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ARTICLE

Analysis of Protein Composition of Red Wine in Comparison with Rosé and White Wines by Electrophoresis and High-Pressure Liquid Chromatography−Mass Spectrometry (HPLC-MS)

Petra Wigand,[†] Stefan Tenzer,[§] Hansjoerg Schild,[§] and Heinz Decker*,[†]

[†]Institute of Molecular Biophysics of the Johannes Gutenberg University Mainz, Germany and [§]Institute of Immunology, University Medical Center of the Johannes Gutenberg University, Mainz, Germany

Wine proteins not only influence wine stability but are also being discussed as potential allergens. Proteins from red, rosé, and white wines were enriched by dialysis and lyophilization followed by separation by SDS-PAGE. Significant differences were detected in the protein compositions of the analyzed wine varieties, and the major protein bands were identified by mass spectrometry after in-gel digestion with trypsin. In German Portugieser red wine, a total of 121 tryptic peptides were identified, which were attributed to 12 grape proteins and 6 proteins derived from yeast. Among the identified constituents are several proteins considered to influence wine stability and previously described potential grape allergens. The pathogenesis-related proteins represent the main proteins in all of the wines, but only some red wines show a band with a molecular mass of 12 kDa, identified as a lipid transfer protein (LTP). The occurrence and distribution of LTP depend on the wine variety.

KEYWORDS: Wine; *Vitis vinifera*; proteins; SDS-PAGE; FPLC; ESI-Q-TOF-MS; LTP; TLP

INTRODUCTION

Proteins are minor constituents in wine. However, they influence the quality of the end product, for example, by affecting haze formation. As a consequence, different methods have been developed to remove proteins during the process of winemaking. Nevertheless, low concentrations of proteins, about 10−200 mg/L, remain in the final wine (*1*−*20*).

The first paper describing proteins in wine was published 1959 (*1*). During the following decades different methods were applied for the quantification, purification, characterization, and identification of wine proteins, but still a consistent, broad overview is missing (*2*−*19*). There is agreement that the majority of wine proteins are found in the molecular mass range of 20−30 kDa. Most of the proteins were attributed to the class of pathogenesis-related proteins, which survive the vinification process due to their resistance against proteolysis and acidic conditions (*6*, *10*, *12*, *14*, *37*). The first extensive identification of proteins in white wine was provided in 2004 (*16*) and further investigations followed in 2006 and 2008 (*18*, *19*).

Even though many factors such as grape variety, vintage, climate, soil, and vinification process are considered to have an influence on the protein composition of a wine, it seems that there is a highly similar set of proteins present in all wines (*1*, *5*, *14*, *20*).

The allergenic potential of wine proteins is an aspect of great actual and political interest. During recent years a couple of case studies reporting allergic reactions against grapes and wine were published (*21*−*27*), and some proteins were already described as potential grape and wine allergens with the lipid transfer protein accepted as an allergen by the International Union of Immunological Societies Allergen Nomenclature Subcommittee (*26*, *27*). Besides grape proteins, potential allergens might be brought in during vinification, for example, as fining agents such as casein, ovalbumin, and lysozyme. These are included in Directive 2007/89/EC, which contains a list of food additives that could be risk factors for allergenic individuals and therefore have to be listed on the product label. Current investigations cannot exclude the presence of dried egg white and lysozyme in wine (*28*), but allergic reactions in sensitized mice could not be detected (*29*).

Due to the problems with haze formation, most research has been done using white wine. This paper focuses on the identification of proteins in red wine (Portugieser) and a comparison between the protein composition of red, rosé (Portugieser Weissherbst), and white wines (Riesling) from Germany using SDS-PAGE and ESI-Q-TOF-MS. To our knowledge this is the first analysis of the protein content of German wine and red wine so far. As we were able to identify a known grape allergen, lipid transfer protein isoform 4, in German red wine, we extended our investigations by anaylzing its occurrence in different red wine varieties of different countries.

MATERIALS AND METHODS

Materials. The chemicals were of high purity and purchased from commercial companies. Acrylamide, ammonium persulfate, 2-mercaptoethanol, SDS, Tris, glycine, disodium hydrogen phosphate, Coomassie brilliant blue, sodium chloride, acetic acid, methanol, Schiff's reagent, and formic acid in acetonitrile (LC-MS grade) were from Roth (Karlsruhe,

*Author to whom correspondence should be addressed (telephone + 0049-6131-39-23570; fax + 0049-6131-39-23557; e-mail hdecker@uni-mainz.de).

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Germany), bovine serum albumin and periodic acid were from Sigma (Steinheim, Germany), protein standard was from Bio-Rad (Munich, Germany), sodium dihydrogen phosphate was from Merck (Darmstadt, Germany), and bromphenol blue and Polyclar^{AT} were from Serva (Heidelberg, Germany).

Wine and Concentration of Proteins. The wines used in this study are Portugieser 2005 from Palatinate (Germany), Dornfelder 2002, 2003, 2004, and 2005 from Rhinehessen (Germany), Pinot Noir 2005 from Rhinehessen (Germany), Riesling 2006 from Rhinehessen (Germany), Portugieser rosé wine 2006 from Rhinehessen (Germany), Cabernet Sauvignon 2005 from Rhinegau (Germany), Shiraz 2006 from Australia, Chianti 2005 from Italy, Cabernet Sauvignon 2006 from California, and Bordeaux 2007 from France. Riesling, Dornfelder, and Pinot Noir were obtained from a winemaker in Mainz (Rhinehessen, Germany), and Cabernet Sauvignon (Rhinegau, Germany) was obtained from the Research Centre Geisenheim. Portugieser red wine, Portugieser rosé wine, Shiraz, Chianti, and Cabernet Sauvignon from California as well as Bordeaux were purchased from commercial supermarkets.

To concentrate the wine it was dialyzed for at least 5 days against water by regenerated cellulose dialysis membranes of 3.5 kDa molecular mass cutoff (Spectra/Por from Roth, Karlsruhe, Germany), and the water was changed twice daily. After the samples had been frozen at −30 °C by spin-freezing, they were fully lyophilized for at least 24 h (Alpha 1-4 LSC from Christ, Osterode, Germany). Two hundred milliliters of Riesling white wine and Portugieser Weissherbst resulted in about 120 mg of lyophilized powder and 200 mL of Portugieser red wine in about 220 mg of lyophilized powder.

Treatment with PVP. To remove substances interacting with proteins, such as polyphenols, the red wine was treated with polyvinylpyrrolidone (PVP). Thirty milligrams of lyophilized wine powder was dissolved in 3 mL of 0.1 M sodium phosphate buffer, pH 7.0; 300 mg of PVP was added, and the mixture was stirred overnight and then centrifuged at 3000*g* for 10 min. Afterward, the procedure was repeated once and remaining PVP was removed by filtering through a 0.22 μm polyethersulfone filter before the sample was applied to SDS-PAGE, which was silver-stained using the method by Blum et al. (*32*). Due to the low amount of protein, the sample was concentrated by fully lyophilizing it before SDS-PAGE separation and Coomassie staining before mass spectrometric analysis.

SDS-PAGE. Discontinuous SDS-PAGE was performed using the method by Laemmli, as modified by See and Jackowski (*30*, *31*). For comparison of the different wine varieties and homemade 12.5% polyacrylamide gels and for the further identification with mass spectrometry, 10−17.5% gradient polyacrylamide gels were used, each with 3% stacking gels.

The lyophilized samples were redissolved in 0.1 M sodium phosphate buffer, pH 7.0, and mixed in equal amounts with SDS sample buffer [25% (v/v) 0.5 M Tris (pH 6.8)/20% (v/v) glycerin/10% (v/v) 2-mercaptoethanol/4% (w/v) SDS with a small amount of bromphenol blue]. Before being subjected to gel electrophoresis, the samples were denaturated for 5 min at 95 °C. As protein standard the Precision Plus Protein Standard from Bio-Rad was used. A voltage of 100 V was applied to the gel for 90 min at room temperature, and the proteins were visualized by staining with Coomassie brilliant blue R-250 or silver nitrate using the method by Blum et al. (*32*).

Glycoprotein Detection. To characterize the presence of carbohydrates, SDS-PAGE was performed as described and the gel stained directly afterward with periodic acid−Schiff (PAS) staining (*33*) modified as follows: after SDS-PAGE, the gel was fixed in 50% (v/v) methanol/40% (v/v) acetic acid/10% (v/v) water. After washing with 7.5% acetic acid in water, it was oxidized with 1% periodic acid for 20 min at 4 °C in the dark. After washing the gel again, it was stained with Schiff's reagent at 4 °C in the dark for 15 min. The gel was destained in 40% (v/v) methanol/10% (v/v) acetic acid.

In-Gel Digest and Mass Spectrometry. SDS-PAGE gels were stained with Coomassie blue R-250 (Sigma) and destained for 30 min, and the relevant protein bands were excised and sliced into small pieces. After destaining and drying, gel slices were reduced with 2 mM DTT at 55 °C and alkylated with 20 mM iodacetamide at room temperature in the dark for 1 h each. After washing and drying, trypsin digests were done at 37 °C overnight (1 μg of trypsin per gel slice). The resulting peptides were transferred into an autosampler vial for peptide analysis via LC-MS/MS.

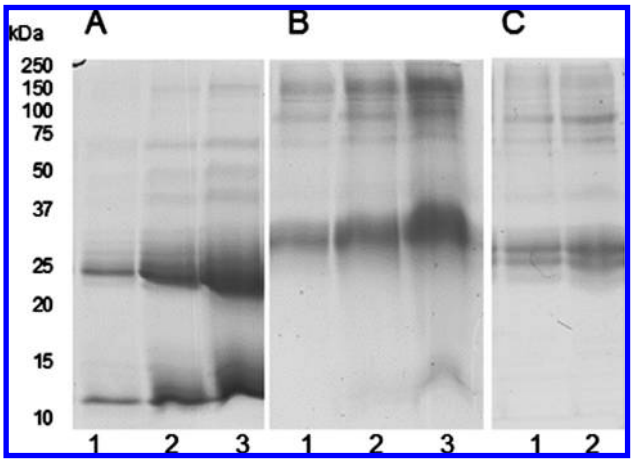

Figure 1. SDS-PAGE (12.5%) analysis of wine proteins: (**A**) Portugieser red wine (lane 1, 0.125 mg; lane 2, 0.625 mg; lane 3, 1.25 mg of lyophilized wine); (**B**) Portugieser Weissherbst (lane 1, 1.875 mg; lane 2, 3.75; lane 3, 7.5 mg of lyophilized wine); (**C**) Riesling white wine (lane 1, 0.5 mg; lane 2, 1 mg of lyophilized wine). Note that the lyophilized wine contains proteins and unknown amounts of pigments as well.

UPLC Configuration. Capillary liquid chromatography of tryptic peptides was performed with a Waters NanoAcquity UPLC system equipped with a 75 μm × 150 mm BEH C18 reversed phase column and a 2.6 μL PEEKSIL-sample loop (SGE, Darmstadt, Germany). The aqueous mobile phase (mobile phase A) was H_2O with 0.1% formic acid. The organic mobile phase (mobile phase B) was 0.1% formic acid in acetonitrile. Samples (2.6 μL injection) were loaded onto the column in direct injection mode with 3% mobile phase B for 15 min at 400 nL/min, followed by an additional 10 min wash (3% B) for 10 min at 300 nL/min. Peptides were eluted from the column with a gradient from 3 to 35% mobile phase B over 90 min at 300 nL/min followed by a 20 min rinse of 80% mobile phase B. The column was immediately re-equilibrated at initial conditions (3% mobile phase B) for 20 min. [Glu^1]fibrinopeptide was used as lockmass at 300 fmol/μL. Lockmass solution was delivered from the auxiliary pump of the NanoAcquity system at 400 nL/min to the reference sprayer of the NanoLockSpray source. Samples were analyzed in triplicate.

Mass Spectrometer Configuration. Mass spectrometry analysis of tryptic peptides was performed using a Waters Q-TOF Premier API system, operated in V-mode with typical resolving power of R = 10.000. All analyses were performed using positive mode ESI using a NanoLockSpray source. The lock mass channel was sampled every 30 s. The mass spectrometer was calibrated with a [Glu1]fibrinopeptide solution (300 fmol/μL) delivered through the reference sprayer of the NanoLockSpray source. For fragment identification, the instrument was run in data-directed acquisition mode, with the three most intense peaks selected for MS-MS fragmentation analysis. Fragmentation of the parent ion was achieved by collision with argon atoms. Collision energy was varied from 15 to 35 eV dependent on precursor ion mass and charge. The integration time for the TOF analyzer was 1 s with an interscan delay of 0.1 s.

Data Processing and Protein Identification. The liquid chromatography tandem mass spectrometry data were processed and searched by using PROTEINLYNX GLOBAL SERVER, ver. 2.3. (Waters). Protein identifications were assigned by searching a hybrid database containing all known protein sequences from *Vitis vinifera* (containing 54988 sequences from the TREMBL database, 904 sequences from the GenBank database, and 158 sequences from the RefSeq database), *Saccharomyces cerevisiae* (6747 sequences from the Swissprot database), and known possible contaminants (trypsin, human keratins) and typical fining reagents (casein, ovalbumin, lysozyme, collagens) with the precursor and fragmentation data afforded by the LC-MS/MS acquisition method. The maximum mass error tolerance values were typically 15 ppm for precursor ions and 30 ppm for fragment ions. Peptide identifications were restricted to tryptic peptides with no more than one missed cleavage and cysteine carbamidomethylation, allowing for the following variable modifications [oxidation (Met), deamidation (Asn, Gln)]. All identified peptide sequences were verified by

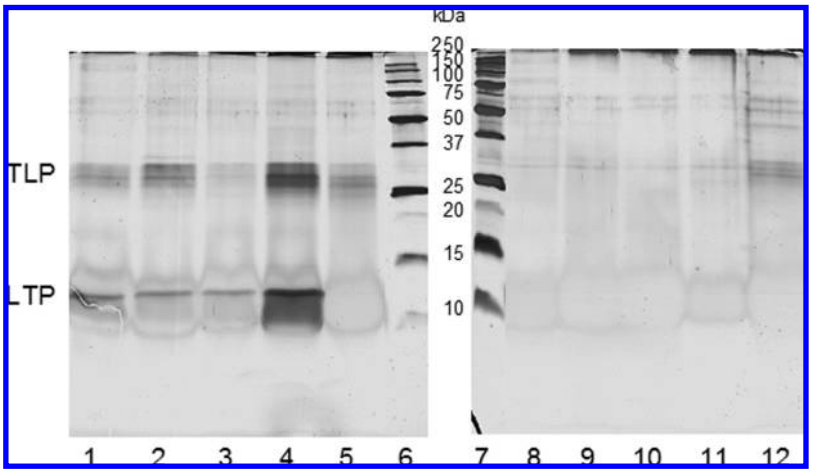

Figure 2. Silver-stained SDS-PAGE (12.5%) analysis of red wines after treatment with PVP: lane 1, Dornfelder 2002 (Germany); lane 2, Dornfelder 2003 (Germany); lane 3, Dornfelder 2004 (Germany); lane 4, Dornfelder 2005 (Germany); lane 5, Pinot Noir (Germany) 2005; lane 6, marker; lane 7, marker; lane 8, Cabernet Sauvignon (Germany); lane 9, Cabernet Sauvignon (USA); lane 10, Shiraz (Australia); lane 11, Chianti (Italy); lane 12, Bordeaux (France). After dialysis and lyophilization, the red wines were treated with PVP to remove interacting substances. The positions of the lipid transfer proteins (LTP) and thaumatin-like proteins (TLP) are indicated.

manual interpretation of the fragment spectra (requiring at least five consecutive *y*-ions and no major unassigned fragment ions). To assess the false-positive rate of protein identification, the database search was repeated using a randomized database generated from the composite database described above. Using the stringent criteria we applied for manual interpretation of the fragment spectra, the false-positive rate of peptide identification was calculated to be $<1\%$.

ELISA. Wines have been tested for the presence of casein using the RIDASCREENFAST Casein ELISA (R-Biopharm AG, Germany) following the test kit description (LOQ = 0.5 mg/kg, LOD = 0.12 mg/kg, recovery = 85−110%) and egg white proteins using the RIDASCREENFAST Ei/Egg ELISA (R-Biopharm AG) according to the leaflet (LOQ = 0.5 mg/kg whole egg powder, LOD = 0.27 mg/kg whole egg powder, recovery = 87−115%).

RESULTS AND DISCUSSION

We analyzed the protein compositions of different varieties of red, rosé, and white wines. In the case of red wine we studied eight different varieties as well as four different vintages of one of these wines. In the overall protein composition we found striking similarities—albeit differences in the protein amounts and composition were found in the final products (**Figure 1**).

All of the red wines contain protein bands with molecular masses ranging from 25 to 30 kDa in different intensities as well as at approximately 60 and 70 kDa (**Figure 2**). However, only two wine varieties (Dornfelder and Portugieser) contain an intense protein band at approximately 12 kDa (**Figure 2**), which was identified as lipid transfer protein by mass spectrometry.

As red wines contain high amounts of coloring polyphenols it is necessary to remove these substances before separation by SDS-PAGE. Therefore, dialyzed and lyophilized red wine samples were treated with polyvinylpyrrolidone (PVP), a substance known to interact with polyphenols. Whereas all other red wine varieties required PVP treatment, solely the Portugieser red wine could be separated by SDS-PAGE without any PVP treatment, which might be due to the lower polyphenol content and a lower protein−polyphenol complexation (*38*). For that reason we chose Portugieser as model wine to analyze the protein composition in detail by mass spectrometry. After excision and in-gel digestion with trypsin, we identified several proteins by ESI-Q-TOF mass spectrometry. The proteins were either derived from the grape or released from the yeast during the process of vinification (**Table 1**; Supporting Information Table 1).

The electrophoretic comparison of the protein composition of Portugieser red wine with that of the rosé wine (Portugieser) in **Figure 3** and the white wine (Riesling) in **Figure 1c** illustrates that a highly similar set of proteins seems to occur in all wine varieties; however, the exact protein composition differs depending on the particular wine (**Figure 1**). Each of the investigated wine varieties contains low amounts of proteins with molecular masses ranging from about 12 to >100 kDa.

Grape Proteins. Several of the identified proteins derived from the grape and could be assigned to the following protein families (**Table 1**; Supporting Information Table 1).

Lipid Transfer Proteins. In Portugieser red wine, the intense band with a M_r of approximately 12 kDa, as determined by SDS-PAGE (band 2 in **Figure 3**), could be identified as LTP isoform 4 from *Vitis vinifera* (AAO33394). To our knowledge this is the first study showing its presence in red wine, whereas its occurrence in grapes (*27*) and Chardonnay white wine has already been reported (*18*). Okuda et al. (*18*) discussed finding a hydrolysis product of LTP (AF 467945) in Chardonnay, because the M_r determined by two-dimensional gel electrophoresis was smaller (9.6 kDa) than the theoretical. Our results let us hypothesize that the LTP isoform 4, which is known as grape allergen (*26*), is not hydrolyzed during the vinification, because the M_r determined by SDS-PAGE is in accordance with the theoretical M_r derived from the database.

In addition to the LTP isoform 4, the investigated Portugieser red wine contains a LTP from *Vitis aestivalis* (AAQ96338) as well as a LTP from *Vitis berlandieri* $\times$ *V. vinifera* (AAO33357) (**Table 1**). The M_r values of these LTPs determined by SDS-PAGE are smaller than predicted from the sequence, so these might be present as hydrolysis products (band 1 in **Figure 3**).

LTP isoform 4 from *V. vinifera* was also identified in Dornfelder red wine of the vintages 2002, 2003, 2004, and 2005 by mass spectrometry, whereas the presence of LTPs could not be detected in all of the investigated wines (**Figure 2**). Several wine varieties (Shiraz, Cabernet Sauvignon, Chianti, Bordeaux, and Pinot Noir) did not show any bands at a molecular mass range below 20 kDa. These results let us hypothesize that LTPs are not universally present in bottled red wine, at least not in amounts detectable with Coomassie staining. The reasons therefore are quite unclear. Wine variety and growing conditions as well as the vinification process can influence the protein composition of the end product wine. Because LTPs are present mainly in the skin of

Table 1. Identified Proteins from Portugieser Wine

band (mass [kDa], exptl)	identified protein	mass [kDa] theor	species	coverage [%]	peptides	accession/gi no.
1 (9)	ns lipid transfer protein	11.7	*V. berlandieri* × *V. vinifera*	14.3	2	AAO33357/8193996
	lipid transfer protein	11.8	*V. aestivalis*	14.3	2	AAQ96338/37625029
2 (12)	lipid transfer protein isoform 4	11.7	*V. vinifera*	53.8	3	AAO33394/28194086
	hypothetical protein	11.7	*V. vinifera*	20.0	2	CAN70512/147802122
	lipid transfer protein	11.8	*V. aestivalis*	14.3	2	AAQ96338/37625029
3 (25)	VVTL1	24.0	*V. vinifera*	36.9	7	AAB61590/2213852
	putative thaumtin-like protein	24.0	*V. vinifera*	36.9	7	CAB85637/7406716
	thaumatin-like protein	23.8	*V. vinifera*	30.7	6	ABD64681/89242712
	putative thaumatin-like protein	20.1	*V. vinifera*	26.8	6	CAB85636/7406714
	class IV endochitinase	27.2	*V. vinifera*	16.5	7	AAB65776/2306811
	hypothetical protein	21.1	*V. vinifera*	14.4	3	CAN67019/147773144
4 (37)	vacuolar invertase 1 GIN 1	71.5	*V. vinifera*	7.2	3	AAB47171/1839578
	protein TOS1 precursor (target of SFB1)	48.0	*S. cerevisiae*	5.3	4	P38288/586301
5 (47)	vacuolar invertase 1 GIN 1	71.5	*V. vinifera*	13.9	7	AAB47171/1839578
	cell wall protein 11 precursor	23.2	*S. cerevisiae*	11.5	3	P47001/1353025
	extracellular matrix protein 33 precursor	48.3	*S. cerevisiae*	6.8	3	P38248/1351738
6 (61)	cell wall protein CWP1 precursor	24.3	*S. cerevisiae*	37.7	7	P28319/465661
	vacuolar invertase 1 GIN 1	71.5	*V. vinifera*	12.8	7	AAB47171/1839578
	cell wall protein 11 precursor	23.2	*S. cerevisiae*	11.5	3	P47001/1353025
	probable glycosidase CRH1 precursor	53.0	*S. cerevisiae*	10.7	6	P53301/1723734
	extracellular matrix protein 33 precursor	48.3	*S. cerevisiae*	6.8	3	P38248/1351738
7 (77)	vacuolar invertase 1 GIN 1	71.5	*V. vinifera*	15.3	10	AAB47171/1839578
	cell wall protein 11 precursor (CIS3 protein)	23.2	*S. cerevisiae*	11.5	3	P47001/1353025
8 (150)	probable glycosidase CRH1 precursor	53.0	*S. cerevisiae*	19.5	11	P53301/1723734
	endochitinase precursor	59.0	*S. cerevisiae*	3.2	2	P29029/1705815

grapes, especially the time of skin contact during the vinification process could be of importance. On the other hand, different kinds and amounts of fining agents, especially bentonite, can have an impact on the appearance of proteins in wine. This will be discussed in detail in a following study. LTPs are part of the defense system of plants, and climatic conditions could influence the occurrence of these proteins in wine as well.

Among the group of pathogenesis-related (PR) proteins, LTPs belong to family 14 (*35*). Because LTPs are resistant against proteolytic activity and acidic conditions, they can survive the vinification, and it seems consistent to find them in the end product wine.

As shown in **Figure 1b**,**c** LTPs are almost absent in Portugieser rosé wine and Riesling white wine. This may be due to exclusion by the vinification process, because for white and rosé wines the alcoholic fermentation takes place on the must, whereas the red wine is fermented on the mash. This in turn means that proteins of the grape skin will be found to a much lower extent in the white and rosé wines compared to red wine. This is in agreement with earlier reports in which LTP is discussed to be a protein present mainly in the skin and seeds of plants (*34*). LTPs have already been described as potential allergens in grapes (*23*, *26*, *27*) and in many other plants, especially within the family of Rosaceae fruits in Mediterranean countries (*35*, *36*). Thus, further tests to evaluate the allergenic potential of LTPs in red wine are in progress.

Thaumatin-like Proteins. Very intense bands were detected with molecular masses of approximately 25 kDa in Portugieser red wine, which contains a couple of TLPs from *V. vinifera* (**Figure 3**; **Table 1**). One band was assigned to the VVTL 1 (AAB61590) by mass spectrometry. In addition, other (putative) TLPs were identified on the basis of known sequences

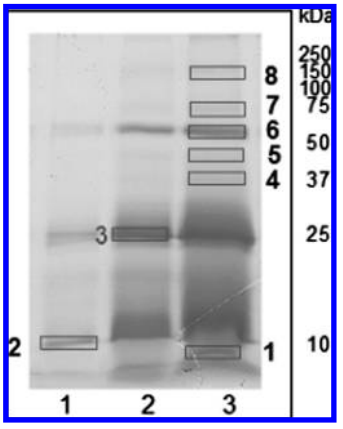

Figure 3. SDS-PAGE (10−17.5%) analysis of Portugieser red wine: lane 1, 1 mg of lyophilized wine; lane 2, 2 mg of lyophilized wine; lane 3, 4 mg of lyophilized wine. Identified proteins are listed in **Table 1**. The marked protein bands were excised for mass spectrometry analysis. SDS-PAGE was performed after dialysis and lyophilization.

(CAB85636, ABD64681, CAB85637). However, one protein could not be directly attributed to TLP, but showed sequence similarity to TLP (CAN67019) (**Table 1**).

Following PVP treatment, in Shiraz from Australia and in Chianti from Italy as well as in Cabernet Sauvignon from California and Germany, protein bands in the molecular mass range of TLPs are detectable by SDS-PAGE in small amounts, whereas the bands for Bordeaux, Pinot Noir, and Dornfelder are more intense. Reasons for these differences could be similar to those discussed before for the LTPs such as differences during the vinification, especially fining with bentonite.

TLPs are grouped in family 5 of the PR proteins (*34*) and have been described in white wine (Sauvignon Blanc, Muscat of Alexandria, Chardonnay) before (*6*, *18*, *19*, *24*, *37*). We could detect TLPs in white (Riesling) and red (Portugieser) wine by mass spectrometry, and the SDS-PAGE suggests its presence in rosé wine (Portugieser Weissherbst) as well (**Figure 1**).

TLPs are known as allergens in some fruits, for example, as a major allergen in apple, and are also discussed as being potential grape allergens (*26*, *34*).

Endochitinase. Class IV endochitinase from *V. vinifera* (AAB65776) could be identified in Portugieser red wine (**Table 1**). The class IV endochitinase belongs to family 3 of PR proteins and, as other chitinases, it acts antimycotically through hydrolyzing chitin, a main component of fungal cells (*34*). The class IV endochitinase was also identified in Sauvignon Blanc and Chardonnay (*16*, *19*), whereas two different chitinases had already been found in Muscat of Alexandria (*37*). Pastorello et al. hypothesized that the class IV endochitinase may act as an allergen of Vino Novello (*V. vinifera*) and Fragolino (*Vitis labrusca*) (*26*), which could not be confirmed for *V. vinifera* grapes by Vassilopoulou in patients with reported grape allergy (*27*). Further tests remain to be done, especially using purified endochitinase.

Vacuolar Invertase. Protein bands with molecular masses of 37, 47, 61, and 77 kDa as determined by SDS-PAGE could be attributed to vacuolar invertase 1 from *V. vinifera* (AAB47171) with a theoretical molecular mass of about 71.5 kDa (**Figure 3**; **Table 1**). It has already been reported that the invertase was hydrolyzed during vinification (*18*), which could be confirmed by our investigations (**Figure 3**; **Table 1**). The vacuolar invertase is located in the pulp and responsible for the accumulation of the hexoses glucose and fructose in the grape berry during ripening. This protein is present in Portugieser red wine as well as Riesling as identified by mass spectrometry. The presence of vacuolar invertase was already described in Sauvignon Blanc and Chardonnay (*16*, *18*, *19*).

Yeast Proteins. A number of yeast proteins were detected in Portugieser red wine. Nowadays, specific yeast strains are added during the vinification to achieve a controlled fermentation process. By mass spectrometry several proteins were identified: the covalently linked cell wall protein 11 precursor (P47001), the cell wall protein CWP1 precursor (P28319), the protein TOS1 precursor (P38288), the extracellular matrix protein 33 precursor (P38248), a probable glycosidase CRH1 precursor (P53301), and an endochitinase precursor (P29029) (**Table 1**). These proteins are part of the cell wall of *Saccharomyces cerevisiae* and are likely to be released into the wine during the process of vinification.

The molecular masses determined by SDS-PAGE differ from the theoretical ones derived from the sequence (**Table 1**). This could be due to different glycosylations, because it is known that the yeast proteins are highly glycosylated.

Glycoproteins. The few proteins above 100 kDa were not further identified, because they are not visible with Coommassie staining, which is used for the following mass spectrometry analysis (**Figure 3**). That is probably due to their high proportion of carbohydrates.

Due to the periodic acid−Schiff staining we hypothesize the proteins above 100 kDa as being glycoproteins (**Figure 4**). Those proteins are likely to be yeast proteins and brought in during the process of vinification, because we do not see them in the same intensity when staining grape proteins with PAS (data not shown). We localized glycosylated proteins at a molecular mass range between 20 and 70 kDa. Thus, a major part of the wine protein seems to be glycosylated (**Figure 4**).

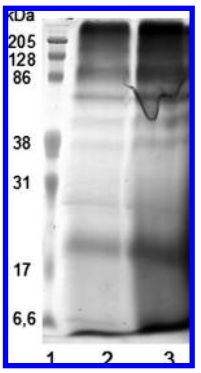

Figure 4. SDS-PAGE (12.5%) analysis of wine glycoproteins from Portugieser red wine, PAS staining after SDS-PAGE: lane1, marker; lane 2, 0.3 mg of lyophilized sample; lane 3, 0.6 mg of lyophilized sample.

Of the yeast proteins detected with Coomassie staining and mass spectrometry cell wall protein 11 precursor (P47001), cell wall protein CWP1 precursor (P28329), and endochitinase precursor (P29029) are known to be extensively O-glycosylated. Protein TOS 1 (P38288), the probable glycosidase CRH1 precursor (P53301), and Protein ECM33 (P38248) have potential glycosylation binding sites.

Fining Agent Proteins. German Portugieser red wine and Dornfelder have been tested for the presence of casein and egg white proteins by ELISA (R-Biopharm AG). In both cases the results are well below the limit of quantification (LOQ) of the test systems; no allergenic proteins have been found. Using mass spectrometry, we were not able to detect the presence of fining agent proteins in the bands analyzed.

In conclusion, 12 grape and 6 yeast proteins were identified with their original function in German Portugieser as an example of red wine (**Table 1**). It should be noted that other proteins might occur in much lower amounts and therefore are not detectable by the applied methods.

Comparison with Portugieser Weissherbst exemplary for rosé wine and Riesling as white wine showed significant differences in the protein composition. Still, PR proteins provide the main contribution in all of the varieties. Their occurrence in wine should be due to their stability and resistance against proteolysis and acidic conditions, which allows them to survive the vinification.

This is the first study to show that LTPs are common for Portugieser and Dornfelder red wines detected in wines from different vintages. However, other investigated red wine varieties, such as Cabernet Sauvignon, Shiraz, and Pinot Noir, do not contain LTPs detectable by SDS-PAGE in combination with mass spectrometry. A proteomic approach for validation and extension of the data is planned.

Thus, proteins that may cause instability, namely, thaumatin-like proteins and chitinase, are found in the red wines tested as are proteins described as potential grape allergens, especially LTPs. Further investigations need to be done to test the relevance for the consumer and allergic patient.

ABBREVIATIONS USED

PR proteins, pathogenesis-related proteins; LTP, lipid transfer proteins; TLP, thaumatin-like proteins; VVTL, *Vitis vinifera* thaumatin-like protein 1; LC, liquid chromatography; HPLC, high-pressure liquid chromatography; MS, mass spectrometry.

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Supporting Information Available: Supplementary table. This material is available free of charge via the Internet at http://pubs.acs.org.

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