

Immunochemical and Mass Spectrometry Detection of Residual Proteins in Gluten Fined Red Wine

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 Supporting Information

ABSTRACT: Recently, wheat gluten has been proposed as technological adjuvant in order to clarify wines. However, the possibility that residual gluten proteins remain in treated wines cannot be excluded, representing a hazard for wheat allergic or celiac disease patients. In this work, commercial wheat glutens, in both partially hydrolyzed (GBS-P51) and nonhydrolyzed (Gluvital 21000) forms, were used as fining agents in red wine at different concentrations. Beside immunochemical analyses using anti-gliadin, anti-prolamin antibodies and pooled sera of wheat allergic patients, a method based on liquid chromatography coupled to mass spectrometry has been proposed to detect residues of gluten proteins. Residual gluten proteins were detected by anti-prolamin antibodies, anti-gliadin antibodies and sera-IgE only in the wine treated with GBS-P51 at concentration 50, 150, and 300 g/hL, respectively, whereas no residual proteins were detected by these systems in the wine treated with Gluvital 21000. In contrast liquid chromatography—mass spectrometry analyses allowed the detection of proteins in red wines fined down to 1 g/hL of Gluvital 21000 and GBS-P51. Our results indicate that MS methods are superior to immunochemical methods in detecting gluten proteins in wines and that adverse reactions against gluten treated wines cannot be excluded.

KEYWORDS: wheat gluten, fining, wine, mass spectrometry

INTRODUCTION

Wheat gluten is a flour fraction, mainly constituted of water-insoluble proteins (gliadins and glutenins that represent about 80% of total wheat proteins), containing also lipids (ca. 3.5–6.8%), minerals (ca. 0.5–0.9%) and carbohydrates (7.0–16.0%), the latter mainly consisting of starch and lesser amounts of nonstarch polysaccharides.¹

Wheat gluten is widely used in the food industry as an ingredient in processed foods, and it could be utilized as a dough strengthener, formulation aid, nutrient supplement, stabilizer and thickener and texturing agent.¹ Recently, gluten has also been proposed as wine fining agent and its use was authorized by Food Standards Australia New Zealand (FSANZ)² and EC REGULATION No. 2165 (2005).³ Fining wine is the process of removing undesirable substances such as phenolic compounds in order to prevent colloidal precipitation phenomena and to reduce bitterness and astringency.⁴ In red wines this is commonly done by adding proteins such as gelatin, isinglass, albumin and casein.⁵ These protein–phenol complexes are then removed by decanting, centrifugation or filtration.

Previous studies showed that commercial wheat gluten, especially when in the form of a hydrolyzed preparation, allows very efficient clarification of the wine,^{6,7} and it has also been demonstrated that wheat gluten selectively precipitates condensed tannins from red wine and from a model one.⁸

Nevertheless, it is well-known that the gluten proteins are involved in both food allergy and celiac disease (CD) that occur in sensitive individuals after consumption of wheat products.⁹ It has been demonstrated that several gluten proteins, such as α/β -, γ -, ω -gliadins, are implicated in wheat allergy.¹⁰ Gliadins, particularly the α -gliadins, are also responsible for celiac disease,

producing the most severe effects.¹¹ The gluten proteins are soluble in alcoholic and acidic solution,¹² and their solubility could increase after hydrolysis due to their conversion in low molecular mass protein derivatives. On the basis of these considerations, the possibility that gluten proteins remain in the wine after treatment cannot be excluded, representing a potential hazard for wheat allergic or CD patients.

As there are no safety threshold values for allergen contents in food, it is necessary to provide consumers with all the information on food composition on the label.¹³ A list of allergenic foods to be declared in food labels has been drafted by legislation in USA and Europe. In particular the Directive 2006/142/EC of the European Commission extended the list to 14 foods, including cereal containing gluten.¹⁴

So, detection of residual gluten proteins in gluten treated wines has become an essential safety and legal issue.

In previous studies no gluten-associated immunochemical reaction was observed, by immunoblotting or dot-blot, in red and white wine treated with raw, hydrolyzed and deamidated gluten by using polyclonal specific antibodies or allergic patients' sera.^{6,15,16} Also, gluten antibodies were not able to recognize their antigens by ELISA in a Muscat must treated with deamidated gluten.¹⁷ These results seem to exclude a risk for subjects with celiac disease or gluten allergy.

Recently, mass spectrometry (MS) methods for the direct and absolute identification and quantification of food allergens have

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Table 1. Analytical Characteristics of the Merlot Wine before Fining

total acidity (g/L tartaric acid)	6.0
pH	3.34
alcohol content (% v/v)	12.1
anthocyanins ^a (mg/L)	498
proanthocyanidins ^a (mg/L)	2257

^a Means of the three determinations. Standard deviation <0.5%.

been developed. MS allows the detection of allergenic proteins in trace amounts, thanks to its high sensitivity. Previously, Weber et al.¹⁸ detected caseins in cookies through a method based on nano liquid chromatography–tandem mass spectrometry (LC–MS/MS), while Monaci and van Hengel¹⁹ developed a method for the quantification of whey allergen traces in mixed-fruit juice by using liquid chromatography coupled to MS. Also the major allergens of peanut were detected by using LC–MS/MS in vanilla ice cream²⁰ and in dark chocolate.²¹ Moreover, a method to detect allergenic milk proteins in fined white wines based on capillary liquid chromatography combined with electrospray ionization tandem mass spectrometry has also been proposed.²² Recently, a method based on combinatorial peptide ligand libraries (CPLLs) coupled to MS has been applied for identifying traces of casein, used as fining agent, in white²³ and red wines.²⁴

Nevertheless, the detection of residual fining agents seems to be particularly complicated in red wines, as proteins are difficult to analyze for the presence of a large quantity of interfering compounds, such as polyphenols and carbohydrates.²⁵

The aim of the present study is to evaluate the presence of gluten residual proteins in a red wine after its clarification with two commercial gluten samples. Beside the classical immunochemical analyses, using a polyclonal antibody against a wide range of gluten proteins and sera of wheat allergic patients, a method based on liquid chromatography coupled to MS for the detection of residual gluten proteins in red wine has been developed.

MATERIALS AND METHODS

Materials. A commercial wheat vital gluten (Gluvtal 21000, Cere-star, Italia), a partially hydrolyzed commercial wheat gluten (GBS-P51, Chamtor, Francia) and a wheat gluten prepared in our laboratory from a commercial wheat semolina (WG) were used.

The red wine (cv. Merlot) used for the fining experiments was produced during the 2008 vintage. Some analytical characteristics of the wine are reported in Table 1.

Wine Analyses. Alcohol, pH and titratable acidity were determined using the OIV methods.²⁶

Anthocyanins were determined using the method of Riberau-Gayon and Stonestreet.²⁷ Proanthocyanidins were quantified according to Pompei et al.²⁸

In-House Wheat Gluten Preparation. 100 g of durum wheat semolina was mixed with 40 mL of water to obtain dough. Gluten was prepared by washing the dough under tap water for 2–3 min in order to eliminate water-soluble proteins and starch. The gluten ball preparation was freeze-dried and grinded into a soft powder (WG).

Nitrogen Quantification and Protein Content Determination. Samples were mineralized according to the method of Hach et al.²⁹ and nitrogen was quantified by the AOAC method 33.056.³⁰ The protein content was calculated as $N \times 5.7$.

Evaluation of Wheat Gluten Solubility. The solubility of the different wheat gluten samples was evaluated in model wine. 50 mg of

each sample was solubilized in 50 mL of model wine (12% (v/v) ethanol, 5% tartaric acid, pH 3.5). After 24 h in continuous stirring, the samples were centrifuged at 12000g for 30 min and the soluble nitrogen was measured according to the method of Hach et al.²⁹

Statistical Analysis. Total protein and protein soluble in model wine were performed in triplicate. Statistical analysis was calculated by Student's *t* test.

Fining Experiments. Gluvtal 21000 and GBS-P51 were used for fining experiments in an unfined Merlot red wine.

Each commercial gluten was suspended in water at a final concentration of 5% (w/v) and stirred for 12 h at room temperature before use. The suspensions were added to 500 mL of wine to final concentrations of 1, 5, 10, 15, 50, 150, 250, 300 g/hL. After settling for 48 h at 20 °C, wine samples were centrifuged at 1900g for 10 min.³¹

Protein Recovery from the Clarified Wine. Protein recovery from the clarified wine was made by the potassium dodecyl sulfate method (KDS method).³² Briefly, 10% (w/v) sodium dodecyl sulfate (SDS) was added to samples (25 mL) to a 0.2% (w/v) final concentration. Samples were heated in boiling water for 10 min. 2 M KCl was then added to reach a final concentration of 400 mM, and samples were gently mixed for a further 45 min at 4 °C. KDS–protein pellets were recovered by centrifugation at 14000g for 15 min at 4 °C.

Electrophoresis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to Laemmli³³ in a Mini Protean II cell (Bio-Rad, Milan, Italy) with a gel containing a total polyacrylamide concentration of 15%.

Ten milligrams of each gluten sample was solubilized in 1 mL of buffer A [0.3 M Tris-HCl, pH 7.4 containing 2% (w/v) SDS and 8% (w/v) glycerol]. After stirring for 1 h, samples were centrifuged at 10000g for 20 min. Fifteen microliter portions of the resulting protein solutions were loaded on the gels.

KDS–protein pellets were resuspended in 500 μ L of buffer A, and 10 μ L of each sample was loaded onto the gels.

Electrophoresis was run at 50 mA constant current until the tracking dye bromophenol blue reached the bottom of the gel. Molecular weight standard proteins (Bio-Rad) were phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa), soy trypsin inhibitor (21.5 kDa) and lysozyme (14.4 kDa).

Dot-Blot. KDS–protein pellets (from 25 mL of clarified wine) were resuspended in 500 μ L of buffer B (0.3 M Tris-HCl pH 6.0) and used for dot-blotting. 45 μ L of resuspended KDS–protein pellets and 10 μ L of a solution (1 mg/mL) of each commercial wheat gluten (as a control) were spotted on the nitrocellulose membrane as described by De Zorzi et al.³⁴

Immunoenzymatic Analysis. Dot-blots were performed according to the method reported by Pasini et al.³⁵ using a polyclonal antibody against wheat prolamins (high molecular weight, low molecular weight and α/β - and γ -gliadins)³⁶ diluted 1:200 as a primary antibody. Anti-rabbit IgG horseradish peroxidase conjugate diluted 1:10000 (Sigma, A-9169) was secondary antibody.

Dot-blots were also developed using an anti-gliadin antibody (Sigma G-9144) diluted 1:3000.

IgE-binding proteins on dot-blots (using pooled sera) were detected by an anti-human IgE peroxidase–conjugate antibody (Sigma) diluted 1:6000. The negative control was a spot of bovine serum albumin (BSA). Peroxidase activity was visualized by chemiluminescence using the Super Signal detection kit (Pierce), following the manufacturer's instructions.

Pooled sera were collected by patients (4 women, 3 men, aged between 25 and 45 years) reporting gastrointestinal symptoms after ingestion of wheat-based foods, selected on the basis of positivity to RAST (radioallergosorbent test) and SPT (skin prick test).

Reverse-Phase LC–MS/MS Analyses. Wines clarified with 25, 15, 10, 5, and 1 g/hL of GBS-P51 and Gluvtal 21000 were used for mass

spectrometry analysis. Proteins recovered from 50 mL of clarified wines by the KDS method were washed with acetone, in order to eliminate SDS, and centrifuged at 12000g for 10 min. The obtained pellets were dried and then dissolved in 50 mM NH_4HCO_3 , 8 M urea, reduced with 10 mM dithiothreitol (DTT) (1 h, 37 °C, in the dark) and alkylated with 30 mM iodoacetamide (1 h, at room temperature, in the dark). One microgram of sequencing grade modified trypsin (Promega, Madison, WI, USA) was added after 1:10 dilution of the samples with 50 mM NH_4HCO_3 to reach a final concentration of 0.8 M urea, and digestion was carried out overnight at 37 °C. Samples were desalted with C18 cartridges (Strata C18-E, 50 mg/1 mL, Phenomenex). Extracted peptides were dissolved in 50 μL of 0.1% formic acid. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) analyses were performed with a 6520 Q-TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) coupled to a chip-based chromatographic interface. A large capacity chip was used, 2 μL samples were injected into the enrichment column (C18, 9 mm, 160 nL volume) at a flow rate of

4 $\mu\text{L}/\text{min}$, and peptides were separated in the C18 nanocolumn (150 mm $\times$ 75 μm) at a flow rate of 0.5 $\mu\text{L}/\text{min}$. Water/formic acid 0.1% and acetonitrile/formic acid 0.1% were used as eluents A and B respectively. The chromatographic separation was achieved by using a gradient of eluent B from 3% to 50% in 50 min. Mass spectra were acquired in a data dependent mode: MS/MS spectra of the 3 most intense ions were acquired for each MS scan in the range of 350–2400 Da. Scan speed was set to 4 MS spectra/s and 3 MS/MS spectra/s. Capillary voltage was set to 1750 V and drying gas to 5 L/s. Raw data files were converted into Mascot Generic Format (MGF) files with MassHunter Qualitative Analysis Software version B.03.01 (Agilent Technologies) and searched using Mascot Search Engine (version 2.2.4 Matrix Science, London, U.K.) against the UniRef100 database (version 15.14, 9757328 sequences, 3429197968 residuals). Enzyme specificity was set to semitrypsin with one missed cleavage using a mass tolerance window of 10 ppm for the precursor ion and 0.05 Da for the fragment ions and carbamidomethyl-cysteine as fixed modification. Proteins with at least 2 peptides with individual significant score ($p < 0.05$) were considered as positively identified.

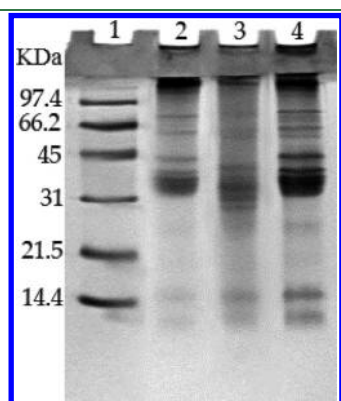


Figure 1. SDS–PAGE in unreducing conditions of wheat glutes used for wine fining, stained with Coomassie Brilliant Blue. Lane 1: MW markers. Lane 2: wheat gluten (WG). Lane 3: GBS-P51 (partially hydrolyzed). Lane 4: Gluvital 21000.

RESULTS

Wheat Gluten Analyses. *Total Protein Content.* Gluvital 21000 contained 89 (SD ± 3.6), GBS-P51 84 (SD ± 2.8) and WG 83 (SD ± 4.3) mg of protein/100 mg of sample, and the remainder of constituents was mainly starch.

Electrophoresis. The protein patterns of the two commercial glutes were analyzed by electrophoresis in nonreducing conditions and compared to WG (Figure 1). As expected, the electrophoretic profile of Gluvital 21000, a gluten retaining its functionality (Figure 1, lane 4), is very similar to WG (Figure 1, lane 2) showing the classical protein components of the wheat flour. These latter comprised α/β - and γ -gliadins (31–45 kDa); ω -gliadins (60–80 kDa) and glutenin polymers remaining blocked at the top of the gel, due to their high molecular mass.³⁷ Protein bands corresponding to members of the protein

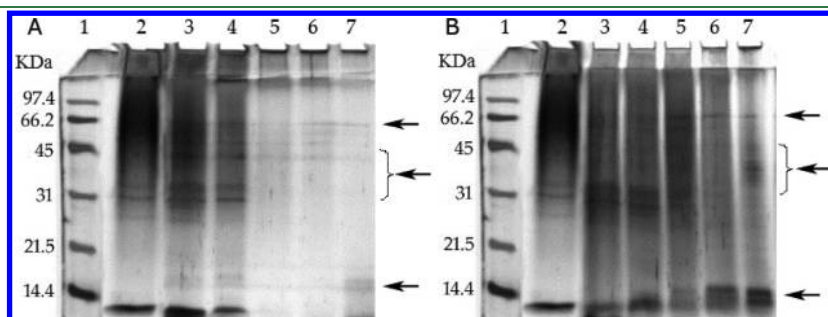


Figure 2. SDS–PAGE of red wine proteins recovered through the KDS method after clarification with wheat glutes (A, GBS-P51; B, Gluvital 21000) at different concentrations, stained with silver. Lane 1: MW markers. Lane 2: unclarified wine. Lane 3–7: wine + 10, 15, 50, 150, 300 g/hL of wheat glutes. The arrows indicate residual gluten proteins with M_r 14 kDa (α -amylase inhibitors), 31–45 kDa (gliadins) and 60 kDa (ω -gliadin).

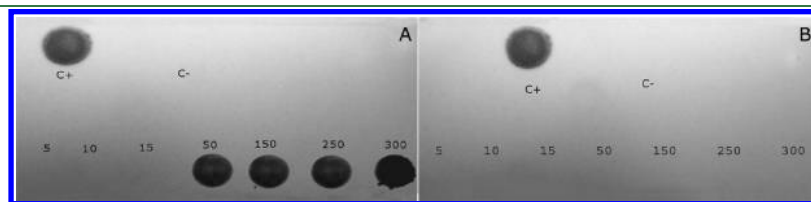


Figure 3. Dot-blot of wine fined with wheat glutes (A, GBS-P51; B, Gluvital 21000) at different concentration (5–300 g/hL), performed with anti-prolamin antibodies. C+ = positive control (GBS-P51 or Gluvital 21000); C- = negative control (untreated wine).

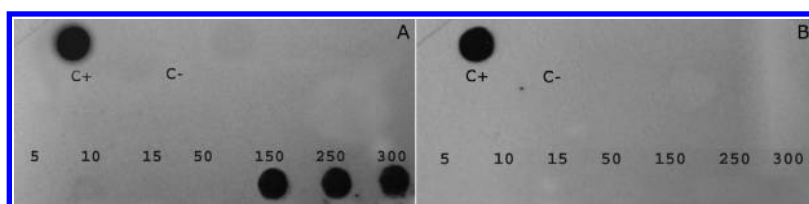


Figure 4. Dot-blot of wine fined with wheat glutens (A, GBS-P51; B, Gluvital 21000) at different concentration (5–300 g/hL), performed with anti-gliadin antibodies. C+ = positive control (GBS-P51 or Gluvital 21000); C– = negative control (untreated wine).

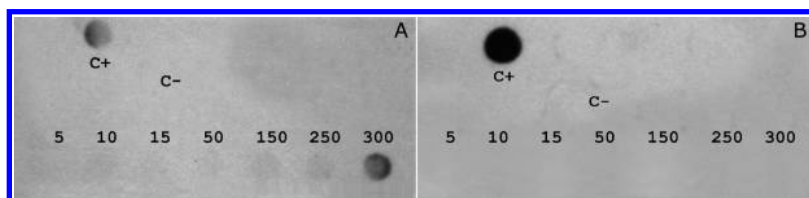


Figure 5. IgE-dot blot of wine fined with wheat glutens (A, GBS-P51; B, Gluvital 21000) at different concentration (5–300 g/hL), performed with pool wheat allergic sera. C+ = positive control (GBS-P51 or Gluvital 21000); C– = negative control (untreated wine).

Table 2. Proteins Identified in Red Wines Fined with 5 g/hL of Gluvital 21000 or GBS-P51^a

accession number	(A) Gluvital 21000 identified protein	species
B2Y2Q1	LMW glutenin subunit	<i>Triticum aestivum</i> (wheat)
Q0GNF9	LMW glutenin	<i>Triticum aestivum</i> (wheat)
B2Y2R4	LMW glutenin subunit	<i>Triticum aestivum</i> (wheat)
Q6QGV8	LMW glutenin pGM107	<i>Triticum aestivum</i> (wheat)
Q1WA40	alpha-gliadin Gli2-LM2-12	<i>Triticum aestivum</i> (wheat)
P17314	alpha-amylase/trypsin inhibitor CM3	<i>Triticum aestivum</i> (wheat)
D0ES81	gamma-gliadin	<i>Triticum aestivum</i> (wheat)
ASJSA7	alpha-gliadin	<i>Triticum aestivum</i> (wheat)
B6UKP6	gamma-gliadin	<i>Triticum aestivum</i> (wheat)
Q4U195	dimeric alpha-amylase inhibitor	<i>Triticum aestivum</i> (wheat)
D2KFG9	gliadin/avenin-like seed protein	<i>Triticum aestivum</i> (wheat)
B5B0D5	major allergen CM16	<i>Triticum aestivum</i> (wheat)
Q2A784	putative avenin-like a	<i>Triticum aestivum</i> (wheat)
A4ZIX1	monomeric alpha-amylase inhibitor	<i>Triticum aestivum</i> (wheat)
B8XU29	alpha-gliadin	<i>Triticum monococcum</i>
Q6UJY7	HMW-glutenin By subunit	<i>Triticum turgidum</i>
A9YSK5	HMW glutenin subunit	<i>Triticum aestivum</i> (wheat)
Q41540	CM 17 protein	<i>Triticum aestivum</i> (wheat)
Q9M4G6	Putative thaumatin-like protein	<i>Vitis vinifera</i> (grape)
Q7XAU7	thaumatin-like protein	<i>Vitis vinifera</i> (grape)
D1IBD6	cysteine-type endopeptidase	<i>Vitis vinifera</i> (grape)
D1IP80	chitinase-like	<i>Vitis vinifera</i> (grape)
ASAYU8	ATP synthase subunit beta	<i>Vitis vinifera</i> (grape)
D1HU00	hydrolase/glycosidase activity	<i>Vitis vinifera</i> (grape)
B6VJY3	ATP synthase subunit alpha	<i>Vitis vinifera</i> (grape)
ASBAX1	putative uncharacterized protein	<i>Vitis vinifera</i> (grape)
D1HDI5	cysteine-type endopeptidase	<i>Vitis vinifera</i> (grape)
D1HTT2	enolase family	<i>Vitis vinifera</i> (grape)
Q7XAU6	class IV chitinase	<i>Vitis vinifera</i> (grape)
ASBUW9	putative uncharacterized protein	<i>Vitis vinifera</i> (grape)
ASAKD8	peptidyl-prolyl cis–trans isomerase	<i>Vitis vinifera</i> (grape)
Q09VU3	phospholipase D alpha	<i>Vitis vinifera</i> (grape)
D1IRB3	ATPase (P-type) family	<i>Vitis vinifera</i> (grape)

Table 2. Continued

(A) Gluvital 21000		
accession number	identified protein	species
B3LP15	protein YGP1	<i>Saccharomyces cerevisiae</i>
B5VJM6	YGR282Cp-like protein	<i>Saccharomyces cerevisiae</i>
Q04E64	60 kDa chaperonin	<i>Oenococcus oeni</i>
(B) GBS-P51		
accession number	identified protein	species
Q0GNF9	LMW glutenin	<i>Triticum aestivum</i> (wheat)
B2Y2Q1	LMW glutenin subunit	<i>Triticum aestivum</i> (wheat)
Q6QGV8	LMW glutenin pGM107	<i>Triticum aestivum</i> (wheat)
BSANT6	LMW glutenin	<i>Triticum aestivum</i> (wheat)
Q1ZZT4	LMW glutenin subunit	<i>Triticum aestivum</i> (wheat)
B2Y2S1	LMW glutenin subunit	<i>Triticum aestivum</i> (wheat)
Q571Q5	putative LMW-glutenin subunit	<i>Triticum aestivum</i> (wheat)
A7LHB3	alpha-gliadin	<i>Triticum aestivum</i> (wheat)
C0SUC3	HMW glutenin subunit x5	<i>Triticum aestivum</i> (wheat)
Q41593	serpin	<i>Triticum aestivum</i> (wheat)
B6UKL5	gamma-gliadin	<i>Triticum turgidum</i>
B6UKM7	gamma-gliadin	<i>Triticum aestivum</i> (wheat)
B9VRI0	gamma-gliadin	<i>Triticum aestivum</i> (wheat)
A7LHA4	alpha-gliadin	<i>Lophopyrum elongatum</i>
P17314	alpha-amylase/trypsin inhibitor CM3	<i>Triticum aestivum</i> (wheat)
D1MJA1	HMW glutenin subunit Ax-dp	<i>Triticum turgidum</i>
A1EHE7	gamma-gliadin	<i>Triticum aestivum</i> (wheat)
Q1WA39	alpha-gliadin	<i>Triticum aestivum</i> (wheat)
Q0Q5D8	Y-type HMW glutenin	<i>Triticum aestivum</i> (wheat)
B2CGM6	tritacin	<i>Triticum aestivum</i> (wheat)
Q1WA40	alpha-gliadin Gli2-LM2–12	<i>Triticum aestivum</i> (wheat)
A4ZIX1	Monomeric alpha-amylase inhibitor	<i>Triticum aestivum</i> (wheat)
Q6UJY5	HMW-glutenin Bx subunit	<i>Triticum turgidum</i>
Q41528	alpha-gliadin	<i>Triticum aestivum</i> (wheat)
B7U6L4	globulin 3	<i>Triticum aestivum</i> (wheat)
BSB0D5	major allergen CM16	<i>Triticum aestivum</i> (wheat)
Q2A784	putative avenin-like a	<i>Triticum aestivum</i> (wheat)
Q41540	CM 17 protein	<i>Triticum aestivum</i> (wheat)
Q9M4G6	putative thaumatin-like protein	<i>Vitis vinifera</i> (grape)
D1HU00	hydrolase/glycosidase activity	<i>Vitis vinifera</i> (grape)
Q7XAU7	thaumatin-like protein	<i>Vitis vinifera</i> (grape)
D1IBD6	cysteine-type endopeptidase	<i>Vitis vinifera</i> (grape)
ASAKD8	peptidyl-prolyl cis–trans isomerase	<i>Vitis vinifera</i> (grape)
ASAYU8	ATP synthase subunit beta	<i>Vitis vinifera</i> (grape)
Q04E64	60 kDa chaperonin	<i>Oenococcus oeni</i>

^a Potential allergens are in bold.

family of the α -amylase/trypsin inhibitors (M_r 14–16 kDa) were also evident.

Since WG and Gluvital 21000 were very similar (Figure 1, lanes 2 and 4), only the latter was considered for the following studies.

In GBS-P51 (Figure 1, lane 3), the partially hydrolyzed wheat gluten, protein bands belonging to gliadins (31–45 kDa) were less evident obviously due to the enzymatic breakdown that gives rise to the accumulation of bands with molecular masses in the range of 31–14 kDa. ω -Gliadins (60–80 kDa) and α -amylase inhibitors (M_r 14–16 kDa),³⁷ were also evident in GBS-P51

(Figure 1, lane 3), indicating their resistance to the enzymatic treatment used to produce this latter gluten preparation.³⁵

Protein Solubility. As gluten proteins are partially soluble in 70% alcohol/water mixture (especially gliadins) and partially soluble in acid solution (especially glutenins),¹² we have evaluated the solubility in model wine. The protein solubility in model wine was 19.9 (SD $\pm$ 1.39) and 49.2 (SD $\pm$ 3.2) mg/100 mg of Gluvital 21000 and GBS-S1, respectively. As GBS-P51 presented higher solubility than Gluvital 21000, this is probably due to the smaller molecular mass of the proteins deriving from hydrolysis process.

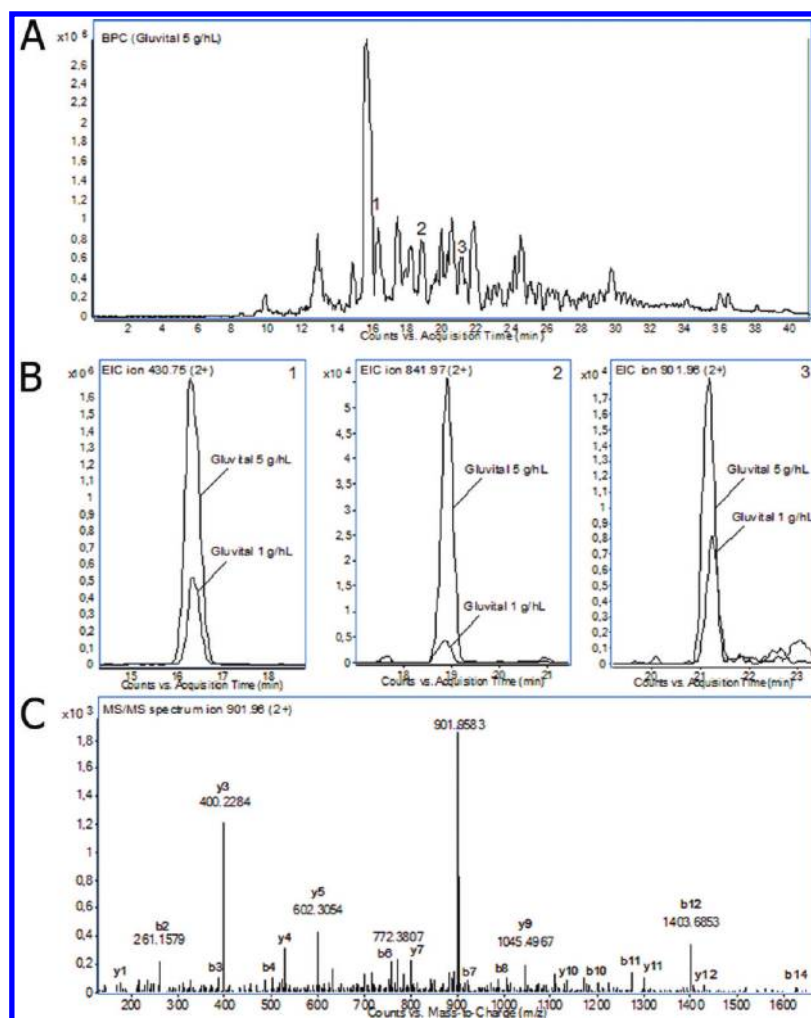


Figure 6. A: Base peak chromatogram (BPC) of a tryptic digest of red wine fined with 5 g/hL Gluvital. B: Extracted ion chromatogram (EIC) of 3 peptide ions arising from gliadin and glutenin, detected in sample fined with 1 or 5 g/hL Gluvital, as indicated. 1: Peptide VNVPLYR (ion 430.75, 2+), low molecular weight glutenin subunit (UniProt accession number B2Y2Q1). 2: Peptide IIQPQQPAQYEVIR (ion 841.97, 2+), gamma gliadin (UniProt accession number B6UKLS). 3: Peptide LFLQQCSPVAMPQR (ion 901.96, 2+), low molecular weight glutenin subunit (UniProt accession number B2LS34). C: MS/MS spectrum of peptide ion 901.96 (2+) (LFLQQCSPVAMPQR) detected in red wine fined with 1 g/hL Gluvital. The main matching b and y product ions are indicated in the spectrum and in Table 3.

Electrophoretic and Dot-Blotting Analyses of Wine Treated with Wheat Glutens. SDS–PAGE analyses of the proteins (with MW > 10 kDa) recovered through the KDS method from the wines treated with wheat glutens at different concentrations (10, 15, 50, 150, 300 g/hL) are reported in Figure 2. The control (untreated wine) (Figure 2A,B, lanes 2) showed the classical profile of the main endogenous wine proteins, including chitinase.³⁸ In general, adding wheat glutens at increasing concentrations resulted in electrophoretic patterns of increasing quality (Figure 2A,B). This can be due to removal of tannins by the gluten proteins. Bands corresponding to gluten proteins were detectable in the wine samples treated at high concentration of fining agents, especially Gluvital 21000. In particular, bands with molecular masses around 14 kDa (α -amylase inhibitors) (arrows in Figure 2) and 31–45 kDa (α/β - and γ -gliadins) (arrows in Figure 2) and a band with electrophoretic mobility corresponding to that of ω -gliadins (around 60 kDa) were revealed by silver staining (arrows in Figure 2).

In order to detect the possible presence of fragments of wheat gluten proteins with a small molecular mass too low to be

retained in the electrophoretic gel, dot-blotting experiments using two antibodies, one specific for the wheat prolamins (high molecular weight, low molecular weight and α/β - and γ -gliadins) and the other for gliadins, were performed. Moreover dot-blotting were also assayed using pooled sera of wheat allergic patients.

Residual wheat proteins were immunodetected by anti-prolamin antibodies only in the wine treated with the hydrolyzed gluten GBS-P51 (Figure 3A) from the 50 g/hL concentration upward; the anti-prolamin antibodies did not react with the wine treated with Gluvital 21000 (Figure 3B).

The commercial anti-gliadin antibody was able to bind residual proteins in the wine treated with GBS-P51 from the 150 g/hL concentration upward (Figure 4A). Also in this case no residual wheat proteins were detectable in the wine treated with Gluvital 21000 (Figure 4B).

IgE-dot-blotting (Figure 5) showed IgE-binding proteins only in the wine treated with GBS-P51 (Figure 5A) at the concentration of 300 g/hL, whereas no IgE-binding was detectable in wine clarified with Gluvital 21000 (Figure 5B).

Reverse-Phase LC–MS/MS Analyses. Liquid chromatography–mass spectrometry (LC–MS/MS) analysis of the wines treated with decreasing concentrations (25, 15, 10, 5, and 1 g/hL) of glutens (Gluvtal 21000 and GBS-P51) led to the identification of many wheat proteins. Grape proteins belonging to thaumatin-like proteins and chitinases were also identified as well as a few yeast proteins (*Saccharomyces cerevisiae*) and a protein from *Oenococcus oeni*. However the majority of identified proteins were from wheat. In particular, it was possible to clearly identify the presence of low and high molecular weight glutenin subunits, as well as components belonging to α/β - and γ -gliadins. Table 2, sections A and B, lists all the proteins that were identified in red wines fined with 5 g/hL of Gluvtal 21000 and GBS-P51, respectively. The complete information on the identifications is provided in Table V (see Supporting Information), reporting the list of peptides identified by MS/MS analysis per each protein. The same kinds of proteins but, as expected, with a lower coverage were also identified in wines fined with Gluvtal 21000 and GBS-P51 at a concentration of 1 g/hL.

The base peak chromatogram (BPC) of the tryptic digest of red wine fined with 5 g/hL of Gluvtal 21000 is reported in Figure 6A. The profiles of three representative ions arising from gliadin and glutenin peptides were extracted from the chromatograms of samples fined with 1 and 5 g/hL Gluvtal 21000 (Figure 6B). Figure 6C shows the MS/MS spectrum of a peptide from glutenin identified in the sample fined with 1 g/hL of Gluvtal 21000. The spectrum is of very good quality, as shown also by the almost complete sequence of y and b ion matches reported in Table 3, indicating that the identification of gluten proteins could be unambiguously achieved also at such a low concentration of fining agent. In parallel, the BPC of the tryptic digest of red wine fined with 5 g/hL of GBS-P51 is reported in Figure 7A. Similar results were obtained for this sample, and three ion profiles arising from glutenin and gliadin peptides are reported in Figure 7B. The MS/MS spectrum of a peptide from gliadin identified in the sample fined with 1 g/hL of GBS-P51 is shown in Figure 7C, together with the list of b and y ions matched (Table 4). Also in the case of GBS-P51, gluten proteins could be clearly detected at this low dosage of fining agent.

DISCUSSION

The use of plant proteins, including wheat gluten, as wine fining agents has been permitted by the Food Standards of Australia and New Zealand (2004)² and by EC REGULATION No. 2165 (2005).³ Nevertheless, it is well-known that gluten proteins are the causal agents in pathologies such as celiac disease (CD) and food allergy.^{9,37} While Codex Alimentarius establishes a limit for gluten quantity in gluten-free foods³⁹ intended for celiac patients, a safe limit cannot be established for food allergies.⁹

Previously,^{6,15} it has been reported that wines fined with wheat glutens could be safe for patients affected by wheat allergy and CD, although only the presence of gliadins¹⁵ and deamidated gluten proteins⁶ was investigated. In our study, vital gluten containing approximately equal amounts of gliadins and glutenins has been considered; so the use of antibodies against the total prolamins seems to be mandatory. As it has been reported that several gluten proteins (α/β -, γ -, ω -gliadins and low molecular weight subunits) can be responsible for wheat allergy^{10,37} while α -gliadin elicits CD,⁹ the role of all the native proteins in such pathologies has to be investigated.

Table 3. Fragment Ions of Precursor 901.96 (2+) (Peptide LFLQQCSPVAMPQR, Low Molecular Weight Glutenin Subunit, Accession Number B2LS34)^a

no.	b	seq	y	no.
1	114.09	L		15
2	261.16	F	1689.82	14
3	374.24	L	1542.75	13
4	502.30	Q	1429.67	12
5	630.36	Q	1301.61	11
6	758.42	Q	1173.55	10
7	918.45	C	1045.49	9
8	1005.48	S	885.46	8
9	1102.53	P	798.43	7
10	1201.60	V	701.38	6
11	1272.64	A	602.31	5
12	1403.68	M	531.27	4
13	1500.73	P	400.23	3
14	1628.79	Q	303.18	2
15		R	175.12	1

^a b and y ions matched by Mascot are indicated in bold, and the amino acid sequence to which they correspond is indicated.

In the present paper, the research of residual wheat proteins was performed after their recovery from treated wine by using the KDS method. It has been demonstrated that this method is more efficient, not only for the white³² but also for the red wines (personal data not shown) than protein precipitation by trichloroacetic acid (TCA) method, which has been previously used.¹⁵ Dot-blotting experiments carried out with both the antibodies and also with wheat allergic patient sera showed residual proteins only in the wine treated with partially hydrolyzed gluten (GBS-P51). As a matter of fact, the anti-prolamin antibodies and the commercial anti-gliadin antibodies recognized residual proteins only for doses of 50 and 150 g/hL of gluten upward, respectively. This indicates that the type of antibody used has a major effect on the possibility to detect residual allergens, raising some doubts on the analyses by immunochemical methods.

A further step to evaluate the potential allergenicity can be the use of wheat allergic patients' sera whose IgE-binding was evident at 300 g/hL treated wine. It is interesting to note that the wine treated with the vital gluten (Gluvtal 21000) which contains native proteins did not show any binding with both the antibodies (anti-prolamins and anti-gliadins) and sera-IgE. This result is obviously due to the low solubility of gluten proteins in wine. In contrast, hydrolyzed gluten proteins (GBS-P51) could remain in the clarified wines to a higher extent with respect to the native ones. Moreover, the possible alteration of the protein epitope structures after their absorption on the membrane surface in dot-blotting experiments might also compromise the results of the immunochemical reaction.⁴⁰ The partially hydrolyzed gluten (GBS-P51), having a good solubility in hydro-alcoholic solution, could remain in higher quantity in wine, and its epitopes could also be better exposed when spotted on solid matrices.

In any case, residual wheat proteins were detectable by the immunological methods only in wines treated with concentrations of gluten (>50 g/hL) higher than those usually employed in wine fining treatments. This could be ascribed to the sensitivity of the immunological techniques,⁴¹ and indicates that these methods could be inappropriate for detection of allergens present in trace amounts.

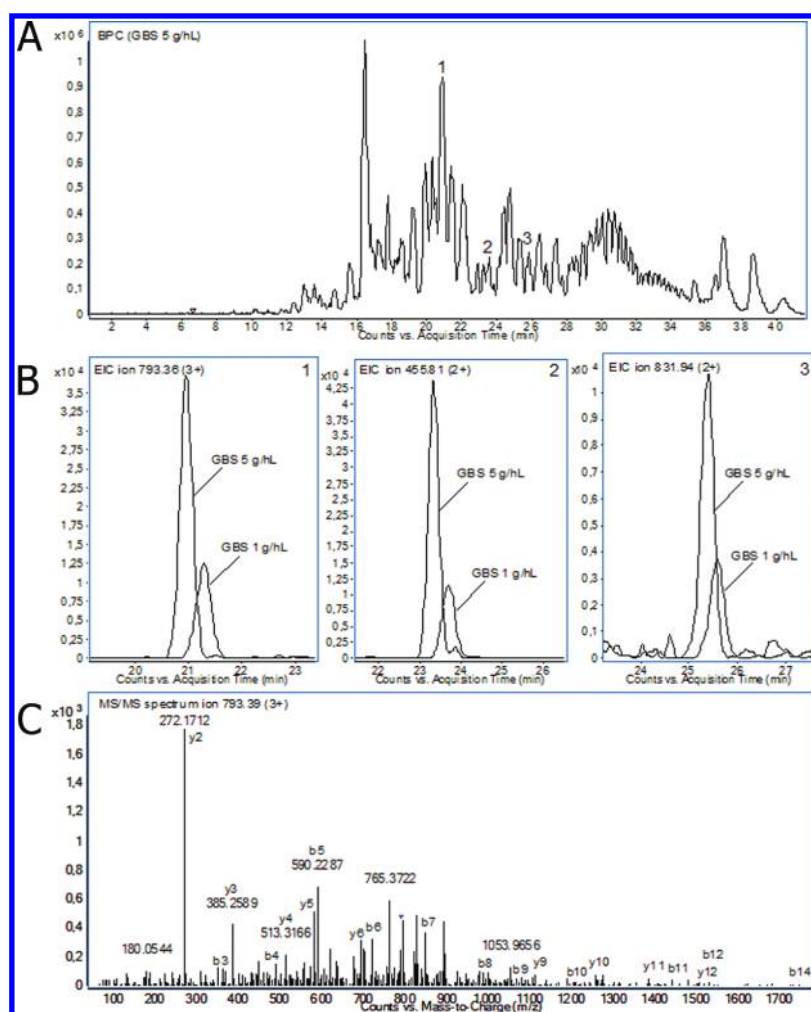


Figure 7. A: Base peak chromatogram (BPC) of a tryptic digest of red wine fined with 5 g/hL GBS. B: Extracted ion chromatogram (EIC) of 3 peptide ions arising from gliadin and glutenin, detected in sample fined with 1 or 5 g/hL GBS, as indicated. 1: Peptide SDCQVMQQCCQQLAQIPR (ion 793.36, 3+), gamma gliadin (UniProt accession number B6UKLS). 2: Peptide LVSIILPR (ion 455.81, 2+), gamma gliadin (UniProt accession number B6UKLS). 3: Peptide ILPTMCSVNPLYR (ion 831.9421, 2+), low molecular weight glutenin pGM107 (UniProt accession number Q6QGV8). C: MS/MS spectrum of peptide ion 793.36 (3+) (SDCQVMQQCCQQLAQIPR) detected in red wine fined with 1 g/hL GBS. The main matching b and y product ions are indicated in the spectrum and in Table 4.

Recently, mass spectrometry (MS) methods for the direct and absolute identification and quantification of food allergens have been developed. Thanks to its high sensitivity MS allows the detection of allergenic proteins in trace, and makes the identification independent of the structure of the allergens^{40,41} which can in contrast affect their detection by immunochemical methods. Here we have shown that KDS protein precipitation combined with the LC–MS/MS approach is a powerful method for the detection of residual proteins in red wines fined with glutes. The detection of proteins can be difficult in red wines, because of the presence of a large quantity of interfering compounds, such as polyphenols and carbohydrates.²⁵

In this study protein samples have been recovered through the KDS method,^{32,42} which is based on protein complexation with dodecyl sulfate followed by their precipitation as potassium salts. The KDS-recovered proteins can be used not only for quantitative and electrophoretic analyses⁴² but, after washing with acetone in order to eliminate SDS, also for the MS analyses.

To the best of our knowledge, a study has been published regarding the analysis of red wine proteins⁴³ by a combination of SDS–PAGE and mass spectrometry.

Recently, a method based on combinatorial peptide ligand libraries (CPLLs) coupled to SDS–PAGE and MS has been applied for identifying traces of casein, used as fining agent, in red wines.²⁴ This method allows the amplification of rare protein signals that normally avoid detection even by MS. The preparation of wine samples provides the use of a different range of wine volumes (from 200 up to 750 mL), a partial removing of phenolic substances by incubation overnight with PVPP, then the absorption (about 3 h), the subsequent desorption of the captured proteins from the ProteoMiner beads, their separation on SDS–PAGE, in-gel digestion, and MS analysis.

In our paper we have also demonstrated a gel-free approach suitable for MS analysis of the proteins present in red wines. Moreover our method allows an easy and rapid sample preparation starting from a lesser quantity of wine and avoiding the removal of phenolic compounds step. Our data clearly showed that many wheat gluten proteins with well established allergenic properties (listed in Table 2, sections A and B) can remain in wines fined with gluten. In conclusion, residual gluten proteins can be clearly detected in red wines treated with as low as 1 g/hL

Table 4. Fragment Ions of Precursor 793.36 (3+) (Peptide SDCQVMQQCCQLAQIPR, Gamma Gliadin, Accession Number B6UKL5)^a

no.	b	seq	y	no.
1	88.04	S		19
2	203.07	D	2291.01	18
3	363.10	C	2175.99	17
4	491.16	Q	2015.96	16
5	590.22	V	1887.90	15
6	721.26	M	1788.83	14
7	849.32	Q	1657.79	13
8	977.38	Q	1529.73	12
9	1105.44	Q	1401.67	11
10	1265.47	C	1273.61	10
11	1425.50	C	1113.58	9
12	1553.56	Q	953.55	8
13	1681.63	Q	825.49	7
14	1794.70	L	697.44	6
15	1865.74	A	584.35	5
16	1993.80	Q	513.31	4
17	2106.88	I	385.26	3
18	2203.94	P	272.17	2
19		R	175.12	1

^ab and y ions matched by Mascot are indicated in bold, and the amino acid sequence to which they correspond is indicated.

of gluten (Table 2, Figures 6 and 7, Table V in the Supporting Information), a dose lower than those usually used for wine fining.

Therefore, it can be concluded that wine samples resulting negative for the presence of gluten proteins by the commonly employed immunochemical methods can potentially trigger an adverse reaction in gluten-sensitized people.

■ ASSOCIATED CONTENT

S Supporting Information. Complete information on the identifications is provided in Table V, reporting the list of peptides identified by MS/MS analysis in wine fined with 5 and 1 g/hL Gluvital 21000 and GBS-PS1. The observed and theoretical molecular weights of peptides are reported, together with the percent of sequence coverage, the difference between observed and calculated molecular weight (in ppm), the Mascot score and *p* value, the ranking of the identification, and the amino acid sequences of the identified peptides. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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GLUVITAL 21000 5 g/hL

prot_acc	prot_desc	prot_score	% prot_cov	pep_rank	pep_exp_mz	pep_exp_mr	pep_calc_mr	pep_delta_ppm	pep_score	pep_expect	pep_seq
B2Y2Q1	Low molecular weight glutenin subunit n=1 Tax=Triticum aestivum RepID=B2Y2Q1_WHEAT	330	22,5	1	430,7553	859,496	859,4916	5,12	33,46	17	VNVPLYR
				2	557,3057	1112,5968	1112,6012	-3,95	22,54	1,70E+02	ILQQLNPCK
				1	511,9479	1532,8219	1532,8133	5,61	37,31	4,7	VHPSILQQLNPCK
				1	816,9011	1631,7877	1631,78	4,72	24,54	1,10E+02	VFLQQQCSPVAMPQ
				1	894,5048	1786,995	1786,9941	0,50	115,44	3,60E-08	AIISIVLQEQQQVR
				1	894,5048	1786,995	1786,9941	0,50	88,92	1,60E-05	AIISIVLQEQQQVR
				1	596,6745	1787,0016	1786,9941	4,20	45,44	0,31	AIISIVLQEQQQVR
				1	596,6745	1787,0016	1786,9941	4,20	28,43	15	AIISIVLQEQQQVR
				1	1030,5271	2059,0396	2059,0343	2,57	70,18	0,0026	VFLQQQCSPVAMPQSLAR
				1	861,6481	3442,5632	3442,5421	6,13	26,32	13	SQMLQQSSCHVMQQQCCQLPQIPQQSR
Q0GNF9	Low molecular weight glutenin n=1 Tax=Triticum aestivum RepID=Q0GNF9_WHEAT	293	28,6	1	430,7553	859,496	859,4916	5,12	33,46	17	VNVPLYR
				2	557,3057	1112,5968	1112,6012	-3,95	22,54	1,70E+02	ILQQLNPCK
				1	683,8491	1365,6837	1365,6777	4,39	53,64	0,16	TTTSVPFGVGTGVGS
				1	765,3828	1528,7511	1528,741	6,61	24,54	1,00E+02	TTTSVPFGVGTGVGSY
				1	511,9479	1532,8219	1532,8133	5,61	37,31	4,7	VHPSILQQLNPCK
				1	816,9011	1631,7877	1631,78	4,72	24,54	1,10E+02	VFLQQQCSPVAMPQ
				1	1030,5271	2059,0396	2059,0343	2,57	70,18	0,0026	VFLQQQCSPVAMPQSLAR
				1	861,6481	3442,5632	3442,5421	6,13	26,32	13	SQMLQQSSCHVMQQQCCQLPQIPQQSR
Q7XAU7	Thaumatococcus-like protein n=1 Tax=Vitis vinifera RepID=Q7XAU7_VITVI	285	34,7	1	728,3243	1454,6341	1454,6283	3,99	92,26	6,20E-06	TTGGCNPCTVFK
				1	728,3243	1454,6341	1454,6283	3,99	23,1	51	TTGGCNPCTVFK
				2	729,7963	1457,5781	1457,5769	0,82	27,41	4,1	CPDAYSYPKDDQ
				1	881,4308	1760,847	1760,8437	1,87	113,07	1,30E-07	GISCTADIVGECPAALK
				1	881,4308	1760,847	1760,8437	1,87	74,34	0,00099	GISCTADIVGECPAALK
				1	720,5957	2158,7653	2158,7626	1,25	32,54	0,051	TDEYCCNSGSCNATDYSR
				1	1164,1652	3489,4737	3489,4476	7,48	20,11	12	CPDAYSYPKDDQTSTFTCPAGTNYEVVFCP
B2Y2R4	Low molecular weight glutenin subunit n=1 Tax=Triticum aestivum RepID=B2Y2R4_WHEAT	273	22,7	1	430,7553	859,496	859,4916	5,12	33,46	17	VNVPLYR
				2	557,3057	1112,5968	1112,6012	-3,95	22,54	1,70E+02	ILQQLNPCK
				1	511,9479	1532,8219	1532,8133	5,61	37,31	4,7	VHPSILQQLNPCK
				1	784,9083	1567,8021	1567,7995	1,66	34,39	10	TTTRVPFGVGTGVGGY

				1	816,9011	1631,7877	1631,78	4,72	24,54	1,10E+02	VFLQQQCSPVAMPQ
				1	559,9572	1676,8498	1676,8378	7,16	24,5	1,10E+02	TLPTMCNVNPLYR
				1	1030,5271	2059,0396	2059,0343	2,57	70,18	0,0026	VFLQQQCSPVAMPQSLAR
				1	861,6481	3442,5632	3442,5421	6,13	26,32	13	SQMLQQSSCHVMQQQCCQLPQIPQQSR
Q6QGV8	LMW glutenin pGM107 n=1 Tax=Triticum aestivum RepID=Q6QGV8_WHEAT	250	21,5	1	430,7553	859,496	859,4916	5,12	33,46	17	VNVPLYR
				2	557,3057	1112,5968	1112,6012	-3,95	22,54	1,70E+02	ILQQLNPCK
				1	511,9479	1532,8219	1532,8133	5,61	37,31	4,7	VHPSILQQLNPCK
				1	816,9011	1631,7877	1631,78	4,72	24,54	1,10E+02	VFLQQQCSPVAMPQ
				1	831,9392	1661,8638	1661,8633	0,30	35,61	8,1	ILPTMCSNVNPLYR
				1	831,9392	1661,8638	1661,8633	0,30	24,2	1,10E+02	ILPTMCSNVNPLYR
				1	554,9657	1661,8753	1661,8633	7,22	22,3	1,60E+02	ILPTMCSNVNPLYR
				1	1030,5271	2059,0396	2059,0343	2,57	70,18	0,0026	VFLQQQCSPVAMPQSLAR
				1	861,6481	3442,5632	3442,5421	6,13	26,32	13	SQMLQQSSCHVMQQQCCQLPQIPQQSR
A9CSP0	Putative thaumatin-like protein n=1 Tax=Vitis hybrid cultivar RepID=A9CSP0_9ROSI	245	39,2	1	461,2582	920,5018	920,4967	5,54	29,36	47	ATFDILNK
				1	729,7963	1457,5781	1457,5769	0,82	32,8	1,2	CPDAYSYPQDDK
				1	777,3657	1552,7169	1552,7093	4,89	51,97	0,13	CTYTVWAAASPGGGR
				1	1009,5041	2016,9936	2016,9865	3,52	45,88	0,68	LDSGQSWTITVNP GTTNAR
				1	1127,9424	2253,8702	2253,8613	3,95	61,86	0,00034	TNEYCCTDGP GSCGPTTYSK
				1	973,7577	2918,2512	2918,2375	4,69	24,12	10	CPDAYSYPQDDKTSLTCTSGTNYK
Q1WA40	Alpha-gliadin Gli2-LM2-12 n=1 Tax=Triticum aestivum RepID=Q1WA40_WHEAT	242	31,3	2	551,307	1100,5995	1100,5978	1,54	21,46	2,80E+02	QLPQFAEIR
				1	569,3058	1136,5971	1136,5866	9,24	26,1	78	TIAPFGIFGTN
				1	634,8594	1267,7042	1267,7071	-2,29	22,56	1,10E+02	ILQQQLIPCR
				1	584,3354	1749,9842	1749,9923	-4,63	23,5	43	ILQQILQQQLIPCR
				1	627,0253	1878,0542	1878,0509	1,76	23,02	51	QILQQILQQQLIPCR
				1	669,7136	2006,119	2006,1095	4,74	33,36	4,2	QQILQQILQQQLIPCR
				1	1173,5872	3517,7396	3517,7197	5,66	27,46	21	NLALQTL PAMCNVYIPPHCSTTIAPFGIFGTN
				1	880,4424	3517,7404	3517,7197	5,88	27,95	18	NLALQTL PAMCNVYIPPHCSTTIAPFGIFGTN
				1	966,8989	4829,4583	4829,4341	5,01	25,19	14	DVVLQQH NIAHASSQVLQQSTYQLLQQLCCQQLLQIPEQSR
				1	1208,373	4829,4631	4829,4341	6,00	49,82	0,049	DVVLQQH NIAHASSQVLQQSTYQLLQQLCCQQLLQIPEQSR
				1	1208,373	4829,4631	4829,4341	6,00	64,04	0,0019	DVVLQQH NIAHASSQVLQQSTYQLLQQLCCQQLLQIPEQSR
P17314	Alpha-amylase/trypsin inhibitor CM3 n=3 Tax=Triticum RepID=IAAC3_WHEAT	221	42,3	4	555,7607	1109,5069	1109,4964	9,46	22,65	1,20E+02	EMQWDFVR
				1	566,9792	1697,9159	1697,9141	1,06	33,78	9,3	YFIALPVPSQPVDPR
				1	849,9665	1697,9184	1697,9141	2,53	23,96	92	YFIALPVPSQPVDPR
				1	864,4274	1726,8402	1726,8308	5,44	57,71	0,049	SGNVGESGLIDLPGCPR

				1	901,428	1800,8415	1800,8353	3,44	35,93	5	DYVLQQTCTGFTPGSK
				1	979,4383	1956,862	1956,8492	6,54	70,84	0,00074	LYCCQELAEISQQCR
D0ES81	Gamma gliadin n=1 Tax=Triticum aestivum ReplID=D0ES81_WHEAT	189	21,7	1	408,2476	814,4806	814,4735	8,72	31,43	14	VSMILPR
				4	623,3282	1244,6418	1244,6401	1,37	30,4	36	APFASIVADIGGQ
				4	623,3282	1244,6418	1244,6401	1,37	28,29	59	APFASIVADIGGQ
				1	851,0948	2550,2626	2550,2532	3,69	49,97	0,2	SLVLGTLPTMCNVFVPPECSTTK
				1	851,0948	2550,2626	2550,2532	3,69	42,95	1	SLVLGTLPTMCNVFVPPECSTTK
				1	1276,1416	2550,2687	2550,2532	6,08	24,19	77	SLVLGTLPTMCNVFVPPECSTTK
				1	675,8867	2699,5175	2699,5123	1,93	23,9	18	PLFQLVQGGGIIQPQQAQLEVIR
				1	952,8821	2855,6246	2855,6134	3,92	28,96	3,7	RPLFQLVQGGGIIQPQQAQLEVIR
				1	714,9156	2855,6335	2855,6134	7,04	53,13	0,013	RPLFQLVQGGGIIQPQQAQLEVIR
A5JSA7	Alpha-gliadin n=1 Tax=Triticum aestivum ReplID=A5JSA7_WHEAT	177	12,5	1	569,3058	1136,5971	1136,5866	9,24	26,1	78	TIAPFGIFGTN
				1	580,3141	1158,6136	1158,6033	8,89	27,44	83	QLPQFEEIR
				1	634,8594	1267,7042	1267,7071	-2,29	22,56	1,10E+02	ILQQQLIPCR
				1	584,3354	1749,9842	1749,9923	-4,63	23,5	43	ILQQILQQQLIPCR
				1	627,0253	1878,0542	1878,0509	1,76	23,02	51	QILQQILQQQLIPCR
				1	669,7136	2006,119	2006,1095	4,74	33,36	4,2	QQILQQILQQQLIPCR
				1	727,3749	2179,103	2179,1022	0,37	20,52	2,10E+02	PQAQGSVQPQQLPQFEEIR
D1IBD6	Whole genome shotgun sequence of line PN40024, scaffold_3.assembly12x (Fragment) n=1 Tax=Vitis vinifera ReplID=D1IBD6_VITVI	176	4,1	1	654,2914	1306,5682	1306,5612	5,36	35,06	3,3	SCEAPSSLDWR
				1	689,8111	1377,6076	1377,5983	6,75	45,15	0,4	ASCEAPSSLDWR
				1	689,8111	1377,6076	1377,5983	6,75	27,36	24	ASCEAPSSLDWR
				1	1108,5112	2215,0079	2214,9925	6,95	60,82	0,0098	NTDLPYGECAINAMASYPTK
				1	791,3753	2371,1041	2371,0936	4,43	34,7	5,1	RNTDLPYGECAINAMASYPTK
B6UKP6	Gamma-gliadin n=1 Tax=Triticum aestivum ReplID=B6UKP6_WHEAT	145	22	1	408,2476	814,4806	814,4735	8,72	31,43	14	VSMILPR
				1	609,3323	1216,6501	1216,6452	4,03	36,1	9,1	APFASIVASIGGQ
				1	755,7501	2264,2285	2264,2277	0,35	21,66	81	LVQGGGIIQPQQAQYEVIR
				1	793,359	2377,0552	2377,0396	6,56	28,72	10	SDCQVMQQQCCQQLAQIPR
				1	917,5041	2749,4906	2749,4915	-0,33	27,21	16	PLFQLVQGGGIIQPQQAQYEVIR
A5AYU8	ATP synthase subunit beta n=1 Tax=Vitis vinifera ReplID=A5AYU8_VITVI	128	10,4	2	488,2858	974,5571	974,5549	2,26	33,82	9,2	IGLFGGAGVGK
				1	631,8248	1261,635	1261,6336	1,11	26,98	72	TIAMDGTEGLVR
				1	746,8926	1491,7706	1491,7681	1,68	35,5	9,7	FTQANSEVSALLGR

				1	498,2651	1491,7736	1491,7681	3,69	25,81	78	FTQANSEVSALLGR
				1	729,3862	2185,1369	2185,1379	-0,46	31,21	16	IPSAVGYQPTLATDLGGLQER
D1HU00	Whole genome shotgun sequence of line PN40024, scaffold_22.assembly12x (Fragment) n=1 Tax=Vitis vinifera ReplD=D1HU00_VITVI	119	8,6	1	602,7963	1203,578	1203,5732	3,99	33,26	15	SSLAVDDVDQR
				1	602,7963	1203,578	1203,5732	3,99	39,64	3,4	SSLAVDDVDQR
				1	655,8423	1309,67	1309,6666	2,60	29,11	37	VYPTEAIYGAAR
				1	567,2966	1698,8681	1698,869	-0,53	25,68	83	VLVDHSIVEGFSQGGGR
				1	645,0093	1932,006	1932,0026	1,76	25	81	IYGSIVPVLDEKPTMR
B6VJY3	ATP synthase subunit alpha n=1 Tax=Vitis vinifera ReplD=B6VJY3_VITVI	116	6,7	1	486,7805	971,5465	971,54	6,69	39,69	5,3	VVSVGDGIAR
				1	602,3328	1202,6511	1202,6506	0,42	52,8	0,17	AAELTTLESR
				1	650,8773	1299,74	1299,7398	0,15	23,06	95	TAIAIDTILNQK
Q4U195	Dimeric alpha-amylase inhibitor n=2 Tax=Triticum aestivum ReplD=Q4U195_WHEAT	116	19,9	1	785,9041	1569,7937	1569,7933	0,25	31,75	21	LQCNGSQVPEAVLR
				1	927,8843	1853,754	1853,7495	2,43	83,78	9,60E-06	ECCQQQLADISEWCR
D1IP80	Whole genome shotgun sequence of line PN40024, scaffold_81.assembly12x (Fragment) n=1 Tax=Vitis vinifera ReplD=D1IP80_VITVI	113	21	1	660,3525	1318,6904	1318,6841	4,78	29,73	37	VTNTATGTQATVR
				1	812,3707	1622,7268	1622,7148	7,40	29,44	15	GHLTVNYQFVNCGD
				1	840,4225	1678,8305	1678,8309	-0,24	53,67	0,13	CLSVTNTATGTQATVR
D2KFG9	Gliadin/avenin-like seed protein n=1 Tax=Triticum aestivum ReplD=D2KFG9_WHEAT	108	18,9	1	650,9876	1949,941	1949,9339	3,64	25,3	76	DALLQQCSPVADMSFLR
				1	975,9781	1949,9417	1949,9339	4,00	62,27	0,015	DALLQQCSPVADMSFLR
				1	879,0645	2634,1717	2634,1811	-3,57	45,69	0,18	SQVVQHSSCLVMWEQCCQQLK
Q04E64	60 kDa chaperonin n=1 Tax=Oenococcus oeni PSU-1 ReplD=CH60_OENOB	106	16,4	1	693,3934	2077,1583	2077,1532	2,46	22,78	46	IGNIQDILPVLQSVVEQGR
				1	774,0728	2319,1966	2319,1958	0,34	23,08	1,00E+02	NVVLEQSYGTPTITNDGVTIK
				1	830,4249	2488,253	2488,2657	-5,10	38,82	2,7	TNDVAGDGTATVLTQAIVNEGLK
				1	831,4465	2491,3178	2491,3394	-8,67	21,52	1,00E+02	ALEEPLRQIVENAGLEGSVIAQR
B5B0D5	Major allergen CM16 n=2 Tax=Triticum ReplD=B5B0D5_WHEAT	96	24,5	1	584,7597	1167,5049	1167,4979	6,00	35,49	3,1	DYVEQQACR
				1	588,3197	1174,6249	1174,6234	1,28	22,76	2,00E+02	IETPGSPYLAK

				1	931,4136	1860,8127	1860,8029	5,27	37,72	1,4	QQCCGELANIPQQCR
				1	621,2791	1860,8153	1860,8029	6,66	22,08	53	QQCCGELANIPQQCR
Q2A784	Putative avenin-like a n=1 Tax=Triticum aestivum RepID=Q2A784_WHEAT	92	15,5	1	768,3848	1534,755	1534,7418	8,60	33,8	13	CQAVCSVAQIIMR
				1	908,929	1815,8434	1815,8356	4,30	58,46	0,027	QQCCQPLAQISEQAR
A5BAX1	Whole genome shotgun sequence of line PN40024, scaffold_11.assembly12x n=1 Tax=Vitis vinifera RepID=A5BAX1_VITVI	92	35,8	1	848,9159	1695,8172	1695,8178	-0,35	40,17	2,5	GEGCLVIWTIEYEK
				1	571,9435	1712,8086	1712,8006	4,67	21,21	1,70E+02	IEVHEGDWETQGSVK
				1	729,6027	2914,3815	2914,358	8,06	30,6	12	HWSLTVGENSESIKETVDQIDEENR
C1IIM6	Beta-amylase n=1 Tax=Hordeum vulgare RepID=C1IIM6_HORVU	91	8,2	1	721,8588	1441,7031	1441,6983	3,33	20,82	2,20E+02	EGLNVACENALPR
				1	876,9504	1751,8862	1751,8842	1,14	40,05	3,2	LSNQLVEGQNYVNFK
				1	590,6478	1768,9217	1768,9108	6,16	29,95	27	SAPEELVQQVLSAGWR
D1HDI5	Whole genome shotgun sequence of line PN40024, scaffold_1.assembly12x (Fragment) n=1 Tax=Vitis vinifera RepID=D1HDI5_VITVI	90	6,2	1	769,9193	1537,8241	1537,8212	1,89	31,61	16	AVANQPVSVAIEAGGR
				1	513,6184	1537,8334	1537,8212	7,93	29,2	25	AVANQPVSVAIEAGGR
				1	773,394	1544,7735	1544,7623	7,25	58,8	0,048	EFQLYQSGIFTGR
D1HTT2	Whole genome shotgun sequence of line PN40024, scaffold_22.assembly12x (Fragment) n=1 Tax=Vitis vinifera RepID=D1HTT2_VITVI	87	6,7	1	754,3869	1506,7593	1506,7565	1,86	42,13	2,1	IEEELGSAAVYAGAK
				1	787,4244	1572,8342	1572,8359	-1,08	44,64	0,96	VNQIGSVTESIEAVK
A4ZIX1	Monomeric alpha-amylase inhibitor (Fragment) n=1 Tax=Triticum aestivum RepID=A4ZIX1_WHEAT	86	24,6	2	525,7851	1049,5556	1049,5506	4,76	26,42	78	SVYQEIGVR
				1	587,8122	1173,6098	1173,6064	2,90	28,16	67	LTAASVPEVCK
				1	778,416	1554,8175	1554,8188	-0,84	30,92	23	LQCVGSQVPEAVLR
B8XU29	Alfa gliadin n=1 Tax=Triticum monococcum RepID=B8XU29_TRIMO	83	14,7	1	569,3058	1136,5971	1136,5866	9,24	26,1	78	TIAPFGIFGTN
				1	836,4274	4177,1005	4177,0797	4,98	27,6	13	DVIVLQQHNIAHESSQVLQSSYQVLQQLCCQQLR
				1	1045,285	4177,111	4177,0797	7,49	56,86	0,015	DVIVLQQHNIAHESSQVLQSSYQVLQQLCCQQLR
B0FZ26	Class IV chitinase n=1 Tax=Vitis pseudoreticulata RepID=B0FZ26_9ROSI	80	14,2	1	638,8199	1275,6253	1275,6241	0,94	39,8	3,7	GIINQAASSCAGK

				1	821,7304	2462,1694	2462,1462	9,42	40,47	1,6	AAFLSALNSYSGFGNDGSTDANKR
Q6UJY7	HMW-glutenin By subunit n=1 Tax=Triticum turgidum RepID=Q6UJY7_TRITU	78	4	1	661,3102	1320,6058	1320,5979	5,98	29	25	ELQESSLEACR
				1	665,9966	1994,9681	1994,9518	8,17	49,11	0,29	QGGQLEQGQQPGQQQQR
A9YSK5	High molecular weight glutenin subunit n=1 Tax=Triticum aestivum RepID=A9YSK5_WHEAT	72	4,4	2	588,7999	1175,5853	1175,5782	6,04	25,06	1,20E+02	LEGSDALSTRQ
				1	651,8406	1301,6666	1301,6616	3,84	21,34	2,70E+02	QYEQPVVPSK
				1	729,3734	1456,7322	1456,7279	2,95	25,28	1,00E+02	AQQLAAQLPAMCR
A5BUW9	Putative uncharacterized protein n=1 Tax=Vitis vinifera RepID=A5BUW9_VITVI	68	10,1	1	500,274	998,5334	998,5284	5,01	46,32	0,66	VTISEPEPK
				1	713,8306	1425,6466	1425,6446	1,40	21,26	1,20E+02	CDEVVPPQPEEK
Q41540	CM 17 protein n=1 Tax=Triticum aestivum RepID=Q41540_WHEAT	63	19,6	1	584,7597	1167,5049	1167,4978	6,08	30,85	9,2	NYVEEQACR
				1	750,02	2247,0382	2247,03	3,65	32,22	8,8	NEDCTPWTSTLITPLPSR
				1	1124,5287	2247,0428	2247,03	5,70	20,48	1,30E+02	NEDCTPWTSTLITPLPSR
B5VJM6	YGR282Cp-like protein (Fragment) n=1 Tax=Saccharomyces cerevisiae AWRI1631 RepID=B5VJM6_YEAS6	61	4,9	1	742,8634	1483,7123	1483,7042	5,46	61,43	0,018	STSDYETELQALK
A5AKD8	Peptidyl-prolyl cis-trans isomerase n=2 Tax=Vitis vinifera RepID=A5AKD8_VITVI	59	13,4	1	567,7794	1133,5442	1133,5387	4,85	27,35	57	AVVVSDCGQLS
				1	696,8629	1391,7113	1391,7119	-0,43	31,84	23	IVMELFADTTTPR
Q09VU3	Phospholipase D alpha n=1 Tax=Vitis vinifera RepID=Q09VU3_VITVI	58	3,3	1	435,5922	1303,7548	1303,75	3,68	20,84	81	IEDVGALHLIPK
				1	767,3812	1532,7478	1532,7392	5,61	37,23	6	IVVVDSEMPSGGSEK
B3LP15	Protein YGP1 n=1 Tax=Saccharomyces cerevisiae RM11-1a RepID=B3LP15_YEAS1	56	5,6	1	432,2315	862,4485	862,4436	5,68	25,83	98	SALESIFP
				1	552,3071	1102,5997	1102,5982	1,36	30,91	25	SSAGAVVVTNAK
D1IRB3	Whole genome shotgun sequence of line PN40024, scaffold_2.assembly12x (Fragment) n=1 Tax=Vitis vinifera RepID=D1IRB3_VITVI	56	3,8	1	482,9274	1445,7605	1445,7548	3,94	29,31	39	DMDKDTVLLAAR
				1	808,7831	2423,3276	2423,3159	4,83	26,65	20	LLEGDPLKIDQSALTGESLPVTK

GBS-P51 5 g/hL

prot_acc	prot_desc	prot_score	% prot_cov	pep_rank	pep_exp_mz	pep_exp_mr	pep_calc_mr	pep_delta_ppm	pep_score	pep_expect	pep_seq
Q0GNF9	Low molecular weight glutenin n=1 Tax=Triticum aestivum RepID=Q0GNF9_WHEAT	655	40,4	2	352,706	703,3974	703,3905	9,81	23,25	1,30E+02	VNVPLY
				1	430,7558	859,497	859,4916	6,28	38,05	5,1	VNVPLYR
				1	461,7299	921,4452	921,4379	7,92	25,33	72	VFLQQQC
				1	481,2778	960,5411	960,5393	1,87	32,73	14	VNVPLYRT
				1	481,2778	960,5411	960,5393	1,87	25,43	78	VNVPLYRT
				5	557,3088	1112,603	1112,6012	1,62	20,97	2,20E+02	ILQQLNPCK
				1	566,8269	1131,6392	1131,6322	6,19	43,18	1,1	LEVMTSIALR
				1	640,335	1278,6554	1278,6456	7,66	26,8	80	TTTSVPFGVGTGVG
				1	683,8486	1365,6827	1365,6777	3,66	58,24	0,054	TTTSVPFGVGTGVGS
				1	722,9145	1443,8144	1443,8119	1,73	67,42	0,0027	IAQLEVMTSIALR
				1	765,3827	1528,7508	1528,741	6,41	24,33	1,10E+02	TTTSVPFGVGTGVGSY
				2	511,9485	1532,8237	1532,8133	6,78	23,88	1,10E+02	VHPSILQQLNPCK
				1	773,931	1545,8475	1545,8402	4,72	41,66	1,4	AIYSILQEQQQ
				1	860,4174	1718,8203	1718,812	4,83	28,52	36	VFLQQQCSPVAMPQS
				1	860,4174	1718,8203	1718,812	4,83	63,44	0,012	VFLQQQCSPVAMPQS
				1	683,3833	2047,1282	2047,1248	1,66	41,62	0,75	LQPHQIAQLEVMTSIALR
				1	1030,526	2059,0374	2059,0343	1,51	59,32	0,03	VFLQQQCSPVAMPQSLAR
				1	667,1212	2664,4558	2664,4421	5,14	41,51	0,57	LAQGTFLQPHQIAQLEVMTSIALR
				2	1033,1479	3096,422	3096,411	3,55	23,1	41	LQSSCHVMQQCCQLPQIPQQSR
B2Y2Q1	Low molecular weight glutenin subunit n=1 Tax=Triticum aestivum RepID=B2Y2Q1_WHEAT	643	36,1	2	352,706	703,3974	703,3905	9,81	23,25	1,30E+02	VNVPLY
				1	430,7558	859,497	859,4916	6,28	38,05	5,1	VNVPLYR
				1	461,7299	921,4452	921,4379	7,92	25,33	72	VFLQQQC
				1	481,2778	960,5411	960,5393	1,87	32,73	14	VNVPLYRT
				1	481,2778	960,5411	960,5393	1,87	25,43	78	VNVPLYRT
				5	557,3088	1112,603	1112,6012	1,62	20,97	2,20E+02	ILQQLNPCK
				1	566,8269	1131,6392	1131,6322	6,19	43,18	1,1	LEVMTSIALR
				1	640,335	1278,6554	1278,6456	7,66	26,8	80	TTTSVPFGVGTGVG
				1	722,9145	1443,8144	1443,8119	1,73	67,42	0,0027	IAQLEVMTSIALR
				1	766,9216	1531,8287	1531,8246	2,68	29,36	28	AIYSIVLQEQQQ
				2	511,9485	1532,8237	1532,8133	6,78	23,88	1,10E+02	VHPSILQQLNPCK
				1	860,4174	1718,8203	1718,812	4,83	28,52	36	VFLQQQCSPVAMPQS
				1	860,4174	1718,8203	1718,812	4,83	63,44	0,012	VFLQQQCSPVAMPQS

				1	914,936	1827,8574	1827,8534	2,19	83,51	9,00E-05	GSIQTQQQQPQQLGQC
				1	683,3833	2047,1282	2047,1248	1,66	41,62	0,75	LQPHQIAQLEVMTSIALR
				1	1030,526	2059,0374	2059,0343	1,51	59,32	0,03	VFLQQQCSPVAMPQSLAR
				1	667,1212	2664,4558	2664,4421	5,14	41,51	0,57	LAQGTFLQPHQIAQLEVMTSIALR
				2	1033,1479	3096,422	3096,411	3,55	23,1	41	LQQSSCHVMQQQCCQLPQIPQQSR
Q6QGV8	LMW glutenin pGM107 n=1 Tax=Triticum aestivum RepID=Q6QGV8_WHEAT	626	35,9	1	430,7558	859,497	859,4916	6,28	38,05	5,1	VNVPLYR
				1	461,7299	921,4452	921,4379	7,92	25,33	72	VFLQQQC
				5	557,3088	1112,603	1112,6012	1,62	20,97	2,20E+02	ILQQLNPCK
				1	566,8269	1131,6392	1131,6322	6,19	43,18	1,1	LEVMTSIALR
				3	704,8581	1407,7016	1407,6882	9,52	34,77	12	TTTSVPFDVGTGVGA
				1	722,9145	1443,8144	1443,8119	1,73	67,42	0,0027	IAQLEVMTSIALR
				2	511,9485	1532,8237	1532,8133	6,78	23,88	1,10E+02	VHPSILQQLNPCK
				1	773,931	1545,8475	1545,8402	4,72	41,66	1,4	AIYSIILQEQQQ
				1	831,9421	1661,8697	1661,8633	3,85	47,43	0,49	ILPTMCSNVNPLYR
				1	554,9664	1661,8775	1661,8633	8,54	20,83	2,30E+02	ILPTMCSNVNPLYR
				1	860,4174	1718,8203	1718,812	4,83	28,52	36	VFLQQQCSPVAMPQS
				1	860,4174	1718,8203	1718,812	4,83	63,44	0,012	VFLQQQCSPVAMPQS
				1	882,4606	1762,9066	1762,911	-2,50	54,38	0,11	ILPTMCSNVNPLYRT
				1	683,3833	2047,1282	2047,1248	1,66	41,62	0,75	LQPHQIAQLEVMTSIALR
				1	1030,526	2059,0374	2059,0343	1,51	59,32	0,03	VFLQQQCSPVAMPQSLAR
				1	667,1212	2664,4558	2664,4421	5,14	41,51	0,57	LAQGTFLQPHQIAQLEVMTSIALR
				2	1033,1479	3096,422	3096,411	3,55	23,1	41	LQQSSCHVMQQQCCQLPQIPQQSR
B5ANT6	LMW-glutenin n=1 Tax=Triticum aestivum RepID=B5ANT6_WHEAT	546	40,6	1	430,7558	859,497	859,4916	6,28	38,05	5,1	VNVPLYR
				5	557,3088	1112,603	1112,6012	1,62	20,97	2,20E+02	ILQQLNPCK
				1	566,8269	1131,6392	1131,6322	6,19	43,18	1,1	LEVMTSIALR
				3	704,8581	1407,7016	1407,6882	9,52	34,77	12	TTTSVPFDVGTGVGA
				1	722,9145	1443,8144	1443,8119	1,73	67,42	0,0027	IAQLEVMTSIALR
				1	773,931	1545,8475	1545,8402	4,72	41,66	1,4	AIYSIILQEQQQ
				1	831,9421	1661,8697	1661,8633	3,85	47,43	0,49	ILPTMCSNVNPLYR
				1	554,9664	1661,8775	1661,8633	8,54	20,83	2,30E+02	ILPTMCSNVNPLYR
				1	882,4606	1762,9066	1762,911	-2,50	54,38	0,11	ILPTMCSNVNPLYRT
				1	901,9587	1801,9028	1801,8967	3,39	47,1	0,58	LFLQQQCSPVAMPQR
				1	683,3833	2047,1282	2047,1248	1,66	41,62	0,75	LQPHQIAQLEVMTSIALR
				1	810,7839	2429,33	2429,3465	-6,79	35,06	3,1	LVQQQIPVVQPSILQQLNPCK
				1	1215,6859	2429,3573	2429,3465	4,45	23,78	26	LVQQQIPVVQPSILQQLNPCK
				1	810,7934	2429,3584	2429,3465	4,90	45,18	0,18	LVQQQIPVVQPSILQQLNPCK
				1	667,1212	2664,4558	2664,4421	5,14	41,51	0,57	LAQGTFLQPHQIAQLEVMTSIALR

				2	1033,1479	3096,422	3096,411	3,55	23,1	41	LQQSSCHVMQQCCQQLPQIPQQSR
Q1ZZT4	Low-molecular-weight glutenin subunit n=1 Tax=Triticum aestivum ReplD=Q1ZZT4_WHEAT	467	27	2	352,706	703,3974	703,3905	9,81	23,25	1,30E+02	VNVPLY
				1	430,7558	859,497	859,4916	6,28	38,05	5,1	VNVPLYR
				1	461,7299	921,4452	921,4379	7,92	25,33	72	VFLQQQC
				1	481,2778	960,5411	960,5393	1,87	32,73	14	VNVPLYRT
				1	481,2778	960,5411	960,5393	1,87	25,43	78	VNVPLYRT
				1	550,3007	1098,5869	1098,5856	1,18	21,41	2,10E+02	VLQQLNPCK
				1	566,8269	1131,6392	1131,6322	6,19	43,18	1,1	LEVMTSIALR
				1	640,335	1278,6554	1278,6456	7,66	26,8	80	TTTSVPFGVGTGVG
				1	722,9145	1443,8144	1443,8119	1,73	67,42	0,0027	IAQLEVMTSIALR
				3	766,9216	1531,8287	1531,8246	2,68	20,39	2,20E+02	AIVYSIILQEQQQ
				1	795,4314	1588,8482	1588,8461	1,32	56,22	0,06	AIVYSIILQEQQQG
				1	868,9655	1735,9164	1735,9145	1,09	29,18	31	AIVYSIILQEQQQGF
				1	683,3833	2047,1282	2047,1248	1,66	41,62	0,75	LQPHQIAQLEVMTSIALR
				1	667,1212	2664,4558	2664,4421	5,14	41,51	0,57	LAQGTFLQPHQIAQLEVMTSIALR
B2Y2S1	Low molecular weight glutenin subunit n=1 Tax=Triticum aestivum ReplD=B2Y2S1_WHEAT	393	21,1	5	557,3088	1112,603	1112,6012	1,62	20,97	2,20E+02	ILQQLNPCK
				1	566,8269	1131,6392	1131,6322	6,19	43,18	1,1	LEVMTSIALR
				1	722,9145	1443,8144	1443,8119	1,73	67,42	0,0027	IAQLEVMTSIALR
				1	788,8647	1575,7149	1575,71	3,11	29,3	20	QLGQCSFQQPQQQ
				1	860,4174	1718,8203	1718,812	4,83	28,52	36	VFLQQQCSPVAMPQS
				1	860,4174	1718,8203	1718,812	4,83	63,44	0,012	VFLQQQCSPVAMPQS
				1	892,4699	1782,9253	1782,9264	-0,62	42,15	1,6	AIISIVLQEQQHGQG
				1	595,3189	1782,935	1782,9264	4,82	24,33	95	AIISIVLQEQQHGQG
				1	683,3833	2047,1282	2047,1248	1,66	41,62	0,75	LQPHQIAQLEVMTSIALR
				1	1030,526	2059,0374	2059,0343	1,51	59,32	0,03	VFLQQQCSPVAMPQSLAR
Q571Q5	Putative LMW-glutenin subunit n=1 Tax=Triticum aestivum ReplD=Q571Q5_WHEAT	350	35,9	1	461,7299	921,4452	921,4379	7,92	25,33	72	VFLQQQC
				5	557,3088	1112,603	1112,6012	1,62	20,97	2,20E+02	ILQQLNPCK
				1	566,8269	1131,6392	1131,6322	6,19	43,18	1,1	LEVMTSIALR
				3	766,9216	1531,8287	1531,8246	2,68	20,39	2,20E+02	AIVYSIILQEQQQ
				2	511,9485	1532,8237	1532,8133	6,78	23,88	1,10E+02	VHPSILQQLNPCK
				1	860,4174	1718,8203	1718,812	4,83	28,52	36	VFLQQQCSPVAMPQS
				1	860,4174	1718,8203	1718,812	4,83	63,44	0,012	VFLQQQCSPVAMPQS
				1	1030,526	2059,0374	2059,0343	1,51	59,32	0,03	VFLQQQCSPVAMPQSLAR

				1	688,7087	2063,1042	2063,1197	-7,51	34,32	6,1	LQPHQISQLEVMTSIALR
				2	1033,1479	3096,422	3096,411	3,55	23,1	41	LQQSSCHVMQQCCQQLPQIPQQSR
				1	1040,8462	3119,5167	3119,5018	4,78	35,61	4	TLPTMCGVNVPLYSSTTIMPFISIGTGVGGY
Q9M4G6	Putative thaumatin-like protein n=1 Tax=Vitis vinifera ReplD=Q9M4G6_VITVI	264	39,2	1	461,2582	920,5018	920,4967	5,54	45,46	1,1	ATFDILNK
				1	729,7966	1457,5786	1457,5769	1,17	25,34	6,6	CPDAYSYPQDDK
				1	740,3502	1478,6858	1478,6712	9,87	30,88	19	TSLFTCTSGTNYK
				1	777,3665	1552,7184	1552,7093	5,86	30,15	19	CTYTVWAAASPGGGR
				1	1009,5029	2016,9913	2016,9865	2,38	52,2	0,15	LDSGQSWTITVNP GTTNAR
				1	1127,9413	2253,868	2253,8613	2,97	81,58	3,50E-06	TNEYCCTDGP GSCGPTTYSK
				1	752,3013	2253,882	2253,8613	9,18	59,03	0,00088	TNEYCCTDGP GSCGPTTYSK
A7LHB3	Alpha gliadin n=1 Tax=Triticum aestivum ReplD=A7LHB3_WHEAT	237	34,5	1	537,959	1610,8551	1610,8416	8,38	23,66	1,10E+02	VQPQQLPQFEEIR
				1	746,7021	2237,0844	2237,0683	7,20	23,61	96	NLALQTL PAMCNVYIPPYC
				1	925,8318	2774,4737	2774,461	4,58	22,54	63	QQQQQQQQLQQLQQQLIPCR
				1	942,1432	2823,4078	2823,3798	9,92	54,23	0,069	NLALQTL PAMCNVYIPPYCTIAPFG
				1	1119,5585	3355,6537	3355,6443	2,80	58,89	0,017	NLALQTL PAMCNVYIPPYCTIAPFGIFGTN
				1	1119,5585	3355,6537	3355,6443	2,80	66,04	0,0032	NLALQTL PAMCNVYIPPYCTIAPFGIFGTN
				1	1219,3619	4873,4186	4873,3916	5,54	34,65	1,6	DVVLQQHSIAYGSSQVLQQSTYQLVQQLCCQQLWQIPEQSR
				1	975,6934	4873,4304	4873,3916	7,96	46,74	0,1	DVVLQQHSIAYGSSQVLQQSTYQLVQQLCCQQLWQIPEQSR
C0SUC3	High-molecular-weight glutenin subunit x5 n=1 Tax=Triticum aestivum ReplD=C0SUC3_WHEAT	235	9,6	1	688,8239	1375,6332	1375,6337	-0,36	39,67	2,3	ACQQVMDQQLR
				1	718,3111	1434,6076	1434,6045	2,16	38,53	0,93	EGEASEQLQCER
				1	718,3513	1434,6881	1434,6813	4,74	30,87	25	DISPECHPVVSP
				1	729,3702	1456,7258	1456,7279	-1,44	35,39	8,8	AQQLAAQLPAMCR
				1	821,052	2460,1342	2460,1186	6,34	40,11	1,2	EGEASEQLQCERELQELQER
				1	837,0842	2508,2307	2508,2317	-0,40	50,64	0,18	LTSPQQSGGQQPGQLQQAQGGK
Q41593	Serpin-Z1A n=1 Tax=Triticum aestivum ReplD=SPZ1A_WHEAT	232	19,1	1	576,3479	1150,6812	1150,675	5,39	40,65	0,77	LVL ANALYFK
				1	627,306	1252,5975	1252,5936	3,11	42,77	1,4	AETQSVDFQTK
				2	646,8617	1291,7088	1291,7023	5,03	26,68	45	ISFGIEASDLLK
				1	781,3996	1560,7846	1560,7784	3,97	57,7	0,055	AAEVTTQVNSWVEK
				1	904,4778	2710,4117	2710,3926	7,05	65,35	0,0044	SAASNAAFSPVSLYSALLAAGAGSATR
B6UKL5	Gamma-gliadin n=1 Tax=Triticum turgidum ReplD=B6UKL5_TRITU	229	21,5	1	399,2686	796,5226	796,5171	6,91	27,15	6,8	VSILPR
				1	399,2686	796,5226	796,5171	6,91	30,25	3,4	VSILPR

				1	455,8111	909,6076	909,6011	7,15	40,7	0,12	LVSILPR
				1	455,8111	909,6076	909,6011	7,15	50,73	0,012	LVSILPR
				1	841,9664	1681,9182	1681,9151	1,84	52,42	0,1	IIQPQQPAQYEVIR
				1	841,9664	1681,9182	1681,9151	1,84	22,17	1,10E+02	IIQPQQPAQYEVIR
				1	561,6483	1681,923	1681,9151	4,70	26,13	41	IIQPQQPAQYEVIR
				1	561,6483	1681,923	1681,9151	4,70	46,09	0,41	IIQPQQPAQYEVIR
				1	873,4392	1744,8639	1744,8567	4,13	24,77	99	NFLLQQCNHVSLSVSS
				1	1189,5343	2377,0541	2377,0396	6,10	70,98	0,00058	SDCQVMQQQCCQQLAQIPR
				1	793,3595	2377,0567	2377,0396	7,19	41,5	0,55	SDCQVMQQQCCQQLAQIPR
B6UKM7	Gamma-gliadin n=1 Tax=Triticum aestivum ReplID=B6UKM7_WHEAT	225	13,5	1	399,2686	796,5226	796,5171	6,91	27,15	6,8	VSILPR
				1	399,2686	796,5226	796,5171	6,91	30,25	3,4	VSILPR
				1	455,8111	909,6076	909,6011	7,15	40,7	0,12	LVSILPR
				1	455,8111	909,6076	909,6011	7,15	50,73	0,012	LVSILPR
				1	795,954	1589,8934	1589,8889	2,83	28,79	19	IIQPQQPAQLEGIR
				1	530,9733	1589,8981	1589,8889	5,79	45,71	0,27	IIQPQQPAQLEGIR
				1	530,9733	1589,8981	1589,8889	5,79	36,06	2,5	IIQPQQPAQLEGIR
				1	873,4392	1744,8639	1744,8567	4,13	24,77	99	NFLLQQCNHVSLSVSS
				1	587,6746	1760,0019	1759,9944	4,26	33,29	3,6	LGIIQPQQPAQLEGIR
				1	904,7922	2711,3549	2711,3485	2,36	39,83	1,9	SLVLQTLPTMCNVYVPPYCSTIR
B9VRI0	Gamma-gliadin n=1 Tax=Triticum aestivum subsp. macha ReplID=B9VRI0_WHEAT	182	16,2	1	636,3441	1270,6736	1270,6703	2,60	30,84	26	NILLQQCKPAS
				1	636,3441	1270,6736	1270,6703	2,60	31,95	20	NILLQQCKPAS
				1	692,8865	1383,7584	1383,7544	2,89	25,2	84	NILLQQCKPASL
				1	544,985	1631,9333	1631,9359	-1,59	24,51	32	IIQPQQPAQLEVIR
				1	829,4489	1656,8832	1656,8869	-2,23	25,16	82	NILLQQCKPASLVSS
				1	829,4489	1656,8832	1656,8869	-2,23	27,42	49	NILLQQCKPASLVSS
				1	845,4876	1688,9607	1688,9573	2,01	23,81	40	GIIQPQQPAQLEVIR
				1	898,7809	2693,3208	2693,3049	5,90	48,63	0,25	SLVLQTLPSMCNVYVPPYPCSIMR
A7LHA4	Alpha gliadin n=1 Tax=Lophopyrum elongatum ReplID=A7LHA4_LOPEL	172	21,8	1	456,7455	911,4765	911,4713	5,71	24,87	84	DVVLQQPN
				1	746,7021	2237,0844	2237,0683	7,20	23,61	96	NLALQTLPAMCNVYIPPYC
				1	843,0779	2526,2118	2526,1957	6,37	36,21	4,5	NLALQTLPAMCNVYIPPYCSTT
				1	925,8318	2774,4737	2774,461	4,58	22,54	63	QQQQQQQQILQQILQQQLIPCR
				1	1004,831	3011,4711	3011,4595	3,85	38,61	2,1	NLALQTLPAMCNVYIPPYCSTTIAPFG
				1	1004,831	3011,4711	3011,4595	3,85	22,28	89	NLALQTLPAMCNVYIPPYCSTTIAPFG
				1	1182,2548	3543,7425	3543,7241	5,19	26,51	26	NLALQTLPAMCNVYIPPYCSTTIAPFGIFGTN

Q7XAU7	Thaumatococcus Tax=Vitis vinifera ReplID=Q7XAU7_VITVI	163	26,7	1	728,3246	1454,6346	1454,6283	4,33	38,36	1,5	TTGGCANNPCTVFK
				1	729,7966	1457,5786	1457,5769	1,17	25,34	6,6	CPDAYSYPKDDQ
				1	881,4344	1760,8543	1760,8437	6,02	70,33	0,0026	GISCTADIVGECPAALK
				1	720,6003	2158,779	2158,7626	7,60	29,02	0,17	TDEYCCNSGSCNATDYSR
P17314	Alpha-amylase/trypsin inhibitor CM3 n=3 Tax=Triticum ReplID=IAAC3_WHEAT	159	35,1	1	568,7823	1135,5501	1135,5444	5,02	27,69	51	SGSCVPGVAFR
				1	849,9689	1697,9233	1697,9141	5,42	25,32	61	YFIALPVPSQPVDPR
				1	566,9833	1697,928	1697,9141	8,19	39,38	2,1	YFIALPVPSQPVDPR
				1	864,4259	1726,8373	1726,8308	3,76	36,56	6	SGNVGESGLIDLPGCPR
				1	864,4259	1726,8373	1726,8308	3,76	25,17	83	SGNVGESGLIDLPGCPR
				1	901,4269	1800,8393	1800,8353	2,22	55,18	0,058	DYVLQQTCTGFTPGSK
D1MJA1	High molecular weight glutenin subunit Ax-dp (Fragment) n=1 Tax=Triticum turgidum subsp. polonicum ReplID=D1MJA1_TRITU	146	3,5	1	729,3702	1456,7258	1456,7279	-1,44	35,39	8,8	AQQLAAQLPAMCR
				1	755,3774	1508,7402	1508,7406	-0,27	43,11	1,6	VSPECQPVGGGPVAR
				1	812,8907	1623,7669	1623,7675	-0,37	68,55	0,0038	DVSPECQPVGGGPVAR
A1EHE7	Gamma-gliadin n=9 Tax=Triticum ReplID=A1EHE7_WHEAT	146	20,2	1	535,6452	1603,9137	1603,9046	5,67	45,67	0,24	IIQPQQPAQLEAIR
				1	535,6452	1603,9137	1603,9046	5,67	24,43	31	IIQPQQPAQLEAIR
				1	802,965	1603,9154	1603,9046	6,73	54,06	0,035	IIQPQQPAQLEAIR
				1	831,4697	1660,9249	1660,926	-0,66	21,47	1,00E+02	GIIQPQQPAQLEAIR
				1	888,1348	2661,3827	2661,358	9,28	42,89	0,79	SLVLQTLPTMCNVYVPPECSI
				1	1051,2074	3150,6004	3150,5804	6,35	27,63	24	SLVLQTLPTMCNVYVPPECSI
Q1WA39	Alpha-gliadin n=1 Tax=Triticum aestivum ReplID=Q1WA39_WHEAT	142	34,6	1	537,959	1610,8551	1610,8416	8,38	23,66	1,10E+02	VQPQQLPQFEEIR
				1	925,8318	2774,4737	2774,461	4,58	22,54	63	QQQQQQQILQQILQQQLIPCR
				1	828,1669	3308,6386	3308,6284	3,08	45,84	0,35	NLAETLPAMCNVYIPPYCTIAPVGIFGTN
				1	1103,8902	3308,6488	3308,6284	6,17	49,33	0,15	NLAETLPAMCNVYIPPYCTIAPVGIFGTN
				1	1103,8902	3308,6488	3308,6284	6,17	45,04	0,41	NLAETLPAMCNVYIPPYCTIAPVGIFGTN
				1	1219,3619	4873,4186	4873,3916	5,54	34,65	1,6	DVVLQQHSIAYGSSQVLQQSTYQLVQQLCCQQLWQIPEQSR
				1	975,6934	4873,4304	4873,3916	7,96	46,74	0,1	DVVLQQHSIAYGSSQVLQQSTYQLVQQLCCQQLWQIPEQSR
Q0Q5D8	Y-type HMW glutenin n=1 Tax=Triticum aestivum ReplID=Q0Q5D8_WHEAT	136	7,4	1	557,3024	1112,5903	1112,5938	-3,15	38,93	3,9	QVVDQQLAGR
				1	661,309	1320,6035	1320,5979	4,24	34,05	7,9	ELQESSLEACR
				1	661,309	1320,6035	1320,5979	4,24	43,53	0,89	ELQESSLEACR

				2	771,4042	1540,7938	1540,7854	5,45	24	1,10E+02	VQQPATQLPIMCR
				1	665,006	1991,9962	1991,9886	3,82	29,34	31	QQPGQQGQIQGQQQLGQGR
B2CGM6	Triticin n=1 Tax=Triticum aestivum ReplID=B2CGM6_WHEAT	136	10,1	3	443,2628	884,5111	884,508	3,50	25,06	1,20E+02	LQASTPLR
				1	503,5991	1507,7754	1507,7671	5,50	22,4	1,80E+02	FSREEELGVFAPK
				1	636,9821	1907,9245	1907,9126	6,24	22,99	1,30E+02	SSQLHSSQNIFSGFDVR
				1	681,7158	2042,1254	2042,116	4,60	65,14	0,0036	NSILGALPVDVIANAYGISR
				1	1022,0712	2042,1279	2042,116	5,83	50,67	0,096	NSILGALPVDVIANAYGISR
Q04E64	60 kDa chaperonin n=1 Tax=Oenococcus oeni PSU-1 ReplID=CH60_OENOB	128	9,9	1	680,3469	1358,6793	1358,679	0,22	49,77	0,36	VADSLQGDVATGAR
				1	693,3972	2077,1696	2077,1532	7,90	52,08	0,046	IGNIQDILPVLQSVVEQGR
				1	737,0954	2208,2644	2208,2617	1,22	26,46	10	SLLIADDITGEALPTLVLNK
Q1WA40	Alpha-gliadin Gli2-LM2-12 n=1 Tax=Triticum aestivum ReplID=Q1WA40_WHEAT	120	27,2	1	834,4106	2500,2101	2500,1913	7,52	33,58	8,7	NLALQTLPAMCNVYIPPHCSTT
				1	925,8318	2774,4737	2774,461	4,58	22,54	63	QQQQQQQQILQQILQQQLIPCR
				1	1208,3682	4829,4436	4829,4341	1,97	64,22	0,0019	DVVLQQHNIHASSQVLQQSTYQLLQQLCCQQLLQIPEQSR
				1	966,8992	4829,4598	4829,4341	5,32	43,51	0,21	DVVLQQHNIHASSQVLQQSTYQLLQQLCCQQLLQIPEQSR
D1HU00	Whole genome shotgun sequence of line PN40024, scaffold_22.assembly12x (Fragment) n=1 Tax=Vitis vinifera ReplID=D1HU00_VITVI	120	6,5	1	602,7964	1203,5782	1203,5732	4,15	30,34	29	SSLAVDDVDQR
				1	567,3004	1698,8794	1698,869	6,12	24,31	1,10E+02	VLVDHSIVEGFSQGGR
				1	567,3004	1698,8794	1698,869	6,12	47,52	0,51	VLVDHSIVEGFSQGGR
				1	879,4161	1756,8176	1756,813	2,62	41,64	1,3	IWEMASADIHPYPLD
A4ZIX1	Monomeric alpha-amylase inhibitor (Fragment) n=1 Tax=Triticum aestivum ReplID=A4ZIX1_WHEAT	119	34,1	1	519,743	1037,4715	1037,4634	7,81	27,37	41	CGDLSSMLR
				1	525,7842	1049,5539	1049,5506	3,14	23,74	1,40E+02	SVYQELGVR
				1	778,4143	1554,8141	1554,8188	-3,02	29,71	32	LQCVGSQVPEAVLR
				1	991,4082	1980,8019	1980,7877	7,17	38,55	0,25	DCCQQLADINNEWCR
Q6UJY5	HMW-glutenin Bx subunit n=1 Tax=Triticum turgidum ReplID=Q6UJY5_TRITU	118	5,5	2	551,2714	1100,5282	1100,5285	-0,27	23	1,30E+02	DVSPGCRPIT
				1	573,7935	1145,5724	1145,5676	4,19	24,95	1,30E+02	LEGSDALSARQ
				1	651,839	1301,6634	1301,6616	1,38	34,24	14	QYEQQPVVPSK
				1	729,3702	1456,7258	1456,7279	-1,44	35,39	8,8	AQQLAAQLPAMCR
Q41528	Alpha-gliadin n=1 Tax=Triticum aestivum ReplID=Q41528_WHEAT	93	15	1	537,959	1610,8551	1610,8416	8,38	23,66	1,10E+02	VQPQQLPQFEEIR

				1	752,0319	2253,0738	2253,0632	4,70	22,3	1,20E+02	NLALQTLPSMCNVYIPPYC
				1	843,9224	3371,6603	3371,6393	6,23	23,41	58	NLALQTLPSMCNVYIPPYCTIAPFGIFGTN
				1	1124,8951	3371,6636	3371,6393	7,21	48,68	0,17	NLALQTLPSMCNVYIPPYCTIAPFGIFGTN
				1	1124,8951	3371,6636	3371,6393	7,21	31,75	8,6	NLALQTLPSMCNVYIPPYCTIAPFGIFGTN
B7U6L4	Globulin 3 n=1 Tax=Triticum aestivum ReplID=B7U6L4_WHEAT	86	4,8	1	514,7845	1027,5544	1027,5484	5,84	48,76	0,36	VAIMEVNPR
				1	738,6598	2212,9575	2212,9468	4,84	38,22	0,81	GSGSESEEEQDQQRVETVR
B5B0D5	Major allergen CM16 n=2 Tax=Triticum ReplID=B5B0D5_WHEAT	81	24,5	1	584,7583	1167,502	1167,4979	3,51	24,47	37	DYVEQQACR
				1	931,4123	1860,81	1860,8029	3,82	32,36	4,5	QQCCGELANIPQQCR
				1	775,716	2324,1262	2324,1107	6,67	23,98	83	DYVEQQACRIETPGSPYLAK
Q2A784	Putative avenin-like a n=1 Tax=Triticum aestivum ReplID=Q2A784_WHEAT	77	18,2	1	908,9273	1815,8401	1815,8356	2,48	56,04	0,045	QQCCQPLAQISEQAR
				1	1012,4845	2022,9544	2022,9391	7,56	20,69	1,70E+02	VNIPQYCTTTPCSTITPA
D1IBD6	Whole genome shotgun sequence of line PN40024, scaffold_3.assembly12x (Fragment) n=1 Tax=Vitis vinifera ReplID=D1IBD6_VITVI	77	1,5	1	654,2914	1306,5682	1306,5612	5,36	30,68	9,1	SCEAPSSLDWR
				1	654,2914	1306,5682	1306,5612	5,36	43,63	0,46	SCEAPSSLDWR
				1	689,8118	1377,609	1377,5983	7,77	32,97	6,3	ASCEAPSSLDWR
				1	689,8118	1377,609	1377,5983	7,77	20,05	1,20E+02	ASCEAPSSLDWR
A5AKD8	Peptidyl-prolyl cis-trans isomerase n=2 Tax=Vitis vinifera ReplID=A5AKD8_VITVI	76	13,4	1	567,78	1133,5455	1133,5387	6,00	35,64	9,3	AVVVSDCGQLS
				1	696,8665	1391,7184	1391,7119	4,67	40,05	4,1	IVMELFADTTTPR
Q41540	CM 17 protein n=1 Tax=Triticum aestivum ReplID=Q41540_WHEAT	61	19,6	1	584,7583	1167,5021	1167,4978	3,68	36,89	2,1	NYVEEQACR
				1	750,0239	2247,05	2247,03	8,90	24,52	59	NEDCTPWTSTLITPLPSCR
A5AYU8	ATP synthase subunit beta n=1 Tax=Vitis vinifera ReplID=A5AYU8_VITVI	57	5,8	1	631,8226	1261,6307	1261,6336	-2,30	27,79	64	TIAMDGTEGLVR
				1	746,8953	1491,776	1491,7681	5,30	29,21	35	FTQANSEVSALLGR

GLUVITAL 21000 1 g/hL

prot_acc	prot_desc	prot_score	%prot_cover	pep_rank	pep_exp_mz	pep_exp_mr	pep_calc_mr	pep_delta	pep_score	pep_expect	pep_seq
B6UKL5	Gamma-gliadin n=1 Tax=Triticum turgidum RepID=B6UKL5_TRITU	169	13,4	1	399,27	796,52	796,52	0	29,74	3,7	VSIILPR
				1	455,81	909,61	909,6	0,01	37,36	0,29	LVSIIILPR
				1	455,81	909,61	909,6	0,01	35,06	0,48	LVSIIILPR
				1	594,33	1186,64	1186,63	0,01	65,06	0,013	APFASIVAGIGGQ
				1	841,97	1681,92	1681,92	0,01	37,06	3,7	IIQPQQPAQYEVIR
				1	561,65	1681,92	1681,92	0,01	25,42	53	IIQPQQPAQYEVIR
D0ES83	Gamma gliadin n=1 Tax=Triticum aestivum RepID=D0ES83_WHEAT	139	20,2	1	594,33	1186,64	1186,63	0,01	65,06	0,013	APFASIVAGIGGQ
				1	636,34	1270,67	1270,67	0	28,05	52	NILLQQCKPAS
				1	535,64	1603,91	1603,9	0,01	37,01	1,9	IIQPQQPAQLEAIR
				1	802,96	1603,91	1603,9	0,01	14,39	3,40E+02	IIQPQQPAQLEAIR
				7	850,95	1699,88	1699,87	0,01	9,08	4,20E+03	FIQPSLQQQLNPCK
B9VRI0	Gamma-gliadin n=1 Tax=Triticum aestivum subsp. macha RepID=B9VRI0_WHEAT	139	15,9	1	594,33	1186,64	1186,63	0,01	65,06	0,013	APFASIVAGIGGQ
				1	636,34	1270,67	1270,67	0	28,05	52	NILLQQCKPAS
				5	544,99	1631,93	1631,94	0	4,65	3,50E+03	IIQPQQPAQLEAIR
				1	816,98	1631,94	1631,94	0,01	36,7	1,4	IIQPQQPAQLEAIR
				7	850,95	1699,88	1699,87	0,01	9,08	4,20E+03	FIQPSLQQQLNPCK
UPI00019 84057	PREDICTED: similar to cysteine protease Cp5 n=1 Tax=Vitis vinifera RepID=UPI0001984057	136	6,9	1	605,31	1208,6	1208,59	0,01	20,89	3,10E+02	VVSIDGYKDVD
				1	653,31	1304,61	1304,6	0,01	18,46	4,00E+02	GVVTGIKDQGDGCG
				1	654,29	1306,57	1306,56	0,01	30,37	12	SCEAPSSLDWR
				3	654,29	1306,57	1306,56	0,01	20,64	1,10E+02	SCEAPSSLDWR
				2	436,53	1306,57	1306,56	0,01	13,8	5,50E+02	SCEAPSSLDWR
				1	1108,51	2215,01	2214,99	0,02	66,58	0,003	NTDLPYGECAINAMASYPTK
D0EMA4	Gamma gliadin n=3 Tax=Triticum RepID=D0EMA4_WHEAT	123	7,9	1	399,27	796,52	796,52	0	29,74	3,7	VSIILPR
				1	455,81	909,61	909,6	0,01	37,36	0,29	LVSIIILPR
				1	455,81	909,61	909,6	0,01	35,06	0,48	LVSIIILPR
				1	530,97	1589,89	1589,89	0,01	14,95	4,00E+02	IIQPQQPAQLEGIR
				1	795,96	1589,9	1589,89	0,01	14,41	4,40E+02	IIQPQQPAQLEGIR
				1	587,67	1760	1759,99	0	40,82	0,88	LGIIQPQQPAQLEGIR
Q0GNF9	Low molecular weight glutenin n=1 Tax=Triticum aestivum RepID=Q0GNF9_WHEAT	122	17,4	1	430,76	859,5	859,49	0,01	23,13	1,70E+02	VNVPLYR
				1	557,31	1112,61	1112,6	0,01	32,83	12	ILQQLNPCK

					1	683,85	1365,69	1365,68	0,01	21,31	2,60E+02	TTTSVPFGVGTGVGS
					1	687,36	2059,05	2059,03	0,02	14,93	9,30E+02	VFLQQQCSPVAMPQSLAR
B2LS34	Low molecular weight glutenin subunit n=1 Tax=Triticum aestivum ReplID=B2LS34_WHEAT	119	12,5	1	430,76	859,5	859,49	0,01	23,13	1,70E+02	VNVPLYR	
				1	557,31	1112,61	1112,6	0,01	32,83	12	ILQQLNPCK	
				1	831,94	1661,87	1661,86	0,01	25,33	90	ILPTMCSVNVPLYR	
				2	554,97	1661,87	1661,86	0,01	13,59	1,30E+03	ILPTMCSVNVPLYR	
				1	901,96	1801,9	1801,9	0,01	38,15	4,9	LFLQQQCSPVAMPQR	
				1	849,97	1697,92	1697,91	0,01	42,84	1,2	YFIALPVPSQPVDPR	
				1	864,43	1726,84	1726,83	0,01	49,91	0,3	SGNVGESGLIDLPGCPR	
D2XT93	ATP synthase subunit alpha n=1 Tax=Allium cepa ReplID=D2XT93_ALLCE	100	12,4	1	486,78	971,54	971,54	0	39,23	5,7	VVSVGDGIAR	
				1	513,8	1025,59	1025,59	0	15,5	4,70E+02	AVDSLPIGR	
				1	522,29	1042,57	1042,57	0	13,55	2,00E+03	TGSIVDVPAGK	
				1	602,34	1202,66	1202,65	0,01	21,58	2,20E+02	AAELTTLLESR	
				3	605,84	1209,68	1209,67	0	10,58	1,80E+03	VVDALGVPIDGR	
A3QRB5	Thaumatococcus-like protein n=1 Tax=Vitis vinifera ReplID=A3QRB5_VITVI	100	13,3	1	728,33	1454,64	1454,63	0,01	33,35	5,8	TTGGCNPCTVFK	
				1	728,33	1454,64	1454,63	0,01	27,96	20	TTGGCNPCTVFK	
				1	587,96	1760,85	1760,84	0,01	20,37	2,60E+02	GISCTADIVGECPAALK	
				1	881,44	1760,86	1760,84	0,01	66,73	0,0066	GISCTADIVGECPAALK	
B2Y2R3	Low molecular weight glutenin subunit n=1 Tax=Triticum aestivum ReplID=B2Y2R3_WHEAT	97	12,8	1	430,76	859,5	859,49	0,01	23,13	1,70E+02	VNVPLYR	
				1	557,31	1112,61	1112,6	0,01	32,83	12	ILQQLNPCK	
				1	784,91	1567,81	1567,8	0,01	15,22	9,80E+02	TTTRVPFGVGTGVGGY	
				1	687,36	2059,05	2059,03	0,02	14,93	9,30E+02	VFLQQQCSPVAMPQSLAR	
D3LAL9	60 kDa chaperonin n=1 Tax=Oenococcus oeni AWRIB429 ReplID=D3LAL9_OENOE	90	8,6	1	421,27	840,52	840,52	0	14,12	5,20E+02	LAGGVAVVR	
				2	493,79	985,56	985,56	0	16,47	9,70E+02	ATAAAVAGLNK	
				2	584,83	1167,64	1167,64	0	16,78	4,60E+02	NVTAGANPVGIR	
				1	680,35	1358,69	1358,68	0,01	31,55	26	VADSLQGDVATGAR	
				1	496,6	1486,78	1486,77	0	11,13	2,90E+03	KVADSLQGDVATGAR	
A4URY8	Dy-type high molecular weight subunit protein n=1 Tax=Triticum aestivum ReplID=A4URY8_WHEAT	87	5,2	1	557,3	1112,59	1112,59	0	25,87	79	QVVDQQLAGR	
				1	661,31	1320,61	1320,6	0,01	41,57	1,6	ELQESSLEACR	

				1	735,38	1468,75	1468,75	0,01	19,96	3,60E+02	AQQPATQLPTVCR
D4HNC8	Y-type HMW glutenin subunit n=1 Tax=Eremopyrum triticeum RepID=D4HNC8_9POAL	79	4,3	1	661,31	1320,61	1320,6	0,01	41,57	1,6	ELQESSLEACR
				1	682,3	1362,59	1362,58	0	37,32	1,8	EGEASGQLQCER
B2LWZ7	Low molecular weight glutenin subunit I2 n=1 Tax=Aegilops comosa RepID=B2LWZ7_AEGCM	68	7,6	1	557,31	1112,61	1112,6	0,01	32,83	12	ILQQLNPCK
				1	573,3	1716,89	1716,88	0,01	12,47	1,80E+03	VFLQQQCIPVAMQR
				1	859,45	1716,89	1716,88	0,01	24,21	1,20E+02	VFLQQQCIPVAMQR
				2	610,35	1218,69	1218,69	0,01	21,66	1,20E+02	VVDLIEYVAKA
B5VLC4	Glyceraldehyde-3-phosphate dehydrogenase (Fragment) n=1 Tax=Saccharomyces cerevisiae AWRI1631 RepID=B5VLC4_YEAS6	64	10,7	1	910,45	1818,89	1818,9	-0,01	43,34	1,4	IVSNASCTTNCLAPLAK
				1	505,8	1009,6	1009,59	0	42,2	0,79	AIDALVPVGR
Q1NFW0	ATP synthase alpha subunit n=1 Tax=Sphingomonas sp. SKA58 RepID=Q1NFW0_9SPHN	64	4,1	1	543,31	1084,61	1084,61	0	21,36	2,90E+02	TGTIVDVPVGK
				1	705,41	1408,8	1408,8	0	32,48	7,8	VLNTGSPITVPVGR
Q0JKB4	ATP synthase subunit beta n=1 Tax=Oryza sativa Japonica Group RepID=Q0JKB4_ORYSJ	63	5	1	746,9	1491,78	1491,77	0,01	30,65	30	FTQANSEVSALLGR
				3	587,97	1760,88	1760,87	0,02	13,59	1,40E+03	GITISTAHVEYETANR
				2	552,31	1102,6	1102,6	0	29,1	45	SSAGAVVVTNAK
B3LP15	Protein YGP1 n=1 Tax=Saccharomyces cerevisiae RM11-1a RepID=B3LP15_YEAS1	56	6,8	1	595,33	1188,65	1188,65	0	26,86	65	NAVAGAGYLSPIK
				1	364,74	727,46	727,46	0	12,58	1,90E+03	VIDLIR
Q0J8A4	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic n=3 Tax=Oryza sativa RepID=G3PC_ORYSJ	56	6,8	1	910,45	1818,89	1818,9	-0,01	43,34	1,4	IVSNASCTTNCLAPLAK
				1	688,82	1375,62	1375,63	-0,01	12,27	1,00E+03	ACQQVMDQQLR
C0SUC3	High-molecular-weight glutenin subunit x5 n=1 Tax=Triticum aestivum RepID=C0SUC3_WHEAT	55	2,7	1	688,83	1375,64	1375,63	0,01	7,92	4,10E+03	ACQQVMDQQLR
				1	718,31	1434,61	1434,6	0,01	42,31	0,5	EGEASEQLQCER

GBS-P51 1 g/hL

prot_acc	prot_desc	prot_score	%prot_cover	pep_rank	pep_exp_mz	pep_exp_mr	pep_calc_mr	pep_delta	pep_score	pep_expect	pep_seq
UPI0001984057	PREDICTED: similar to cysteine protease Cp5 n=1 Tax=Vitis vinifera ReplD=UPI0001984057	120	5,5	1	605,3	1208,59	1208,59	0	10,86	2,60E+03	VVSIDGYKDVD
				1	654,29	1306,56	1306,56	0	9,17	1,30E+03	SCEAPSSLDWR
				1	654,29	1306,56	1306,56	0	24	43	SCEAPSSLDWR
				1	436,53	1306,57	1306,56	0,01	13,99	5,30E+02	SCEAPSSLDWR
				5	689,81	1377,61	1377,6	0,01	8,44	2,10E+03	ASCEAPSSLDWR
				3	689,81	1377,61	1377,6	0,01	24,91	46	ASCEAPSSLDWR
				1	739,34	2215	2214,99	0,01	53,57	0,048	NTDLPYGECAINAMASYPTK
				1	1108,51	2215,01	2214,99	0,01	12,95	6,20E+02	NTDLPYGECAINAMASYPTK
				2	791,38	2371,11	2371,09	0,01	6,71	3,60E+03	RNTDLPYGECAINAMASYPTK
A3QRB5	Thaumatococcus-like protein n=1 Tax=Vitis vinifera ReplD=A3QRB5_VITVI	113	18,7	1	642,77	1283,52	1283,52	0	13,04	2,00E+02	TNCNFDASGNKG
				1	728,32	1454,63	1454,63	0	84,22	4,00E-05	TTGGCNPCTVFK
				1	728,32	1454,63	1454,63	0	5,33	3,10E+03	TTGGCNPCTVFK
				1	881,43	1760,84	1760,84	0	15,46	8,10E+02	GISCTADIVGECPAALK
				1	587,96	1760,85	1760,84	0,01	7,41	5,20E+03	GISCTADIVGECPAALK
B6UKL5	Gamma-gliadin n=1 Tax=Triticum turgidum ReplD=B6UKL5_TRITU	65	15,7	1	455,81	909,61	909,6	0,01	23,54	6,9	LVSILPR
				1	841,96	1681,91	1681,92	0	11,66	1,50E+03	IIQPQQPAQYEVIR
				2	561,65	1681,92	1681,92	0,01	8,66	2,50E+03	IIQPQQPAQYEVIR
				1	793,36	2377,04	2377,04	0,01	29,85	6,9	SDCQVMQQQCCQLAQIPR
D0ES83	Gamma gliadin n=1 Tax=Triticum aestivum ReplD=D0ES83_WHEAT	59	9,7	1	636,34	1270,67	1270,67	0	33,33	16	NILLQQCKPAS
				1	802,96	1603,9	1603,9	0	25,76	35	IIQPQQPAQLEAIR
				1	535,64	1603,91	1603,9	0	8,88	1,60E+03	IIQPQQPAQLEAIR

Table V (supporting information): List of proteins identified by MS/MS in wine fined with 5 and 1 g/hL Gluvital 21000 and GBS-P51. For each protein the score and percentage of sequence coverage are reported, together with the list of all peptides identified, their observed and theoretical molecular weight, the difference between observed and calculated molecular weight (in ppm), the Mascot score and *p* value, the ranking of the identification, and the amino acid sequences.