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Quantitative Colorimetric Assay for Total Protein Applied to the Red Wine Pinot Noir

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ABSTRACT: A standard method for assaying protein in red wine is currently lacking. The method described here is based on protein precipitation followed by dye binding quantification. Improvements over existing approaches include minimal sample processing prior to protein precipitation with cold trichloroacetic acid/acetone and quantification based on absorbance relative to a commercially available standard representative of proteins likely to be found in wine, the yeast mannoprotein invertase. The precipitation method shortened preparation time relative to currently published methods and the mannoprotein standard yielded values comparable to those obtained by micro-Kjeldahl analysis. The assay was used to measure protein in 48 Pinot noir wines from 6 to 32 years old. The protein content of these wines was found to range from 50 to 102 mg/L with a mean value of 70 mg/L. The availability of a simple and relatively rapid procedure for assaying protein provides a practical tool to quantify a wine component that has been overlooked in routine analyses of red wines.

KEYWORDS: Pinot noir, red wine protein, mannoprotein, glycoprotein, grape, yeast, invertase

1. INTRODUCTION

The role of proteins in red wine quality is largely unknown. One well-characterized interaction is their binding to tannins that can lead to formation of insoluble complexes. This interaction is widely exploited in the use of certain proteins as fining agents that are deliberately added to red wine to remove excess tannin and improve sensory quality by reducing the associated astringency and bitterness.[1] In spite of this, a number of studies have convincingly shown that red wines also contain soluble proteins that avoid tannin-mediated precipitation.[2−10] Many of these proteins copurify with polysaccharides and have been reported to be glycoproteins.[6,8] Glycoproteins have been shown to bind less tannin than nonglycosylated proteins.[11,12] Because measurement of protein in red wine is not a standard analysis, relatively few studies have surveyed protein content. In contrast, proteins in white wines have been widely measured and characterized.[13]

A number of methods have been used to concentrate, fractionate, and quantify or identify protein in red wines. The following examples illustrate that this is not a trivial undertaking. In one report,[2] a Carignan noir wine was dialyzed extensively, concentrated, chromatographed on Sephadex LH 20 to remove pigment, and dialyzed again. The retentate was then lyophilized, solubilized, and fractionated by size exclusion on a Sephadex G-75 column prior to protein quantification by a modified Lowry method. Muscat Bailey A, Cabernet sauvignon, Pinot noir, and Merlot wines were dialyzed for 3 days prior to nitrogen (micro-Kjeldahl) and amino acid analyses.[4] In a subsequent study, Muscat Bailey A wines were concentrated and subjected to ammonium sulfate precipitation.[6] The resulting precipitates were dissolved and dialyzed, and protein in the dialysates was precipitated by addition of 5 volumes of acetone at pH 3.5. Protein was then quantified as in the prior study. Proteins were further characterized by additional chromatographic steps and by electrophoresis. In more recent work, proteins were identified but not quantified in Portugieser red wines.[10] Briefly, wine was dialyzed for at least five days, lyophilized, solubilized, treated with polyvinylpyrrolidone to remove polyphenols, filtered, lyophilized again, and concentrated prior to SDS-PAGE analysis. Protein bands visualized by Coomassie Brilliant Blue R-250 staining were subjected to in-gel trypsin digestion prior to analysis of peptides by LC-MS/MS which resulted in identification of 121 peptides attributed to 12 grape and 6 yeast proteins.

The present work was motivated by the need for a simple method to quantify protein in red wine. A number of reports suggest that yeast mannoproteins and grape glycoproteins contribute to improved "texture" of red wine,[14−16] a difficult sensory attribute to define.[17] On the basis of the importance that consumers and winemakers ascribe to wine texture, a better understanding of the contributing factors would be helpful. The assay described here is expected to facilitate routine analysis of protein in red wine and allow a direct determination of its contribution to improved sensory quality.

2. MATERIALS AND METHODS

2.1. Chemicals. All chemicals were reagent grade. Yeast invertase (catalog no. I4504) and mannan (catalog no. M7504) were from Sigma-Aldrich (St. Louis, MO).

2.2. Wines. Table 1 provides a description of the wines that were analyzed.

2.3. Protein Precipitation Procedures. *2.3.1. KDS Precipitation.* Protein was precipitated and assayed essentially as described.[9] Briefly, 10.1 μL of a 10% SDS solution were added to 1 mL of wine prefiltered through a 0.45 μm filter in a 1.7 mL screw-capped tube, vortexed vigorously, and placed in a 100 °C

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Table 1. Protein Content of Pinot Noir Wines

wine	description[a]	protein[b] (mg/L invertase equiv.)
1	CH 2004 Coral Creek 4 L yeast lees	78.9 ± 13.1
2	CH 2004 Coral Creek 4 L yeast lees, pectinase	78.6 ± 8.7
3	CH 2004 Coral Creek 4 L lees, stirred	81.5 ± 9.9
4	CH 2004 Coral Creek 4 L lees, stirred, pectinase	78.4 ± 6.5
5	CH 2004 Stoller 4 L yeast lees	55.4 ± 7.6
6	CH 2004 Stoller 4 L lees, pectinase	49.8 ± 6.0
7	CH 2004 Stoller 4 L lees, stirred	63.1 ± 8.7
8	CH 2004 Stoller 4 L lees, stirred, pectinase	60.7 ± 2.1
9	WI 2003 no yeast lees	56.9 ± 10.0
10	WI 2004 no yeast lees	64.1 ± 4.7
15	WI 2003 4 L yeast lees	60.9 ± 11.1
16	WI 2004 4 L yeast lees	74.0 ± 8.6
17	WI 2004 4 L yeast lees from 2003	67.1 ± 10.3
18	WI 2004 4 L lees, pectinase	60.9 ± 4.4
19	WI 2004 8 L yeast lees from 2003	71.9 ± 2.4
20	WI 2004 8 L yeast lees	67.2 ± 5.1
21	BH 2004 pectinase	62.3 ± 5.0
22	BH 2004 no yeast lees	66.0 ± 1.9
25	BH 2004 12 L yeast lees	65.7 ± 7.9
28	BH 2004 4 L yeast lees	85.2 ± 4.8
29	BH 2004 4 L yeast lees, pectinase	62.0 ± 4.7
30	BH 2004 8 L yeast lees	78.8 ± 4.7
31	BH 2004 8 L yeast lees, pectinase	71.9 ± 6.1
33	BH 2004 (lot 2) no yeast lees	67.2 ± 7.6
34	BH 2004 (lot 2) 12 L yeast lees from 2003	71.2 ± 5.6
35	BH 2004 (lot 2) 2 L yeast lees from 2003	58.8 ± 7.5
36	BH 2004 (lot 2) 4 L yeast lees from 2003	65.9 ± 5.4
37	BH 2004 (lot 2) 8 L yeast lees from 2003	58.9 ± 8.2
38	EY 1979	63.0 ± 12.0
39	EY 1980	74.2 ± 8.7
40	EL 1983	80.6 ± 12.4
41	AM 1978	102.3 ± 15.3
42	AL 1980	64.9 ± 3.0
43	ER 2001	69.8 ± 12.9
44	BL 2002 L-block barrel sample	82.9 ± 1.6
45	CA nonvintage	56.7 ± 9.2
46	BY 1999 yeast BRL97	72.7 ± 11.3
47	BY 1999 yeast D254	81.1 ± 8.1
48	BY 1999 yeast BGY	74.4 ± 6.0
49	BY 1999 yeast BM45	85.1 ± 9.3
50	BY 1999 yeast RC212	62.8 ± 7.8
51	AM 1983 Sunnyside vineyard	72.5 ± 13.6
52	AM 1985 Wadensvil clone of Pinot noir	55.6 ± 3.5
53	AM 1993 Winter's Hill Farm	75.5 ± 11.0
54	AM 1996	64.8 ± 3.3
55	AM 1998 Zielinski vineyard	74.2 ± 14.1
56	KR 2001 Rebecca's Reserve	77.9 ± 7.8
57	KR 2002 Estate	73.2 ± 1.7

[a] Wineries are given 2-letter codes (AM, Amity; AL, Alpine; BH, Bethel Heights; BL, Benton Lane; BY, Byron; CA, Cameron; CH, Chehalem; EL, Ellendale; ER, Erath; EY, Eyrie; KR, Kramer; WI, Willakenzie). Additional information is also provided when available: vintage, vineyard name, clone of Pinot noir, strain of *Saccharomyces cerevisiae* used in fermentation, volume of yeast lees added during barrel aging per 228-L barrel, whether lees were stirred, use of pectinase. All wines were made in Oregon except for #46−50 which were from California. Wines #1−30 were commercially made experimental lots. [b] Data are means ± standard deviations from triplicate measurements using the TCA/acetone precipitation procedure in combination with the Bradford assay as described in the Materials and Methods.

water bath for 5 min. Tubes were cooled quickly to room temperature on ice, and to each, 252.2 μL of 1 M KCl were added. The tubes were then mixed gently for 30 min at room temperature. The resulting mixture of wine and precipitate was centrifuged at 22 000 $\times g$ at 4 °C in a microcentrifuge for 15−20 min. The pellet was then washed twice with 1 M KCl at 4 °C and solubilized in 1 mL of distilled water.

2.3.2. Acetone Precipitation. Protein was precipitated by adding 2 volumes of −20 °C acetone to one volume of wine which had been prefiltered through a 0.45 μm polyethersulfone (PES) syringe filter. Samples were incubated for 45 min at −20 °C and centrifuged for 15 min at 22 000 $\times g$ at 4 °C. The pellet was washed once with −20 °C acetone, air-dried, and solubilized in distilled water. Solubilized material in specified samples was subjected to additional washing to eliminate non-protein, BCA-reactive material using centrifugal membrane filters. In this case, the solubilized solutions were washed repeatedly with distilled water on 10 kDa MW cutoff PES centrifugal membrane filters using ∼450 μL per wash. After each wash, retentates were returned to their original volumes with distilled water.

2.3.3. TCA/Acetone Precipitation. Protein was precipitated by adding 2 volumes of −20 °C acetone containing 10% (w/v) freshly prepared tricholoroacetic acid (TCA) to one volume of wine which had been prefiltered through a 0.45 μm PES syringe filter. Samples were incubated for 45 min at −20 °C and centrifuged for 15 min at 22 000 $\times g$ and 4 °C. The pellet was washed once with −20 °C acetone, air-dried, and solubilized in distilled water. Wine samples up to 0.5 mL in volume were processed in 1.7 mL polypropylene microfuge tubes. Larger wine samples (up to 15 mL) were processed in 50 mL screw-capped polypropylene tubes which were initially centrifuged for 30 min at lower speed, 2000 $\times g$, in a benchtop centrifuge at 4 °C to pellet the TCA/acetone precipitate. The pellets from these larger samples were then washed once with −20 °C acetone, centrifuged at 4 °C for 10 min at 2000 $\times g$, air-dried, and solubilized in distilled water. Protein concentrations determined in wine samples subjected to either the 2000 or 22000 $\times g$ centrifugation step were not significantly different.

2.4. Protein Quantification. *2.4.1. BCA Assay.* Protein was measured in the solubilized wine precipitates based on the bicinchoninic acid (BCA) assay,[18] using a commercial kit (Pierce Laboratories, Rockford, IL) following the manufacturer's

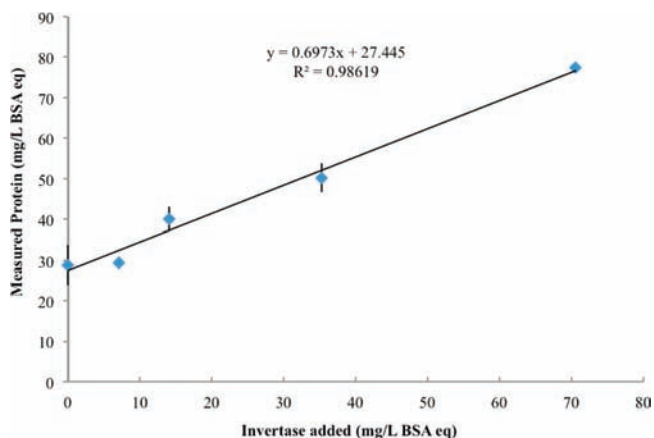

Figure 1. Recovery of invertase in a solubilized acetone precipitate from a 2003 Adelsheim Vineyard Pinot noir washed 5 times on a 10 kDa centrifugal filter and measured by the BCA assay. Data are means ± standard deviations from duplicate measurements.

instructions. A bovine serum albumin (BSA) standard was supplied by the manufacturer. A yeast invertase standard was included for comparison.

2.4.2. Bradford Assay. Protein was measured in the solubilized wine precipitates based on the Bradford method,[19] using a commercial kit (Bio-Rad Laboratories, Hercules, CA) following the manufacturer's instructions for the microassay procedure. A BSA standard was supplied by the manufacturer. A yeast invertase standard was included for comparison.

2.4.3. Micro-Kjeldahl Assay. Total nitrogen was measured in solubilized wine precipitates following the protocol outlined in AOAC Official Method 960.52.[20] Protein concentration was then estimated by multiplying nitrogen content by 6.25.

2.5. Dialysis of Wine. Wine (15 mL) was placed in 3500 Da M.W. cutoff dialysis tubing (Spectrapor, Spectrum Medical Industries, Inc., LA, CA) and dialyzed with stirring at 4 °C with 6 changes of 2 L of distilled water over 30 h.

2.6. Mannan Analysis. Mannan was quantified in the TCA/acetone solubilized wine precipitates by lectin blotting, essentially as described,[21] except that 4 μg/mL rather than 9.5 μL/mL of the mannose-specific, biotinylated *Narcissus pseudonarcissus* lectin (Vector Laboratories, Burlingame, CA) were used, the nitrocellulose membrane was rinsed after substrate addition for 5 min rather than 2−3 min, and the membrane was exposed immediately to X-ray film for 30−60 s rather than after overnight storage at 4 °C. Mannan standards (400, 200, 100, and 50 nanograms) and a positive control (yeast invertase) were blotted with samples onto each nitrocellulose membrane (0.45 μm Bio-Rad Laboratories Hercules, CA) in a 48-well slot blot apparatus. The mannan content of the samples was quantified in mannan equivalents. Triplicate wine samples were blotted in duplicate, resulting in 6 spots for each wine.

3. RESULTS AND DISCUSSION

3.1. Protein Analysis. *3.1.1. KDS Method.* Initially, wine protein was precipitated using the KDS method and quantified with the BCA assay,[9] as done previously to measure the protein content of model wine fermentations.[21] To test the validity of applying this method to red wines, Pinot noir samples were supplemented to various extents with yeast invertase prior to the precipitation step and then the assay was applied to determine percent invertase recovery. The results were inconsistent, but on average, less than 15% of the added invertase was recovered based on the BCA-determined equivalents added to the wine. For example, when wines containing 9 ± 1.0 mg/L endogenous protein (BSA equivalents in the BCA assay) were supplemented with 8, 17, or 42 mg/L BSA equivalents of yeast invertase, only an additional 1.5 ± 2.8, 0 ± 2.4, or 6.2 ± 3.3 mg/L were measured, respectively. These results agree with a recent report that showed that the KDS precipitation method did not consistently precipitate glycoproteins from white wines.[22] In that study, glycoproteins were detected but not quantified by periodic acid-Schiff (PAS) staining of solubilized precipitates run on SDS-PAGE gels.

3.1.2. Acetone Precipitation. The above results suggested that other protein precipitation methods may be better suited for the quantification of protein in red wines, such as precipitation with −20 °C acetone.[23] This method had been ruled out in a previous study [9] because a deproteinized wine, i.e., analysis of a wine filtrate that had passed a 1 kDa ultrafiltration membrane, gave an unacceptably high value for protein content (250 mg/L, quantified using the BCA assay). Our analyses with wines not subjected to ultrafiltration also resulted in protein contents well above that expected (~850 mg/L) based on previous measurements of protein in red wine.[2,4] The results from both the present work and that of Vincenzi et al. [9] are consistent with the presence of nonprotein BCA-reactive material, possibly wine-derived phenolic compounds [24] and/or reducing sugars,[18] in the preparations used in the BCA assays. In agreement with this presumption is our finding that this precipitation approach, when applied to the quantification of supplemental protein, allowed recoveries approaching 100% of the supplemental invertase added to Pinot noir (data not shown). While this implies a significant background of BCA-reactive components in the wine, this background reactivity did not appear to affect protein quantification, provided it was accounted for.

Initial attempts to circumvent the problem of the low molecular weight, nonprotein, BCA-reactive material associated with the solubilized protein-precipitates were based on washing the protein preparations using 10 kDa cutoff centrifugal membrane filters. After each wash, retentates were returned to their original volumes with distilled water and refiltered. Individual washes were monitored to determine when the filtrate no longer contained BCA-reactive components. The results indicated 5 washes were required to remove low molecular weight BCA-reactive material. A second parameter of relevance was the ratio of acetone-to-wine for the initial precipitation step. To determine this parameter, retentates from the final wash of solubilized wine precipitates resulting from precipitations at acetone-to-wine ratios of 2, 3, or 4 were compared for protein content and were found to be nearly identical. This result, combined with the observation that the lower amount of acetone was associated with the least amount of low molecular weight BCA-reactive contaminant, suggested that a 2:1 ratio of −20 °C acetone to wine would be appropriate. Hence, in subsequent testing, 2 volumes of acetone were used for protein precipitation and low molecular weight BCA-reactive contaminants were removed through 5 sequential washes using a 10 kDa cutoff centrifugal membrane filter.

Protein recovery studies, based on supplementing wine with known amounts of yeast invertase, were conducted on representative wines to assess quantification and reproducibility of the combined −20 °C acetone precipitation and BCA quantification method. Recoveries of supplemented invertase were ~70%, with

Table 2. Effect of Quantification Method on Measured Wine Protein Content, (mg/L)[a,b]

coded wine[c]	BCA assay—BSA standard[d]	BCA assay— invertase standard[e]	Bradford assay—BSA standard[d]	Bradford assay—invertase standard[e]	micro-Kjeldahl protein[f]
1	53.4 ± 4.5	302.5 ± 25.5	7.1 ± 1.2	78.9 ± 13.1	81.0 ± 9.3
2	49.3 ± 4.6	279.0 ± 26.2	7.1 ± 0.8	78.6 ± 8.7	89.7
3	60.0 ± 0.8	339.7 ± 4.5	7.3 ± 0.9	81.5 ± 9.9	nd[g]
4	45.5 ± 0.7	257.4 ± 4.0	7.1 ± 0.6	78.4 ± 6.5	nd

[a] Data are means ± SD from triplicate assays for the BCA and Bradford measurements and for duplicate assays for the micro-Kjeldahl analysis of wine #1. The micro-Kjeldahl analysis of wine #2 was unreplicated. [b] Colorimetric assays were based on measurements of protein solubilized from TCA/acetone wine precipitates (see text). [c] Wines are described in Table 1. [d] Bovine serum albumin calibration standard, values are mg BSA equivalents per L. [e] Invertase calibration standard, values are mg invertase equivalents per L. [f] Protein estimated as 6.25 × measured nitrogen (reported as mg protein/L). [g] Not determined.

Table 3. Nitrogen Content of Pinot noir Fractions from TCA/acetone Precipitation[a]

micro-Kjeldahl nitrogen	prior to TCA/acetone precipitation		protein-containing pellet from TCA/acetone precipitation		wash from TCA/acetone precipitation pellet	
	native	dialyzed	native	dialyzed	native	dialyzed
nitrogen (mg/L)	314.8 ± 27.2	37.1 ± 5.0	13.0 ± 1.5	14.0 ± 2.5	309.4 ± 34.9	21.1 ± 0.5
percentage of total nitrogen[b]	100	100	4.1	37.7	98.3	58.5

[a] Wine #1 was used for this experiment (Table 1). Data are means ± standard deviations from duplicate measurements. [b] Percentages are based on mean values.

CV values less than 20%. Figure 1 shows a representative result for one such Pinot noir that contained ~30 mg/L of endogenous protein. The next step in assay development was application of the method for the determination of protein content in a selection of Pinot noir wines. The results were somewhat surprising in that the reproducibility of the assay (coefficient of variation ~32% based on triplicate measurements) was significantly higher than that observed in the invertase recovery experiments. One plausible interpretation for this phenomenon is that the reproducibility of the precipitation/washing steps was in some way related to protein size or character. Native secreted yeast invertase is a homodimer containing 50% mannan and a molecular weight of 270 kDa,[25,26] while the molecular weight of the majority of wine proteins has been reported to be in the range of 20−30 kDa.[13] The differences in protein size or character may be particularly important with respect to the random permeation/adsorption of the lower molecular weight proteins during repeated ultrafiltration.

3.1.3. TCA/Acetone Precipitation. A third precipitation method was tested, the TCA/acetone method, which involved adding two volumes of −20 °C acidified acetone (10% [w/v] TCA) to one volume of wine followed by one wash with −20 °C acetone as described in the Materials and Methods. In an initial analysis of 4 wines by this method (Table 2), the mean coefficient of variation for the subsequent BCA and Bradford protein assays was reduced to 8.6 ± 5.1%. On the basis of this improvement, the TCA/acetone precipitation method was evaluated further.

3.2. Choice of Protein Assay and Standard Protein. An important determinant when choosing a protein quantification method is the relative merits of the different colorimetric assays, including consideration of the nature of the color-forming reagent and the protein chosen as the calibration standard. In this work, the BCA and Bradford assays were compared with either BSA or yeast invertase as the calibration standard. The two assays were chosen based on their previous use in this field.[9,27] Mannosylated yeast invertase was considered as an alternative calibration standard because it seemed more representative of the glycoproteins in wine,[6,7] whereas BSA is not a glycoprotein. The results of the comparative experiments (Table 2) demonstrate that the estimated amount of protein in a given wine varied widely depending on which assay was used. For example, the protein content of wine #3 ranged from ~7 to ~340 mg/L depending on the protein assay used; this is nearly a 50-fold difference between the lowest and highest values. The spread in the data is attributable to both the nature of the color-forming reagent and the protein chosen as the calibration standard. It is relatively straightforward to compare the relevance of the chosen calibration standard. The protein values in Table 2 reflect the fact that the color yield per mg invertase is considerably lower than that for BSA in both the BCA and the Bradford assays. The result of the different color yields is that, within a given assay, the measured protein content will be considerably higher when using invertase rather than BSA as the calibration standard. The discrepancy in calculated protein contents within an assay is directly proportional to the difference in color yields of the calibration standards, which was higher in the Bradford assay (difference ≈ 11-fold) than in the BCA assay (difference ≈ 5.5-fold). It is also important to consider the nature of the color-forming reagents. The two reagents in this study differ in that one is based on the change in the absorbance properties of the Coomassie Brilliant Blue G-250 dye when it associates with protein (Bradford assay) and the other is based on the change in absorbance resulting from the reduction of copper and the subsequent chelation of the reduced copper by bicinchoninic acid (BCA assay). The relative color yield of the combined wine proteins and the two calibration standards are likely to differ under the different assay conditions. The magnitude of this difference will be translated to the measured protein contents

Table 4. Quantification of Wine Protein Based on Assay of Solubilized TCA/Acetone Wine Precipitates by Micro-Kjedlahl and Bradford Analyses[a]

coded wine	micro-Kjeldahl protein (mg/L)	Bradford protein invertase eq (mg/L)
6	62.4 ± 4.6	49.8 ± 6.0
35	77.7 ± 1.6	58.8 ± 7.5
41	104.0 ± 10.8	102.3 ± 15.3

[a] Data are means ± standard deviations from duplicate measurements for the micro-Kjeldahl analyses and from triplicate measurements for the Bradford assay.

shown in Table 2. It is relevant to consider the extent to which the wine proteins reflect the behavior of the calibration standards. Theoretically, if the combined wine proteins were an exact match to a calibration standard (e.g., purified wine proteins were used as the calibration standard), then the ratio of the measured protein contents obtained using two different assays (e.g., the BCA and Bradford assays) would be 1.0. This is because the difference in the color yield of the calibration protein in the two assays would be an exact match to the analogous change in color yield of the wine proteins. In the present case, the wine proteins are obviously not an exact match to either BSA or invertase. Thus, it is not surprising that the ratios of the measured protein contents obtained using the same calibration standard in the different assays do not equal 1. The extent to which these ratios differ from 1 provides an indication as to which of the two calibration standards most closely reflects the behavior of the wine proteins. The quotient obtained by dividing the protein content determined by the BCA assay with BSA as the calibration standard by the protein content determined by the Bradford assay with the BSA calibration standard is ~7.2. The analogous quotient for the protein contents obtained in assays using invertase as the calibration standard is ~3.7. The implication is that the invertase standard better reflects the difference in the color yield chemistry of the wine proteins under the BCA and Bradford assay conditions.

The large assay-dependent variation in the measured protein content of a given wine leads to the obvious question of which assay is closest to the true value (i.e., which method provides the best estimate). While Kjeldahl nitrogen has its limitations as a measure of protein content,[28] it is often used as the reference method against which other methods are compared. Hence, the protein content of wines #1 and #2 were measured by the micro-Kjeldahl method for comparison with values obtained by the colorimetric methods. The values that most closely agreed with micro-Kjeldahl analysis were those measured by the Bradford assay and expressed in invertase equivalents. For example, the solubilized TCA/acetone pellet from wine #1 contained 81 mg/L protein based on micro-Kjeldahl analysis of duplicate samples, and ~79 mg/L invertase equivalents by the Bradford assay. The solubilized TCA/acetone pellet from wine #2 contained ~90 mg/L protein based on a single micro-Kjeldahl assay, and ~79 mg/L invertase equivalents by the Bradford assay.

3.3. Further Evaluation of the TCA/Acetone Precipitation Method. To test our assumption that the nitrogen measured in the solubilized TCA/acetone precipitates was of high molecular weight, total nitrogen was measured both in wine #1, the TCA/acetone pellet obtained from wine #1, and in the pellet wash solution. In addition, total nitrogen was measured in dialyzed wine #1, in the TCA/acetone pellet obtained from the dialyzed wine, and in the pellet wash solution. Total wine nitrogen decreased ~90% due to dialysis (315 mg/L prior to dialysis and 37 mg/L after dialysis, Table 3), presumably representing the loss of low molecular weight nitrogen. Prior to dialysis, the nitrogen in the pellet accounted for only 4% of the total nitrogen in the wine, whereas after dialysis, the same amount of nitrogen was recovered, 13−14 mg/L, but it accounted for nearly 40% of total wine nitrogen. We speculate that the 60% nitrogen in the dialyzed wine that did not precipitate upon addition of TCA/acetone was either >3500 Da and soluble in TCA/acetone, or <3500 Da and protein-bound under aqueous conditions, but not in TCA/acetone. The amount of nitrogen detected in the TCA/acetone pellet after a single acetone wash (13 mg/L) was about the same as detected in the pellet following dialysis (14 mg/L), which indicates that the nitrogen in the pellet is of high molecular weight and that the single acetone wash step was as effective in removing low molecular weight nitrogen as the 30 h dialysis.

On the basis of these promising results, an additional 3 wines were analyzed to compare results from the Bradford assay (with invertase as a standard) to those obtained by micro-Kjeldahl analysis of the washed and solublized TCA/acetone precipitates (Table 4). The 3 wines chosen for this comparison represented the range of protein contents observed among the collection of wines analyzed. The mean coefficient of variation for the Bradford protein assay for these 3 samples was 13.3 ± 1.6%. In each case, the Bradford values were lower than the micro-Kjeldahl values, suggesting that the Bradford values represent minimum estimates of wine protein content. It is of interest that the Bradford assay has been reported to consistently underestimate protein content of glycoproteins when compared to amino acid analyses as well.[29]

The TCA/acetone method for precipitating protein coupled with the Bradford assay (using yeast invertase as a protein standard) was thus chosen to estimate protein content in 48 Pinot noir wines ranging in age from 6 to 32 years. Table 1 shows the protein content of all 48 wines tested. Protein content varied from 50 to 102 mg/L in invertase equivalents with a mean value of 69.7 ± 9.9. No simple correlations were observed between protein concentration and either wine age or tannin content (tannin measurements[30] not shown.) The oldest wine tested, #41, had the highest amount of protein (~100 mg/L), while five wines (#5, 6, 9, 45, and 52) that had close to the least amount of protein (~55 mg/L), ranged in age from 6 to 15 yr. Brillouet et al.,[2] reported that following extensive dialysis, concentration, separation on a Sephadex LH 20 column, and subsequent dialysis and lyophilization, a Carignan noir red wine was found to have 63 mg/L of protein by a modified Lowry method. Yokotsuka et al., 1994[4] subjected red wines to extensive dialysis, followed by centrifugation, and then determined protein concentration by micro-Kjeldahl analysis. A Pinot noir and Merlot were found to contain 77 and 48 mg/L protein, respectively. Five different vintages of Cabernet sauvignon were found to contain 45 to 86 mg/L and Muscat Bailey A wines aged 1 to 12 years contained 33 to 87 mg/L protein (mean 57 mg/L). No correlation was found between protein content and wine age. All protein fractions were presumed to be glycoproteins based on finding from ~2 to 11% (w/w) associated carbohydrate. Considering the differences in wine age, grape variety, and methods used to isolate and quantify protein, it is striking that the values detected in the present work and those reported in the aforementioned studies[2,4] are so similar.

Mannan was measured in the solubilized TCA/acetone precipitates from a subset of wines because of the presumed contributions wine mannoproteins make to sensory quality.[14−16] Mannan concentrations in wines #51−57 were 335.7 ± 38.4, 254.7 ± 34.5, 263.7 ± 59.8, 319.3 ± 45.1, 290.4 ± 21.6, 370.9 ± 36.9, and 351.7 ± 39.3 mg/L, respectively (n = 3). Although yeast mannoproteins are the single known source of mannan in wine, we found that TCA/acetone also induced precipitation of mannan alone (data not shown), and therefore, cannot conclude that the mannan detected was necessarily protein-bound. Nonetheless, yeast mannoproteins have also been identified in red wine on the basis of amino acid sequence.[10,31] Vidal et al., 2004 [8] found that mannoproteins accounted for about 1/3 of total polysaccharides in a Carignan noir wine while total wine polysaccharides have been reported to range from 300 to 1000 mg/L.[32] The mannan concentrations detected here are consistent with these other studies.

The relatively rapid procedure described here for estimating protein content in Pinot noir provides a convenient assay for a component that has largely been overlooked in systematic chemical analyses of red wines. The values obtained with minimal sample processing were in general agreement with values measured by others, but that involved lengthy fractionation steps.[2,4]

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