Mechanisms of Adsorbent Action in Beverage Stabilization

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Unstabilized apple juice (unfermented cider clarified by centrifugation and filtration) was treated with various amounts of bentonite, silica gel, or polyvinylpolypyrrolidone (PVPP). The effectiveness of these adsorbents was assessed by measurements of haze-active (HA) protein and HA polyphenol. Bentonite was very effective in removing HA protein, taking out about ~92% at the highest treatment level, while silica gel removed only ~25%. Bentonite and silica gel had little effect on HA polyphenol. PVPP took out essentially all of the HA polyphenol at the highest treatment level and reduced HA protein somewhat. These results are quite different from those obtained previously with beer but can be explained by the large differences in protein to polyphenol ratio between the two beverages.

Keywords: *Haze; protein; polyphenol; silica gel; bentonite; PVPP*

INTRODUCTION

A concept of protein−polyphenol interactions has been described previously (Siebert et al., 1996b; see Figure 1). Polyphenols bind only to specific sites in haze-active (HA) proteins. These have been shown to be proline residues, and peptides that lack proline are not haze-active (Asano et al., 1982; Siebert et al., 1996b). HA polyphenols have two or more points that can attach to proteins, joining them together. The largest amount of haze forms in situations when there are roughly equal numbers of polyphenol binding sites in the proteins and binding ends of polyphenol molecules (Siebert et al., 1996b). This results in a large network, corresponding to large particles and considerable light scattering. In polyphenol-poor beverages such as beer, in which there is a large excess of HA protein to HA polyphenol (Siebert et al., 1996a), most of the polyphenols are able to bridge two proteins together, but there are few additional polyphenols to link these protein "dimers". This results in small particles and less light scattering. In polyphenol-rich beverages such as apple juice, there is a large excess of HA polyphenol to HA protein (Siebert et al., 1996a) and nearly all of the binding sites on the proteins are occupied by polyphenols. However, few of these can find a free site on another protein to bridge to. This also results in relatively small particles and little haze.

In beer it is necessary to have good foam, and this is associated with a class of proteins different from those that are haze-active (Asano and Hashimoto, 1980; Asano et al., 1982; Lusk et al., 1995; Sørensen et al., 1993). Treatments used for beer stabilization must, therefore, not impair foam performance. Bentonite, a naturally occurring clay that is mainly aluminum silicate, functions as a protein adsorbent. Its use for beer stabilization has been evaluated, but it has generally not been deemed satisfactory because of its negative impact on foam (Coors, 1977). Recent work showed that bentonite is very nonspecific and that it removes foam-active and HA protein equally effectively from beer (Siebert and Lynn, 1997b). This is in agreement with previous studies which have shown that bentonite does not discriminate on the basis of protein molecular size (Sitters, 1988) or isoelectric point (Dawes et al., 1994) or between proteins associated with polyphenols or free (Somers and Ziemelis, 1973), and it even reduces the concentration of free amino acids in wine (Ganeva and Gorinova, 1980).

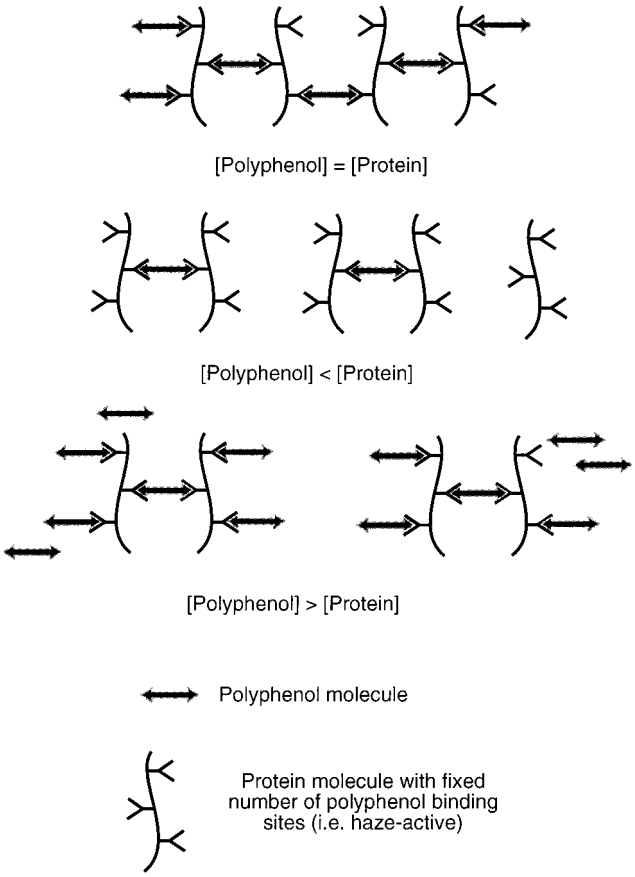

Figure 1. Concept of protein−polyphenol interaction (Siebert et al., 1996b).

Silica gels and sols, on the other hand, are commonly employed to stabilize beer because they have been found to remove HA proteins with little effect on foam (Coors, 1977; Weyh, 1987). This remarkable specificity has generally been explained by the effects of silica particle and pore size; it has been claimed that by manipulating these properties, foam-active proteins can be excluded from much of the surface area, while HA proteins are admitted and bound (Hough and Lovell, 1979). How-

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ever, it was recently demonstrated that silica attaches to the same sites in HA proteins as do HA polyphenols, making it highly specific for HA protein (Siebert and Lynn, 1997b) and better accounting for its minimal effect on foam.

In juice processing and winemaking there is no functional need to preserve any protein and bentonite is widely used, often in combination with gelatin fining (to reduce HA polyphenols) and frequently also silica sol. Bentonite has been used to stabilize a variety of fruit juices (Heatherbell, 1984; Goertges and Haubrich, 1992; Guenther and Junker, 1995), including apple (Heatherbell, 1984; Goertges and Dickmann, 1982; Jepsen et al., 1981; Wucherpfennig and Possmann, 1979), pear (Grampp, 1978; Nagel and Schobinger, 1985), grape (Rentschler, 1969), and guava (Montenegro Brasil et al., 1995), as well as wine (Main and Morris, 1994; Giacomini and Giacomini, 1991; Baldwin, 1992).

Another treatment used for achieving colloidal stabilization of beer is removal of polyphenols by polyvinylpolypyrrolidone (PVPP) adsorption (Hums, 1981); this is used either by itself or in combination with silica gel (McMurrough et al., 1992), typically when particularly long shelf lives are desired. PVPP has, however, somewhat limited effectiveness in beer, removing at most only ~50% of the HA polyphenol (Sibert and Lynn, 1997b). This is thought to occur because most of the beer HA polyphenol is involved in linking two HA protein molecules together (Siebert et al., 1996a; see Figure 1) and is thus unavailable to combine with PVPP. PVPP has also been applied to fruit juices (Gierschner et al., 1982; Heatherbell, 1984; Wucherpfennig and Possmann, 1979), including apple (Hums et al., 1980; Ritter and Dietrich, 1996; Schobinger et al., 1995) and pear (Cornwell and Wrolstad, 1981), and wines (Sims et al., 1995). While PVPP has been shown to remove essentially all polyphenols from apple juice at very high dosage levels (Siebert and Lynn, 1997a), i.e. it can be quite nonspecific, it is considerably more specific at low dosage rates with model systems (McMurrough et al., 1995). It is quite likely that PVPP binds to HA polyphenols in a manner similar to the binding of HA proteins to polyphenols (Siebert and Lynn, 1997b) since PVPP bears great resemblance to polyproline (both have five-member, saturated, nitrogen-containing rings, amide bonds, and no other functional groups), which is one of the most HA peptides known (Asano et al., 1982; Siebert et al., 1996b).

It was of interest to treat apple juice with these adsorbents and compare the results with those obtained previously for beer.

EXPERIMENTAL PROCEDURES

Chemicals and Materials. Tannic acid (TA) was purchased from Mallinckrodt Chemical. TA stock solution was prepared fresh daily by dissolving TA in HPLC grade (deionized, distilled, and filtered) water. Gelatin (bovine, type B, 75 bloom) was purchased from Sigma Chemical (St. Louis, MO).

Cider (cloudy, nonstabilized, unfermented apple juice) was purchased locally and clarified by centrifugation (20 min at 12200*g*), followed by filtration through glass fiber filter circles with vacuum. It was then filtered through Whatman No. 2V followed by Whatman No. 5 filter paper.

Silica gel (Britesorb L 900) was donated by PQ Corp. (Philadelphia, PA). Insoluble PVPP (Polyclar AT) was a gift from International Specialty Products (Wayne, NJ). Bentonite was obtained from Fisher Scientific (Pittsburgh, PA).

Adsorbent Treatments. Clarified, unstabilized apple juice was treated with an adsorbent at a number of addition rates

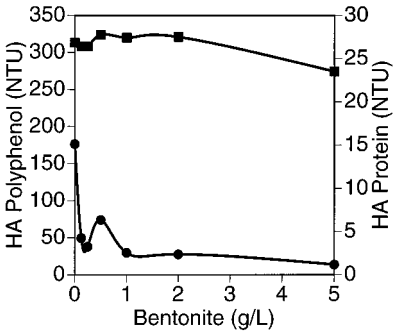

Figure 2. Effect of treating apple juice with bentonite on HA protein (●) and polyphenol (■).

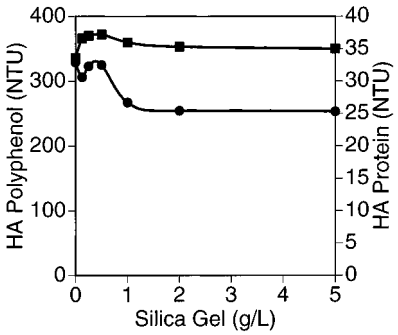

Figure 3. Effect of treating apple juice with silica gel on HA protein (●) and polyphenol (■).

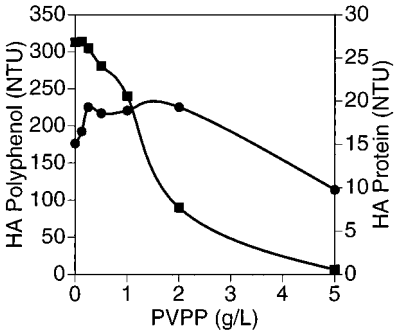

Figure 4. Effect of treating apple juice with PVPP on HA protein (●) and polyphenol (■).

(0, 0.125, 0.25, 0.5, 1, 2, and 5 g/L). The mixtures were stirred for 30 min and then refrigerated overnight. They were filtered through glass fiber circles (Fisher) and then Whatman No. 2 and 5 filter paper with the aid of vacuum. They were then refiltered through Whatman No. 2V and 5 filter paper by gravity.

Determination of HA Protein. Clarified apple juice samples (100 mL) were placed in a 200 mL beaker. TA stock solution was then added to achieve a final concentration of 1.67 g/L. The beaker was then placed in a 25 °C water bath for 30 min to develop haze, which was assessed by light scattering.

Determination of HA Polyphenol. Clarified apple juice samples (100 mL) were placed in 200 mL beakers, and gelatin stock solution was added to achieve a final concentration of 240 mg/L. The beakers were then placed in a 25 °C water bath for 30 min to develop haze, which was assessed by light scattering.

Light Scattering Measurement. Light scattering was observed in 24 mm diameter cuvettes using a Hach Model 2100AN ratio turbidimeter (Hach Co., Loveland, CO). Results were expressed in nephelos turbidity units (NTU).

RESULTS AND DISCUSSION

Treatment with Adsorbents. Cider (cloudy, unfermented apple juice) was obtained and clarified by

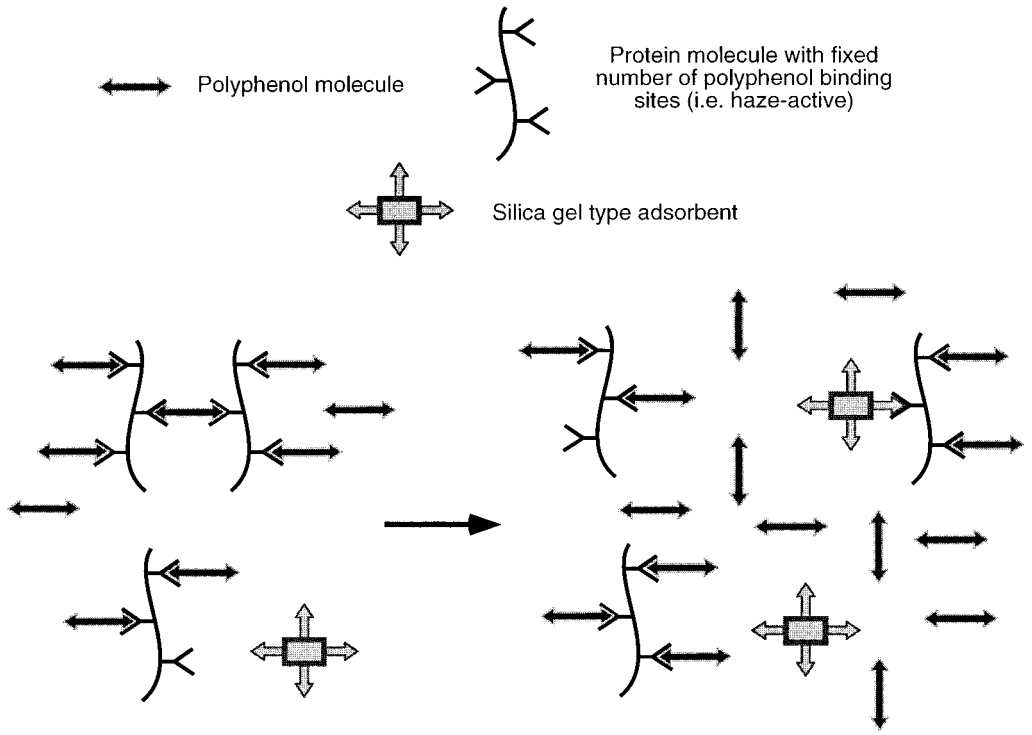

Figure 5. Concept of silica gel adsorption in the high-polyphenol/protein situation in apple juice. Silica gel finds few unoccupied proline sites in the HA protein to which it can bind.

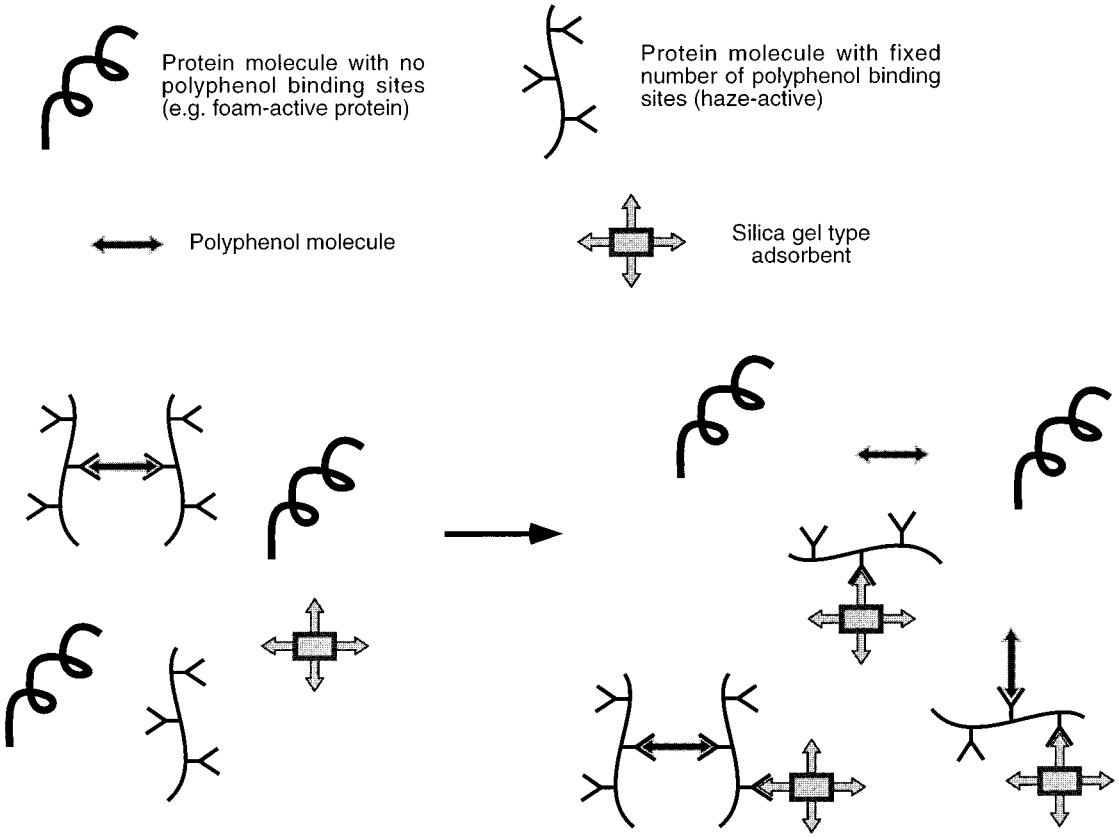

Figure 6. Concept of silica gel adsorption in the high-protein/polyphenol situation in beer. Silica gel binds to the plentiful unoccupied proline residues in HA proteins. It does not bind to proteins lacking proline sites.

centrifugation and filtration. Cider was used so that any HA materials that would have been removed by commercial stabilization treatments would still be present. Clarification was needed to produce a low haze background against which an increase in turbidity in the assessments of HA materials could readily be observed. The clarified cider was separately treated with each of three adsorbents, bentonite, silica gel, and PVPP, at levels ranging from 0 to 5 g/L. The higher end of this range is an excessive treatment level well beyond the range normally employed commercially, but these are the same levels used to treat beer in a previous study (Siebert and Lynn, 1997b). The results are shown in Figures 2−4.

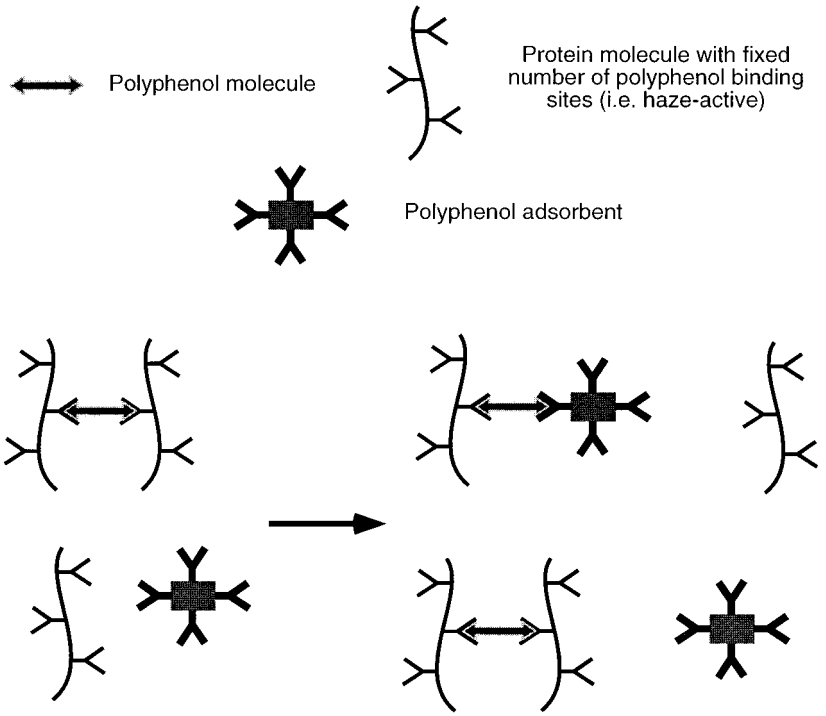

Figure 7. Concept of PVPP adsorption in beer. In this high-protein/polyphenol environment much of the polyphenol is bound at both ends to beer HA protein and not readily accessible for binding to PVPP unless the complex opens up.

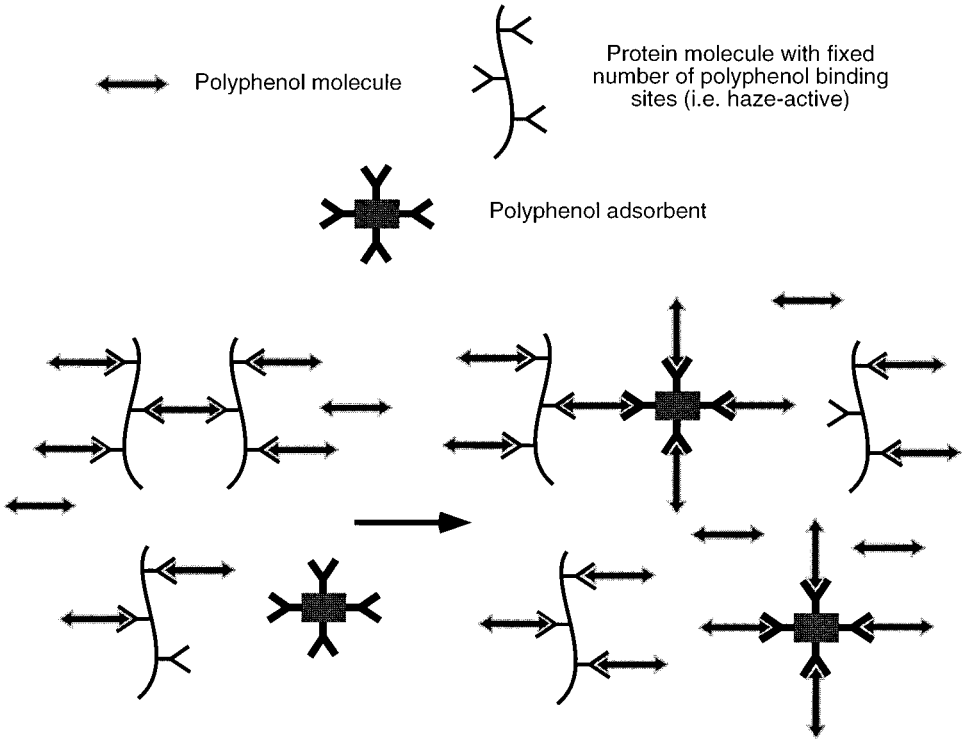

Figure 8. Concept of the situation when apple juice is treated with PVPP. The high polyphenol/protein ratio ensures a plentiful amount of free polyphenol and exposed ends of polyphenol molecules in protein−polyphenol complexes is available for attachment to PVPP.

The HA protein method is based on adding TA, a known HA polyphenol, to the sample. There it combines with just the protein able to bind to it to form a haze that is assessed turbidimetrically (Thompson and Forward, 1969). The HA polyphenol method is analogous (Siebert et al., 1996a). A known HA protein, gelatin, is added to the sample. There it combines with any polyphenol able to bind to it to generate a haze that is assessed turbidimetrically.

Bentonite (Figure 2) was very effective in removing juice HA protein, taking out ~92%. In beer, results of the Bradford method (Bradford, 1976), which is based on Coomassie blue dye binding and which is heavily biased toward proteins rich in basic and aromatic amino acids (Compton and Jones, 1985), provide a reasonable estimate of foam-active protein, which is fairly rich in these amino acids (Siebert and Knudson, 1989). Coomassie blue gives very little response to beer HA protein, which is known to be derived from barley hordein (Asano et al., 1982) and which contains roughly

30% glutamine and 20% proline (Shewry, 1993). It has been shown that neither polyproline nor polyglutamine produces any response with the Bradford method (Siebert and Lynn, 1997b). Bentonite at the highest treatment level removed essentially all of the Coomassie blue responsive and HA protein in beer (Siebert and Lynn, 1997b). Bentonite had very little effect on HA polyphenol in apple juice (see Figure 2).

Silica gel (Figure 3) at the highest treatment levels removed only ~25% of the HA protein from apple juice. This is in contrast to beer, in which it removed ~85% of the HA protein but virtually none of the Coomassie blue response (Siebert and Lynn, 1997b). Since silica gel binds to the polyphenol binding sites of HA proteins, in a polyphenol-rich environment such as apple juice in which most of these sites are occupied by polyphenols, the silica can find few places to attach, making it much less effective (see Figure 5) than it is in a high protein/polyphenol beverage such as beer (see Figure 6). Silica gel had very little effect on HA polyphenol in apple juice (see Figure 3).

PVPP (Figure 4) was quite effective in removing HA polyphenol from apple juice, taking out ~100% at the highest treatment level. This stands in contrast to the situation in beer in which it removed at most only 50% (Siebert and Lynn, 1997b). The explanation for this difference again appears to be due to the major difference in the ratio of protein to polyphenol in the two beverages. In beer, most of the polyphenol is attached to HA protein (see Figure 7) and not readily accessible to the PVPP. In apple juice, however, (see Figure 8) much of the HA polyphenol is free or attached to protein at only one end. As a result it is far more accessible to the adsorbent. The results in Figure 4 show an initial rise in HA protein followed by a small decline with the higher treatment levels. The initial rise is likely due to a shift toward a more even polyphenol/protein ratio (corresponding to larger particles and more haze) as small amounts of polyphenol are removed by the PVPP treatment. The complementary effect was seen with the bentonite and silica gel treatments of beer (Siebert and Lynn, 1997b). The decline in HA protein suggests that PVPP removed protein−polyphenol complexes from apple juice to some extent (see the mechanism in Figure 8).

The results are quite consistent with the usage pattern of commercial beverage producers. Silica gels and PVPP are typically used to stabilize beer; this spares the foam-active protein, and silica gels are quite effective in removing HA protein from beer. Bentonite, which is unspecific but effective in both high- and low-polyphenol environments, is commonly applied to stabilize fruit juices and wine. Another treatment commonly applied to fruit juices is gelatin fining in combination with silica sol. It appears that the silica in this case is effective in removing residual HA protein (particularly excess gelatin); perhaps the silica acts before the binding sites on the gelatin can all be saturated with polyphenols.

Conclusions. Bentonite is quite effective in removing HA protein from apple juice. Silica gel, on the other hand, is much less effective, because the protein sites to which it binds are to a large extent blocked in the polyphenol-rich environment of apple juice. In the low-polyphenol environment of beer, however, silica gel is effective in removing HA protein and spares foam-active protein. PVPP is much more effective in removing HA polyphenols from apple juice than it is in beer. Once again, the explanation lies in the difference in the ratio of protein to polyphenol in the two beverages.

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