Isolation and Partial Characterization of a Natural Haze Protective Factor from Wine

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A haze protective factor (HPF) reduced the visible haziness induced by heating wine proteins in wine, by decreasing the particle size of the haze. The relationship between the decrease in particle size and the concentration of the HPF was exponential. The HPF, which was detectable by SDS-PAGE with the periodic acid-Schiff reagent stain and was evident as a smear in the high M_r region of the gel, was separated from other macromolecules of wine by a combination of concanavalin A and anion-exchange and cation-exchange chromatography. The macromolecule was made up of a polysaccharide component (96%) that was dominated by mannose (78%) and glucose (13%) and a protein component (4%) that was dominated by serine (31%) and threonine (13%).

INTRODUCTION

Clarity is of vital importance to wine quality because this property makes the first impression on the consumer. A major cause of white wine turbidity is the instability of the grape proteins that occur naturally in wine. Protein haze in wine is due to the denaturation of grape proteins leading to their aggregation. The aggregated protein can subsequently precipitate to give an amorphous sediment or flocculate and thus produce a suspended and unattractive haze.

In a recent study of fractionated wine macromolecules, we demonstrated that a polysaccharide-rich fraction apparently protected some wine proteins against heat-induced haze formation (Waters et al., 1991). The polysaccharide-rich fraction was isolated from wine by ammonium sulfate fractionation and ultrafiltration. When heated in wine, the polysaccharide-rich fraction gave little turbidity, and this response was observed regardless of whether the polysaccharide-rich material was present in isolation or together with the proteins which normally accompanied it in wine. This was significant because the wine proteins, in the absence of the polysaccharide-rich fraction, were capable of producing a substantial amount of haze. Thus, the components in the polysaccharide-rich fraction had a stabilizing effect on the proteins, protecting them from hazing.

This study describes the isolation of this haze protective polysaccharide fraction and investigates its properties and the mechanism of its action in decreasing visible haze. Recognition of the presence and the properties of this natural polysaccharide fraction as a haze protective factor (HPF) opens up a totally new method for avoiding protein haze spoilage in winemaking.

MATERIALS AND METHODS

Materials. The Muscat of Alexandria (syn. Muscat Gordo Blanco or Gordo) wines used in this study were described previously (Waters et al., 1992). Chromatography gels and columns were purchased from Pharmacia Australia Pty. Ltd. Water used was purified by a Milli-Q reagent water system (Millipore Pty. Ltd.). All other chemicals were of reagent grade.

Ultrafiltration. Samples were ultrafiltered as previously described (Waters et al., 1992).

Micromethod for the Measurement of Heat-Induced Haze. The effect of polysaccharide additions on the haze potential of proteins was determined according to the micromethod described by Waters et al. (1991), modified in the following manner. To a constant, known amount of protein [5 μL of either wine protein precipitated by 0–70% saturation of $(NH_4)_2SO_4$ or bovine serum albumin (BSA)] were added aqueous solutions of polysaccharide-rich fractions (0–50 μL made up to 50 μL with water) to ultrafiltered wine (1050 μL). After sparging with nitrogen, samples were sealed and heated for 6 h at 80 °C followed by 16 h at 4 °C using the procedure of Pocock and Rankine (1973). The induced haze was measured by the absorbance at 540 nm. Values were corrected by subtraction of A_{540} for a control (no protein or polysaccharide added before heat testing). Data were subjected to linear regression analysis. The protein concentration of BSA was determined spectrophotometrically using the published extinction coefficient of 0.663 L g^{-1} cm^{-1} (Gill and von Hippel, 1989), while the protein concentration of wine proteins was determined from the sum of the masses of the amino acids generated by acid hydrolysis in hermetically sealed microcapillary tubes (Waters et al., 1991).

Spectrophotometry. Normal and derivative spectrophotometric measurements in the range 250–300 nm were made with a Varian DMS 200 instrument.

Particle Size Measurement. The particle size of hazes was measured by photon correlation spectroscopy using a Malvern Autosizer IIc (Malvern Instruments). Samples (4 mL) were analyzed at 25 °C after vigorous shaking to disperse the flocculated haze.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS–PAGE). Discontinuous SDS–PAGE was performed according to the method of Laemmli (1970). Proteins were stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 in 10% (v/v) acetic acid and 40% (v/v) methanol and destained in 10% (v/v) acetic acid and 40% (v/v) methanol. Polysaccharide material was detected with the periodic acid-Schiff reagent (PAS) stain procedure of Allen et al. (1976). M_r values of unknown samples were estimated from the linear regression equation of log M_r vs mobility of molecular weight standards (Bio-Rad Laboratories Pty. Ltd.).

Polysaccharide Quantification. The total neutral carbohydrate content of samples was estimated by the resorcinol assay (Monsigny et al., 1988). For the analysis of sugar composition the samples, containing the internal standard *myo*-inositol, were hydrolyzed in 2 M trifluoroacetic acid at 110 °C for 6 h. After removal of the acid, the sugars were converted to their tri-

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methylsilylates with TriSil reagent (Pierce) and then separated by gas chromatography on a BP1 fused silica capillary column (20 m × 0.33 mm i.d., 0.25-μm film thickness, SGE International Pty. Ltd.). The relative amount of the component sugars was determined by comparing their peak areas. The amino sugars, galactosamine and glucosamine, coeluted, and they are reported as "hexosamine".

Purification of the Haze Protective Factor (HPF). The following chromatographic steps were performed at room temperature. All samples were diluted 10-fold in the appropriate buffer and centrifuged (10000*g*, 5 min) before loading onto the columns. Protein was detected by monitoring the eluant at A_{280}. Anion- and cation-exchange chromatographies were performed on a medium-pressure Pharmacia FPLC system.

(*a*) *Concanavalin A Affinity Chromatography.* Concanavalin A–Sepharose was packed into a 70 mm × 15 mm column (volume approximately 7 mL) and equilibrated with 50 mM sodium acetate–HCl buffer (pH 5.6) containing 150 mM NaCl and 1 mM each of $CaCl_2$, $MgCl_2$, and $MnCl_2$ (starting buffer). Elution of bound material was achieved by addition of 500 mM methyl α-D-mannoside to the starting buffer. For preparative separations, the unretained fractions were rechromatographed until no more material eluted with the elution buffer. The retained fractions from these repetitive runs were pooled and desalted by ultrafiltration.

(*b*) *Anion-Exchange Chromatography.* Samples were loaded onto a Mono-Q (HR 5/5) column which had been equilibrated with 20 mM piperazine–HCl buffer (pH 5.5). The NaCl concentration was increased from 0 to 60 mM over a period of 3 min and held at that concentration until the absorbance of the eluant decreased to a constant value. The NaCl concentration gradient was subsequently increased in two steps, (a) from 60 to 250 mM over a period of 10 min and (b) from 250 mM to 1.0 M over 1 min. At the end of each of these steps the NaCl concentration was held at the higher level until the absorbance decreased to a constant value.

(*c*) *Cation-Exchange Chromatography.* Samples were loaded onto a Mono-S (HR 5/5) column which had been equilibrated with 20 mM sodium citrate buffer (pH 3.0). The NaCl concentration was increased from 0 to 50 mM over a period of 2 min and held at this concentration until the absorbance decreased to a constant value. The NaCl gradient concentration was subsequently increased in three steps, (a) from 50 to 120 mM in 4 min, (b) from 120 to 150 mM over a period of 2 min, and (c) from 150 mM to 1.0 M over a period of 1 min. At the end of each of these steps the NaCl concentration was held at the higher level until the absorbance decreased to a constant value.

RESULTS

Investigation of the Mechanism of Action of the Crude HPF. (*a*) *Effect on the Haze Potential of Wine Proteins.* In a previous study (Waters et al., 1991), the effect of a polysaccharide-rich fraction (containing crude HPF) on heat-induced haze was assessed on those proteins (predominantly M_r 26 000 and 24 000 species) which accompanied the polysaccharide-rich fraction in the initial steps of its isolation. Also, these tests were made at a concentration of crude HPF that was naturally present in the fractions obtained in the purification scheme. To extend these results, the effect of increasing the concentration of crude HPF on the haze induced from a constant level of total wine proteins was determined (see Figure 1). Total wine proteins included M_r 26 000 and 24 000 species in addition to another major wine protein with M_r 32 000 and other minor protein components. The data in Figure 1 indicate that the protection afforded by crude HPF was apparently extended to all wine proteins, and this protection became greater, reducing the visible haziness of the proteins, as the ratio of added crude HPF to wine protein increased. The relationship between the extent of haze observed and concentration of added crude HPF was exponential, and a residual low level of haze was still detected by the spectrophotometric assay at the highest level of crude HPF tested.

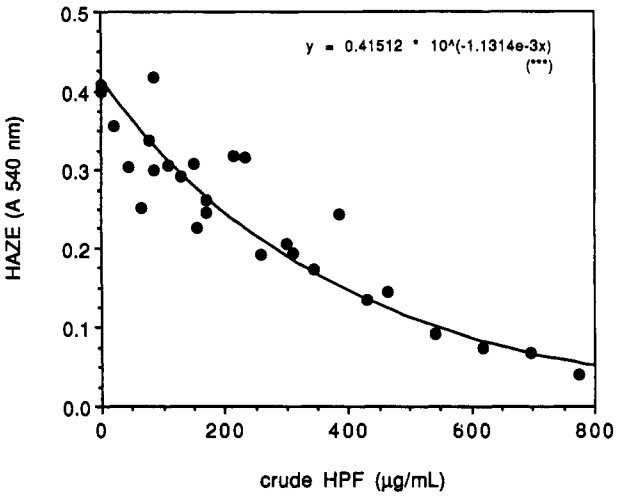

Figure 1. Effect of increasing concentration of crude HPF on heat-induced protein haze formation in wine. An increasing concentration (expressed as micrograms of galactose equivalents) of crude HPF [isolated by a combination of ammonium sulfate precipitation and ultrafiltration as described by Waters et al. (1991)] was back-added to wine containing 100 μg/mL wine protein and incubated as detailed under Materials and Methods. (***) Significant correlation at 0.001% level.

(*b*) *Haze and Supernatant Protein and Polysaccharide Composition.* Hazes and supernatant samples from the experiments described under (a) were analyzed by SDS–PAGE (see Figure 2). The electrophorograms showed that although there was a small decrease in band intensities as the level of polysaccharide addition increased, this decrease was not significant. The sample with the highest level of HPF addition (Figure 2, lane B3), although exhibiting only 10% of the visible haze given by the sample with no HPF addition (Figure 2, lane A1), had protein band intensities similar to that of the latter sample. Figure 2, lane B4, shows the protein band intensities that might be expected for a haze reduction to 10% resulting from a decrease in protein precipitation. Furthermore, all of the proteins present before heating (i.e., M_r 32 000, 26 000, and 24 000 species), as well as some lower M_r bands, were found in all hazes, regardless of the level of polysaccharide addition (Figure 2, lanes A1–7 and B1–3). The supernatants from the samples with no, low, or high levels of added crude HPF (Figure 2, lanes B5–7) all showed no measurable protein.

Some of the hazes and supernatants analyzed by SDS–PAGE were visualized with both the protein stain and the PAS carbohydrate stain (data not shown). All of the haze samples gave a poor response to the carbohydrate stain, indicating that none contained a large amount of polysaccharide material. In contrast, the supernatant samples responded increasingly to the PAS stain as the concentration of added crude HPF was raised.

(*c*) *Haze Particle Size.* The particle size of suspended haze samples from the experiment under (*a*) were measured by photon correlation spectroscopy (Figure 3). The data showed that the size of the haze particles decreased as the concentration of added crude HPF increased. The relationship between the haze particle size and crude HPF concentration was also exponential, with the decrease in particle size of the precipitate diminishing to less than 5 μm at a high addition of crude HPF. A replot of the data in Figures 1 and 2 showed a linear correlation between visible haziness and haze particle size (data not shown).

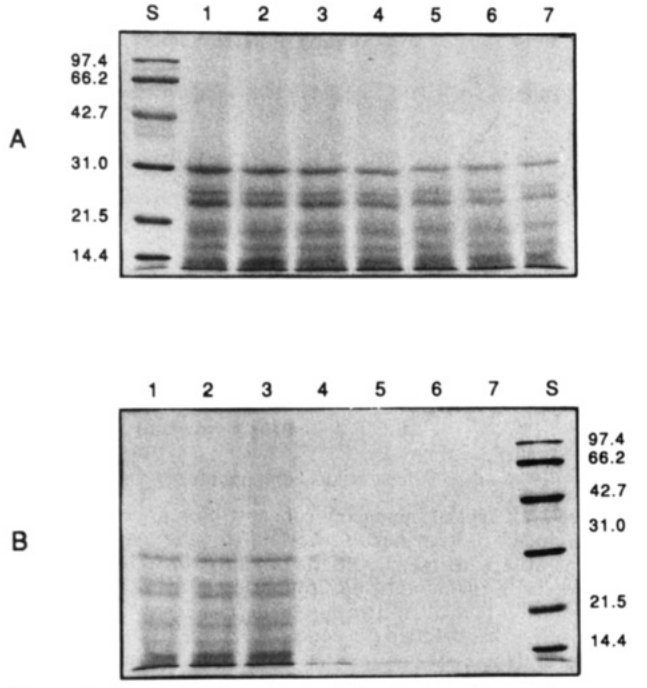

Figure 2. SDS–PAGE analysis of wine hazes and supernatants. Samples (1 mL) from the previous experiment (Figure 1) were centrifuged (10000*g*, 10 min), the supernatant was removed, and the pellet was washed twice with water (250 μL). The hazes, redissolved in SDS–PAGE sample buffer, and the supernatants were analyzed by SDS–PAGE as described under Materials and Methods. Relative molecular mass ($M_r \times 10^{-3}$) of protein standards (S) are given on the left side of gel A and the right side of gel B. The other lanes were as follows: (lane A1) haze from sample containing 100 μg/mL wine protein but no added crude HPF; (lanes A2–7 and B1–3) hazes from samples containing 100 μg/mL wine protein and crude HPF at 150, 230, 310, 390, 460, 540, 620, 700, and 780 μg, respectively; (lane B4) haze from sample containing 100 μg/mL wine protein but no added crude HPF and at 10% of the loading in lanes A1–7 and B1–3; (lanes B5 and B6) supernatants from samples containing 100 μg/mL wine protein and crude HPF at 390 and 780 μg, respectively; (lane B7) supernatant from sample containing 100 μg/mL wine protein but no added crude HPF.

Purification of the HPF. (*a*) *Affinity Chromatography.* A small-scale separation of crude HPF by concanavalin A (Con A) affinity chromatography demonstrated that the HPF could be further purified by this technique (data not shown).

Preparative affinity chromatography of a wine ultrafiltration retentate (containing the full complement of wine polysaccharides and proteins) on a Con A column separated the majority of the wine proteins from a fraction containing the HPF. This latter fraction was retained by the affinity column and was eluted with methyl mannoside. On SDS–PAGE, the retained fraction showed a profile similar to that of crude HPF which had been isolated by an ammonium sulfate fractionation procedure (Waters et al., 1991). In particular, the Con A retained fraction appeared as a smear with the sugar-specific PAS stain at the high M_r region of the gel and also contained a PAS-staining band at M_r 54 000, as well as a PAS- and protein–positive band at M_r 63 000 and a faintly staining protein band at M_r 23 000 (gel not shown).

The effects of an increasing concentration of the Con A retained fraction on the haze induced from total wine proteins, and from BSA, are shown in Figure 4. The Con A retained fraction showed a similar reduction in the visible haziness produced by heating both of these protein fractions in wine. BSA can thus be used as an alternative to wine proteins in assessing the haze protective activity of polysaccharides in wine.

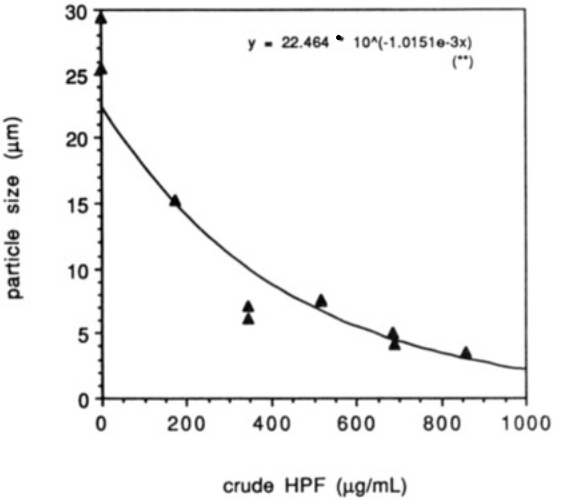

Figure 3. Effect of increasing concentration of crude HPF on protein haze particle size. An increasing concentration (expressed as micrograms of galactose equivalents) of crude HPF was back-added to wine containing 100 μg/mL wine protein and then heated. The particle size of the resultant haze was measured by photon correlation spectroscopy. Details of the methods for heat-inducing haze and for determining its particle size are given under Materials and Methods. (**) Significant correlation at 0.01% level.

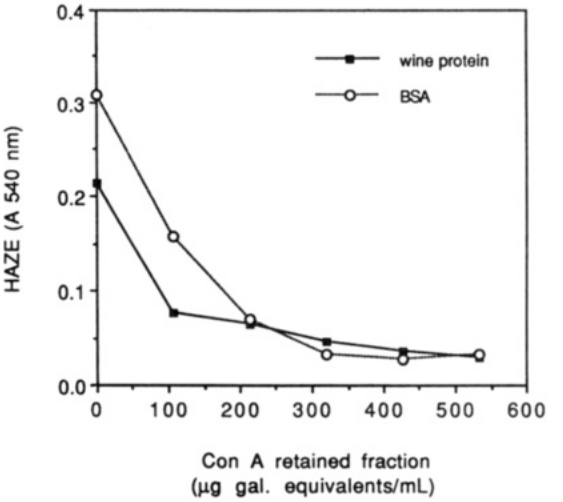

Figure 4. Effect of an increasing concentration of the Con A retained fraction on heat-induced protein haze formation. An increasing concentration (expressed as micrograms of galactose equivalents) of Con A retained fraction was back-added to wine containing 50 μg/mL wine protein, or 100 μg/mL BSA, and heated as described under Materials and Methods.

(*b*) *Anion-Exchange Chromatography.* The HPF in the Con A retained fraction was further purified by anion-exchange chromatography on an FPLC Mono Q column at pH 5.5 (Figure 5). Most of the material loaded onto the column was retained and eluted with an increase in NaCl concentration.

The haze protective properties of the fractions isolated by anion-exchange chromatography were assessed by determining their effect on the heat-induced haze of BSA (Figure 6). The response of each fraction can be compared to that of the Con A retained starting material, because each fraction was concentrated to the same volume as that of the starting material loaded onto the column. Neither the unretained fraction nor fraction IIIa showed a significant haze protective effect. Fraction Ia showed some haze protection, but the majority of HPF appeared to be

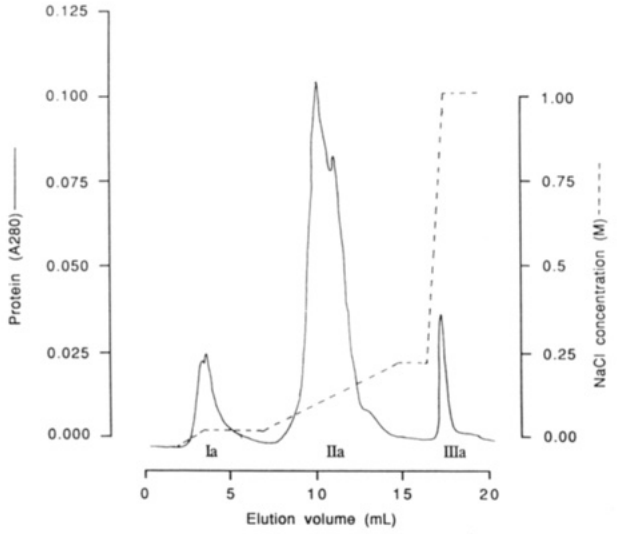

Figure 5. Separation of the Con A retained fraction by anion-exchange chromatography. The Con A retained fraction was separated by anion-exchange chromatography on a FPLC Mono Q column at pH 5.5 as described under Materials and Methods.

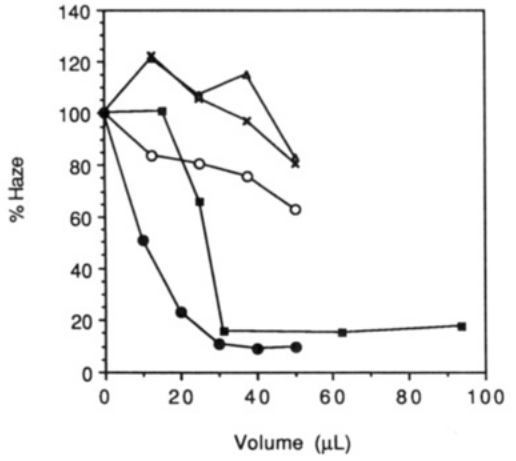

Figure 6. Effect of an increasing concentration of fractions isolated by anion-exchange chromatography on the heat-induced haze of BSA. The fractions isolated in Figure 5, and also the Con A retained starting material, were back-added to ultrafiltered wine containing 100 μg/mL BSA and then heated. The Con A retained starting material contained the equivalent of 10.68 μg of Gal/μL. Details of the method for determining heat-induced haze are described under Materials and Methods. (●) Starting material; (△) unretained fraction; (○) fraction Ia; (■) fraction IIa; (×) fraction IIIa.

localized in fraction IIa. Analysis of this latter fraction by SDS–PAGE revealed that it contained some of the PAS-staining components of the starting material that smeared at the high M_r region of the gel in addition to a PAS-staining band at M_r 54 000, a PAS- and protein-positive band at M_r 63 000, and a protein band at M_r 23 000 (gel not shown).

(c) *Cation-Exchange Chromatography.* Fraction IIa from anion-exchange chromatography (Figure 5) was further separated by cation-exchange chromatography on a FPLC Mono S column at pH 3.0 (Figure 7). Most of the material loaded onto the column was retained and eluted with an increase in NaCl concentration.

The protein and charged polysaccharide composition of these fractions, as revealed by SDS–PAGE, is shown in Figure 8. The material not retained by the cation-exchange column (lane 1) contained most of the PAS-staining components of the starting material that smeared at the high M_r region of the gel. Fraction Ic (lane 2) contained

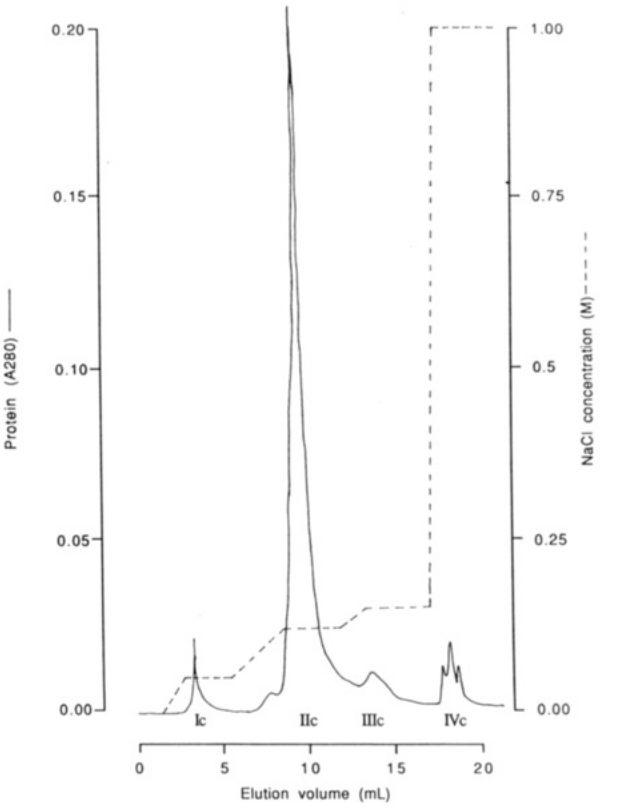

Figure 7. Separation of fraction IIa by cation-exchange chromatography. Fraction IIa, isolated by anion-exchange chromatography (Figure 5), was further fractionated by cation-exchange chromatography as described under Materials and Methods.

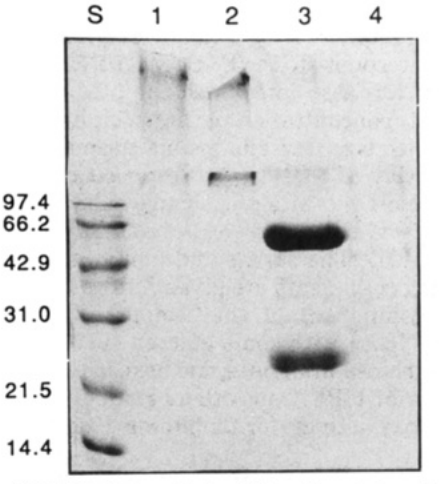

Figure 8. SDS–PAGE analysis of fractions taken during the cation-exchange chromatographic purification of HPF. Relative molecular masses ($M_r \times 10^{-3}$) of protein standards (S) are given on the left side of the gel. The other lanes are as follows: (lane 1) unretained fraction; (lane 2) fraction Ic; (lane 3) fraction IIc; (lane 4) fraction IVc. The gel was stained for protein with Coomassie Brilliant Blue and for carbohydrate with the PAS stain.

only smeared PAS-staining material and no detectable protein. Fraction IIc (lane 3) contained all of the protein bands of the starting material. These proteins were also present in fraction IIIc, although at reduced concentration (data not shown). The strongly retained fraction (IVc, lane 4) contained the PAS-staining band at M_r 54 000.

The effect of these cation-exchange fractions on the haze induced from BSA was determined as described under (*b*) (data not shown). Fraction Ic showed a high degree of haze protection and neither the unbound fraction, fraction

Table I. Composition of HPF

	rel %
carbohydrate	96
protein	4

Neutral Sugar Composition[a]

sugar	% molar ratio	sugar	% molar ratio
mannose[b]	78	galactose[c]	3
glucose[b]	13	arabinose[c]	2
apiose[c]	3	hexosamine[c]	1

Amino Acid Composition[d]

amino acid	% molar ratio	amino acid	% molar ratio
Ser	31	Ile	5
Thr	13	Tyr	5
Asx	9	Leu	4
Glx	9	Phe	2
Gly	9	Lys	1
Val	6	Pro	nd[e]
Ala	5		

[a] Determined as described under Materials and Methods. [b] Confirmed by co-injection. [c] Peaks eluting at the same retention times as standards. [d] Determined as described in Waters et al. (1991).[e] nd, not determined.

IIc, nor IVc showed a haze protective effect. On the contrary, fraction IVc increased the haze potential of added BSA.

Hazes and supernatants from the samples containing fractions Ic and IVc were analyzed by SDS–PAGE (data not shown). None of the supernatants contained any protein, nor were there any significant differences in protein composition among the haze samples, similar to that observed above with crude HPF. The hazes could be differentiated only with the PAS reagent. Haze samples containing an increasing amount of fraction Ic gave an increasing response to the PAS reagent, clearly indicating that HPF was associated with the precipitated protein and that its concentration in the precipitate increased in tandem with a decrease in visible turbidity.

Properties of HPF. (*a*) *Composition.* Analysis of fraction Ic off the cation-exchange column showed that the HPF was comprised of polysaccharide (96%) and protein (4%). The sugars and amino acids making up these two components are given in Table I. The carbohydrate component of the factor was dominated by mannose (78%) with some glucose (13%) and traces of apiose, galactose, arabinose, and hexosamine. Incomplete separation of HPF from other, grape-derived, polysaccharides may account for the presence of the latter three sugars.

The amino acid composition of the protein component of the factor was determined as described by Waters et al. (1991). The protein component (Table I) was dominated by serine (31%) and threonine (13%), with major contributions from glycine (9%), aspartic acid and or asparagine (Asx) (9%), and glutamic acid and or glutamine (Glx) (9%).

(*b*) *UV Spectrum.* In the second-order derivative spectrum of HPF (Figure 9), minima were clearly observable at 281 and 255 nm. These wavelengths correspond to the primary minima of tyrosine and phenylalanine, respectively, confirming their presence in HPF (see also Table I). Minima at 275 and 285 nm and the intersection of the zero-ordinate axis at 273 nm are also reportedly characteristic of tyrosine, as are minima at 268 and 260 nm of phenylalanine (Palladino and Cohen, 1991).

Second-order derivative spectroscopy also allowed examination for the presence of tryptophan. No tryptophan was detected in acid hydrolysates of HPF, but this amino

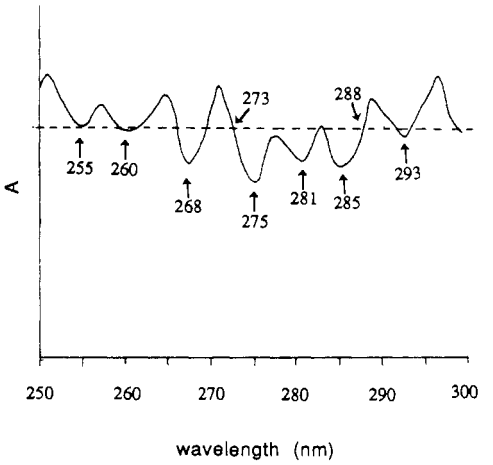

Figure 9. Second-order derivative spectrum of HPF. The second-order derivative spectrum of HPF in pH 5.0 buffer (50 mM sodium acetate) was measured in a 10-mm path length cell, at a scan rate of 200 nm/s and slit width of 2 nm. Minima and zero-ordinate axis intersections are arrowed.

acid can be destroyed during acid hydrolysis especially when carbohydrate is present (Ng et al., 1987). Unfortunately, its identification in HPF by second-order derivative spectroscopy was hampered by the large proportion of tyrosine present in the factor (5%, Table I). In proteins with a tyrosine:tryptophan ratio greater than 4:1, it has been shown that the primary minimum of tryptophan (290 nm) is obscured by the primary minimum of tyrosine (281 nm). The contribution of tryptophan to the spectrum is thus reduced to a characteristic intersection of the zero-ordinate axis upscale from 282 nm (Palladino and Cohen, 1991). Such a feature is evident in Figure 9 at 288 nm, suggesting that there may be some tryptophan present but the quantity is only minor compared to that of tyrosine present in HPF.

DISCUSSION

The polysaccharide-rich fraction described in a previous paper (Waters et al., 1991) has now been shown to extend its haze protective effect to all wine proteins and to the mammalian protein BSA. The visible haziness of the heated proteins was reduced as the ratio of added polysaccharide-rich fraction to protein increased, and the relationship between the extent of haze protection and the concentration of the added polysaccharides was exponential. A low level of haze was still detectable by the spectrophotometric assay at the highest concentration tested of added HPF.

The mechanism of haze protection was to decrease the particle size of the haze rather than to prevent either a specific or a general protein precipitation. This had the visual effect of making the wine appear less hazy. The sizes of particles detected in wine hazes studied here were broadly comparable to those observed in beer spoiled by haze. It has been reported that visually hazy beer contained particles from 8 to 12 μm in size; furthermore, it has been proposed that particles greater than 5 μm were commercially significant (Leedham and Carpenter, 1977). This is consistent with the findings in this study where, at the highest additions of crude HPF studied, wine haze particle size had decreased to 5 μm and was barely detectable with the naked eye.

Some of the added polysaccharide material was present in the hazes, but most of the polysaccharide in the crude HPF appeared to remain soluble after heating. With the purified HPF, however, polysaccharide material was

clearly associated with the haze, and its concentration increased in tandem with a decrease in visible turbidity.

The retention of crude HPF on a Con A affinity column was used to advantage. When Con A, a lectin, is immobilized on a Sepharose column, selective retention of mannose- and glucose-containing components can be effected. The Con A retained fraction contained most of the high M_r carbohydrate material, including a PAS- and protein-positive band at M_r 63 000 and other proteins at M_r 40 000, 32 000, and 23 000. Passage of this fraction through an anion-exchange column separated HPF from some of the high M_r carbohydrate material, but it still retained the PAS- and protein-positive band at M_r 63 000 and other proteins at M_r 40 000 and 23 000. The protein components were not responsible for the haze protective effect, however, because they were separated from HPF by cation-exchange chromatography and found to precipitate normally when heated.

Purified HPF did not stain with the Coomassie Brilliant Blue protein stain after SDS–PAGE but was visualized with the carbohydrate-specific PAS stain and showed as a diffuse smear in the stacking gel and in the high M_r region of the separating gel. Such smearing may be an indication of its size heterogeneity. HPF was composed predominantly of carbohydrate (96%) with some protein (4%).

Although the protein component of HPF was minor, the amino acid composition of the protein was significant because of the dominance by serine (31%) and threonine (13%). The presence of such high quantities of these two amino acids indicates that there may be covalent linkages between the protein and polysaccharide parts of the factor. This could be directly via O-glycosidic linkages to serine and threonine or through asparagine and a di-*N*-acetylglucosamine linker, because N-glycosylation sites have been identified as the sequence Asn-X-Ser/Thr (Orlean et al., 1991). That at least some of the polysaccharide chains could be linked in this way seems possible since hexosamines were detected in the HPF. A similarly high proportion of the two hydroxylated amino acids was also reported for the protein component of yeast structural cell wall mannoprotein, although that macromolecule contained more threonine (30%) than serine (15%) (Frevert and Ballou, 1985).

The amino acid composition of HPF was investigated further by derivative spectrophotometry, which offers the advantage of producing sharper spectral features than conventional absorbance (i.e., zero-order derivative) spectrophotometry and allows the identification of aromatic amino acids in proteins and peptides (Palladino and Cohen, 1991). The presence of the UV-absorbing amino acids, tyrosine (5%) and phenylalanine (2%), in the protein component of HPF explains the UV absorbance of the factor, enabling its detection at 280 nm in chromatographic eluants. The most intensely UV-absorbing amino acid, tryptophan, was not detected in the acid hydrolysates, although a hydrolysis method designed to minimize its loss was employed (Lui and Boykins, 1989); nor was there conclusive evidence by UV derivative spectrophotometry for the presence of tryptophan in the intact HPF. This result indicates that the UV absorbance of HPF is largely due to the presence of phenylalanine and tyrosine.

The carbohydrate portion of the factor was dominated by mannose (78%) with some glucose (13%), and this composition suggests a yeast cell wall origin for the HPF, similar to that of other yeast-derived mannoproteins (Ballou, 1982). The SDS–PAGE results are also consistent with a yeast cell wall origin for the HPF, because purified yeast mannoprotein is reported to have smeared in SDS–PAGE stacking gels and stained only with the PAS stain and not with Coomassie Brilliant Blue or the more sensitive silver stain procedure (Novick and Schekman, 1983; Frevert and Ballou, 1985).

The wine used for the isolation of HPF had not been produced by a method designed to increase yeast autolysis (e.g., stored on yeast lees), but it is likely that the fermentation process alone released some yeast products. Saulnier et al. (1991) have shown that storage on yeast lees was not necessary for colloid release and that by the end of fermentation yeasts had released about 150 mg/L of polysaccharide material. These workers have further suggested that yeast mannoproteins were released into wine from the yeast cell wall by hydrolytic enzymes. This mechanism may be responsible for the occurrence of HPF in the wines used in this study.

This research shows that it may be possible to enhance the natural haze protection of wine by enriching the wine in yeast cell wall polysaccharides. This enrichment could be achieved through prolonged aging of the wine on yeast lees—a procedure relevant to a few specific wine styles. Alternatively, the enrichment could be applied to all white wine varieties during normal wine production by adding a haze protection factor obtained from yeast cells. Such procedures hold promise for a new generation of protein stabilization techniques in winemaking. Further studies are in process to test these hypotheses.

ABBREVIATIONS USED

Asx, aspartic acid or asparagine (undefined); BSA, bovine serum albumin; Con A, concanavalin A; Gal, galactose; Glx, glutamic acid or glutamine (undefined); Gordo, Muscat Gordo Blanco; HPF, haze protective factor; M_r, relative molecular mass; nd, not determined; PAS, periodic acid-Schiff reagent; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

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