Aggregation of Protein and Precipitation by Polyphenol in Mashing[1]

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ABSTRACT

In a grain-out mashing system that contained 0.900 mg/ml of dissolved protein, 0.400–0.500 mg/ml of protein precipitated close to the conversion temperature of 70°C; this precipitation was accompanied by a decrease in total phenols of about 0.100 mg/ml, measured as gallic acid. Protein precipitation in the mash depended on the presence of polyphenols from malt; when polyphenol was partially removed, the amount of protein precipitating at 70°C was substantially reduced. Adding 0.100 mg/ml of gallic acid back to low-polyphenol grain-out mashes caused the mash to again precipitate 0.400 mg/ml protein at 70°C but not below. In low-polyphenol mashes, the mashing process appeared to create protein molecules that were larger than usual. A combination of protein aggregation and high temperature appeared to be necessary for the precipitation of protein by polyphenol during mashing.

Key words: Aggregation, Malt, Mashing, Polyphenol, Precipitation, Protein

The importance of protein–polyphenol reactions in brewing is well known. For example, during kettle boiling and wort cooling, hop and malt tannins react with proteins to form hot and cold breaks, and in finished beer polymerization reactions between proteins and polyphenols cause chill and permanent hazes (4).

Lewis et al (5), using a colorimetric method described by Bradford (2), demonstrated that 0.400–0.500 mg of proteinaceous material per milliliter precipitated during mashing when the temperature was raised from a 30-min protein rest at 45°C to a conversion temperature of 70°C. This reaction was not previously recognized. Lewis and Wahnon (6) used a grain-out mash (in which the insoluble portions of ground malt were filtered out before mashing) to isolate a sample of the precipitate formed at 70°C; it contained protein, carbohydrate, and polyphenol.

Because protein–polyphenol reactions are so important in beer stability, we thought that further study of such reactions might be beneficial, especially if this reaction could be made to occur more completely. By lowering the levels of reactive proteins and polyphenols in wort during mashing, lower levels should then appear in beer and be unavailable to adversely influence beer stability. The purpose of this work was to show that polyphenols are necessary for the precipitation of protein during mashing.

EXPERIMENTAL

Assays for Protein and Phenols

A colorimetric method for the determination of dissolved protein in wort and beer described by Lewis et al (5) was used in this study. The method is based on the binding of Coomassie brilliant blue G-250 to protein in acidic solution, causing a spectral shift that can be measured at 595 nm (2). Bovine serum albumin was used to produce standard curves. A method described by Singleton and Slinkard (7) was used to measure total phenols. This is a colorimetric method that uses phosphomolybdicphosphotungstic acid (Folin Ciocalteau) reagent. Gallic acid was used to create a standard curve.

Raw Materials and Equipment

A commercial two-row malt was used for all the experiments described. All mashes used deionized water with an 8:1 ratio of water to malt in a six-cup laboratory-scale mashing apparatus.

Spectrophotometric analysis was done with a Bausch and Lomb Spectronic 20. Wort haze was measured with a Radiometer UKMID haze meter.

Wort samples were applied to Sephadex G-100 columns that were eluted with 0.1 *M* phosphate buffer, pH 5.5, containing 0.02% sodium azide (as bacteriostat).

Grain-Out Mashing

Fifty grams of malt, finely ground according to ASBC Methods of Analysis MALT-4, (1) was mixed with 400 ml of deionized water at room temperature (22°C). The mixture was stirred every 10 min with a glass rod. After 30 min, the contents of the beaker were filtered through S&S no. 597 32-cm paper to obtain 300 ml of clear filtrate. The phrase "before mashing" refers to samples or actions taken at this time.

These extracts were mashed according to the following program and are referred to here as grain-out mashes: 30 min at 45°C, raised to 70°C in 15 min and held 30 min, and finally raised to 75°C and held 5 min. The phrase "after mashing" refers to samples or actions taken at this time. Samples (5 ml) were also taken at suitable intervals during mashing for analysis of protein and phenols.

Low-Polyphenol Mashing

Polyphenol was removed from grain-out extract by adding polyvinylpyrolidone (PVP) in various amounts and filtering. This was done at room temperature before mashing.

Low-polyphenol mash was produced by screening coarse-ground malt with a no. 30 screen to produce for mashing 50 g of malt flour with little husk. Alternatively, malt was washed 30 min with 0.01 *N* NaOH to reduce husk polyphenol, drained, washed in water, and dried in a vacuum oven before use. In this case, wort pH was 0.1–0.2 units higher than usual.

Mash Additions

Polyphenol was added back to PVP-treated grain-out mashes at room temperature and before mashing, in the form of gallic acid (0.100 mg/ml). Also, methanol washings of PVP recovered from treatment of wort were added to PVP-treated mashes. Finally, portions of untreated grain-out extract were added to PVP-treated ones.

Hydrogen peroxide, with or without peroxidase, was added at 100 mg/L to grain-out mashes at room temperature, before mashing, and samples were taken during the mash at suitable

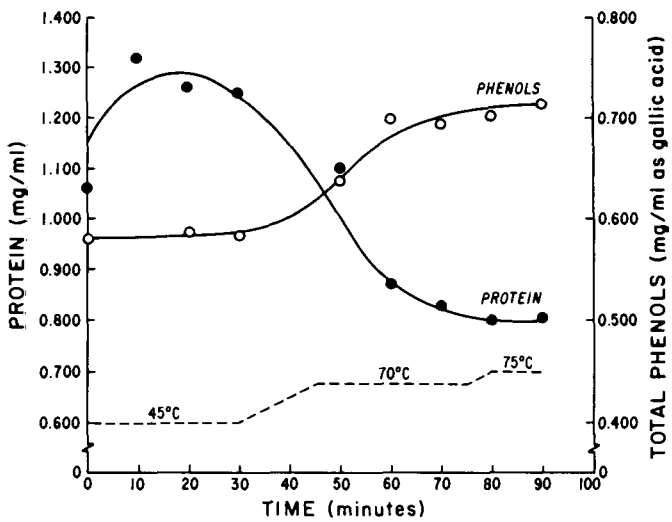

Fig. 1. Solution of protein and total phenols during an ASBC mash.

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intervals. Alternatively, 100 mg of potassium metabisulphite per liter was added.

RESULTS

Proteins and Phenols During Mashing

During the initial stages of mashing finely ground malt at 45°C, protein dissolved rapidly to a maximum concentration of 1.30 mg/ml (Fig. 1). As the mash temperature was raised, the dissolved protein decreased to about 0.800 mg/ml. In contrast, total phenols increased with higher mash temperature, presumably because of the more efficient solution of polyphenols from the husk materials.

In a grain-out mash, in contrast, the amount of dissolved material present was at a maximum before the application of heat; data therefore are not confounded by continuous leaching of material from the spent grain. Also, the formation of protein flocs was visible (6,8). The grain-out mash, therefore, has advantages in some applications. During a grain-out mash, protein decreased from 0.950 mg/ml to 0.500 mg/ml, and total phenols decreased from 0.350 mg/ml to 0.280 mg/ml (Fig. 2). As Wahnon (8) and Lewis and Wahnon (6) observed, in such mashes, a nonfilterable wort haze formed as mash temperature was raised; this haze flocculated and became readily filterable at 70°C (Fig. 3, right).

Treatment for Polyphenol Reduction

Treatment of a grain-out extract before mashing with 0.09 kg/L of PVP removed approximately 0.100 mg/ml of total phenol and caused the behavior of proteins and phenols to change from that shown in Fig. 2. At the end of mashing, protein concentration remained about twice as high (0.800 mg/ml) in PVP-treated worts as in untreated ones, and phenol concentration did not decrease but remained almost constant at about 0.300 mg/ml. Also, the haze that formed when the temperature was raised never flocculated but remained unfilterable throughout mashing in the PVP-treated extract (Fig. 3, left).

Figure 4 shows the concentration of protein during mashing of malt flour that contained comparatively little husk. After 90 min of mashing, the dissolved protein remained 0.350 mg/ml higher in the mash deficient in husk material than in the mash containing husk. Similar results were obtained when malt was washed with NaOH, to remove polyphenol, before use in mashing.

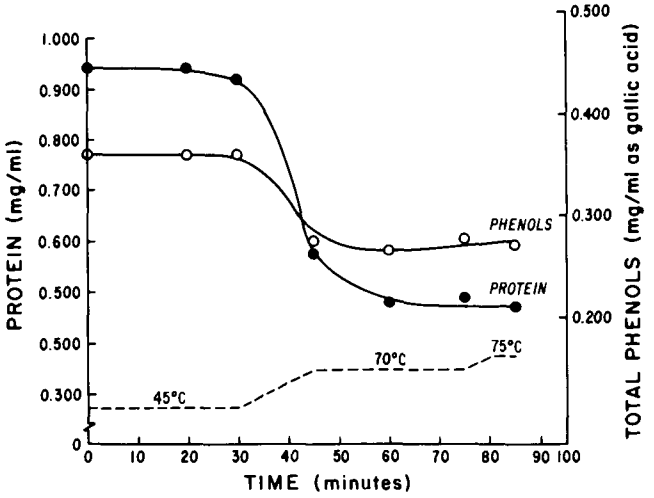

Fig. 2. Precipitation of protein and total phenols during mashing of a grain-out extract.

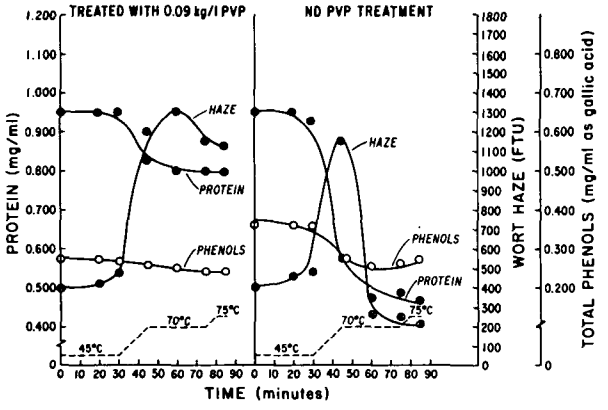

Fig. 3. Precipitation of protein, total phenols, and the appearance and flocculation of haze materials in a grain-out mash. **Right,** control; **left,** grain-out extract treated with PVP 0.09 kg/L before mashing.

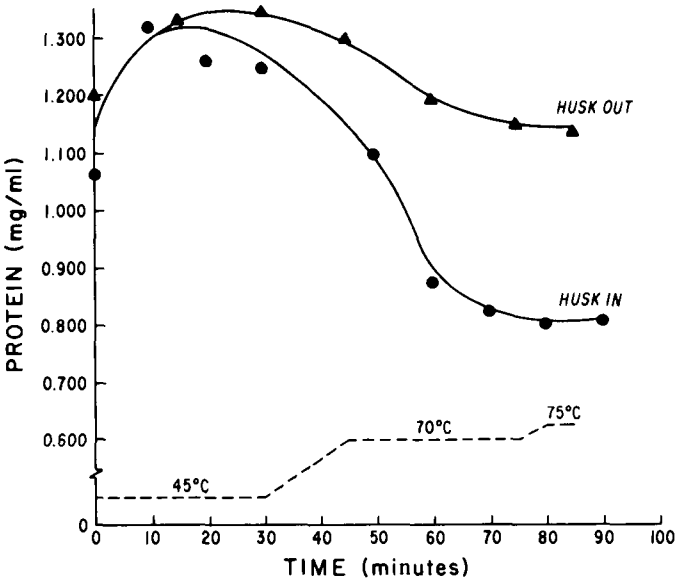

Fig. 4. Solution and precipitation of protein in an ASBC mash in the presence or substantial absence of malt husk.

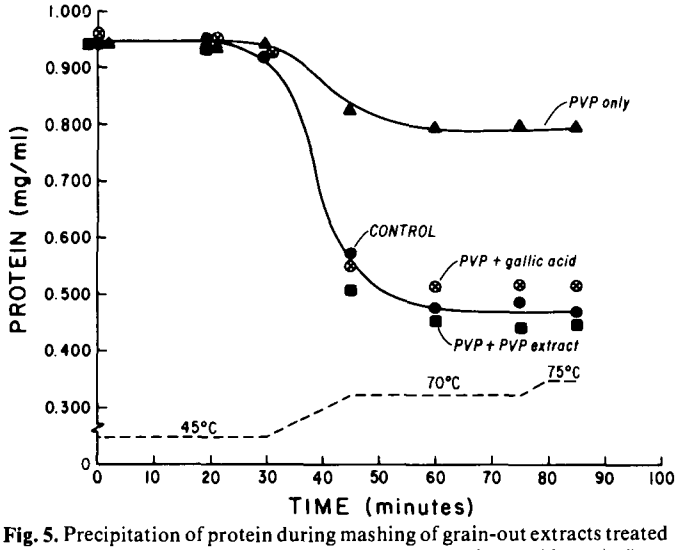

Fig. 5. Precipitation of protein during mashing of grain-out extracts treated with PVP before mashing (▲) to which gallic acid (0.100 mg/ml) or methanol extract of the PVP used in treatment of the extract (■) have been added. The control (●) was not treated with PVP or in any other manner.

Addition of Polyphenol

Adding 0.100 mg/ml of gallic acid to PVP-treated grain-out extract, before mashing, caused protein to precipitate at 70°C as completely as in the control (Fig. 5). The methanol extract of PVP recovered from the PVP treatment of extract also caused protein to precipitate as completely as in the control (Fig. 5). When a 10% volume of untreated grain-out extract was added to the PVP-treated extract, protein precipitation equal to the control was again observed (Fig. 6). These additions, before or after mashing, did not cause protein precipitation at room temperature.

Oxidized and Reduced Mashes

In a grain-out extract to which 100 mg/L of hydrogen perioxide was added before mashing, the amount of protein and phenol precipitating was considerably reduced compared to that in the control; total phenols did not change in concentration during mashing, and the haze formed at 70°C remained unfilterable. Protein was even less precipitated when H_2O_2 and peroxidase were added immediately before mashing (Fig. 7).

In contrast, a reduced mash (containing 100 mg/L potassium metabisulphite) precipitated 0.150 mg/ml (33%) more protein than the control (Fig. 8). Total phenol concentration in wort after the test mash was slightly less than that in the control.

Sephadex Chromatography in Wort Proteins

The protein elution profiles of grain-out worts before and after mashing, as separated on Sephadex G-100, are shown in Fig. 9. Before mashing, the control and PVP-treated samples were identical. After mashing, there was less total protein in the control extract than in the PVP-treated extract, as expected (Fig. 3), and

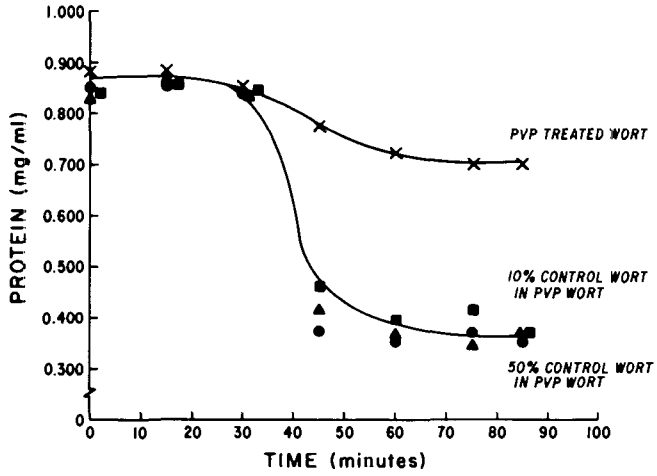

Fig. 6. Protein precipitation during mashing of grain-out extracts treated with PVP before mashing (x) to which a 50% volume (▲) or 10% volume (■) of untreated (control) extract was added. Control extract (•) was not treated in any way.

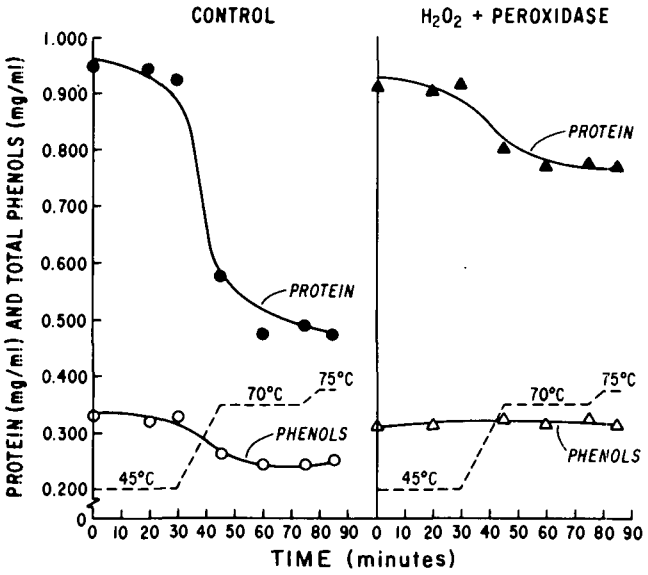

Fig. 7. Precipitation of protein and total phenols during mashing of grain-out extract to which **(right)** hydrogen peroxide (100 mg/L) and peroxidase were added before mashing.

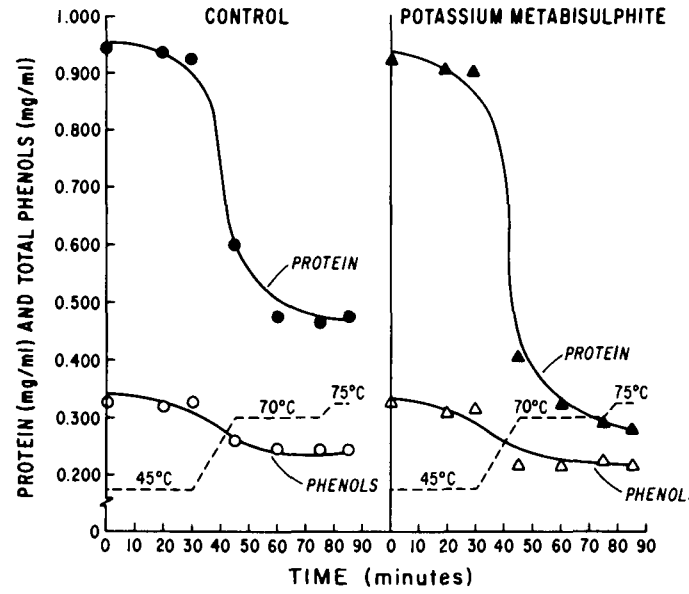

Fig. 8. Precipitation of protein and total phenols during mashing of grain-out extracts to which **(right)** potassium metabisulphite (100 mg/L) was added.

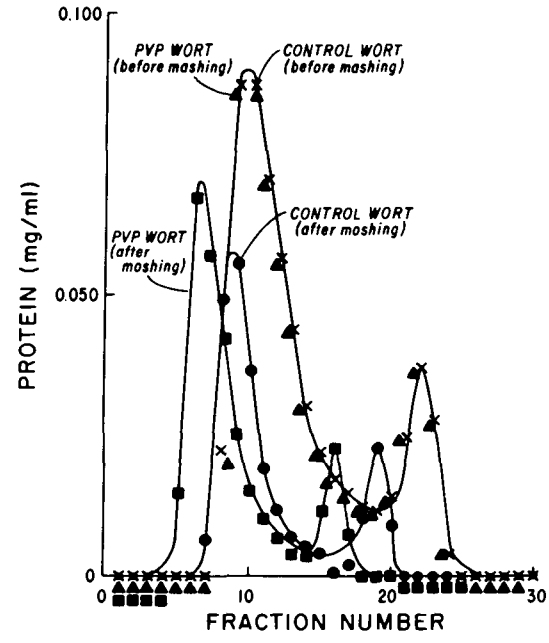

Fig. 9. Separation of protein on Sephadex G-100 from grain-out extract before mashing, which was untreated (x) or treated with PVP (0.09 kg/L) (▲). After mashing, the same comparison between untreated (•) and PVP-treated extract is made (■).

the protein of the PVP-treated sample eluted earlier from the column, indicating an increase in molecular weight in this case.

DISCUSSION

During a temperature-programmed mash, protein precipitated as the temperature was raised (6); our present observations confirm that although heat is the catalyst for this precipitation reaction, two other conditions must exist: the aggregation of proteins and the presence of polyphenol.

Removal of polyphenol from the mash either by adsorption with PVP, or by removing (or otherwise treating) the husk, which contains most of the polyphenol in malt (3), prevented the typical precipitation of protein (Figs. 3, 4). The effects of polyphenol removal can be reversed by adding back a source of polyphenol. Although this study did not explore the type of polyphenol involved, our observations indicate that it must be similar in reactivity to gallic acid, which caused protein to precipitate in a PVP-treated mash (Fig. 5). There appears to be more than an adequate supply of this material in wort, because only 10% volume of untreated extract added to PVP-treated extract promoted normal protein precipitation.

During mashing, as the temperature was increased, there was an aggregation of particles that eventually became too large to remain soluble and formed a visible colloidal haze (Fig. 3). At 70°C, the control mashing precipated these aggregates as they grew too large to stay in suspension. Polyphenols in solution simultaneously decreased. In PVP-treated mashes, however, the final step in this aggregation reaction (ie, precipitation or flocculation) was not completed, and a strong haze persisted in the wort. This haze material was not precipitated after cooling to room temperature by any source of polyphenol tested. Polyphenols thus appear to react with proteins in mashing only at comparatively high temperature and only after substantial aggregation has occurred. Protein aggregation to form haze in grain-out mashes is the result of the well-known heat denaturation (unfolding) of proteins; this is not necessarily associated with polyphenol reactions, because such aggregation occurs equally well in PVP-treated extracts. That polyphenol reacts with the protein aggregates after they reach a certain size is suggested because in the absence of reactive polyphenols, the protein aggregates in solution can grow larger than usual. On Sephadex G-100, these large molecules eluted with the solvent front, indicating (in PVP-treated extract) the presence of larger molecular weight protein aggregates than in the control. We had thus created in the absence of polyphenol some protein aggregates with a higher molecular weight than are normally present in wort. This observation may even raise questions about whether protein aggregation is a prelude to protein-polyphenol reactions in beer to form chill haze.

Alteration of the oxidation-reduction state of a grain-out mash affected the amount of dissolved protein present after mash-off. An oxidized environment caused the protein and polyphenol to remain in solution (Fig. 7), while the reduced mash precipitated more protein and phenols (Fig. 8). During kettle boiling, the more reduced environment produces more trub, whereas an oxidized one produces less (3), and we seem to have uncovered a similar situation during mashing. This, however, was not entirely expected because we anticipated that oxidative polymerization of phenols would promote protein–polyphenol reaction and hence protein precipitation. Although we may not have expected an increase in precipitation caused by this treatment, we did not expect less precipitation. This suggests that the reactive species of phenols may be of comparatively low molecular weight, a suggestion supported by the relatively large amount of PVP required to show the phenomenon of Fig. 3.

Although polyphenols are partly responsible for the formation of beer haze, removing them too soon in the process may be detrimental to beer stability; without polyphenols in the mash (or in the kettle boil), more protein might remain soluble and be carried through to the finished product. This might occur when a low-polyphenol barley is used in brewing or when adjuncts are used that contain protein but no husk.

Further research might look at the types of polyphenols that enhance protein precipitation and whether or not a more complete precipitation of protein–polyphenol during mashing could produce a beer with enhanced physical stability.

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