Approaches to prevent the light-struck taste in white wine

D. FRACASSETTI[1], M. GABRIELLI[1], J. ENCINAS[1], M. MANARA[2], L. PELLEGRINO[1] and A. TIRELLI[1]

[1]Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, 20133 Milan, Italy; [2]Research & Developments, Dal Cin Gildo SpA, 20863 Concorezzo (MB), Italy

Corresponding author: Prof. Antonio Tirelli, email antonio.tirelli@unimi.it

Short title: Prevention of light-struck taste

Abstract

Background and Aims: The light-struck taste is a fault occurring in light-exposed white wine containing methionine and a high concentration of riboflavin (RF) and bottled in clear bottles. These conditions induce the formation of methanethiol and dimethyl disulfide, responsible for a cabbage-like aroma. In order to decrease the risk of wine spoilage, a low concentration of RF should be obtained in wine either by preventing RF release from yeast during winemaking or by removing RF from wine.

Methods and Results: Fifteen commercial *Saccharomyces* strains intended for the wine industry were tested for RF production which was also evaluated when two yeast-based nutrients were added into the must for one of these strains. The RF released during vinification was strain-dependent and a concentration from 30 to 170 μg/L was found in wine. A high concentration of RF was released in presence of the yeast-based nutrients due to either the yeast metabolism or the RF contained in the nutrient itself. The ability of different inorganic (bentonite, charcoal, zeolite, kaolin) and organic adjuvants (egg-white proteins, polyvinylpolypyrrolidone) to deplete RF in wine was evaluated. A relatively low level of charcoal (50 mg/L) removed up to 60% of RF in wine, though its effectiveness was related to the charcoal source. A high concentration of bentonite (1 g/L) was needed to effectively decrease the risk of wine spoilage.

Conclusion: A critical RF concentration in white wine can be prevented by applying one or more approaches in winemaking: using low RF-producing yeast strains in fermentation, selecting suitable yeast nutrients or adsorbing RF by insoluble charcoal or bentonite.

Significance of the study: The research highlights suitable tools to prevent light-struck taste in white winemaking.

***Keywords**: bentonite, charcoal, light-struck taste, riboflavin, white wine*

Introduction

Light can induce major changes to wine components with detrimental effects on the sensory attributes. When wine is packaged in clear bottles, light can degrade anthocyanins in red wine (Maccarone et al. 1987, Sisa et al., 2010) as well as leading to the browning of white wine (Dias et al., 2012, 2013). Moreover, the light-struck taste can appear when white wine bottled in clear glass is exposed to light. Photochemical oxidations are mainly involved in such spoilage, which may affect phenolic substances, acids, alcohols and other wine compounds (Clark et al. 2007, 2011). In particular, riboflavin (RF), a highly photosensitive molecule, can undergo photochemical degradation through several pathways. Among these, the intermolecular photo-reduction is the most relevant to the formation of the light-struck taste. The degradation process described by Maujean and Seguin (1983a) involves the photoxidative degradation of methionine (Met) to give methional with the reduction of RF. Methional is unstable under intense light exposure and promptly decomposes into acrolein and methanethiol. Two molecules of the latter eventually yield dimethyl disulfide (DMDS). Methanethiol and DMDS are the main compounds responsible for the ‘cooked cabbage’ aroma of white wine exposed to light though hydrogen sulfide may have a role (Haye et al. 1977, Maujean et al., 1978, Maujean and Seguin 1983b). Light exposure can also influence the production of other undesirable aroma compounds such as furfural (Jung et al. 2007) which has been positively correlated to the ‘cooked vegetable’ aroma of white wines stored under oxidative conditions (Escudero et al., 2000, 2002). Even light-induced acetaldehyde production by an increased oxidation rate of ethanol was observed under model conditions (Clark et al. 2007) and a perceivable acetaldehyde odour was detected in light-exposed white wine in colorless glass bottles (Dias et al. 2013). Not all white wines are prone to this light-induced fault, and though not all the factors triggering the light-struck taste are well known, the role of a high RF concentration has been proved (Pichler 1996, Mattivi et al. 2000) and a concentration lower than 80–100 µg/L could limit the risk of the light-struck defect development. The concentration of RF in grape must hardly exceeds a few tens of micrograms per litre, however, a concentration higher than 200 µg/L can be found in white wine due to the additional RF produced by *Saccharomyces cerevisiae* during fermentation (Ournac

1968). The production of RF by yeast is affected by temperature and availability of other vitamins (Pichler 1996, Paalme et al. 2014). The metabolic pathway of *S. cerevisiae* to produce RF involves riboflavin synthase as a last step, which is encoded by the gene RIB5 (Santos et al. 1995). Ournac (1968) measured the RF produced by three *S. cerevisiae* strains in wine and by four grape cultivars. He observed that a similar RF concentration (210–259 μg/L) was released by the three yeast strains, whereas a variable amount was obtained (110–250 μg/L) in spontaneously fermented grape juices. Therefore, there is a lack of information concerning the ability of different yeast strains to produce RF.

Exposure of wine to light at wavelengths close to 370 nm or 442 nm, corresponding to the highest visible light absorption of RF, is particularly effective in inducing the light-struck taste (Maujean and Seguin 1983a) when clear glass bottles are used (Dias et al. 2012). Under such conditions of lighting or bottling, RF depletion in wine can be a suitable approach for reducing the spoilage risk. The removal of RF by means of adsorption on bentonite, a fining material usually employed for protein stabilisation in wine, was proposed by Pichler (1996). The significant amount (e.g. 1 g/L) of this clay, however, needed to decrease RF by 60% can be detrimental to the sensory properties of white wine.

Our research aimed to investigate the role of different oenological approaches in lowering the RF concentration in wine in order to decrease the risk of light-struck taste in white wine. The behaviour of either several wine strains of *Saccharomyces* or of nutrients derived from yeast fractions, in the release of RF during must fermentation was assessed. Moreover, the effectiveness of several fining agents and food adjuvants in removing RF from white wine was assayed and compared to the behaviour of bentonite.

Materials and methods

Chemicals and reagents

Methanol, ethanol, acetonitrile, riboflavin (RF), citric acid, tetrahydrofuran (THF), boric acid, mercaptoethanol, *o*-ptaldehyde (OPA), amino acids and hydrochloric acid were purchased from Sigma-Aldrich (St Louis, MO, USA). All the chemicals were at least of analytical grade. Water of HPLC grade was obtained by a Milli-Q system (Millipore Filter, Bedford, MA, USA).

Fermentation trials

Fifteen commercial freeze-dried yeast strains [nine *Saccharomyces cerevisiae,* five *S. bayanus* and one *S. uvarum* (Dal Cin, Concorrezzo, Italy)] intended for winemaking were tested in laboratory-scale fermentations in triplicate using 200 mL of a Chardonnay must produced in vintage 2014. Diammonium phosphate (300 mg/L) was added to increase the yeast assimilable nitrogen at least up to 250 mg/L and, so to ensure regular alcoholic fermentation. The chemical composition of must is reported in Table 1. The 15 yeast strains were rehydrated by dispersing 2 g in 20 mL of water at 38°C, stirred for 10 min and left to stand for 10 min. Finally, each yeast strain was inoculated into the must (10^6 CFU/mL). The temperature of the fermenting musts was controlled at 18°C. Two additional fermentation trials were carried out using the *S. cerevisiae* 8 after addition of either a partially soluble yeast nitrogen source (YL) consisting of inactivated yeast cells (300 mg/L) (Dal Cin) or a soluble nutrient (YE) containing only the cytoplasmic part of yeast (300 mg/L) (Dal Cin). The fermentations were monitored daily as mass loss.

Removal of riboflavin

The performance of the fining materials and adjuvants was tested in model wine solution [5.0 g/L tartaric acid, 12% ethanol (v/v), adjusted to pH 3.2] with 350 µg/L of RF added. The fining materials and adjuvants included six samples of bentonite, two samples of charcoal, zeolite, polyvinylpolypyrrolidone (PVPP), kaolin and a colloidal suspension of pure silica (Dal Cin).

The relevant characteristics of the bentonite (BEN) samples were:

- BEN1: calcium bentonite from European raw material;
- BEN6: calcium bentonite from Asian raw material;
- BEN3: highly (chemically) activated sodium bentonite from European raw material;

- BEN4: highly (chemically) activated sodium bentonite. Asian raw material;
- BEN5: highly (chemically and physically) activated sodium bentonite from European raw material; and
- BEN6: low (chemically and physically) activated sodium bentonite from Asian raw material.

The relevant characteristics of the samples of charcoal (CHA) samples were:

- CHA1: large pore-size, chemically activated; and
- CHA2: small pore-size, physically activated.

Preliminary assays were carried out as follows. The 50 g/L water suspensions of bentonite or zeolite or kaolin were stirred for at least 1 h to obtain a good dispersion/dissolution and hydration of the product, then different volumes were added to the model wine solution (250 mL) in order to obtain 1 g/L of bentonite or kaolin or colloidal silica. Solid PVPP was also added at 100, 400 and 800 mg/L straight to the model wine solution. All the samples were stirred for 15 min and then kept in the dark up to 24 h. Further triplicate tests were carried out with BEN6 at 100, 500 and 1000 mg/L, zeolite at 1 g/L, both samples of charcoal (as straight addition of the solid material to the model wine solution) at 5, 10, 20, 50 and 100 mg/L.

Preliminary tests were carried out with egg albumin. Powdered egg albumin was hydrated and dispersed (50 g/L) either into a 1% sodium carbonate solution (final pH 10.4) or into water (final pH 6.7) and then added to the model wine solution (100 mg/L). The pH condition of the latter was even replicated by sequentially dispersing the hydrated egg albumin and the hydrated BEN6 (1 g/L as dry material) into the model wine solution, in order to remove any residual egg protein and RF.

The effectiveness of zeolite (1 g/L), BEN6 (1 g/L) and CHA1 (5–100 mg/L) in removing RF was tested in white wine applying the above mentioned conditions. The model wine solution was replaced with the Chardonnay wine samples obtained by blending of all the fermentation trials. The wine was stored refrigerated for a week, centrifuged to remove the suspended material, and then RF added up to 350 μg/L. Triplicate trials were carried out.

Determination of riboflavin

The method of Amidžić et al. (2008) with some modifications was adopted for assessing the RF concentration in both model wine solution and in white wine. An Ultra High Pressure Liquid Chromatography (UPLC) Acquity HClass system equipped with a photo diode array detector 2996 (Waters, Milford, MA, USA) was used. The detection wavelength was 440 nm. Samples were passed through a 0.22 µm filter and directly injected (50 µL) into a Hypersil column ODS C18 (100 mm x 3.0 mm, 3 µm particle size) (CPS Analitica, Milan, Italy) maintained at 25°C. The eluting solvents were: (A) 90% 50 mmol citrate buffer at pH 2.5 and 10% methanol (v/v), and (B) 10% 50 mmol citrate buffer at pH 2.5 and 90% methanol (v/v). The gradient was from 0 to 70% B in 8 min at flow-rate of 0.6 mL/min. Calibration curves were prepared for an RF concentration in the range 20 to 500 µg/L and RF was quantified according to the external standard method. Data were acquired and processed with Empower 2 software (Waters)

Determination of free amino acids

Amino acids were determined according to the method of Pereira et al. (2008) with some modifications with a Waters 2695 HPLC system (Waters) and a FP-920 Intelligent Fluorescence Detector (JASCO, Easton, MD, USA). Amino acids were separated in a Nova-Pak C18, 150 mm x 3.9 mm column, with 4 µm particle size (Waters). Eluting solvents were as follows: (A) 1% tetrahydrofuran, 8% methanol, 91% citrate buffer (10 mmol, pH 7.3) (v/v/v), and (B): 80% methanol and 20% citrate buffer (10 mmol, pH 7.3) (v/v). The gradient program was 6 min, 0% B; 6–17 min, 0–15% B; 17–25 min, 15–20% B; 25–33 min, 20–30% B; 33–45 min, 30–40% B; 45–61 min, 40–80% B. After the separation run the column was rinsed and conditioned for 13 min. The flow rate was set to 1 mL/min and the column temperature was 35°C. The OPA derivatives were detected by monitoring fluorescence at 335 nm and 440 nm as excitation and emission wavelengths, respectively. The amino acids were quantified according to the external standard method. Data were acquired and processed with Empower 2 software.

Statistical analysis

STATISTICA software (Statsoft, Tulsa, OK, USA) was used for statistical analysis. Differences were evaluated by the *t*-test ($P < 0.05$).

Results and discussion

Fermentation trials

The contribution of the grape to the RF concentration in wine is usually minor, since 3–60 μg/L were found in grape must (Ribéreau-Gayon et al. 1975), whereas the greatest amount can be related to the *Saccharomyces* metabolism. Therefore, the yeast-mediated production of this vitamin was evaluated with 15 *Saccharomyces* strains used as starter in winemaking. The strains were used to ferment a Chardonnay grape juice containing 5 μg/L RF. The yeast strains showed variable lag phase time and fermentation rate: 13 strains completed the alcoholic fermentation within 14 days, whereas two strains (*S. bayanus* 2 and *S. cerevisiae* 7) needed 18–20 days (Figure 1). Half of the yeast strains produced a low concentration of RF (less than 50 μg/L), while *S. bayanus* 2 and 5 and *S. cerevisiae* 7 produced 102, 116 and 170 μg/L, respectively, which could potentially lead to the development of the light-struck taste in wine (Figure 2). No significant difference was found in the RF production by *S. cerevisiae* and *S. bayanus,* since both species included strains that produced a low and high concentration of RF. In contrast to the data of Ournac (1968), our data highlighted the major role of the yeast strain in the final concentration of RF in wine, since the lowest RF producer strains (*S. bayanus 3* and *S. cerevisiae* 2) released less than 30 μg/L.

The ability of certain oenological yeast nutrients, usually employed to avoid sluggish or stuck fermentation, to affect the release of RF during fermentation was assessed. Nutrients based on yeast-extract usually contain vitamins, including RF, which thus might add up to the amount released during the fermentation. Yeast lysate can be also employed as an additive in white winemaking to prevent the anti-fermentative activity of medium-chain fatty acids (Ribéreau-Gayon et al. 2006). In order to assess whether nutrients derived from yeast fractions could affect the RF concentration in vinification, a trial of alcoholic fermentation was carried out by providing *S. cerevisiae* 8 (an average RF producer) with either YE or YL. Both the additives slightly increased the nitrogen concentration of the must (6 and 12 mg/L for YL and YE, respectively). No difference of the alcoholic fermentation rate was found (data not shown), however, a higher concentration in RF was detected in the final wines where the yeast fractions were added (76 and 72 μg/L, for YE and YL, respectively) in

196 comparison to the Control trial (55 μg/L). The excess of 21 μg/L detected in the presence of YE can
197 be ascribed to the RF in the nutrient (70 μg/g). The occurrence of RF in YE was expected, since YE
198 is composed of the soluble compounds contained in the yeast cytosol. An increased RF
199 concentration was also detected in the sample added with YL though no RF was found in YL. The
200 lipid fraction naturally occurring in yeast lysate may have affected the ability of the fermenting yeast
201 to produce purines, the RF precursors in yeast metabolism (Yatsyshyn et al. 2009, Kato and Park
202 2012). The rationale behind the observed effect of yeast fractions on RF release during fermentation
203 cannot be fully explained by our data and is outside the scope of this work. Our preliminary results,
204 however, highlight the potential role exerted by yeast nutrients commonly used in winemaking,
205 especially when the presence of RF in their composition is not taken into account.
206 The amount of oxidised Met in light-exposed wine is related to several physical and chemical
207 factors, including the concentration of RF, oxygen, Met and other amino acids. The photosensitised
208 RF can oxidise Met as well as other amino acids. Then the reduced RF can be oxidised back to RF
209 by oxygen (Cardoso et al. 2012). Eventually, these two oxidation-reduction cycles can involve a high
210 amount of Met depending on the amount of other substrates (e.g. other amino acids) capable of
211 reducing RFto 8.0 mg/L with 1.9 mg/L as mean value (Figure 2). The lower RF producer strains (i.e.
212 *S. bayanus* 3 and 5 and *S. cerevisiae 2*) were also low Met producers (< 1.1 mg/L) and they can
213 effectively decrease the spoilage risk.
214 *Removal of riboflavin*
215 Several fining materials and adjuvants were tested in order to understand their effectiveness in
216 decreasing the RF concentration in model wine solution containing RF 350 μg/L. Such a high
217 concentration was chosen in order to match the highest concentration found in white wine according
218 to the literature (Ournac 1968 Pichler, 1996). The highest concentration in wine can be found
219 following fermentation of musts that contain a high RF concentration (e.g. 30–50 μg/L), as well as
220 following ageing of wine on yeast lees (Ournac 1968). All six bentonites tested removed a similar
221 amount of RF within 2 h of the addition (Figure 3). The average residual RF was 60% and similar
222 results were obtained by Pichler (1996). No difference was found comparing the behaviour of sodium

and calcium bentonites. Zeolite decreased the RF concentration by 50%. Although this fining material is not included among the adjuvants permitted by the Organisation Internationale de la Vigne et du Vin, it was investigated as a possible replacement of bentonite in white wine clarification to improve protein and tartaric stability (Mercurio et al. 2010).

Egg albumin is commonly employed as a clarifying agent (Ribéreau-Gayon et al. 2006) and is commercially available as a partially soluble dry powder which needs to be dispersed into water or buffer solution before addition to wine. It was reported to contain about 1% of riboflavin-binding proteins (flavoproteins) (Tillotson and Bashor 1980), therefore, we tested its ability to remove RF. Whatever the pH of the medium used for dissolving the protein, no RF removal was obtained in the model wine solution unless bentonite was used to deplete egg albumin (Figure 3). The denaturing effect of the drying process and the low RF-binding ability at acidic pH could explain the lack of effectiveness we observed (Becvar and Palmer 1982, Campbell et al. 2003) and ethanol could have a further detrimental role.

Polyvinylpolypyrrolidone was not effective in removing RF, whatever the concentration tested. Tests with silica and kaolin gave similar findings..

The effect of different dosage level was also evaluated for selected fining materials. The bentonite BEN6 was assayed at 100, 500 and 1000 mg/L, while charcoal was tested at a much lower concentration (5, 10, 20 and 50 mg/L) due to its detrimental effect on the flavour-related compounds (Figure 4). Charcoal1 was chosen for the full test since CHA2 proved to be less effective in a preliminary trial. The removal of RF achieved in the model wine solution increased with the level of bentonite or charcoal added. Our findings showed that the decrease of RF was not proportional to the increase of bentonite added (Figure 4), indicating an almost asymptotic behavior (Figure 4) and eventually 35% RF could be adsorbed when 1 g/L was used. Charcoal was considerably more effective than bentonite since even the lowest added level (5 and 10 mg/L) achieved 70 and 94% depletion, respectively. Charcoal2 confirmed that it was less active than CHA1 leading to a RF decrease by 50% when 10 mg/L was used.

The depletion of RF by CHA1 at several concentration values was also assessed after 2 and 24 h of treatment. Removal was concentration dependent and occurred almost entirely within 2 h (Table 2). As a high concentration of charcoal can be detrimental for the sensory characteristics of wine, a concentration as low as 5 and 10 mg/L would be preferable.

As CHA1, bentonite and zeolite proved to be the most effective in removing RF, they were tested in the Chardonnay wine containing RF 350 μg/L in order to evaluate their capability in adsorbing RF under commercial winemaking conditions. The ability of the fining materials tested to deplete RF was lower in wine; the levels removed were 10% with zeolite (1 g/L), 25% with BEN6 (1 g/L) and 60% with CHA1 (50 mg/L). Charcoal1 was even assayed at several concentration values and times resulting in 70% adsorbed RF when 100 mg/L of CHA1 was added (Table 2). Again, longer treatment did not lead to a more effective removal of RF. The lower extent of RF absorption on charcoal in wine, compared to that in the model wine solution, highlights the major role exerted by the wine components. Charcoal can adsorb a variety of compounds, such as proteins, phenols and colloids, which occur in wine at a relatively high amount compared to that of RF. They can all shield competitively the active sites of charcoal thus preventing RF from being effectively adsorbed (Ribéreau-Gayon et al. 2006). A higher concentration of charcoal is expected to improve RF removal in wine with the drawback of the negative side effect on sensory attributes.

Conclusions

The risk of development of light-struck taste in white wine can be reduced either by using a low RF and Met producer yeast strain in fermentation or by removing RF with adsorbing materials such as charcoal or bentonite. The former approach avoids the use of material detrimental to wine flavour, however, its feasibility implies the availability of commercial strains selected for this purpose. The effectiveness of charcoal in removing RF proved to be lowered in wine by the competitive adsorption of minor wine components. Conditions such as pH value, ionic strength and the concentration of phenolic substances, may vary in wine. Since these conditions can also influence the effectiveness of bentonite or charcoal, a suitable dosage should be optimised under winemaking conditions.

References

Amidžić, D., Klarić, I., Jasprica, I. and Vedrina-Dragojević, I. (2008) Determination of water soluble vitamins in blackberry wines using high-performance liquid chromatography. Šegudović, N., ed. Book of abstract of the fourteenth International symposium on separation sciences: new achievements in chromatography; 30 September–03 October 2008; Primošten, Hrvatska, Croatia (Croatian Society of Chemical Engineers: Zagreb, Croatia) p. 144.

Becvar, J. and Palmer, G. (1982) The binding of flavin derivatives to the riboflavin-binding protein of egg-white. The Journal of Biological Chemistry **257**, 5607-5617.

Bekbölet, M. (1990) Light effects on food. Journal of Food Protection **53**, 430-440.

Campbell, L., Raikos, V. and Euston, S. R. (2003) Modification of functional properties of egg-white proteins. Nahrung **6**, 369-376.

Cardoso, D.R., Libardi, S.H. and Skibsted, L.H. (2012) Riboflavin as a photosensitizer. Effects on human health and food quality. Food & Function **3**, 487-502

Clark, A.C., Prenzler, P.D. and Scollary, G.R. (2007) Impact of the condition of storage of tartaric acid solutions on the production and stability of glyoxylic acid. Food Chemistry **102**, 905-916.

Clark, A.C., Dias, D.A., Smith, T.A., Ghiggino, K.P. and Scollary, G.R. (2011) Iron(III) tartrate as a potential precursor of light induced oxidative degradation of white wine: studies in a model wine system. Journal of Agricultural and Food Chemistry **59**, 3575-3581.

Dias, D.A., Smith, T.A., Ghiggino, K.P. and Scollary, G.R. (2012) The role of light, temperature and wine bottle colour on pigment enhancement in white wine. Food Chemistry **135**, 2934-2941.

Dias, D.A., Clark, A.C., Smith, T.A., Ghiggino, K.P. and Scollary, G R. (2013) Wine bottle color and oxidative spoilage: whole bottle light exposure experiments under controlled and uncontrolled temperature conditions. Food Chemistry **138**, 2451-2459.

299 Escudero, A., Asensio, E., Cacho, J. and Ferreira, V. (2002) Sensory and chemical changes of
300 young white wines stored under oxygen. An assessment of the role played by aldehydes and
301 some other important odorants. Food Chemistry **77**, 325-331.

302 Escudero, A., Hernández-Orte, P., Cacho, J. and Ferreira, V. (2000) Clues about the role of
303 methional as character impact odorant of some oxidized wines. Journal of Agricultural and Food
304 Chemistry **48**, 4268-4272.

305 Grant-Preece, P., Barril, C., Schmidtke, L. M., Scollary, G. R. and Clark A. C. (2017) Light-
306 induced changes in bottled white wine and underlying photochemical mechanisms. Critical
307 Reviews in Food Science and Nutrition **57**, 743-754

308 Haye, B., Maujean, A., Jacquemin, C. and Feuillat, M. (1977) Contribution a l'étude des "goûts
309 de lumière" dans le vin de Champagne. I. Aspects analytiques. Dosage des mercaptans et des
310 thiols dans les vins. Connaissance de la Vigne et du Vin **11**, 243-254.

311 Jung, R., Hey, M., Hoffmann, D., Leiner, T., Patz, C.D., Rauhut, D., Schüssler, C. and Wirsching,
312 M. (2007) Lichteinfluss bei der Lagerrung von Wein. Mitteilungen Klosterneuburg **57**, 224-231.

313 Kato, T. and Park, E.Y. (2012) Riboflavin production by *Ashbya gossypii*. Biotechnology Letters
314 **34**, 611–618.

315 Maccarone, E., Ferrigno, V., Longo, M. L. and Rapisarda, P. (1987) Effects of light on
316 anthocyanins. Kinetics and photodegradation in acidic aqueous solutions. Annali di Chimica **77**,
317 499-508.

318 Mattivi, F., Monetti, A., Vrhovšek, U., Tonon, D. and Andrés-Lacueva, C. (2000) High-
319 performance liquid chromatographic determination of the riboflavin concentration in white wines
320 for predicting their resistance to light. Journal of Chromatography A **888**, 121-127.

321 Maujean, A. and Seguin, N. (1983a) Contribution à l'étude des goûts de lumière dans les vins
322 de Champagne. 3. Les réactions photochimiques responsables des goûts de lumière dans le vin
323 de Champagne. Sciences des Aliments **3**, 589-601.

Maujean, A. and Seguin, N. (1983b) Contribution à l'étude des goûts de lumière dans les vins de Champagne. 4. Approaches a une solution œnologique des moyens de prévention des goûts de lumière. Science des Aliments **3**, 603-613.

Maujean, A., Haye, M. and Feuillat, M. (1978) Contribution a l'étude des "goûts de lumière" dans le vin de Champagne. II. Influence de la lumière sur le potentiel d'oxydoreduction. Correlation avec la teneur en thiols du vin. Connaissance de la Vigne et du Vin **12**, 277-290.

Mercurio, M, Mercurio, V., de Gennaro, B., de Gennaro, M., Grifa, C., Langella, A. and Morra, V. (2010) Natural zeolites and white wines from Campania region (Southern Italy): a new contribution for solving some oenological problems. Periodico di Mineralogia **79**, 95-112.

Ournac, A. (1968) Riboflavine pendant la fermentation du jus de raisin et la conservation du vin sur lies. Annales de Technologie Agricole **17**, 67-75.

Paalme, T., Kevvai, K., Vilbaste, A., Halvin, K. and Nisamedtinov, I. (2014) Uptake and accumulation of B-group vitamers in *Saccharomyces cerevisiae* in ethanol-stat fed-batch culture. World Journal of Microbiology and Biotechnology **30**, 2351-2359.

Pereira, V., Pontes, M., Câmara, J.S. and Marques, J.C. (2008) Simultaneous analysis of free amino acids and biogenic amines in honey and wine samples using in loop orthophtaladeyde derivatization procedure. Journal of Chromatography A **1189**, 435-443.

Pichler, U. (1996) Analisi della riboflavina nei vini bianchi e influenza della sua concentrazione. L'Enotecnico **32**, 57-62.

Ribéreau-Gayon, J., Peynaud, E. and Ribéreau-Gayon, P. (1975) Sciences et techniques du vin (Dunod Publishing: Paris, France).

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., Lonvaud, A. (2006). Handbook of enology, The microbiology of wine and vinifications (John Wiley & Sons: Chichester, England).

Santos, M.A., García-Ramírez, J.J. and Revuelta, J.L. (1995) Riboflavin biosynthesis in *Saccharomyces cerevisiae*. Cloning, characterization, and expression of the RIB5 gene encoding riboflavin synthase. The Journal of Biological Chemistry **270**, 437-444.

Sisa, M., Bonnet S.L., Ferreira D. and Van der Westhuizen, J.H. (2010) Photochemistry of flavonoids. Molecules **15**, 5196-5245.

Tillotson, J.A. and Bashor, M.M. (1980) Fluorometric apoprotein titration of urinary riboflavin. Analytical Biochemistry **107**, 214-219.

Yatsyshyn, V.Y., Ishchuk, O.P., Voronovsky, A.Y., Fedorovych, D.V. and Sibirny, A.A. (2009) Production of flavin mononucleotide by metabolically engineered yeast *Candida famata*. Metabolic Engineering **11**,163-167.

Figure captions

Figure 1. Alcoholic fermentation trend of *Saccharomyces* strains 1,3,4,6–15 (●), 2 (Δ) and 5 (○) in Chardonnay grape must.

Figure 2. Release of riboflavin (□) and methionine (■) by five strains of *Saccharomyces bayanus*, nine strains of *S. cerevisiae* and one strain of *S. uvarum* in Chardonnay grape must.

Figure 3. Relative residual concentration of riboflavin (RF) in model wine solution (350 μg RF/L) treated with polyvinylpolypyrrolidone (PVPP), bentonites (BEN1–6), silica, kaolin, zeolite, albumin + bentonite and albumin.

Figure 4. Relative residual concentration of riboflavin (RF) in model wine solution (350 μg RF/L) treated with bentonite (BEN6) and charcoal (CHA1 and CHA2) at several concentration values.

368 **Table 1.** Composition of Chardonnay must (vintage 2014) used for the fermentation trials.

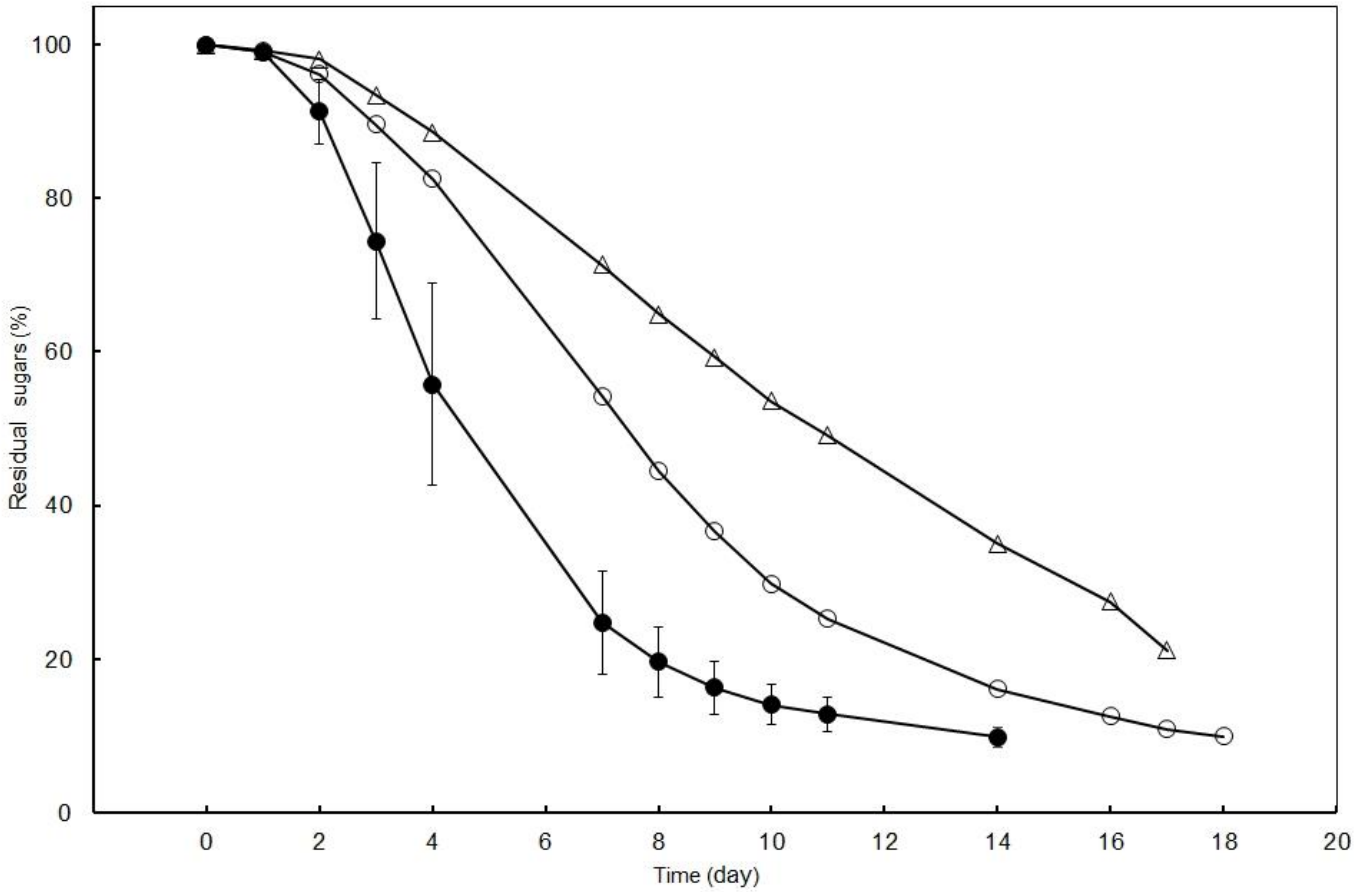
Parameter	
Sugars (g/L)	213
TA (g tartaric acid/L)	8.8
pH	3.28
Yeast assimilable nitrogen (mg N/L)	168
Tartaric acid (g/L)	5.6
Malic acid (g/L)	3.1
K^+ (g/L)	2.2
Riboflavin (µg/L)	5.2

369

Table 2. Relative residual amount of riboflavin in model wine solution (350 μg RF/L) and Chardonnay wine (350 μg RF/L) treated with different concentrations of charcoal (CHA1) and tested after 2 and 24 hours of treatment.

	Residual riboflavin (%)			
Charcoal	Chardonnay wine		Model wine solution	
(mg/L)	2 h	24 h	2 h	24 h
0	100	100	100	100
5	88	85	43	35
10	84	83	13	8
50	46	42	0	0
100	30	29	0	0

375 Figure 1



376

377

Figure 2

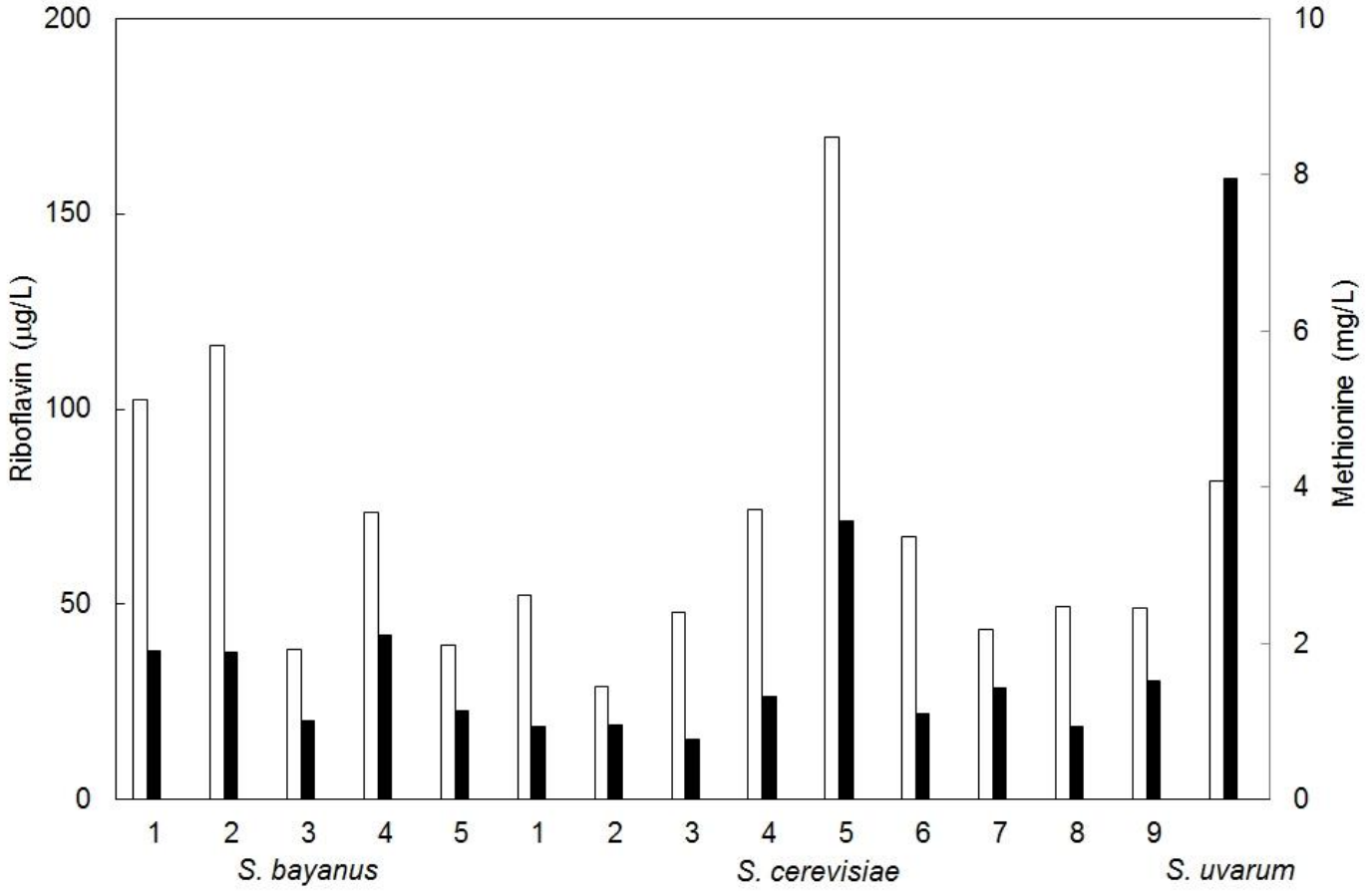
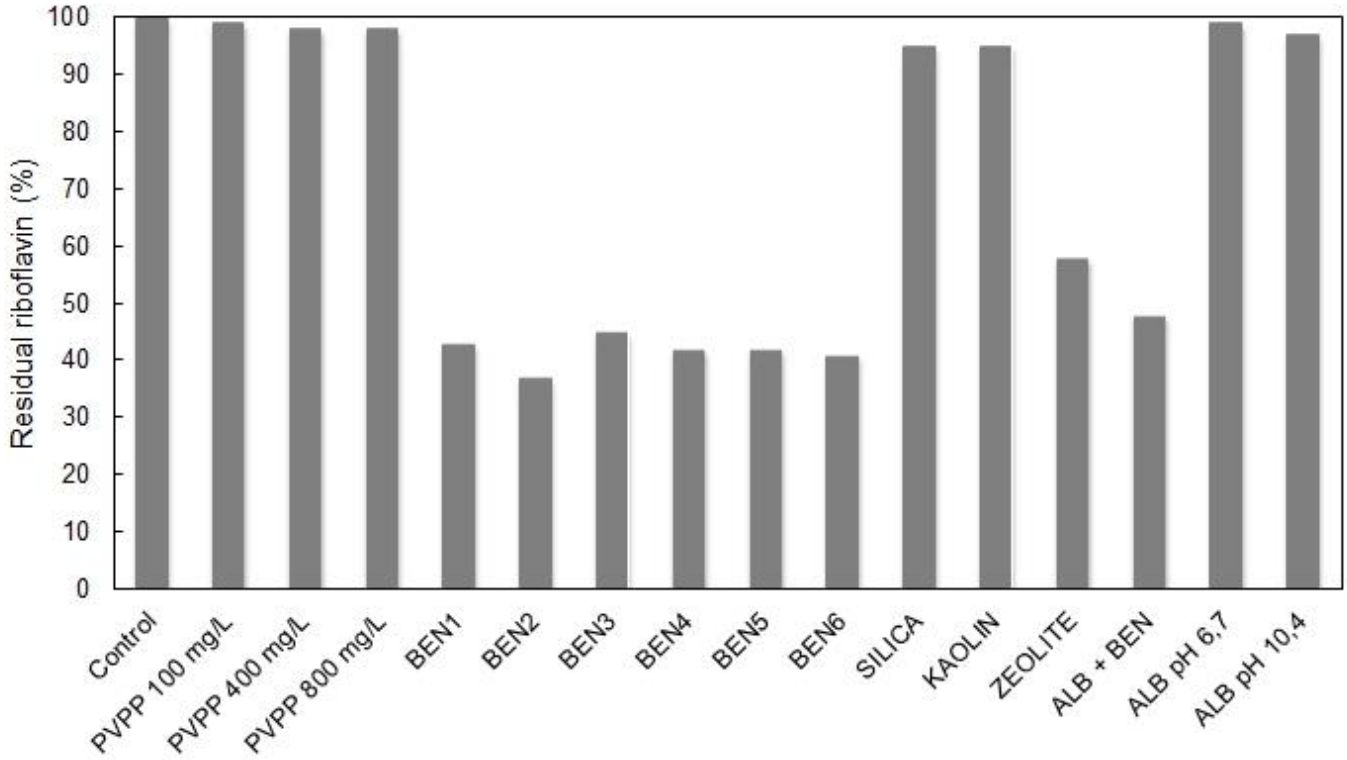
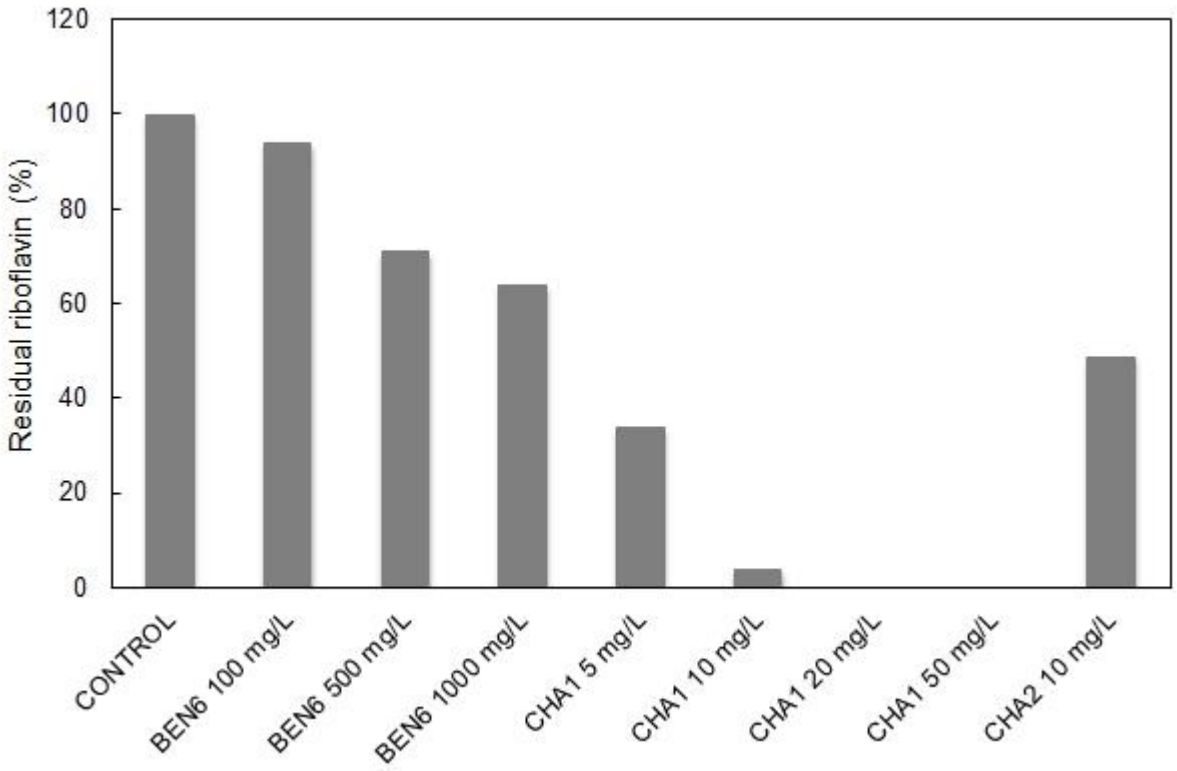

200
150
100
50
0
Riboflavin (µg/L)
10
8
6
4
2
0
Methionine (mg/L)
1 2 3 4 5 1 2 3 4 5 6 7 8 9
S. bayanus
S. cerevisiae
S. uvarum

Figure 3


Residual riboflavin (%)
100
90
80
70
60
50
40
30
20
10
0
Control
PVPP 100 mg/L
PVPP 400 mg/L
PVPP 800 mg/L
BEN1
BEN2
BEN3
BEN4
BEN5
BEN6
SILICA
KAOLIN
ZEOLITE
ALB + BEN
ALB pH 6,7
ALB pH 10,4

384 Figure 4



385