

Determination of Reduced Cysteine in Oenological Cell Wall Fractions of *Saccharomyces cerevisiae*

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Compounds containing cysteine residues, such as glutathione, can affect the redox potential of must and wine by reduction of *o*-quinones and hydrogen peroxide. The oenological yeast cell wall fractions contain cysteine residues in their protein structure, and they could affect both oxidative and odor properties of wine. An analytical approach based on the derivatization of cysteinyl residues with *p*-benzoquinone followed by reversed-phase high-performance liquid chromatography separation was developed to quantify glutathione and free and protein cysteine in 16 *Saccharomyces cerevisiae* strains and 12 commercial samples of yeast mannoproteins, hulls, and lysates. The chemical modifications induced by the Maillard reaction following the industrial preparation of such fractions were evaluated as well. Lysates showed the highest protein cysteine content and high contents of glutathione and free cysteine. Mannoproteins showed an intense Maillard reaction (furosine >60 mg/100 g protein), and most of the samples were able to bind thiol compounds with a potentially detrimental effect toward the thiol-related odors in wine.

KEYWORDS: Cysteine; glutathione; mannoprotein; yeast; hull; lysate; HPLC; quinone

INTRODUCTION

Thiol compounds of grape and yeast can strongly affect the sensorial properties of wine. Some aliphatic thiols are involved in the varietal aroma of wine even though their perception threshold is in the order of a few tens of nanomoles per liter (1). Barrel aging and bottle storage can decrease the concentration of such compounds below the perception level in wine because of their reaction with *o*-quinones and hydrogen peroxide arising from phenol oxidation (2). The reaction of quinones with the odor-related thiols, as well as other thiol compounds, produces thiol-substituted hydroquinones (3) or disulfide compounds lacking of aromatic properties (4, 5). Glutathione (GSH) can preserve wine odor since it can compete against the odor-related thiols, for oxidation if its concentration exceeds a few micromoles per liter (6). Cysteine (Cys) residues can also hinder the alterative or atypical aging of white wine by preventing the formation of Maillard reaction-related compounds such as sotolone (7) and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (8). The reducing property of GSH toward the *o*-quinones produced by the tyrosinase activity in must is well-known as well (9).

The mercaptans responsible for the reduced odors in wine represent a further group of thiol compounds, and they can be removed by racking wine under aerating conditions or by barrel aging on yeast lees. Mercaptans are converted to thiol-substituted hydroquinones by reaction with the *o*-quinones or with the Cys residues of the yeast cell wall to give disulfides (4). The antioxidant properties of the reducing protein Cys (RPC) from yeast lees have not been extensively investigated in wine, but some results

show the Cys residues of yeast mannoproteins (MPs) to be effective antioxidants if they are accessible to oxidizing molecules (10). Moreover, it is well-known that white wines and sparkling wines develop faster nonenzymatic browning, oxidation, and loss of odor-related thiols after yeast lees are removed (6, 11). The release of MPs from yeast lees as well as the addition of yeast glycoproteins to wine could increase the antioxidant Cys content. Nevertheless, little is known about the Cys content of yeast cell wall fractions as well as about the antioxidant behavior of yeast lysates, hulls, and MPs in wine.

The chemical modifications of the oenological yeast fractions following their preparation procedures have not been evaluated to date. Indeed, the technologies applied to produce oenological yeast fractions can chemically modify the MP moiety of the yeast cell wall. Heat treatments and drying conditions, as those adopted to produce yeast fractions, can promote browning due to Maillard reaction (12). The formation of α -dicarbonyl compounds (12) and degradation of Cys residues (13) occur as well. Such reactions likely modify the RPC content of the yeast fractions, affecting their antioxidant properties. Despite the growing interest in exploiting such properties of yeast cell wall fractions in winemaking, a reliable analytical method is not currently available to quantify RPC. The adoption of classical analytical approaches such as the Ellman's method (14) for the determination of glycoprotein Cys, is hindered by the dark-yellow color produced by the MP solutions. On these bases, the present paper describes a reliable analytical method to quantify both the glycoprotein and the nonproteinaceous Cys content of yeast fractions. The method was applied to assess the antioxidant properties of commercial samples of yeast lysates, hulls, and MPs on the basis of their Cys content. The furosine index was

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applied for assessing the heat damage promoted by the technological processes adopted for the production of yeast fractions.

MATERIALS AND METHODS

Chemicals. Cys, 3-mercaptopropionic acid (3MPA), and *p*-benzoquinone (pBQ) were purchased from Fluka (Switzerland). GSH, starch, and trifluoroacetic acid (TFA) were from Sigma-Aldrich (St. Louis, MO). Citric acid was purchased from J. T. Baker (Phillipsburg, NJ), and the oenological lysozyme was from Intec (Verona, Italy). High-performance liquid chromatography (HPLC) grade methanol was purchased from Panreac (Barcelona, Spain), and HPLC water was obtained by Milli-Q system (Millipore Filter Corp., Bedford, MA).

Samples. The following commercial samples of yeast fractions for oenological use supplied by six different producers as dried products with unknown compositions were evaluated: four MPs, four hulls, four lysates, and two extracts. Additionally, 16 commercial dried yeast starters employed in winemaking were evaluated: 11 strains were from Lallemand Inc. (Ontario, Canada), and 5 strains were from Dal Cin (Milan, Italy).

Derivatization of Cys Residues in the Samples. Samples were added with 100 μ L of 400 μ M methanol solution of pBQ. After 1 min of mixing, 1 mL of 500 μ M 3MPA dissolved in 0.3 M citrate buffer, pH 3.5, was added.

Determination of Cys Residues in Yeast Fractions. At least 50 mg of insoluble sample (active dried yeast, yeast hulls, and yeast lysates) was dispersed in 50 mM of citrate buffer, pH 5.0, to obtain a 2 mL suspension with sample concentrations in the range of 20–100 g/L. The dispersed sample was centrifuged at 5000g for 15 min at 15 °C by a thermostatted Sorvall centrifuge (Thermo, Waltham, MA). The supernatants were diluted 1–10 fold, and 2 mL was submitted to derivatization. The precipitated material was resuspended in 5 mL of 50 mM citrate buffer, pH 5.0, and submitted to the derivatization reaction. After derivatization, 1 mL of the suspension was centrifuged at 14000g for 5 min at 25 °C by a thermostatted benchtop centrifuge (Hettich, Tuttlingen, Germany), and the supernatant was submitted to HPLC separation. To recover the derivatized 3MPA potentially adsorbed on the insoluble yeast fraction, the precipitated material was rinsed with 2 mL of 0.1% hydrochloric ethanol as described by Ummarino et al. (15), carefully resuspended, and centrifuged at 14000g for 5 min. The supernatant was dried under vacuum, redissolved in 1 mL of water, and injected into HPLC.

MP and yeast extract samples were prepared by dissolving 100–200 mg of sample in 2 mL of 50 mM citrate buffer, pH 5.0. The solution was submitted to a derivatization reaction, and then, it was ultrafiltered using 3 kDa cutoff Microcon membranes (Millipore, Billerica, MA) at 14000g for 100 min at 25 °C by the thermostatted benchtop centrifuge. The permeate was submitted to HPLC separation.

The retentate was added with 1 mL of 0.1% hydrochloric ethanol and centrifuged at 14000g for 5 min. The supernatant was dried under vacuum, and the dried material was redissolved in 500 μ L of water before the HPLC separation. Each sample was analyzed in at least duplicate.

Precision Parameters. The repeatability of the Cys residues evaluation method was assessed by submitting one sample of yeast hulls and two samples of yeast lysates to five determinations each. The response linearity of the method was assessed for RPC concentrations up to 180 μ M by dispersing 27–100 g/L of a yeast hull sample containing 0.18 mmol RPC/100 g product in the 50 mM citrate buffer. The response linearity of the method for higher RPC concentrations (210–800 μ M) was attained by analyzing dispersions (10–40 g/L) of a yeast hull sample containing 2.1 mmol RPC/100 g product.

Evaluation of Matrix Effects on the Cys Residues Determination.

To evaluate possible interferences arising from high polysaccharide contents, one sample of yeast lysate (L2) containing GSH and protein Cys was assayed with or without addition of 30 or 80 g/L of amylose or caramelized amylose. The caramelization was obtained by exposing 2 g of amylose suspended in 2 mL of 0.1% HCl at 110 °C for 24 h. Interference of protein disulfides (cystine) was evaluated by submitting 5 g/L lysozyme solution in 50 mM citrate buffer, pH 5.0, to protein Cys evaluation.

HPLC Separation of Derivatized Thiol Compounds. The reversed-phase (RP)-HPLC of the thiol-substituted hydroquinones and *p*-hydroquinone (pHQ) was performed with a Waters Alliance 2695 (Milford, MA) equipped with a photodiode array detector Waters 2996. The separation column was a hexyl-phenyl column, 250 mm \times 4.6 mm, 5 μ m, 110 Å

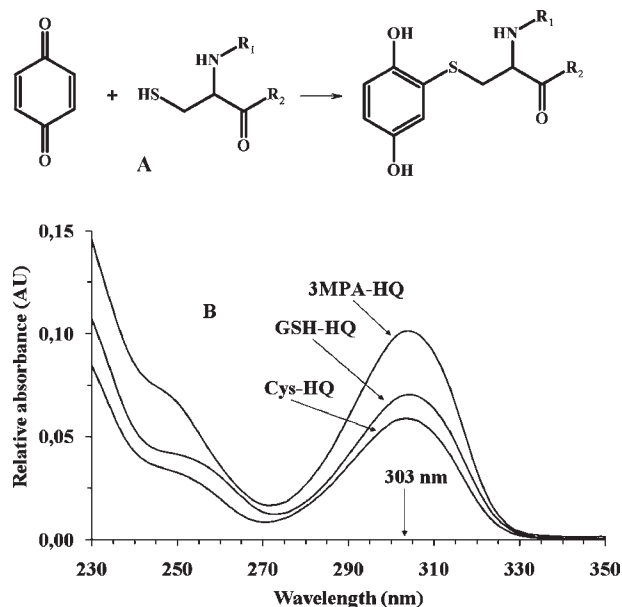


Figure 1. (A) General scheme of the derivatization reaction of Cys thiols with pBQ and (B) UV spectra obtained for derivatized Cys (Cys-HQ), GSH (GSH-HQ), and 3-mercaptopropionic acid (3MPA-HQ).

(Phenomenex, Torrance, CA). Eluting solvents were water/trifluoroacetic acid (0.05% v/v) and methanol; the concentration of the latter increased from 10 to 35% in 18 min during the elution gradient at 1.0 mL/min flow. Chromatographic data were acquired and processed by Millennium software v. 4.0 (Waters).

HPLC/Electrospray Ionization–Mass Spectrometry (ESI-MS).

For MS detection, the LCQ Deca XP spectrometer, controlled by the Excalibur software (Thermo Finnigan, San Jose, CA), was operated in positive ion mode. A postcolumn flow splitter was used to introduce 1:15 of the HPLC flow stream into the ESI source. The ESI interface and the ion optics settings were as follows: spray potential, 5.0 kV; nebulization gas (nitrogen) relative flow value, 10; capillary temperature, 275 °C; and cone voltage, 30 V. Full-scan mass spectra were acquired scanning the range 50–800 *m/z*. Mass accuracy was ensured by calibration with a mixture of caffeine, reserpine, and the tripeptide PFK (in methanol:water 1:1, 0.1% acetic acid) infused separately.

Quantification of Thiol Compounds. Cys and GSH were quantified chromatographically by the external standard method in both the soluble and the insoluble fractions. Standard solutions containing Cys and GSH concentrations up to 100 μ M were prepared in 50 mM citrate buffer, pH 5.0.

The concentration of RPC in the evaluated sample fraction was calculated as follows:

$$[\text{RPC}] = [\text{pBQ}] - ([\text{Cys}] + [\text{GSH}] + [\text{pHQ}] + [3\text{MPA-HQ}])$$

where [pBQ] = concentration of pBQ used for the derivatization, [Cys] = concentration of Cys quantified chromatographically, [GSH] = concentration of GSH quantified chromatographically, [3MPA-HQ] = concentration of *S*-3-mercaptopropionyl-hydroquinone quantified chromatographically, and [pHQ] = concentration of the underivatized hydroquinone quantified chromatographically.

Evaluation of Protein Content and Furosine Level. The protein content and furosine level were determined as described by Resmini et al. (16) with the following modification: 200 mg of sample (corresponding to about 20 mg of protein) was added with 8 mL of 8 M HCl and submitted to acid hydrolysis at 110 °C for 23 h before solid-phase extraction (SPE).

Total Cys Content. The total amount of Cys (i.e., Cys + 2 \times cystine) was assayed on the acid-hydrolyzed sample obtained from the furosine determination. After purification with SPE on a C18 cartridge, the sample was dried under vacuum and redissolved in 0.15 M sodium carbonate buffer, pH 8.6. The buffered solution was then submitted to Cys determination according to Krause et al. (17).

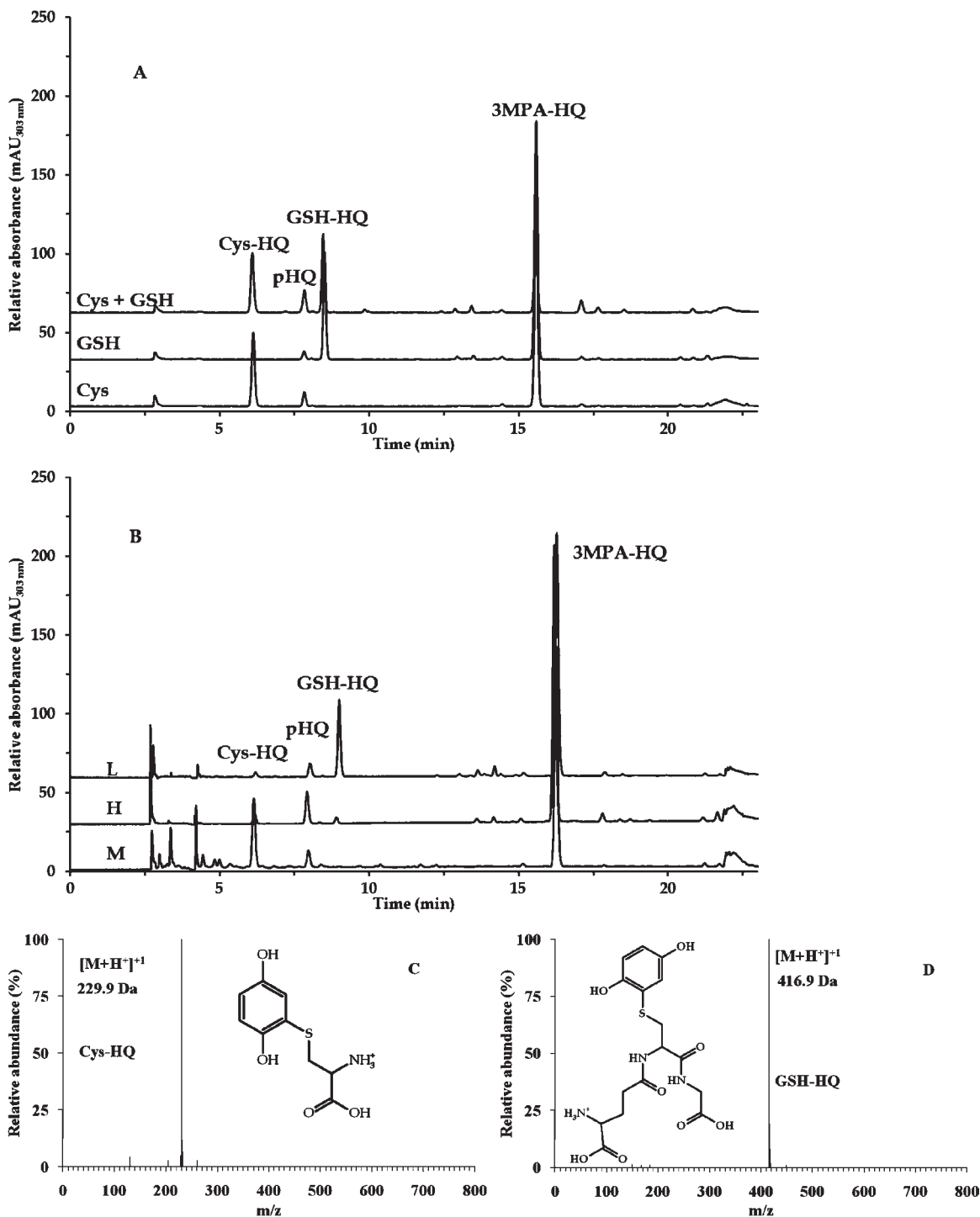


Figure 2. HPLC separation of Cys-HQ, GSH-HQ, 3MPA-HQ, and pHQ obtained from derivatization with pBQ of (A) standard water solutions of Cys, GSH, and their mixture and (B) MP sample added with Cys (M), yeast lysate (L), and yeast hull (H). HPLC-ESI/MS spectra of Cys-HQ (C) and GSH-HQ (D) are reported.

RESULTS AND DISCUSSION

The thiol property to reduce quinones to thiol-substituted hydroquinones, as it spontaneously occurs for the *o*-quinones in must and wine, was adopted to assess the Cys residues in the yeast fractions. For this purpose, the symmetric pBQ was used as a derivatizing agent to prevent the formation of multiple hydroquinone derivatives and to obtain the single mono derivative for each thiol molecule. The molecular size of pBQ is comparable to that of the *o*-quinones detectable in must and wine, and it can react with the thiol residues actually accessible in the glycoprotein structures. The formation of thiol-substituted hydroquinones

(Figure 1A) is fast and stoichiometric at room temperature, and it allows Cys and GSH to be detected spectrophotometrically as *S*-cysteinyl-*p*-hydroquinone (Cys-HQ) and *S*-glutathionyl-*p*-hydroquinone (GSH-HQ), respectively (Figure 1B). The thiol derivatization procedure was followed by the addition of 3MPA to remove the exceeding amount of pBQ. The latter could be reduced to pHQ by oxidation of the thiol-substituted hydroquinones to thiol-substituted *p*-quinones, which are able to bind a further thiol molecule, so forming a dithiol-substituted hydroquinone (18). Moreover, pBQ can readily polymerize to produce brown compounds.

The formation of Cys-HQ and GSH-HQ in citrate buffer solution/suspension containing Cys and GSH (**Figure 2A**) or yeast fractions (**Figure 2B**) was confirmed by HPLC-ESI/MS (**Figure 2C, D**). Such thiol-substituted hydroquinones, including *S*-mercaptopropionyl-*p*-hydroquinone (3MPA-HQ), showed a maximum absorption at 303 nm wavelength (**Figure 1B**). The HPLC pattern of **Figure 2A** also shows the formation of both pHQ and minor amounts of dithio-substituted hydroquinones following the derivatization reaction. The exceeding amount of pBQ could be effectively removed by the addition of sulfur dioxide, but the amount of the products obtained after such a reaction is affected by a number of factors (19). On the contrary,

Table 1. Analytical Values (μM) and RSDs Obtained from Five Replicated Determinations Performed on One Sample of Yeast Hull (S4) and Two Samples of Yeast Lysates (L2 and L4)

	Cys			GSH			RPC		
	S4	L2	L4	S4	L2	L4	S4	L2	L4
	43.3	0.0	1.8	13.4	10.0	92	173	266	246
	43.0	0.0	2.1	15.0	9.7	101	171	299	241
	43.8	0.0	2.9	16.3	10.4	102	165	311	254
	44.2	0.0	2.9	13.8	11.0	103	164	286	269
	43.1	0.0	1.9	14.6	11.3	89	186	280	248
mean	43.0		2.3	14.6	10.5	97	172	288	252
RSD (%)	1.2		23.4	7.7	6.4	6.9	5.1	6.0	4.2

the addition of 3MPA allows us to verify whether the amount of pBQ exceeds the content of Cys residues and to assess the level of residual pBQ after thiol derivatization. As a result of the developed analytical approach, the amount of the RPC was calculated by the difference between the amount of added pBQ and the amounts of GSH, Cys, pHQ, and 3MPA-HQ chromatographically evaluated.

Because the insoluble cell wall fractions were submitted to derivatization with pBQ after removal of the soluble Cys residues, the amount of pBQ reacted with γ -glutamyl-Cys and cysteinyl-glycine potentially present in yeast preparations was assumed as negligible. Such dipeptides represent about 4% of GSH in yeast (20); therefore, their contribution was not taken into account also for the MP samples where the nonproteinaceous Cys thiols were never detected. The amounts of dithiol-substituted hydroquinones were not included in the calculation since their values were close to the limit of quantification (signal-to-noise ratio > 10). The removal of the soluble material from the samples allows an effective RPC determination in wine yeast lees since sulfur dioxide interferences are minimized (data not shown).

The precision parameters for the quantification of Cys, GSH, and RPC were assessed by analyzing one sample of yeast hull and two samples of yeast lysates (**Table 1**). An average value of 5.4% can be assumed for the relative standard deviation (RSD) of Cys thiol concentrations exceeding $10 \mu\text{M}$.

The calibration curves of Cys and GSH (**Figure 3A**) showed linear and similar analytical responses for concentrations up to

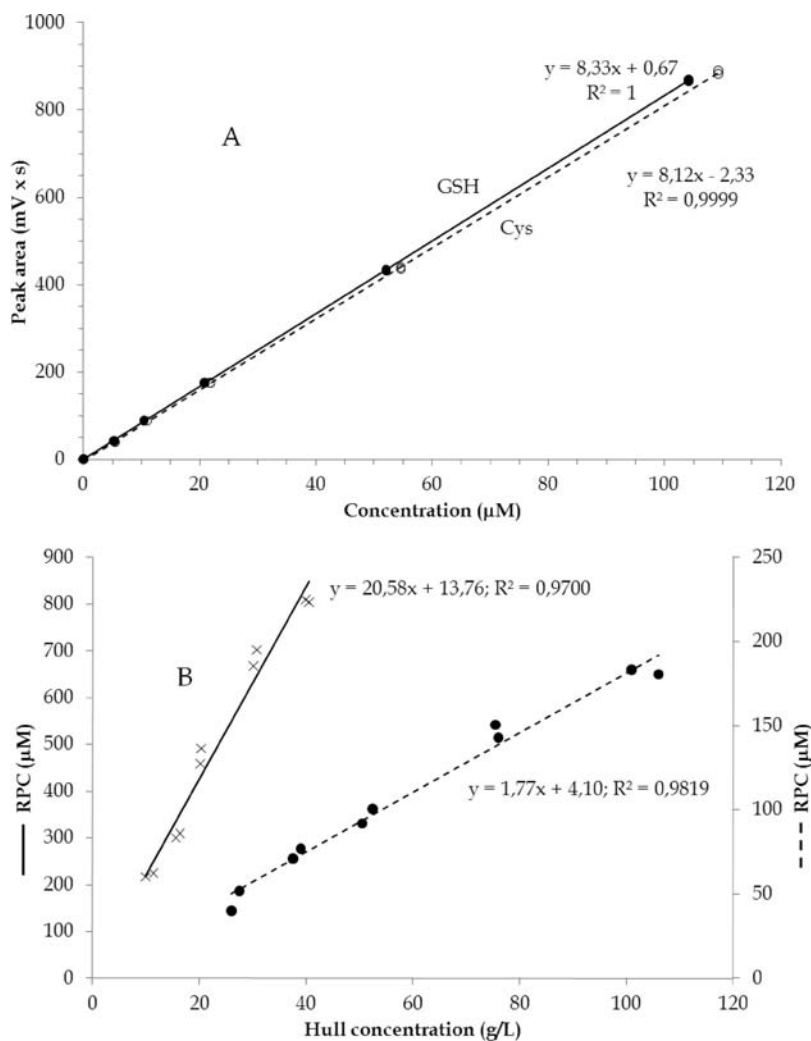


Figure 3. Analytical response of the proposed analytical method obtained for (A) standard solutions of Cys and GSH and (B) high (solid line) and low (dashed line) RPC concentrations. Data of duplicated determinations are reported.

Table 2. Effect of Amylose or Caramelized Amylose on the Quantification of the Cys Residues in a Sample of Yeast Lysate^a

added amount (g/L)	amount (mmol/100 g sample)			
	amylose		caramelized amylose	
	GSH	RPC	GSH	RPC
0	0.22–0.21	1.59–1.44	0.23–0.23	1.38–1.35
30	0.22–0.22	1.52–1.56	0.25–0.26	1.35–1.33
80	0.21–0.22	1.57–1.60	0.27–0.28	1.38–1.35

^a Results of duplicated determinations are reported.

100 μM . Because no standard material is commercially available for RPC quantification, the range of linear response was evaluated in yeast hull suspensions containing RPC levels up to 800 μM . Suspensions containing RPC amounts higher than 210 μM were prepared by dispersing 10–40 g/L of a yeast hull sample containing 2.1 mmol RPC/100 g product. To not affect the representativeness of the sampling, amounts higher than 50 mg were used to prepare the suspensions intended for linear response assessment. For this reason, the linear response for low (< 180 μM) RPC concentrations was evaluated analyzing suspensions (27–100 g/L) of a yeast hull sample containing 0.18 mmol RPC/100 g product. Under the adopted conditions, linear responses were observed for the entire range tested (**Figure 3B**).

According to the signal-to-noise ratio, the detection limits for Cys and GSH by means of HPLC analysis were 0.30 and 0.26 μM , respectively; for the same compounds, the quantification limits were 1.0 and 0.85 μM , respectively. To assess whether RPC quantification was affected by matrix effects arising from polysaccharides or disulfide bonds, the quantification was performed after the addition of increasing amounts of amylose or caramelized amylose to a yeast lysate. No significant difference ($p < 0.05$) was found for RPC and GSH quantification in the presence of either amylose or caramelized amylose (**Table 2**). Similarly, no interference was observed when 5 g/L lysozyme, a protein containing four disulfide bonds, was added to water solutions containing GSH or Cys.

The analytical approach was first applied to the characterization of 16 commercial samples of oenological dry active yeasts to evaluate their natural thiol content. Because the yeast samples tested were aimed to both effective propagation and alcoholic fermentation, a very low intensity of Maillard reaction was expected. Indeed, the furosine values were lower than 8 mg/100 g protein (**Table 3**), and they agree with the values detected in other unprocessed biological material (21–23), although no data are reported in the literature for dry active yeasts. Amounts of RPC in the range 0.76–1.28 mmol/100 g were detected in yeast samples as well as GSH levels up to 0.92 mmol/100 g (**Table 3**). GSH is a cytoplasmatic metabolite, and it likely arises from the lysis of yeast cells during drying. None of the studied samples contained free Cys. Because the MP fractions represent about 10% of the yeast cell wall and they are mainly linked to the outer layer of the glucan backbone (24), oenological glycoprotein fractions with a higher content of Cys residues can be potentially obtained. The commercial MP samples showed levels of RPC very different from each other. Samples M1 and M2 had the lowest Cys levels (**Table 4**). Sample M2 presented the highest furosine level, thus suggesting a strong heat damage, which agreed with the dark brown color and the burnt odor characterizing the sample. The RPC level of sample M4 was close to the values found in the dry yeast samples. Overall, the RPC levels were far from those needed to obtain effective antioxidant activity if the usual amounts of MPs added to wine (200–500 mg/L) are taken into account. Neither GSH nor Cys was detected in the

Table 3. Characterization of 16 Samples of Oenological Dry *Saccharomyces cerevisiae* Strains According to Their Content of Cys Forms, Protein Content, and Intensity of the Maillard Reaction Expressed as Furosine Level^a

sample	protein (g/100 g)	furosine (mg/100 g protein)	reducing Cys		overall Cys (mmol/100 g)
			GSH	RPC	
1	11.3	2.7	0.92	0.76	7.1
2	11.0		0.45	0.80	
3	10.5		0.71	0.86	
4	12.0	3.6	0.39	0.88	6.8
5	12.0		0.73	0.89	
6	10.3	7.6	0.63	0.90	6.9
7	12.0		0.77	0.91	
8	12.0		0.60	0.91	
9	11.2		0.83	0.91	
10	13.9	7.8	0.55	0.95	4.5
11	11.0		0.45	0.96	
12	9.2		0.54	0.97	
13	16.9	2.3	0.58	0.99	7.8
14	13.6	6.4	0.82	1.02	7.8
15	14.9		0.58	1.05	
16	11.2		0.63	1.28	
average	12.1	5.1	0.64	0.94	6.8

^a Overall Cys refers to Cys + 2 \times cystine.

Table 4. Characterization of Commercial Yeast Cell Wall Fractions and Yeast Extracts According to Their Contents of Free Cys, GSH, RPC, and Overall Cys (Cys + 2 \times Cystine)^a

sample	protein (g/100 g)	furosine (mg/100 g protein)	Cys				
			unrecovered	free	GSH	RPC	overall
M1	2.86	28	<0.01	0	0	0.03	0.55
M2	10.51	254	<0.01	0	0	0	0.54
M3	9.02	62	0.04	0	0	0.03	2.3
M4	8.77	67	0.41	0	0	0.51	3.1
H1	7.48	17	0.04	0	0	0.08	3.3
H2	8.75	12	0.03	0	0	0.07	4.4
H3	8.87	6	0.04	0	0	0.14	3.3
H4	11.88	12	0	2.6	0.85	0.86	10.6
L1	9.96	3	0	0	0.45	0.73	6.9
L2	17.94	5	0	0	0.33	1.4	15.8
L3	16.84	38	0	0.32	4.6	0.81	12
L4	14.33	3	0	0.07	2.8	1.3	8.1
E1	21.68	154	0.46	0	0	1.1	37.4
E2	23.87	20	0.29	0	0	0.76	25.4

^a The protein content and the intensity of the Maillard reaction expressed as furosine level are reported. The unrecovered Cys refers to the amount of analytically missing Cys after the addition of known amounts of Cys to samples lacking in nonproteinaceous Cys residues (M, MP; H, hull; L, lysate; and E, extract).

commercial MP samples. Surprisingly, the amount of 3MPA-HQ increased when Cys was added to samples before derivatization. Such a behavior occurred with most of the samples not containing free Cys and GSH, and it was likely due to the presence of oxidized phenol aminoacids in the protein structure (10). The sensorial properties of wines containing odor-related thiols can be detrimentally affected by the addition of MPs capable of binding high amounts of thiols like sample M4. In this regard, 200–400 mg/L of such MP could deplete up to 250–500 μM of thiols from wine. Contrarily, such MPs could be usefully added to wine as an early treatment for removing the reduced odors since they can link

mercaptans, so avoiding exposure of wine to oxygen or the addition of copper sulfate. The high furosine values of MP samples indicate a strong extent of the Maillard reaction. These commercial MPs are capable of rapidly increasing the formation of atypical aging-related compounds in wine. Therefore, their addition to wine to improve colloidal and tartaric stabilities or to modify the astringency and the viscosity can also deplete wine odor and decrease wine shelf life.

Yeast hulls having a high RPC content could protect wine during barrel aging from the oxidative effect of micro-oxygenation. The analyzed commercial samples presented RPC levels close to the values detected for MPs as well as similar capability to bind free Cys. The amounts of GSH and free Cys revealed in sample H4 were likely due to GSH and Cys addition during manufacturing to increase the reducing properties of the product.

Amounts of RPC effective as antioxidant were detected in the yeast lysates L2 and L4. The former also contained high levels of GSH. The amounts of GSH detected in samples L2 and L3 accounted for 0.8–1.2% of yeast dry weight as reported in the literature (25). Nevertheless, their overall Cys content was about twice higher than the level found in the dry yeast samples, and likely, an enrichment with exogenous GSH occurred.

Because the cytoplasmic GSH represents about 0.5–1% of yeast dry weight, high amounts of GSH were expected to be found in yeast extracts. Moreover, in spite of the high value of total Cys (about 30 mmol/100 g sample), no GSH was detected in the two extract samples, and up to 0.50 mmol Cys/100 g sample was combined after the addition of Cys to the same samples. Such data suggest an intense oxidation of the yeast extracts likely due to the chemical/heat damage arising from the industrial production as supported by the high furosine values observed.

A wide range of oenological yeast fractions are commercially available, and yeast hulls, lysates, and MPs effective against wine oxidation and wine atypical aging can be potentially obtained. The data reported in this work show that a number of such oenological samples do not have useful reducing properties. Moreover, the technologies applied for their production are not suitable for preserving the RPC content of the yeast fractions. On these bases, both odor and antioxidant properties of wine could be potentially endangered by using most of the studied samples, which cannot be considered profitable in winemaking.

The RPC content of yeasts suggests that more useful fractions could be obtained to protect the odor-related thiols and to increase the shelf life of wine. In this regard, the adoption of specific culture media to increase GSH and RPC contents in yeasts, the selection of yeast strains with improved MP release properties, and the use of specific enzymatic procedures could greatly enhance the oenological properties of yeast fractions.

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