

AN ABSTRACT OF THE THESIS OF

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Abstract approved:

Alan T. Bakalinsky

Yeast mannoproteins released during the process of aging wine on the yeast lees have been reported to make important contributions to wine quality. However, few mannoproteins have been identified in wine and their lifespan during aging is unknown. As a first step towards better understanding the contributions of yeast mannoproteins to wine quality, a model system was used to follow changes in yeast protein release, and to identify the released proteins over a 9-month time course following completion of fermentation. Model musts were fermented in duplicate by a number of commercial yeast strains, including BM45 and RC212, and were stored on the yeast lees post-fermentation with monthly stirring at 15°C. Wine samples were taken during and after fermentation, and following removal of suspended solids, total protein and total mannoproteins were measured, and individual proteins were identified—but not quantified—by HPLC-MS of tryptic fragments.

The total number of identified proteins in all samples increased from between 3-15 following inoculation, to between 70-80 after one month on the lees, and decreased to about 20 after 6 months on the lees. Over 50% of the identified proteins were shared among all yeast samples. For most strains, protein and mannoprotein concentrations increased during, but decreased by the end of fermentation. Both protein and mannoprotein concentrations were found to increase again post-fermentation, reaching values about 2- and 6-fold higher than values measured at 2 days, respectively. Consistent with the increase in mannoprotein concentration, cell wall mannoproteins were the predominant proteins identified after 6 months of aging on the lees. Most cytosolic proteins found during and soon after fermentation were not found after 6 months of aging on the lees.

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Yeast Protein Release during Fermentation and Aging in a Model Wine

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I understand that my thesis will become a part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Jeffrey D. Rowe, Author

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Introduction

Wine is typically evaluated for quality based on aroma, texture, and color, not for protein content, but certain proteins may, in fact, ultimately contribute to aroma, texture, and color. Protein is a minor constituent in wine, ranging from undetectable levels to over 200 mg/L. Typical protein concentrations in white wines range from 10 to 50 mg/L. Wine proteins can have significant positive and negative effects. Highly undesirable heat-induced protein hazes have been attributed in part, to grape proteins present in the must that survive the winemaking process (Ferreira et al. 2002, Waters et al. 1996). It has been reported that some yeast proteins contribute positively to wine quality by forming tannin-protein complexes (Escot et al. 2001; Riou et al. 2002; Charpentier et al. 2004) that may attenuate astringency (Vidal et al. 2004a&b), by interacting with aroma compounds (Lubbers et al. 1994, Ramirez-Ramirez et al. 2004), and by enhancing foam stability in sparkling wine (Moreno-Arribas et al. 2000). Considerable attention has been paid to yeast mannoproteins released during fermentation and aging (Escot et al. 2001; Riou et al. 2002; Charpentier et al. 2004; Vidal et al. 2004a&b). It has been suggested that aging wine on the yeast lees may lead to an increase in yeast mannoproteins (Dupin et al. 2000).

A limited number of studies have identified yeast- and grape-derived proteins in commercial wines by HPLC-mass spectrometry (Cilindre et al. 2007, Kwon 2004). Proteins of grape origin have included VVTL, class IV chitinase, and vacuolar invertase, while yeast-derived proteins have included Suc4, Cwp1, Gas1, Ecm33, and Crh1. Much current research regarding wine yeast proteins has focused on quantification of protein and polysaccharides released during fermentation and aging

(Luguera et al. 1998; Martinez-Rodriguez 2002; Nunez 2005). Differences in levels of proteins and polysaccharides released by yeast strains have been observed, but as of this writing, a critical accounting of yeast-derived proteins extracted into wine during aging has not been published. The goal of the present study was to determine the kinetics of protein extraction from multiple wine yeast strains. Specifically, changes in protein and mannoprotein concentrations were monitored over a 9-month time course, and individual proteins extracted into model wines were identified.

Literature Review

I. Aging of wine on the yeast lees

A) Definition of aging on the lees and description of process

The French term “sur lies,” which means “on the (yeast) sediment” refers to the aging of wine in barrel, tank, or bottle in the presence of yeast for an extended period of time after completion of the alcoholic fermentation. The “sur lies” process is defined and regulated by the Institut National des Appellations d'Origine (INAO) governmental statutes in France, but not in the United States (Fornairon et al. 2002). The duration of aging on the lees varies. In Burgundy, the highest quality “Grand Cru” white wines may age for 12 to 15 months. In contrast, aging on the lees may only last between 2 to 6 months, namely in Switzerland, Australia or other new world wine regions (Fornairon et al. 2002). Still table wines are aged in barrels or in large tanks while sparkling wines age in the bottle after undergoing a second alcoholic fermentation. In the case of sparkling wines, aging in the bottle, known as “en tirage,” may range from months to many years (Fornairon et al. 2002). Lees are defined as either gross or fine—gross lees include the grape insoluble solids and much of the yeast cell mass. Fine lees are defined as the yeast biomass remaining in the wine after an initial racking. The proportion of fine lees is small in comparison to the total biomass created throughout fermentation (Fornairon et al. 2002). The lees and wine are typically separated after an initial settling and racking, after which the remaining lees are separated into gross and fine lees. The fine lees are then added back to the wine in barrel. Periodically, the sediment is agitated through the use of a stirring wand or some other device. The stirring regimen varies in timing and duration

based on the preferences of the winemaker. Stirring increases surface contact between the wine and sediment, hastening extraction of yeast components. Increased exposure to the sediment increases the rate of release of proteins, polysaccharides, and other yeast metabolites, some of which contribute to the overall quality of the wine.

B) Composition of the lees

Among the microorganisms found in the lees, *Saccharomyces cerevisiae* is the predominant species. Other native microflora including *Kloeckera apiculata* and *Candida stellata* may be present along with malolactic bacteria *Oenococcus oeni* (as cited by Fornairon-Bonnefond 2000). Sciancapelore et al. (1983) determined total nitrogen of fine lees from Italian red wines to be 4.2% dry weight using the Kjeldahl method. Similarly, using the Kjeldahl method, Ferrari et al. (1988) monitored nitrogen content of lees from Burgundian white wines at different stages of the aging process by sampling the yeast lees (solids) during aging. Nitrogen content was 1.55% dry weight at the beginning, and then dropped to 1.15% dry weight after 5 months of aging and stirring. Lipids from the plasma membrane and other membranes within the cell have been shown to be present in yeast lees in small amounts ranging from 1-6% as dry weight (Fornairon-Bonnefond 2000). Gomez et al. (2004) found lipids present at 5.4% as dry weight in the lees of sherry wine. Palmitic (C16:0), linoleic (C18:2), and stearic acids (C18:0) were the major fatty acids, comprising 33.3, 21.3, and 10.4% of the total fatty acid pool, respectively. These findings are similar to those reported by Sciancapelore et al. (1983) for Italian red wines, in which palmitic, linoleic, oleic (C18:1), and stearic acids were the most abundant, comprising 29, 28, 15.3, and 10.0% of the total fatty acid pool, respectively.

C) Yeast autolysis under winemaking conditions

1) Overview

Salkowski coined the definition of yeast autolysis more than a century ago—the enzymatic degradation of the cell brought about by cell death (as cited in Charpentier & Feuillat 1992). A more detailed definition of autolysis is the set of reactions that hydrolyze intracellular biopolymers via endohydrolases (Fornairon et al. 2002). Cell damage due to elevated levels of ethanol is thought to be the main cause of death during and after fermentation. Autolysis can generally be divided into four stages:

- Initial disruption of cytoplasmic membrane, induced by cell death, leads to intracellular hydrolysis of polymeric constituents.
- Increased hydrolysis of the cell wall glucans and mannoproteins leads to increased porosity of the cell wall.
- The size of the hydrolyzed proteins and polysaccharides decrease enough to diffuse into the wine. (Fornairon et al. 2002; Charpentier & Feuillat 1992).

While the cell is being hydrolyzed from the inside out by endohydrolases, the polysaccharides and mannoproteins making up the cell wall are degraded by glucanases and proteases, further increasing the porosity of the cell wall. Increasing porosity releases more macromolecules into the extracellular medium. Autolysis in sparkling wine has been shown to begin after a 6-12 month latent period following the second fermentation (Feuillat and Charpentier, 1982).

2) Proteolysis

Proteolysis is the hydrolysis of peptide bonds. Proteolysis is carried out by endoproteases throughout the aging process and appears to have a variable activity curve. Protease activities were found to change over the course of 5 months of aging on the lees (Ferrari & Feuillat 1988). Activity increased slightly over the first two months followed by a month of almost no activity. The activity then dramatically increased following the latent month. Similarly, protease activity in sparkling wine decreased for the first few months of aging, followed by increased protease activity for several years. Ultimately protease activity reached its maximum after six years (Feuillat and Charpentier, 1982; Leroy et al. 1990). Alexandre et al. (2001) later showed that protease activity decreased before cell death was complete and continued to decrease during an additional two months of aging. It is interesting to note that the study by Alexandre et al. (2001) did not measure protease activity after several months to years where it has been suggested to increase.

3) Degradation of the cell wall

The cell wall (discussed in more detail below) consists of β 1-3 glucan, β 1-6 glucan, mannoproteins, and chitin. The respective percentages of each constituent are: 50-55%, 5-10%, 35-40%, and 1-2% with the mannoproteins associated with the outer layer consisting of up to 90% glycan (Klis et al. 2002). The initial effects of autolysis begin with the β -1-3 glucans associated with mannoproteins on the outer layer of the cell. β -1-3 glucanases hydrolyze bonds connecting the β -1-3 glucan portion of the cell wall to the so-called Pir-mannoproteins. Following this step, β -glucanases further hydrolyze the β -1-3 glucans that make up the cell wall (Fornairon et al. 2002). Based on electron micrographs the inner layer of the cell wall has been shown to all but

disappear within the first six months of aging sparkling wine on yeast lees (Piton et al. 1988).

D) Effects on wine quality

1) Haze protection in white wines

Protein haze is caused by proteins present in white wines that precipitate and aggregate to form a visible haze (Ferreira et al. 2002). Elevated storage temperatures typically trigger protein haze formation. Currently, bentonite fining is considered the method of choice for insuring protein haze stability in white wine. Fining is the addition of an adsorptive compound, followed by the settling of the adsorptive compound and the compounds removed from the medium. The initial addition of bentonite is followed by the separation of the bentonite lees from the wine (Ferreira et al 2002). Bentonite is a non-specific cation exchanger, which at wine pH adsorbs positively charged ions, including proteins. However, positively charged solutes other than proteins, including flavor and aroma compounds can also be removed, which can negatively influence the finished wine (Ferreira et al 2002). It has also been found that the voluminous lees produced by bentonite fining contain 5 to 20% of the wine volume (Lagace & Bisson, 1990). All of these factors have led many winemakers to search for alternatives to bentonite fining. The observation that white wine aged on lees requires less bentonite to be stabilized has led to study of the haze-protective activity of certain wine glycoproteins.

Pocock et al. (2007) suggested that sulfate, a ubiquitous wine component, was a contributing factor in the formation of protein haze. They found that model wines containing chitinase and thaumatin-like proteins produced no haze when heated if

sulfate were absent. Conversely, when sulfate was present, haze increased in proportion to increasing sulfate concentration. Sulfate is listed in the Hofmeister series of anions, which ranks the ability of ions to precipitate protein. Its presence in wine, combined with temperature stress, suggests the ability to ‘salt out’ positively charged proteins, which then forms a haze (Pocock et al. 2007). Brown et al. (2007) stated “...haze protective factors act by competing with the wine proteins for other wine components required for the formation of aggregations of protein large enough to be visually detected as haze.”

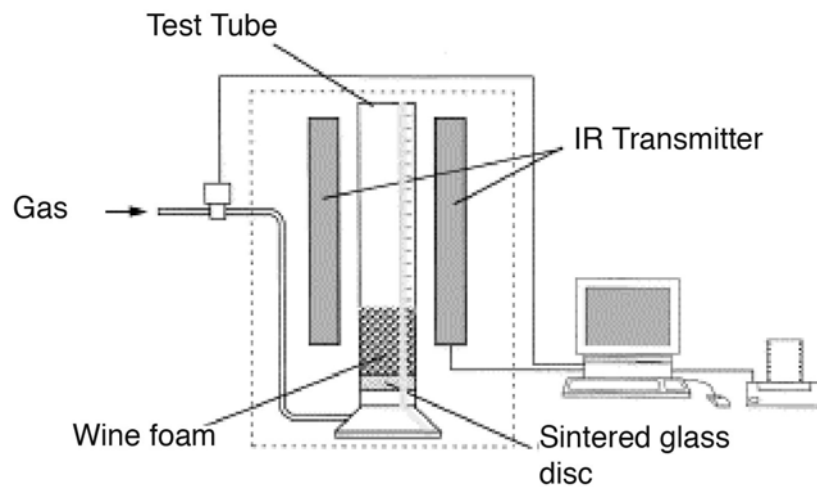
Ledoux et al. (1992) first established the protective effect of yeast mannoproteins by showing that a mannoprotein fragment isolated from yeast invertase reduced the incidence of haze in white wine (Dupin et al. 2000). Waters et al. (1994a) discovered that an approximately 400-ppm addition of grape-derived arabinogalactan–protein (AGP) reduced wine haze by 20% relative to a control, to which no protective factors had been added. Hpf1p, a 420kD polysaccharide-rich yeast mannoprotein isolated from red wine was found to have significant haze protective activity (Waters et al. 1994b). Brown et al. (2007) demonstrated that yeast strains overexpressing Hpf1p or another mannoprotein, Hpf2p, produced more haze protective activity than wild type strains. Wine to which a 6xHis-Hpf2p-fusion protein was added produced 40% less haze relative to a control wine containing no haze protection factors. The 6xHis-Hpf2p-fusion protein added to wine and stored at 25°C for two years showed good haze protective activity for the first 12 months. Over the two-year storage period the wine containing the 6xHis-Hpf2p-fusion protein produced less haze than equivalent concentrations of invertase.

Lomolino and Curioni (2007) investigated several methods to extract yeast cell wall proteins and evaluated their ability to reduce heat-induced haze in wine. Yeast cells were treated with EDTA, a chelating agent, DTT, a reducing agent, or β -glucanase preparations (Zymolyase, Glucanex, and Finizym 250L). Following the treatments the extracts were either added in increasing concentrations to ultrafiltered wines, or they were subjected to Con A affinity chromatography. Con A is a plant lectin that has high affinity for mannan and is commonly used to isolate mannoproteins. The extracts that were added to the ultrafiltered wine were named wine soluble fractions (WSF), while the extracts subjected to Con A treatment and retained by the lectin were named the mannoprotein fraction (MP). Following the Con A treatment a portion of the MP was added to ultrafiltered wine and subjected to a heat treatment of 90°C for 1 hour. The heat-treated wine containing MP was centrifuged and the supernatant was found to contain a new fraction named heat-stable mannoproteins (HSMP). Each fraction (WSF, MP, HSMP) was then added to white wine in increasing quantities and heat tests were carried out. The WSF wines treated by DTT and Zymolyase were found to have 90 and 80% less haze than control wine containing no cell wall proteins, respectively. In contrast, protein haze was higher in the wines that contained WSF treated by Glucanex, Finizym, and EDTA than the control wine (Lomolino and Curioni 2007). Wines that contained the fractions MP and HSMP treated with DTT and Zymolase always produced less haze than the control.

2) Enhanced foam quality in sparkling wine

Sparkling wine is known for its bubbles, which form a foam ring on the surface wine. Foam quality in sparkling wine has been studied using a ‘Mosulux’ device (**Fig 1.**), which measures foam volume (Peron et al. 2000; Senee et al. 1999). In such a device, the volume is measured either as static or dynamic foam, while being sparged with a steady flow of gas exiting a sintered plate at the bottom of a glass tube through a de-gassed wine. Static foam is not continuously subject to gas sparging, while the dynamic form is continuously sparged until the foam has reached equilibrium between formation and decay (Senee et al. 1999; Moreno-Arribas et al. 2000). Visual evaluation by trained judges has also been used to assess foam quality.

Figure 1. Mosulux device image adapted from Senee et al. (1999).



Despite the relatively low concentration of protein in sparkling wine, between 4-20 mg/L, proteins are viewed as the most important macromolecule in sparkling wine foam formation (Marchal et al. 1997). Polysaccharides have also been found to affect foam quality (Moreno-Arribas et al. 2000). Proteins are important for the foam characteristics of other foodstuffs, namely beer, due largely to their surface properties (Brissonnet & Maujean 1993). Roberts (1975) found that beers containing

deglycosylated protein fractions had less foam than beer with glycosylated proteins. Senee et al. (1999) found that model wines containing low glycoprotein concentrations (3 mg/L) had significantly less stable foams than commercial sparkling wines containing greater protein concentrations (14.2 mg/L). Dambrouck speculated that glycoproteins have a higher capacity for stabilizing foam than non-glycosylated proteins due to hydrophilic polysaccharide moieties that preferentially associate with the liquid at the bubble surface. When the liquid film between bubbles begins to thin, the viscosity of the liquid increases due to the polysaccharide moiety. The increased viscosity slows the drainage of the liquid thereby stabilizing the foam (Dambrouck et al. 2005). The protein moiety acts equally on the liquid surface tension providing a critical stabilization factor in foam (Dambrouck et al. 2005).

Glycoproteins found in wine originate from both the grape and the yeast. Most sparkling wines are aged on the lees for an extended period of time. This period of “tirage” leads to a higher concentration of yeast glycoproteins, which affects foam quality (Nunez et al. 2005). Nunez et al. (2005) found that glycoprotein release was greater in wine fermented with a mutant yeast strain, IFI473I, which undergoes accelerated autolysis after the secondary fermentation. The foam quality of the wine fermented with the yeast mutant improved with respect to a control wine fermented with a yeast strain that did not undergo accelerated autolysis. In fact, foam quality improved as early as six months into the aging process in wines fermented with the mutant strain. In a separate study, Nunez et al. (2006) examined the foam quality of sparkling wine treated with thermally and enzymatically processed yeast cell wall extracts. Both cell wall fractions were added to model and commercial wines in

amounts ranging from 0 to 0.50 g/L. The foam quality of wine treated with the thermal extract improved in proportion to increasing additions. The control wines were found to be no different than the enzymatically-treated wines. To establish a relationship between the composition of the extract and foam quality, Con A affinity chromatography was performed on the thermal extract. Interestingly, wines containing the presumably mannoprotein-free eluted fraction (3% protein) of the extract had higher foam quality than wine containing the Con A retained fraction (28% protein). The authors speculated that other yeast colloids must contribute significantly to foam quality in sparkling wine. Another interesting observation was the low quantity of extract needed to significantly affect foam quality. This finding is similar to observations made by Moreno-Arribas et al. (2000) who found no relationship between foam characteristics and concentrations of wine peptides. These findings suggest that specific proteins and other wine colloids, not the quantity of protein contributes to foam quality.

3) Aroma retention enhancement

Aroma is a critical indicator of wine quality. Proteins from yeast cell walls have been claimed to bind volatile aroma compounds when added to a model wine (Lubbers et al. 1994). The addition of yeast cell walls increased the amount of ethyl octanoate, β -ionone, isoamyl alcohol, octanol, hexanoate, and octanol retained in the wine. The aroma compounds were measured using headspace detection at 25° C where known concentrations of added compounds are measured in the headspace and compared to the concentration retained in solution at equilibrium. This comparison

gives a percentage that is “bound” or left in solution. Binding percentage was determined by:

$$Lb = \frac{n_1 - n_2}{n_0} \times 100 (\%)$$

where n_1 = number of moles of bound ligand and free ligand, n_2 = number of moles of free ligand at equilibrium, and n_0 = moles of ligand at the beginning. The retention of aroma compounds in the model wine was found to increase as a function of their increasing hydrophobicity. To determine what yeast cell wall component was responsible for retention of the aroma compounds, Lubbers et al. (1994) isolated glycoproteins present in cell wall material. Four fractions were obtained using DEAE Sephadex A25 ion exchange chromatography. Fraction 1 (F1) contained protein (18.4%), and neutral polysaccharides. Fractions 2 (F2) and 3 (F3) contained glucan and mannoproteins, with protein comprising 12.2 and 8.6% of the mass, respectively. Fraction 4 (F4) contained mostly complex polysaccharides of ribose, mannose and glucose along with some proteins (10%). Fraction 1 was further fractionated into two separate fractions using Con A sepharose affinity chromatography with a bound fraction (R-F1) and an unbound fraction (N-F1). N-F1 contained glycan (39% mannose and glucose) and a large protein fraction (61%). R-F1 contained highly glycosylated mannoproteins bound to the lectin with protein comprising 10% of the mass. The resulting fractions were added to a model wine with several volatile aroma compounds. The wines supplemented with purified glycoproteins containing the highest percentage of protein as opposed to mannan (N-F1) bound the most β -ionone (17.5%) and the most ethyl hexanoate (2.7%). Wine containing the glycoprotein fraction with greater glycan content and less protein (R-F1) bound β -ionone to a

lesser extent (6.2%). Highly glycosylated mannoproteins present in R-F1 bound β -ionone poorly, and did not associate with ethyl hexanoate. Interestingly, polysaccharide fractions and monomeric rhamnogalacturonans II in an aqueous mixture had the opposite effect on aroma compounds—in fact, the volatility of aroma compounds increased when sugars were present (Dufour & Bayonove 1999). Ramirez-Ramirez et al. (2004) conducted a study of aroma compounds involving yeast mannoproteins and oak wood in a model system. The authors observed that as mannoprotein concentrations increased from 0.1 to 5.0 g/L, hydrophobic volatile sorption by the wood increased correspondingly. The opposite was observed for more hydrophilic compounds.

4) Tannin-mannoprotein interactions

Mannoproteins released into wine during aging on the lees are believed to interact with grape polyphenols. Riou et al. (2002) used dynamic light scattering (DLS) to observe a reduction of tannin aggregation in model wine when increasing amounts of wine polysaccharides were added. DLS measures light scattering 90° from the incidence beam, giving an estimate of mean particle diameter, in this case, the diameter of tannin-tannin colloids. Riou et al. (2002) speculated that large tannin molecules with a high mean degree of polymerization (mDP) attract one another because of their relative hydrophobicity in wine. This hydrophobic attraction may lead to formation of tannin-tannin, colloidal complexes that eventually become insoluble. It is speculated that mannoproteins compete with the high mDP tannins to form smaller and more soluble tannin-mannoprotein complexes, thus reducing interactions between the high mDP tannins themselves. High mDP tannins are

thought to be less 'harsh' than low mDP tannins, which has encouraged work focused on mannoprotein and tannin colloid formation and its effect on sensory attributes in red wine.

Charpentier et al. (2004) investigated the 'stability coefficient' of tannins in solution supplemented with glycoproteins derived from yeast strains BM45 and RC212. The authors defined a stability coefficient as "the ratio between the absorbance of tannin alone and absorbance of tannin with glycosylated proteins: $A_{700\text{nm}}(\text{tannins}) / A_{700\text{nm}}(\text{tannins} + \text{glycosylated proteins})$." The authors observed a reduction in A_{700} values for mixtures of tannin and glycoproteins relative to the tannin alone. A synthetic medium was fermented with the yeast strains BM45 and RC212 and glycoproteins were extracted by cold 95% ethanol precipitation. A solution that contained tannin and the BM45-derived glycoproteins produced a stability coefficient 9 times greater than the control (tannin in solution with no glycoproteins). The solution that contained the RC212-derived glycoproteins produced a stability coefficient 4.5 times greater than the control. Lu & Bennick (1998) investigated the tannin-protein binding capacity of human salivary proteins, which are glycosylated. The authors found that there was very little binding of condensed tannin to the native glycoproteins, but there was significantly more binding of tannin following a deglycosylation treatment of the proteins. The authors speculated that the lack of tannin binding is a result of the interaction between the glycosylated proteins and the tannins.

It has been suggested that complexes between tannin and yeast derived-proteins are formed during aging on the lees through non-covalent interaction, such

as, hydrophobic association (adsorption) and hydrogen bonding (Verge et al. 2002; Riou et al 2002; Morata et al. 2003). Polyphenols are partially hydrophobic (aromatic ring structure) and hydrophilic (hydroxyl groups) (Riou et al. 2002). Mannoproteins are also comprised of a hydrophilic (polysaccharide) fraction and slightly hydrophobic (protein) moieties (Ramirez-Ramirez 2004).

The interaction of mannoproteins with polyphenols in red wine has been suggested to modify “texture” and mouth-feel by attenuating astringency associated with skin-derived tannins. Vidal et al. (2004a&b) reported a relationship between mannoproteins and modification of mouth-feel. A trained sensory panel tasted model wine containing glycoproteins [mannoproteins and arabinogalactan proteins (MP+AGP), or rhamanogalacturonan II (RG-II)] and wine polyphenols (procyanidins, anthocyanins). The panel perceived more “fullness” in wines containing the glycoproteins than in the control wine, which contained no glycoproteins. The panel perceived less “dry” character (after expectoration) in the wine containing MP+AGP than in the control wine. Wines containing RG-II had a significantly lower perceived astringency than the base wine (Vidal et al. 2004a). In a second study, Vidal et al. (2004b) reported that wines containing MP+AGP were scored as less bitter than the control wine. Wines containing RG-II were rated less intense in several categories including, ‘chalky’, ‘pucker’, ‘coarse’, and overall astringency.

Escot et al. (2001) measured astringency in red wines supplemented with different amounts of mannoproteins derived from different yeast strains. Astringency was determined by gelatin index, an in vitro assay that measures tannin reactivity with proteins. Increasing gelatin values correspond to increasing astringency.

Mannoproteins derived from three yeast strains (BM45, RC212, BRG) were isolated and added to a base wine that had been previously fined with gelatin. The polysaccharide and protein make-up of the isolated mannoproteins varied between yeast strains. Protein comprised 15.7 and 38.3% of the mass of extracts obtained from BM45 and RC212, respectively. The mannose to glucose ratio for the BM45 and RC212 extracts was 94:6 and 53:47, respectively (Escot et al. 2001). Astringency decreased upon addition of the mannoproteins. A greater decrease in astringency was observed wine containing mannoproteins from BM45 and BRG than in the wine containing the RC212-derived mannoproteins. The authors suggest that yeast strain influences astringency in red wine. One weakness of this study is that it is predicated on the assumption the both the protein and the polyphenolic content of the base wine was unaltered by the initial gelatin-fining step.

5) Interaction of yeast lees with other phenolic compounds

In addition to soluble tannin-mannoprotein complexes that appear to form in wine, adsorption of anthocyanins and polymeric anthocyanins by the yeast lees is commonly observed in winemaking. The yeast lees of red wines following fermentation are typically tinted red or purple. Several studies have documented anthocyanin adsorption onto yeast lees (Morata et al. 2003; Mazauric & Salmon (2005, 2006). Palomero et al. (2007) measured the monomeric anthocyanin content in wine treated with β -glucanase, which accelerates autolysis, and in wine in which the yeast underwent conventional autolysis. Wines containing yeast lees (conventional autolysis), lees and β -glucanase (enzyme assisted autolysis), and a control containing no lees were aged for 420 days. Anthocyanin content was measured by HPLC

photodiode array detection (PDAD) and quantified using an external standard. Wines treated with the commercial β -glucanase were found to have 30% less monomeric anthocyanin than the control wine and the wines in which the yeast underwent conventional autolysis. The authors speculated that the loss of anthocyanin was due to β -glucosidase impurities within the enzyme preparation. β -glucosidase hydrolyzes the glucose from anthocyanin glycosides, thus producing an unstable aglycone. Wine in which the yeast underwent conventional autolysis was found to have higher concentrations of monomeric anthocyanins than the control wine. The authors speculated that the apparent stabilization of monomeric anthocyanin was due to the “protective effect” of aging on the lees. The study did not take into account the direct effect of wine glycoprotein on polyphenolic content.

In an experiment to characterize interactions between yeast lees and wine polyphenols, Mazauric & Salmon (2005) analyzed adsorption of polyphenols onto lees during simulated aging. Polyphenolic adsorption was biphasic in nature, with rapid binding in the first hours leading to protracted and maximal removal of polyphenolics after a week. Approximately one-third of the total free anthocyanins were no longer detectable, and condensed tannins were reduced by 70%. Mazauric & Salmon (2006) analyzed polyphenolic fractions adsorbed by the lees. Different fractions (F1-F5) were recovered using several, increasingly intensive, extraction procedures to ensure a mass balance. Approximately 62, 91, 83, and 97% of the total anthocyanins, monomeric phenolics, condensed tannins, and phenolic acids, respectively, were eventually recovered either from the wine itself or the fractions extracted from yeast lees. The majority of the adsorbed phenolic acids were recovered

from the first fraction (F1), while the majority of condensed tannins were extracted by the second fractionation step (F2). Approximately 11% of the total anthocyanins found in wine prior to aging were recovered from F1, with the majority of anthocyanins recovered from the wine itself (F0). Reflectance tristimulus measurements show F5 still retaining blue- and red-colored compounds; suggesting that trace anthocyanins remained tightly bound to the lees even after successive extraction procedures. Reflectance tristimulus measures color using a reflectance spectrophotometer. Low amounts of acetyl derivatives of anthocyanins were recovered (38%) relative to the glucosyl (74%) and p-coumaroyl (71%) derivatives. Morata et al. (2003) also found that acetyl and p-coumaroyl derivatives adsorbed more tightly to yeast lees than non-acyl adducts. Based on reflectance tristimulus analysis, the acetyl derivatives were suggested to be tightly bound to the lees and thus not recoverable. This conclusion does not take into account the possibility of loss due to oxidation of the anthocyanins. The remaining anthocyanins may have been tightly bound to the lees and not extractable, but they may also have been oxidized and thus would have been undetectable.

Mazauric and Salmon (2005, 2006) claimed that polyphenolic adsorption onto yeast lees is not related to the polarity of the polyphenol. They found that adsorption of anthocyanins by lees did not correlate with increasing polarity as was originally thought (Vasserot et al. 1997). This argument is supported by the discovery that condensed tannins were not preferentially bound onto lees based on size or polarity. The percentages of anthocyanins adsorbed by the lees in order of decreasing polarity are delphinidin, 29%; cyanidin, 34%; petunidin, 33%; peonidin, 38%; and malvidin

32%. Mazauric and Salmon (2005, 2006) suggest that anthocyanin adsorption within the complex polyphenolic environment, present in wine, is restricted by adsorption potential at the cell surface or by the reactivity of the anthocyanin itself and not related to polarity of the given anthocyanin.

Morata et al. (2003) found that wine fermented by different yeast strains contained different amounts of anthocyanin. The yeast lees were isolated and adsorbed anthocyanin was recovered and measured. Anthocyanin adsorbed by the lees differed between the yeast strains and the wines fermented by the different yeasts. Similarly, Caridi et al. (2004) found significant differences in color intensity, total polyphenols, and non-anthocyanic flavonoids in wines fermented by two different strains of yeast. These findings suggest that differences between yeast strains may affect adsorption of wine polyphenols and polymeric pigment onto yeast lees.

6) Stimulation of malo-lactic bacteria

The malo-lactic fermentation (MLF) is the conversion of L-malate to L-lactate and CO₂ in the process. The MLF normally occurs after the alcoholic fermentation and is important because it lowers acidity and may add complexity to aroma and flavor profiles. In red wines, the MLF is difficult to avoid and its occurrence prior to bottling increases the microbiological stability of wine. Guilloux-Benatier et al. (1995) investigated the effect of ethanol-precipitated material (polysaccharides and mannoproteins, EPM) derived from yeast, must, and finished wine on the growth of *Oenococcus oeni* and on MLF activity. Model media containing cellulose, must colloids (from Chardonnay grapes), yeast EPM (from Chardonnay wine), yeast

mannoproteins (from commercial preparation), and a control (containing no additions) underwent alcoholic fermentation and were subsequently inoculated with *O. oeni*. The treatments, containing yeast EPM and yeast mannoproteins were found to have significantly more bacterial biomass than the treatments that contained must colloids and the control. There were no significant differences in MLF activity between treatments.

In a separate study Guilloux-Benatier & Chassagne (2003) investigated the effect that nitrogen-containing wine components had on growth of *Oenococcus oeni* following the alcoholic fermentation. Wine was fermented and aged for 8 weeks, followed by centrifugation to remove solids. The supernatant was removed and fractionated into four molecular weight classes (>10, 1-10, 0.5-1, and < 0.5 kDa), and amino acids, peptides, sugars, protein, and nitrogen were measured. Most amino acids (>90%) were found in the < 0.5 kDa fraction. Protein was not found in high quantities in the >10 kDa fraction. The largest amount of mannose was found in the >10 kDa fraction. The influence of the different fractions on bacterial growth was investigated. Each fraction was added to a model media in equivalent amounts of free amino nitrogen (50 mg/L N) in order to meet N requirements. The wines were subsequently inoculated with *O. oeni*. No growth occurred in the control medium that contained no nitrogen. The most bacterial growth was observed in media containing lower molecular weight components (< 1 kDa), growth was observed in model media that contained higher molecular weight components (>10 kDa) as well. The authors suggested that the nitrogen-starved bacteria secreted proteases that hydrolyze the proteins and peptides in this fraction into amino acids.

II) Wine Proteins

A) Grape proteins

The proteins present in wine are derived from two main sources, *Saccharomyces cerevisiae* (yeast) and *Vitis vinifera* (grape). The proteins of grape origin are abundant in the juice prior to fermentation, but decrease throughout fermentation and aging. Ethanol, low pH, tannins (in red wine), and proteases released by the grapes and yeast degrade or denature and precipitate the proteins. The majority of grape proteins that remain soluble in the finished wine are typically known as pathogenesis related proteins (PR) (Robinson & Davies 2000). PR proteins include chitinases, β -1, 3 glucanases, osmotin-like proteins and thaumatin-like proteins (Ferreira et al. 2002). These proteins are associated with defense against pathogens, and begin to accumulate in the berries at the onset of berry ripening (Robinson & Davies 2000). PR proteins are the major proteins implicated in heat-induced haze in white wines. PR proteins are acid-stable and resist proteolysis, which means that they survive the winemaking process and can eventually cause haze in bottled white wines (Waters et al. 1996).

Chitinases and thaumatin-like proteins have been identified as the most abundant proteins in free run juice (Waters et al. 1998). HPLC was employed to analyze the juice from Muscat grapes. The chitinases comprised 50% of the total protein found in the free run juice, while thaumatin-like proteins accounted for the other 50%. According to Ferreira et al. (2002), PR proteins are present in low levels

in healthy grapes. The authors suggested that the free run juice of freshly pressed grapes would contain elevated levels of PR proteins, synthesized as a response to wounding.

Chitinase is a 32-kDa enzyme present in leaves, roots, stems, and berries. Chitinase exists in several isoforms that are believed to have the same function, to protect against fungal infection (Ferreira et al. 2002). Chitinases derive their antifungal properties from their ability to hydrolyze chitin, which is present in the fungal cell walls (Ferreira et al. 2002). *Vitis vinifera* thaumatin-like protein-1 (VVTL1) has a molecular weight of 24-kDa and belongs to the thaumatin-like protein family (Tattersall et al. 1997). Tattersall et al. (1997) isolated VVTL1 and found that it was expressed exclusively in the berry at the onset of ripening, and was encoded by a single gene. Ferreira et al. (2002) proposed that the antifungal properties of thaumatin proteins arise from their ability to permeabilize cell membranes.

Cilindre et al. (2008) analyzed wines made from healthy and botrytized (fungal infection) Chardonnay grapes. The wines were concentrated and then subjected to ultrafiltration through a 10-kDa molecular weight cut off (MWCO) filter, followed by precipitation of the wine proteins using cold ethanol containing 15% trichloroacetic acid. The solution was then centrifuged and the pellet obtained was rinsed and resolubilized in buffer. The wine protein was then digested by trypsin, and tandem HPLC/MS was performed on the tryptic peptides. Several grape proteins were identified in the wine made from healthy grapes, including VVTL1, class IV chitinase, class IV endochitinase, osmotin-like proteins, vacuolar invertase, and β -1,3 glucanase. These findings are in agreement with the work of Kwon (2004), who

found many of the same proteins (class IV endochitinase, putative TL protein, vacuolar invertase, and β -1,3 glucanase precursor) in a Sauvignon Blanc wine using a similar isolation procedure and tandem mass spectrometry analysis. Monteiro et al. (2001) also found similar PR proteins (VVTL1, osmotin-like proteins, and chitinase) in wine made from Moscatel and Arinto grapes.

B) Yeast proteins

Yeast-derived wine proteins are a minor constituent of a finished wine, but as described above they play a major role in haze-stability, texture, aroma in white and red table wine, as well as foam quality in sparkling wine. Some yeast-derived proteins are resistant to proteolysis and acidic conditions and survive the winemaking process. The majority of yeast proteins found in wine are localized to the yeast cell wall (Kwon 2004; Monteiro et al. 2001; Cilindre et al. 2008). The majority of yeast cell wall proteins are mannoproteins—in fact, mannoproteins comprise 35-40% of the dry weight of the cell wall (Klis et al 2002). Kwon (2004) identified 12 yeast-derived proteins in a Sauvignon Blanc wine, 10 of which were localized to the wall or plasma membrane. The list included mannosylated β -1,3 glucanases (Eng1, Bgl2, and Exg2), glycosidases (Crh1 and Utr2) cell wall mannoproteins (Cwp1, Ygp1, Suc4, Tos1), plasma membrane proteins (Gas1, Ecm33), and the phosphatase (Pho3). β -1,3 glucanases and glycosidases are released by the mother cells during cell growth for the purpose of “softening up” the cell wall locally to allow the bud to emerge (Dupin et al. 2000). Bgl2 and Gas1 are mannosylated proteins attached to the β -glucan network via a glycosylphosphatidyinositol (GPI) anchor. The GPI anchor serves as a link between the protein and the β -1,3 or 1,6 glucan networks or plasma membrane

(Klis et al. 1998, 2002). Cilindre et al. (2008) also found the cell wall mannoprotein Cwp1 in a Chardonnay wine, but it was the only yeast-derived protein found in the wine. Moine-Ledoux and Dubourdieu (1999) isolated the yeast mannoprotein invertase (Suc2) from wine using tandem DEAE ion exchange chromatography and Con A affinity chromatography. Protoplast secreted protein 1 (Pst1 or Hpf2p) was isolated from a white wine by Waters et al. 1994b). Pst1 is a membrane and cell wall associated mannoprotein—containing 5 occupied N-glycosylation sites. Occupied sequons were confirmed using PNGase F. The amino acid sequence was analyzed following enzymatic deglycosylation and any Asp that replaced an Asn indicated an occupied sequon.

C) Glycoproteins

Many of the yeast proteins isolated from wine are glycosylated (mannan) and localized to the cell wall. Glycosylation of proteins in eukaryotic cells is a significant post-translational modification of many membrane-bound and secreted proteins. Glycoproteins contain either *N*-linked or *O*-linked carbohydrate residues, or both, discussed in further detail below. Some functions of the attached glycan include resistance to proteolysis, increasing solubility and assisting protein folding. Cell-cell communication as well as other cellular interactions may be affected by glycosylation (Creighton 1992). Glycoproteins are known for having variable structure, which allows for interaction between molecules based on shape and the nature of the glycosylation.

1) *N*-Linkages:

N-linked glycans are linked to an asparagine (N) residue within a conserved sequence of three amino acids, named a sequon (NXS or NXT, where X is any amino acid but proline) (Creighton 1992; Reddy et al.1988). Not all sequons are glycosylated. For example, although yeast invertase has 14 known sequons, only 9-10 sites are typically glycosylated (Reddy et al.1988). *N*-linked glycans are first assembled as core structures in the endoplasmic reticulum (ER). The core oligosaccharide (Glc3-Man9-GlcNAc2) is attached at the sequon after translation. The protein is further modified as it travels from the ER through the Golgi apparatus where more mannan is added, some of which is phosphorylated (Creighton 1992).

2) *O*-Linkages

O-linked glycosylation is the attachment of a glycan to a serine or threonine residue. This attachment is catalyzed by the enzyme UDP-N-acetyl-D-galactosamine polypeptide N-acetylgalactosaminyltransferase, and the attachment usually takes place in the ER. The specific carbohydrate added is species dependent, as for *N*-glycosylation. N-acetyl-galactosamine is added following protein folding and probably occurs in the Golgi (Creighton 1992). Other carbohydrates like galactose and sialic acid are added following the addition of N-acetyl-galactosamine. No definite sequon or rules for *O*-glycosylation have been discovered.

D) Wine protein quantification

A wide range of protein concentrations reported in the literature, from 1 g/L to 1 mg/L (Vincenzi et al. 2005). The wide range of protein concentration has been attributed to interfering substances present in wine (ethanol, phenolics, and sugars). Several protein quantification methods have been used to measure wine protein, such

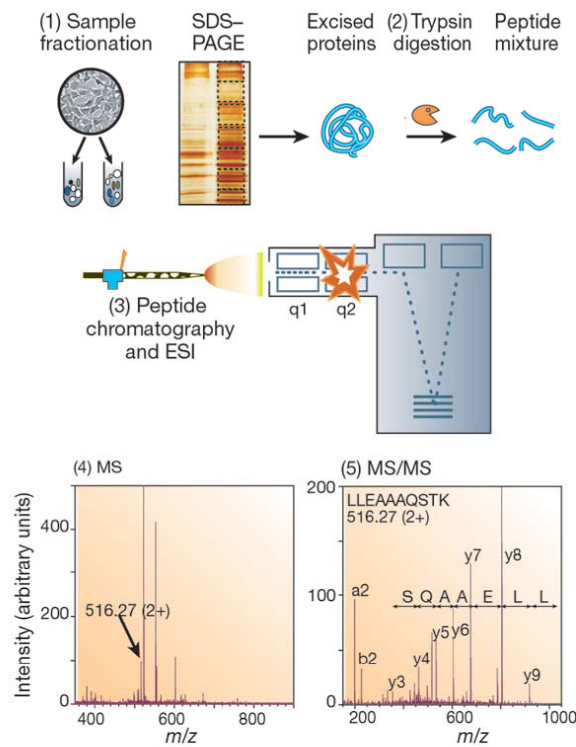
as the Bradford assay, Lowry method and amido black (Kwon 2005, Escot et al. 2001, Weiss and Bisson 2001). In order to simplify and standardize wine protein quantification Vincenzi et al. (2005) developed a method combining protein isolation and quantification. Several different protein isolation methods (KDS, ethanol, TCA, acetone, and dialysis followed by lyophilization) along with several protein detection methods were compared (Lowry, Bradford and Smith). Known amounts of BSA were added to a deproteinized wine and the proteins were subsequently recovered and analyzed. The most effective and consistent procedure was a combination of protein isolation (KDS method) and quantification with the Smith assay (described in Materials and Methods). The authors adapted the KDS method from previous work and optimized it for isolation of wine proteins (Carraro et al. 1994).

E) Mass spectrometry-based proteomics

Mass spectrometry (MS) is a powerful tool in the fields of proteomics and molecular and cellular biology. The emergence of complete gene and genome databases along with advances in protein separation and ionization methods have been invaluable for identifying proteins by MS (Aebersold & Mann, 2003). **Figure 2** outlines a generic MS experiment. The initial steps in MS analysis include protein isolation and possible pre-treatments. Some MS may require pre-treatment of the proteins with a detergent (acid-labile surfactant) or deglycosylation. Following the pre-treatment proteins are generally subjected to enzymatic digestion with trypsin, which specifically cleaves the primary sequence at the carboxyl side of lysine and arginine residues. Tryptic peptides are smaller than the intact protein and are easier to separate and ionize. The peptides are injected into an HPLC column where they are

separated by size and eluted into an electrospray ion source creating a fine spray of charged droplets. The protonated peptide fragments enter the MS and a mass spectrum of the eluting peptides is generated. The computer generates a list of the peptide fragments that were identified. Further fragmentation of the peptide by energetic collision of gas and tandem MS/MS of specific peptide ions is followed by analysis of the raw data using database-searching software.

Figure 2. Generic MS experiment adapted from Aebersold & Mann (2003)



1) Deglycosylation

There are several reasons to remove the glycan moiety from a glycoprotein. For example, reasons to remove the glycan moiety can include clean-up prior to mass spectrometry, identification and structural analysis of the oligosaccharides, identification of the sequons, biosynthetic/molecular studies, and further study of the

protein moiety. When analyzing glycoproteins by mass spectrometry (MS) it is usually necessary to remove the glycan prior to analysis (Yu et al. 2005). The sometimes-large glycan groups interfere with the analysis of the peptides and often produce an adulterated or skewed mass-to-charge ratio giving the appearance of a larger amino acid in sequence (Morelle et al. 2006).

Endoglycosidase H (Endo H) specifically removes N-linked hybrid or high-mannose oligosaccharides as free glycans or as linked glycans. The enzyme hydrolyzes the *N*-linked oligosaccharides from the end of the glycan stopping at the core trimannosyl group. In other words, the trimannosyl core group added at the beginning of the glycosylation process is not a good substrate for Endo H, while any high-mannose containing glycan chains are excellent substrates for the enzyme (Tarentino et al 1989).

PNGase F, or peptide-N-glycosidase F, is highly specific to the Asn linkage present in all N-linked glycans. The enzyme cleaves the glycan at Asn, leaving an Aspartic acid (Asp). PNGase F removes the entire glycan and leaves the glycoprotein devoid of its N-linked carbohydrate moiety. It is of particular interest to those who are interested in studying fully intact N-linked oligosaccharides from glycoproteins. The highly specific cleavage occurs between the Asn and the proximal N-acetylglucosamine.

O-linked glycoproteins are removed by an O-glycosidase that hydrolyzes at the Ser or Thr residue. The enzyme will only remove the Gal- β (1-3)-GalNAc core structure attached to the amino acid residues; it does not recognize modified core structures (Iwase et al 1993). Common modifications to the O-linked Gal- β (1-3)-

GalNAc include mono, di, or trisialylation. The typical approach for removal of these carbohydrates is use of exoglycosidases—such as α -2-(3,6,8,9)-neuraminidase, β (1-4)-specific galactosidase, and N-acetylglucosaminidase to shorten the chain until the core is left exposed

Materials and Methods

Yeast strains

Saccharomyces cerevisiae wine strains RC212 and BM45 were obtained in active-dry form and five coded wine strains were provided on YEPD slants: 262, 170, 142, 98, 116 (Lallemand, Inc., lallemandwine.com)

Starter culture preparation

Strains was transferred from isolated colonies on fresh YEPD plates (2% w/v glucose, 2% w/v peptone, 1% w/v yeast extract, US Biologicals, Swampscott, MA) to 100 mL of broth containing 10% w/v glucose, 1% w/v peptone, and 1% w/v yeast extract and were incubated on an incubator-shaker at 25°C for 48 hr. After the 48-hr incubation, cells were concentrated 10-fold by centrifugation at 2,000 x g for 35 min, removal of the supernatant, and resuspension of cells in 10 ml of sterile distilled water to yield 10⁹ cfu/ml.

Fermentations

Duplicate 3 liter model wine fermentations were conducted at 25°C in pre-sterilized 1 gallon glass jugs fitted with fermentation locks using a synthetic must (Table 11) similar to that described by Wang et al. (2003). The must was sterilized by filtration through a 0.45 µm nominal filter (Ultipor® N66 filter device, Pall Co., East Hills, NY). To assist flocculation, three grams of sterile cellulose powder suspended in 25 ml of sterile distilled water were added to each fermentation prior to inoculation of the 3-liter musts with 3 ml of the concentrated 48-hr yeast cultures to yield an

initial concentration of about 10^6 cfu/ml. The fermenting musts were stirred daily (10 min at mid-range speed setting #5 on a magnetic stirrer). When glucose levels were <2% (Clinitest® tablets, Bayer, Elkhart, IN), the fermenters were transferred to 15°C storage for periodic post-fermentation sampling and were stirred every month as described above.

Sampling and determination of viable yeast cells

Ten ml samples were removed every other day until the fermentations were complete using sterile-filtered N₂ gas to force samples through an aseptic sampling device and into sterile 15 ml screw-capped polypropylene tubes which were immediately placed on ice. The fermentations were stirred just prior to sampling. Viable cell counts were determined by plating duplicate dilutions that yielded 50-400 colonies per YEPD plate. Colonies were counted after 48-hr incubation of plates at 30°C. Following removal of samples needed to determine viable cell counts, the remaining sample was centrifuged (2,000 x g for 35 min) and the supernatant was filtered through a sterile 0.45 µm PVDF filter (Fisher Scientific, Ireland). Filtrates were stored at -20°C in sterile 15 mL screw-capped polypropylene tubes until further analysis for 2 weeks following fermentation.

Chemical analyses

Glucose and ethanol.

Glucose and ethanol in the filtrates were separated by HPLC and detected by refractometry. The analytical conditions included a Bio-Rad HPX-87C (Bio-Rad Laboratories, Hercules, CA) column running a mobile phase of H₂O with 200mg/L Ca(NO₃)₂ at 85°C. The flow rate was 0.7 ml/min with an injection volume of 15µl.

The refractive index (RI) at 1×10^{-5} 35°C was detected on an HP-1047A (Hewlett-Packard, Palo Alto, CA).

Protein by the KDS method.

Protein was isolated and assayed as described by Vincenzi et al. (2005). Briefly, 10.1 μ l of a 10% SDS solution were added to 1 ml of sample filtrate in a 1.7 ml screw-capped microfuge tube, vortexed vigorously, and placed in a 100°C water bath for 5 minutes. Tubes were cooled to room temperature and to each, 252.2 μ L of 1 M KCl were added. The tubes were then mixed gently for 30 min at room temperature. The resulting mixture of wine and protein precipitate was centrifuged at 16,000 x g at 4°C. The pellet was washed twice with 1 M KCl, solubilized in 1 ml of distilled water, diluted 2-fold in water and filtered through a 0.45 μ m PVDF filter. Protein was measured by the Smith BCA assay (Smith 1985) using a commercial kit (Pierce Laboratories, Rockford, IL).

Immunoblotting.

Mannoproteins were quantified by immunoblotting performed according to the manufacturer's instructions (Bio-Dot® SF Microfiltration apparatus instruction manual, section 4.2, Bio-Rad Laboratories, Hercules, CA). Briefly, 50 μ l samples containing 1 to 500 ng of protein (model wine proteins isolated by KDS extraction) and standards (yeast invertase, #I4504, and mannan, #M7504, Sigma-Aldrich, St. Louis, MO) and nisin (Aplin & Barrett Ltd, Beaminster, UK) were blotted onto nitrocellulose membranes (0.45 μ m Bio-Rad Laboratories Hercules, CA) in a 48-well slot blot apparatus. After the samples and standards were loaded onto the membrane by gravity flow, 250 μ l per well of Tris-buffered saline (20 mM Tris, pH 7.5, 500

mM NaCl, TBS) were added under vacuum. The membrane was then removed from the apparatus, placed in a plastic box, and rinsed with a blocking/wash solution of Tris-buffered saline containing Tween 20 (Tris-buffered saline + 0.1% Tween 20, TBST) for five minutes. This rinse was repeated once with fresh TBST. The membrane was then incubated 30 minutes at room temperature with continuous mixing with 9.5 $\mu\text{l/ml}$ (final concentration) of the mannose-specific primary antibody, biotinylated *Narcissus pseudonarcissus* lectin (Vector Labs, Burlingame, CA) in TBST. The membrane was then washed twice with TBST and subsequently placed in TBST containing 1 $\mu\text{g/ml}$ of streptavidin-conjugated alkaline phosphatase (Vector Labs) for 30 minutes at room temperature with continuous mixing. The membrane was then washed twice in TBST and rinsed once in TBS for five minutes to remove residual Tween 20. The membrane was then equilibrated in 100 mM Tris pH 9.5 for five minutes. After equilibration, the membrane was removed, shaken to remove excess liquid, and placed—blotted side up—on top of plastic wrap within a dry plastic box under subdued light. The chemiluminescent/fluorescent alkaline phosphatase substrate DuoLux™ (Vector Labs) was then added at a rate of 50 $\mu\text{l/cm}^2$ (4.45 ml per membrane) onto the membrane, which was covered with plastic wrap to uniformly spread the substrate under subdued light for 5 minutes. The membrane was then removed and rinsed in 100 mM Tris pH 9.5 for an additional 2-3 minutes. This rinse step was found to minimize background on the X-ray film. The membrane was then stored in a plastic Ziploc™ bag and kept in a light-proof and sealed container at 4°C overnight. Exposure to X-ray film (Hyperfilm™, Amersham Biosciences, Buckinghamshire, UK) was done the next day in a darkroom with the membrane

sandwiched between new or clean used 8.5 x 11" plastic sheets to protect the film from the moisture on the membrane. The film was exposed between 2 to 5 minutes. After developing the film it was scanned as a transparency (to make the light background dark and the dark bands light) into a data file and the integrated densities were determined using ImageJ image analysis software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <http://rsb.info.nih.gov/ij/>, 1997-2007).

Sample processing for identification of proteins by HPLC-MS of tryptic peptides.

Model fermentation samples (100 mL) were concentrated 100-200-fold by ultrafiltration using a centrifugal filter cartridge (5 kDa regenerated cellulose membrane, Millipore, Billerica, MA) following initial removal of suspended solids by centrifugation (2,000 x g for 35 min) and filtration through a 0.45 µm PVDF filter. In order to dilute low molecular weight wine solutes (<5 kDa), the concentrated samples were diluted with about 50 ml of distilled water and concentrated back to the original 100-200-fold level by ultrafiltration.

Aliquots (160 µl) of the concentrated samples generated by ultrafiltration were adjusted to 50 mM ammonium bicarbonate pH 8.5, and 0.1% Acid labile surfactant, a long chain derivative of 1,3-dioxolane sodium propyloxy sulfate (RapiGest™, Waters Co. Milford, MA) in a final volume of 200 µl and were reduced and alkylated using final concentrations of 5 mM DTT and 15 mM iodoacetamide, respectively. The reduction step was done at 60°C for 30 minutes, followed by the addition of iodoacetamide and incubation in the dark for 30 minutes at room

temperature. Enzymatic deglycosylation was subsequently performed with PNGase F (N-Glycanase™, Prozyme®, San Leandro, CA), which cleaves the entire asparagine-linked glycan by hydrolyzing the bond between the asparagine and the proximal *N*-acetyl glucosamine, leaving an aspartic acid residue. The deglycosylation reaction was performed overnight at 37°C at a final concentration of 2 µg/ml enzyme in a total volume of 200 µl 50 mM ammonium bicarbonate pH 8.5. Following the overnight incubation, the ammonium bicarbonate concentration was increased to 200 mM and trypsin was added to a final concentration of about 1 µg/mL. The mixture was then incubated at 37°C for 8-12 hours. Ten µl aliquots were subjected to subsequent HPLC-MS analysis.

HPLC-MS identification of tryptic peptides

Samples were run by HPLC/MS-MS using a Waters (Millford, MA) nano Acquity HPLC connected to a Waters Q-ToF Ultima Global. One µl of sample (about 1 µg total protein) was loaded onto a Waters Symmetry C-18 180 µm x 20 mm trap at 6 µl/min for 3 minutes. Peptides were then eluted from the trap onto a 15 or 20 cm x 75 µm Waters BEH analytical column at 260 nl/min. The HPLC gradient went from 2% to 40% B in 60 minutes, then to 93% B in 5 minutes and held for 12 minutes. Solvent A was 0.1% formic acid in water, and B was 0.1% formic acid in acetonitrile. Data were acquired for 80 minutes.

Peptide precursor ions were monitored as they eluted from the analytical column with 0.6 second survey scans from *m/z* 500-1990. Up to 3 parent ions per scan that had sufficient intensity, and 2 or 3 positive charges were chosen for MS/MS. The MS/MS scans were 2.4 seconds from *m/z* 50-1990.

The mass spectrometer was calibrated using the MS/MS spectrum from glu-fibrinopeptide. Masses were corrected during each run using a lock mass scan every 60 seconds of glu-fibrinopeptide.

The raw data were processed with Waters Protein Lynx Global Server 2.3 software to produce pkl files, a set of smoothed and centroided parent ion masses with the associated fragment ion masses. The pkl files were searched with Mascot 2.2 (Matrix Science Ltd., London, UK) database searching software, using mass tolerances of 35 ppm for the precursors and 0.1 Da for fragments. The Mascot results were combined and reviewed using Scaffold software (Proteome Software, Inc.). To validate results, Scaffold uses an independent implementation of Bayesian statistical algorithms. Using these algorithms, the software determines the distributions of the Mascot scores, which depend on database size and specific sample characteristics. From these distributions, Scaffold translates the Mascot scores into probabilities that a given identification is correct. The probability that a protein has been correctly identified increases as more fragments are identified and subsequently assigned to a particular protein.

The only database searched to identify of model wine proteins was *S. cerevisiae* genome, and thus the search was more limited than a standard Mascot search, which further increased the probability of a match. Mascot provides a probability estimate based on database size, but not on sample characteristics. By incorporating the sample-specific distribution, Scaffold provides better estimates of the probability of a correct identification. In this study, 80% probability was used as a cut-off for all time points except for the 9-month sample for which the cut-off was lowered to 65%. The

lower cut-off was used because a few additional proteins were identified at that probability. At earlier time-points, no new proteins were detected when the probability cut-off was lowered to 65%.

Fisher's least significance difference (LSD)

Statistical analysis was performed using Statsgraphics (Statsgraphics® Centurion, Herndon, VA, USA) software. Multiple range tests on duplicate samples between time points and yeast strains were performed by the Fisher's least significance difference (LSD) procedure discriminating between the means at a 95% confidence interval.

Results

Recent studies have investigated wine protein composition and quantification of protein or mannan independently. As of this writing no studies have reported differences in protein extraction between multiple yeast strains during aging on lees. Thus protein composition, and quantity of protein and mannoprotein were monitored. Significant differences in protein and mannoprotein concentration were observed between most yeast strains during aging. Both protein and protein-associated mannan concentrations were found to increase again post-fermentation, reaching values about 2- and 6-fold higher than values measured at 2 days, respectively. Consistent with the increase in mannoprotein concentration, cell wall mannoproteins were the predominant proteins identified after 6 months of aging on the lees. Most cytosolic proteins found during and soon after fermentation were not found after 6 months of aging on the lees.

1) Fermentations

After inoculation into synthetic must, all yeast strains reached populations of 10^7 cfu mL⁻¹ or greater by day 2 (**Table 1, Figure 3**). Wines fermented by strains 98 and 262 reached maximum populations after two days. Nearly all yeast strains increased about 10-fold from their starting inoculums. The viable cell count for both of these yeast strains declined slowly until reaching a minimum by day 12. These two strains were quite different from strains 142 and 170, which reached maximum population between 3 and 4 days, ultimately reaching minimum population on day 12. Interestingly, strain 98 reached the second lowest minimum growth, well above the minimum growth of RC212. RC212 reached its maximum at day 2, but the

population was approximately 10 fold less than 262 at the same time point. The population of RC212 declined to 6×10^6 cfu mL⁻¹ after day 4. The populations of all other strains remained 10^7 cfu mL⁻¹ or greater after day 4. Yeast populations steadily declined over the course of the fermentations. The largest decrease in viable cells during fermentation was detected in wine fermented by RC212, which ultimately declined to 10^4 cfu mL⁻¹. Once glucose concentrations were below 2%, the populations of all other strains, with the exception of 98, had declined but retained viable populations of 5×10^5 or greater. Large variation between replicates was observed in strain 98 near the end of fermentation. One replicate declined rapidly from 5×10^6 to 4×10^4 cfu mL⁻¹ between days 8 and 10, while the other replicate decreased from 3×10^6 to 2×10^6 in the same period.

Figure 3. Yeast viability during fermentation

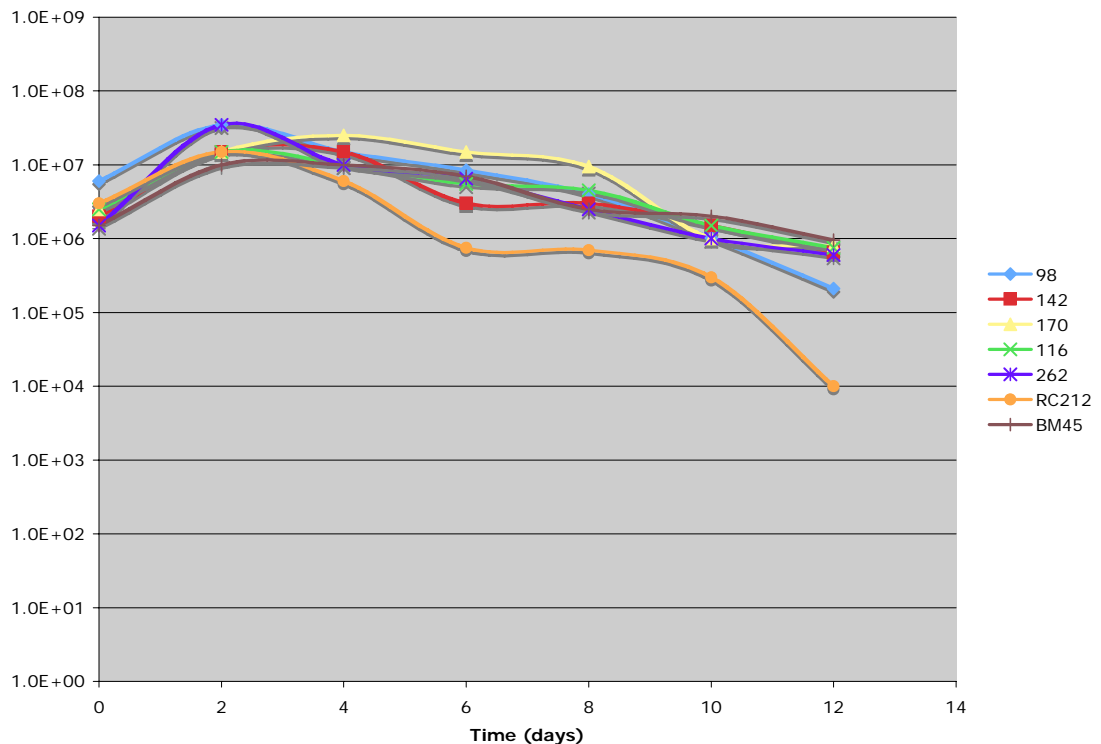


Table 1. Change in yeast viable cells during fermentation¹

	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12
Strain							
98	$6 \times 10^6 \pm 0$	$3.5 \times 10^7 \pm 7 \times 10^6$	$1.5 \times 10^7 \pm 7 \times 10^6$	$8.5 \times 10^6 \pm 2.1 \times 10^6$	$4.0 \times 10^6 \pm 1.4 \times 10^6$	$1.0 \times 10^6 \pm 1.3 \times 10^6$	$2.1 \times 10^5 \pm 2.7 \times 10^5$
142	$2 \times 10^6 \pm 0$	$1.5 \times 10^7 \pm 7 \times 10^6$	$1.5 \times 10^7 \pm 7 \times 10^6$	$3 \times 10^6 \pm 1.4 \times 10^6$	$3.0 \times 10^6 \pm 1.4 \times 10^6$	$1.5 \times 10^6 \pm 7 \times 10^5$	$6.5 \times 10^5 \pm 2 \times 10^5$
170	$2.5 \times 10^6 \pm 7 \times 10^5$	$1.5 \times 10^7 \pm 7 \times 10^6$	$2.5 \times 10^7 \pm 7 \times 10^6$	$1.5 \times 10^7 \pm 7 \times 10^6$	$9.5 \times 10^6 \pm 7 \times 10^5$	$1 \times 10^6 \pm 0$	$3 \times 10^5 \pm 0$
116	$2.5 \times 10^6 \pm 7 \times 10^5$	$1.5 \times 10^7 \pm 7 \times 10^6$	$1 \times 10^7 \pm 0$	$5.5 \times 10^6 \pm 2.1 \times 10^6$	$4.5 \times 10^6 \pm 7 \times 10^5$	$1.5 \times 10^6 \pm 7 \times 10^5$	$7.5 \times 10^5 \pm 7.1 \times 10^4$
262	$1.5 \times 10^6 \pm 7 \times 10^5$	$3.5 \times 10^7 \pm 7 \times 10^6$	$1 \times 10^7 \pm 0$	$6.5 \times 10^6 \pm 7 \times 10^5$	$2.5 \times 10^6 \pm 7 \times 10^5$	$1 \times 10^6 \pm 0$	$6 \times 10^5 \pm 0$
RC212	$3 \times 10^6 \pm 0$	$1.5 \times 10^7 \pm 7 \times 10^6$	$6 \times 10^6 \pm 0$	$7.5 \times 10^5 \pm 7 \times 10^4$	$7 \times 10^5 \pm 2.8 \times 10^5$	$3 \times 10^5 \pm 0$	$1 \times 10^4 \pm 0$
BM45	$1.5 \times 10^6 \pm 7 \times 10^5$	$1 \times 10^7 \pm 0$	$1 \times 10^7 \pm 0$	$7 \times 10^6 \pm 2.8 \times 10^6$	$2.5 \times 10^6 \pm 7 \times 10^5$	$2 \times 10^6 \pm 0$	$9.5 \times 10^5 \pm 2.1 \times 10^5$

¹Data are means \pm standard deviations of colony-forming units (cfu)/ml

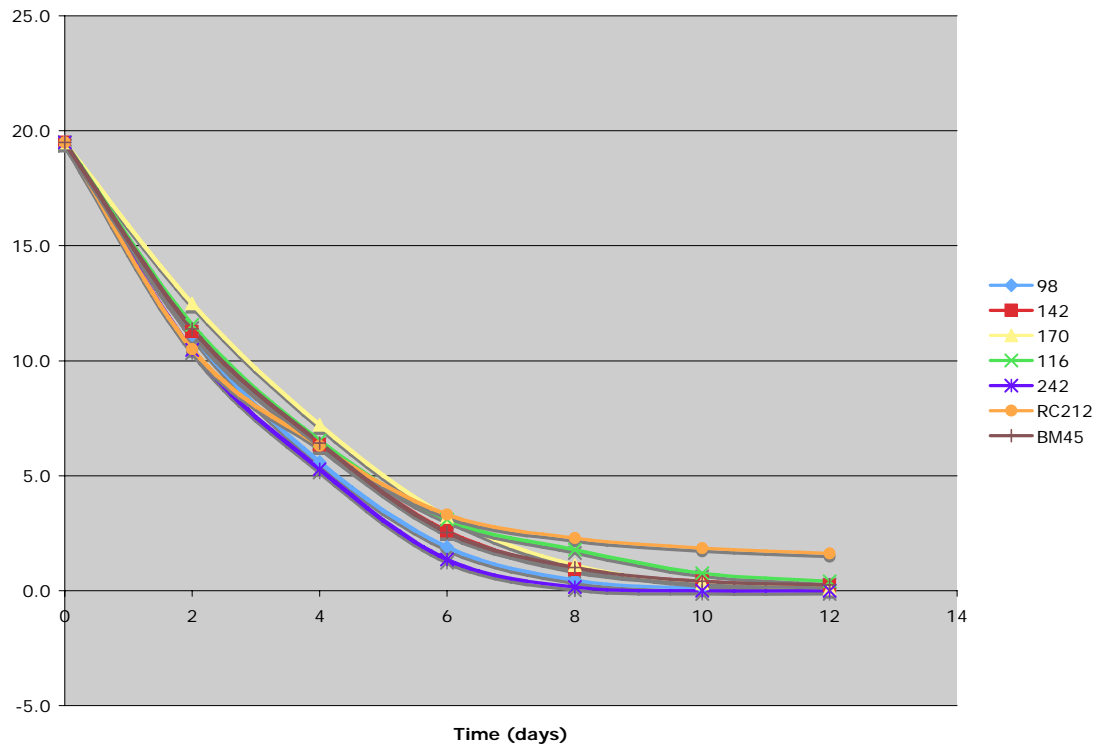
Glucose consumption was generally similar in all wines, with the exception of wine fermented by RC212. By day12, glucose concentration in the wine fermented by RC212 was 1.6g/100mL glucose, while concentrations for all other wines were less than 0.4g/100mL. (Table 2 and Figure 4).

Table 2. Change in glucose concentration during fermentation¹

	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12
Strain							
98	19.5 ± 0	11.1 ± 0.3	5.6 ± 1.1	1.9 ± 1.0	0.5 ± 0.5	0.1 ± 0.2	0.3 ± 0
142	19.5 ± 0	11.3 ± 0.9	6.3 ± 1.4	2.6 ± 1.2	0.9 ± 0.8	0.4 ± 0.4	0.2 ± 0
170	19.5 ± 0	12.5 ± 0.3	7.2 ± 0.0	3.2 ± 0.4	1.1 ± 0.5	0.3 ± 0.2	0.1 ± 0.1
116	19.5 ± 0	11.6 ± 0.6	6.5 ± 1.8	3.1 ± 1.3	1.8 ± 0.5	0.8 ± 0.6	0.4 ± 0.4
262	19.5 ± 0	10.5 ± 0.1	5.3 ± 0.2	1.4 ± 0.1	0.2 ± 0.0	0	0
RC212	19.5 ± 0	10.5 ± 0.5	6.3 ± 0.2	3.3 ± 0.1	2.3 ± 0.0	1.8 ± 0.1	1.6 ± 0.3
BM45	19.5 ± 0	11.4 ± 1.0	6.4 ± 1.2	2.5 ± 1.2	1.0 ± 0.8	0.4 ± 0.6	0.2 ± 0.3

¹Data are means \pm standard deviations (g/100ml).

Figure 4. Change in glucose concentration during fermentation



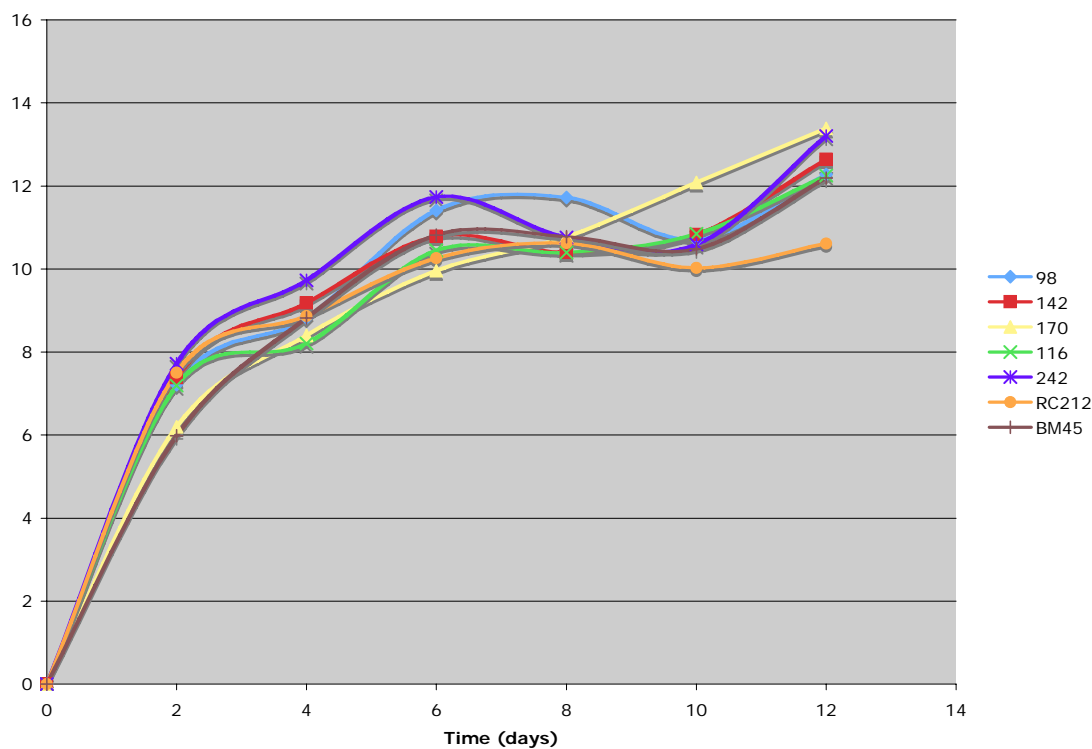
Ethanol production increased comparably in all wines, excluding wines fermented by strains BM45 and 170. These two strains fermented more slowly than the others with ethanol reaching ~6% (v/v) on day 2. In contrast, ethanol reached ~8% (v/v) in the other wines on day 2. By day 4, the ethanol content was between 8-10% in all wines. It is interesting to note that all yeast strains except BM45 and 170 produced the most ethanol during the rapid growth phase from inoculation to day 2, while stationary-phase cells produced the most ethanol in the fermentations with BM45 and 170. Between days 4 and 12, ethanol concentrations in all other wines were similar (**Table 3** and **Figure 5**).

Table 3. Change in ethanol concentration during fermentation¹

	Day	Day	Day	Day	Day	Day	Day
Strain	0	2	4	6	8	10	12
98	0	7.2± 0.4	8.8± 0.1	11.4± 0.9	11.7± 0.5	10.7± 0.4	12.3± 0.6
142	0	7.4± 0.5	9.2± 0.4	10.8± 0.8	10.4± 0.9	10.8± 0.5	12.6± 0.8
170	0	6.2± 0.3	8.4± 0.0	10.0± 0.8	10.8± 0.3	12.1± 0.5	13.4± 0.0
116	0	7.2± 0.5	8.2± 0.2	10.4± 0.7	10.4± 0.5	10.8± 0.3	12.3± 0.6
262	0	7.7± 0.0	9.7± 0.2	11.7± 0.1	10.8± 0.1	10.6± 0.5	13.2± 0.5
RC212	0	7.5± 0.2	8.9± 0.4	10.3± 0.1	10.6± 0.2	10.0± 0.4	10.6± 1.1
BM45	0	6.0± 0.5	8.8± 0.8	10.8± 0.9	10.8± 1.3	10.5± 0.1	12.2± 1.1

¹Data are means ± standard deviations of % (v/v) ethanol

Figure 5. Change in ethanol concentration during fermentation



2) Changes in protein concentration

Changes in protein during fermentation peaked for all strains at day 6, which represents a 3-4 fold increase relative to day 2 (**Table 4**).

Table 4. Change in protein concentration during fermentation¹

	Day	Day	Day	Day	Day	Day
Strain	2	4	6	8	10	12
98	7.3± 0.5	5.2± 1.3	20.4± 8.3	16.7± 4.9	17.1± 6.7	14.7± 1.0
142	8.6± 1.7	4.2± 1.8	27.5± 2.8	14.1± 3.0	9.4± 3.7	14.4± 3.7
170	5.4± 2.1	4.8± 1.8	34.9± 4.9	13.7± 0.2	13.2± 0.2	12.6± 0.5
116	7.0± 0.1	7.0± 6.5	21.1±1 3.1	19.9±1 6.5	13.3± 8.5	16.3±1 5.0
262	4.3± 3.5	8.6± 4.0	16.6± 2.3	7.2± 1.1	15.6± 2.2	14.3± 4.5
RC212	7.1± 0.8	3.8± 2.1	34.4± 1.3	24.2± 7.9	35.0± 8.1	32.0± 0.5
BM45	6.5± 0.4	3.5± 0.8	19.3± 9.6	19.7± 4.5	17.4± 1.8	13.7± 2.2

¹Data are means ± standard deviations (µg/mL)

Protein concentrations decreased by the end of fermentation, but were still higher than at the beginning. Minimal concentrations were reached for most wines 45 days post-inoculation, while some wines reached the minimum 14 days post-inoculation. Protein concentration in wine fermented by RC212 increased from its minimum by day 45, while protein increased in the remaining wines after the day 105. The spike of protein midway through fermentation may be caused by release of cytosolic proteins by cells that have ruptured due to osmotic shock.

Changes in protein concentration for each yeast strain during aging are indicated in **Table 5a** and **Figure 6**.

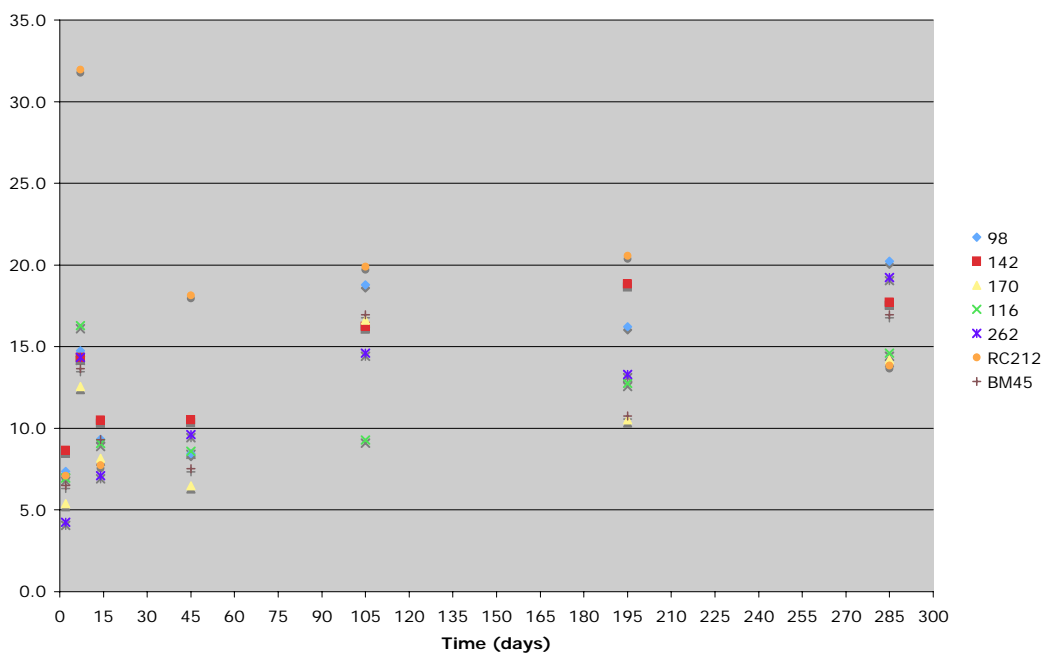
Table 5a. Change in protein concentration over time¹

	2	7	14	45	105	195	290
Strain	Days	Days	Days	Days	Days	Days	Days
98	7.4 ^a ±0.5	14.8 ^a ±0.9	9.3 ^{ab} ±1.6	8.4 ^a ±1.0	18.8 ^{ad} ±0.1	16.2 ^{ab} ±5.7	20.2 ^a ±5.0
142	8.7 ^a ±5.9	14.4 ^a ±4.3	10.5 ^a ±1.3	10.5 ^a ±4.0	16.2 ^{ad} ±1.0	18.8 ^{ab} ±6.2	17.7 ^a ±1.3
170	5.4 ^a ±2.1	12.6 ^a ±0.5	8.2 ^{ab} ±0.6	6.5 ^a ±1.6	16.6 ^{ad} ±2.5	10.5 ^a ±1.1	14.2 ^a ±4.6
116	7.0 ^a ±0.5	16.3 ^a ±3.1	9.1 ^{ab} ±0.5	8.6 ^a ±1.6	9.3 ^b ±0.6	12.7 ^{ab} ±2.6	14.6 ^a ±5.4
262	4.3 ^a ±3.5	14.4 ^a ±4.5	7.1 ^{ab} ±2.5	9.6 ^a ±2.1	14.6 ^{ac} ±4.1	13.3 ^{ab} ±3.2	19.2 ^a ±0.4
RC212	7.1 ^a ±0.8	32.0 ^b ±0.5	7.7 ^b ±0.6	18.1 ^b ±1.4	19.9 ^d ±0.0	20.6 ^b ±5.7	13.8 ^a ±1.9
BM45	6.5 ^a ±0.4	13.7 ^a ±2.2	9.3 ^{ab} ±0.9	7.5 ^a ±1.6	17.0 ^{ad} ±0.8	10.8 ^{ab} ±1.4	17.0 ^a ±1.0

¹Data are means ± standard deviations (µg/ml) for 2 replicates.

abcd Means at any given time point with no common superscripts are significantly different (Fishers LSD procedure p<0.05).

Figure 6. Change in protein concentration over time



Protein increased in all wines after 195 and 285 days and in some cases doubled or nearly doubled from the minimum values reached at 14 or 45 days. Wine fermented by RC212 increased in protein until 195 days and then decreased slightly after 285 days. Fisher's least significance difference (LSD) procedure was used to determine significant differences in protein concentration for each yeast strain at successive time points as well as differences between yeast strains at the same time points. Significant changes in protein concentration at successive time points were observed in wine fermented by many of the yeast strains. Wine fermented by 142 exhibited no significant changes in protein over time (**Table 5b**).

Table 5b. Significant changes in protein during aging¹

Strain	2 Days	7 Days	14 Days	45 Days	105 Days	195 Days	2 Days
	through	through	through	through	through	through	through
	7 Days	14 Days	45 Days	105 Days	195 Days	290 Days	290 Days
98	↑	-	-	↑	-	-	↑
142	-	-	-	-	-	-	-
170	↑	-	-	↑	↓	-	↑
116	↑	↓	-	-	-	-	↑
262	↑	-	-	-	-	-	↑
RC212	↑	↓	↑	-	-	↓	↑
BM45	↑	↓	-	↑	↓	↑	↑

↑ Denotes a significant increase between indicated time points within strains.

↓ Denotes a significant decrease between indicated time points within strains.

- Denotes no significant change between indicated time points within strains. (Fishers LSD procedure p<0.05).

Protein increased significantly between 2 and 7 days for all wines except wine fermented by 142. Protein concentration in all wines decreased between 7 and 14 days, and decreases were statistically significant for wines fermented by 116, RC212, and BM45. Very little change was observed for any strains between 14 and 45 days of

aging. The only significant increase in protein was observed in wine fermented by RC212. Protein increased significantly in wines fermented by strains 98, 170, and BM45 between 45 and 105 days of aging, while protein increases were insignificant in wines fermented by 142 and 262. Protein concentrations in wines fermented by 116 and RC212 did not change significantly between these two time points. Protein did not change significantly in wines fermented by 98, 142, 262, and RC212 between 105 and 195 days. Significant decreases in protein were observed in wine fermented by 170 and BM45. In contrast, protein increased in wine fermented by 116 between 3 and 6 months, but not significantly. Protein levels did not change significantly in wines fermented by 142 and 116 between 195 and 285 days. Insignificant increases in protein occurred in wines fermented by 98, 170, and 262, while wine fermented by BM45 increased significantly. In contrast, the protein concentrations decreased significantly in wine fermented by RC212 between the two time points.

Significant differences in protein concentration between yeast strains were observed at several points during aging (**Table 5a**). No significant differences between strains were observed after 2 days. No significant differences between yeast strains were observed at day 7, with the exception of RC212, which was significantly different from all other strains. The only significant difference between strains at 14 days was between 142 and 262. Significant differences were observed between RC212 and all other yeast strains after 45 days, but similar to day 7, no significant differences were observed between the other strains. After 105 days significant differences were observed between 116 and all other yeast strains and between 262 and RC212. The differences between the remaining strains were insignificant.

Following 195 days the only significant difference was between RC212 and 170, and no significant differences were observed after 285 days.

3) Changes in protein associated mannan (mannan equivalents)

Changes in protein-associated mannan concentration for each yeast strain during aging are displayed in **Table 6a** and **Figure 7**.

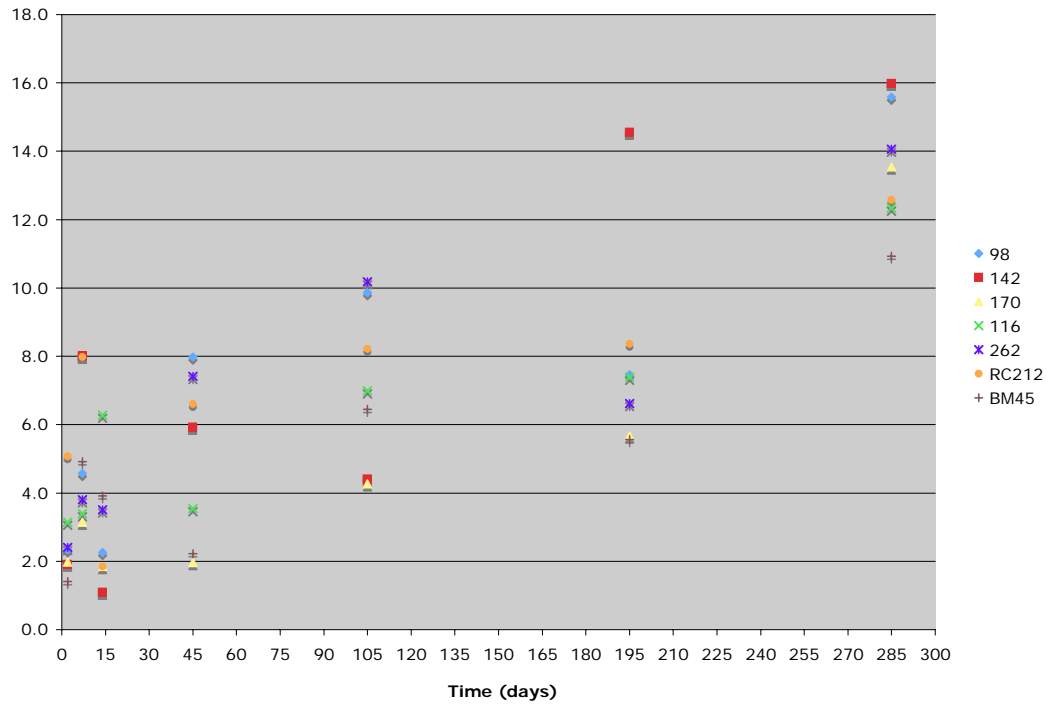
Table 6a. Change in concentration of protein associated mannan during aging¹

	2	7	14	45	105	195	290
Strain	Days	Days	Days	Days	Days	Days	Days
98	2.3 ^{ab} ±1.4	4.6 ^{ab} ±2.9	2.3 ^{ae} ±0.3	8.0 ^a ±0.5	9.9 ^a ±0.8	7.5 ^a ±0.4	15.6 ^a ±1.4
142	1.9 ^{ab} ±1.4	8.0 ^a ±1.3	1.1 ^{cde} ±0.4	5.9 ^{ae} ±1.9	4.4 ^b ±0.1	14.6 ^b ±0.6	16.0 ^a ±0.1
170	2.0 ^{ab} ±0.5	3.1 ^b ±0.9	1.9 ^{ad} ±0.5	2.0 ^b ±0.4	4.3 ^b ±4.4	5.7 ^a ±0.5	13.5 ^a ±3.8
116	3.1 ^{ab} ±2.6	3.4 ^b ±1.7	6.3 ^b ±0.0	3.5 ^{bce} ±1.7	7.0 ^{ab} ±1.9	7.4 ^a ±0.3	12.3 ^a ±7.2
262	2.4 ^{ab} ±0.8	3.8 ^{ab} ±0.1	3.5 ^{ae} ±2.6	7.4 ^{ae} ±1.7	10.2 ^a ±0.6	6.6 ^a ±0.7	14.1 ^a ±6.1
RC212	5.1 ^a ±0.6	8.0 ^a ±3.2	1.8 ^{ae} ±0.7	6.6 ^{acefg} ±2.7	8.2 ^{ab} ±0.9	8.4 ^a ±5.4	12.6 ^a ±2.6
BM45	1.4 ^b ±1.0	4.9 ^{ab} ±1.1	3.9 ^{ab} ±0.6	2.2 ^{bcde} ±1.0	6.4 ^{ab} ±0.7	5.6 ^a ±0.6	10.9 ^a ±3.3

¹Data are means ± standard deviations (µg/ml mannan equivalents) for 2 replicates. abcdefg Means at any given time point with no common superscripts are significantly different (Fishers LSD procedure p<0.05).

Protein-associated mannan concentration did not change much during fermentation in most wines, but did increase in wine fermented by 142 and RC212 after 7 days followed by a decrease after 14 days. Protein-associated protein, like protein concentration, reached minimums after 14 or 45 days for all wines. After minimums were reached following fermentation, protein-associated mannan concentration peaked after 285 days in all wines. In fact, between 4 and 16 fold increases in concentration were observed in all wines.

Figure 7. Change in protein associated mannan over time



Fisher's least significance difference (LSD) procedure was used again to determine significant differences in protein-associated mannan concentration. Significant changes in protein-associated concentration between time points were observed in wine fermented by all of the yeast strains (**Table 6b**). Between 2 and 7 days, increases in protein-associated mannan were found in wines fermented by strains 142, RC212, and BM45, significant increases were observed in wines fermented by 142 and BM45. The only significant change between 7 and 14 days was a decrease in protein-associated mannan found in wine fermented by 142. Protein increased significantly in wines fermented by 98 and 142 between 14 and 45 days.

The only significant change between 45 and 105 days was an increase of protein-associated mannan found in wine fermented by BM45.

Table 6b. Change in protein associated mannan during aging¹

Strain	2 Days	7 Days	14 Days	45 Days	105 Days	195 Days	2 Days
	through	through	through	through	through	through	through
	7 Days	14 Days	45 Days	105 Days	195 Days	290 Days	290 Days
98	-	-	↑	-	-	↑	↑
142	↑	↓	↑	-	↑	-	↑
170	-	-	-	-	-	↑	↑
116	-	-	-	-	-	-	↑
262	-	-	-	-	-	↑	↑
RC212	-	-	-	-	-	-	↑
BM45	↑	-	-	↑	-	↑	↑

↑ Denotes a significant increase between indicated time points within strains.

↓ Denotes a significant decrease between indicated time points within strains.

- Denotes no significant change between indicated time points within strains.

(Fishers LSD procedure $p < 0.05$)

Between 105 and 195 days the only significant change was an increase in protein-associated mannan found in wine fermented by 142. Significant increases of protein-associated mannan were observed in wines fermented by yeast strains 98, 170, 262, and BM45 between 195 and 285 days, while protein-associated mannan increases in wines fermented by 262 and RC212 were not significant.

Significant differences in protein-associated mannan concentration released between yeast strains at individual points were observed during aging (**Table 6a**). Only one significant difference between yeast strains was observed at day 2 and that was between RC212 and BM45. Significant differences were observed between strain 142 and the strains 170 and 116, while RC212 differed significantly from both 116 and 170 after day 7. Strain 116 differed significantly from all other yeast strain along

with another significant difference between 142 and BM45 following 14 days.

Several significant differences were observed between strains after 45 days, see

Table 6a. Following 105 days, significant differences between strain 98 and the strains 142 and 170 were observed, while 262 differed significantly from both 142 and 170. There were significant differences between 142 and all other yeast strains after 195 days, and no significant differences between strains after 285 days.

The ratio of mannan to protein (**Table 7**) was relatively low at the early stages of aging and during fermentation, but increased for most strains over time.

Table 7. Ratio of mannan to protein¹

	2	7	14	45	105	195	290
Strain	Days	Days	Days	Days	Days	Days	Days
98	31.2± 17.2	31.7± 21.5	24.9± 7.9	94.8± 5.7	52.6± 4.2	48.6± 14.7	78.5± 12.7
142	21.4± 1.6	54.8± 8.1	22.3± 4.9	76.6± 3.9	38.6± 0.8	61.7± 24.0	82.1± 6.0
170	41.5± 24.8	25.2± 7.8	22.5± 3.8	30.5± 1.7	28.1± 30.6	54.5± 10.6	96.1± 4.2
116	44.0± 33.8	20.2± 6.5	69.3± 3.1	43.8± 27.4	74.7± 15.7	59.0± 9.4	81.0± 19.4
262	73.0± 39.9	27.6± 7.6	45.8± 20.5	77.2± 0.3	73.2± 24.5	51.8± 17.7	73.4± 33.2
RC212	72.4± 16.7	25.1± 10.3	23.6± 7.3	35.9± 12.0	41.3± 4.4	38.5± 15.3	90.5± 6.3
BM45	21.1± 14.7	35.8± 2.1	42.0± 2.1	31.7± 20.7	38.1± 6.2	52.5± 12.5	64.0± 15.7

¹Data are means ± standard deviations for the ratio of mannoprotein to protein concentration for 2 replicates.

4) Identification of proteins

In general, for all samples, the numbers of proteins increased from about 3 at the time of inoculation to 60 to 70. A drastic increase in proteins was observed in all wines after 7 days of fermentation. Additionally, the number of proteins drastically

decreased between 105 and 195 days in all wines. Proteins shared between replicates were consistently above 60%, and were often higher between 70 and 80% (**Table 8**).

Table 8. Shared proteins between replicates and samples

	Sample	Total proteins	Shared between replicates	% shared between replicates	Common to all four samples
0 Days	<i>RC212a</i>	3		100	
	<i>RC212b</i>	3	3	100	
	<i>BM45a</i>	3		100	
	<i>BM45b</i>	3	3	100	3
6 Days	<i>RC212a</i>	48		75.0	
	<i>RC212b</i>	59	36	61.0	
	<i>BM45a</i>	48		60.4	
	<i>BM45b</i>	47	29	61.7	24
14 Days	<i>RC212a</i>	68		69.1	
	<i>RC212b</i>	57	47	82.5	
	<i>BM45a</i>	61		62.3	
	<i>BM45b</i>	67	38	56.7	26
45 Days	<i>RC212a</i>	66		75.8	
	<i>RC212b</i>	59	50	84.7	
	<i>BM45a</i>	65		78.5	
	<i>BM45b</i>	67	51	76.1	35
105 Days	<i>RC212a</i>	55		69.1	
	<i>RC212b</i>	55	38	69.1	
	<i>BM45a</i>	63		71.4	
	<i>BM45b</i>	56	45	80.4	29
195 Days	<i>RC212a</i>	19		78.9	
	<i>RC212b</i>	19	15	78.9	
	<i>BM45a</i>	26		65.4	
	<i>BM45b</i>	21	17	81.0	10
285 Days	<i>RC212a</i>	34		76.5	
	<i>RC212b</i>	29	26	89.7	
	<i>BM45a</i>	35		62.9	
	<i>BM45b</i>	27	21	81.5	15

Roughly 50% of proteins found at each time point were common to all samples (**Table 8**). Proteins identified in all samples and replicates, and at all time points are tabulated in **Appendices 1-7**. **Appendix 9** is a master list of all proteins detected in all wines and their cellular localization. Because protein composition was so similar in all wines at each time point, only data for RC212 and BM45 are presented below.

The numbers of proteins found in the wines fermented by RC212 and BM45 drastically increased after 7 days (**Table 9**). The number of proteins continued to increase after day 14. The number of proteins found in wines fermented by RC212 and BM45 remained unchanged until day 195, when far fewer proteins were found. The number of proteins found after 285 days was slightly higher than after 195 days. Secreted proteins constituted the majority of proteins found in wines fermented by RC212 and BM45. The most cytosolic (non-secreted) proteins were found during fermentation and immediately following fermentation (**Table 9**). Cytosolic proteins, as a percentage of the total number of proteins, gradually decreased after 45 days, and by 195 days was nearly zero for both wines. **Table 10** presents the cellular localization of the secreted proteins found in wines fermented by RC212 and BM45. Mitochondrial proteins were the largest class of secreted proteins at most time points. Cell wall proteins accounted for a considerable proportion of the total proteins, roughly 40% after 195 days. Multi-compartmental proteins, associated with both the cell wall and cell membrane, were found at all time points and were also found in high proportion at the later stages of aging. Pst1, a protein associated with heat-induced haze reduction in white wines and previously detected in wine was also found.

Table 9. Protein Localization

	Sample	Total Proteins	Secreted Proteins % (#)	Cytosolic Proteins % (#)	Unknown % (#)
0 Days	<i>RC212a</i>	3	100 (3)	0	0
	<i>RC212b</i>	3	100 (3)	0	0
	<i>BM45a</i>	3	100 (3)	0	0
	<i>BM45b</i>	3	100 (3)	0	0
6 Days	<i>RC212a</i>	48	89.6 (43)	8.3 (4)	2.1 (1)
	<i>RC212b</i>	59 ¹	75.9 (44)	20.3 (12)	1.7 (1)
	<i>BM45a</i>	48 ¹	76.6 (36)	19.1 (9)	2.1 (1)
	<i>BM45b</i>	47	80.9 (38)	17.0 (8)	2.1 (1)
14 Days	<i>RC212a</i>	68 ¹	62.1 (41)	33.3 (22)	4.4 (3)
	<i>RC212b</i>	57 ¹	75.9 (41)	18.5 (10)	5.3 (3)
	<i>BM45a</i>	61 ¹	75.0 (45)	23.3 (14)	1.6 (1)
	<i>BM45b</i>	67 ¹	76.9 (50)	21.5 (14)	1.5 (1)
45 Days	<i>RC212a</i>	66	84.8 (56)	9.1 (6)	6.1 (4)
	<i>RC212b</i>	59	84.7 (50)	10.2 (6)	5.1 (3)
	<i>BM45a</i>	65	81.5 (53)	16.9 (11)	1.5 (1)
	<i>BM45b</i>	67	85.1 (57)	13.4 (9)	4.5 (3)
105 Days	<i>RC212a</i>	55	92.7 (51)	5.5 (3)	1.8 (1)
	<i>RC212b</i>	55	85.5 (47)	10.9 (6)	3.6 (2)
	<i>BM45a</i>	63	93.7 (58)	7.9 (5)	0.0
	<i>BM45b</i>	56 ¹	89.1 (49)	9.1 (5)	1.8 (1)
195 Days	<i>RC212a</i>	19	94.7 (18)	0.0	5.3 (1)
	<i>RC212b</i>	19	89.5 (17)	0.0	10.5 (2)
	<i>BM45a</i>	26	88.5 (23)	3.8 (1)	7.7 (2)
	<i>BM45b</i>	21	100.0 (21)	0.0	0.0
285 Days	<i>RC212a</i>	34	85.3 (29)	2.9 (1)	11.8 (4)
	<i>RC212b</i>	29	89.7 (26)	3.4 (1)	6.9 (2)
	<i>BM45a</i>	35	88.6 (31)	5.7 (2)	5.7 (2)
	<i>BM45b</i>	27	88.9 (24)	0.0	11.1 (3)

¹Indicates proteins that are both secreted and not secreted, which are excluded from the list. These constitute 1-5 % of the total proteins in any one sample.

Table 10. Localization of secreted proteins

	Sample	Secreted Proteins	Mito-chondria % (#)	Cell wall % (#)	Plasma memb % (#)	Nucleus % (#)	Multi compart. % (#)	Other ¹ % (#)
0 Days	<i>RC212a</i>	3	0	0	0	0	100 (3)	0
	<i>RC212b</i>	3	0	0	0	0	100 (3)	0
	<i>BM45a</i>	3	0	0	0	0	100 (3)	0
	<i>BM45b</i>	3	0	0	0	0	100 (3)	0
6 Days	<i>RC212a</i>	43	40 (18)	14 (6)	2 (1)	9 (4)	23 (9)	12 (5)
	<i>RC212b</i>	44	30 (14)	14 (6)	0	20 (9)	27 (11)	11 (4)
	<i>BM45a</i>	36	33 (13)	17 (6)	0	14 (5)	25 (8)	11 (4)
	<i>BM45b</i>	38	32 (13)	24 (9)	0	8 (3)	29 (10)	8 (3)
14 Days	<i>RC212a</i>	41	24 (11)	14 (6)	2 (1)	24 (10)	22 (8)	12 (5)
	<i>RC212b</i>	41	32 (14)	15 (6)	2 (1)	15 (6)	24 (9)	12 (5)
	<i>BM45a</i>	45	36 (17)	15 (7)	2 (1)	15 (7)	22 (9)	9 (4)
	<i>BM45b</i>	50	38 (20)	16 (8)	4 (2)	12 (6)	20 (9)	10 (5)
45 Days	<i>RC212a</i>	56	27 (15)	21 (12)	2(1)	13 (7)	27 (15)	11 (6)
	<i>RC212b</i>	50	26 (13)	24 (12)	4 (2)	6 (3)	28 (14)	12 (6)
	<i>BM45a</i>	53	34 (18)	17 (9)	4 (2)	15 (8)	19 (10)	11 (6)
	<i>BM45b</i>	57	42 (24)	19 (11)	4 (2)	5 (3)	21 (12)	9 (5)
105 Days	<i>RC212a</i>	51	31 (16)	16 (8)	4 (2)	10 (5)	27 (13)	12 (7)
	<i>RC212b</i>	47	26 (13)	21 (10)	4 (2)	6 (3)	30 (13)	13 (6)
	<i>BM45a</i>	58	38 (22)	14 (8)	3 (2)	10 (6)	21 (12)	14 (8)
	<i>BM45b</i>	48	35 (17)	17 (8)	4 (2)	13 (6)	22 (11)	10 (5)
195 Days	<i>RC212a</i>	18	17 (3)	33 (6)	0	0	39 (7)	11 (2)
	<i>RC212b</i>	17	18 (3)	41 (7)	0	0	41 (6)	0
	<i>BM45a</i>	23	13 (3)	43 (10)	0	4 (1)	26 (6)	13 (3)
	<i>BM45b</i>	21	14 (3)	43 (9)	0	5 (1)	38 (8)	0
285 Days	<i>RC212a</i>	29	28 (8)	31 (9)	0	7 (2)	31 (8)	3 (2)
	<i>RC212b</i>	26	23 (6)	42 (11)	0	4 (1)	27 (7)	4 (1)
	<i>BM45a</i>	31	42 (13)	23 (7)	0	6 (2)	26 (8)	3 (1)
	<i>BM45b</i>	24	21 (5)	38 (9)	0	4 (1)	33 (8)	4 (1)

¹Other compartments include the Golgi, endoplasmic reticulum, ribosome, and vacuole

Discussion

1) Cytosolic protein release during fermentation and aging

The observed initial increase and rapid decrease in protein concentration during fermentation may be due to transiently soluble cytosolic proteins. Not only did the protein concentration increase after 7 days, the number of proteins increased correspondingly in all wines. Following the end of fermentation, protein concentrations in most wines decreased from the peak that was reached after 7 days. Protein concentrations remained low in all wines after 45 days, with the exception of wine fermented by RC212, which increased. Martinez-Rodriguez et al. (2002), Arizumi et al. (1994), and Alexandre et al. (2001) measured protein during and after fermentation in a sparkling wine, wine fermented from Koshu grapes, and a synthetic wine, respectively. The authors observed similar initial increases in protein during fermentation, followed by rapid decreases post-fermentation and during the initial weeks of aging. Large numbers of proteins were detected in wines after 14 days regardless of the decrease in protein concentration. This may be due to the limited solubility of certain proteins extracted during fermentation. Most cytosolic proteins were found during and immediately following fermentation. The number of cytosolic proteins found in the wines decreased after 45 days and continued to decrease until the number of cytosolic proteins was near 0 after 195 days.

2) Secreted protein release during fermentation and aging

Secreted proteins were found in wine fermented by RC212 and BM45 at all time points from inoculation until 285 days. Secreted protein means the protein, which is secreted outside of the cell membrane. Cell wall proteins, mitochondrial

proteins, nuclear, and multi-compartmental proteins are considered secreted proteins. Multi-compartmental proteins can be found in more than one cellular compartment. For example, Pst1 is a multi-compartmental protein associated with the cell wall and cell membrane. The ratio of secreted proteins to total number of proteins ranged from 62 to 100% throughout aging. After 14 days, the ratio of secreted proteins to total number of proteins was lowest, from 62 to 76% in all replicates. The ratio steadily increased, reaching from 89 to 100% in all replicates after 195 days. The increase in the ratio of secreted proteins to total number of proteins coincided with a decrease of cytosolic proteins. The ratio of mitochondrial and nuclear proteins to the total number of proteins was consistent, until the ratios decreased after 195 days. This may be caused by proteases that degrade proteins over time or it may be caused by the wine itself. High alcohol and low pH combine to create an inhospitable environment for these proteins. Alexandre et al. (2001) measured no detectable protease A (an acid protease) activity in a synthetic wine for the first month of aging, followed by increases in activity after 54 and 73 days of aging. This suggests that the initial observed decreases in protein concentration are not directly linked to protease activity. It also suggests that proteins that are not degrading and appear to be increasing in concentration, such as cell wall proteins, are resistant to protease degradation. Proteins found in other compartments like the ER, the Golgi, and the vacuole shared similar life spans to the mitochondria and nuclear proteins. The secreted proteins that did not follow this trend were the cell wall proteins and the proteins associated with multiple compartments.

3) Cell wall protein release during fermentation and aging

The ratios of cell wall proteins to total secreted proteins found in wines fermented by RC212 and BM45 were similar, ranging from 14 to 24% between 7 and 105 days of aging. Cell wall proteins were the dominant protein found in the wines after 285 days of aging. The ratio of multi-compartmental proteins to total secreted proteins found in wines remained consistent during aging, from 19 to 40%. Proteins associated with both the cell wall and cell membrane were the predominant multi-compartmental protein found after 285 days. Multi-compartmental proteins that were not associated with the cell wall were not found as much after 285 days. Significant increases in protein concentration were observed after 195 days in nearly all wines. This observation combined with a decrease in cytosolic and other secreted proteins may suggest that cell wall proteins are more apt to remain soluble in wine, while simultaneously increasing in concentration over time. Comparable increases in wine protein concentration during aging have been observed by Martinez-Rodriguez et al. (2002), who measured protein concentration increase in sparkling wine from 150 to 250 days. Similarly, Languera et al. (1998) measured an increase in protein concentration in sparkling wine from 6 to 9 months.

Not only did protein concentration increase during aging, protein-associated mannan (mannan equivalents) increased as well. More protein-associated mannan was found as the wine aged on the lees. The ratio of mannan to protein concentrations increased in most wines during aging, suggesting a greater proportion of

mannoproteins present in the aged wine. Similarly, Dupin et al. (2000) observed polymeric mannose steadily increase as wine aged on the lees for 8 weeks.

4) Proteins of interest

Many proteins, including cell wall and cell membrane localized proteins, were found in the wines. One protein, Pst1 or Hpf2p, was of particular interest because of its potential for haze protection in white wines. Pst1 was found in all samples at all time points and has 5 known *N*-linked glycosylation sites (Brown et al. 2007). Pst1 is a mannosylated GPI-dependent protein associated with both the plasma membrane and cell wall. The protein retained haze protective effects in wine aged for 2 years suggesting that the protein remains soluble in wine for some time. Our discovery of Pst1 after at least 9 months of aging shows its robust solubility over time.

Other proteins of interest include proteins previously found in wine. Including, mannosylated β -1, 3 glucanases (Eng1, Bgl2, and Exg2), glycosidases (Crh1 and Utr2) cell wall mannoproteins (Cwp1, Ygp1, Suc4, Tos1), plasma membrane proteins (Gas1, Ecm33), and the phosphatase (Pho3) (Kwon 2004, and Cilidre et al. (2007). All of the aforementioned proteins were found in the model wines fermented by RC212 and BM45. Gas1 and Ecm33 are considered multi-compartmental proteins that are associated with both the plasma membrane and cell wall. Gas1 is potentially involved in extending and rearranging β -1,3 glucan chains (Klis et al. 2002). Other proteins within similar families were found in the model wines. For example, two other Gas-family proteins including Gas5 and Gas3 were found. Exg2 and Bgl2 were found in nearly all of the time points and wines, suggesting that they too remain soluble during aging. Exg2 and Bgl-2 are GPI-

anchored mannosylated β -1,3 glucanases involved in autolysis, specifically the hydrolysis of the cell wall (Klis et al. (2002). Eng1 is a β -1,3 endo-glucanase associated with cell division that degrades the cell wall from the daughter-cell side causing daughter to separate from mother (Colman-Lerner et al. 2001). Crh1 and Utr2 are both cell wall glycoproteins that function in the cross linking of chitin to β -1,6 glucan. Utr2 and Crh1 are GPI-linked protein, expressed at different growth stages. Utr2 is only expressed during vegetative growth, while Crh1 is induced by cell stress and is expressed during sporulation (Cabib et al. 2007). Ygp1 is a cell wall Pir protein induced by nutrient limitation. Pir proteins are directly linked to β -1,3 glucan through alkali sensitive linkages (Klis et al. 2002). Several other Pir linked proteins were found in the model wines including, Pau-family of proteins, Pir3, and Hsp150.

5) Does glycosylation increase solubility of mannoproteins?

The vast majority of proteins (~85%) that remained soluble in the model wines after both 195 and 285 days were glycoproteins or contain potential sites of *N*-glycosylation. The ratio of glycoproteins to total proteins increased from ~75% of the total proteins after 7 days. The yeast proteins that have been found in commercial wines including commercial sparkling wine we have analyzed are also glycosylated cell wall proteins (**Appendix 8**). Several grape proteins that have been found in wine are glycosylated. One of the major proteins commonly found in wine, VVTL1, does not appear to be glycosylated (Tattersal et al. 1997). Another thaumatin-like protein found in wine contains no potential *N*-glycosylation sites in its primary sequence of amino acids. Several common grape proteins found in wine contain sequons of potential *N*-glycosylation. For example, vacuolar invertase, a class IV chitinase, an

unnamed protein, and an endo-chitinase all contain at least one, if not multiple potential sites for N-glycosylation. It appears that there may be some predisposition for glycoproteins to remain soluble in wine. *N*-glycosylation is not a prerequisite for protein stability in wine as it ages. VVTL1 a non-glycosylated grape protein was found in sparkling wine that we measured from the 2000 vintage **Appendix 8**. Proteins that do not contain potential sites of *N*-glycosylation were found in the model wines after 195 days, including Tef1, Mic14, Rps3, Trx2, Trx1, Ahp1, Hsp12, and Hsp26. The presence of a glycan moiety is not all-important to remain soluble in wine over a long time. It seems unlikely that the increase of glycosylated and potentially glycosylated proteins at the end of the fermentation is a coincidence. The glycan moiety guards the protein against proteolytic degradation, and may prevent unfolding and non-polar association with other proteins that may lead to aggregation and precipitation. Perhaps a phosphorylated glycan moiety increases the proteins solubility in the wine.

Table 11. Composition of model must, pH 3.5**Sugars and salts**

	g/L
Glucose	200
Potassium hydroxide	1.17
Potassium tartrate	6

CaCl ₂ 2 H ₂ O	0.44
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K ₂ HPO ₄	1.14
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Nitrogen

	g/L
Diammonium Phosphate	1.5

Vitamins

	mg/L
Myo-Inositol	100
Pyridoxine HCl	2
Nicotinic acid	2
Ca ⁺ Pantothenate	0.25
Thiamin HCl	0.5
p-amino benzoic acid	0.2
Riboflavin	0.2
Folic acid	0.2
Biotin	0.01

Minerals

	mg/L
MnCl ₂ 4 H ₂ O	0.198
ZnCl ₂	0.136
FeCl ₂	0.050
CuCl ₂	0.014
Boric Acid	0.006
CoCl ₂ 6 H ₂ O	0.001
NaMoO ₄ 2 H ₂ O	0.024
KI	0.010

Conclusion

The goal of our work was to better understand yeast protein and mannoprotein content and composition in a model wine as it ages on the lees. The cell wall mannoproteins, which have been suggested to positively affect wine quality, were of particular interest. Chemical analyses were modified and developed in order to measure quantities of protein and mannoprotein present in wine. HPLC-MS was employed to study the protein composition of model wines containing several yeast strains at various time points during fermentation and 9 months of aging.

In nearly all wines, protein and mannoprotein concentrations increased during, but decreased by the end of fermentation. The protein and mannoprotein concentrations in all wines increased again after 3 months of aging, with the exception of wine fermented by RC212. Protein and protein-associated mannan concentrations increased after 1 month in wine fermented by RC212. For all wines, the protein and protein-associated mannan concentrations ultimately reached maximal values about 2- and 6-fold higher than values measured at 2 days.

Consistent with increases in protein-associated mannan concentration, cell wall mannoproteins were the predominant proteins found in wines after 285 days of aging. Most cytosolic proteins were found immediately following fermentation and one month after fermentation. Non-cell wall secreted proteins also decreased during aging. This suggests that cell wall mannoproteins are extracted and remain soluble long into the aging process.

The mannan to protein ratio increased as the wines aged. In fact, approximately 85% of the proteins found in wines fermented by RC212 and BM45

were glycosylated or contained potential sites for *N*-glycosylation. The exact mechanism of cell wall mannoprotein solubility in wine as it ages is an interesting area of further research.

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Appendices

Appendix 1. Proteins identified in wines made by strain 98¹**0 Days**

Protein name	Gene ID	98a	98b
Protoplast secreted protein 1 precursor	PST1	100% (3)	100% (4)
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH2		99% (2)
Protein ECM33 precursor	ECM33	90% (1)	85% (1)
Phosphoglycerate kinase	PGK1	90% (1)	85% (1)

7 days

Protein name	Gene ID	98a	98b
Protoplast secreted protein 1 precursor	PST1	100% (13)	100% (10)
Enolase 1	ENO1	100% (4)	100% (4)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (5)	100% (9)
Phosphoglycerate kinase	PGK1	100% (7)	100% (5)
Cell wall protein DAN2 precursor	DAN2	99% (2)	90% (1)
Fructose-bisphosphate aldolase	FBA1	100% (3)	100% (3)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (3)	90% (1)
Heat shock protein SSA2	HSP72	100% (5)	100% (3)
Enolase 2	ENO2	90% (1)	99% (2)
Phosphoglycerate mutase 1	PMG1	99% (2)	100% (3)
Pyruvate decarboxylase isozyme 1	PDC1	100% (6)	100% (5)
Glyceraldehyde-3-phosphate dehydrogenase 3	TDH3	100% (4)	100% (4)
Heat shock protein 26	HSP26	100% (3)	100% (4)
Pyruvate kinase 1	PYK1	100% (3)	99% (2)
Probable family 17 glucosidase SCW4 precursor	SCW4	99% (2)	99% (2)
Triosephosphate isomerase	TPI1	100% (4)	100% (3)
Thioredoxin I	TRX1	99% (2)	90% (1)
Thioredoxin II	TRX2	90% (1)	90% (1)
12 kDa heat shock protein	HSP12	90% (1)	90% (1)
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	100% (3)	90% (1)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	90% (1)	
Glucan 1,3-beta-glucosidase precursor	BGL2	90% (1)	90% (1)
Protein YGP1 precursor	YGP1	90% (1)	90% (1)
Protein ECM33 precursor	ECM33	90% (1)	90% (1)
Peroxiredoxin TSA1	TSA1	99% (2)	99% (2)
Actin	ACT1	99% (2)	97% (1)
Lysophospholipase 2 precursor	PLB2	90% (1)	
Alcohol dehydrogenase 1	ADH1	99% (2)	90% (1)
Outer mitochondrial membrane protein porin 1	POR1	90% (1)	90% (1)
Ketol-acid reductoisomerase, mitochondrial precursor	ILV5	99% (2)	99% (2)
ATP synthase subunit beta, mitochondrial precursor	ATP2	90% (1)	90% (1)
Elongation factor 1-alpha	TEF1	90% (1)	90% (1)
Alcohol dehydrogenase 4	ADH4	99% (2)	99% (2)
Malate dehydrogenase, mitochondrial precursor	MDH1	90% (1)	90% (1)

Peroxiredoxin type-2	AHP1	90% (1)	90% (1)
5-methyltetrahydropteroyltriglutamate-- homocysteine methyltransferase	MET6	90% (1)	99% (2)
Heat shock protein SSA1	HSP71	90% (1)	90% (1)
60S ribosomal protein L8-A	RPL8A	90% (1)	90% (1)
NADP-specific glutamate dehydrogenase 1	DHE4		99% (2)
30 kDa heat shock protein	HSP30	90% (1)	90% (1)
Aminopeptidase Y precursor	APE3	90% (1)	90% (1)
Plasma membrane ATPase 1	PMA1	90% (1)	99% (2)
60S ribosomal protein L19	RPL19A	90% (1)	90% (1)
Protein HMF1	HMF1	90% (1)	90% (1)
Histone H2A.1	HTA1		90% (1)
40S ribosomal protein S5	RPS5	90% (1)	
Heat shock protein SSC1, mitochondrial precursor	HSP77	90% (1)	
Protein MMF1, mitochondrial precursor	MMF1	90% (1)	
60S ribosomal protein L13-A	RPL13A	90% (1)	90% (1)
60S ribosomal protein L4-A	RPL4A		
Hexokinase-1	HXK1	90% (1)	100% (3)
Vacuolar ATP synthase subunit B	VMA2	90% (1)	
40S ribosomal protein S6	RPS6A	90% (1)	
60S acidic ribosomal protein P2-beta	RPP2B	90% (1)	90% (1)
40S ribosomal protein S3	RPS3		90% (1)
Dihydroxy-acid dehydratase, mitochondrial precursor	ILV3		90% (1)
Reticulon-like protein 1	RTN1	88% (1)	90% (1)
2-oxoglutarate dehydrogenase E1 component, mitochondrial precursor	ODO1	90% (1)	
40S ribosomal protein S22-A	RPS22A	90% (1)	90% (1)
Hypothetical 41.2 kDa protein in FPR1-TOM22 intergenic region	YNN4	90% (1)	
Adenosine kinase	ADO1		90% (1)
Homoserine dehydrogenase	HOM6	90% (1)	
Protein MET17 [Includes: O-acetylhomoserine sulfhydrylase	MET17	90% (1)	
Metallothionein precursor	CUP1-1	90% (1)	

14 days

Protein name	Gene ID	98a	98b
Protoplast secreted protein 1 precursor	PST1	100% (12)	100% (13)
Enolase 1	ENO1	100% (7)	100% (8)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (8)	100% (7)
Phosphoglycerate kinase	PGK1	100% (7)	100% (9)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (6)	100% (6)
Cell wall protein DAN2 precursor	DAN2	100% (3)	99% (2)
Fructose-bisphosphate aldolase	FBA1	100% (3)	100% (5)
Enolase 2	ENO2	100% (3)	100% (3)
Glyceraldehyde-3-phosphate dehydrogenase 3	TDH3	100% (5)	100% (6)
Pyruvate decarboxylase isozyme 1	PDC1	100% (6)	100% (4)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (3)	100% (3)
Heat shock protein 26	HSP26	100% (3)	99% (2)

Thioredoxin I	TRX1	100% (4)	100% (4)
Pyruvate kinase 1	PYK1	100% (5)	100% (3)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (3)	100% (3)
5-methyltetrahydropteroyltriglutamate-- homocysteine methyltransferase	MET6	100% (6)	100% (5)
Protein YGP1 precursor	YGP1	99% (2)	99% (2)
Thioredoxin II	TRX2	100% (3)	99% (2)
Heat shock protein SSA2	HSP72	100% (3)	100% (5)
Phosphoglycerate mutase 1	PMG1	100% (2)	100% (3)
Protein ECM33 precursor	ECM33	90% (1)	100% (3)
Triosephosphate isomerase	TPI1	100% (3)	100% (3)
Lysophospholipase 2 precursor	PLB2	100% (3)	90% (1)
Alcohol dehydrogenase 4	ADH4	99% (2)	99% (2)
6-phosphogluconate dehydrogenase, decarboxylating 1 -	GND1	100% (3)	100% (3)
12 kDa heat shock protein	HSP12		90% (1)
Elongation factor 1-alpha	TEF1	99% (2)	99% (2)
Alcohol dehydrogenase 1	ADH1	90% (1)	90% (1)
40S ribosomal protein S17-A	RPS17A	90% (1)	90% (1)
Probable glycosidase CRH1 precursor	CRH1	90% (1)	
Transaldolase	TAL1	99% (2)	99% (2)
Peroxiredoxin TSA1	TSA1	99% (2)	90% (1)
Seripauperin-5	PAU5	90% (1)	90% (1)
30 kDa heat shock protein	HSP30	90% (1)	90% (1)
Glycolipid-anchored surface protein 5 precursor	GAS5	90% (1)	90% (1)
NADP-specific glutamate dehydrogenase 1	DHE4	99% (2)	90% (1)
Uncharacterized protein YGL258W	YGL258W	90% (1)	90% (1)
Histone H2A.1	HTA1	90% (1)	
40S ribosomal protein S14-A	RPS14A	90% (1)	99% (2)
60S ribosomal protein L19	RPL19A	90% (1)	
Aminopeptidase Y precursor	APE3	90% (1)	90% (1)
Peroxiredoxin type-2	AHP1	90% (1)	90% (1)
Protein PRY1 precursor	PRY1	90% (1)	90% (1)
Vacuolar ATP synthase catalytic subunit A	VMA1	90% (1)	90% (1)
60S ribosomal protein L13-A	RPL13A	90% (1)	90% (1)
Ketol-acid reductoisomerase, mitochondrial precursor	ILV5	99% (2)	90% (1)
60S ribosomal protein L8-B	RPL8B		90% (1)
40S ribosomal protein S16	RPS16	90% (1)	90% (1)
40S ribosomal protein S5	RPS5	90% (1)	90% (1)
Ubiquitin	UBI1		99% (2)
40S ribosomal protein S19-A	RPS19A	99% (2)	90% (1)
Aspartate-semialdehyde dehydrogenase	HOM2	93% (1)	
Plasma membrane ATPase 1	PMA1	90% (1)	
3-isopropylmalate dehydrogenase	LEU3	90% (1)	
Heat shock protein SSA1	HSP71	90% (1)	
(DL)-glycerol-3-phosphatase 1	GPP1	90% (1)	90% (1)
Probable inosine-5'-monophosphate dehydrogenase IMD1	IMDH1	90% (1)	90% (1)
Aspartic proteinase yapsin-3 precursor	YPS3	90% (1)	
60S ribosomal protein L4-A	RL4A	90% (1)	

Vacuolar ATP synthase subunit B	VMA2		90% (1)
Mannose-1-phosphate guanyltransferase	MPG1	90% (1)	
60S acidic ribosomal protein P2-beta	RLA4	90% (1)	90% (1)
Uncharacterized protein YNL200C	YNL200C	90% (1)	90% (1)
Heat shock protein SSC1, mitochondrial precursor	HSP77		90% (1)
40S ribosomal protein S27-A	RPS27A	90% (1)	
Hexokinase-1	HXK1	90% (1)	90% (1)
Homoserine dehydrogenase	HOM6	90% (1)	
Adenosine kinase	ADO1	90% (1)	
Reticulon-like protein 1	RTN1	90% (1)	90% (1)
60S ribosomal protein L28	RPL28	90% (1)	
40S ribosomal protein S18	RPS18		90% (1)
Protein disulfide-isomerase precursor	PDI1		99% (2)
Dihydroxy-acid dehydratase, mitochondrial precursor	ILV3	90% (1)	
Protein ZEO1	ZEO1	90% (1)	
Nicotinamidase	PNC1	90% (1)	
Acetyl-CoA acetyltransferase	ERG10		90% (1)
Homoisocitrate dehydrogenase, mitochondrial precursor	LYS12	81% (1)	
60S ribosomal protein L18	RPL18	80% (1)	

1 month

Protein name	Gene ID	98a	98b
Protoplast secreted protein 1 precursor	PST1	100% (14)	100% (15)
Phosphoglycerate kinase	PGK1	100% (6)	100% (11)
Enolase 1	ENO1	100% (5)	100% (6)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (7)	100% (5)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (6)	100% (5)
Cell wall protein DAN2 precursor	DAN2	100% (4)	99% (2)
Glucan 1,3-beta-glucosidase precursor	BGL2	99% (2)	100% (3)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (3)	99% (2)
Fructose-bisphosphate aldolase	FBA1	100% (6)	100% (7)
Lysophospholipase 2 precursor	PLB2	100% (4)	100% (3)
Protein YGP1 precursor	YGP1	100% (3)	87% (1)
Phosphoglycerate mutase 1	PMG1	100% (3)	100% (3)
Peroxiredoxin type-2	AHP1	100% (4)	100% (5)
Seripauperin-5	PAU5	99% (2)	87% (1)
Thioredoxin-2	TRX2	100% (3)	87% (1)
Protein ECM33 precursor	ECM33	90% (1)	87% (1)
Aspartic proteinase yapsin-3 precursor	YPS3	100% (3)	87% (1)
Glycolipid-anchored surface protein 5 precursor	GAS5	100% (2)	
Thioredoxin-1	TRX1	99% (2)	87% (1)
Heat shock protein SSA1	HSP71	100% (4)	
Pyruvate decarboxylase isozyme 1	PDC1		100% (3)
Enolase 2	ENO2	90% (1)	87% (1)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	90% (1)	87% (1)
Heat shock protein 26	HSP26	99% (2)	

30 kDa heat shock protein	HSP30		87% (1)
12 kDa heat shock protein	HSP12	90% (1)	
Elongation factor 1-alpha	TEF1	99% (2)	
Pyruvate kinase 1	PYK1	90% (1)	87% (1)
Aminopeptidase Y precursor	APE3	90% (1)	87% (1)
Triosephosphate isomerase	TPI1	99% (2)	87% (1)
Transaldolase	TAL1	90% (1)	
5-methyltetrahydropteroyltriglutamate-- homocysteine methyltransferase	MET6	90% (1)	99% (2)
Lysophospholipase 1 precursor	PLB1	99% (2)	
Glycogen debranching enzyme	GDB1	90% (1)	87% (1)
FK506-binding protein 1	FKBP	90% (1)	87% (1)
ATP synthase subunit beta, mitochondrial precursor	ATP2	90% (1)	87% (1)
60S ribosomal protein L4-A	RPL4A	90% (1)	87% (1)
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH3	99% (2)	87% (1)
Homoserine dehydrogenase	HOM6	99% (2)	87% (1)
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	90% (1)	99% (2)
NADP-specific glutamate dehydrogenase 1	DHE4	90% (1)	
150 kDa heat shock glycoprotein precursor	HS150	90% (1)	
Protein disulfide-isomerase precursor	PDI1	90% (1)	87% (1)
Plasma membrane ATPase 1	PMA1	99% (2)	87% (1)
Superoxide dismutase [Cu-Zn]	SOD1	99% (2)	
Saccharopine dehydrogenase [NAD+, L-lysine- forming]	LYS1	90% (1)	87% (1)
Histone H2A.1	HTA1	90% (1)	87% (1)
Protein HMF1	HMF1	90% (1)	87% (1)
Dihydroxy-acid dehydratase, mitochondrial precursor	ILV3		87% (1)
Alcohol dehydrogenase 4	ADH4		87% (1)
Alcohol dehydrogenase 1	ADH1	90% (1)	87% (1)
40S ribosomal protein S20	RPS20	90% (1)	87% (1)
Malate dehydrogenase, mitochondrial precursor	MDH1	90% (1)	
Peroxiredoxin TSA1	TSA1	90% (1)	
Protein EGT2 precursor	EGT2		87% (1)
40S ribosomal protein S3	RPS3		87% (1)
60S ribosomal protein L8-B	RPL8B	90% (1)	
Actin	ACT1		98% (2)
Protein MMF1, mitochondrial precursor	MMF1		87% (1)
40S ribosomal protein S17-A	RPS17A	90% (1)	
Endo-1,3(4)-beta-glucanase 1 precursor	ENG1	90% (1)	
Eukaryotic translation initiation factor 5A-1	HYP1		87% (1)
40S ribosomal protein S5	RPS5	90% (1)	87% (1)
Seripauperin-2	PAU2	90% (1)	
Transcriptional modulator WTM1	WTM1	90% (1)	
Ketol-acid reductoisomerase, mitochondrial precursor	ILV5	90% (1)	
Mannose-1-phosphate guanyltransferase	MPG1	90% (1)	87% (1)
40S ribosomal protein S22-A	RPS22A		87% (1)
Reticulon-like protein 1	RTN1		87% (1)

Phospho-2-dehydro-3-deoxyheptonate aldolase, tyrosine-inhibited	ARO4	90% (1)	
Hexokinase-1	HXK1	90% (1)	87% (1)
Protein ZEO1	ZEO1	90% (1)	
10 kDa heat shock protein, mitochondrial	HSP10		87% (1)
60S ribosomal protein L15-A	RPL15A	90% (1)	
Vacuolar aminopeptidase 1 precursor	APE1	90% (1)	
60S ribosomal protein L20	RPL20	90% (1)	
Bifunctional purine biosynthesis protein ADE17 [Includes: Phosphoribosylaminoimidazolecarboxamide formyltransferase	PUR92		87% (1)
UBX domain-containing protein 1	UBX1	90% (1)	
3-isopropylmalate dehydrogenase	LEU3	90% (1)	
Heat shock protein homolog SSE1	HSP7F		87% (1)

3 months

		98a	98b
Protein name	Gene ID		
Protoplast secreted protein 1 precursor	PST1	100% (11)	100% (9)
Phosphoglycerate kinase	PGK1	100% (5)	100% (5)
Enolase 1	ENO1	100% (4)	100% (6)
Cell wall protein DAN2 precursor	DAN2	87% (1)	87% (1)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (4)	100% (4)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	99% (2)	87% (1)
Fructose-bisphosphate aldolase	FBA1	100% (4)	100% (4)
Glucan 1,3-beta-glucosidase precursor	BGL2	87% (1)	98% (2)
Probable family 17 glucosidase SCW4 precursor	SCW4	99% (2)	87% (1)
Lysophospholipase 2 precursor	PLB2	87% (1)	87% (1)
Protein YGP1 precursor	YGP1	87% (1)	87% (1)
Enolase 2	ENO2	87% (1)	98% (2)
Thioredoxin-2	TRX2		98% (2)
Elongation factor 1-alpha	EF1A	87% (1)	87% (1)
Phosphoglycerate mutase 1	PMG1	87% (1)	87% (1)
Pyruvate decarboxylase isozyme 1	PDC1	100% (3)	87% (1)
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	99% (2)	87% (1)
ATP synthase subunit alpha, mitochondrial precursor	ATP1		87% (1)
30 kDa heat shock protein	HSP30	87% (1)	87% (1)
Glycolipid-anchored surface protein 5 precursor	GAS5	87% (1)	87% (1)
40S ribosomal protein S3	RPS3	87% (1)	87% (1)
Glycogen debranching enzyme	GDB1	87% (1)	87% (1)
Triosephosphate isomerase	TPI1		87% (1)
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH2		87% (1)
Alcohol dehydrogenase 4	ADH4		87% (1)
FK506-binding protein 1	FPR1	87% (1)	
NADP-specific glutamate dehydrogenase 1	DHE4		87% (1)
Magnesium-activated aldehyde dehydrogenase, cytosolic	ALDH6	87% (1)	87% (1)
Plasma membrane ATPase 1	PMA1	87% (1)	

Protein HMF1	HMF1	87% (1)	87% (1)
Saccharopine dehydrogenase [NAD ⁺ , L-lysine-forming]	LYS1	87% (1)	87% (1)
Trehalose synthase complex regulatory subunit TSL1	TSL1	87% (1)	
Vacuolar aminopeptidase 1 precursor	APE1	87% (1)	
60S ribosomal protein L20	RPL20	87% (1)	
60S ribosomal protein L8-A	RPL8A		87% (1)
40S ribosomal protein S5 -	RPS5	87% (1)	
40S ribosomal protein S19-A	RPS19A	87% (1)	
Bifunctional purine biosynthesis protein ADE17 [Includes: Phosphoribosylaminoimidazolecarboxamide formyltransferase	PUR92		87% (1)
Heat shock protein homolog SSE1	HSP7F	87% (1)	
Fatty acid synthase subunit alpha	FAS2	87% (1)	
Stress protein DDR48	DDR48		87% (1)
40S ribosomal protein S15	RPS15	87% (1)	
40S ribosomal protein S8	RPS8	87% (1)	87% (1)
Vacuolar proton pump subunit G	VMA10	87% (1)	
40S ribosomal protein S27-A	RPS27A	99% (2)	
RNA polymerase II holoenzyme cyclin-like subunit	SSN8		87% (1)

6 months

Protein name	Gene ID	98a	98b
Protoplast secreted protein 1 precursor	PST1	100% (12)	100% (15)
Cell wall protein DAN2 precursor	DAN2	100% (4)	100% (7)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (9)	100% (8)
Phosphoglycerate kinase	PGK1	99% (2)	99% (2)
Lysophospholipase 2 precursor	PLB2	100% (8)	100% (9)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (5)	100% (7)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (5)	100% (4)
Protein YGP1 precursor	YGP1	100% (3)	100% (5)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (4)	100% (4)
Protein ECM33 precursor	ECM33	100% (3)	100% (4)
Glycolipid-anchored surface protein 5 precursor	GAS5	100% (3)	99% (2)
Probable glycosidase CRH1 precursor	CRH1	100% (4)	100% (3)
Repressible acid phosphatase precursor	PPA5	100% (3)	100% (3)
Fructose-bisphosphate aldolase	FBA1		99% (2)
12 kDa heat shock protein	HSP12	99% (2)	99% (2)
Aminopeptidase Y precursor	APE3	99% (2)	99% (2)
Lysophospholipase 1 precursor	PLB1	99% (2)	100% (4)
Elongation factor 1-alpha	TEF1		99% (2)
Covalently-linked cell wall protein 14 precursor	CCW14	99% (2)	
Thioredoxin-2	TRX2	100% (3)	100% (2)
Phosphoglycerate mutase 1	PMG1		99% (2)
Thioredoxin-1	TRX1	99% (2)	100% (3)
Enolase 2	ENO2	100% (4)	
Uncharacterized protein YJL171C precursor	YJR1		99% (2)

Uncharacterized protein YGL258W	YGL258W	99% (2)	99% (2)
Heat shock protein 26	HSP26	99% (2)	
Reticulon-like protein 1	RTN1	99% (2)	
Protein PRY3	PRY3		99% (2)

9 months

Protein name	Gene ID	98a	98b
Seripauperin-17 precursor	PAU17	100% (9)	100% (6)
Cell wall mannoprotein PST1 precursor	PST1	100% (13)	100% (15)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (13)	100% (9)
Phosphoglycerate kinase	PGK1	100% (3)	100% (5)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (5)	100% (8)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (9)	100% (9)
Protein YGP1 precursor	YGP1	100% (2)	100% (5)
Phosphoglycerate mutase 1	PMG1	100% (5)	100% (6)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (6)	100% (4)
Cell wall protein DAN3 precursor	DAN3	100% (3)	100% (3)
Cell wall protein ECM33 precursor	ECM33	100% (4)	100% (4)
Glycolipid-anchored surface protein 5 precursor	GAS5	100% (4)	100% (4)
Pyruvate kinase 1	PYK1	100% (5)	
Seripauperin-5	PAU5	77% (1)	99% (2)
Glycolipid-anchored surface protein 1 precursor	GAS1	100% (4)	
Uncharacterized protein YJL171C precursor	YJR1	77% (1)	100% (2)
Probable glycosidase CRH1 precursor	CRH1	99% (2)	
Elongation factor 1-alpha	TEF1	77% (1)	99% (2)
12 kDa heat shock protein	HSP12		95% (1)
Fructose-bisphosphate aldolase	FBA1	100% (3)	
Lysophospholipase 2 precursor	PLB2	77% (1)	100% (3)
Septation protein SUN4 precursor	SUN4	99% (2)	100% (3)
Covalently-linked cell wall protein 14 precursor	CCW14	99% (2)	99% (2)
Protein PIR3 precursor	PIR3	77% (2)	98% (2)
Heat shock protein SSA1	HSP71		93% (1)
Mitochondrial intermembrane space cysteine motif-containing protein MIC14	MIC14	77% (1)	100% (3)
Pyruvate decarboxylase isozyme 1	PDC1	77% (1)	
6-phosphofructokinase subunit beta	PFK2	77% (1)	
Protein VEL1 precursor	VEL1	77% (1)	99% (2)
Probable family 17 glucosidase SCW10 precursor	SCW10	97% (2)	99% (2)
Glycogen debranching enzyme	GDB1		49% (1)
Thioredoxin-2	TRX2	98% (2)	
Protein SIM1 precursor	SIM1	77% (1)	
Cell wall protein CWP1 precursor	CWP1	99% (2)	
Glycolipid-anchored surface protein 3 precursor	GAS3	99% (2)	

¹ The percentage adjacent to the Gene Id is the Bayesian probability that the protein is correctly identified; the number of peptides attributed to the protein is in parentheses.

Appendix 2. Proteins identified in wines made by strain 142¹**0 Days**

		142a	142b
Protein name	Gene Id		
Protoplast secreted protein 1 precursor	PST1		100% (3)
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH2	99% (2)	100% (4)
Protein ECM33 precursor	ECM33		80% (1)
Pyruvate decarboxylase isozyme 1	PDC1	86% (1)	100% (3)
Enolase 2	ENO2		100% (6)
Phosphoglycerate kinase	PGK1		99% (2)
Fructose-bisphosphate aldolase	FBA1		99% (2)
Pyruvate kinase 1	PYK1		100% (3)
Alcohol dehydrogenase 1	ADH1		99% (2)
Phosphoglycerate mutase 1	PMG1		99% (2)
Protein ZEO1	ZEO1		80% (1)
Peroxiredoxin type-2	TSA2		80% (1)
Triosephosphate isomerase	TPI1		80% (1)
Elongation factor 1-alpha	TEF1		80% (1)
12 kDa heat shock protein	HSP12		80% (1)

7 days

		142a	142b
Protein name	Gene Id		
Protoplast secreted protein 1 precursor	PST1	100% (16)	100% (16)
Enolase 1	ENO1	100% (4)	100% (7)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (6)	100% (7)
Phosphoglycerate kinase	PGK1	100% (6)	100% (8)
Fructose-bisphosphate aldolase	FBA1	100% (4)	100% (4)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (5)	99% (2)
Heat shock protein SSA2	HSP72	100% (5)	100% (4)
Enolase 2	ENO2	90% (1)	90% (1)
Phosphoglycerate mutase 1	PMG1	100% (3)	100% (3)
Pyruvate decarboxylase isozyme 1	PDC1	100% (5)	100% (4)
Glyceraldehyde-3-phosphate dehydrogenase 3	TDH3	99% (2)	100% (3)
Heat shock protein 26	HSP26	100% (5)	100% (5)
Pyruvate kinase 1	PYK1	100% (3)	100% (3)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (3)	99% (2)
Triosephosphate isomerase	TPI1	99% (2)	99% (2)
Thioredoxin I	TRX1	100% (4)	99% (2)
Thioredoxin II	TRX2	100% (4)	99% (2)
12 kDa heat shock protein	HSP12	99% (2)	100% (3)
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	100% (2)	90% (1)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	100% (3)	100% (4)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (3)	99% (2)
Protein YGP1 precursor	YGP1	99% (2)	99% (2)
Protein ECM33 precursor	ECM33	100% (3)	99% (2)
Peroxiredoxin TSA1	TSA1	90% (1)	99% (2)
Actin	ACT1		99% (2)
Lysophospholipase 2 precursor	PLB2	100% (3)	90% (1)
Alcohol dehydrogenase 1	ADH1	90% (1)	90% (1)

Outer mitochondrial membrane protein porin 1	POR1	90% (1)	90% (1)
Ketol-acid reductoisomerase, mitochondrial precursor	ILV5	99% (2)	90% (1)
ATP synthase subunit beta, mitochondrial precursor	ATP2	90% (1)	99% (2)
Elongation factor 1-alpha	TEF1	90% (1)	90% (1)
Alcohol dehydrogenase 4	ADH4	90% (1)	90% (1)
Malate dehydrogenase, mitochondrial precursor	MDH1	99% (2)	90% (1)
Peroxiredoxin type-2	AHP1	99% (2)	90% (1)
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	MET6	90% (1)	90% (1)
Heat shock protein SSA1	HSP71	90% (1)	90% (1)
60S ribosomal protein L8-A	RPL8A	90% (1)	90% (1)
30 kDa heat shock protein	HSP30	90% (1)	90% (1)
Seripauperin-5	PAU5	90% (1)	90% (1)
Plasma membrane ATPase 1	PMA1	90% (1)	90% (1)
60S ribosomal protein L19	RPL19A	90% (1)	90% (1)
Transaldolase	TAL1	90% (1)	90% (1)
Protein HMF1	HMF1	90% (1)	
Histone H2A.1	HTA1	90% (1)	90% (1)
Heat shock protein SSC1, mitochondrial precursor	HSP77		99% (2)
Glycolipid-anchored surface protein 1 precursor	GAS1	99% (2)	90% (1)
60S ribosomal protein L13-A	RPL13A		84% (1)
60S ribosomal protein L4-A	RPL4A	90% (1)	90% (1)
Hexokinase-1	HXK1		90% (1)
Glycolipid-anchored surface protein 5 precursor	GAS5	90% (1)	99% (2)
40S ribosomal protein S6	RPS6A		90% (1)
60S acidic ribosomal protein P2-beta	RPP2B		90% (1)
40S ribosomal protein S3	RPS3	90% (1)	
40S ribosomal protein S14-A	RPS14A		90% (1)
Aconitate hydratase, mitochondrial precursor	ACO1	90% (1)	
Lysophospholipase 1 precursor	PLB1	90% (1)	
Mannose-1-phosphate guanyltransferase	MPG1		90% (1)
2-oxoglutarate dehydrogenase E1 component, mitochondrial precursor	ODO1		90% (1)
40S ribosomal protein S20	RPS20	90% (1)	
Hypothetical 41.2 kDa protein in FPR1-TOM22 intergenic region	YNN4	90% (1)	
10 kDa heat shock protein, mitochondrial	HSP10	86% (1)	90% (1)
Eukaryotic translation initiation factor 5A-1	IF5A1	90% (1)	
Uncharacterized protein YJL171C precursor	YJR1		90% (1)
Transketolase 1	TKL1	90% (1)	
3-isopropylmalate dehydrogenase	LEU3		90% (1)
Cell wall protein DAN3 precursor	DAN3		90% (1)
FK506-binding protein 1	FPR1	90% (1)	
40S ribosomal protein S18	RPS18	86% (1)	

14 days

		142a	142b
Protein name	Gene Id		
Protoplast secreted protein 1 precursor	PST1	100% (11)	100% (10)
Enolase 1	ENO1	100% (8)	100% (5)

Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (7)	100% (6)
Phosphoglycerate kinase	PGK1	100% (9)	100% (6)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (5)	100% (5)
Fructose-bisphosphate aldolase	FBA1	100% (5)	100% (5)
Enolase 2	ENO2	100% (2)	99% (2)
Glyceraldehyde-3-phosphate dehydrogenase 3	TDH3	100% (4)	100% (3)
Pyruvate decarboxylase isozyme 1	PDC1	100% (4)	100% (4)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (3)	100% (3)
Heat shock protein 26	HSP26	100% (4)	100% (4)
Thioredoxin I	TRX1	99% (2)	100% (4)
Pyruvate kinase 1	PYK1	100% (5)	100% (3)
Glucan 1,3-beta-glucosidase precursor	BGL2	99% (2)	100% (4)
5-methyltetrahydropteroyltriglutamate-- homocysteine methyltransferase	MET6	100% (3)	100% (3)
Protein YGP1 precursor	YGP1	100% (3)	100% (5)
Thioredoxin II	TRX2	99% (2)	100% (3)
Heat shock protein SSA2	HSP72	100% (6)	
Phosphoglycerate mutase 1	PMG1	99% (2)	96% (1)
Protein ECM33 precursor	ECM33	98% (1)	100% (4)
Triosephosphate isomerase	TPI1	90% (1)	88% (1)
Lysophospholipase 2 precursor	PLB2	100% (3)	88% (1)
Alcohol dehydrogenase 4	ADH4	90% (1)	99% (2)
6-phosphogluconate dehydrogenase, decarboxylating 1 -	GND1	99% (2)	88% (1)
12 kDa heat shock protein	HSP12		99% (2)
Glycolipid-anchored surface protein 1 precursor	GAS1	90% (1)	99% (2)
Elongation factor 1-alpha	TEF1	90% (1)	88% (1)
Alcohol dehydrogenase 1	ADH1		99% (2)
40S ribosomal protein S17-A	RPS17A	97% (1)	99% (2)
Probable glycosidase CRH1 precursor	CRH1	99% (2)	88% (1)
Transaldolase	TAL1	90% (1)	99% (2)
Peroxiredoxin TSA1	TSA1	90% (1)	
Seripauperin-5	PAU5	90% (1)	88% (1)
30 kDa heat shock protein	HSP30	90% (1)	88% (1)
Glycolipid-anchored surface protein 5 precursor	GAS5	90% (1)	88% (1)
NADP-specific glutamate dehydrogenase 1	DHE4	90% (1)	
40S ribosomal protein S14-A	RPS14A	90% (1)	88% (1)
60S ribosomal protein L19	RPL19A	90% (1)	88% (1)
Aminopeptidase Y precursor	APE3	90% (1)	
Peroxiredoxin type-2	AHP1	90% (1)	88% (1)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	90% (1)	88% (1)
Protein PRY1 precursor	PRY1		88% (1)
Suppressor protein STM1	STM1	99% (2)	
Protein SIM1 precursor	SIM1	90% (1)	
Vacuolar ATP synthase catalytic subunit A	VMA1	90% (1)	
Aconitate hydratase, mitochondrial precursor	ACO1	90% (1)	88% (1)
60S ribosomal protein L13-A	RPL13A	90% (1)	88% (1)
Ketol-acid reductoisomerase, mitochondrial precursor	ILV5	90% (1)	
40S ribosomal protein S16	RPS16	90% (1)	
40S ribosomal protein S5	RPS5	90% (1)	
Outer mitochondrial membrane protein porin 1	POR1		88% (1)
Ubiquitin	UBI1		99% (2)

40S ribosomal protein S19-A	RPS19A		88% (1)
Heat shock protein SSA1	HSP71		88% (1)
(DL)-glycerol-3-phosphatase 1	GPP1		88% (1)
Probable inosine-5'-monophosphate dehydrogenase IMD1	IMDH1		88% (1)
Aspartic proteinase yapsin-3 precursor	YPS3	90% (1)	
ATP synthase subunit beta, mitochondrial precursor	ATP2	90% (1)	88% (1)
60S ribosomal protein L4-A	RL4A		88% (1)
Vacuolar ATP synthase subunit B	VMA2	90% (1)	
60S acidic ribosomal protein P2-beta	RLA4	90% (1)	
Trehalose synthase complex regulatory subunit TSL1	TSL1	90% (1)	88% (1)
Uncharacterized protein YNL200C	YNL200C		88% (1)
40S ribosomal protein S3	RPS3	90% (1)	
Hypothetical 41.2 kDa protein in FPR1-TOM22 intergenic region	YNN4	90% (1)	
Heat shock protein SSC1, mitochondrial precursor	HSP77		88% (1)
10 kDa heat shock protein, mitochondrial	HSP10	90% (1)	
Protein PIR3 precursor	PIR3	90% (1)	
40S ribosomal protein S27-A	RPS27A	90% (1)	
Hexokinase-1	HXK1	90% (1)	
Homoserine dehydrogenase	HOM6	90% (1)	
Elongation factor 2	EFT2		88% (1)
FK506-binding protein 1	FPR1	90% (1)	88% (1)
60S ribosomal protein L28	RPL28	90% (1)	
Coproporphyrinogen III oxidase	HEM6		88% (1)
RNA polymerase II transcription factor B subunit 4	TFB4	90% (1)	
Protein TOS1 precursor	TOS1	90% (1)	
Actin	ACT1	90% (1)	
26S proteasome regulatory subunit RPN10	RPN10	90% (1)	
Protein MMF1, mitochondrial precursor	MMF1	90% (1)	
Cerevisin precursor	PRB1	90% (1)	
Glucokinase GLK1	GLK1	90% (1)	
Protein HMF1	HMF1	90% (1)	

1 month

		142a	142b
Protein name	Gene Id		
Protoplast secreted protein 1 precursor	PST1	100% (14)	100% (14)
Phosphoglycerate kinase	PGK1	100% (7)	100% (10)
Enolase 1	ENO1	100% (5)	100% (6)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (9)	100% (6)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (4)	100% (5)
Cell wall protein DAN2 precursor	DAN2	99% (2)	
Glucan 1,3-beta-glucosidase precursor	BGL2	99% (2)	99% (2)
Probable family 17 glucosidase SCW4 precursor	SCW4	99% (2)	100% (3)
Fructose-bisphosphate aldolase	FBA1	100% (4)	100% (5)
Lysophospholipase 2 precursor	PLB2	89% (1)	99% (2)
Protein YGP1 precursor	YGP1	99% (2)	99% (2)
Phosphoglycerate mutase 1	PMG1	99% (2)	100% (3)
Peroxiredoxin type-2	AHP1	100% (5)	100% (3)
Seripauperin-5	PAU5	89% (1)	98% (2)

Thioredoxin-2	TRX2		99% (2)
Protein ECM33 precursor	ECM33		100% (4)
Aspartic proteinase yapsin-3 precursor	YPS3	99% (2)	89% (1)
Glycolipid-anchored surface protein 5 precursor	GAS5	100% (4)	100% (4)
Thioredoxin-1	TRX1	89% (1)	89% (1)
Heat shock protein SSA1	HSP71	100% (4)	100% (3)
Pyruvate decarboxylase isozyme 1	PDC1	99% (2)	99% (2)
Enolase 2	ENO2		89% (1)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	99% (2)	99% (2)
Glycolipid-anchored surface protein 1 precursor	GAS1	97% (1)	99% (2)
Heat shock protein 26	HSP26	100% (4)	99% (2)
30 kDa heat shock protein	HSP30	89% (1)	89% (1)
12 kDa heat shock protein	HSP12	100% (4)	
Elongation factor 1-alpha	TEF1		89% (1)
Pyruvate kinase 1	PYK1	100% (3)	99% (2)
Aminopeptidase Y precursor	APE3		89% (1)
Triosephosphate isomerase	TPI1	89% (1)	
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	MET6	89% (1)	99% (2)
Glycogen debranching enzyme	GDB1	89% (1)	89% (1)
FK506-binding protein 1	FKBP	89% (1)	99% (2)
ATP synthase subunit beta, mitochondrial precursor	ATP2		89% (1)
60S ribosomal protein L4-A	RPL4A	98% (1)	
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH3		89% (1)
Homoserine dehydrogenase	HOM6	99% (2)	99% (2)
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	89% (1)	89% (1)
150 kDa heat shock glycoprotein precursor	HS150	89% (1)	89% (1)
Protein disulfide-isomerase precursor	PDI1	89% (1)	
Plasma membrane ATPase 1	PMA1	99% (2)	89% (1)
Superoxide dismutase [Cu-Zn]	SOD1		89% (1)
Saccharopine dehydrogenase [NAD+, L-lysine-forming]	LYS1	89% (1)	89% (1)
Protein HMF1	HMF1	89% (1)	89% (1)
Dihydroxy-acid dehydratase, mitochondrial precursor	ILV3	89% (1)	89% (1)
Alcohol dehydrogenase 1	ADH1		89% (1)
Malate dehydrogenase, mitochondrial precursor	MDH1	89% (1)	89% (1)
Fatty acid synthase subunit alpha	FAS2	89% (1)	89% (1)
Peroxiredoxin TSA1	TSA1	89% (1)	89% (1)
Actin	ACT1	99% (2)	89% (1)
40S ribosomal protein S17-A	RPS17A	89% (1)	89% (1)
Ribonucleoside-diphosphate reductase small chain 1	RIR2	89% (1)	89% (1)
Uncharacterized protein YHR087W	YHR087W	88% (1)	89% (1)
ATP-dependent molecular chaperone HSC82	HSC82	89% (1)	89% (1)
Heat shock protein SSB1	HSP75	89% (1)	89% (1)
40S ribosomal protein S5	RPS5		89% (1)
Ketol-acid reductoisomerase, mitochondrial precursor	ILV5		89% (1)
40S ribosomal protein S22-A	RPS22A	89% (1)	89% (1)
Reticulon-like protein 1	RTN1	89% (1)	

Heat shock protein SSC1, mitochondrial precursor	HSP77	99% (2)	
60S ribosomal protein L15-A	RPL15A	89% (1)	
Vacuolar aminopeptidase 1 precursor	APE1	89% (1)	
Sporulation-specific wall maturation protein precursor	SPS100	89% (1)	
Acetolactate synthase catalytic subunit, mitochondrial precursor	ILV2		89% (1)
Phosphoribosylaminoimidazole-succinocarboxamide synthase	PUR7	89% (1)	89% (1)
60S acidic ribosomal protein P2-beta	RLA4		89% (1)
Bifunctional purine biosynthesis protein ADE17 [Includes: Phosphoribosylaminoimidazolecarboxamide formyltransferase	PUR92	89% (1)	
Glucose-6-phosphate isomerase	PGI1		89% (1)
60S ribosomal protein L11-B	RPL11B		89% (1)
Protein MET17 [Includes: O-acetylhomoserine sulfhydrylase	MET17		89% (1)
Probable chaperone protein HSP31	HSP31		89% (1)
60S ribosomal protein L7-A	RPL7A	89% (1)	

3 months

Protein name	Gene Id	142a	142b
Protoplast secreted protein 1 precursor	PST1	100% (8)	100% (12)
Phosphoglycerate kinase	PGK1	100% (5)	100% (6)
Enolase 1	ENO1	100% (7)	100% (7)
Cell wall protein DAN2 precursor	DAN2	80% (1)	
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (5)	100% (4)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (3)	100% (6)
Fructose-bisphosphate aldolase	FBA1	100% (5)	99% (2)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (3)	100% (3)
Probable family 17 glucosidase SCW4 precursor	SCW4	80% (1)	95% (1)
Lysophospholipase 2 precursor	PLB2		100% (3)
Protein YGP1 precursor	YGP1	80% (1)	99% (2)
Enolase 2	ENO2	80% (1)	89% (1)
Thioredoxin-2	TRX2		99% (2)
Protein ECM33 precursor	ECM33	100% (3)	89% (1)
Elongation factor 1-alpha	EF1A	80% (1)	89% (1)
Phosphoglycerate mutase 1	PMG1	80% (1)	89% (1)
Pyruvate decarboxylase isozyme 1	PDC1	80% (1)	99% (2)
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	80% (1)	99% (2)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	80% (1)	100% (3)
30 kDa heat shock protein	HSP30	80% (1)	89% (1)
Glycolipid-anchored surface protein 5 precursor	GAS5	80% (1)	89% (1)
Aminopeptidase Y precursor	APE3		89% (1)
40S ribosomal protein S3	RPS3	80% (1)	99% (2)
Thioredoxin-1	TRX1		89% (1)
Heat shock protein 26	HSP26		94% (1)
Glycogen debranching enzyme	GDB1	80% (1)	89% (1)
Triosephosphate isomerase	TPI1		89% (1)

Pyruvate kinase 1	PYK1		89% (1)
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH2	99% (2)	89% (1)
Glycolipid-anchored surface protein 1 precursor	GAS1	80% (1)	99% (2)
Alcohol dehydrogenase 4	ADH4		89% (1)
FK506-binding protein 1	FPR1		89% (1)
NADP-specific glutamate dehydrogenase 1	DHE4	80% (1)	89% (1)
Seripauperin-5	PAU5		84% (1)
Magnesium-activated aldehyde dehydrogenase, cytosolic	ALDH6	80% (1)	89% (1)
Aspartic proteinase yapsin-3 precursor	YPS3		89% (1)
12 kDa heat shock protein	HSP12		99% (2)
Repressible acid phosphatase precursor	PPA5	80% (1)	
Plasma membrane ATPase 1	PMA1	80% (1)	
Protein HMF1	HMF1	80% (1)	89% (1)
40S ribosomal protein S20	RPS20		89% (1)
Uncharacterized protein YJL171C precursor	YJR1		89% (1)
Protein MMF1, mitochondrial precursor	MMF1	80% (1)	
ATP synthase subunit beta, mitochondrial precursor	ATP2	80% (1)	89% (1)
Saccharopine dehydrogenase [NAD ⁺ , L-lysine-forming]	LYS1	80% (1)	89% (1)
Actin	ACT1		100% (3)
Trehalose synthase complex regulatory subunit TSL1	TSL1		89% (1)
Vacuolar aminopeptidase 1 precursor	APE1	80% (1)	89% (1)
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	MET6	80% (1)	
60S ribosomal protein L20	RPL20		
Transcriptional modulator WTM1	WTM1		89% (1)
Endo-1,3(4)-beta-glucanase 1 precursor	ENG1		89% (1)
40S ribosomal protein S9-A	RPS9A		89% (1)
60S ribosomal protein L8-A	RPL8A	80% (1)	89% (1)
10 kDa heat shock protein, mitochondrial	CH10	80% (1)	89% (1)
40S ribosomal protein S5 -	RPS5	80% (1)	89% (1)
Glucose-6-phosphate isomerase	PGI1	80% (1)	
40S ribosomal protein S19-A	RPS19A		89% (1)
Uncharacterized protein YNL208W	YNU8		89% (1)
ATP-dependent molecular chaperone HSC82	HSC82		89% (1)
Bifunctional purine biosynthesis protein ADE17 [Includes: Phosphoribosylaminoimidazolecarboxamide formyltransferase	PUR92	80% (1)	
Heat shock protein homolog SSE1	HSP7F	80% (1)	
Alcohol dehydrogenase 1	ADH1		89% (1)
Fatty acid synthase subunit alpha	FAS2	99% (2)	
Mitochondrial intermembrane space cysteine motif-containing protein MIC14	MIC14		89% (1)
Stress protein DDR48	DDR48		89% (1)
Uncharacterized protein YJL066C	YJG6		89% (1)
26S protease regulatory subunit 8 homolog	PRS8		89% (1)
60S ribosomal protein L28	RPL28	80% (1)	
Single-stranded DNA-binding protein RIM1, mitochondrial precursor	RIM1		89% (1)
60S ribosomal protein L19	RPL19		89% (1)

6-phosphofructokinase subunit beta	PFK2		
Adenosine kinase	ADO1		89% (1)
Peroxiredoxin TSA1	TSA1	80% (1)	
Heat shock protein SSA1	HSP71		99% (2)
Malate dehydrogenase, mitochondrial precursor	MDH1		89% (1)
Cytochrome c iso-1	CYC1		89% (1)
Heat shock protein SSC1, mitochondrial precursor	HSP77		89% (1)
40S ribosomal protein S17-A	RPS17A		89% (1)
Dolichyl-P-Man:Man(5)GlcNAc(2)-PP-dolichyl mannosyltransferase	ALG3	80% (1)	
Probable aconitate hydratase 2	ACO2	80% (1)	
60S ribosomal protein L15-A	RPL15A	80% (1)	
Probable chaperone protein HSP31	HSP31	80% (1)	

6 months

Protein name	Gene Id	142a	142b
Protoplast secreted protein 1 precursor	PST1	100% (15)	100% (15)
Cell wall protein DAN2 precursor	DAN2	100% (5)	99% (2)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (10)	100% (7)
Phosphoglycerate kinase	PGK1	100% (4)	100% (5)
Lysophospholipase 2 precursor	PLB2	100% (7)	100% (3)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (7)	100% (6)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (3)	100% (4)
Protein YGP1 precursor	YGP1	100% (5)	100% (3)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (4)	100% (3)
Protein ECM33 precursor	ECM33	100% (6)	
Glycolipid-anchored surface protein 5 precursor	GAS5	100% (6)	100% (5)
Probable glycosidase CRH1 precursor	CRH1	100% (5)	99% (2)
Repressible acid phosphatase precursor	PPA5	100% (7)	
Fructose-bisphosphate aldolase	FBA1	99% (2)	99% (2)
12 kDa heat shock protein	HSP12	99% (2)	100% (3)
Aminopeptidase Y precursor	APE3	99% (2)	
Lysophospholipase 1 precursor	PLB1	99% (2)	
Elongation factor 1-alpha	TEF1	99% (2)	99% (2)
Glycolipid-anchored surface protein 1 precursor	GAS1	100% (3)	99% (2)
Covalently-linked cell wall protein 14 precursor	CCW14	100% (5)	100% (3)
Phosphoglycerate mutase 1	PMG1		99% (2)
Covalently-linked cell wall protein 11 precursor	CIS3	99% (2)	
Thioredoxin-1	TRX1	99% (2)	
Uncharacterized protein YJL171C precursor	YJR1	99% (2)	
ATP synthase subunit alpha, mitochondrial precursor	ATP1		99% (2)
Protein TOS1 precursor	TOS1	100% (3)	
Pyruvate kinase 1	PYK1		99% (2)
Mitochondrial intermembrane space cysteine motif-containing protein MIC14	MIC14		99% (2)
Protein MMF1, mitochondrial precursor	MMF1		99% (2)
Actin-binding protein	ABP1	98% (2)	
Heat shock protein SSA2	HSP72		99% (2)
Metallothionein precursor	CUP1-1	99% (2)	
Cell wall protein CWP1 precursor	CWP1	99% (2)	

9 months

Protein name	Gene Id	142a	142b
Seripauperin-17 precursor	PAU17	100% (5)	100% (3)
Cell wall mannoprotein PST1 precursor	PST1	100% (16)	100% (14)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (7)	100% (7)
Phosphoglycerate kinase	PGK1	100% (5)	100% (4)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (12)	100% (9)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (9)	100% (10)
Protein YGP1 precursor	YGP1	100% (6)	100% (10)
Phosphoglycerate mutase 1	PMG1	100% (8)	100% (6)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (5)	100% (4)
Cell wall protein DAN3 precursor	DAN3	98% (2)	96% (1)
Cell wall protein ECM33 precursor	ECM33	100% (5)	100% (3)
Glycolipid-anchored surface protein 5 precursor	GAS5	100% (6)	100% (4)
Pyruvate kinase 1	PYK1	100% (5)	
Seripauperin-5	PAU5	80% (1)	99% (2)
Glycolipid-anchored surface protein 1 precursor	GAS1	100% (4)	99% (2)
Uncharacterized protein YJL171C precursor	YJR1	98% (2)	99% (2)
Probable glycosidase CRH1 precursor	CRH1	95% (1)	100% (3)
Enolase 1	ENO1		100% (4)
Elongation factor 1-alpha	TEF1	99% (2)	100% (3)
12 kDa heat shock protein	HSP12	80% (2)	100% (5)
Fructose-bisphosphate aldolase	FBA1	100% (3)	93% (1)
Lysophospholipase 2 precursor	PLB2	80% (1)	100% (3)
Septation protein SUN4 precursor	SUN4	80% (1)	99% (2)
Covalently-linked cell wall protein 14 precursor	CCW14	100% (3)	100% (3)
Protein transport protein SEC31	SEC31	80% (1)	
Protein PIR3 precursor	PIR3	97% (2)	94% (1)
Heat shock protein SSA1	HSP71	93% (1)	96% (1)
Mitochondrial intermembrane space cysteine motif-containing protein MIC14	MIC14	80% (1)	99% (2)
6-phosphofructokinase subunit beta	PFK2	80% (1)	
Probable family 17 glucosidase SCW10 precursor	SCW10	99% (2)	
Protein TOS1 precursor	TOS1	99% (2)	
Glycogen debranching enzyme	GDB1	80% (1)	89% (1)
Heat shock protein 26	HSP26	80% (1)	100% (2)
Thioredoxin-2	TRX2	99% (2)	
Triosephosphate isomerase	TPI1	75% (1)	
Cell wall protein CWP1 precursor	CWP1	100% (3)	
Glycolipid-anchored surface protein 3 precursor	GAS3	72% (1)	
Plasma membrane ATPase 1	PMA1	99% (2)	

¹ The percentage adjacent to the Gene Id is the Bayesian probability that the protein is correctly identified; the number of peptides attributed to the protein is in parentheses.

Appendix 3. Proteins identified in wines made by strain 170¹**0 Days**

		170a	170b
Protoplast secreted protein 1 precursor	PST1	99% (2)	99% (2)
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH2	99% (2)	99% (2)
Protein ECM33 precursor	ECM33		90% (1)
Pyruvate decarboxylase	PDC1		90% (1)

isozyme 1			
Phosphoglycerate kinase	PGK1		90% (1)
7 days			
		170a	170b
Protoplast secreted protein 1 precursor	PST1	100% (12)	100% (13)
Enolase 1	ENO1	100% (6)	100% (5)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (10)	100% (8)
Phosphoglycerate kinase	PGK1	100% (7)	100% (5)
Cell wall protein DAN2 precursor	DAN2	90% (1)	
Fructose-bisphosphate aldolase	FBA1	100% (5)	100% (4)
Heat shock protein SSA2	HSP72	100% (3)	100% (5)
Enolase 2	ENO2	90% (1)	90% (1)
Phosphoglycerate mutase 1	PMG1	100% (4)	100% (4)
Pyruvate decarboxylase isozyme 1	PDC1	100% (4)	100% (3)
Glyceraldehyde-3-phosphate dehydrogenase 3	TDH3	100% (4)	100% (3)
Heat shock protein 26	HSP26	100% (3)	90% (1)
Pyruvate kinase 1	PYK1	100% (3)	99% (2)
Probable family 17 glucosidase SCW4 precursor	SCW4	90% (1)	99% (2)
Triosephosphate isomerase	TPI1	99% (2)	90% (1)
Thioredoxin I	TRX1		90% (1)
Thioredoxin II	TRX2		90% (1)
12 kDa heat shock protein	HSP12	90% (1)	90% (1)
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	90% (1)	99% (2)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	100% (4)	90% (1)
Glucan 1,3-beta-glucosidase precursor	BGL2	90% (1)	90% (1)
Protein YGP1 precursor	YGP1	99% (2)	99% (2)
Protein ECM33 precursor	ECM33	99% (2)	90% (1)
Peroxiredoxin TSA1	TSA1	90% (1)	99% (2)
Actin	ACT1	99% (2)	99% (2)
Alcohol dehydrogenase 1	ADH1	99% (2)	90% (1)
Outer mitochondrial membrane protein porin 1	POR1	90% (1)	90% (1)
Ketol-acid reductoisomerase, mitochondrial precursor	ILV5		90% (1)
ATP synthase subunit beta, mitochondrial precursor	ATP2		90% (1)
Elongation factor 1-alpha	TEF1	90% (1)	90% (1)
Alcohol dehydrogenase 4	ADH4		90% (1)
Malate dehydrogenase, mitochondrial precursor	MDH1	90% (1)	90% (1)
5-methyltetrahydropteroyltriglutamate-- homocysteine methyltransferase	MET6	90% (1)	90% (1)
Heat shock protein SSA1	HSP71	90% (1)	90% (1)
60S ribosomal protein L8-A	RPL8A	90% (1)	90% (1)
NADP-specific glutamate dehydrogenase 1	DHE4	90% (1)	99% (2)
30 kDa heat shock protein	HSP30	90% (1)	90% (1)
Plasma membrane ATPase 1	PMA1	90% (1)	
Saccharopine dehydrogenase [NAD+, L-lysine- forming]	LYS1_	90% (1)	90% (1)
60S ribosomal protein L19	RPL19A	90% (1)	90% (1)
Transaldolase	TAL1	99% (2)	
Histone H2A.1	HTA1	90% (1)	
40S ribosomal protein S5	RPS5	97% (1)	90% (1)

Heat shock protein SSC1, mitochondrial precursor	HSP77	90% (1)	
Protein MMF1, mitochondrial precursor	MMF1	90% (1)	90% (1)
60S ribosomal protein L13-A	RPL13A		90% (1)
60S ribosomal protein L4-A	RPL4A	99% (2)	
Glycolipid-anchored surface protein 5 precursor	GAS5	90% (1)	
40S ribosomal protein S6	RPS6A	90% (1)	
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH2	90% (1)	
Dihydroxy-acid dehydratase, mitochondrial precursor	ILV3		90% (1)
Glucose-6-phosphate isomerase	PGI1	99% (2)	
40S ribosomal protein S14-A	RPS14A	90% (1)	
Peptidyl-prolyl cis-trans isomerase	CPR1	99% (2)	
Heat shock protein SSB1	HSP75	90% (1)	
Heat shock protein homolog SSE1	SSE1	90% (1)	
60S ribosomal protein L26-B	RPL26B	90% (1)	90% (1)
Nascent polypeptide-associated complex subunit beta-1	NACB1	99% (2)	

14 days

		170a	170b
Protoplast secreted protein 1 precursor	PST1	100% (8)	100% (6)
Enolase 1	ENO1	100% (8)	100% (5)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (8)	100% (3)
Phosphoglycerate kinase	PGK1	100% (7)	100% (4)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (2)	90% (1)
Cell wall protein DAN2 precursor	DAN2	99% (2)	99% (2)
Fructose-bisphosphate aldolase	FBA1	100% (3)	100% (3)
Enolase 2	ENO2	99% (2)	
Glyceraldehyde-3-phosphate dehydrogenase 3	TDH3	100% (4)	100% (4)
Pyruvate decarboxylase isozyme 1	PDC1	99% (2)	100% (4)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (3)	90% (1)
Heat shock protein 26	HSP26	99% (2)	
Thioredoxin I	TRX1	100% (3)	90% (1)
Pyruvate kinase 1	PYK1	99% (2)	90% (1)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (3)	90% (1)
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	MET6	99% (2)	90% (1)
Protein YGP1 precursor	YGP1	90% (1)	
Thioredoxin II	TRX2	100% (3)	100% (4)
Phosphoglycerate mutase 1	PMG1	99% (2)	100% (4)
Protein ECM33 precursor	ECM33	98% (2)	
Triosephosphate isomerase	TPI1	100% (3)	99% (2)
Lysophospholipase 2 precursor	PLB2	90% (1)	
Alcohol dehydrogenase 4	ADH4	99% (2)	90% (1)
6-phosphogluconate dehydrogenase, decarboxylating 1 -	GND1	90% (1)	90% (1)
12 kDa heat shock protein	HSP12	99% (2)	100% (3)
Elongation factor 1-alpha	TEF1	99% (2)	90% (1)
Alcohol dehydrogenase 1	ADH1	90% (1)	
40S ribosomal protein S17-A	RPS17A	99% (2)	
Transaldolase	TAL1	90% (1)	90% (1)
Peroxiredoxin TSA1	TSA1	90% (1)	
Seripauperin-5	PAU5	90% (1)	

30 kDa heat shock protein	HSP30	90% (1)	
Glycolipid-anchored surface protein 5 precursor	GAS5	90% (1)	
NADP-specific glutamate dehydrogenase 1	DHE4	100% (3)	
Uncharacterized protein YGL258W	YGL258W	90% (1)	
Histone H2A.1	HTA1	90% (1)	
40S ribosomal protein S14-A	RPS14A	90% (1)	90% (1)
60S ribosomal protein L19	RPL19A	90% (1)	
ATP synthase subunit alpha, mitochondrial precursor	ATP1	90% (1)	
Protein PRY1 precursor	PRY1	90% (1)	90% (1)
Vacuolar ATP synthase catalytic subunit A	VMA1	90% (1)	
Aconitate hydratase, mitochondrial precursor	ACO1	90% (1)	
Ketol-acid reductoisomerase, mitochondrial precursor	ILV5	90% (1)	
60S ribosomal protein L8-B	RPL8B	90% (1)	
Cell wall protein DAN3 precursor	DAN3	90% (1)	90% (1)
40S ribosomal protein S19-A	RPS19A	90% (1)	90% (1)
Aspartate-semialdehyde dehydrogenase	HOM2		90% (1)
Plasma membrane ATPase 1	PMA1	90% (1)	
Protein MET17 [Includes: O-acetylhomoserine sulfhydrylase	MET17		90% (1)
Heat shock protein SSA1	HSP71	90% (1)	
Vacuolar ATP synthase subunit B	VMA2	90% (1)	
Heat shock protein SSB1	HSP75	90% (1)	90% (1)
Uncharacterized protein YNL200C	YNL200C	90% (1)	
Elongation factor 2	EFT2	90% (1)	
Protein UTH1	UTH1		90% (1)
Uncharacterized protein YOR387C precursor	YOR387C		90% (1)
Actin	ACT1		90% (1)
Rho-GTPase-activating protein LRG1	LRG1		90% (1)
60S ribosomal protein L5	RL5	90% (1)	

1 month

		170a	170b
Protoplast secreted protein 1 precursor	PST1	100% (11)	100% (11)
Phosphoglycerate kinase	PGK1	100% (7)	100% (7)
Enolase 1	ENO1	100% (5)	100% (8)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (6)	100% (7)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (6)	100% (6)
Cell wall protein DAN2 precursor	DAN2	100% (7)	100% (3)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (4)	99% (2)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (3)	100% (3)
Fructose-bisphosphate aldolase	FBA1		100% (3)
Lysophospholipase 2 precursor	PLB2	99% (2)	99% (2)
Protein YGP1 precursor	YGP1	100% (3)	99% (2)
Phosphoglycerate mutase 1	PMG1	100% (3)	100% (3)
Peroxiredoxin type-2	AHP1	99% (2)	100% (3)
Seripauperin-5	PAU5	99% (2)	99% (2)
Thioredoxin-2	TRX2	100% (3)	100% (3)
Protein ECM33 precursor	ECM33	90% (1)	88% (1)
Aspartic proteinase yapsin-3 precursor	YPS3	99% (2)	88% (1)
Glycolipid-anchored surface protein 5 precursor	GAS5	100% (2)	88% (1)
Thioredoxin-1	TRX1	90% (1)	99% (2)
Heat shock protein SSA1	HSP71		100% (3)

Pyruvate decarboxylase isozyme 1	PDC1		100% (5)
Enolase 2	ENO2	90% (1)	88% (1)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	90% (1)	100% (4)
Glycolipid-anchored surface protein 1 precursor	GAS1	96% (1)	
Heat shock protein 26	HSP26		88% (1)
30 kDa heat shock protein	HSP30	90% (1)	88% (1)
12 kDa heat shock protein	HSP12	90% (1)	
Elongation factor 1-alpha	TEF1	90% (1)	88% (1)
Pyruvate kinase 1	PYK1	90% (1)	88% (1)
Aminopeptidase Y precursor	APE3	90% (1)	88% (1)
Triosephosphate isomerase	TPI1	90% (1)	100% (3)
Transaldolase	TAL1		88% (1)
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	MET6	90% (1)	99% (2)
Lysophospholipase 1 precursor	PLB1		88% (1)
Glycogen debranching enzyme	GDB1	90% (1)	88% (1)
FK506-binding protein 1	FKBP	90% (1)	88% (1)
ATP synthase subunit beta, mitochondrial precursor	ATP2	90% (1)	88% (1)
60S ribosomal protein L4-A	RPL4A	90% (1)	
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH3	90% (1)	88% (1)
Homoserine dehydrogenase	HOM6	99% (2)	88% (1)
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	90% (1)	88% (1)
NADP-specific glutamate dehydrogenase 1	DHE4	90% (1)	88% (1)
150 kDa heat shock glycoprotein precursor	HS150	90% (1)	
Plasma membrane ATPase 1	PMA1	99% (2)	88% (1)
Superoxide dismutase [Cu-Zn]	SOD1	90% (1)	
Saccharopine dehydrogenase [NAD+, L-lysine-forming]	LYS1	90% (1)	88% (1)
Histone H2A.1	HTA1	90% (1)	88% (1)
1 month			
		170a	170b
Protein HMF1	HMF1	90% (1)	88% (1)
Dihydroxy-acid dehydratase, mitochondrial precursor	ILV3		88% (1)
Alcohol dehydrogenase 4	ADH4		88% (1)
Alcohol dehydrogenase 1	ADH1	99% (2)	99% (2)
Probable glycosidase CRH1 precursor	CRH1	88% (1)	
40S ribosomal protein S20	RPS20		88% (1)
Covalently-linked cell wall protein 11 precursor	CIS3	90% (1)	
Malate dehydrogenase, mitochondrial precursor	MDH1	90% (1)	
Fatty acid synthase subunit alpha	FAS2		88% (1)
Peroxiredoxin TSA1	TSA1	90% (1)	99% (2)
40S ribosomal protein S3	RPS3	90% (1)	
Vacuolar ATP synthase subunit B	VAT2	90% (1)	88% (1)
Protein MMF1, mitochondrial precursor	MMF1		97% (1)
40S ribosomal protein S17-A	RPS17A	90% (1)	88% (1)
Ribonucleoside-diphosphate reductase small chain 1	RIR2	90% (1)	88% (1)
Uncharacterized protein YHR087W	YHR087W	90% (1)	88% (1)
ATP-dependent molecular chaperone HSC82	HSC82	90% (1)	88% (1)
Endo-1,3(4)-beta-glucanase 1 precursor	ENG1	90% (1)	

Eukaryotic translation initiation factor 5A-1	HYP1	90% (1)	88% (1)
Heat shock protein SSB1	HSP75		88% (1)
Transcriptional modulator WTM1	WTM1	90% (1)	
Isocitrate dehydrogenase [NADP], mitochondrial precursor	IDP1	99% (2)	
Ketol-acid reductoisomerase, mitochondrial precursor	ILV5		88% (1)
40S ribosomal protein S22-A	RPS22A		88% (1)
Hexokinase-1	HXK1		88% (1)
Uncharacterized protein YJL066C	YJL066C	90% (1)	
10 kDa heat shock protein, mitochondrial	HSP10		88% (1)
Sporulation-specific wall maturation protein precursor	SPS100	90% (1)	
60S acidic ribosomal protein P2-beta	RLA4		88% (1)
Homoisocitrate dehydrogenase, mitochondrial precursor	LYS12		88% (1)
Phosphoglucomutase-2	PGM2		99% (2)
Origin recognition complex subunit 2	ORC2		88% (1)

3 months

		170a	170b
Protoplast secreted protein 1 precursor	PST1	100% (12)	100% (8)
Phosphoglycerate kinase	PGK1	100% (6)	100% (6)
Enolase 1	ENO1	100% (9)	100% (7)
Cell wall protein DAN2 precursor	DAN2	100% (3)	90% (1)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (5)	100% (8)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (3)	90% (1)
Fructose-bisphosphate aldolase	FBA1	100% (3)	100% (5)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (3)	
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (3)	90% (1)
Lysophospholipase 2 precursor	PLB2	100% (3)	
Protein YGP1 precursor	YGP1	100% (3)	90% (1)
Enolase 2	ENO2	90% (1)	99% (2)
Thioredoxin-2	TRX2	100% (2)	99% (2)
Protein ECM33 precursor	ECM33	99% (2)	90% (1)
Elongation factor 1-alpha	EF1A	99% (2)	90% (1)
Phosphoglycerate mutase 1	PMG1	99% (2)	100% (3)
Pyruvate decarboxylase isozyme 1	PDC1	100% (4)	90% (1)
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	99% (2)	99% (2)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	99% (2)	90% (1)
30 kDa heat shock protein	HSP30	90% (1)	90% (1)
Glycolipid-anchored surface protein 5 precursor	GAS5	90% (1)	
Aminopeptidase Y precursor	APE3	99% (2)	
40S ribosomal protein S3	RPS3	90% (1)	90% (1)
Thioredoxin-1	TRX1	90% (1)	
Heat shock protein 26	HSP26	90% (1)	
Glycogen debranching enzyme	GDB1	90% (1)	90% (1)
Triosephosphate isomerase	TPI1	99% (2)	
Pyruvate kinase 1	PYK1	90% (1)	
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH2	99% (2)	97% (1)
Alcohol dehydrogenase 4	ADH4	90% (1)	90% (1)
FK506-binding protein 1	FPR1	90% (1)	

NADP-specific glutamate dehydrogenase 1	DHE4	90% (1)	100% (3)
Seripauperin-5	PAU5	90% (1)	
Magnesium-activated aldehyde dehydrogenase, cytosolic	ALDH6		90% (1)
Aspartic proteinase yapsin-3 precursor	YPS3	90% (1)	
12 kDa heat shock protein	HSP12	90% (1)	90% (1)
Plasma membrane ATPase 1	PMA1	90% (1)	
Protein HMF1	HMF1		99% (2)
40S ribosomal protein S20	RPS20	90% (1)	
Uncharacterized protein YJL171C precursor	YJR1	90% (1)	
Protein MMF1, mitochondrial precursor	MMF1		90% (1)
ATP synthase subunit beta, mitochondrial precursor	ATP2	90% (1)	90% (1)
Saccharopine dehydrogenase [NAD ⁺ , L-lysine-forming]	LYS1		90% (1)
Actin	ACT1		90% (1)
Trehalose synthase complex regulatory subunit TSL1	TSL1	90% (1)	90% (1)
Vacuolar aminopeptidase 1 precursor	APE1	90% (1)	90% (1)
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	MET6	90% (1)	90% (1)
60S ribosomal protein L20	RPL20	90% (1)	
Transcriptional modulator WTM1	WTM1	90% (1)	
Endo-1,3(4)-beta-glucanase 1 precursor	ENG1	90% (1)	
40S ribosomal protein S9-A	RPS9A	90% (1)	
40S ribosomal protein S5 -	RPS5	90% (1)	
Phosphatidylinositol 4-kinase LSB6	LSB6	90% (1)	
40S ribosomal protein S19-A	RPS19A		99% (2)
Uncharacterized protein YNL208W	YNU8	90% (1)	
ATP-dependent molecular chaperone HSC82	HSC82	90% (1)	
Bifunctional purine biosynthesis protein ADE17 [Includes:			
Phosphoribosylaminoimidazolecarboxamide formyltransferase	PUR92		90% (1)
Heat shock protein homolog SSE1	HSP7F		90% (1)
Alcohol dehydrogenase 1	ADH1	90% (1)	
Fatty acid synthase subunit alpha	FAS2		90% (1)
60S ribosomal protein L28	RPL28		90% (1)
Elongation factor 2	EFT2		90% (1)
Vacuolar proton pump subunit G	VMA10		90% (1)
Isocitrate dehydrogenase [NADP], mitochondrial precursor	IDP1	99% (2)	
Peroxiredoxin type-2 -	AHP1	99% (2)	
Heat shock protein SSB1	HSP75	90% (1)	
Protein URA1 [Includes: Glutamine-dependent carbamoyl-phosphate synthase	PYR1	90% (1)	
Acetolactate synthase catalytic subunit, mitochondrial precursor	ILV2	90% (1)	
60S ribosomal protein L26-A	RPL26A		90% (1)
6 months			
Protoplast secreted protein 1 precursor	PST1	170a 100% (15)	170b 100% (16)
Cell wall protein DAN2	DAN2	100% (3)	100% (5)

precursor			
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (7)	100% (7)
Phosphoglycerate kinase	PGK1	100% (5)	99% (2)
Lysophospholipase 2 precursor	PLB2	100% (4)	100% (6)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (5)	100% (5)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (3)	100% (5)
Protein YGP1 precursor	YGP1	100% (4)	100% (3)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (3)	100% (4)
Protein ECM33 precursor	ECM33	99% (2)	99% (2)
Glycolipid-anchored surface protein 5 precursor	GAS5	98% (2)	99% (2)
Probable glycosidase CRH1 precursor	CRH1		100% (3)
Repressible acid phosphatase precursor	PPA5		99% (2)
Fructose-bisphosphate aldolase	FBA1	100% (3)	100% (3)
12 kDa heat shock protein	HSP12		99% (2)
Aminopeptidase Y precursor	APE3		99% (2)
Lysophospholipase 1 precursor	PLB1		100% (3)
Elongation factor 1-alpha	TEF1		99% (2)
Seripauperin-5	PAU5		99% (2)
Enolase 2	ENO2	100% (3)	99% (2)
Protein SIM1 precursor	SIM1		99% (2)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	99% (2)	99% (2)
Pyruvate kinase 1	PYK1	99% (2)	99% (2)
Peptidyl-prolyl cis-trans isomerase	CPR1	99% (2)	
Heat shock protein SSA2	HSP72	99% (2)	99% (2)

9 months

		170a	170b
Seripauperin-17 precursor	PAU17	100% (4)	100% (5)
Cell wall mannoprotein PST1 precursor	PST1	100% (13)	100% (12)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (8)	100% (7)
Phosphoglycerate kinase	PGK1	100% (4)	100% (5)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (10)	100% (8)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (9)	100% (7)
Protein YGP1 precursor	YGP1	100% (5)	100% (6)
Phosphoglycerate mutase 1	PMG1	100% (5)	100% (9)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (6)	100% (4)
Cell wall protein DAN3 precursor	DAN3	99% (2)	100% (3)
Cell wall protein ECM33 precursor	ECM33	69% (1)	100% (3)
Glycolipid-anchored surface protein 5 precursor	GAS5	99% (2)	99% (2)
Pyruvate kinase 1	PYK1	100% (6)	100% (5)
Seripauperin-5	PAU5	69% (1)	100% (3)
Glycolipid-anchored surface protein 1 precursor	GAS1		99% (2)
Uncharacterized protein YJL171C precursor	YJR1	96% (1)	93% (1)
Probable glycosidase CRH1 precursor	CRH1	69% (1)	
Enolase 1	ENO1	99% (3)	100% (3)
Elongation factor 1-alpha	TEF1	69% (1)	94% (1)
12 kDa heat shock protein	HSP12	69% (2)	62% (1)
Fructose-bisphosphate aldolase	FBA1		100% (3)
Lysophospholipase 2 precursor	PLB2	69% (1)	100% (2)
Septation protein SUN4 precursor	SUN4	69% (1)	
Protein transport protein SEC31	SEC31	69% (1)	93% (1)
Protein PIR3 precursor	PIR3	69% (1)	

Heat shock protein SSA1	HSP71	99% (2)	97% (2)
Mitochondrial intermembrane space cysteine motif-containing protein MIC14	MIC14	69% (1)	62% (1)
Pyruvate decarboxylase isozyme 1	PDC1	100% (3)	
Protein VEL1 precursor	VEL1	95% (1)	
Probable family 17 glucosidase SCW10 precursor	SCW10	69% (1)	
Thioredoxin-2	TRX2	99% (2)	
Alcohol dehydrogenase 4	ADH4	99% (2)	99% (2)
Putative uncharacterized protein YIL169C precursor	YIQ9	97% (2)	
Protein NCA3, mitochondrial precursor	NCA3	99% (2)	

¹ The percentage adjacent to the Gene Id is the Bayesian probability that the protein is correctly identified; the number of peptides attributed to the protein is in parentheses.

Appendix 4. Proteins identified in wines made by strain 116¹

0 Days

		116a	116b
Protoplast secreted protein 1 precursor	PST1	99% (2)	100% (3)
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH2	99% (2)	99% (2)
Protein ECM33 precursor	ECM33	90% (1)	81% (1)
Pyruvate decarboxylase isozyme 1	PDC1	90% (1)	99% (2)
Phosphoglycerate kinase	PGK1	90% (1)	
Fructose-bisphosphate aldolase	FBA1		81% (1)

7 days

		116a	116b
Protoplast secreted protein 1 precursor	PST1	100% (12)	100% (12)
Enolase 1	ENO1	100% (6)	99% (2)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (7)	100% (6)
Phosphoglycerate kinase	PGK1	100% (6)	100% (6)
Cell wall protein DAN2 precursor	DAN2	99% (2)	99% (2)
Fructose-bisphosphate aldolase	FBA1	100% (4)	99% (2)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (3)	100% (4)
Heat shock protein SSA2	HSP72	100% (8)	100% (6)
Enolase 2	ENO2	90% (1)	90% (1)
Phosphoglycerate mutase 1	PMG1	95% (1)	100% (4)
Pyruvate decarboxylase isozyme 1	PDC1	100% (3)	90% (1)
Glyceraldehyde-3-phosphate dehydrogenase 3	TDH3	100% (3)	100% (3)
Heat shock protein 26	HSP26	90% (1)	99% (2)
Pyruvate kinase 1	PYK1	100% (3)	99% (2)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (3)	90% (1)
Triosephosphate isomerase	TPI1	99% (2)	99% (2)
Thioredoxin I	TRX1	90% (1)	90% (1)
Thioredoxin II	TRX2	100% (3)	90% (1)
12 kDa heat shock protein	HSP12	99% (2)	99% (2)
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	90% (1)	90% (1)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	100% (3)	90% (1)
Glucan 1,3-beta-glucosidase precursor	BGL2	90% (1)	90% (1)
Protein YGP1 precursor	YGP1	100% (3)	90% (1)
Protein ECM33 precursor	ECM33	98% (2)	

Peroxioredoxin TSA1	TSA1	99% (2)	99% (2)
Actin	ACT1	90% (1)	90% (1)
Alcohol dehydrogenase 1	ADH1	94% (1)	97% (1)
Outer mitochondrial membrane protein porin 1	POR1	90% (1)	90% (1)
Ketol-acid reductoisomerase, mitochondrial precursor	ILV5	99% (2)	99% (2)
ATP synthase subunit beta, mitochondrial precursor	ATP2	90% (1)	
Elongation factor 1-alpha	TEF1	90% (1)	90% (1)
Alcohol dehydrogenase 4	ADH4	90% (1)	90% (1)
Malate dehydrogenase, mitochondrial precursor	MDH1	90% (1)	90% (1)
Peroxioredoxin type-2	AHP1	99% (2)	90% (1)
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	MET6	90% (1)	90% (1)
Heat shock protein SSA1	HSP71	90% (1)	90% (1)
60S ribosomal protein L8-A	RPL8A		90% (1)
NADP-specific glutamate dehydrogenase 1	DHE4	90% (1)	99% (2)
30 kDa heat shock protein	HSP30	90% (1)	90% (1)
Seripauperin-5	PAU5	90% (1)	90% (1)
Amino-peptidase Y precursor	APE3	90% (1)	90% (1)
Plasma membrane ATPase 1	PMA1	90% (1)	
Saccharopine dehydrogenase [NAD+, L-lysine-forming]	LYS1_	90% (1)	90% (1)
60S ribosomal protein L19	RPL19A	90% (1)	90% (1)
Protein HMF1	HMF1	90% (1)	90% (1)
Histone H2A.1	HTA1	90% (1)	90% (1)
40S ribosomal protein S5	RPS5		90% (1)
Heat shock protein SSC1, mitochondrial precursor	HSP77	90% (1)	90% (1)
Protein MMF1, mitochondrial precursor	MMF1	90% (1)	90% (1)
60S ribosomal protein L13-A	RPL13A	90% (1)	90% (1)
60S ribosomal protein L4-A	RPL4A	90% (1)	
Hexokinase-1	HXK1		90% (1)
Vacuolar ATP synthase subunit B	VMA2		90% (1)
40S ribosomal protein S6	RPS6A	90% (1)	
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH2		90% (1)
Glucose-6-phosphate isomerase	PGI1	90% (1)	
Glucokinase GLK1	GLK1	99% (2)	90% (1)
40S ribosomal protein S14-A	RPS14A	90% (1)	
Reticulon-like protein 1	RTN1		90% (1)
Peptidyl-prolyl cis-trans isomerase	CPR1		90% (1)
Uncharacterized protein YNL208W	YNU8		99% (2)
Heat shock protein SSB1	HSP75		90% (1)
Vacuolar aminopeptidase 1 precursor	AMPL	90% (1)	90% (1)
Heat shock protein homolog SSE1	SSE1	90% (1)	90% (1)
Adenosine kinase	ADO1	90% (1)	
Pyruvate decarboxylase isozyme 2	PDC5	90% (1)	
Magnesium-activated aldehyde dehydrogenase, cytosolic	ALDH6		90% (1)
Vacuolar ATP synthase catalytic subunit A	VMA1	93% (1)	
60S ribosomal protein L1	RPL1A	90% (1)	
Protein PRY3	PRY3	90% (1)	
Invertase 2 precursor	SUC2		90% (1)
60S ribosomal protein L25	RPL25		90% (1)

Superoxide dismutase [Cu-Zn]	SOD1	90% (1)	
Probable chaperone HSP31	HSP31	88% (1)	
Suppressor protein STM1	STM1		86% (1)

14 days

		116a	116b
Protoplast secreted protein 1 precursor	PST1	100% (11)	100% (10)
Enolase 1	ENO1	100% (5)	100% (5)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (6)	100% (6)
Phosphoglycerate kinase	PGK1	100% (8)	100% (8)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (6)	100% (5)
Cell wall protein DAN2 precursor	DAN2	100% (3)	99% (2)
Fructose-bisphosphate aldolase	FBA1	100% (3)	100% (4)
Enolase 2	ENO2	100% (3)	99% (2)
Glyceraldehyde-3-phosphate dehydrogenase 3	TDH3	100% (3)	100% (3)
Pyruvate decarboxylase isozyme 1	PDC1	90% (1)	100% (3)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (4)	100% (3)
Heat shock protein 26	HSP26	90% (1)	99% (2)
Thioredoxin I	TRX1	100% (3)	100% (4)
Pyruvate kinase 1	PYK1	100% (4)	100% (3)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (3)	100% (3)
5-methyltetrahydropteroyltriglutamate-- homocysteine methyltransferase	MET6	100% (3)	99% (2)
Protein YGP1 precursor	YGP1	90% (1)	
Thioredoxin II	TRX2	94% (1)	99% (2)
Heat shock protein SSA2	HSP72	100% (3)	
Phosphoglycerate mutase 1	PMG1	95% (1)	100% (2)
Protein ECM33 precursor	ECM33	99% (2)	99% (2)
Triosephosphate isomerase	TPI1	99% (2)	99% (2)
Lysophospholipase 2 precursor	PLB2	99% (2)	100% (3)
Alcohol dehydrogenase 4	ADH4	99% (2)	99% (2)
6-phosphogluconate dehydrogenase, decarboxylating 1 -	GND1		99% (2)
12 kDa heat shock protein	HSP12	90% (1)	90% (1)
Glycolipid-anchored surface protein 1 precursor	GAS1	90% (1)	
Elongation factor 1-alpha	TEF1	90% (1)	
Alcohol dehydrogenase 1	ADH1	90% (1)	99% (2)
40S ribosomal protein S17-A	RPS17A		90% (1)
Probable glycosidase CRH1 precursor	CRH1	90% (1)	90% (1)
Transaldolase	TAL1	90% (1)	
Peroxisome protein TSA1	TSA1		90% (1)
Seripauperin-5	PAU5	90% (1)	90% (1)
30 kDa heat shock protein	HSP30	90% (1)	90% (1)
Glycolipid-anchored surface protein 5 precursor	GAS5		90% (1)
NADP-specific glutamate dehydrogenase 1	DHE4	99% (2)	90% (1)
Uncharacterized protein YGL258W	YGL258W	99% (2)	90% (1)
Histone H2A.1	HTA1		90% (1)
60S ribosomal protein L19	RPL19A	90% (1)	
Aminopeptidase Y precursor	APE3	99% (2)	90% (1)
ATP synthase subunit alpha, mitochondrial precursor	ATP1		90% (1)
Suppressor protein STM1	STM1	90% (1)	90% (1)

Protein SIM1 precursor	SIM1		90% (1)
Vacuolar ATP synthase catalytic subunit A	VMA1		90% (1)
Aconitate hydratase, mitochondrial precursor	ACO1	90% (1)	90% (1)
60S ribosomal protein L13-A	RPL13A	90% (1)	
14 days		116a	116b
Ketol-acid reductoisomerase, mitochondrial precursor	ILV5		90% (1)
40S ribosomal protein S16	RPS16	90% (1)	
Ubiquitin	UBI1	90% (1)	97% (1)
Cell wall protein DAN3 precursor	DAN3	90% (1)	
Aspartate-semialdehyde dehydrogenase	HOM2		90% (1)
Plasma membrane ATPase 1	PMA1		93% (1)
Protein MET17 [Includes: O-acetylhomoserine sulfhydrylase	MET17		90% (1)
3-isopropylmalate dehydrogenase	LEU3		90% (1)
Heat shock protein SSA1	HSP71		90% (1)
(DL)-glycerol-3-phosphatase 1	GPP1	90% (1)	
Probable inosine-5'-monophosphate dehydrogenase IMD1	IMDH1	90% (1)	
60S ribosomal protein L4-A	RL4A		90% (1)
Trehalose synthase complex regulatory subunit TSL1	TSL1	90% (1)	
Hypothetical 41.2 kDa protein in FPR1-TOM22 intergenic region	YNN4	90% (1)	
40S ribosomal protein S6	RPS6	90% (1)	
FK506-binding protein 1	FPR1	90% (1)	
Adenosine kinase	ADO1	90% (1)	
6-phosphofructokinase subunit beta	PFK2	90% (1)	90% (1)
Reticulon-like protein 1	RTN1		90% (1)
Histidine biosynthesis trifunctional protein [Includes: Phosphoribosyl-AMP cyclohydrolase	HIS2		90% (1)
1 month		116a	116b
Protoplast secreted protein 1 precursor	PST1	100% (14)	100% (11)
Phosphoglycerate kinase	PGK1	100% (7)	100% (8)
Enolase 1	ENO1	100% (5)	100% (4)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (7)	100% (5)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (6)	100% (7)
Cell wall protein DAN2 precursor	DAN2		100% (3)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (4)	100% (3)
Probable family 17 glucosidase SCW4 precursor	SCW4	99% (2)	99% (2)
Fructose-bisphosphate aldolase	FBA1	100% (4)	
Lysophospholipase 2 precursor	PLB2	99% (2)	100% (3)
Protein YGP1 precursor	YGP1	99% (2)	100% (3)
Phosphoglycerate mutase 1	PMG1	100% (4)	94% (1)
Peroxiredoxin type-2	AHP1	100% (3)	99% (2)
Seripauperin-5	PAU5	99% (2)	87% (1)
Thioredoxin-2	TRX2	87% (1)	100% (3)
Protein ECM33 precursor	ECM33	87% (1)	87% (1)
Aspartic proteinase yapsin-3 precursor	YPS3	87% (1)	99% (2)
Glycolipid-anchored surface protein 5 precursor	GAS5		87% (1)
Thioredoxin-1	TRX1	87% (1)	87% (1)

Heat shock protein SSA1	HSP71	100% (3)	
Pyruvate decarboxylase isozyme 1	PDC1	96% (1)	100% (2)
Enolase 2	ENO2	87% (1)	87% (1)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	87% (1)	87% (1)
Glycolipid-anchored surface protein 1 precursor	GAS1	87% (1)	
Heat shock protein 26	HSP26	87% (1)	
30 kDa heat shock protein	HSP30	87% (1)	87% (1)
12 kDa heat shock protein	HSP12		
Elongation factor 1-alpha	TEF1	87% (1)	99% (2)
Pyruvate kinase 1	PYK1	87% (1)	99% (2)
Aminopeptidase Y precursor	APE3	87% (1)	87% (1)
Triosephosphate isomerase	TPI1	100% (3)	87% (1)
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	MET6	99% (2)	87% (1)
Lysophospholipase 1 precursor	PLB1	87% (1)	87% (1)
Glycogen debranching enzyme	GDB1	87% (1)	87% (1)
FK506-binding protein 1	FKBP	87% (1)	87% (1)
ATP synthase subunit beta, mitochondrial precursor	ATP2	87% (1)	87% (1)
60S ribosomal protein L4-A	RPL4A	87% (1)	87% (1)
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH3	93% (1)	99% (2)
Homoserine dehydrogenase	HOM6	99% (2)	87% (1)
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	87% (1)	87% (1)
NADP-specific glutamate dehydrogenase 1	DHE4	87% (1)	
150 kDa heat shock glycoprotein precursor	HS150		87% (1)
Protein disulfide-isomerase precursor	PDI1	87% (1)	87% (1)
Plasma membrane ATPase 1	PMA1		87% (1)
Saccharopine dehydrogenase [NAD+, L-lysine-forming]	LYS1	87% (1)	87% (1)
Histone H2A.1	HTA1	94% (1)	87% (1)
Repressible acid phosphatase precursor	PPA5		
Protein HMF1	HMF1	87% (1)	87% (1)
Dihydroxy-acid dehydratase, mitochondrial precursor	ILV3	87% (1)	87% (1)
Alcohol dehydrogenase 4	ADH4	87% (1)	
Alcohol dehydrogenase 1	ADH1	87% (1)	
40S ribosomal protein S20	RPS20	87% (1)	87% (1)
Fatty acid synthase subunit alpha	FAS2	87% (1)	87% (1)
Peroxiredoxin TSA1	TSA1		87% (1)
40S ribosomal protein S3	RPS3	87% (1)	
Actin	ACT1	87% (1)	
ATP-dependent molecular chaperone HSC82	HSC82		87% (1)
Endo-1,3(4)-beta-glucanase 1 precursor	ENG1		87% (1)
Eukaryotic translation initiation factor 5A-1	HYP1	87% (1)	87% (1)
Heat shock protein SSB1	HSP75		87% (1)
Adenosine kinase	ADO1		87% (1)
40S ribosomal protein S5	RPS5	87% (1)	
Isocitrate dehydrogenase [NADP], mitochondrial precursor	IDP1		99% (2)
Ketol-acid reductoisomerase, mitochondrial precursor	ILV5	87% (1)	
Reticulon-like protein 1	RTN1	87% (1)	

Pyruvate decarboxylase isozyme 2	PDC5	87% (1)	
Phospho-2-dehydro-3-deoxyheptonate aldolase, tyrosine-inhibited	ARO4	87% (1)	
60S ribosomal protein L20	RPL20		87% (1)
Homoisocitrate dehydrogenase, mitochondrial precursor	LYS12	87% (1)	
Protein PRY1 precursor	PRY1	87% (1)	87% (1)
Magnesium-activated aldehyde dehydrogenase, cytosolic	ALDH6		87% (1)
40S ribosomal protein S14-A	RPS14A	87% (1)	
Vacuolar proton pump subunit G	VMA10	87% (1)	

3 months

		116a	116b
Protoplast secreted protein 1 precursor	PST1	100% (15)	100% (15)
Phosphoglycerate kinase	PGK1	100% (4)	100% (5)
Enolase 1	ENO1	100% (4)	100% (7)
Cell wall protein DAN2 precursor	DAN2	100% (5)	100% (3)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (3)	100% (4)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (8)	100% (6)
Fructose-bisphosphate aldolase	FBA1	100% (3)	100% (3)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (7)	100% (4)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (4)	100% (3)
Lysophospholipase 2 precursor	PLB2	100% (7)	100% (7)
Protein YGP1 precursor	YGP1	100% (3)	100% (3)
Enolase 2	ENO2	84% (1)	84% (1)
Thioredoxin-2	TRX2	100% (2)	99% (2)
Protein ECM33 precursor	ECM33	99% (2)	99% (2)
Elongation factor 1-alpha	EF1A	99% (2)	84% (1)
Phosphoglycerate mutase 1	PMG1	99% (2)	84% (1)
Pyruvate decarboxylase isozyme 1	PDC1	84% (1)	84% (1)
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	84% (1)	84% (1)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	84% (1)	99% (2)
30 kDa heat shock protein	HSP30	84% (1)	84% (1)
Glycolipid-anchored surface protein 5 precursor	GAS5	84% (1)	84% (1)
Aminopeptidase Y precursor	APE3	99% (2)	99% (2)
40S ribosomal protein S3	RPS3	84% (1)	84% (1)
Thioredoxin-1	TRX1	100% (3)	84% (1)
Heat shock protein 26	HSP26	84% (1)	84% (1)
Glycogen debranching enzyme	GDB1	84% (1)	84% (1)
Triosephosphate isomerase	TPI1	84% (1)	84% (1)
Pyruvate kinase 1	PYK1	84% (1)	84% (1)
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH2	84% (1)	
Glycolipid-anchored surface protein 1 precursor	GAS1	84% (1)	84% (1)
Alcohol dehydrogenase 4	ADH4	84% (1)	84% (1)
FK506-binding protein 1	FPR1	84% (1)	98% (2)
NADP-specific glutamate dehydrogenase 1	DHE4		84% (1)
Seripauperin-5	PAU5	99% (2)	99% (2)
Lysophospholipase 1 precursor	PLB1	100% (3)	100% (2)
Magnesium-activated aldehyde dehydrogenase,	ALDH6	84% (1)	84% (1)

cytosolic			
Aspartic proteinase yapsin-3 precursor	YPS3	84% (1)	
12 kDa heat shock protein	HSP12	84% (1)	84% (1)
Repressible acid phosphatase precursor	PPA5	94% (1)	84% (1)
Plasma membrane ATPase 1	PMA1	84% (1)	84% (1)
40S ribosomal protein S20	RPS20	84% (1)	84% (1)
Uncharacterized protein YJL171C precursor	YJR1	84% (1)	84% (1)
Peptidyl-prolyl cis-trans isomerase	CPR1	99% (2)	
Metallothionein precursor	CUP1-1	84% (1)	84% (1)
60S ribosomal protein L20	RPL20		84% (1)
Transcriptional modulator WTM1	WTM1	84% (1)	
Endo-1,3(4)-beta-glucanase 1 precursor	ENG1	84% (1)	
40S ribosomal protein S9-A	RPS9A	84% (1)	84% (1)
Protein disulfide-isomerase precursor	PDI1	84% (1)	84% (1)
150 kDa heat shock glycoprotein precursor	HS150	84% (1)	84% (1)
60S ribosomal protein L8-A	RPL8A		84% (1)
Glucose-6-phosphate isomerase	PGI1	84% (1)	84% (1)
Phosphatidylinositol 4-kinase LSB6	LSB6		80% (1)
Covalently-linked cell wall protein 11 precursor	CIS3	84% (1)	
ATP-dependent molecular chaperone HSC82	HSC82		84% (1)
Protein PRY1 precursor	PRY1	84% (1)	84% (1)
Actin-binding protein	ABP1	84% (1)	84% (1)
Uncharacterized protein YGL258W	YGZC	99% (2)	84% (1)
Protein ZPS1 precursor	ZPS1		
Vacuolar ATP synthase subunit B -	VAT2	84% (1)	
26S protease regulatory subunit 8 homolog	PRS8		84% (1)
26S proteasome regulatory subunit RPN10	RPN10	84% (1)	84% (1)
Reticulon-like protein 1	RTN1	84% (1)	
Transaldolase	TAL1		84% (1)
6-phosphofructokinase subunit beta	PFK2	84% (1)	
Adenosine kinase	ADO1		84% (1)
Protein TOS1 precursor	TOS1	84% (1)	
Endochitinase precursor	CTS1	84% (1)	
Acid phosphatase PHO11 precursor	PHO11	84% (1)	
6 months			
		116a	116b
Protoplast secreted protein 1 precursor	PST1	100% (14)	100% (13)
Cell wall protein DAN2 precursor	DAN2	100% (6)	100% (4)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (8)	100% (9)
Phosphoglycerate kinase	PGK1	100% (3)	100% (3)
Lysophospholipase 2 precursor	PLB2	100% (7)	100% (5)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (7)	100% (4)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (4)	100% (4)
Protein YGP1 precursor	YGP1	100% (4)	100% (4)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (3)	100% (3)
Protein ECM33 precursor	ECM33	100% (3)	100% (4)

Glycolipid-anchored surface protein 5 precursor	GAS5	98% (2)	
Probable glycosidase CRH1 precursor	CRH1	100% (3)	
Repressible acid phosphatase precursor	PPA5	99% (2)	99% (2)
Fructose-bisphosphate aldolase	FBA1	99% (2)	99% (2)
Aminopeptidase Y precursor	APE3	99% (2)	99% (2)
Lysophospholipase 1 precursor	PLB1	100% (3)	
Elongation factor 1-alpha	TEF1	99% (2)	
Glycolipid-anchored surface protein 1 precursor	GAS1		99% (2)
Seripauperin-5	PAU5	99% (2)	
Covalently-linked cell wall protein 11 precursor	CIS3		99% (2)
Uncharacterized protein YJL171C precursor	YJR1	99% (2)	
Protein SIM1 precursor	SIM1	99% (2)	
Uncharacterized protein YGL258W	YGL258W	100% (3)	
Protein TOS1 precursor	TOS1	99% (2)	

9 months

		116a	116b
Seripauperin-17 precursor	PAU17	100% (8)	100% (3)
Cell wall mannoprotein PST1 precursor	PST1	100% (15)	100% (11)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (8)	100% (6)
Phosphoglycerate kinase	PGK1	100% (6)	100% (5)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (8)	100% (10)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (9)	100% (9)
Protein YGP1 precursor	YGP1	100% (3)	100% (5)
Phosphoglycerate mutase 1	PMG1	100% (9)	100% (6)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (6)	100% (4)
Cell wall protein DAN3 precursor	DAN3	100% (3)	
Cell wall protein ECM33 precursor	ECM33	100% (4)	100% (3)
Glycolipid-anchored surface protein 5 precursor	GAS5	65% (1)	100% (3)
Pyruvate kinase 1	PYK1	100% (9)	100% (7)
Seripauperin-5	PAU5	99% (2)	
Glycolipid-anchored surface protein 1 precursor	GAS1	99% (2)	100% (3)
Uncharacterized protein YJL171C precursor	YJR1	96% (1)	
Probable glycosidase CRH1 precursor	CRH1	99% (2)	99% (2)
Enolase 1	ENO1	100% (2)	100% (2)
Elongation factor 1-alpha	TEF1	100% (2)	99% (2)
12 kDa heat shock protein	HSP12	65% (2)	100% (4)
Fructose-bisphosphate aldolase	FBA1		100% (3)
Lysophospholipase 2 precursor	PLB2	100% (3)	99% (2)
Septation protein SUN4 precursor	SUN4	100% (2)	
Covalently-linked cell wall protein 14 precursor	CCW14	99% (2)	100% (3)
Protein transport protein SEC31	SEC31	65% (1)	
Protein PIR3 precursor	PIR3	65% (1)	97% (2)
Heat shock protein SSA1	HSP71	99% (2)	99% (2)
Mitochondrial intermembrane space cysteine motif-containing protein MIC14	MIC14	65% (1)	98% (2)
6-phosphofructokinase subunit beta	PFK2	99% (2)	
Protein VEL1 precursor	VEL1	100% (5)	
Protein TOS1 precursor	TOS1	99% (2)	
Glycogen debranching enzyme	GDB1	89% (1)	79% (1)
Heat shock protein 26	HSP26	99% (2)	99% (2)
Alcohol dehydrogenase 4	ADH4	100% (3)	
Peptidyl-prolyl cis-trans isomerase	CPR1	97% (2)	

Triosephosphate isomerase TPI1 93% (1)
¹The percentage adjacent to the Gene Id is the Bayesian probability that the protein is correctly identified; the number of peptides attributed to the protein is in parentheses.

Appendix 5. Proteins identified in wines made by strain 262¹

0 Days

		262a	262b
Protoplast secreted protein 1 precursor	PST1	90% (1)	90% (1)
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH2	99% (2)	99% (2)
Protein ECM33 precursor	ECM33	90% (1)	90% (1)

7 days

		262a	262b
Protoplast secreted protein 1 precursor	PST1	100% (11)	100% (12)
Enolase 1	ENO1	100% (5)	100% (3)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (7)	100% (7)
Phosphoglycerate kinase	PGK1	100% (9)	100% (5)
Cell wall protein DAN2 precursor	DAN2		99% (2)
Fructose-bisphosphate aldolase	FBA1	100% (4)	100% (3)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	96% (1)	100% (10)
Heat shock protein SSA2	HSP72	100% (6)	100% (4)
Enolase 2	ENO2	99% (2)	90% (1)
Phosphoglycerate mutase 1	PMG1	100% (3)	100% (3)
Pyruvate decarboxylase isozyme 1	PDC1	100% (5)	100% (4)
Glyceraldehyde-3-phosphate dehydrogenase 3	TDH3	100% (4)	90% (1)
Heat shock protein 26	HSP26	99% (2)	100% (3)
Pyruvate kinase 1	PYK1	99% (2)	90% (1)
Probable family 17 glucosidase SCW4 precursor	SCW4	99% (2)	100% (3)
Triosephosphate isomerase	TPI1	100% (3)	100% (3)
Thioredoxin I	TRX1	90% (1)	100% (4)
Thioredoxin II	TRX2	100% (3)	100% (4)
12 kDa heat shock protein	HSP12	90% (1)	99% (2)
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	100% (2)	99% (2)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	90% (1)	99% (2)
Glucan 1,3-beta-glucosidase precursor	BGL2	90% (1)	100% (3)
Protein YGP1 precursor	YGP1	90% (1)	99% (2)
Protein ECM33 precursor	ECM33	90% (1)	100% (3)
Peroxiredoxin TSA1	TSA1	99% (2)	90% (1)
Actin	ACT1	99% (2)	90% (1)
Lysophospholipase 2 precursor	PLB2		100% (5)
Alcohol dehydrogenase 1	ADH1	99% (2)	90% (1)
Outer mitochondrial membrane protein porin 1	POR1	90% (1)	90% (1)
Ketol-acid reductoisomerase, mitochondrial precursor	ILV5	99% (2)	90% (1)
ATP synthase subunit beta, mitochondrial precursor	ATP2		99% (2)
Elongation factor 1-alpha	TEF1	90% (1)	90% (1)
Alcohol dehydrogenase 4	ADH4	90% (1)	90% (1)
Malate dehydrogenase, mitochondrial precursor	MDH1	90% (1)	90% (1)

Peroxiredoxin type-2	AHP1	90% (1)	99% (2)
5-methyltetrahydropteroyltriglutamate-- homocysteine methyltransferase	MET6	90% (1)	90% (1)
Heat shock protein SSA1	HSP71	90% (1)	
60S ribosomal protein L8-A	RPL8A	90% (1)	90% (1)
NADP-specific glutamate dehydrogenase 1	DHE4	90% (1)	90% (1)
30 kDa heat shock protein	HSP30	90% (1)	90% (1)
Seripauperin-5	PAU5	90% (1)	90% (1)
Aminopeptidase Y precursor	APE3	90% (1)	90% (1)
Plasma membrane ATPase 1	PMA1	90% (1)	
Saccharopine dehydrogenase [NAD ⁺ , L-lysine- forming]	LYS1_	90% (1)	90% (1)
60S ribosomal protein L19	RPL19A	90% (1)	
Transaldolase	TAL1	90% (1)	90% (1)
Histone H2A.1	HTA1		90% (1)
40S ribosomal protein S5	RPS5		90% (1)
Heat shock protein SSC1, mitochondrial precursor	HSP77		
Glycolipid-anchored surface protein 1 precursor	GAS1		99% (2)
Protein MMF1, mitochondrial precursor	MMF1	90% (1)	90% (1)
60S ribosomal protein L13-A	RPL13A	90% (1)	
Vacuolar ATP synthase subunit B	VMA2	90% (1)	
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH2	90% (1)	
60S acidic ribosomal protein P2-beta	RPP2B		90% (1)
40S ribosomal protein S3	RPS3		90% (1)
Dihydroxy-acid dehydratase, mitochondrial precursor	ILV3	90% (1)	
Glucose-6-phosphate isomerase	PGI1	99% (2)	
Glucokinase GLK1	GLK1	99% (2)	
40S ribosomal protein S14-A	RPS14A	90% (1)	
Aconitate hydratase, mitochondrial precursor	ACO1		90% (1)
Peptidyl-prolyl cis-trans isomerase	CPR1	90% (1)	
Uncharacterized protein YNL208W	YNU8		90% (1)
Lysophospholipase 1 precursor	PLB1		99% (2)
Heat shock protein SSB1	HSP75	90% (1)	
Mannose-1-phosphate guanyltransferase	MPG1	90% (1)	
Vacuolar aminopeptidase 1 precursor	AMPL	90% (1)	
2-oxoglutarate dehydrogenase E1 component, mitochondrial precursor	ODO1		90% (1)
40S ribosomal protein S20	RPS20		90% (1)
Heat shock protein homolog SSE1	SSE1		
60S ribosomal protein L26-B	RPL26B	90% (1)	
40S ribosomal protein S22-A	RPS22A	90% (1)	
Eukaryotic translation initiation factor 5A-1	IF5A1	90% (1)	
Protein disulfide-isomerase precursor	PDI1		90% (1)
Repressible acid phosphatase precursor	PHO5		90% (1)
Protein PRY3	PRY3		90% (1)
Superoxide dismutase [Cu-Zn]	SOD1	90% (1)	
Probable chaperone HSP31	HSP31		90% (1)
14 days			
Protoplast secreted protein 1 precursor	PST1	262a 100% (11)	262b 100% (12)

Enolase 1	ENO1	100% (6)	100% (6)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (5)	100% (7)
Phosphoglycerate kinase	PGK1	100% (8)	100% (8)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (6)	100% (8)
Cell wall protein DAN2 precursor	DAN2	100% (3)	100% (3)
Fructose-bisphosphate aldolase	FBA1	100% (4)	100% (3)
Enolase 2	ENO2	99% (2)	90% (1)
Glyceraldehyde-3-phosphate dehydrogenase 3	TDH3	100% (5)	99% (2)
Pyruvate decarboxylase isozyme 1	PDC1	99% (2)	90% (1)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (5)	100% (6)
Heat shock protein 26	HSP26	100% (4)	100% (4)
Thioredoxin I	TRX1	100% (3)	100% (3)
Pyruvate kinase 1	PYK1	99% (2)	99% (2)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (3)	100% (4)
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	MET6	99% (2)	99% (2)
Protein YGP1 precursor	YGP1	100% (4)	100% (5)
Thioredoxin II	TRX2	100% (4)	100% (3)
Heat shock protein SSA2	HSP72	100% (3)	99% (2)
Phosphoglycerate mutase 1	PMG1	99% (2)	90% (1)
Protein ECM33 precursor	ECM33	100% (4)	100% (3)
Triosephosphate isomerase	TPI1	90% (1)	90% (1)
Lysophospholipase 2 precursor	PLB2	100% (5)	100% (5)
Alcohol dehydrogenase 4	ADH4	99% (2)	99% (2)
6-phosphogluconate dehydrogenase, decarboxylating 1 -	GND1	90% (1)	
12 kDa heat shock protein	HSP12		99% (2)
Glycolipid-anchored surface protein 1 precursor	GAS1	99% (2)	99% (2)
Elongation factor 1-alpha	TEF1	100% (3)	90% (1)
Alcohol dehydrogenase 1	ADH1	90% (1)	
40S ribosomal protein S17-A	RPS17A	99% (2)	
Probable glycosidase CRH1 precursor	CRH1	99% (2)	100% (3)
Transaldolase	TAL1	90% (1)	
Peroxiredoxin TSA1	TSA1		90% (1)
Seripauperin-5	PAU5	90% (1)	90% (1)
30 kDa heat shock protein	HSP30	90% (1)	90% (1)
Glycolipid-anchored surface protein 5 precursor	GAS5	90% (1)	99% (2)
Uncharacterized protein YGL258W	YGL258W	99% (2)	90% (1)
Histone H2A.1	HTA1	90% (1)	90% (1)
60S ribosomal protein L19	RPL19A	86% (1)	
Aminopeptidase Y precursor	APE3	90% (1)	
Peroxiredoxin type-2	AHP1	90% (1)	
ATP synthase subunit alpha, mitochondrial precursor	ATP1	90% (1)	
Protein PRY1 precursor	PRY1	90% (1)	90% (1)
Suppressor protein STM1	STM1	90% (1)	90% (1)
Protein SIM1 precursor	SIM1	99% (2)	100% (3)
Vacuolar ATP synthase catalytic subunit A	VMA1	90% (1)	
Aconitate hydratase, mitochondrial precursor	ACO1	90% (1)	
60S ribosomal protein L13-A	RPL13A		90% (1)
40S ribosomal protein S16	RPS16	90% (1)	

40S ribosomal protein S5	RPS5	90% (1)	
Outer mitochondrial membrane protein porin 1	POR1	90% (1)	90% (1)
Cell wall protein DAN3 precursor	DAN3	90% (1)	90% (1)
Aspartate-semialdehyde dehydrogenase	HOM2	90% (1)	
Protein MET17 [Includes: O-acetylhomoserine sulfhydrylase	MET17	90% (1)	
3-isopropylmalate dehydrogenase	LEU3	90% (1)	90% (1)
(DL)-glycerol-3-phosphatase 1	GPP1	90% (1)	
Aspartic proteinase yapsin-3 precursor	YPS3	99% (2)	
Repressible acid phosphatase precursor	PHO5		100% (3)
Mannose-1-phosphate guanyltransferase	MPG1	90% (1)	
Heat shock protein SSB1	HSP75	90% (1)	
Trehalose synthase complex regulatory subunit TSL1	TSL1	90% (1)	
60S ribosomal protein L35	RPL35	90% (1)	
40S ribosomal protein S20	RPS20	90% (1)	
Adenosine kinase	ADO1	90% (1)	
6-phosphofructokinase subunit beta	PFK2		90% (1)
Protein UTH1	UTH1		90% (1)
Endochitinase precursor	CTS1	90% (1)	90% (1)
Uncharacterized protein YOR387C precursor	YOR387C		90% (1)
Probable glycosidase CRH2 precursor	CRH2		90% (1)
Protein TOS1 precursor	TOS1		90% (1)
Metallothionein precursor	CUP1-1	90% (1)	
Uncharacterized protein YHR138C precursor	YHR138C		90% (1)
Eukaryotic translation initiation factor 5A-2	IF5A2	90% (1)	
Protein PRY3	PRY3		90% (1)
Covalently-linked cell wall protein 11 precursor	CIS3		90% (1)
Protein NCA3, mitochondrial precursor	NCA3		90% (1)

1 month

		262a	262b
Protoplast secreted protein 1 precursor	PST1	100% (14)	100% (14)
Phosphoglycerate kinase	PGK1	100% (8)	100% (7)
Enolase 1	ENO1	100% (7)	100% (6)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (7)	100% (6)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (8)	100% (7)
Cell wall protein DAN2 precursor	DAN2	100% (4)	100% (3)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (6)	100% (4)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (3)	100% (4)
Fructose-bisphosphate aldolase	FBA1	100% (3)	100% (3)
Lysophospholipase 2 precursor	PLB2	100% (6)	100% (6)
Protein YGP1 precursor	YGP1	100% (3)	99% (2)
Phosphoglycerate mutase 1	PMG1	100% (4)	87% (1)
Peroxiredoxin type-2	AHP1	100% (3)	99% (2)
Seripauperin-5	PAU5	99% (2)	100% (2)
Thioredoxin-2	TRX2	100% (4)	100% (4)
Protein ECM33 precursor	ECM33	100% (4)	99% (2)
Aspartic proteinase yapsin-3 precursor	YPS3	100% (4)	100% (3)
Glycolipid-anchored surface protein 5 precursor	GAS5	96% (1)	87% (1)
Thioredoxin-1	TRX1	100% (3)	99% (2)
Heat shock protein SSA1	HSP71	86% (1)	100% (4)

Pyruvate decarboxylase isozyme 1	PDC1	86% (1)	99% (2)
Enolase 2	ENO2	86% (1)	87% (1)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	86% (1)	99% (2)
Glycolipid-anchored surface protein 1 precursor	GAS1	99% (2)	87% (1)
Heat shock protein 26	HSP26	99% (2)	99% (2)
30 kDa heat shock protein	HSP30	86% (1)	87% (1)
12 kDa heat shock protein	HSP12	99% (2)	99% (2)
Elongation factor 1-alpha	TEF1	99% (2)	99% (2)
Pyruvate kinase 1	PYK1	99% (2)	99% (2)
Aminopeptidase Y precursor	APE3	99% (2)	87% (1)
Triosephosphate isomerase	TPI1	86% (1)	87% (1)
Transaldolase	TAL1	86% (1)	99% (2)
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	MET6	86% (1)	87% (1)
Lysophospholipase 1 precursor	PLB1	99% (2)	87% (1)
Glycogen debranching enzyme	GDB1	86% (1)	87% (1)
FK506-binding protein 1	FKBP	86% (1)	99% (2)
ATP synthase subunit beta, mitochondrial precursor	ATP2	86% (1)	87% (1)
60S ribosomal protein L4-A	RPL4A	86% (1)	93% (1)
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH3	86% (1)	87% (1)
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	86% (1)	87% (1)
NADP-specific glutamate dehydrogenase 1	DHE4	99% (2)	99% (2)
150 kDa heat shock glycoprotein precursor	HS150	86% (1)	87% (1)
Protein disulfide-isomerase precursor	PDI1	86% (1)	87% (1)
Superoxide dismutase [Cu-Zn]	SOD1	86% (1)	99% (2)
Saccharopine dehydrogenase [NAD+, L-lysine-forming]	LYS1	86% (1)	
Histone H2A.1	HTA1	86% (1)	87% (1)
Repressible acid phosphatase precursor	PPA5	99% (2)	
Dihydroxy-acid dehydratase, mitochondrial precursor	ILV3	86% (1)	87% (1)
Alcohol dehydrogenase 4	ADH4	86% (1)	87% (1)
40S ribosomal protein S20	RPS20	86% (1)	
Covalently-linked cell wall protein 11 precursor	CIS3	86% (1)	87% (1)
Malate dehydrogenase, mitochondrial precursor	MDH1		87% (1)
Protein TOS1 precursor	TOS1	86% (1)	
Fatty acid synthase subunit alpha	FAS2	86% (1)	
Protein SIM1 precursor	SIM1	86% (1)	
Protein EGT2 precursor	EGT2	86% (1)	87% (1)
40S ribosomal protein S3	RPS3		87% (1)
Vacuolar ATP synthase subunit B	VAT2	86% (1)	
Uncharacterized protein YGL258W	YGL258W	86% (1)	87% (1)
Protein MMF1, mitochondrial precursor	MMF1		100% (3)
Uncharacterized protein YHR087W	YHR087W		87% (1)
Heat shock protein SSB1	HSP75	86% (1)	
Adenosine kinase	ADO1		87% (1)
40S ribosomal protein S5	RPS5		83% (1)
Seripauperin-2	PAU2		87% (1)
Aconitate hydratase, mitochondrial precursor	ACO1	86% (1)	87% (1)
Isocitrate dehydrogenase [NADP],	IDP1		87% (1)

mitochondrial precursor			
Mannose-1-phosphate guanyltransferase	MPG1	86% (1)	
Uncharacterized protein YJL171C precursor	YJR1	86% (1)	87% (1)
Pyruvate decarboxylase isozyme 2	PDC5		87% (1)
Heat shock protein SSC1, mitochondrial precursor	HSP77		87% (1)
Glucose-6-phosphate isomerase	PGI1	86% (1)	
Magnesium-activated aldehyde dehydrogenase, cytosolic	ALDH6		87% (1)
Uncharacterized protein YNL208W	YNL208W	86% (1)	87% (1)
Thioredoxin-3, mitochondrial precursor	TRX3		87% (1)
Reticulon-like protein 2	RTN2	86% (1)	

3 months

		262a	262b
Protoplast secreted protein 1 precursor	PST1	100% (13)	100% (13)
Phosphoglycerate kinase	PGK1	100% (5)	100% (5)
Enolase 1	ENO1	100% (8)	100% (6)
Cell wall protein DAN2 precursor	DAN2		99% (2)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (4)	
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (5)	100% (5)
Fructose-bisphosphate aldolase	FBA1	100% (5)	99% (2)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (4)	100% (3)
Probable family 17 glucosidase SCW4 precursor	SCW4	96% (1)	100% (3)
Lysophospholipase 2 precursor	PLB2	100% (3)	100% (5)
Protein YGP1 precursor	YGP1	99% (2)	100% (3)
Enolase 2	ENO2	90% (1)	87% (1)
Thioredoxin-2	TRX2	99% (2)	99% (2)
Protein ECM33 precursor	ECM33	100% (3)	99% (2)
Elongation factor 1-alpha	TEF1	90% (1)	87% (1)
Phosphoglycerate mutase 1	PGM1	99% (2)	99% (2)
Pyruvate decarboxylase isozyme 1	PDC1		87% (1)
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	99% (2)	87% (1)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	99% (2)	99% (2)
30 kDa heat shock protein	HSP30	90% (1)	87% (1)
Glycolipid-anchored surface protein 5 precursor	GAS5	90% (1)	87% (1)
Aminopeptidase Y precursor	APE3	99% (2)	87% (1)
40S ribosomal protein S3	RPS3	99% (2)	97% (1)
Thioredoxin-1	TRX1	90% (1)	87% (1)
Heat shock protein 26	HSP26	99% (2)	99% (2)
Glycogen debranching enzyme	GDB1	90% (1)	87% (1)
Triosephosphate isomerase	TPI1	90% (1)	87% (1)
Pyruvate kinase 1	PYK1	99% (2)	87% (1)
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH2	90% (1)	99% (2)
Alcohol dehydrogenase 4	ADH4	90% (1)	87% (1)
FK506-binding protein 1	FPR1	90% (1)	87% (1)
NADP-specific glutamate dehydrogenase 1	GDH1	90% (1)	87% (1)
Seripauperin-5	PAU5		87% (1)
Magnesium-activated aldehyde dehydrogenase, cytosolic	ALD6	90% (1)	87% (1)

Aspartic proteinase yapsin-3 precursor	YPS3	90% (1)	99% (2)
Protein HMF1	HMF1	90% (1)	87% (1)
40S ribosomal protein S20	RPS20	90% (1)	
Protein MMF1, mitochondrial precursor	MMF1	99% (2)	87% (1)
ATP synthase subunit beta, mitochondrial precursor	ATP2	90% (1)	87% (1)
Saccharopine dehydrogenase [NAD+, L-lysine-forming]	LYS1		87% (1)
Actin	ACT1	90% (1)	99% (2)
Trehalose synthase complex regulatory subunit TSL1	TSL1	90% (1)	87% (1)
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	MET6	82% (1)	87% (1)
60S ribosomal protein L20	RPL20A	90% (1)	
Transcriptional modulator WTM1	WTM1	90% (1)	
Endo-1,3(4)-beta-glucanase 1 precursor	ENG1	90% (1)	
60S ribosomal protein L8-A	RPL8A	90% (1)	
40S ribosomal protein S5 -	RPS5	90% (1)	
Phosphatidylinositol 4-kinase LSB6	LSB6	81% (1)	
40S ribosomal protein S19-A	RPS19A	90% (1)	
Uncharacterized protein YNL208W	YNU8	90% (1)	87% (1)
ATP-dependent molecular chaperone HSC82	HSC82	90% (1)	
Bifunctional purine biosynthesis protein ADE17 [Includes:			
Phosphoribosylaminoimidazolecarboxamide formyltransferase	ADE17	90% (1)	
Stress protein DDR48	DDR48	90% (1)	
40S ribosomal protein S15	RPS15	90% (1)	
Protein MET17 [Includes: O-acetylhomoserine sulfhydrylase	MET17	90% (1)	87% (1)
40S ribosomal protein S8	RPS8A	90% (1)	
26S protease regulatory subunit 8 homolog	RPT6	90% (1)	
60S ribosomal protein L28	RPL28	90% (1)	
Elongation factor 2	EFT2	90% (1)	
Single-stranded DNA-binding protein RIM1, mitochondrial precursor	RIM1	90% (1)	
Polyadenylate-binding protein, cytoplasmic and nuclear	PAB1	90% (1)	
60S ribosomal protein L19	RPL19A		87% (1)
Peroxiredoxin TSA1	TSA1	90% (1)	
Phosphoglucomutase-2	PGM2	90% (1)	
Transketolase 1	TKL1	90% (1)	
60S ribosomal protein L38	RPL38	90% (1)	
Ketol-acid reductoisomerase, mitochondrial precursor	ILV5	90% (1)	
Glutathione reductase	GLR1	90% (1)	
Homoisocitrate dehydrogenase, mitochondrial precursor	LYS12	90% (1)	

6 months

		262a	262b
Protoplast secreted protein 1 precursor	PST1	100% (13)	100% (15)
Cell wall protein DAN2 precursor	DAN2	99% (2)	100% (5)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (9)	100% (9)

Phosphoglycerate kinase	PGK1	100% (4)	100% (3)
Lysophospholipase 2 precursor	PLB2	100% (7)	100% (9)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (4)	100% (5)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (4)	100% (5)
Protein YGP1 precursor	YGP1	100% (4)	100% (3)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (3)	100% (3)
Protein ECM33 precursor	ECM33	100% (4)	100% (4)
Glycolipid-anchored surface protein 5 precursor	GAS5	100% (3)	100% (3)
Probable glycosidase CRH1 precursor	CRH1		99% (2)
Repressible acid phosphatase precursor	PPA5	99% (2)	99% (2)
Fructose-bisphosphate aldolase	FBA1	100% (4)	
12 kDa heat shock protein	HSP12		99% (2)
Aminopeptidase Y precursor	APE3		99% (2)
Lysophospholipase 1 precursor	PLB1		100% (3)
Elongation factor 1-alpha	TEF1	99% (2)	99% (2)
Glycolipid-anchored surface protein 1 precursor	GAS1		100% (3)
Phosphoglycerate mutase 1	PMG1	99% (2)	
Thioredoxin-1	TRX1	99% (2)	
Enolase 2	ENO2	100% (4)	
Uncharacterized protein YGL258W	YGL258W		99% (2)
Peptidyl-prolyl cis-trans isomerase	CPR1	99% (2)	
Aspartic proteinase yapsin-3 precursor	YPS3		99% (2)
Protein MMF1, mitochondrial precursor	MMF1	99% (2)	

9 months

		262a	262b
Seripauperin-17 precursor	PAU17	100% (6)	100% (6)
Cell wall mannoprotein PST1 precursor	PST1	100% (17)	100% (15)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (10)	100% (12)
Phosphoglycerate kinase	PGK1	100% (5)	100% (5)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (5)	100% (3)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (8)	100% (7)
Protein YGP1 precursor	YGP1	100% (3)	100% (6)
Phosphoglycerate mutase 1	PMG1	100% (6)	100% (3)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (5)	99% (2)
Cell wall protein DAN3 precursor	DAN3	99% (2)	99% (2)
Cell wall protein ECM33 precursor	ECM33	100% (4)	99% (2)
Glycolipid-anchored surface protein 5 precursor	GAS5	100% (4)	93% (1)
Pyruvate kinase 1	PYK1		100% (3)
Seripauperin-5	PAU5	99% (2)	99% (2)
Glycolipid-anchored surface protein 1 precursor	GAS1	99% (2)	99% (2)
Uncharacterized protein YJL171C precursor	YJR1	98% (2)	97% (1)
Probable glycosidase CRH1 precursor	CRH1	98% (2)	97% (2)
Lysophospholipase 2 precursor	PLB2	100% (3)	66% (1)
Septation protein SUN4 precursor	SUN4	97% (2)	100% (3)
Protein ZPS1 precursor	ZPS1		100% (5)
Heat shock protein SSA1	HSP71	99% (2)	
Pyruvate decarboxylase	PDC1		100% (3)

isozyme 1			
6-phosphofructokinase subunit beta	PFK2		99% (2)
Protein VEL1 precursor	VEL1	100% (5)	
Probable family 17 glucosidase SCW10 precursor	SCW10		99% (2)
Protein TOS1 precursor	TOS1	99% (2)	
Protein SIM1 precursor	SIM1		99% (2)
Peptidyl-prolyl cis-trans isomerase	CPR1	99% (2)	
Trehalose synthase complex regulatory subunit TSL1	TSL1	99% (2)	

¹ The percentage adjacent to the Gene Id is the Bayesian probability that the protein is correctly identified; the number of peptides attributed to the protein is in parentheses.

Appendix 6. Proteins identified in wines made by strain RC212¹

0 Days

		RC212a	RC212b
Protoplast secreted protein 1 precursor	PST1	90% (1)	90% (1)
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH2	99% (2)	90% (1)
Protein ECM33 precursor	ECM33	90% (1)	90% (1)

7 days

		RC212a	RC212b
Protoplast secreted protein 1 precursor	PST1	100% (11)	100% (12)
Enolase 1	ENO1	100% (6)	99% (2)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (7)	100% (7)
Phosphoglycerate kinase	PGK1	100% (5)	100% (4)
Cell wall protein DAN2 precursor	DAN2	100% (3)	100% (3)
Fructose-bisphosphate aldolase	FBA1	100% (5)	100% (4)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (8)	100% (7)
Heat shock protein SSA2	HSP72		100% (6)
Enolase 2	ENO2	90% (1)	90% (1)
Phosphoglycerate mutase 1	PMG1	94% (1)	99% (2)
Pyruvate decarboxylase isozyme 1	PDC1	100% (3)	99% (2)
Glyceraldehyde-3-phosphate dehydrogenase 3	TDH3	98% (1)	100% (3)
Heat shock protein 26	HSP26	100% (4)	99% (2)
Pyruvate kinase 1	PYK1	90% (1)	99% (2)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (3)	99% (2)
Triosephosphate isomerase	TPI1	90% (1)	99% (2)
Thioredoxin I	TRX1	100% (5)	100% (4)
Thioredoxin II	TRX2	99% (2)	90% (1)
12 kDa heat shock protein	HSP12	99% (2)	90% (1)
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	90% (1)	99% (2)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	100% (3)	
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (4)	90% (1)
Protein YGP1 precursor	YGP1	99% (2)	90% (1)
Protein ECM33 precursor	ECM33	99% (2)	90% (1)
Actin	ACT1	90% (1)	

Lysophospholipase 2 precursor	PLB2	100% (4)	90% (1)
Alcohol dehydrogenase 1	ADH1		98% (1)
Outer mitochondrial membrane protein porin 1	POR1	90% (1)	90% (1)
Ketol-acid reductoisomerase, mitochondrial precursor	ILV5	90% (1)	90% (1)
ATP synthase subunit beta, mitochondrial precursor	ATP2	99% (2)	99% (2)
Elongation factor 1-alpha	TEF1	90% (1)	90% (1)
Alcohol dehydrogenase 4	ADH4	90% (1)	90% (1)
Malate dehydrogenase, mitochondrial precursor	MDH1	90% (1)	90% (1)
Peroxiredoxin type-2	AHP1	90% (1)	90% (1)
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	MET6		90% (1)
Heat shock protein SSA1	HSP71	90% (1)	90% (1)
60S ribosomal protein L8-A	RPL8A		90% (1)
NADP-specific glutamate dehydrogenase 1	DHE4	90% (1)	90% (1)
Seripauperin-5	PAU5	90% (1)	90% (1)
Aminopeptidase Y precursor	APE3	95% (1)	90% (1)
Plasma membrane ATPase 1	PMA1		90% (1)
Transaldolase	TAL1	90% (1)	
Protein HMF1	HMF1	90% (1)	90% (1)
Histone H2A.1	HTA1		90% (1)
40S ribosomal protein S5	RPS5		90% (1)
Glycolipid-anchored surface protein 1 precursor	GAS1	90% (1)	
Vacuolar ATP synthase subunit B	VMA2	90% (1)	90% (1)
40S ribosomal protein S6	RPS6A		90% (1)
60S acidic ribosomal protein P2-beta	RPP2B		90% (1)
40S ribosomal protein S3	RPS3		90% (1)
Protein ZPS1 precursor	ZPS1		100% (3)
Reticulon-like protein 1	RTN1		90% (1)
Aconitate hydratase, mitochondrial precursor	ACO1	90% (1)	
Uncharacterized protein YNL208W	YNU8	90% (1)	
Mannose-1-phosphate guanyltransferase	MPG1		90% (1)
40S ribosomal protein S20	RPS20		90% (1)
Hypothetical 41.2 kDa protein in FPR1-TOM22 intergenic region	YNN4		90% (1)
Adenosine kinase	ADO1		87% (1)
6-phosphofructokinase subunit beta	PFK2	90% (1)	
Protein disulfide-isomerase precursor	PDI1	90% (1)	
Transcriptional modulator WTM1	WTM1		90% (1)
Homoserine dehydrogenase	HOM6		90% (1)
Uncharacterized protein YJL171C precursor	YJR1		90% (1)
Aspartic proteinase yapsin-3 precursor	YPS3	90% (1)	
60S ribosomal protein L11-A	RPL11A	90% (1)	
Probable glycosidase CRH2 precursor	CRH2		90% (1)
40S ribosomal protein S15	RPS15		90% (1)
Serine hydroxymethyltransferase, cytosolic	SHM2		90% (1)
40S ribosomal protein S17-A	RPS17A		90% (1)
Actin-binding protein	ABP1	90% (1)	

14 days

		RC212a	RC212b
Protoplast secreted protein 1 precursor	PST1	100% (11)	100% (10)
Enolase 1	ENO1	100% (7)	100% (7)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (9)	100% (7)
Phosphoglycerate kinase	PGK1	100% (8)	100% (9)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (7)	100% (4)
Cell wall protein DAN2 precursor	DAN2	99% (2)	100% (2)
Fructose-bisphosphate aldolase	FBA1	100% (3)	100% (5)
Enolase 2	ENO2	99% (2)	89% (1)
Glyceraldehyde-3-phosphate dehydrogenase 3	TDH3	100% (5)	100% (3)
Pyruvate decarboxylase isozyme 1	PDC1	99% (2)	100% (5)
Probable family 17 glucosidase SCW4 precursor	SCW4	99% (2)	100% (4)
Heat shock protein 26	HSP26	100% (3)	100% (3)
Thioredoxin I	TRX1	100% (4)	100% (3)
Pyruvate kinase 1	PYK1	100% (3)	100% (4)
Glucan 1,3-beta-glucosidase precursor	BGL2	90% (1)	99% (2)
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	MET6	100% (4)	100% (3)
Protein YGP1 precursor	YGP1	99% (2)	89% (1)
Thioredoxin II	TRX2	99% (2)	94% (1)
Heat shock protein SSA2	HSP72	100% (5)	100% (4)
Phosphoglycerate mutase 1	PMG1	95% (1)	100% (3)
Protein ECM33 precursor	ECM33	99% (2)	89% (1)
Triosephosphate isomerase	TPI1	99% (2)	89% (1)
Lysophospholipase 2 precursor	PLB2	90% (1)	
Alcohol dehydrogenase 4	ADH4	99% (2)	99% (2)
6-phosphogluconate dehydrogenase, decarboxylating 1 -	GND1	100% (3)	100% (3)
12 kDa heat shock protein	HSP12	90% (1)	89% (1)
Glycolipid-anchored surface protein 1 precursor	GAS1		89% (1)
Elongation factor 1-alpha	TEF1	99% (2)	
Alcohol dehydrogenase 1	ADH1	90% (1)	89% (1)
40S ribosomal protein S17-A	RPS17A	99% (2)	89% (1)
Probable glycosidase CRH1 precursor	CRH1		89% (1)
Peroxiredoxin TSA1	TSA1	99% (2)	89% (1)
Seripauperin-5	PAU5	90% (1)	89% (1)
30 kDa heat shock protein	HSP30	90% (1)	89% (1)
NADP-specific glutamate dehydrogenase 1	DHE4	90% (1)	99% (2)
Uncharacterized protein YGL258W	YGL258W	90% (1)	89% (1)
Histone H2A.1	HTA1	90% (1)	
40S ribosomal protein S14-A	RPS14A	90% (1)	89% (1)
60S ribosomal protein L19	RPL19A	90% (1)	
Aminopeptidase Y precursor	APE3	90% (1)	89% (1)
Peroxiredoxin type-2	AHP1	90% (1)	
Protein PRY1 precursor	PRY1		89% (1)
Suppressor protein STM1	STM1	90% (1)	89% (1)
Vacuolar ATP synthase catalytic subunit A	VMA1	90% (1)	89% (1)
Aconitate hydratase, mitochondrial precursor	ACO1		89% (1)
60S ribosomal protein L13-A	RPL13A	90% (1)	89% (1)
Ketol-acid reductoisomerase, mitochondrial precursor	ILV5		99% (2)
60S ribosomal protein L8-B	RPL8B	90% (1)	

40S ribosomal protein S16	RPS16	90% (1)	89% (1)
40S ribosomal protein S5	RPS5	90% (1)	
Cell wall protein DAN3 precursor	DAN3	90% (1)	
Aspartate-semialdehyde dehydrogenase	HOM2	90% (1)	
Plasma membrane ATPase 1	PMA1	93% (1)	89% (1)
Protein MET17 [Includes: O-acetylhomoserine sulphydrylase	MET17	90% (1)	
3-isopropylmalate dehydrogenase	LEU3	90% (1)	89% (1)
Heat shock protein SSA1	HSP71	90% (1)	
(DL)-glycerol-3-phosphatase 1	GPP1	90% (1)	
Probable inosine-5'-monophosphate dehydrogenase IMD1	IMDH1	90% (1)	89% (1)
Protein ZPS1 precursor	ZPS1	100% (3)	100% (3)
Vacuolar ATP synthase subunit B	VMA2	90% (1)	
Mannose-1-phosphate guanyltransferase	MPG1	90% (1)	
60S acidic ribosomal protein P2-beta	RLA4	90% (1)	
40S ribosomal protein S3	RPS3	88% (1)	89% (1)
60S ribosomal protein L35	RPL35	90% (1)	89% (1)
40S ribosomal protein S27-A	RPS27A	90% (1)	
Hexokinase-1	HXK1	90% (1)	
40S ribosomal protein S6	RPS6	90% (1)	89% (1)
Homoserine dehydrogenase	HOM6	90% (1)	
Endochitinase precursor	CTS1		89% (1)
40S ribosomal protein S18	RPS18		89% (1)
Coproporphyrinogen III oxidase	HEM6		89% (1)
RNA polymerase II transcription factor B subunit 4	TFB4	90% (1)	
Adenosylhomocysteinase	SAH1	98% (1)	
Invertase 2 precursor	INV2	90% (1)	89% (1)
Phospho-2-dehydro-3-deoxyheptonate aldolase, tyrosine-inhibited	ARO4		89% (1)
Pyruvate decarboxylase isozyme 2	PDC5	90% (1)	85% (1)
40S ribosomal protein S15	RS15	90% (1)	
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH2		89% (1)

1 month

		RC212a	RC212b
Protoplast secreted protein 1 precursor	PST1	100% (17)	100% (15)
Phosphoglycerate kinase	PGK1	100% (7)	100% (4)
Enolase 1	ENO1	100% (5)	100% (3)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (5)	100% (5)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (8)	100% (9)
Cell wall protein DAN2 precursor	DAN2	100% (6)	100% (7)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (5)	100% (4)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (3)	100% (4)
Fructose-bisphosphate aldolase	FBA1	100% (3)	99% (2)
Lysophospholipase 2 precursor	PLB2	100% (3)	99% (2)
Protein YGP1 precursor	YGP1	99% (2)	100% (3)
Phosphoglycerate mutase 1	PMG1	89% (1)	99% (2)
Peroxiredoxin type-2	AHP1	100% (3)	
Seripauperin-5	PAU5	100% (3)	100% (2)
Thioredoxin-2	TRX2	100% (3)	100% (3)

Protein ECM33 precursor	ECM33	100% (4)	100% (5)
Aspartic proteinase yapsin-3 precursor	YPS3	89% (1)	100% (3)
Glycolipid-anchored surface protein 5 precursor	GAS5	89% (1)	95% (1)
Thioredoxin-1	TRX1	100% (4)	100% (4)
Heat shock protein SSA1	HSP71	99% (2)	
Pyruvate decarboxylase isozyme 1	PDC1		86% (1)
Enolase 2	ENO2	89% (1)	86% (1)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	99% (2)	86% (1)
Glycolipid-anchored surface protein 1 precursor	GAS1	99% (2)	100% (3)
Heat shock protein 26	HSP26	89% (1)	86% (1)
30 kDa heat shock protein	HSP30		86% (1)
12 kDa heat shock protein	HSP12	89% (1)	86% (1)
Elongation factor 1-alpha	TEF1	99% (2)	86% (1)
Aminopeptidase Y precursor	APE3	99% (2)	99% (2)
Transaldolase	TAL1	99% (2)	100% (3)
Lysophospholipase 1 precursor	PLB1	99% (2)	100% (3)
Glycogen debranching enzyme	GDB1	89% (1)	86% (1)
ATP synthase subunit beta, mitochondrial precursor	ATP2	89% (1)	86% (1)
60S ribosomal protein L4-A	RPL4A	89% (1)	
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	89% (1)	
NADP-specific glutamate dehydrogenase 1	DHE4	99% (2)	
150 kDa heat shock glycoprotein precursor	HS150	89% (1)	86% (1)
Protein disulfide-isomerase precursor	PDI1	89% (1)	86% (1)
Repressible acid phosphatase precursor	PPA5	96% (1)	99% (2)
Protein HMF1	HMF1	89% (1)	
Alcohol dehydrogenase 4	ADH4	89% (1)	86% (1)
Probable glycosidase CRH1 precursor	CRH1	99% (2)	100% (4)
Protein ZPS1 precursor	ZPS1	100% (5)	100% (4)
40S ribosomal protein S20	RPS20	89% (1)	
Covalently-linked cell wall protein 11 precursor	CIS3	89% (1)	86% (1)
Protein TOS1 precursor	TOS1	100% (3)	100% (3)
Protein SIM1 precursor	SIM1	99% (2)	99% (2)
Protein EGT2 precursor	EGT2	89% (1)	86% (1)
Vacuolar ATP synthase subunit B	VAT2	89% (1)	86% (1)
Uncharacterized protein YGL258W	YGL258W	93% (1)	86% (1)
60S ribosomal protein L8-B	RPL8B	89% (1)	
6-phosphofructokinase subunit beta	PFK2	89% (1)	86% (1)
Ribonucleoside-diphosphate reductase small chain 1	RIR2	89% (1)	
Endo-1,3(4)-beta-glucanase 1 precursor	ENG1	89% (1)	
Adenosine kinase	ADO1	89% (1)	86% (1)
Seripauperin-2	PAU2	89% (1)	86% (1)
Aconitate hydratase, mitochondrial precursor	ACO1	89% (1)	
Transcriptional modulator WTM1	WTM1	89% (1)	

Reticulon-like protein 1	RTN1		86% (1)
Protein UTH1	UTH1	89% (1)	86% (1)
Uncharacterized protein YJL171C precursor	YJR1		86% (1)
Pyruvate decarboxylase isozyme 2	PDC5	80% (1)	
Heat shock protein SSC1, mitochondrial precursor	HSP77	89% (1)	
Protein ZEO1	ZEO1	89% (1)	
Cytochrome c oxidase copper chaperone	COX17		86% (1)
Probable family 17 glucosidase SCW10 precursor	SCW10	89% (1)	99% (2)
Protein PRY3	PRY3	89% (1)	99% (2)
Invertase 2 precursor	SUC2	89% (1)	99% (2)
Endochitinase precursor	CTS1	99% (2)	86% (1)
Cell wall protein AWA1 precursor	AWA1	89% (1)	86% (1)
Metallothionein precursor	CUP1-1		86% (1)
Actin-binding protein	ABP1		86% (1)
Homoserine kinase	THR1	89% (1)	
6-phosphofructokinase subunit alpha	PFK1	89% (1)	
Suppressor protein STM1	STM1		86% (1)
GrpE protein homolog, mitochondrial precursor	MGE1		86% (1)

3 months

		RC212a	RC212b
Protoplast secreted protein 1 precursor	PST1	100% (15)	100% (14)
Phosphoglycerate kinase	PGK1	100% (5)	100% (4)
Enolase 1	ENO1	100% (7)	100% (4)
Cell wall protein DAN2 precursor	DAN2	100% (3)	100% (4)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (3)	100% (4)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (6)	100% (8)
Fructose-bisphosphate aldolase	FBA1	100% (2)	100% (5)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (3)	100% (5)
Probable family 17 glucosidase SCW4 precursor	SCW4	99% (2)	100% (3)
Lysophospholipase 2 precursor	PLB2		100% (3)
Protein YGP1 precursor	YGP1	100% (3)	100% (4)
Enolase 2	ENO2	90% (1)	88% (1)
Thioredoxin-2	TRX2	90% (1)	99% (2)
Protein ECM33 precursor	ECM33	99% (2)	100% (2)
Elongation factor 1-alpha	EF1A	90% (1)	88% (1)
Phosphoglycerate mutase 1	PMG1	99% (2)	88% (1)
Pyruvate decarboxylase isozyme 1	PDC1	99% (2)	
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	90% (1)	88% (1)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	90% (1)	
30 kDa heat shock protein	HSP30	90% (1)	88% (1)
Glycolipid-anchored surface protein 5 precursor	GAS5	94% (1)	96% (1)
Aminopeptidase Y precursor	APE3	99% (2)	99% (2)
40S ribosomal protein S3	RPS3		88% (1)
Thioredoxin-1	TRX1	90% (1)	99% (2)

Heat shock protein 26	HSP26	90% (1)	88% (1)
Glycogen debranching enzyme	GDB1	90% (1)	
Triosephosphate isomerase	TPI1	90% (1)	88% (1)
Pyruvate kinase 1	PYK1	99% (2)	88% (1)
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH2		88% (1)
Glycolipid-anchored surface protein 1 precursor	GAS1	99% (2)	88% (1)
Alcohol dehydrogenase 4	ADH4	90% (1)	88% (1)
FK506-binding protein 1	FPR1	90% (1)	88% (1)
NADP-specific glutamate dehydrogenase 1	DHE4	90% (1)	
Seripauperin-5	PAU5	99% (2)	99% (2)
Lysophospholipase 1 precursor	PLB1	95% (1)	99% (2)
Magnesium-activated aldehyde dehydrogenase, cytosolic	ALDH6		
Aspartic proteinase yapsin-3 precursor	YPS3	90% (1)	99% (2)
Repressible acid phosphatase precursor	PPA5	100% (3)	88% (1)
Plasma membrane ATPase 1	PMA1	90% (1)	88% (1)
Protein HMF1	HMF1	90% (1)	
40S ribosomal protein S20	RPS20	90% (1)	88% (1)
Uncharacterized protein YJL171C precursor	YJR1	99% (2)	
Peptidyl-prolyl cis-trans isomerase	CPR1	99% (2)	99% (2)
ATP synthase subunit beta, mitochondrial precursor	ATP2	90% (1)	
Metallothionein precursor	CUP1-1	99% (2)	
Vacuolar aminopeptidase 1 precursor	APE1	90% (1)	
Transcriptional modulator WTM1	WTM1	90% (1)	
Endo-1,3(4)-beta-glucanase 1 precursor	ENG1		88% (1)
40S ribosomal protein S9-A	RPS9A		88% (1)
Protein disulfide-isomerase precursor	PD11	90% (1)	88% (1)
150 kDa heat shock glycoprotein precursor	HS150	90% (1)	88% (1)
10 kDa heat shock protein, mitochondrial	CH10	90% (1)	
Glucose-6-phosphate isomerase	PGI1	90% (1)	
Phosphatidylinositol 4-kinase LSB6	LSB6	89% (1)	
Covalently-linked cell wall protein 11 precursor	CIS3		88% (1)
Protein PRY1 precursor	PRY1	90% (1)	88% (1)
Actin-binding protein	ABP1	90% (1)	
Uncharacterized protein YGL258W	YGZC		88% (1)
Protein ZPS1 precursor	ZPS1	99% (2)	99% (2)
40S ribosomal protein S15	RPS15		88% (1)
Uncharacterized protein YJL066C	YJG6		88% (1)
Protein MET17 [Includes: O-acetylhomoserine sulfhydrylase	MET17		88% (1)
Reticulon-like protein 1	RTN1	90% (1)	
Invertase 2 precursor	SUC2	90% (1)	99% (2)
Transaldolase	TAL1		98% (2)
6-phosphofructokinase subunit beta	PFK2	90% (1)	
Protein TOS1 precursor	TOS1		88% (1)
Probable glycosidase CRH1 precursor	CRH1		88% (1)
Protein transport protein SEC31	SEC31	90% (1)	
60S ribosomal protein L4-A	RPL4A		88% (1)
Cell wall protein DAN3 precursor	DAN3		88% (1)
LAS seventeen-binding protein 3	LSB3		88% (1)

Homoserine dehydrogenase	HOM6		88% (1)
6 months			
Protoplast secreted protein 1 precursor	PST1	RC212a 100% (13)	RC212b 100% (13)
Cell wall protein DAN2 precursor	DAN2	100% (4)	100% (3)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (7)	100% (9)
Phosphoglycerate kinase	PGK1	100% (3)	99% (2)
Lysophospholipase 2 precursor	PLB2	99% (2)	
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (6)	100% (5)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (4)	100% (5)
Protein YGP1 precursor	YGP1	100% (3)	99% (2)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (3)	100% (3)
Protein ECM33 precursor	ECM33	99% (2)	100% (3)
Repressible acid phosphatase precursor	PHO5	100% (2)	
Fructose-bisphosphate aldolase	FBA1	99% (2)	99% (2)
Aminopeptidase Y precursor	APE3	99% (2)	99% (2)
Lysophospholipase 1 precursor	PLB1		99% (2)
Elongation factor 1-alpha	TEF1	99% (2)	99% (2)
Covalently-linked cell wall protein 14 precursor	CCW14	99% (2)	99% (2)
Seripauperin-5	PAU5	99% (2)	99% (2)
Thioredoxin-2	TRX2	99% (2)	
Phosphoglycerate mutase 1	GMP1	99% (2)	
Uncharacterized protein YJL171C precursor	YJR1		100% (3)
Protein SIM1 precursor	SIM1		99% (2)
Uncharacterized protein YGL258W	YGL258W		99% (2)
Protein ZPS1 precursor	ZPS1	100% (4)	100% (6)
9 months			
Seripauperin-17 precursor	PAU17	RC212a 100% (5)	RC212b 100% (6)
Cell wall mannoprotein PST1 precursor	PST1	100% (15)	100% (15)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (11)	100% (12)
Phosphoglycerate kinase	PGK1	100% (6)	100% (5)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (6)	100% (3)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (8)	100% (7)
Protein YGP1 precursor	YGP1	100% (4)	100% (6)
Phosphoglycerate mutase 1	GMP1	100% (6)	100% (3)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (4)	99% (2)
Cell wall protein DAN3 precursor	DAN3	100% (3)	99% (2)
Cell wall protein ECM33 precursor	ECM33	100% (4)	99% (2)
Glycolipid-anchored surface protein 5 precursor	GAS5	65% (1)	93% (1)
Pyruvate kinase 1	PYK1	100% (4)	100% (3)
Seripauperin-5	PAU5	99% (2)	99% (2)
Glycolipid-anchored surface protein 1 precursor	GAS1	99% (2)	99% (2)

Uncharacterized protein YJL171C precursor	YJR1	99% (3)	97% (1)
Probable glycosidase CRH1 precursor	CRH1	96% (1)	97% (2)
Enolase 1	ENO1	100% (3)	
Elongation factor 1-alpha	TEF1	99% (2)	
Fructose-bisphosphate aldolase	FBA1	99% (2)	
Septation protein SUN4 precursor	SUN4	65% (1)	100% (3)
Protein transport protein SEC31	SEC31	99% (2)	66% (1)
Protein ZPS1 precursor	ZPS1	100% (6)	100% (5)
Pyruvate decarboxylase isozyme 1	PDC1	100% (5)	100% (3)
6-phosphofructokinase subunit beta	PFK2	65% (1)	99% (2)
Protein VEL1 precursor	VEL1	100% (3)	
Probable family 17 glucosidase SCW10 precursor	SCW10	99% (2)	99% (2)
Protein TOS1 precursor	TOS1	100% (3)	
Protein SIM1 precursor	SIM1		99% (2)
Putative uncharacterized protein YIL169C precursor	YIQ9	94% (1)	
Pyruvate decarboxylase isozyme 2	PDC5	98% (2)	

¹ The percentage adjacent to the Gene Id is the Bayesian probability that the protein is correctly identified; the number of peptides attributed to the protein is in parentheses.

Appendix 7. Proteins identified in wines made by strain BM45¹

0 Days

		BM45a	BM45b
Protoplast secreted protein 1 precursor	PST1	90% (1)	90% (1)
Glyceraldehyde-3-phosphate dehydrogenase 2	TDH2	99% (2)	99% (2)
Protein ECM33 precursor	ECM33	90% (1)	90% (1)

7 days

		BM45a	BM45b
Protoplast secreted protein 1 precursor	PST1	100% (11)	100% (15)
Enolase 1	ENO1	100% (4)	100% (4)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (7)	100% (6)
Phosphoglycerate kinase	PGK1	100% (5)	100% (4)
Cell wall protein DAN2 precursor	DAN2	99% (2)	
Fructose-bisphosphate aldolase	FBA1	100% (4)	100% (3)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (3)	100% (5)
Heat shock protein SSA2	HSP72	100% (3)	
Enolase 2	ENO2	90% (1)	85% (1)
Phosphoglycerate mutase 1	PMG1	100% (3)	100% (2)
Pyruvate decarboxylase isozyme 1	PDC1	90% (1)	85% (1)
Glyceraldehyde-3-phosphate dehydrogenase 3	TDH3	90% (1)	
Heat shock protein 26	HSP26	99% (2)	100% (3)
Pyruvate kinase 1	PYK1	100% (4)	85% (1)
Probable family 17 glucosidase SCW4 precursor	SCW4	99% (2)	100% (3)
Triosephosphate isomerase	TPI1	90% (1)	99% (2)
Thioredoxin I	TRX1	90% (1)	100% (3)

Thioredoxin II	TRX2	90% (1)	100% (5)
12 kDa heat shock protein	HSP12		99% (2)
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	90% (1)	
ATP synthase subunit alpha, mitochondrial precursor	ATP1	90% (1)	99% (2)
Glucan 1,3-beta-glucosidase precursor	BGL2	90% (1)	100% (4)
Protein YGP1 precursor	YGP1	90% (1)	99% (2)
Protein ECM33 precursor	ECM33	90% (1)	100% (4)
Peroxiredoxin TSA1	TSA1	90% (1)	
Actin	ACT1	99% (2)	
Lysophospholipase 2 precursor	PLB2	90% (1)	100% (3)
Alcohol dehydrogenase 1	ADH1		99% (2)
Outer mitochondrial membrane protein porin 1	POR1	90% (1)	
Ketol-acid reductoisomerase, mitochondrial precursor	ILV5	90% (1)	
ATP synthase subunit beta, mitochondrial precursor	ATP2	90% (1)	99% (2)
Elongation factor 1-alpha	TEF1	90% (1)	85% (1)
Alcohol dehydrogenase 4	ADH4	90% (1)	85% (1)
Malate dehydrogenase, mitochondrial precursor	MDH1		85% (1)
Peroxiredoxin type-2	AHP1	90% (1)	85% (1)
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	MET6	90% (1)	85% (1)
Heat shock protein SSA1	HSP71		85% (1)
60S ribosomal protein L8-A	RPL8A	90% (1)	85% (1)
Seripauperin-5	PAU5	90% (1)	85% (1)
Aminopeptidase Y precursor	APE3		85% (1)
Saccharopine dehydrogenase [NAD+, L-lysine-forming]	LYS1_	90% (1)	
Transaldolase	TAL1		85% (1)
Protein HMF1	HMF1	90% (1)	
40S ribosomal protein S5	RPS5	90% (1)	
Heat shock protein SSC1, mitochondrial precursor	HSP77		85% (1)
Glycolipid-anchored surface protein 1 precursor	GAS1		99% (2)
60S ribosomal protein L4-A	RPL4A	90% (1)	85% (1)
Hexokinase-1	HXK1	90% (1)	
Glycolipid-anchored surface protein 5 precursor	GAS5		99% (2)
40S ribosomal protein S3	RPS3	90% (1)	
Protein ZPS1 precursor	ZPS1	99% (2)	
Aconitate hydratase, mitochondrial precursor	ACO1		85% (1)
Lysophospholipase 1 precursor	PLB1		85% (1)
6-phosphofructokinase subunit beta	PFK2	90% (1)	85% (1)
10 kDa heat shock protein, mitochondrial	HSP10		85% (1)
Pyruvate decarboxylase isozyme 2	PDC5	90% (1)	
Protein disulfide-isomerase precursor	PDI1	90% (1)	
Transcriptional modulator WTM1	WTM1	90% (1)	
Transketolase 1	TKL1		85% (1)
Repressible acid phosphatase precursor	PHO5		99% (2)

Probable glycosidase CRH2 precursor	CRH2	90% (1)	
Suppressor protein STM1 - <i>Saccharomyces cerevisiae</i> (Baker's yeast)	STM1		85% (1)
Protein SIM1 precursor	SIM1		85% (1)
Protein UTH1	UTH1		85% (1)
Cell wall protein CWP1 precursor	CWP1		85% (1)

14 days

		BM45a	BM45b
Protoplast secreted protein 1 precursor	PST1	100% (13)	100% (13)
Enolase 1	ENO1	100% (6)	100% (7)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (6)	100% (7)
Phosphoglycerate kinase	PGK1	100% (7)	100% (6)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (5)	100% (4)
Cell wall protein DAN2 precursor	DAN2		100% (3)
Fructose-bisphosphate aldolase	FBA1	100% (5)	100% (4)
Enolase 2	ENO2	90% (1)	90% (1)
Glyceraldehyde-3-phosphate dehydrogenase 3	TDH3	100% (3)	90% (1)
Pyruvate decarboxylase isozyme 1	PDC1	100% (4)	100% (5)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (4)	90% (1)
Heat shock protein 26	HSP26	100% (4)	100% (3)
Thioredoxin I	TRX1	100% (4)	99% (2)
Pyruvate kinase 1	PYK1	100% (3)	99% (2)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (4)	100% (4)
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	MET6	100% (4)	90% (1)
Protein YGP1 precursor	YGP1	100% (5)	100% (4)
Thioredoxin II	TRX2	99% (2)	99% (2)
Heat shock protein SSA2	HSP72	100% (4)	100% (4)
Phosphoglycerate mutase 1	PMG1	90% (1)	88% (1)
Protein ECM33 precursor	ECM33	99% (2)	90% (1)
Triosephosphate isomerase	TPI1	90% (1)	
Lysophospholipase 2 precursor	PLB2	99% (2)	93% (1)
Alcohol dehydrogenase 4	ADH4	99% (2)	99% (2)
6-phosphogluconate dehydrogenase, decarboxylating 1 -	GND1	99% (2)	99% (2)
12 kDa heat shock protein	HSP12	99% (2)	99% (2)
Glycolipid-anchored surface protein 1 precursor	GAS1	100% (4)	99% (2)
Elongation factor 1-alpha	TEF1	90% (1)	
Alcohol dehydrogenase 1	ADH1	99% (2)	90% (1)
40S ribosomal protein S17-A	RPS17A	90% (1)	90% (1)
Probable glycosidase CRH1 precursor	CRH1	97% (1)	
Transaldolase	TAL1		90% (1)
Peroxiredoxin TSA1	TSA1	90% (1)	99% (2)
Seripauperin-5	PAU5		88% (1)
30 kDa heat shock protein	HSP30	90% (1)	
Glycolipid-anchored surface protein 5 precursor	GAS5	100% (3)	
Uncharacterized protein YGL258W	YGL258W	83% (1)	
Histone H2A.1	HTA1	90% (1)	90% (1)
60S ribosomal protein L19	RPL19A	90% (1)	

Aminopeptidase Y precursor	APE3	97% (1)	
Peroxiredoxin type-2	AHP1	90% (1)	99% (2)
ATP synthase subunit alpha, mitochondrial precursor	ATP1		90% (1)
Protein PRY1 precursor	PRY1	85% (1)	
Suppressor protein STM1	STM1	99% (2)	
Protein SIM1 precursor	SIM1	90% (1)	
Vacuolar ATP synthase catalytic subunit A	VMA1	90% (1)	
Aconitate hydratase, mitochondrial precursor	ACO1	90% (1)	
60S ribosomal protein L13-A	RPL13A	90% (1)	
40S ribosomal protein S16	RPS16	90% (1)	
40S ribosomal protein S5	RPS5	90% (1)	99% (2)
Outer mitochondrial membrane protein porin 1	POR1	90% (1)	
Cell wall protein DAN3 precursor	DAN3		90% (1)
Aspartate-semialdehyde dehydrogenase	HOM2	90% (1)	
Aspartic proteinase yapsin-3 precursor	YPS3	90% (1)	90% (1)
ATP synthase subunit beta, mitochondrial precursor	ATP2	90% (1)	99% (2)
60S ribosomal protein L4-A	RL4A		99% (2)
Mannose-1-phosphate guanyltransferase	MPG1		90% (1)
60S acidic ribosomal protein P2-beta	RLA4		90% (1)
Heat shock protein SSB1	HSP75		90% (1)
Uncharacterized protein YNL200C	YNL200C	90% (1)	
40S ribosomal protein S3	RPS3	90% (1)	
Hypothetical 41.2 kDa protein in FPR1-TOM22 intergenic region	YNN4	90% (1)	90% (1)
Eukaryotic translation initiation factor 5A-1	HYP1	90% (1)	90% (1)
Heat shock protein SSC1, mitochondrial precursor	HSP77	90% (1)	
10 kDa heat shock protein, mitochondrial	HSP10	90% (1)	90% (1)
Protein PIR3 precursor	PIR3		90% (1)
Glucose-6-phosphate isomerase	PGI1	99% (2)	
40S ribosomal protein S27-A	RPS27A	90% (1)	
40S ribosomal protein S6	RPS6	90% (1)	
Homoserine dehydrogenase	HOM6		90% (1)
FK506-binding protein 1	FPR1		90% (1)
40S ribosomal protein S20	RPS20	90% (1)	
Superoxide dismutase [Cu-Zn]	SOD1	96% (1)	
Phosphoglucomutase-2	PGM2		100% (3)
60S ribosomal protein L28	RPL28		90% (1)
Saccharopine dehydrogenase [NAD+, L-lysine-forming]	LYS1	90% (1)	90% (1)
Probable glycosidase CRH2 precursor	CRH2	90% (1)	
26S proteasome regulatory subunit RPN10	RPN10		90% (1)
2-oxoglutarate dehydrogenase E1 component, mitochondrial precursor	ODO1	90% (1)	90% (1)
Phospho-2-dehydro-3-deoxyheptonate aldolase, tyrosine-inhibited	ARO4		90% (1)
Dihydroxy-acid dehydratase, mitochondrial precursor	ILV3	88% (1)	
Intermembrane space import and assembly protein 40, mitochondrial precursor	MIA40		90% (1)
Magnesium-activated aldehyde	ALDH6	90% (1)	

dehydrogenase, cytosolic -			
Fatty acid synthase subunit alpha	FAS2	90% (1)	
Endo-1,3(4)-beta-glucanase 1 precursor	ENG1		90% (1)
Glycogen debranching enzyme	GDB1		90% (1)
Malate dehydrogenase, mitochondrial precursor	MDH1		90% (1)
Protease A inhibitor 3	IPA3		90% (1)
Uncharacterized protein YJL066C	YJL066C		90% (1)
ATP-dependent molecular chaperone HSC82	HSC82		90% (1)

1 month

		BM45a	BM45b
Protoplast secreted protein 1 precursor	PST1	100% (12)	100% (13)
Phosphoglycerate kinase	PGK1	100% (9)	100% (6)
Enolase 1	ENO1	100% (9)	100% (3)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (7)	100% (5)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (6)	100% (8)
Cell wall protein DAN2 precursor	DAN2	100% (6)	100% (3)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (7)	100% (7)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (3)	100% (3)
Fructose-bisphosphate aldolase	FBA1	100% (2)	100% (3)
Lysophospholipase 2 precursor	PLB2	100% (6)	100% (5)
Protein YGP1 precursor	YGP1	100% (4)	100% (3)
Phosphoglycerate mutase 1	PMG1	89% (1)	83% (1)
Peroxiredoxin type-2	AHP1	100% (3)	100% (3)
Seripauperin-5	PAU5	100% (3)	99% (2)
Thioredoxin-2	TRX2	100% (3)	100% (3)
Protein ECM33 precursor	ECM33	99% (2)	100% (4)
Aspartic proteinase yapsin-3 precursor	YPS3	100% (4)	100% (3)
Glycolipid-anchored surface protein 5 precursor	GAS5	100% (4)	100% (3)
Thioredoxin-1	TRX1	100% (3)	100% (3)
Heat shock protein SSA1	HSP71		100% (4)
Pyruvate decarboxylase isozyme 1	PDC1	89% (1)	83% (1)
Enolase 2	ENO2	89% (1)	83% (1)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	89% (1)	99% (2)
Glycolipid-anchored surface protein 1 precursor	GAS1	100% (4)	100% (3)
Heat shock protein 26	HSP26	99% (2)	100% (3)
30 kDa heat shock protein	HSP30	89% (1)	83% (1)
12 kDa heat shock protein	HSP12	99% (2)	100% (4)
Elongation factor 1-alpha	TEF1	99% (2)	99% (2)
Pyruvate kinase 1	PYK1	89% (1)	83% (1)
Aminopeptidase Y precursor	APE3	89% (1)	83% (1)
Triosephosphate isomerase	TPI1	89% (1)	83% (1)
Transaldolase	TAL1	100% (3)	100% (3)
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	MET6	89% (1)	
Lysophospholipase 1 precursor	PLB1	89% (1)	83% (1)
Glycogen debranching enzyme	GDB1	89% (1)	83% (1)

FK506-binding protein 1	FKBP	89% (1)	83% (1)
ATP synthase subunit beta, mitochondrial precursor	ATP2	89% (1)	83% (1)
60S ribosomal protein L4-A	RPL4A	89% (1)	83% (1)
NADP-specific glutamate dehydrogenase 1	DHE4	99% (2)	
150 kDa heat shock glycoprotein precursor	HS150	89% (1)	83% (1)
Protein disulfide-isomerase precursor	PDI1	89% (1)	83% (1)
Plasma membrane ATPase 1	PMA1		83% (1)
Superoxide dismutase [Cu-Zn]	SOD1		99% (2)
Saccharopine dehydrogenase [NAD ⁺ , L-lysine-forming]	LYS1	89% (1)	
Histone H2A.1	HTA1	89% (1)	
Repressible acid phosphatase precursor	PPA5	93% (1)	94% (1)
Dihydroxy-acid dehydratase, mitochondrial precursor	ILV3	89% (1)	
Alcohol dehydrogenase 4	ADH4	89% (1)	83% (1)
Probable glycosidase CRH1 precursor	CRH1		99% (2)
40S ribosomal protein S20	RPS20	89% (1)	
Covalently-linked cell wall protein 11 precursor	CIS3	89% (1)	83% (1)
Malate dehydrogenase, mitochondrial precursor	MDH1	89% (1)	83% (1)
Protein TOS1 precursor	TOS1	89% (1)	
Covalently-linked cell wall protein 14 precursor	CCW14	100% (3)	99% (2)
Protein SIM1 precursor	SIM1	89% (1)	83% (1)
Protein EGT2 precursor	EGT2	89% (1)	
40S ribosomal protein S3	RPS3	89% (1)	83% (1)
Vacuolar ATP synthase subunit B	VAT2		83% (1)
Uncharacterized protein YGL258W	YGL258W		83% (1)
60S ribosomal protein L8-B	RPL8B	99% (2)	83% (1)
6-phosphofructokinase subunit beta	PFK2	89% (1)	83% (1)
Endo-1,3(4)-beta-glucanase 1 precursor	ENG1		83% (1)
Adenosine kinase	ADO1	89% (1)	
Seripauperin-2	PAU2		83% (1)
Aconitate hydratase, mitochondrial precursor	ACO1	89% (1)	83% (1)
Transcriptional modulator WTM1	WTM1	89% (1)	
Mannose-1-phosphate guanyltransferase	MPG1	89% (1)	
Protein UTH1	UTH1	89% (1)	83% (1)
Uncharacterized protein YJL171C precursor	YJR1		83% (1)
Phospho-2-dehydro-3-deoxyheptonate aldolase, tyrosine-inhibited	ARO4	89% (1)	
Protein ZEO1	ZEO1		83% (1)
Uncharacterized protein YJL066C	YJL066C	89% (1)	83% (1)
Cytochrome c oxidase copper chaperone	COX17	89% (1)	83% (1)
10 kDa heat shock protein, mitochondrial	HSP10		83% (1)
Acetolactate synthase catalytic subunit, mitochondrial precursor	ILV2	89% (1)	
Thioredoxin-3, mitochondrial precursor	TRX3		83% (1)
Metallothionein precursor	CUP1-1		83% (1)

Actin-binding protein	ABP1		83% (1)
Glucokinase GLK1	GLK1	89% (1)	
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial precursor	ODP2		83% (1)
Cell wall protein CWP1 precursor	CWP1		83% (1)
Mitochondrial intermembrane space cysteine motif-containing protein MIC14	MIC14		83% (1)

3 months

		BM45a	BM45b
Protoplast secreted protein 1 precursor	PST1	100% (15)	100% (15)
Phosphoglycerate kinase	PGK1	100% (5)	100% (5)
Enolase 1	ENO1	100% (7)	100% (5)
Cell wall protein DAN2 precursor	DAN2	99% (2)	100% (3)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (6)	100% (4)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (6)	100% (4)
Fructose-bisphosphate aldolase	FBA1	100% (3)	100% (3)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (5)	100% (5)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (3)	100% (2)
Lysophospholipase 2 precursor	PLB2	100% (5)	100% (3)
Protein YGP1 precursor	YGP1	100% (3)	99% (2)
Enolase 2	ENO2	88% (1)	90% (1)
Thioredoxin-2	TRX2	100% (3)	100% (3)
Protein ECM33 precursor	ECM33	100% (3)	99% (2)
Elongation factor 1-alpha	EF1A	99% (2)	99% (2)
Phosphoglycerate mutase 1	PMG1	99% (2)	90% (1)
Pyruvate decarboxylase isozyme 1	PDC1	88% (1)	90% (1)
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	88% (1)	90% (1)
ATP synthase subunit alpha, mitochondrial precursor	ATP1	99% (2)	99% (2)
30 kDa heat shock protein	HSP30	88% (1)	90% (1)
Glycolipid-anchored surface protein 5 precursor	GAS5	100% (4)	97% (1)
Aminopeptidase Y precursor	APE3	99% (2)	90% (1)
40S ribosomal protein S3	RPS3	88% (1)	95% (1)
Thioredoxin-1	TRX1	100% (3)	99% (2)
Heat shock protein 26	HSP26	100% (3)	99% (2)
Glycogen debranching enzyme	GDB1	88% (1)	90% (1)
Triosephosphate isomerase	TPI1	99% (2)	90% (1)
Pyruvate kinase 1	PYK1	99% (2)	90% (1)
Glycolipid-anchored surface protein 1 precursor	GAS1	100% (3)	99% (2)
Alcohol dehydrogenase 4	ADH4	88% (1)	90% (1)
FK506-binding protein 1	FPR1	88% (1)	90% (1)
NADP-specific glutamate dehydrogenase 1	DHE4	88% (1)	
Seripauperin-5	PAU5		90% (1)
Lysophospholipase 1 precursor	PLB1	99% (2)	
Magnesium-activated aldehyde dehydrogenase, cytosolic	ALDH6	88% (1)	90% (1)
Aspartic proteinase yapsin-3 precursor	YPS3	88% (1)	90% (1)

12 kDa heat shock protein	HSP12	88% (1)	90% (1)
Repressible acid phosphatase precursor	PPA5	88% (1)	90% (1)
Plasma membrane ATPase 1	PMA1	88% (1)	90% (1)
40S ribosomal protein S20	RPS20	88% (1)	
Uncharacterized protein YJL171C precursor	YJR1	88% (1)	90% (1)
Protein MMF1, mitochondrial precursor	MMF1	99% (2)	98% (2)
Peptidyl-prolyl cis-trans isomerase	CPR1	93% (1)	90% (1)
ATP synthase subunit beta, mitochondrial precursor	ATP2	88% (1)	
Metallothionein precursor	CUP1-1	88% (1)	
Trehalose synthase complex regulatory subunit TSL1	TSL1	88% (1)	
Vacuolar aminopeptidase 1 precursor	APE1	88% (1)	
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	MET6		90% (1)
60S ribosomal protein L20	RPL20	88% (1)	
Transcriptional modulator WTM1	WTM1		90% (1)
Endo-1,3(4)-beta-glucanase 1 precursor	ENG1		90% (1)
40S ribosomal protein S9-A	RPS9A		90% (1)
Protein disulfide-isomerase precursor	PDI1	88% (1)	90% (1)
150 kDa heat shock glycoprotein precursor	HS150	88% (1)	90% (1)
60S ribosomal protein L8-A	RPL8A		90% (1)
10 kDa heat shock protein, mitochondrial	CH10	88% (1)	90% (1)
Glucose-6-phosphate isomerase	PGI1	88% (1)	
Covalently-linked cell wall protein 11 precursor	CIS3	88% (1)	90% (1)
Heat shock protein homolog SSE1	HSP7F		90% (1)
Actin-binding protein	ABP1	88% (1)	
Alcohol dehydrogenase 1	ADH1		99% (2)
Vacuolar ATP synthase subunit B -	VAT2	88% (1)	
Mitochondrial intermembrane space cysteine motif-containing protein MIC14	MIC14	88% (1)	90% (1)
Uncharacterized protein YJL066C	YJG6	88% (1)	
26S proteasome regulatory subunit RPN10	RPN10	88% (1)	
Superoxide dismutase [Cu-Zn]	SOD1	88% (1)	90% (1)
Polyadenylate-binding protein, cytoplasmic and nuclear	PAB1	88% (1)	
Hexokinase-1	HXK1		90% (1)
Histone H2A.1	HTA1		90% (1)
Anaphase-promoting complex subunit 2	APC2	88% (1)	
Heat shock protein SSA4	HSP74	88% (1)	
Intermembrane space import and assembly protein 40, mitochondrial precursor	MIA40	88% (1)	
Aconitate hydratase, mitochondrial precursor	ACO1	88% (1)	
6 months			
		BM45a	BM45b
Protoplast secreted protein 1 precursor	PST1	100% (15)	100% (15)
Cell wall protein DAN2 precursor	DAN2		99% (2)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (8)	100% (8)
Phosphoglycerate kinase	PGK1	100% (4)	100% (3)
Lysophospholipase 2 precursor	PLB2	100% (6)	100% (7)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (7)	100% (6)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (4)	100% (5)

Protein YGP1 precursor	YGP1	100% (5)	100% (3)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (4)	100% (4)
Protein ECM33 precursor	ECM33	100% (4)	100% (4)
Glycolipid-anchored surface protein 5 precursor	GAS5	99% (2)	100% (5)
Probable glycosidase CRH1 precursor	CRH1	100% (5)	100% (4)
Repressible acid phosphatase precursor	PPA5	100% (3)	99% (2)
Fructose-bisphosphate aldolase	FBA1	100% (4)	99% (2)
12 kDa heat shock protein	HSP12	99% (2)	100% (3)
Aminopeptidase Y precursor	APE3	99% (2)	
Lysophospholipase 1 precursor	PLB1	100% (2)	99% (2)
Elongation factor 1-alpha	TEF1		99% (2)
Glycolipid-anchored surface protein 1 precursor	GAS1	100% (3)	100% (4)
Covalently-linked cell wall protein 14 precursor	CCW14	99% (2)	99% (2)
Seripauperin-5	PAU5	99% (2)	
Thioredoxin-2	TRX2	100% (3)	
Phosphoglycerate mutase 1	GPM1		99% (2)
Thioredoxin-1	TRX1	99% (2)	
Protein SIM1 precursor	SIM1	99% (2)	
Uncharacterized protein YGL258W	YGL258W	99% (2)	
Mitochondrial intermembrane space cysteine motif-containing protein MIC14	MIC14		99% (2)
Metallothionein precursor	CUP1-1		
Peroxiredoxin type-2	AHP1	100% (3)	
40S ribosomal protein S3	RPS3	99% (2)	
Intermembrane space import and assembly protein 40, mitochondrial precursor	MIA40	99% (2)	

9 months

		BM45a	BM45b
Seripauperin-17 precursor	PAU17	100% (5)	100% (5)
Cell wall mannoprotein PST1 precursor	PST1	100% (14)	100% (16)
Glucan 1,3-beta-glucosidase I/II precursor	EXG1	100% (6)	100% (11)
Phosphoglycerate kinase	PGK1	100% (8)	100% (5)
Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	100% (11)	100% (3)
Glucan 1,3-beta-glucosidase precursor	BGL2	100% (9)	100% (8)
Protein YGP1 precursor	YGP1	100% (8)	100% (4)
Phosphoglycerate mutase 1	PMG1	100% (11)	100% (6)
Probable family 17 glucosidase SCW4 precursor	SCW4	100% (2)	99% (2)
Cell wall protein DAN3 precursor	DAN3	95% (1)	99% (3)
Cell wall protein ECM33 precursor	ECM33	99% (2)	100% (5)
Glycolipid-anchored surface protein 5 precursor	GAS5	100% (4)	99% (2)
Pyruvate kinase 1	PYK1	100% (5)	
Seripauperin-5	PAU5	99% (2)	99% (2)
Glycolipid-anchored surface protein 1 precursor	GAS1	100% (5)	97% (2)
Uncharacterized protein YJL171C precursor	YJR1	95% (1)	94% (1)

Probable glycosidase CRH1 precursor	CRH1	100% (3)	99% (2)
Enolase 1	ENO1	100% (7)	
Elongation factor 1-alpha	TEF1	100% (4)	98% (2)
12 kDa heat shock protein	HSP12	100% (7)	65% (1)
Fructose-bisphosphate aldolase	FBA1	100% (4)	99% (2)
Lysophospholipase 2 precursor	PLB2		99% (2)
Septation protein SUN4 precursor	SUN4		99% (2)
Covalently-linked cell wall protein 14 precursor	CCW14		99% (2)
Protein transport protein SEC31	SEC31	94% (1)	65% (1)
Protein PIR3 precursor	PIR3		65% (1)
Heat shock protein SSA1	SSA1	99% (2)	
Pyruvate decarboxylase isozyme 1	PDC1		100% (3)
Glycogen debranching enzyme	GDB1	94% (2)	
Heat shock protein 26	HSP26	100% (4)	
Thioredoxin-2	TRX2	99% (2)	
Alcohol dehydrogenase 4	ADH4	99% (2)	
Protein SIM1 precursor	SIM1		
Peptidyl-prolyl cis-trans isomerase	CPR1	93% (1)	95% (1)
Triosephosphate isomerase	TPI1	100% (3)	
40S ribosomal protein S3	RPS3	99% (2)	
Actin-binding protein	ABP1	99% (2)	
ATP synthase subunit alpha, mitochondrial precursor	ATP1	100% (3)	
6-phosphogluconate dehydrogenase, decarboxylating 1	GND1	99% (2)	

¹ The percentage adjacent to the Gene Id is the Bayesian probability that the protein is correctly identified; the number of peptides attributed to the protein is in parentheses.

Appendix 8. Wine protein compositions of sparkling wines from Argyle winery

To determine the order of protein release from yeast during bottle aging of sparkling wine prior to disgorging, samples were taken from multiple vintages, including 2005, 2003, 2002, and 2000, and analyzed by the nearly the same methods used for the model wines. Some differences were an initial degassing of the wine followed by filtration through a 0.45 μ m filter. Another difference involved an 80% Ammonium sulfate precipitation and resolubilization of the wine protein following concentration. The samples were analyzed in February of 2008, which means that each sample was 1 year and 8 months older than its vintage. For example, the 2000 vintage was 6 years and 8 months post-disgorging when it was analyzed.

Mannoprotein and protein concentration in 4 vintage sparkling wines

Mannoprotein equivalents	2000	2002	2003	2005
	μ g/mL	μ g/mL	μ g/mL	μ g/mL
replicate 1	0.8	1.4	4.1	6.4
replicate 2	1.2	2.7	5.7	10.1
replicate 3	0.4	3.4	6.5	5.5
mean	0.8	2.5	5.4	7.3
std dev	0.4	1.0	1.2	2.4
CV	48.5	39.7	22.8	33.2

Protein concentration	2000	2002	2003	2005
	μ g/mL	μ g/mL	μ g/mL	μ g/mL
replicate 1	2.00	25.17	45.79	38.44
replicate 2	3.57	25.71	48.15	34.02
replicate 3	3.16	29.21	45.19	32.40
mean	2.91	26.69	46.38	34.96
std dev	0.81	2.19	1.57	3.13
CV	27.82	8.22	3.38	8.95

Protein compositions of four vintage sparkling wines

2000

Protein identified	Family	Homolog	Replicate 1	Replicate 2	Replicate 3
unnamed protein product	<i>Vitis vinifera</i>	VVTL1	100% (7)		
hypothetical protein [Vitis vinifera]	<i>Vitis vinifera</i>		99% (2)	99% (2)	99% (2)
unnamed protein product [Vitis vinifera]	<i>Vitis vinifera</i>		100% (4)		
phosphoribosylglycinamide formyltransferase	<i>Alkaliphilus metalliredigens</i>				90% (1)
hypothetical protein	<i>Oceanobacter</i>				90% (1)

2002

Protein identified	Family	Homolog	Replicate 1	Replicate 2	Replicate 3
unnamed protein product	<i>Vitis vinifera</i>	VVTL1	100% (21)	100% (23)	100% (19)
hypothetical protein	<i>Vitis vinifera</i>		100% (15)	100% (16)	100% (14)
PR-4 type protein	<i>Vitis vinifera</i>		100% (4)	100% (5)	100% (5)
unnamed protein product	<i>Vitis vinifera</i>		100% (14)	100% (16)	100% (13)
lipid transfer protein isoform 4	<i>Vitis vinifera</i>		100% (3)	100% (3)	100% (3)
putative thaumatin-like protein	<i>Vitis hybrid cultivar</i>	<i>Vitis vinifera</i>	99% (2)	100% (4)	100% (4)
lipid transfer protein isoform 1	<i>Vitis vinifera</i>		100% (6)	100% (4)	100% (3)
hypothetical protein	<i>Vitis vinifera</i>		100% (3)	100% (4)	100% (3)
class IV chitinase	<i>Vitis pseudoreticulata</i>	putative class iv <i>V. vinifera</i>	100% (4)	100% (6)	100% (5)
unnamed protein product	<i>Vitis vinifera</i>		96% (1)	99% (2)	76% (1)
PAU1	<i>S. cerevisiae</i>		68% (1)	79% (1)	76% (1)
thaumatin-like protein	<i>Vitis vinifera</i>		98% (1)	99% (2)	100% (2)
thaumatin-like protein	<i>Vitis vinifera</i>		100% (4)	100% (4)	100% (2)
thaumatin-like protein	<i>Vitis vinifera</i>		68% (1)	79% (1)	76% (1)
hypothetical protein	<i>Vitis vinifera</i>		99% (2)	99% (2)	99% (2)
thaumatin-like protein	<i>Actinidia deliciosa</i>		68% (1)	99% (2)	99% (2)
unknown	<i>Populus trichocarpa x Populus deltoides</i>		68% (1)	79% (1)	76% (1)
Os03g0663500	<i>Oryza sativa</i>	PR related		97% (1)	76% (1)
hypothetical protein	<i>Vitis vinifera</i>		68% (1)	79% (1)	76% (1)
hypothetical protein	<i>Vitis vinifera</i>		68% (1)	79% (1)	76% (1)
thaumatin-like protein	<i>Sambucus nigra</i>			99% (2)	99% (2)
unnamed protein product	<i>Vitis vinifera</i>		68% (1)	79% (1)	76% (1)
osmotin-like protein	<i>Vitis vinifera</i>			99% (2)	76% (1)
thaumatin-like protein	<i>Vitis vinifera</i>		68% (1)	79% (1)	76% (1)
cell wall mannoprotein	<i>S. cerevisiae</i>	CCW14		100% (3)	99% (2)

2002

Protein identified	Family	Homolog	Replicate 1	Replicate 2	Replicate 3
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putative thaumatin-like protein	<i>Solanum tuberosum</i>		94% (1)		
osmotin-like protein	<i>Quercus suber</i>		95% (1)	100% (3)	76% (1)
pathogenesis-related thaumatin-like protein	<i>Coffea arabica</i>		97% (1)	79% (1)	99% (2)
hypothetical protein Cwp1p	<i>Vitis vinifera</i>	unnamed protein product	99% (2)	96% (1)	
hypothetical protein	<i>S. cerevisiae</i>			79% (1)	100% (3)
hypothetical protein	<i>Vitis vinifera</i>		68% (1)	79% (1)	76% (1)
hypothetical protein Copii Coat Subunit, Sec24	<i>Vitis vinifera</i>			79% (1)	
putative fructosyltransferase 2	<i>S. cerevisiae</i>		94% (1)	79% (1)	97% (0)
vacuolar invertase 1	<i>Lolium perenne</i>		68% (1)	79% (1)	76% (1)
RNA polymerase II second largest subunit	<i>Vitis vinifera</i>	GIN1	68% (1)	79% (1)	76% (1)
cell wall protein	<i>Sphagnum sp.</i>			79% (1)	
non-specific lipid transfer protein	<i>S. cerevisiae</i>	CRH1	99% (2)		
hypothetical protein Q0125	<i>Vitis vinifera</i>				99% (2)
beta-fructofuranosidase	<i>S. cerevisiae</i>			95% (1)	
sporulation-specific septin	<i>Citrus sinensis</i>		99% (2)		
					93% (1)

2003

Protein identified	Family	Homolog	Replicate 1	Replicate 2	Replicate 3
unnamed protein product	<i>Vitis vinifera</i>	VVTL1	100% (23)	100% (21)	100% (19)
hypothetical protein	<i>Vitis vinifera</i>		100% (18)	100% (17)	100% (18)
PR-4 type protein	<i>Vitis vinifera</i>		100% (5)	100% (5)	100% (5)
unnamed protein product	<i>Vitis vinifera</i>		100% (11)	100% (12)	100% (13)
lipid transfer protein isoform 4	<i>Vitis vinifera</i>		100% (3)	100% (4)	100% (3)
putative thaumatin-like protein	<i>Vitis hybrid cultivar</i>	<i>Vitis vinifera</i>	100% (4)	100% (5)	100% (5)
lipid transfer protein isoform 1	<i>Vitis vinifera</i>		100% (4)	100% (4)	100% (3)
hypothetical protein	<i>Vitis vinifera</i>		100% (3)	100% (4)	100% (4)
unnamed protein product	<i>Vitis vinifera</i>		98% (1)	94% (1)	99% (2)
PAU1	<i>S. cerevisiae</i>		77% (1)	71% (1)	82% (1)
thaumatin-like protein	<i>Vitis vinifera</i>		100% (3)	100% (3)	100% (3)
thaumatin-like protein	<i>Vitis vinifera</i>		100% (4)	100% (3)	100% (6)
thaumatin-like protein	<i>Vitis vinifera</i>		77% (1)	71% (1)	82% (1)
hypothetical protein	<i>Vitis vinifera</i>		77% (1)	71% (1)	82% (1)
thaumatin-like protein	<i>Actinidia deliciosa</i>		99% (2)	71% (1)	99% (2)

2003

Protein identified	Family	Homolog	Replicate 1	Replicate 2	Replicate 3
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unknown	<i>Populus trichocarpa x Populus deltoides</i>		77% (1)	71% (1)	96% (1)
Os03g0663500	<i>oryza sativa</i>	PR related		99% (2)	99% (2)
osmotin-like protein	<i>Fagus sylvatica</i>		77% (1)		99% (2)
hypothetical protein	<i>Vitis vinifera</i>		77% (1)		82% (1)
thaumatin-like protein	<i>Sambucus nigra</i>		77% (1)	99% (2)	99% (2)
pathogenesis-related thaumatin-like protein	<i>[Coffea arabica]</i>		77% (1)	71% (1)	82% (1)
unnamed protein product	<i>Vitis vinifera</i>		99% (2)	99% (2)	
osmotin-like protein	<i>Vitis vinifera</i>			99% (2)	99% (2)
thaumatin-like protein	<i>Vitis vinifera</i>		77% (1)	71% (1)	82% (1)
putative thaumatin-like protein	<i>Solanum tuberosum</i>		99% (2)	99% (2)	82% (1)
osmotin-like protein	<i>Quercus suber</i>		100% (3)	71% (1)	99% (2)
putative thaumatin-like protein	<i>Vitis vinifera</i>			71% (1)	82% (1)
pathogenesis-related thaumatin-like protein	<i>Coffea arabica</i>		77% (1)	71% (1)	82% (1)
hypothetical protein	<i>Vitis vinifera</i>	unnamed protein product		99% (2)	82% (1)
hypothetical protein	<i>Vitis vinifera</i>			99% (2)	
Copii Coat Subunit, Sec24	<i>S. cerevisiae</i>		64% (1)		98% (1)
suppressor of rho3	<i>S. cerevisiae</i>				76% (1)
putative outer membrane protein	<i>Pedobacter</i>	BAL39			82% (1)
Twin-arginine translocation pathway signal	<i>Roseobacter litoralis</i>				80% (1)
vacuolar invertase 1	<i>Vitis vinifera</i>	GIN1	77% (1)	71% (1)	82% (1)
RNA polymerase II second largest subunit	<i>Sphagnum sp.</i>		77% (1)	71% (1)	82% (1)
unknown	<i>Picea sitchensis</i>			71% (1)	82% (1)
GCYC	<i>Anodiscus xanthophyllus</i>			61% (1)	82% (1)
Pathogenesis-related protein R major form precursor	<i>Nicotiana tabacum</i>		77% (1)		82% (1)
conserved protein [YJM789]	<i>S. cerevisiae</i>		77% (1)	96% (1)	

2005

Protein identified	Family	Homolog	Replicate 1	Replicate 2	Replicate 3
unnamed protein product	<i>Vitis vinifera</i>	VVTL1	100% (21)	100% (18)	100% (20)
hypothetical protein	<i>Vitis vinifera</i>		100% (14)	100% (15)	100% (13)
PR-4 type protein	<i>Vitis vinifera</i>		100% (6)	100% (5)	100% (5)
unnamed protein product	<i>Vitis vinifera</i>		100% (12)	100% (12)	100% (12)

2005

Protein identified	Family	Homolog	Replicate 1	Replicate 2	Replicate 3
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lipid transfer protein isoform 4	<i>Vitis vinifera</i>		100% (5)	100% (3)	100% (3)
putative thaumatin-like protein	<i>Vitis hybrid cultivar</i>	Vitis vinifera	100% (3)	100% (4)	100% (4)
lipid transfer protein isoform 1	<i>Vitis vinifera</i>		100% (6)	100% (4)	100% (5)
hypothetical protein	<i>Vitis vinifera</i>		100% (3)	100% (4)	100% (3)
class IV chitinase unnamed protein product	<i>Vitis pseudoreticulata</i>	putative class iv V. <i>vinifera</i>	100% (7)		100% (6)
PAU1	<i>Vitis vinifera</i>		99% (2)	81% (1)	99% (2)
thaumatin-like protein	<i>S. cerevisiae</i>		99% (2)	81% (1)	76% (1)
thaumatin-like protein	<i>Vitis vinifera</i>		100% (3)	100% (3)	100% (3)
thaumatin-like protein	<i>Vitis vinifera</i>		100% (3)	100% (4)	100% (4)
thaumatin-like protein	<i>Vitis vinifera</i>		73% (1)	81% (1)	76% (1)
hypothetical protein	<i>Vitis vinifera</i>		100% (3)	81% (1)	99% (2)
thaumatin-like protein	<i>Actinidia deliciosa</i>		99% (2)	99% (2)	97% (1)
unknown	<i>Populus trichocarpa x Populus deltoides</i>		73% (1)	81% (1)	76% (1)
Os03g0663500	<i>Oryza sativa</i>	PR related	98% (2)	99% (2)	97% (1)
osmotin-like protein	<i>Fagus sylvatica</i>		73% (1)	99% (2)	99% (2)
hypothetical protein	<i>Vitis vinifera</i>		73% (1)		76% (1)
hypothetical protein	<i>Vitis vinifera</i>		73% (1)	81% (1)	76% (1)
thaumatin-like protein	<i>Sambucus nigra</i>		99% (2)	99% (2)	95% (0)
pathogenesis-related thaumatin-like protein	<i>Coffea arabica</i>		73% (1)	81% (1)	76% (1)
unnamed protein product	<i>Vitis vinifera</i>		73% (1)		76% (1)
osmotin-like protein	<i>Vitis vinifera</i>			99% (2)	76% (1)
thaumatin-like protein	<i>Vitis vinifera</i>		73% (1)	81% (1)	76% (1)
putative thaumatin-like protein	<i>Solanum tuberosum</i>		73% (1)		76% (1)
osmotin-like protein	<i>Quercus suber</i>		98% (1)		97% (1)
putative thaumatin-like protein	<i>Vitis vinifera</i>		93% (1)	81% (1)	76% (1)
pathogenesis-related thaumatin-like protein	<i>Coffea arabica</i>		73% (1)	99% (2)	76% (1)
hypothetical protein	<i>Vitis vinifera</i>	unnamed protein product [Vitis vinifera]	67% (1)	76% (1)	
hypothetical protein	<i>Vitis vinifera</i>		73% (1)	99% (2)	99% (2)
hypothetical protein	<i>Vitis vinifera</i>		99% (2)		
Copii Coat Subunit, Sec24	<i>S. cerevisiae</i>				96% (1)
hypothetical protein	<i>Vitis vinifera</i>			81% (1)	
nicotinate-nucleotide pyrophosphorylase	<i>Desulfotomaculum reducens</i>		73% (1)	81% (1)	
suppressor of rho3	<i>S. cerevisiae</i>				97% (1)
2005					
Protein identified	Family	Homolog	Replicate 1	Replicate 2	Replicate 3

putative				
fructosyltransferase 2	<i>Lolium perenne</i>		73% (1)	81% (1) 76% (1)
osmotin-like	<i>Theobroma cacao</i>			99% (2) 76% (1)
vacuolar invertase 1	<i>Vitis vinifera</i>	GIN1		81% (1) 76% (1)
hypothetical protein	<i>Coxiella burnetii</i>		99% (2)	
Pathogenesis-related				
protein R major form				
precursor	<i>Nicotiana tabacum</i>			81% (1) 76% (1)
osmotin-like protein	<i>Quercus robur</i>		94% (1)	
	<i>Arabidopsis</i>			
thaumatin-like protein	<i>thaliana</i>			80% (1)
Pol I core factor CF				
[YJM789]	<i>S. cerevisiae</i>		92% (1)	

Appendix 9. Master list of proteins and localization

Gene name	Compartment	0 day	7 days	14 days	45 days	105 days	195 days	285 days
APC2	anaph prompt comp core TFIIH					7		
TFB4	comp			2 6				
ABP1	cyto		6		6 7	4 6 7	2	7
ACT1	cyto		1 2 3 4 5 6 7	2 3	1 2 4	2 3 5		
ADE17	cyto				1 2	1 2 3 5		
ADH1	cyto	2	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4	2 3 7		
AHP1	cyto		1 4 5 6 7	1 2 5 6 7	1 2 3 5 6 7	2 3	7	
CUP1-1	cyto		1	5	6 7	4 6 7	2	
DDR48	cyto					1 2 5		
ERG10	cyto			1				
GLK1	cyto		4 5	2	7			
LYS1	cyto		3 4 5 7	7	1 2 3 4 5 7	1 2 3 4		
MET17	cyto		1	3 4 5 6	2	5 6		
MET6	cyto		1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 7	2 3 5 7		
PGM2	cyto	2			3	5		
PSA1	cyto		2 5 6	1 5 6 7				
PYK1	cyto	2	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 7	2 3 4 5 6 7	2 3	1 2 3 4 5 6 7
RPL5	cyto			5				
RPL1A	cyto		4					
RPL13A	cyto		1 2 3 4 5	1 2 4 5 6 7				
RPL15A	cyto				1 2	2		
RPL19A	cyto		1 2 3 4 5	1 2 3 4 5 6 7		2 5		
RPL18	cyto			1				

Gene name	Compartment	0 day	7 days	14 days	45 days	105 days	195 days	285 days
RPL20	cyto				1 4	1 2 3 4 7		
RPL20A	cyto					5		
RPL26A	cyto					3		
RPL26B	cyto		3 5					
RPL25	cyto		4					
RPL35	cyto			5 6				
RPL38	cyto					5		
					1 2 3 4 5 6			
RPL4A	cyto		2 3 4 7	1 2 4 6 7	7			
RPL7A	cyto				2			
RPL8A	cyto		1 2 3 4 5 6 7			1 2 4 5 7		
RPL8B	cyto			1 3 6	1 6 7			
RPL11A	cyto		6					
RPL11B	cyto				2			
UBI1	cyto			1 2 4				
RPP2B	cyto		1 2 5 6 7					
RPS14A	cyto		2 3 4 5	1 2 3 6	4			
RPS15	cyto		6	6		1 5 6		
RPS16	cyto			1 2 4 5 6 7				
RPS17A	cyto		6	1 2 3 4 5 6 7	1 2 3	2		
RPS18	cyto		2	1 6				
RPS19A	cyto			1 2 3 5		1 2 3 5		
RPS20	cyto		2 5 6	5 7	1 3 4 5 6 7	2 3 4 5 6 7		
RPS22A	cyto		1 5		1 2 3			
RPS27A	cyto			1 2 6 7		1		
RPS5	cyto		1 3 4 5 6 7	1 2 5 6 7	1 2 4 5	1 2 3 5		

Gene name	Compartment	0 day	7 days	14 days	45 days	105 days	195 days	285 days
RPS6A	cyto		1 2 3 4 6	4 6 7				
RPS8A	cyto					1 5		
RPS9A	cyto					2 3 4 6 7		
SAH1	cyto			6				
SSE1	cyto		3 4 5					
STM1	cyto		4 6 7	2 5 6 7	6			
TAL1	cyto		1 2 3 5 6 7	1 2 3 4 5 7	1 3 5 6 7	4 6		
TKL1	cyto		2 7			5		
TSA1	cyto	2	1 3 4 5 7	1 2 3 4 5 6 7	1 2 3 4	2 5		
TSA2	cyto							
VMA2	cyto		1 4 5 6	1 2 3 6	3 5 6 7	4 7		
	DNA rep							
ORC2	preint complx				2 3			
ALG3	ER					2		
ALG12	ER							
PDI1	ER		5 6 7	1	1 2 4 5 6 7	4 6 7		
RTN2	ER				5			
CTS1	extra			5 6	6	4		
PHO11	extra					4		
HIS2	intracellular			4				
ACO1	mito		2 5 6 7	2 3 4 5 6 7	5 6 7	7		
ACO2	mito					2		
ADH4	mito		1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 3 4 5 6 7 1 2 3 4 5 6	1 2 3 4 5 6 7		3 4 7
ATP2	mito		1 2 3 4 5 6 7	2 7	7	2 3 5 6 7		
CPR1	mito		3 4 5			4 6 7	3 5	4 5 7
CYC1	mito					2		

Gene name	Compartment	0 day	7 days	14 days	45 days						105 days	195 days	285 days
					1	2	3	4	5	6			
ENO2	mito	2	1 2 3 4 5 6 7	1 2 3 4 5 6 7							1 2 3 4 5 6 7		
FAS2	mito			7				2 3 4 5		1 2 3			
FBA1	mito	2 4	1 2 3 4 5 6 7	1 2 3 4 5 6 7				7		1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 6 7	
GDB1	mito			7				7		1 2 3 4 5 6 7		1 2 4 7	
GND1	mito		1 2 3 4 5 6 7	1 2 3 4 5 6 7				1 2 3 4 5 6		1 2 3 4 5 6 7		7	
HSC82	mito			7				2 3 4		2 3 4 5			
HSP10	mito		2 7	2 7				1 3 7		2 6 7			
HSP77	mito		1 2 3 4 5 7	1 2 6 7				2 5 6		1 2			
IDP1	mito							3 4 5		3			
IDH1	mito			1 2 4 6									
ILV2	mito							2 7		3			
ILV5	mito		1 2 3 4 5 6 7	1 2 3 4 5 6				1 2 3 4		5			
LAS3	mito												
LYS12	mito			1				3 4		5			
MDH1	mito		1 2 3 4 5 6 7	7				1 2 3 5 7		2			
MGE1	mito							6					
MIA40	mito			7						7	7		
MMF1	mito		1 3 4 5	2				1 3 5		2 3 5 7	2 5		
KGD1	mito		1 2 5	7									
ODP2	mito							7					
PFK1	mito							6					
PFK2	mito		6 7	4 5				6 7		2 4 6		1 2 4 5 6	
PGI1	mito		3 4 5	7				2 5		2 4 6 7			

Gene name	Compartment	0 day	7 days	14 days	45 days	105 days	195 days	285 days
					1 2 3 4 5 6			1 2 3 4 5 6
PGK1	mito	1 2 3 4	1 2 3 4 5 6 7	1 2 3 4 5 6 7	7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	7
PMG1	mito		1 2 3 5 6 7	1 2 3 4 5 6 7	1 3 4 5 6 7	1 2 3 4 5 6	1 2 5 6 7	1 2 3 4 5 6 7
POR1	mito		1 2 3 4 6 7	2 5 7				
RIM1	mito					2 5		
SOD1	mito		4 5	7	1 2 3 5 7	7		
TPI1	mito		1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 7	1 2 3 4 5 6 7		2 4 7
TRX3	mito				5 7			
TSL1	mito			2 4 5		1 2 3 5 7		5
URA1	mito					3		
YJR1	mito		2 6		5 6 7	2 3 4 6 7	1 2 4 6	1 2 3 4 5 6 7
YNL200 C	mito			1 2 3 7				
ADO1	multi		1 4 6	1 4 5	1 4 5 6 7	2 4		
ALD6	multi		4		4 5	1 2 3 4 5 6 7		
ARO4	multi			6 7	1 3 4 6 7			
					1 2 3 4 5 6			
ATP1	multi		1 2 3 4 5 6 7	2 3 4 5 7	7	1 2 3 4 5 6 7	2 3	7
CIS3	multi			5	3 5 6 7	4 6 7	2	
COX17	multi				6 7			
ECM33	multi	1 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 7	2 3 4 5 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7
ENG1	multi			7	1 3 4 6 7	2 3 4 5 6 7		
					1 2 3 4 5 6			
ENO1	multi		1 2 3 4 5 6 7	1 2 3 4 5 6 7	7	1 2 3 4 5 6 7	1 3 5	2 3 4 6 7
FPR1	multi		2	2 4 7	1 2 3 4 5 7	1 2 3 4 5 6 7		
GAS1	multi		1 2 5 6 7	2 4 5 6 7	2 3 4 5 6 7	1 2 3 4 6 7	2 4 5 7	1 2 3 4 5 6 7
GLR1	multi					5		

Gene name	Compartment	0 day	7 days	14 days	45 days	105 days	195 days	285 days
HSP12	multi	2	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	2 3 4 7	1 2 3 5 7	1 2 3 4 7
HSP71	multi		1 2 3 4 5 6 7	1 2 3 4 6	7	2		1 2 3 4 5
HSP72	multi		1 2 3 4 5 6 7	1 2 4 5 6 7			2 3	
HYP2	multi			5 7	1 3 4			
HXK1	multi		1 2 4 7	1 2 6	1 3	7		
ILV3	multi		1 3 5	1 7	1 2 3 4 5 7			
LSB3	multi					6		
LSB6	multi					2 3 4 5 6		
LRG1	multi			3				
MPG1	multi				1 5 7			
PAB1	multi					5 7		
PDC1	multi	2 3 4	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7		1 3 5 6 7
PLB2	multi		1 2 5 6 7	1 3 4 5 6 7	7	1 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 7
PMA1	multi		1 2 3 4 5 6	1 3 4 6	1 2 3 4 7	1 2 3 4 6		2
PNC1	multi			1				
PRY1	multi			1 2 3 5 7	4 6	3 6		
PST1	multi	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7
RPL28	multi			1 2 7		2 3 5		
RPS3	multi		1 2 5 6 7	2 6 7	1 3 4 5 7	1 2 3 4 5 6 7	7	7
RTN1	multi		1 4 6	1 4	1 2 4 6	4 6	1	
SEC31	multi					6		2 3 4 6 7
SSA1	multi							7

Gene name	Compartment	0 day	7 days	14 days	45 days	105 days	195 days	285 days
SUC2	multi		4	6	6	6		
SUN4	multi							1 2 3 4 5 6 7
					1 2 3 4 5 6			
TDH1	multi	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7
TDH2	multi		3 4 5	6		1 2 3 4 5 6		
TDH3	multi	2	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5			
TOS1	multi	2		2 5	5 6 7	4 6	2 4	2 4 5 6
UTH1	multi		7	3 5	6 7			
ZEO1	multi			1	1 6 7			
ZPS1	multi		6 7	6	6	6	6	5 6
RPN10	nuc			2 7		4 7		
ADE1	nucl				1			
DHE4	nucl		1 3 4 5 6	1 2 3 4 5	1 2 4 5 6 7	1 2 3 4 6 7		
GDH1	nucl					5		
RHR2	nucl			1 2 4 5 6				
HEM12	nucl			2 6				
HMF1	nucl		1 2 4 6 7	2	1 2 3 4 6	1 2 3 5 6		
HOM2	nucl			1 3 4 5 6 7				
HOM6	nucl		1 6	1 2 6 7	1 2 3 4	6		
					1 2 3 4 5 6			
HSP26	nucl		1 2 3 4 5 6 7	1 2 3 4 5 6 7	7	2 3 4 5 6 7	1	2 4 7
HSP74	nucl					7		
HTA1	nucl		1 2 3 4 5 6	1 2 4 5 6	1 3 4 5 7	7		
LEU3	nucl		2	1 4 5 6	1			
MIC14	nucl				7	2 7	2 7	1 2 3 4
PDC5	nucl		4 7	6	5 4 3			6
RNR2	nucl				2 3 6			

Gene name	Compartment	0 day	7 days	14 days	45 days	105 days	195 days	285 days
RPT6	nucl					2 4 5		
SHP1	nucl				1			
WTM1	nucl		6 7		1 3 6 7 1 2 3 4 5 6	2 3 4 5 6 7		
HSP30	pla memb		1 2 3 4 5	1 2 3 4 5 6 7	7 1 2 3 4 5 6	1 2 3 4 5 6 7		
YPS3	pla memb	2	6	1 2 4 7	7	2 3 4 5 6 7	5	
HSP75	polysome		3 4 5	3 5 7	1 2 3 4 5	1 2 3 7		
ANB1	ribo							
EFT1	ribo					1 2 3 4 6 7		
EFT2	ribo			2 3		3 5		
TEF1	ribo transcription factor		1 2 3 4 5 6 7	1 2 3 4 5 6 7	7 1 2 3 4 5 6	5	1 2 3 4 5 6 7	1 2 3 4 6 7
SSN8	complex					1		
IPA3	unkwn			7				
NCA3	unkwn			5				3
PAU17	unkwn							1 2 3 4 5 6 7
PAU2	unkwn				1 5 6 1 2 3 4 5 6			
PAU5	unkwn		2 4 5 6 7	1 2 3 4 5 6 7	7	2 3 4 5 6 7	3 4 6 7	1 2 3 4 5 6 7
THR1	unkwn				6			
YIQ9	unkwn							3 6
YNN4	unkwn		1 2 6	2 4 7				
YNU8	unkwn		4 5 6		5	2 3 5		
YOR387 C	unkwn			3 5				

Gene name	Compartment	0 day	7 days	14 days	45 days	105 days	195 days	285 days
YHR138 C	unkwn			5				
YJL066C	unkwn			7	3 7	2 6 7		
YHR087 W	unkwn				2 3 5			
YGL258 W	unkwn					4 6		
APE1	vac		4 5		1 2 1 2 3 4 5 6	1 2 3 6 7		
APE3	vac		1 4 5 6 7	1 2 4 5 6 7	7	2 3 4 5 6 7	1 2 3 4 5 6 7	
PRB1	vac			2				
TFP1	vac			1 2 3 4 5 6 7	1 2 3 4 5 6			
TRX1	vac		1 2 3 4 5 6 7	1 2 4 5 6 7	7 1 2 3 4 5 6	2 3 4 5 6 7	1 2 5 7	
TRX2	vac		1 2 3 4 5 6 7	1 2 3 4 5 6 7	7	1 2 3 4 5 6 7	1 6 7	1 2 3 7
VMA10	vac				4	1 3		
VPH1	vac							
AWA1	wall				6 1 2 3 4 5 6			
BGL2	wall		1 2 3 4 5 6 7	1 2 3 4 5 6 7	7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7
CCW14	wall				7		1 2 6 7	1 2 3 4 7
CRH1	wall			1 2 4 6 7	3 6 7	6	1 2 3 4 5 7	1 2 3 4 5 6 7
CWP1	wall		7		7 1 2 3 4 5 6		2	1 2
DAN2	wall		1 3 4 5 6 7	1 3 4 5 6 7	7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	
DAN3	wall		2	3 4 5 6 7		6		1 2 3 4 5 6 7
EGT2	wall				1 4 5 7			

Gene name	Compartment	0 day	7 days	14 days	45 days						105 days	195 days	285 days
					1	2	3	4	5	6			
EXG1	wall		1 2 4 5 6 7	1 2 3 4 5 6 7					7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	
GAS3	wall											1 2	
GAS5	wall		2 3 7	1 2 3 4 5 7					7	1 2 3 4 5 6 7	1 2 3 4 5 7	1 2 3 4 5 6 7	
HSP150	wall								7	4 6 7			
HSP31	wall		4 5						2	2			
NCA1	wall		3										
PHO5	wall		5 7	5				5 6 7		2 4 6 7	1 2 3 4 5 6 7		
PIR3	wall			2 7								1 2 3 4 7	
PLB1	wall		2 5 7					1 3 4 5 6 7		4 6 7	1 2 3 4 5 6 7		
PRY3	wall		4 5	5							1		
SCW10	wall								6			1 2 3 5 6	
SCW4	wall		1 2 3 4 5 6 7	1 2 3 4 5 6 7				7		1 2 4 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	
SIM1	wall		7	2 3 4 5 7				5 6 7			3 4 6 7	1 5 6	
SPS100	wall							2 3					
UTR2	wall		5 6 7	4 5 7									
VEL1	wall			1 3 4 5 6 7				5 6 7			1 4 5 6 7	1 3 4 5 6	
YGP1	wall		1 2 4 5 6 7	1 2 3 4 5 6 7				7		1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7	

¹ 1, 2, 3, 4, 5, 6, 7 are 98, 142, 170, 116, 262, RC212, BM45, respectively. Numbers in bold indicate that the protein was identified in both sample replicates.

