis and volume of sample per container.

Extraction of polyphenols

Degassed beer (100 mL) was measured and spiked with protocatechuic acid as internal standard (500 µL of 75 mg/100 mL in ethanol) prior to extraction. Procatechuic acid was chosen because it did not co-elute with other polyphenolics and it demonstrated a consistent recovery of ~78%. Extractions were performed using Sephadex LH-20 (Amersham Biosciences). The Sephadex LH-20 was equilibrated overnight in water prior to being packed into chromatography columns (25 × 2.5 cm). Samples (100 mL) were loaded onto columns at a rate of 0.5 mL/min. The columns were next rinsed with 500 mL of nanopure water at a rate of 0.5 mL/min. Polyphenols were eluted from the column using a solution (100 mL) of acetone, water and acetic acid (70:29.5:0.5, v/v/v). Acetone was removed by rotary evaporation, and the aqueous solution was freeze-dried and stored at –80°C until LC/MS analysis. All samples were analysed in duplicate.

LC/MS identification and relative quantification of procyanidins

Samples were reconstituted in 500 µL of acetone/water/acetic acid (70:29.5:0.5, v/v/v) and filtered (0.45 µm) prior to LC/MS analysis. The procyanidin oligomers were separated using normal-phase HPLC (Shimadzu Scientific, Columbia, MD) on a Phenomenex (Torrance, CA) 5-µ Luna silica column (25 cm × 2.0 mm). The binary mobile phase consisted of solvent A composed of methylene chloride, methanol, water and acetic acid (82:14:2:2 v/v) and solvent B composed of methanol, water and acetic acid (96:2:2 v/v). Separations were performed by linear gradients of B into A at a flow rate of 0.2 mL/min as follows: time 0–30 min 0–17.6% B; time 30–45 min 17.6–30.7% B; and 45–50 min 30.7–87.8% B. In all cases, the columns were re-equilibrated between injections with the equivalent of 10 mL of the initial mobile phase. The HPLC system was interfaced through an electrospray interface (ESI) to a *ZSPRAY* Micromass Quattro LC (Beverley, MA). Conditions for LC/MS were optimised in (–)-mode ESI against a procyanidin standard extracted from cocoa and include a capillary voltage and a cone voltage of –3.2 kV and –30 V respectively using a source temperature of 150°C and a desolvation gas temperature of 300°C. Post-column addition of 10 mM ammonium acetate at a flow

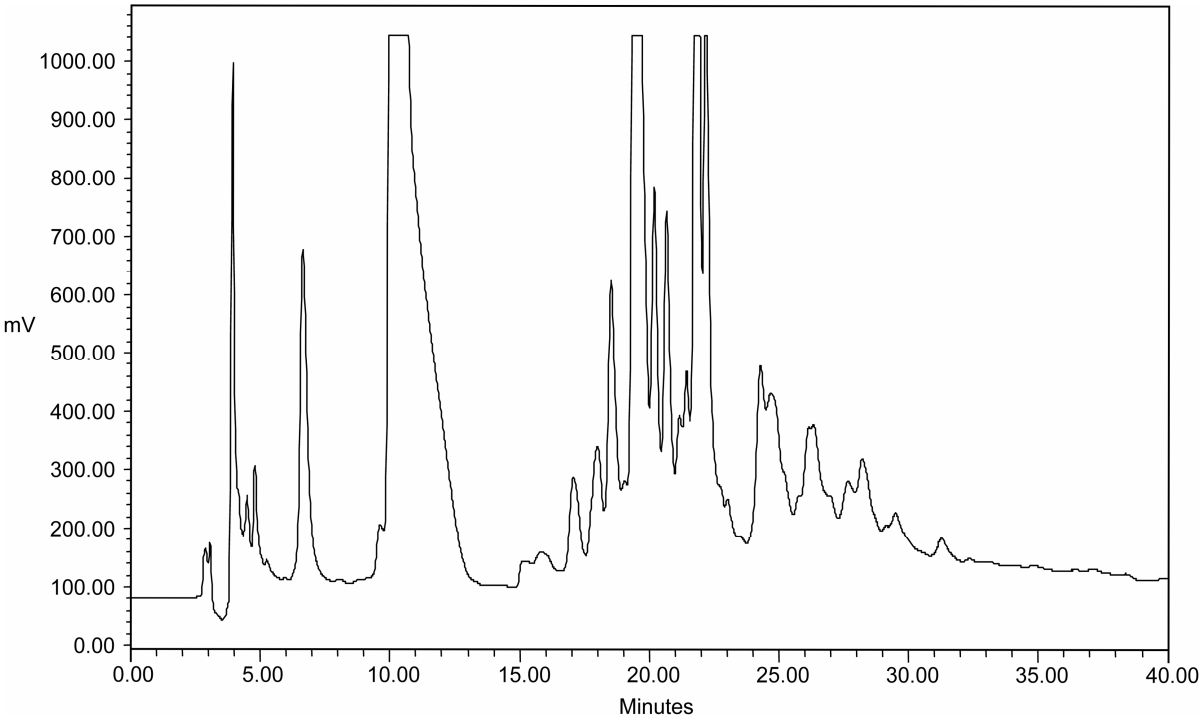

Fig. 2. Representative normal-phase HPLC chromatogram of lager extract monitoring fluorescence at excitation 276 nm and emission 316 nm.

rate of 0.03 mL/min was required for ionisation. Chromatograms were obtained simultaneously using UV-vis at 280 nm (λ_{max} for catechin), fluorescence (excitation 276 nm and emission 316 nm) and mass spectrometry collecting the total ion chromatogram (TIC) from 100 to 3500 m/z. Mass spectra were processed using MassLynx v 3.5. Relative comparisons of the proanthocyanidins in treated beer samples were made by integrating the area under the curve, based upon fluorescence, corresponding to groupings of monomers, dimers and trimers. Values reported are normalised to the untreated beer control and reported as the average of two samples.

RESULTS AND DISCUSSION

LC/MS analysis of beer extracts

Normal-phase HPLC allows for the separation of proanthocyanidins based upon their degree of polymerisation (DP) through decamer, with polymeric forms eluting as a single peak at the end of chromatogram[5]. To date, quantitation of proanthocyanidins by normal-phase HPLC is not possible since galloylated forms can elute in the region of other oligomers, and because galloylated forms give a greater UV-response than non-galloylated forms. In this study the composition of proanthocyanidins in beer extracts was investigated using normal-phase HPLC monitoring fluorescence excitation at 276 nm and emission at 316 nm in conjunction with negative mode mass spectrometry (LC/MS). This approach allows for the identification, and relative comparison, of proanthocyanidins in samples. HPLC chromatograms demonstrate that beer extracts contain proanthocyanidin monomers through trimers (Fig. 2). Mass spectral analysis of these peaks indicates that there are twenty identifiable proanthocyanidins in beer extracts (Table I). The resolution of the dimers and trimers was complicated by the presence of galloylated species; however, the galloylated species did elute in expected regions based on their DP. Epicatechin and catechin and their galloylated counterparts were poorly resolved by this method. MS spectra indicate that there are no proanthocyanidins with DP above tetramer. Results obtained by normal-phase LC/MS compare favourably with those obtained by Whittle et al.[16] using reverse-phase LC/MS analysis.

Table I. Identification of polyphenols.

Retention time (min)	Class	$[M-H]^-$ m/z	Identification
10.21	Monomer	289	EC (epicatechin)
11.58	Monomer	289	C (catechin)
15.21/15.99	Monomer	305	GC/GEC (gallocatechin/ galloepicatechin)
17.68	Dimer	593	G-C (prodelphinidin dimer)
18.01	Dimer	593	G-C (prodelphinidin dimer)
18.23	Dimer	577	C-C (procyanidin dimer)
19.87	Dimer	593	G-C (prodelphinidin dimer)
20.11	Dimer	593	G-C (prodelphinidin dimer)
21.19	Dimer	593	G-C (prodelphinidin dimer)
21.99	Dimer	609	G-G (gallocatechin dimer)
22.00	Dimer	609	G-G (gallocatechin dimer)
22.12	Dimer	609	G-G (gallocatechin dimer)
22.39	Trimer	865	C-C-C (procyanidin trimer)
25.55	Trimer	865	C-C-C (procyanidin trimer)
26.32	Trimer	881	C-C-C (prodelphinidin trimer)
27.85	Trimer	881	G-C-C (prodelphinidin trimer)
28.01	Trimer	881	G-C-C (prodelphinidin trimer)
29.88	Trimer	897	G-G-C (prodelphinidin trimer)
31.52	Trimer	897	G-G-C (prodelphinidin trimer)

Table II. Comparison of proanthocyanidins in lager after adsorption using 3 different contact times and concentrations. Figures indicate (on a percentage basis) the proportion of each oligomer class present in treated beer as compared to the level in untreated control.

		5 g/hL				10 g/hL				15 g/hL			
	T*	Monomer	Dimer	Trimer	Total	Monomer	Dimer	Trimer	Total	Monomer	Dimer	Trimer	Total
Silica	5	92.9	84.7	83.8	87.9	74.7	77.6	74.7	75.9	86.9	87.5	62.4	82.5
	10	79.3	80.3	80.3	79.9	70.1	88.7	92.5	83.4	82.6	88.5	58.9	80.5
	30	82.1	77.0	67.9	77.3	70.1	76.9	91.1	76.9	82.8	76.7	54.3	74.9
PVP-silica	5	84.5	71.5	82.5	76.0	74.3	72.9	69.0	72.7	92.5	86.7	85.6	88.8
	10	94.3	64.7	72.3	78.1	71.8	76.4	60.9	71.6	84.6	84.1	57.0	79.2
	30	86.0	62.5	70.2	73.5	70.3	78.1	67.2	72.9	72.0	73.3	52.5	68.9
PVPP	5	68.8	58.1	43.6	59.7	70.8	54.6	57.7	61.8	77.8	59.5	70.4	69.0
	10	63.2	55.4	43.5	56.3	44.3	34.0	27.9	37.0	57.6	48.1	55.0	53.3
	30	61.0	49.9	35.5	51.7	51.3	37.7	30.9	41.9	53.7	43.5	33.2	45.7

*T: Contact time (min) with adsorbents. "Total" refers to the sum of all polyphenolic species detectable in the method.

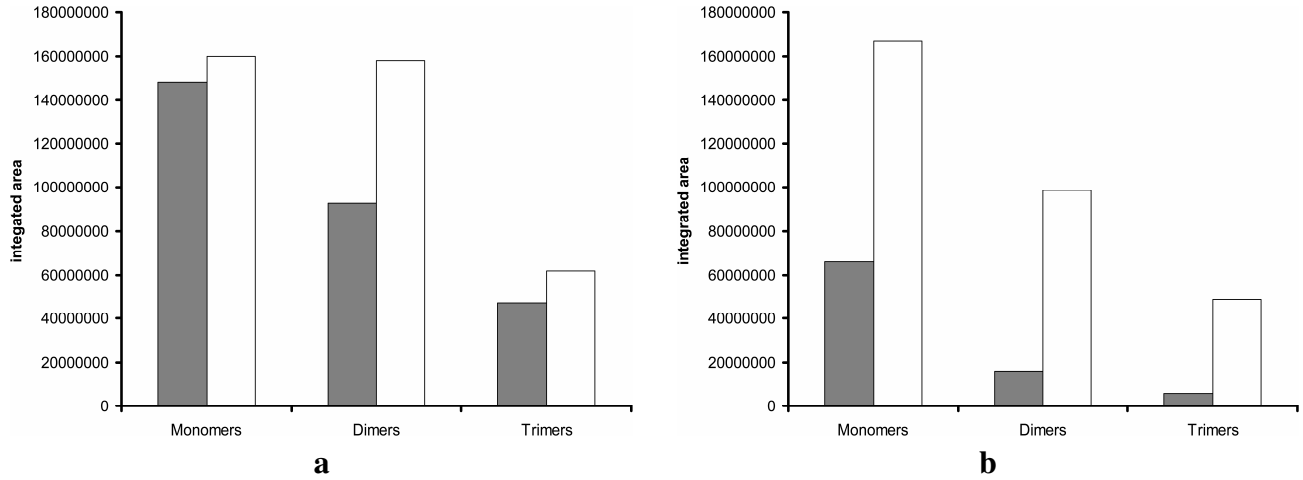

Fig. 3. Comparison of the extent to which PVPP and PVP-silica remove polyphenolics from a lager-style beer at (a) 20°C and (b) 2°C. Treatment was at 15 g/hL agent and for a contact time of 30 min. Values on the *y*-axis indicate the extent of polyphenol remaining in beer after treatment – i.e. the higher is the value the less is the adsorptive capability of the agent. This was a different commercial brand of lager than that examined in Table II. Shaded, PVPP; Open, PVP-silica.

Treatment of beers with adsorbents

Results as shown in Table II indicate that at 5 min contact time with the lager beer, the removal of polyphenolics was most efficient using PVPP (59.7% polyphenol remaining unbound as compared to the control) followed by PVP-silica (76%) and silica (87.9%). Increasing contact times resulted in relatively small increases in polyphenol removal in the case of the PVP-silica composite, though the magnitude was somewhat greater for PVPP. At all treatment times, and concentrations, PVPP was the most efficient in reducing polyphenols whereas silica was the least efficient. The latter was entirely as predicted as this preparation is devoid of the groupings that specifically bind polyphenols. Removal of polyphenol by this agent reflects non-specific adsorption of polyphenolics. This material was included in the study as a control, in view of the fact that silica forms a part of the composite adsorbent. In the 5 and 10 g/hL treatments groups, PVPP and PVP-silica tended to remove a greater proportion of trimers and dimers as compared to silica. This trend was less apparent in the 15 g/hL treatment. Overall, increasing the concentration of adsorbent did not lead to an increased removal of polyphenolics using any of the agents.

The silica tested here may have some structural differences when compared to that involved in the PVP-silica product, however the key thesis is that there can be some non-specific binding of polyphenolic species by silica-based materials. Against this background, the most relevant comparison is that of PVPP and PVP-silica. This being the case, and in view of the fact that commercial stabilisation treatments are customarily effected under colder conditions than those on which we have based the present work, a comparison was made of the adsorptive capabilities of PVPP and PVP-silica at 2°C and 20°C (Fig. 3). Except for monomer removal by PVP-silica, in all instances there was greater removal of polyphenols at the lower temperature. The selfsame preferential binding of trimers > dimers > monomers is observed at both temperatures. However the superior binding capability of PVPP becomes especially marked at the lower temperature, indicating that brewers intent on maximizing removal of polyphenols will achieve this far more readily with PVPP.

Comparison of the data in Table II with that in Fig. 3 (allowing for the fact that different lager beers were used) might suggest that temperature has at least as much sig-

Table III. Comparison of proanthocyanidins in ale after adsorption using 3 different contact times and concentrations. Figures indicate (on a percentage basis) the proportion of each oligomer class present in treated beer as compared to the level in untreated control.

		10 g/hL				15 g/hL				20 g/hL			
	T*	Monomer	Dimer	Trimer	Total	Monomer	Dimer	Trimer	Total	Monomer	Dimer	Trimer	Total
Silica	5	90.6	70.6	92.4	83.3	86.9	67.1	48.6	68.3	86.8	62.9	39.7	64.0
	10	86.7	70.7	68.2	75.2	83.6	74.4	48.6	70.1	89.3	74.5	42.1	70.1
	30	84.4	59.9	49.7	64.9	85.2	60.8	48.3	65.1	78.6	63.3	32.6	59.5
PVP-silica	5	92.8	69.9	78.5	79.7	90.5	59.3	46.0	65.6	89.4	72.1	55.7	73.1
	10	76.6	52.0	71.8	65.6	91.5	74.4	36.1	69.1	92.1	62.6	50.4	68.7
	30	74.7	55.0	68.2	65.1	82.2	55.5	32.5	57.6	85.0	62.2	47.6	65.5
PVPP	5	74.0	51.2	59.0	60.8	55.8	59.1	18.6	46.5	62.6	44.5	33.7	47.3
	10	77.3	46.9	58.1	59.9	61.0	33.2	15.8	37.3	60.3	40.1	19.1	40.7
	30	65.0	43.5	47.3	51.5	69.5	35.1	15.7	40.7	56.8	33.6	13.8	35.5

*T: Contact time (min) with adsorbents. "Total" refers to the sum of all polyphenolic species detectable in the method.

nificance as dosage rate and contact time for the extent of polyphenol binding.

Results as shown in Table III indicate significant trends of polyphenolic removal from the ale based upon both contact times and concentrations. Results indicate that at 5 min contact time, the reduction of polyphenolics was most efficient using PVPP (60.8%) followed by PVP-silica (79.7%) and silica (83.3%) as compared to the control. Increasing contact times (10 g/hL) resulted in only small increases (3–18%) in polyphenol removal, again with the impact being greater for PVPP. Again, PVPP was the most efficient in removing polyphenols. The performance of silica and PVP-silica were comparable, which clearly suggests that the association of the PVP with silica hinders its ability to bind polyphenols. In all treatments groups, the agents tended to remove a greater proportion of trimers and dimers as compared to the monomers. As seen for the lager beer, increasing the concentration of agent within the range investigated did not increase the extent of removal of polyphenolics using any of the adsorbents.

It is important to stress that the treatment rates selected are within the range customarily employed for one-trip PVPP usage[13]. As was emphasized by McMurrough and his co-workers[11], at lower doses of PVPP there is preferred removal of dimers and higher oligomers, with much higher doses needed for the substantive removal of monomers. Within the range of treatment rates examined here we did not identify a firm relationship between dose rate and removal of polyphenols, probably due to the differences being of the same order as inherent variations in the experimental protocol. It would be valuable to examine higher dosage rates beyond those routinely employed, bearing in mind that there are restrictions in some countries concerning how much may be used (e.g. there is a maximum of 50 g PVPP/hL in Germany).

Whilst condensed tannins contribute to the immediate colloidal instability, it is the monomeric polyphenols that contribute to instability after packaging. It has been stressed that it is the dimers such as prodelphinidin and procyanidin in beer that present the biggest risk to immediate haze instability[9]. The present study has demonstrated that, whilst both PVPP and the PVP-silica efficiently reduce the levels of dimers from both lager and ale, it is the PVPP which is the more effective. Thompson et al.[15] also showed that PVPP had the superior ability to adsorb polyphenols, although they claimed that a PVP-silica product was equal to PVPP in its ability to bind "tannoids", the latter assessed nephelometrically by interaction with polyvinylpyrrolidone.

It must be stressed that the present study has been specifically devoted to a comparison of the polyphenol binding capabilities of PVPP and PVP-silica. Strategies for beer stabilization can also involve removal of haze-sensitive polypeptide, with the use of silica-based materials being well studied in that context. A direct comparison of the various stabilizing agents for their efficacy in lengthening the shelf life of beers with different compositions is the subject of ongoing study.

CONCLUSIONS

Beer contains monomeric, dimeric and trimeric proanthocyanidins, but no oligomers with a higher degree of polymerisation could be detected. The higher the degree of polymerisation ($(n = 3) > (n = 2) > (n = 1)$), the greater the tendency of the proanthocyanidins to bind to adsorbents. Of those adsorbents specific for the binding of polyphenols, PVPP displayed better binding capabilities than did a PVP-silica co-product.

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