Potential of high pressure homogenization to induce autolysis of wine yeasts

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Corresponding Author: Dr. Piergiorgio Comuzzo, Ph.D.

Corresponding Author's Institution: Università degli Studi di Udine

First Author: Piergiorgio Comuzzo, Ph.D.

Order of Authors: Piergiorgio Comuzzo, Ph.D.; Sonia Calligaris; Lucilla Iacumin; Federica Ginaldi; Anthony E Palacios Paz; Roberto Zironi

Abstract: High pressure homogenization (HPH) was tested for inducing autolysis in a commercial strain of Saccharomyces bayanus for winemaking. The effects on cell viability, the release of soluble proteins, glucidic colloids and amino acids in wine-like medium and the volatile composition of the autolysates were investigated after processing, in comparison with thermolysis. HPH seemed a promising technique for inducing autolysis of wine yeasts. One pass at 150 MPa was the best operating conditions. Soluble colloids, proteins and free amino acids were similar after HPH and thermolysis, but the former gave a more interesting volatile composition after processing, with higher concentrations of ethyl esters (fruity odors) and lower fatty acids (potential off-flavors). This might allow different winemaking applications for HPH, such as the production of yeast derivatives for wine ageing. In the conditions tested, HPH did not allow the complete inactivation of yeast cells; the treatment shall be optimized before winemaking use.

Cover Letter

Yeast derivatives (YDs) are perhaps the most used enological products in the wineries, after active dry yeast. They are basically inactive dry yeasts or yeast autolysates, promoted by the suppliers for a wide series of applications: fermentation enhancers, flavor and aroma modulators, mouthfeel enhancers, scavengers against undesired compounds (e.g. off-odors or toxic compounds) and, the most recent one, antioxidant preparations.

Despite their wide utilization, very few commercial preparations are specifically tailored for winemaking use (especially for what concerns wine ageing) and winemakers are often forced to use formulations developed for the processing other food products. The problem is that, in food industry, YDs are generally used as flavoring and aromatizing agents, so they may release off-flavors when they are added to the wine. These off-flavors are mostly linked with the thermal treatments that occur during the processing of YDs (e.g. compounds from Maillard reaction or from oxidative breakdown of lipids). For this reason, the development of non-thermal alternative processes for the obtainment of YDs, may open new opportunities for the production of commercial preparations suitable for winemaking use.

This paper is a preliminary investigation to assess the potential of high pressure homogenization (HPH) to be used for this purpose. Results demonstrated that HPH induces autolysis of wine yeasts and the autolysates obtained had interesting characteristics for winemaking. This may open a series of applications for HPH technology in wine sector, such as the production of specific preparations of yeast derivatives for wine storage and ageing, but also the treatment of yeast lees in the winery, to shorten the time needed for *sur lie* maturation. As far as we know, at this time, these aspects have been poorly taken into account.

Highlights

High pressure homogenization (HPH) induced autolysis in *S. bayanus* wine yeast

Release of soluble macromolecules and amino acids was comparable to thermolysis

Higher amounts of ethyl esters and lower fatty acids in autolysates produced by HPH

This makes HPH suitable for the production of yeast derivatives for wine ageing

Tested conditions didn’t inactivate all the viable cells: further optimization needed

Potential of high pressure homogenization to induce autolysis of wine yeasts

Piergiorgio Comuzzo*, Sonia Calligaris, Lucilla Iacumin, Federica Ginaldi, Anthony Efrain Palacios Paz, Roberto Zironi

Università degli Studi di Udine, Dipartimento di Scienze degli Alimenti, via Sondrio 2/A, 33100 Udine – Italy

* Corresponding Author
Piergiorgio Comuzzo
Tel: + 39 0432 55 8166
Fax: + 39 0432 55 8130
e-mail: piergiorgio.comuzzo@uniud.it

Abbreviated running title

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Abstract

High pressure homogenization (HPH) was tested for inducing autolysis in a commercial strain of *Saccharomyces bayanus* for winemaking. The effects on cell viability, the release of soluble proteins, glucidic colloids and amino acids in wine-like medium and the volatile composition of the autolysates were investigated after processing, in comparison with thermolysis. HPH seemed a promising technique for inducing autolysis of wine yeasts. One pass at 150 MPa was the best operating conditions. Soluble colloids, proteins and free amino acids were similar after HPH and thermolysis, but the former gave a more interesting volatile composition after processing, with higher concentrations of ethyl esters (fruity odors) and lower fatty acids (potential off-flavors). This might allow different winemaking applications for HPH, such as the production of yeast derivatives for wine ageing. In the conditions tested, HPH did not allow the complete inactivation of yeast cells; the treatment shall be optimized before winemaking use.

KEYWORDS: high pressure homogenization; thermolysis; *Saccharomyces bayanus*; autolysis; wine; inactive dry yeasts

1 Introduction

Yeast autolysis is an enzymatic self-degradation of cellular constituents that begins after cell death (Charpentier & Feuillat, 1993). Autolysis is an important technological tool during the ageing of certain wine typologies, such as white wines aged on the lees (e.g. French wines from Burgundy) or sparkling wines produced by *Champenoise* method. During ageing on the lees, wine composition varies as a consequence of the release of soluble polysaccharides (Charpentier & Feuillat, 1993), proteins (Perrot, Charpentier, Charpentier, Feuillat, & Chassagne, 2002), peptides and free amino acids (Alexandre, Heintz, Chassagne, Guilloux-Benatier, Charpentier, & Feuillat, 2001; Perrot et al., 2002), lipids (Pueyo, Martínez-Rodríguez, Polo, Santa-María, & Bartolomé, 2000), nucleotides and nucleosides (Charpentier, Aussenac, Charpentier, Prome, Duteurtre, & Feuillat, 2005) and these compositional modifications lead to changes in wine volatile profile (Pozo-Bayón, Pueyo, Martín-Álvarez, Martínez-Rodríguez, & Polo, 2003) and sensory characters (Carrascosa, Martínez-Rodríguez, Cebollero, & Gonzalez, 2011). Moreover, yeast lees are powerful oxygen scavengers (Salmon, Fornairon-Bonnefond, Mazauric, & Moutounet, 2000) and this allows the protection of white wines against oxygen spoilage during barrel ageing.

Despite these positive effects, the long time required for *sur lie* maturation increases the risk of microbial spoilage, such as *Brettanomyces* growth (Guilloux-Benatier, Chassagne, Alexandre, Charpentier, & Feuillat, 2001) and biogenic amine pollution (Martín-Álvarez, Marcobal, Polo, & Moreno-Arribas, 2006; González-Marco, & Ancín-Azpilicueta, 2006). For these reasons, different technological strategies have been suggested for accelerating yeast autolysis and *sur lie* ageing.

The most widely proposed tool is the use of commercial preparations of β-glucanases (Rodriguez-Nogales, Fernández-Fernández, & Vila-Crespo, 2012; Torresi, Frangipane, Garzillo, Massantini, & Contini, 2014); these enzymes are able to hydrolyze β-glucans from

yeast cell walls, increasing the rate of cell degradation and the release of soluble compounds. Another common practice is the addition of yeast derivatives (YD). These products are basically inactive dry yeasts, containing cell wall residues and metabolites released during production process (i.e. induced autolysis) (Pozo-Bayón, Andújar-Ortiz, & Moreno-Arribas, 2009 a). YDs (extracts and autolysates) have been described, even if with some controversial results, as additives for accelerating natural autolysis (Carrascosa et al., 2011), because of their ability to release macromolecules and soluble compounds (Pozo-Bayón et al., 2009 a). These substances can also modify the volatility of wine aroma, generally improving fruity and flowery characters (Rodriguez-Nogales et al., 2012); in a previous study (Comuzzo, Tat, Liessi, Brotto, Battistutta, & Zironi, 2012), a thermally-produced yeast autolysate was compared with a product obtained by enzyme-assisted autolysis (β-glucanase treatment) of the same *S. cerevisiae* strain: thermolysis led to lower levels of non-glycosylated proteins and higher amounts of soluble glycoproteins, and this seemed connected, respectively, with a lower capacity to retain wine aromas and with a higher ability of thermolysates to increase the volatility of certain wine compounds (e.g. esters). Unfortunately, the thermal treatments used during YD's manufacturing can lead to the formation of off-flavors that may be released into the wine, negatively affecting its sensory properties (Pozo-Bayon, Andujar-Otiz, & Moreno-Arribas, 2009c).

Besides β-glucanases and YD supplementation, other techniques are available to accelerate natural autolysis. Recently, ultrasounds (US) have been tested from this point of view, with positive results: ultrasound-assisted autolysis reduced yeast cell viability and increased the release of proteins (García Martín, Guillemet, Feng, & Sun, 2013), total colloids and glycoproteins (Cacciola, Ferran Batllò, Ferraretto, Vincenzi, & Celotti, 2013), even if US effects seemed generally less intense than the use of β-glucanases (Cacciola et al., 2013).

High pressure homogenization (HPH) could also be a good alternative to accelerate *sur lie* ageing. In fact, HPH has already been reported as effective tool for promoting the disruption

of *Saccharomyces* cells and the recovery of yeast intracellular components, such as enzymes and proteins (Middelberg, 1995; Follows, Hetherington, Dunnill, & Lilly, 1971; Hetherington, Follows, Dunnill, & Lilly, 1971). The microbial cell disruption during HPH has been associated to the occurrence of phenomena, such as cavitation, shear and turbulence that occur when the fluid is forced to pass through the narrow gap in the homogenizer valve (Popper & Knorr, 1990). Recently, the ability of HPH in destroying yeast cells has also been tested for the microbial stabilization of fruit juices (Campos, & Cristianini, 2007; Patrignani, Vannini, Kamdem, Lanciotti, & Guerzoni, 2009; Maresca, Donsi, & Ferrari, 2011) and beer (Franchi, Tribst, & Cristianini, 2013). Beside these results, HPH has been poorly considered in winemaking and the few applications reported are limited to the reduction of the indigenous flora in grape musts (Puig, Olmos, Quevedo, Guamis, & Minguez, 2008), or the modulation of autolysis in yeast starter *tirage* cultures during sparkling wine production (Patrignani et al., 2013). Based on literature results, HPH could be particularly interesting as a system to improve natural autolysis, because of the possibility to use it for promoting a non-thermal inactivation of yeasts during the manufacture of YDs, or to replace expensive or noisy technologies (e.g. enzymes or ultrasounds respectively) for processing the lees before wine ageing.

The aim of this work was to study the performances of HPH treatments, in promoting autolysis of a commercial strain of *Saccharomyces bayanus* for winemaking use, in comparison with a thermally-induced cell lysis. The effects of different HPH treatments, as well as thermolysis, were studied by measuring cell viability and the release of free amino acids, proteins and glucidic colloids in wine-like medium. Finally, the impact of the different processes on the development of volatile compounds was also investigated.

2 Materials and Methods

111 *2.1 Chemicals*

112 Sodium chloride, tartaric acid, sodium hydroxide and ethanol (96 % v/v) were purchased from
113 Carlo Erba Reagents (Milan, Italy); *o*-phthaldialdehyde, bovine serum albumin (BSA)
114 fraction V and HPLC grade isoleucine (Ile) were from Sigma-Aldrich (St. Louis, MO, USA);
115 bacteriological peptone and Malt Extract Agar were from Oxoid (Basingstoke, UK).

116 *2.2 Yeast and lysis treatments*

117 A commercial *Saccharomyces bayanus* active dry yeast (ADY) preparation (Mycoferm Cru-
118 05, from EverIntec – Pramaggiore, VE, Italy) was used for the experimental trials. 20 g of
119 ADY was suspended in 200 ml of distilled water and the suspension was immediately
120 subjected to high pressure homogenization and thermolysis, as reported below.
121 HPH was carried out by using a two stage high pressure homogenizer (Panda PLUS 2000,
122 Gea Niro Soavi, Parma, Italy) provided with cylindrical tungsten carbide homogenizing
123 valves. The first valve, which is the actual homogenization stage, was set at 0, 50, 100 and
124 150 MPa; whereas the second valve was fixed at 5 MPa. Samples (200 ml) were homogenized
125 *via* one single pass at 10.8 l h^{-1} flow rate. The homogenizer inlet and outlet were connected to
126 a heat exchanger (Julabo F70, Julabo GmbH, Seelbach, Germany) set at 4 °C. The sample
127 temperature was measured just before and immediately after homogenization by a copper-
128 constantan thermocouple probe (Ellab, Hillerød, Denmark) connected to a portable data
129 logger (mod. 502A1, Tersid, Milan, Italy). As control, thermolysates (200 ml) were prepared
130 by heating the yeast suspension at 121 °C for 2 h in autoclave.
131 The HPH treated samples as well as the thermolysates were stored overnight at 0/+4 °C and
132 then analyzed for microbial viability. The remaining samples were immediately after
133 treatments arranged in food-grade aluminum trays (approx. in a 1 cm layer), frozen at -18 °C,
134 and freeze-dried by using a pilot plant model Mini Fast 1700 (Edwards Alto Vuoto, Milan,
135 Italy). At the end of the process, the samples were finally ground in a ceramic mortar and

136 stored in sealed glass containers (0/+4 °C), until chemical and GC-MS analyses. The active
137 dry yeast preparation used for the experiments, was also subjected to all the analytical
138 determinations reported below, as a reference sample.

139 *2.3 Soluble proteins and free amino acids*

140 The amounts of proteins and free amino acids soluble in wine-like solution, were determined
141 on freeze-dried samples, respectively by Lowry method and *o*-phthaldialdehyde (OPA)
142 derivatization. Aliquots of 1.00 g of powder were suspended in 100 ml of a hydroalcoholic-
143 tartaric buffer (12 % v/v ethanol, in 0.03 M tartaric acid, buffered at pH 3.20 with 4 M sodium
144 hydroxide); after 10 min, the suspensions were centrifuged (5000 rpm for 10 min) and the
145 supernatant was analyzed as reported below.
146 Concerning soluble proteins, 400 μl of limpid solution was subjected to the Lowry assay, as
147 reported by Regenstein & Regenstein (1984); results were given in mg g^{-1} of dried powder,
148 according to a calibration line prepared with bovine serum albumin (BSA).
149 Free amino acids were determined on the supernatant, by OPA derivatization, according to the
150 method published by Dukes & Butzke (1998); the results were expressed in mg g^{-1} of dried
151 powder, on the basis of a calibration line obtained with isoleucine (Ile).

152 *2.4 Glucidic colloids*

153 The amount of glucidic colloids soluble in wine-like medium were determined by ethanol
154 precipitation, modifying the method reported by Usseglio-Tomasset & Castino (1975);
155 aliquots of 1.00 g of freeze-dried powder were suspended in 10 ml of hydroalcoholic-tartaric
156 buffer; after 10 min, the suspensions were centrifuged (5000 rpm for 10 min) and 5 ml of the
157 supernatant was added to 25 ml of 96 % (v/v) ethanol. Samples were stored at 0/+4 °C for 24
158 h; glucidic colloids were recovered by vacuum filtration on a 0.45 μm pore size nylon
159 membrane (Albet-Hahnemühle, Barcelona, Spain) and then determined by weighing, after

160 evaporation of ethanol (at 50 °C), until constant weight; results were given in mg of total
161 colloids per g of freeze-dried powder.

162 *2.5 Microbiological analyses*

163 One (1) ml of each treated sample was transferred into a sterile tube, 9 ml of saline-peptone
164 water (8 g l^{-1} sodium chloride, 1 g l^{-1} bacteriological peptone) were added and mixed for 1.5
165 min using a vortex mixer (VWR International PBI, Milan, Italy). Further decimal dilutions
166 were made in the same solution and yeasts were counted in triplicate agar plates on Malt
167 Extract Agar, incubated at 25°C for 48-72 h under aerobic conditions.

168 *2.6 SPME-GC-MS analyses*

169 The analysis of volatile compounds in the headspace of the freeze-dried powders was
170 performed on a GC-17A gas chromatograph, coupled with a QP-5000 mass spectrometer
171 (both from Shimadzu, Kyoto, Japan), as reported elsewhere (Comuzzo et al., 2012).
172 Aliquots of 2.00 g of the freeze-dried powders were introduced in 50 ml amber glass vials and
173 closed with PTFE/silicone septa. Solid-phase microextraction was carried out at 40 °C by
174 using a 2 cm 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane fiber (Supelco,
175 Bellefonte, PA, USA), with a sampling time of 15 min. Vials were previously pre-conditioned
176 for 15 min before microextraction, to allow the thermal equilibration of the samples.
177 Volatile compounds were separated on a J&W DB-Wax capillary column (30 m x 0.25 mm
178 i.d., 0.25 μm film thickness), purchased from Agilent Technologies Inc. (Santa Clara, CA,
179 USA), with the following operating conditions: 40 °C for 1 min, then 4 °C min^{-1}, up to 240
180 °C, with a final holding of time of 15 min. Injection was performed in splitless mode (60 s of
181 splitless time); the temperature of the injection port and the transfer line was 250 and 240 °C
182 respectively. The carrier gas was helium, at a linear flow rate of 35 cm s^{-1}.

Electron impact mass spectra were recorded at 70 eV and the identification of volatile compounds was carried out by comparison of their mass spectra and retention times with those of standard compounds, or by comparison of mass spectrum, with those reported in the mass spectrum libraries Wiley 6 and NIST 107; linear retention indexes were also calculated on the basis of the retention times of *n*-alkanes, and compared with those reported in literature.

2.7 Statistical analyses

The results are averages of at least three measurements taken from three experiment replications. One-way ANOVA was carried out on the values found for the different parameters analyzed, as well as on the absolute areas of the volatile compounds detected in the headspace of the freeze-dried powders. Means and standard deviations were calculated and significant differences were assessed by Tukey HSD Test at $p < 0.05$.

Concerning SPME-GC-MS analyses, the aroma compounds were grouped, sample by sample, on the basis of their chemical class and the total absolute area obtained for each group was subjected to Principal Component Analysis (PCA). All the elaborations were carried out by the software Statistica for Windows (StatSoft, Tulsa, OK, USA), Version 8.0.

3 Results and Discussion

3.1 Yeast viability

The effect of HPH and thermolysis on yeast cell viability is reported in Fig. 1. As expected, thermolysis caused the complete inactivation of the viable cells in the sample (< 10 CFU g^{-1}). On the contrary, HPH processing led to a 1 log unit reduction at 0 and 50 MPa and a 1.6 and 2.2 log units decrease at 100 and 150 MPa respectively. These results are consistent with literature, highlighting the high resistance of yeasts to HPH treatments (Patrignani et al., 2013 and 2009). Anyway, even without achieving a complete inactivation of the viable cells, it is

expected that HPH treatments may accelerate yeast autolysis during wine aging, as well reported by Patrignani et al. (2013). Moreover, it is interesting to observe that, despite HPH treatments promoted a progressive increase of the sample temperature as pressure increases, the extent of heating was far from that obtained by thermolysis: the sample temperature, measured at the homogenizer outlet, ranged from 20±0.5 °C (untreated sample, 0 MPa) to 39±1.0 °C (150 MPa).

3.2 Release of proteins, free amino acids and glucidic colloids in wine-like solution

As aforementioned, the performances of HPH treated yeasts are strictly related not only to cell viability but also to the release of soluble compounds in wine-like medium. Fig. 2 reports the levels of soluble proteins and free amino acids in the active dry yeast preparation (L) and in the samples obtained by HPH (0-150MPa) and thermolysis (T_121).

Concerning proteins (Fig. 2a), one can observe that the simple passage through the homogenization valve increased their release, so that the 0 MPa treated samples showed a significantly higher content of soluble proteins respect to the ADY preparation (L). For the other treatments, the levels detected increased linearly as the applied pressure increases and the treatment at 150 MPa led to a protein release not significantly different from that of the thermolysates. Generally speaking, the concentrations reported in Fig. 2a are quite low and this might be a positive factor for an eventual winemaking application of the products tested; in fact, high levels of proteins may negatively affect wine aroma perception, since they can bind wine volatile compounds reducing their volatility (Voilley, Beghin, Charpentier, & Peyron, 1991).

The release of free amino acids (Fig. 2b) followed the same behavior of soluble proteins, showing the highest values for the thermally treated samples and for those processed at 100 and 150 MPa. A good extent of proteolysis and thus a high amount of free amino acids are highly desired when yeast derivatives are used as alcoholic fermentation enhancers. On the

232 contrary, they could represent a problem if such products are added during wine ageing, since
233 free amino acids can become substrates for bacterial growth, leading to the production of
234 undesired compounds (e.g. biogenic amines) (González-Marco, & Ancín-Azpilicueta, 2006).
235 Moreover, amino acids are important flavor precursors in yeast derivatives (Münch, &
236 Schieberle, 1998) and it is well known that some commercial YD products can negatively
237 affect wine aroma, releasing off-odors (Comuzzo, Tat, Tonizzo, & Battistutta, 2006); this was
238 also highlighted by Charpentier & Feuillat (1993), who affirmed that YDs commonly used in
239 the food industry have undergone excessive proteolysis and can give rise to off-flavors when
240 added to wine. For this reason, the low amounts reported in Fig. 2b, might represent a positive
241 factor for the use of such products during wine ageing.
242 The content of soluble glucidic colloids is also an important factor for wine quality, since
243 polysaccharides and mannoproteins have been reported as good enhancers of aroma volatility
244 (Dufour, & Bayonove, 1999). As previously observed (Comuzzo et al., 2012), this index is
245 connected with the release of glycosylated proteins (e.g. mannoproteins) from autolyzed
246 yeasts. Figure 3 shows the amount of soluble glucidic colloids in the considered samples.
247 Also in this case, HPH determined a progressive increase of their release in wine-like
248 medium, as the pressure increases. According to ANOVA analysis, the samples processed at
249 150 MPa did not differ significantly from thermolysates. The concentrations found are in
250 good agreement with those reported in literature (Comuzzo et al., 2006; Pozo-Bayón,
251 Andújar-Ortiz, Alcaide-Hidalgo, Martin-Álvarez, & Moreno-Arribas, 2009 b; Comuzzo et al.,
252 2012), so that, if the products obtained by thermolysis and HPH at 150 MPa were added to
253 wine at a concentration of 0.5 g l^{-1} (normal amounts for YD supplementation), they would be
254 able to release approx. 100 mg l^{-1} of soluble colloids, that represent practically a good
255 amount.
256 In conclusion of this part, HPH seems a promising technique for promoting autolysis of wine
257 yeasts, at least for obtaining derivatives to be used during wine ageing. Contrarily, the low

levels of free amino acids detected, makes the conditions tested not suitable for the production of fermentation enhancers. Anyway, further investigations might be useful for optimizing the process, even for the obtainment of fermentation nutrient preparations coming from HPH-autolyzed wine yeasts.

In particular, the treatment at 150 MPa behaved very similarly to thermolysis, leading to low amounts of soluble proteins (potentially involved in wine aroma retention), low concentrations of free amino acids (involved in off-flavor formation) and good levels of glucidic colloids (potential enhancers of wine aroma volatility). The advantage of HPH, compared to thermal treatments (e.g. thermolysis), is that the lytic process occurs without heating and thermal damage to the products. As mentioned above, this could be a real benefit for this technology, because the high temperatures applied during the manufacturing of yeast-derived products have been linked with the formation of unpleasant odorant compounds that may be released into the wine (Münch, & Schieberle, 1998, Pozo-Bayón et al., 2009c). To confirm this hypothesis, the volatile composition of the tested products was studied.

3.3 Volatile composition of autolysates

Twenty-six volatile compounds were tentatively identified in the active dry yeast preparation and in the samples obtained after HPH and thermolysis (Table 1). They were mainly products of yeast metabolism, such as alcohols, short-chain free fatty acids and ethyl esters, with a minor presence of diols, hetetocyclic compounds and carbonyls. Surprisingly, no alkyl-pyrazines, pyrroles, or other compounds typically found in yeast-derived products were detected (Münch, & Schieberle, 1998).

The results of Principal Component Analysis (PCA) are reported in Fig. 4. As one can observe, HPH treated products (0-150 MPa) are well separated respect to both the active dry yeast preparation (L) and the thermolysates (T_121). The latters seemed mostly characterized by the presence of short-chain free fatty acids and some carbonyl compounds. On the

283 contrary, higher alcohols and ethyl esters had an average higher concentration in high
284 pressure treated samples. Untreated yeast suspension (L) was qualitatively more similar to the
285 thermolysates, even if the three repetitions are grouped separately from T_121 products.
286 ANOVA analyses confirmed the behaviors highlighted by PCA, giving also additional
287 information: significant differences among the treatments were marked for the most of the
288 compounds detected (Table 2). As one can observe, higher alcohols and particularly esters
289 were significantly higher in HPH treated samples, and their absolute area in the headspace of
290 the tested products increased by increasing the pressure applied. Thermolysis probably led to
291 the breakdown of such compounds, so that their amount in the thermally-treated products was
292 generally lower. According to Alexandre, & Guilloux-Benatier (2006), esters are the major
293 family of volatile compounds released during autolysis; they are characterized by fruity and
294 positive odors and their higher presence in the products obtained by HPH could be an
295 interesting perspective concerning the application of this technology for winemaking. Higher
296 alcohols are also released during yeast autolysis (Alexandre, & Guilloux-Benatier, 2006).
297 Particularly, 2-phenylethanol has a typical rose odor and the higher concentrations detected in
298 HPH products may represent a positive character for wine aroma. The presence of higher
299 amounts of alcohols and ethyl esters represents further evidence about the ability of HPH to
300 induce autolysis in wine yeast.
301 Fatty acids (particularly acetic and 2-methylpropanoic) were significantly more present in
302 samples L and T_121. In HPH treated products, a slight increase of their mean concentrations
303 (even if not statistically evident) can be observed when the pressure increased. These short-
304 chain fatty acids are produced by yeast metabolism (e.g. acetic acid), but some of them (e.g.
305 2-methylpropanoic acid) can also be formed from the oxidation of Streker's aldehydes (Ames,
306 & McLeod, 1985), derived from the degradation of amino acids during Maillard reaction
307 (Münch, & Schieberle, 1998); this may justify the reason why 2-methylpropanoic acid is
308 generally higher in T_121 samples. Fatty acids are connected with pungent and cheese-like

olfactory notes, and, as previously observed, they could be released into the wine, jeopardizing its global quality (Comuzzo et al., 2006).

Concerning carbonyl compounds, the most abundant were hexanal and acetoin: their concentrations were higher in sample L, while they decreased after processing. As well-known acetoin is a yeast metabolite, characterized by a buttery odor, whereas hexanal is a widely used marker of the development of oxidation of the lipid fraction. The reason of their decrease during both thermal treatment and HPH remains unclear, but reasonably, it might be linked to their involvement in chemical reactions during processing. Among carbonyls, 6-methyl-5-hepten-2-one and acetylcarbinol were characteristic of the thermally treated samples. The former is reported as a breakdown product of carotenoids (Schreier, Drawert, & Junker, 1977), but, as other carbonyls, it might also derive from the oxidative breakdown of lipids, due to the high temperatures reached during the thermal treatment (Grosch, 1982). The lower amounts of carbonyls found in the HPH-treated products could be a positive factor for their winemaking use, because of the negative impact that some of these compounds may have on wine aroma (e.g. hexanal and herbaceous characters). In a previous experiment carried out by gas chromatography – olfactometry (GC-O) we detected 6-methyl-5-hepten-2-one in a chromatographic zone characterized by pungent, potato and cabbage-like odors (Comuzzo et al., 2006).

Finally, diols and heterocyclic compounds were detected in quite low concentrations in all the samples analyzed; they were averagely more present in the ADY preparation and thermolysates, and some of them (e.g. 2,3-butanediol and γ-butyrolactone) are well-known *Saccharomyces* metabolites.

4 Conclusions

In conclusion, HPH treatment was able to induce autolysis in the wine yeast strain (*Saccharomyces bayanus*) used in the experiment, promoting the release of

macromolecules in wine-like medium. The treatment at 150 MPa seemed the most promising, leading to a release of soluble glucidic colloids and proteins similar to those produced by thermally induced autolysis, and a volatile profile expected to be better than that of the thermally treated product. In fact, HPH induced a more interesting composition of the volatile fraction of the autolysates obtained, with a lower concentration of short-chain free fatty acids and higher amounts of ethyl esters. This characteristic is really interesting in the attempt of producing YDs for winemaking, because the use of commercially available products has been linked to the release of off-flavors (e.g. fatty acids) in wine, and this may negatively affect sensory properties.

These evidences allow to imagine different applications for HPH technology in winemaking, such as the production of specific preparations of yeast derivatives for wine storage and ageing. In particular, by modulating the homogenization pressure it would be possible to obtain YDs with defined performances in terms of protein, free amino acid and colloid content and a volatile profile tailored for specific winemaking applications.

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Figure Captions

Fig. 1. Viable yeasts in the active dry yeast preparation (**L**) and in the powders obtained by HPH (**0-150 MPa**) and thermolysis (**T_121**). Means and standard deviations of three repetitions are reported. Different letters represent significant differences according to ANOVA and Tukey HSD test ($p < 0.05$). For sample T_121, data are reported in CFU g^{-1} (< 10 CFU g^{-1}).

Fig. 2. Levels of soluble proteins **(a)** and free amino acids **(b)** in the active dry yeast preparation (**L**) and in the powders obtained by HPH (**0-150 MPa**) and thermolysis (**T_121**). Means and standard deviations of three repetitions are reported. Different letters represent significant differences according to ANOVA and Tukey HSD test ($p < 0.05$).

Fig. 3. Glucidic colloids content in the active dry yeast preparation (**L**) and in the powders obtained by HPH (**0-150 MPa**) and thermolysis (**T_121**). Means and standard deviations of three repetitions are reported. Different letters represent significant differences according to ANOVA and Tukey HSD test ($p < 0.05$).

Fig. 4. PCA of the total absolute areas, obtained by grouping the volatile compounds detected in the headspace of the freeze-dried powders, on the basis of their chemical class. The projection of cases (samples) (**a**) and variables (chemical classes) (**b**) on the factor-plane are both reported. **L**: active dry yeast preparation; **0-150 MPa**: HPH treated samples; **T_121**: thermolysates.

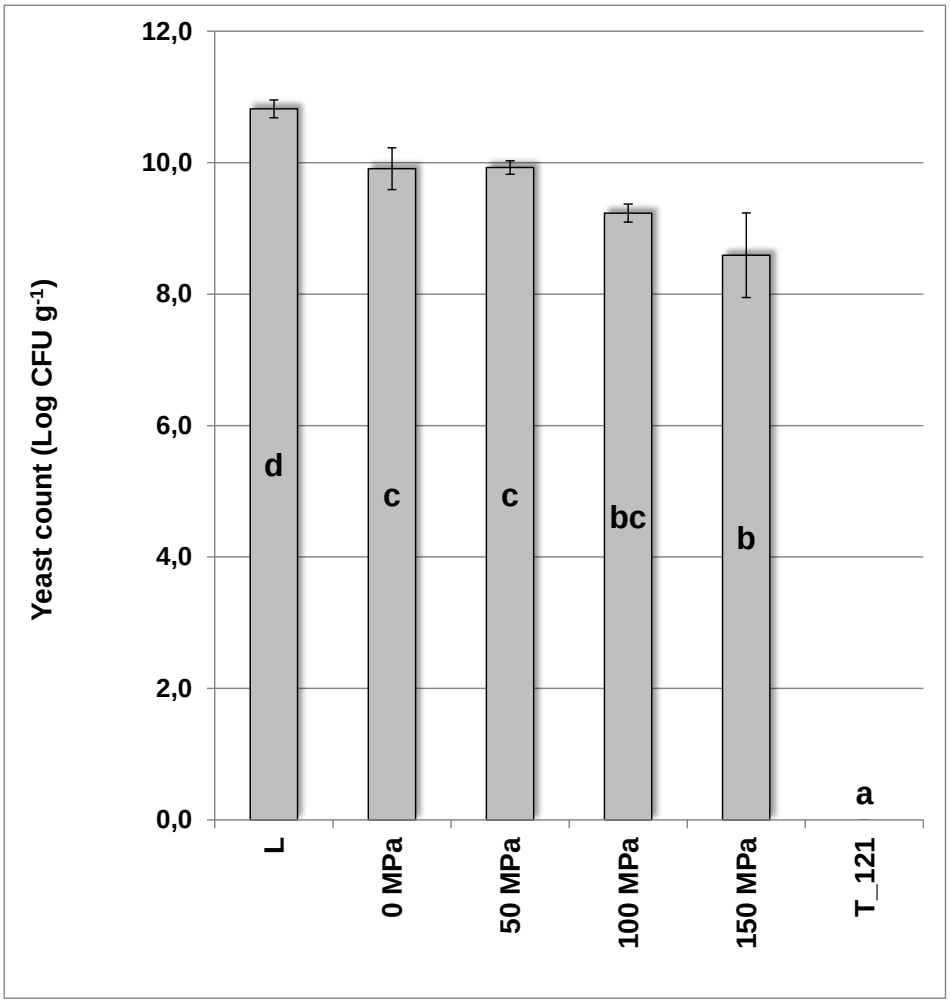

12,0
10,0
8,0
6,0
4,0
2,0
0,0
Yeast count (Log CFU g-1)
d
c
c
bc
b
a
L
0 MPa
50 MPa
100 MPa
150 MPa
T_121

Fig. 1

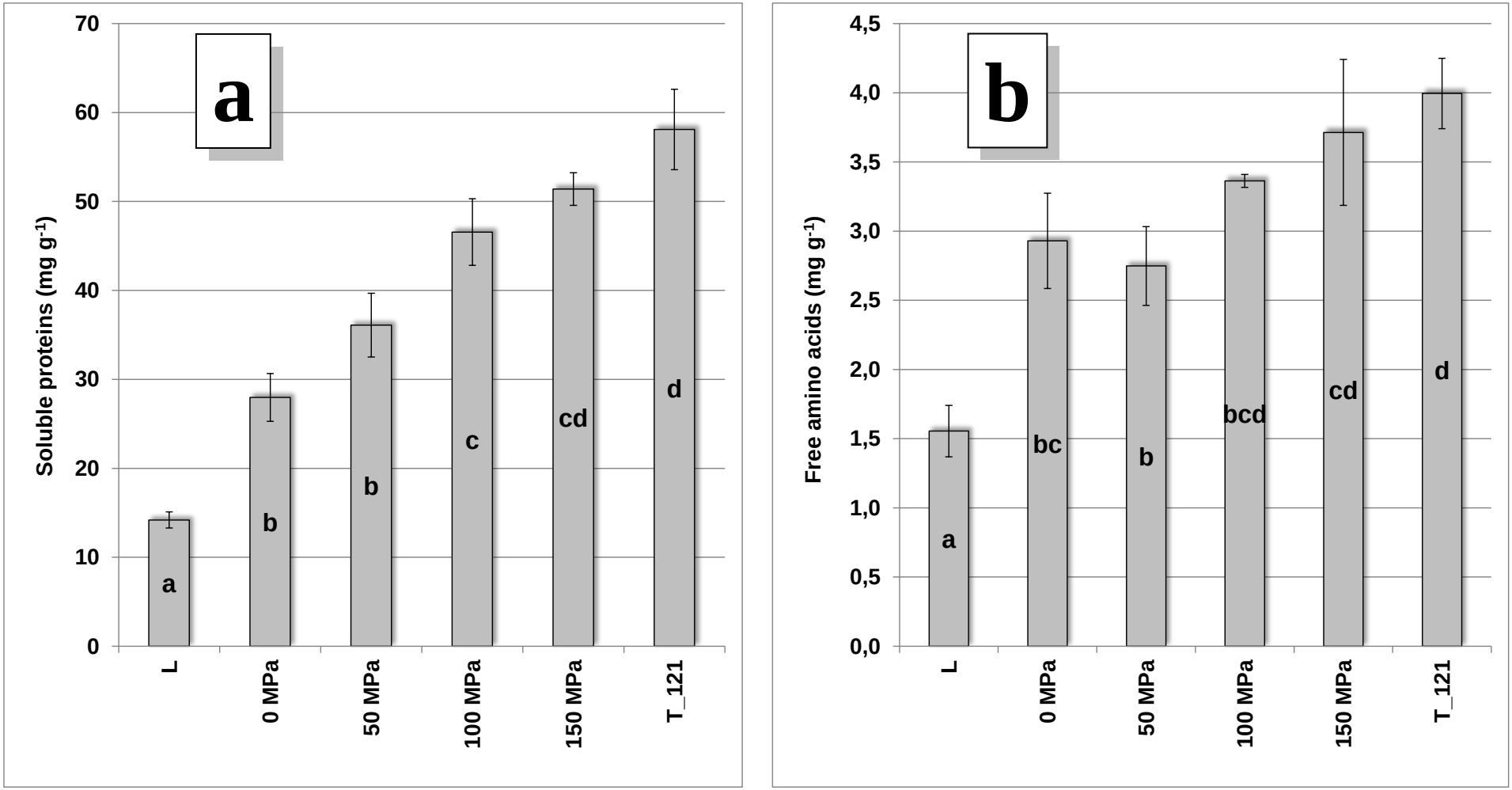
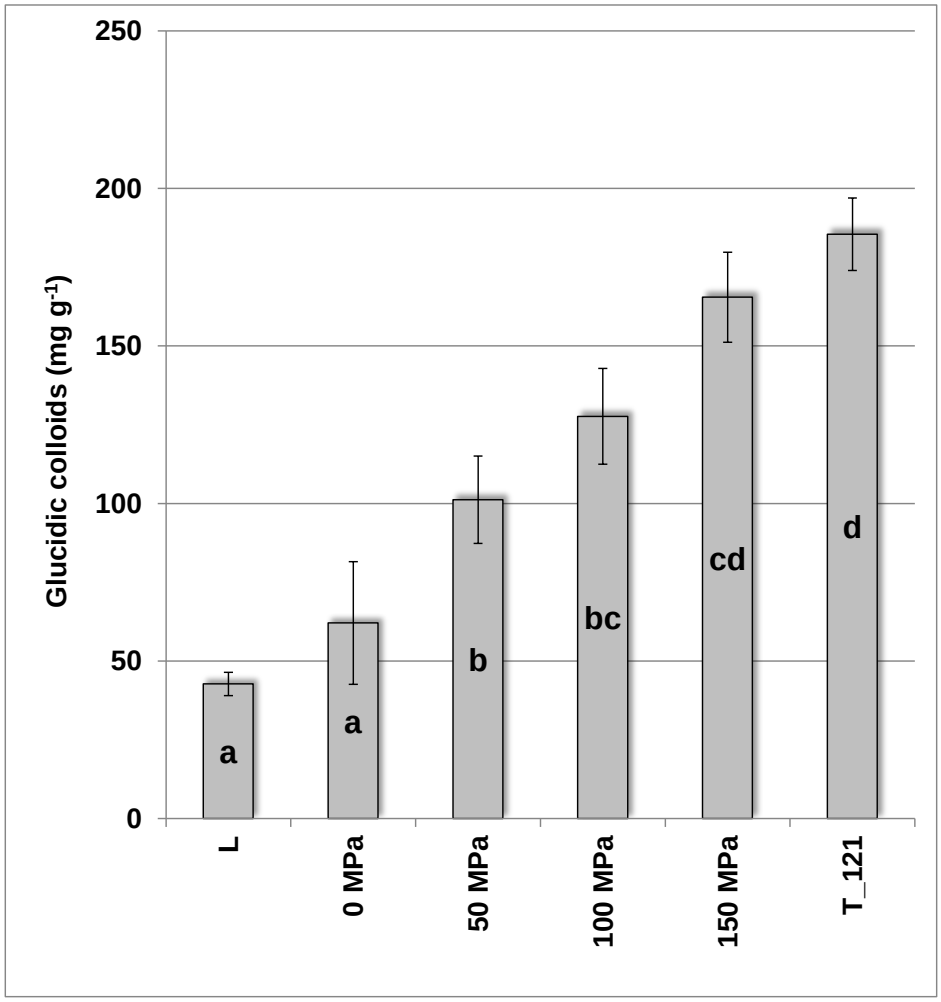

a
Soluble proteins (mg g-1)
70
60
50
40
30
20
10
0
a
b
b
c
cd
d
L
0 MPa
50 MPa
100 MPa
150 MPa
T_121
b
Free amino acids (mg g-1)
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
a
bc
b
bcd
cd
d
L
0 MPa
50 MPa
100 MPa
150 MPa
T_121

Fig. 2

554

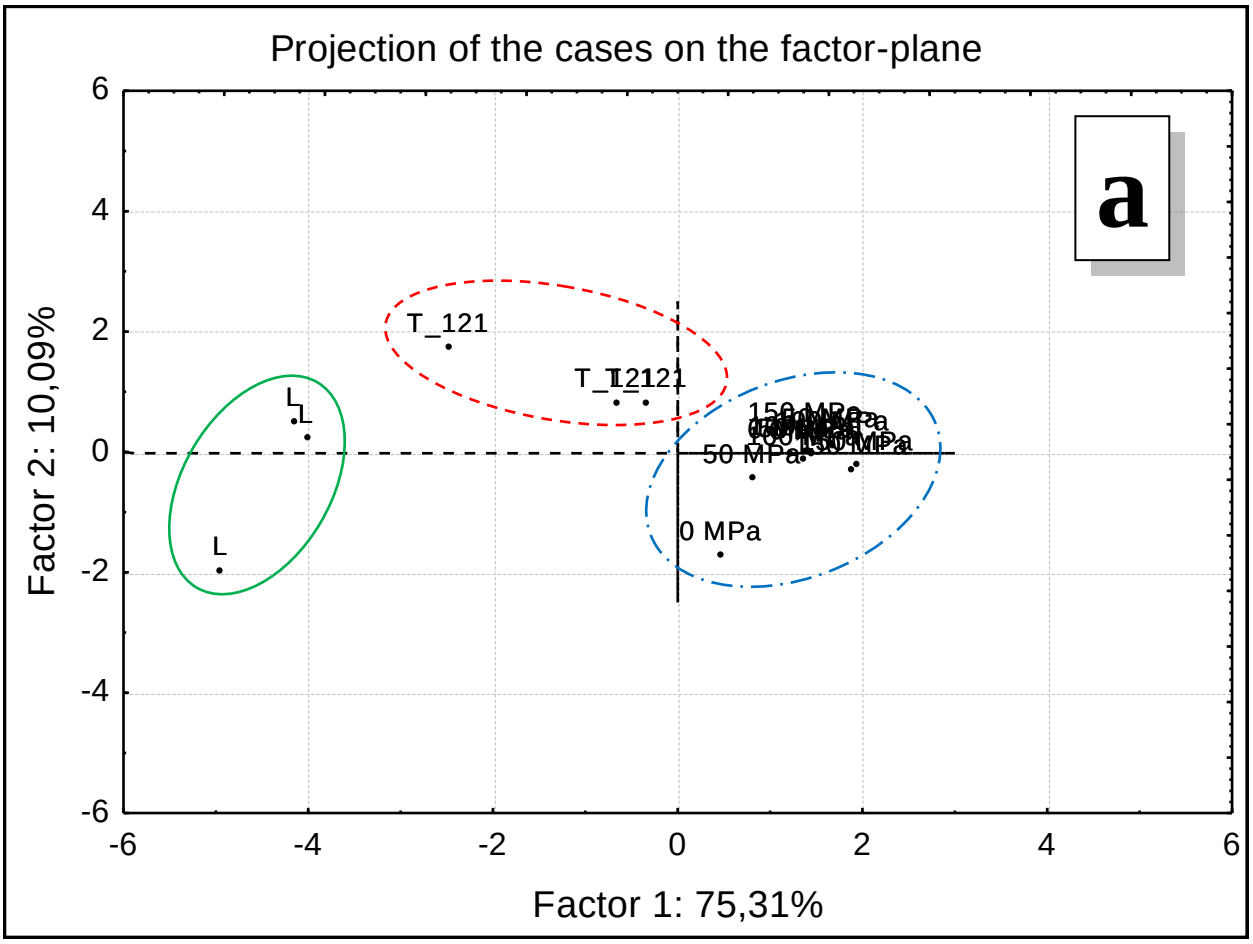
555



556

557

558 **Fig. 3**


Projection of the cases on the factor-plane
a
T_121
T_121
L
L
L
50 MPa
0 MPa
Factor 2: 10,09%
Factor 1: 75,31%
6
4
2
0
-2
-4
-6
-6
-4
-2
0
2
4
6

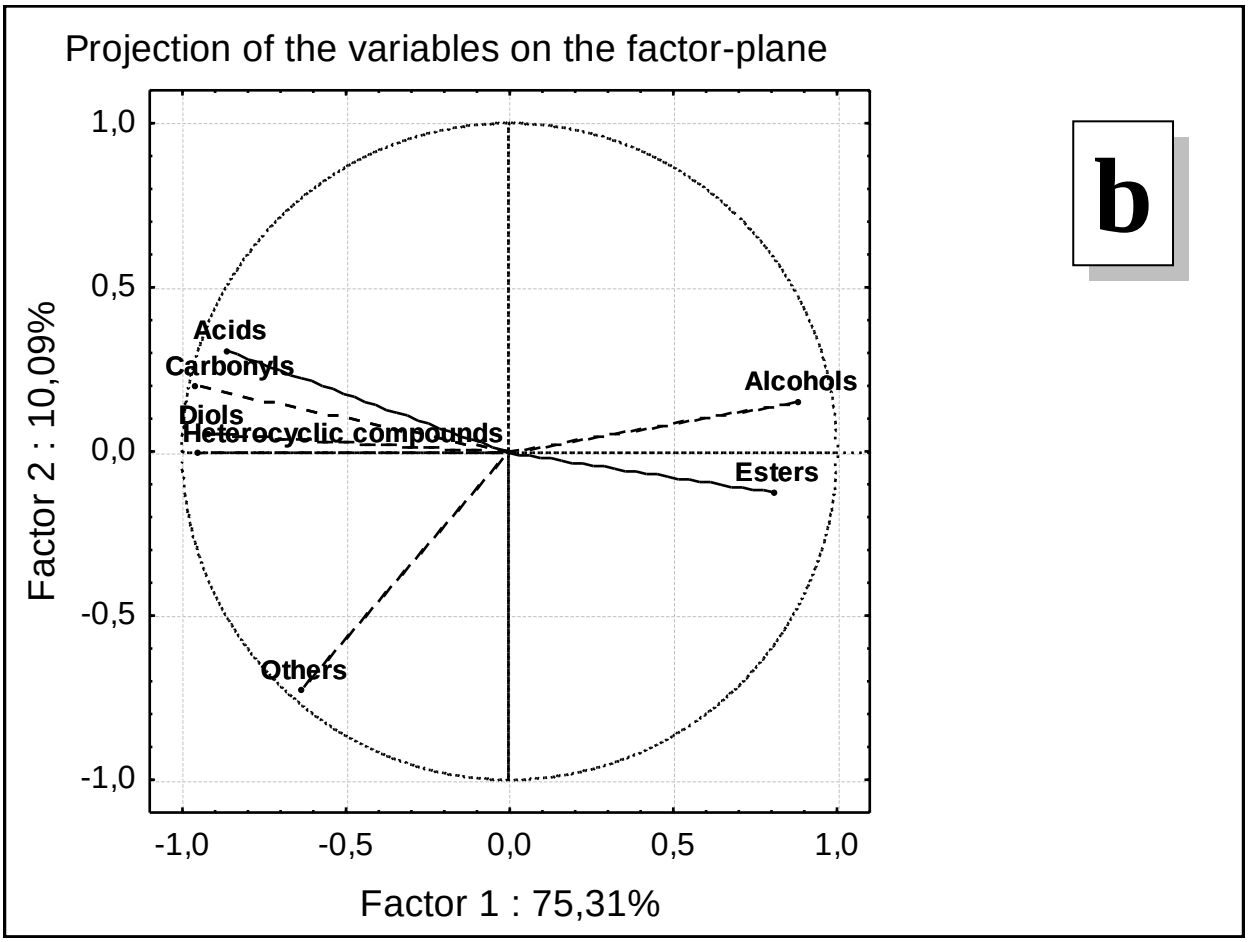

Projection of the variables on the factor-plane
b
Acids
Carbonyls
Diols
Heterocyclic compounds
Alcohols
Esters
Others
Factor 2 : 10,09%
Factor 1 : 75,31%
1,0
0,5
0,0
-0,5
-1,0
-1,0
-0,5
0,0
0,5
1,0

Fig. 4

Table 1

Volatile compounds tentatively identified in the headspace of the active dry yeast preparation and the autolysates obtained by HPH and thermolysis.

	Compound	Ri [a]	$Ri_{(lit)}$ [b]	IM [c]	Reference
1	ethanol	936	929	MS, IR	http://www.flavornet.org/flavornet.html (2014)
2	hexanal	1080	1084	MS, IR, S	Jennings & Shibamoto (1980). New York: Academic Press
3	2-methyl-1-propanol	1093	1096	MS, IR	Lopez, Ferreira, Hernandez & Cacho (1999). J. Sci. Food Agric., 7, 1461-1467
4	2- and 3-methyl-1-butanol	1212	1210	MS, IR, S	Baek & Cadwallader (1999). J. Food Sci., 64, 441-444
5	ethyl hexanoate	1237	1234	MS, IR, S	Baek & Cadwallader (1999). J. Food Sci., 64, 441-444
6	unknown	1252			
7	3-hydroxy-2-butanone (acetoin)	1281	1290	MS, IR	Baek & Cadwallader (1999). J. Food Sci., 64, 441-444
8	1-hydroxy-2-propanone (acetylcarbinol)	1290	1300	MS, IR	Gonzalez-Rios, Suarez-Quiroz, Boulanger, Barel, Guyot, Guiraud & Schorr-Galindo (2007). J. Food Comp. Anal. 20, 297-307
9	6-methyl-5-hepten-2-one	1333	1336	MS, IR	Comuzzo, Tat, Tonizzo & Battistutta (2006). Food Chem., 99, 217-230.
10	1-hexanol	1359	1359	MS, IR, S	Lopez, Ferreira, Hernandez & Cacho (1999). J. Sci. Food Agric., 7, 1461-1467
11	ethyl octanoate	1432	1435	MS, IR, S	Baek & Cadwallader (1999). J. Food Sci., 64, 441-444
12	acetic acid	1448	1451	MS, IR, S	Baek & Cadwallader (1999). J. Food Sci., 64, 441-444
13	2-ethyl-1-hexanol	1493	1490	MS, IR, S	Madruga & Mottram (1998). J. Braz. Chem. Soc., 9, 261-271
14	2,3-butanediol	1545	1545	MS, IR	Baek & Cadwallader (1999). J. Food Sci., 64, 441-444
15	2-methylpropanoic acid	1567	1548	MS, IR, S	Münch, Hofmann & Schieberle (1997). J. Agric. Food Chem., 45, 1338-1344
16	1,2-propanediol	1582	1594	MS, IR	Wong & Bernhard (1988). J. Agric. Food Chem., 36, 123-129
17	dihydro-2(3H)-furanone (γ-butyrolactone)	1618	1632	MS, IR, S	Jennings & Shibamoto (1980). New York: Academic Press
18	butanoic acid	1627	1612	MS, IR, S	Münch, Hofmann & Schieberle (1997). J. Agric. Food Chem., 45, 1338-1344
19	ethyl decanoate	1635	1634	MS, IR, S	Lopez, Ferreira, Hernandez & Cacho (1999). J. Sci. Food Agric., 7, 1461-1467
20	3-methylbutanoic acid	1669	1672	MS, IR, S	Baek & Cadwallader (1999). J. Food Sci., 64, 441-444
21	diethyl succinate	1676	1642	MS	Jennings & Shibamoto (1980). New York: Academic Press
22	5,6-dihydro-2H-pyran-2-one	1688		MS	
23	hexanoic acid	1848	1854	MS, IR, S	Lopez, Ferreira, Hernandez & Cacho (1999). J. Sci. Food Agric., 7, 1461-1467
24	2-phenylethanol	1902	1922	MS, IR, S	Baek & Cadwallader (1999). J. Food Sci., 64, 441-444
25	1,4-butanediol	1924	1861	MS	Jennings & Shibamoto (1980). New York: Academic Press
26	2-ethylhexanoic acid	1947	1974	MS, IR	Welke, Manfroi, Zanus, Lazarotto & Alcaraz Zini (2012). J. Chromatogr. A, 1226 124-139

[a] Calculated linear retention index

[b] Linear retention index from literature

[c] IM: identification method:

S comparison of mass spectra and retention time with those of standard compounds; **RI** comparison of order of elution with those reported in literature; **MS** comparison of mass spectra with those reported in Wiley 6 and NIST 107 mass spectrum libraries

Table 2

Volatile compounds (absolute area / 1000) in the headspace of the active dry yeast preparation (L) and the powders obtained by HPH (0-150 MPa) and thermolysis (T_121). Means and standard deviations of three repetitions are reported. Different letters represent significant differences according to ANOVA and Tukey HSD test ($p < 0.05$).

Compound	Ri [a]	L		0 MPa		50 MPa		100 MPa		150 MPa		T_121	
		Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD	
Alcohols													
ethanol	936	61216 ± 12054	a	567490 ± 53496	b	581152 ± 50167	b	637790 ± 70917	b	669040 ± 81147	b	629121 ± 6478	b
2-methyl-1-propanol	1093	0 ± 0	a	13116 ± 4292	a	41503 ± 8147	ab	72669 ± 9847	bc	107685 ± 39811	c	12051 ± 5460	a
2- and 3-methyl-1-butanol	1212	2220 ± 898	a	229989 ± 44545	b	416358 ± 105722	c	568392 ± 50513	cd	693265 ± 72269	d	214472 ± 75572	b
1-hexanol	1359	2705 ± 283	c	1400 ± 773	ab	1134 ± 493	ab	800 ± 302	ab	1405 ± 218	b	190 ± 329	a
2-ethyl-1-hexanol	1493	2273 ± 192	a	1255 ± 352	a	1195 ± 359	a	1291 ± 301	a	1242 ± 305	a	1938 ± 758	a
2-phenylethanol	1902	899 ± 113	a	1340 ± 721	ab	1442 ± 451	ab	1824 ± 417	ab	2221 ± 324	b	1677 ± 349	ab
Esters													
ethyl hexanoate	1237	0 ± 0	a	1267 ± 400	ab	2287 ± 1025	bc	1909 ± 822	abc	3799 ± 1278	c	0 ± 0	a
ethyl octanoate	1432	5795 ± 5762	a	43638 ± 16063	ab	60086 ± 25653	b	58124 ± 8121	b	84672 ± 21063	b	5817 ± 6899	a
ethyl decanoate	1635	0 ± 0	a	2444 ± 867	bc	3001 ± 1179	cd	2879 ± 583	cd	4671 ± 889	d	656 ± 539	ab
diethyl succinate	1676	0 ± 0	a	694 ± 154	a	1418 ± 681	ab	1321 ± 527	ab	2206 ± 1014	b	0 ± 0	a
Acids													
acetic acid	1448	70496 ± 2444	c	2712 ± 1194	a	3372 ± 1961	a	2855 ± 732	a	4370 ± 474	a	23213 ± 9604	b
2-methylpropanoic acid	1567	21025 ± 7224	ab	4695 ± 3535	a	12123 ± 8661	ab	9474 ± 9198	ab	16790 ± 4267	ab	41589 ± 25135	b
butanoic acid	1627	6245 ± 877	c	0 ± 0	a	721 ± 750	ab	475 ± 577	ab	1046 ± 78	ab	2646 ± 1766	b
3-methylbutanoic acid	1669	7781 ± 1900	a	1882 ± 1716	a	5777 ± 5294	a	4494 ± 5094	a	7634 ± 3243	a	25647 ± 19910	a
hexanoic acid	1848	3585 ± 212	bc	72 ± 125	a	701 ± 611	a	671 ± 482	a	1106 ± 445	ab	3674 ± 2041	c
2-ethylhexanoic acid	1947	875 ± 258	a	548 ± 489	a	887 ± 186	a	1020 ± 1126	a	1144 ± 840	a	8894 ± 10798	a
Carbonyls													
hexanal	1080	12270 ± 7759	b	2517 ± 900	a	2585 ± 1795	a	2028 ± 1733	a	2454 ± 368	a	2423 ± 2161	a
3-hydroxy-2-butanone (acetoin)	1281	31240 ± 1210	c	220 ± 196	a	437 ± 385	a	1030 ± 941	a	2010 ± 1113	ab	3833 ± 1283	b
1-hydroxy-2-propanone (acetylcarbinol)	1290	0 ± 0	a	0 ± 0	a	0 ± 0	a	0 ± 0	a	0 ± 0	a	15679 ± 10055	b
6-methyl-5-hepten-2-one	1333	0 ± 0	a	0 ± 0	a	0 ± 0	a	0 ± 0	a	0 ± 0	a	2778 ± 814	b
Heterocyclic compounds													
5,6-dihydro-2H-pyran-2-one	1688	1453 ± 66	b	359 ± 99	a	665 ± 197	a	683 ± 168	a	592 ± 153	a	1665 ± 237	b
dihydro-2(3H)-furanone (γ-butyrolactone)	1618	9838 ± 1223	c	582 ± 234	a	767 ± 238	ab	986 ± 216	ab	944 ± 141	ab	2418 ± 967	b
Diols													
2,3-butanediol	1545	5944 ± 449	b	2450 ± 821	a	2608 ± 479	a	3008 ± 363	a	2797 ± 652	a	2590 ± 968	a
1,2-propanediol	1582	4552 ± 424	c	1782 ± 255	ab	1834 ± 411	ab	1506 ± 71	a	1737 ± 326	ab	2975 ± 1021	b
1,4-butanediol	1924	574 ± 32	bc	167 ± 155	a	353 ± 56	ab	295 ± 89	ab	403 ± 55	ab	813 ± 155	c
Others													
unknown	1252	53315 ± 36016	a	21822 ± 27130	a	12141 ± 12423	a	11407 ± 8489	a	15637 ± 8677	a	10383 ± 3374	a

[a] Calculated linear retention index

591