Interactions between yeast autolysates and volatile compounds in wine
and model solution

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A B S T R A C T

The addition of a commercial yeast autolysate to a model solution of five typical wine aroma compounds (ethyl octanoate, linalool, 2-phenylethanol, β-ionone and octanoic acid) was investigated considering different variables, such as temperature, pH and the presence of highly concentrated natural volatile substances in wine (e.g., 3-methyl-1-butanol). The interactions of such compounds with both yeast colloids and released colloids were studied using gas chromatography, with liquid–liquid extraction and solid-phase microextraction. The results were compared with those obtained by adding the commercial product to a white table wine, spiked with the five standard compounds. The data confirmed that yeast walls mainly bind less polar molecules: their loss in synthetic medium seemed to increase at higher pH values. Temperature and pH affected differently the interactions between yeast colloids and volatile compounds in wine and model solution: in complex solutions (as the addition of 3-methyl-1-butanol demonstrated) the interaction mechanisms could be influenced by competitive or other matrix-related effects, which can reduce the binding of single compounds, or even enhance their volatility.

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1. Introduction

Yeast extracts and autolysates are rich in free amino acids (Ames & Elmore, 1992; Höhn & Solms, 1975; Münch & Schieberle, 1998), peptides (Ames & Elmore, 1992), colloids and macromolecules (Comuzzo, 2003; Comuzzo, Tat, Tonizzo, & Battistutta, 2006) and they have found widespread use in winemaking, for managing fermentations, wine stabilisation and ageing for a number of years. The enological applications of such products were recently reviewed by Pozo-Bayón, Andújar-Ortiz, and Moreno-Arribas (2009).

The traditional use of yeast derivatives in oenology (as inactive yeasts or yeast ghosts) was their addition to manage yeasts and lactic bacteria growth, as well as to prevent stuck and sluggish fermentations, supplying assimilable nitrogen and reducing the presence of toxic metabolites, such as C6–C10 free fatty acids (Bisson & Butzke, 2000; Edward & Beelman, 1987).

Nevertheless, in the last decade other uses have been suggested for these products, mainly based on the role played by yeast mannoproteins in increasing wine colloidal stability (Ledoux, Dulau, & Dubourdieu, 1997; Waters, Wallace, Tate, & Williams, 1993). However, despite their ability to significantly increase wine colloidal content, the stabilising effect of commercially available yeast autolysates is strongly dependent on the kind of product and on the dosage; moreover, in opposition to what has been reported for purified mannoproteins (Moine-Ledoux & Dubourdieu, 2002), it seems not to be long-lasting, being generally lost in the months following the treatment (Comuzzo, Tat, Battistutta, & Zironi, 2004).

Besides their effects on the fermentation behaviour and wine stability, different research works in the early 1990s focused on other interesting aspects related to yeast macromolecules, such as their ability to affect wine sensory properties.

The hypothesis that wine polysaccharides could have a positive role in reducing wine astringency (Feuillat, Escot, Charpentier, & Dulau, 2001; Glories, 1978; Saucier, Glories, & Roux, 2000) has been confirmed by Vidal et al. (2004), working on two wine polysaccharide fractions (the first one was a mixture of arabinogalactan–proteins and mannoproteins, while the second contained rhamnogalacturonan II). They found that both tended to decrease the intensity of astringent sensory attributes, significantly increasing the ‘fullness’ sensation of a model wine. Guadalupe, Palacios, and Ayestarán (2007) also highlighted similar effects, adding yeast mannoproteins to Tempranillo during maceration: these macromolecules did not maintain the extracted polyphenols in a colloidal dispersion and neither ensured colour stability, but they clearly modified the gustative structure of the wines, enhancing the sweetness and roundness.

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Furthermore, even the ability of yeast walls and macromolecules to bind volatile compounds in wine-like solution was reported by several authors (Chalier, Angot, Delteil, Doco, & Gunata, 2007; Lubbers, Charpentier, Feuillat, & Voilley, 1994; Lubbers, Voilley, Feuillat, & Charpentier, 1994; Pradelles, Alexandre, Ortiz-Julien, & Chassagne, 2008; Voilley, Begin, Charpentier, & Peyron, 1991; Voilley, Lamer, Dubois, & Feuillat, 1990). According to these papers the major binding sites for aroma compounds in yeasts are connected with the lipidic fraction of yeast walls (Lubbers, Charpentier et al., 1994), and with mannoproteins containing a high proportion of proteins (Dufour & Bayonove, 1999; Lubbers, Voilley et al., 1994); for this reason the intensity of such interactions is generally higher for the most hydrophobic compounds.

All these studies, which generally focused on the ability of purified yeast macromolecules to affect wine sensory perception, led the winemaking sector to suppose similar effects even for non-purified yeast derived products, such as extracts and autolysates, which were traded on the market for these purposes; nevertheless, despite their widespread practical use, very few scientific papers report specific results about their real effects in wine.

As regards the ability of yeast derivatives to improve the mouth-feel characteristics of red wines, it was highlighted that the addition of a yeast autolysate can actually improve the interactions between tannins and polysaccharides during storage, but this observation was not related to significant differences between the astringency of the treated and untreated products (Comuzzo, Tat, Battistutta, & Tasso, 2005).

On the other hand, the effects of such additives on wine volatile fraction seemed more interesting: the ability of yeast derivatives to volatile esters. However, despite this negative behaviour, the use of yeast derivatives on wine sensory perception is related to different aspects: these products are normally used in the food industry as flavouring and aromatising agents (Münch, Hofmann, & Schieberle, 1997; Nagodawithana, 1992) and the release of volatile compounds by certain commercial formulates can strongly affect wine sensory profile with cheese-like, broth-like or other unpleasant odours; in this way, but without unpleasant odours; nevertheless, despite this negative behaviour, the use of yeast derivatives can also increase the fruity and flowery perceptions of some volatile esters.

According to our previous results (Comuzzo et al., 2006), the balance between these opposite trends seemed connected to the dosage: only the lowest additions (200 mg/l) increased the volatility of esters, while for the highest amounts (1,000 mg/l), the release of short-chain carboxylic acids from the powders (e.g. butanoic acid) determined some negative cheese-like notes in the olfactory profile of the treated wines.

On the basis of these considerations, the effects of yeast derivatives on wine aroma perception are often unpredictable, and further investigations are needed to better clarify their mechanisms of action in wine; moreover, the ability of yeast macromolecules to bind aroma compounds was mainly investigated in model solution (Chalier et al., 2007; Dufour & Bayonove, 1999; Lubbers, Charpentier et al., 1994; Lubbers, Voilley et al., 1994; Voilley et al., 1990, 1991) and by considering purified mannoprotein extracts, while we found no scientific papers reporting specific data as regards more complex matrices.

For these reasons, the addition of a commercial yeast autolysate was at first investigated on a model solution of five typical wine volatile compounds (ethyl octanoate, linalool, 2-phenylethanol, β-ionone and octanoic acid), also considering the effects of temperature, pH and the presence of 3-methyl-1-butanol (as a possible interfering compound). Capillary gas chromatography (GC) with liquid–liquid extraction (LLE) and solid-phase microextraction (SPME) was used to, respectively, evaluate the fixation of such compounds on yeast walls, and their interaction with the colloids released by the commercial powder. Finally, the results were compared with those obtained by adding the product to a white table wine, spiked with the five standard compounds.

### 2. Materials and methods

#### 2.1. Materials

A commercially available yeast autolysate, prepared from Saccharomyces cerevisiae, was purchased from Pascal Biotech (Paris, France). Standard volatile compounds (3-methyl-1-butanol, ethyl octanoate, linalool, 2-phenylethanol, β-ionone, octanoic acid and ethyl heptanoate), GC grade pentane and dichloromethane were obtained from Sigma–Aldrich (St. Louis, MO, USA); 1 cm length SPME 85 mm polyacrylate coating fibres were supplied by Supelco (Bellefonte, PA, USA). Finally, tartaric acid, ethanol, sodium hydroxide, hydrochloric acid, sodium chloride and anhydrous sodium sulphate were from Carlo Erba Reagents (Milano, Italy).

#### 2.2. Sample preparation: wine and model solutions

For the preparation of model solutions, 5 g/l of tartaric acid was dissolved in a hydroalcoholic mixture (ethanol 10% v/v) and afterwards buffered at different pH values, adding small amounts of a 4 M NaOH solution; these hydroalcoholic-tartaric buffers were used to dissolve the volatile compounds and the commercial autolysate, following the details reported below.

#### 2.2.1. Effect of yeast walls on the fixation of aroma compounds in model solution

To evaluate the effects of yeast walls on the fixation of aromas, the pH was adjusted at two different values: pH 3.00 and 4.00 were selected as extremes, to remain in a range close to the oenological conditions. Concentrated ethyl octanoate, linalool, 2-phenylethanol, β-ionone and octanoic acid were dissolved in both buffers, at the concentrations reported in Table 1; aliquots of both solutions were then treated with 450 mg/l of the commercial yeast autolysate and stored for 1 week at 15 °C, in 2.5 l screw capped glass bottles; three replicates were carried out for each pH level. After this time, the buffers were racked to separate the yeast walls (the insoluble fraction of the autolysate) that settled at the bottom of the bottles. Three additional repetitions of both solutions were prepared in the same way, but without autolysate addition, to be used as a comparison (reference test).

To evaluate eventual competitive effects for the yeast walls binding sites, from wine volatile compounds which normally occur in high concentration, the same experimental protocol was repeated adding 100 mg/l of 3-methyl-1-butanol to the buffers before the autolysate addition; even in such a case, a reference test was prepared without yeast derivative addition and all the trials were repeated three times.

After the separation of the particulate matter (and of the aromas eventually bound), the clean racked solutions were analysed by liquid–liquid extraction and gas-chromatography (LLE–GC) as reported below.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Concentration of volatile compounds in wine and model solutions.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compound</strong></td>
<td><strong>Chemical class</strong></td>
</tr>
<tr>
<td>Ethyl octanoate</td>
<td>Esters</td>
</tr>
<tr>
<td>Linalool</td>
<td>Terpenes</td>
</tr>
<tr>
<td>2-Phenylethanol</td>
<td>Higher alcohols</td>
</tr>
<tr>
<td>β-Ionone</td>
<td>Norisoprenoids</td>
</tr>
<tr>
<td>Octanoic acid</td>
<td>Fatty acids</td>
</tr>
</tbody>
</table>
2.2.2. Interactions between aroma compounds and autolysate colloids in model solution

To evaluate the interactions between the aroma compounds and the colloids released by the autolysate, 450 mg/l of the commercial product was suspended in a hydroalcoholic-tartaric buffer with a pH of 3.2; the samples were then stored for 1 week at 15 °C, to allow the extraction of the colloidal substances from the powder. After this time, the solutions were racked to eliminate the particulate matter (yeast walls), and the pH was adjusted to the values of 3.00 and 4.00; the concentrated standard compounds were then added in the same amounts reported in Table 1. At the same time and for both pH levels, a reference test was prepared by simply dissolving the aromas in the buffers, without any preliminary contact with the autolysate. All the trials were replicated three times.

Finally, to evaluate the eventual competitive effects from highly concentrated wine volatile compounds, the whole experimental design was repeated adding 100 mg/l of 3-methyl-1-butanol to the model solutions.

The effects of the released colloids on the volatility of aroma compounds were evaluated by headspace analysis, using SPME-GC, and working at two different temperatures: 20 °C to simulate the temperature of the wine in the glass (room temperature), and 37 °C which is near to the temperature of the wine in the mouth during tasting.

2.2.3. Global effects of the autolysate addition on a white wine

In a third part of the study, an Italian white table wine (alcoholic degree 11.00% v/v; pH 3.20) was purchased on a local market and spiked with the five standard compounds at the same concentrations reported in Table 1. In this part of the work, the effects of yeast walls and the released colloids were jointly considered at the same time: so, the commercial yeast autolysate was added (450 mg/l), and the wines were stored for 1 week at 15 °C in 2.5 l glass bottles. A reference test was prepared using an aliquot of the same wine spiked with the standard compounds, but without the addition of the commercial powder. All the trials were replicated three times.

Finally, to evaluate eventual effects of the concentration on the interactions between aromas and colloids, a second set of trials was repeated on the same wine, but without any addition of standard compounds.

After the racking and the separation of the particulate, the headspaces of the wine samples were analysed by SPME-GC, working at the two different temperatures of 20 and 37 °C reported for the model solutions.

2.3. Liquid–liquid extraction

To evaluate the effects of the insoluble fraction of the autolysate (yeast walls) on the fixation of the five volatile compounds, 200 ml of the clean buffer solutions obtained after the racking, was placed in a 500 ml volumetric flask; 1 ml of ethyl heptanoate (0.7 g/l in ethanol) was added as internal standard and the volume was adjusted to 500 ml with a 30% (w/v) sodium chloride solution, which was previously acidified (pH 3.00) with few drops of concentrated hydrochloric acid.

The mixture was then extracted with 5 ml of a pentane: dichloromethane (2:1 v/v) solution, shaking the flask vigorously for 5 min; the extraction procedure was repeated five times. The organic phase was collected in a glass tube and dried with anhydrous sodium sulphate; the extract was then transferred into a conical glass tube and stored at −18 °C until GC injection.

2.4. Solid-phase microextraction

1 cm length 85 μm polyacrylate coating fibre (Supelco, Bellefonte, PA, USA) was used for the SPME of both wines and model solutions; 25 ml of the clean samples prepared as reported above, were introduced in a 50 ml glass vial, closed with a PTFE/silicone septum, and stored at 15 °C until analysis.

The microextraction was run both at 20 and 37 °C for 15 min, using the equipment reported elsewhere (Tat, Comuzzo, Stolfo, & Battistutta, 2005); the two different temperatures were chosen to simulate both the temperature of the wine in the glass (20 °C) and the temperature of the mouth during tasting (37 °C). Before the fibre exposition, the vials were pre-conditioned for 15 min in a water bath to reach thermal equilibration (Tat et al., 2005); SPME was immediately followed by GC injection.

2.5. Gas chromatographic conditions

For all the trials, GC analyses were performed using a Carlo Erba (Milano, Italy) HRGC 8560 Mega Series 2 gas chromatograph equipped with a flame ionisation detector (FID) and with a split–splitless injector, both set at 240 °C; helium was the carrier gas, at a linear flow rate of 28 cm/s. Compounds were separated on an Econo-Cap Ec-Wax capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness), purchased from Alltech (State College, PA, USA); the column temperature was programmed as follows: 40 °C for 5 min, then at 4 °C/min up to 240 °C, with a final holding time of 15 min.

The injection was performed in splitless mode, with 1 min of splitless time: the samples obtained by LLE were re-equilibrated at room temperature, concentrated under nitrogen flow (to less than 1 ml) and directly injected (1 μl) in the GC system; for SPME-GC analyses, the fibre remained into the injector for 5 min after the microextraction, to allow the complete desorption of the analytes.

Quantitative analysis was carried out only for LLE–GC, and it was performed by calculating the relative response factor of each compound (calibration curve) considering ethyl heptanoate as internal standard; for the samples processed by SPME-GC, the absolute areas were directly used for the data elaboration.

2.6. Statistical analysis

One way Analysis of Variance (ANOVA) was carried out on the absolute areas and the concentrations, which were detected by SPME-GC and LLE-GC, respectively; means and standard deviations were calculated, and significant differences were evaluated by Tukey Honest Significant Difference (HSD) Test. Variances were homogeneous according to Levene and Brown-Forsyte Tests; significant results were considered at p < 0.05. All the statistical evaluations were performed using the specific software Statistica for Windows (StatSoft, Tulsa, OK, USA), Version 8.0.

3. Results and discussion

3.1. Effect of yeast walls on the fixation of aroma compounds in model solution

The addition of the yeast derivative affected the concentration of the volatile compounds in the model buffers differently. The results are reported in Table 2.

Considering the solutions without 3-methyl-1-butanol addition, the higher fixation on the insoluble fraction of the autolysate was observed for ethyl octanoate, for which a significant decrease in the concentration was highlighted at both pH values. It is interesting to note that at pH 4.00 the amount of aroma eliminated by fixation on the yeast walls was generally higher with respect to that bound at pH 3.00; in fact, at the higher pH, all the volatile compounds, except 2-phenylethanol, showed statistically significant
diminutions after the treatment (and such significant reductions were marked by Analysis of Variance (ANOVA) even at p < 0.01).

A simplified, tentative method for quantifying the interaction between yeast walls and volatile compounds can be done on the basis of the data reported in Fig. 1, where the relative diminutions of the mean concentration of the aroma compounds, as determined by the autolysate addition, are reported; it is possible to point out that ethyl octanoate and β-ionone were the most retained compounds, while linalool, octanoic acid and 2-phenylethanol were less affected by the treatment. Despite these data were not all supported by statistically significant evidences, the percent diminutions reported are generally in agreement with those published in other papers (Voilley et al., 1990). Nevertheless, some differences can be highlighted: for example, in the manuscript published by Lubbers, Charpentier et al. (1994), ethyl octanoate had a lower affinity for the yeast walls if compared with β-ionone; the same authors, however, worked with higher concentrations of both compounds and moreover, in that experience, the norisoprenoid was even more concentrated than the ester (100 μl/l of β-ionone and 40 μl/l of ethyl octanoate). So, it is possible that the slightly higher concentration that we have added for ethyl octanoate, with respect to β-ionone, could have determined its prevalence in the interaction with the insoluble fraction, and, as a consequence, the resulting greater diminution of the ester. However, the data obtained confirmed what was reported by the same authors (Lubbers, Charpentier et al., 1994): the compounds which are mostly involved in the interaction with yeast walls are the less polar ones.

Nevertheless, the fixation of such molecules was affected by the pH of the buffer too. As already stated, the interaction with the yeast walls was higher at pH 4.00, and at this pH, it became significant for almost all the analysed compounds; however, the effect of the pH was not the same for all of them. We have calculated the percent difference between the relative diminutions of the mean concentration of the analysed aroma compounds, as determined by the treatment at the two different pH values; this calculation was done according to the Eq. (1).

$$\Delta \% = \left( \frac{\% \text{ diminution pH} 4.00 - \% \text{ diminution pH} 3.00}{\% \text{ diminution pH} 4.00} \right) \times 100$$  (1)

where the “% diminutions” at pH 3.00 and 4.00 are the relative diminutions reported for the two pHs in Fig. 1. Briefly, $\Delta \%$ represents the greater percentage of each aroma which is fixed on the insoluble fraction of the autolysate at pH 4.00, with respect to pH 3.00 (Fig. 2). Despite this kind of elaboration had not a direct confirmation from the statistical point of view, it seems that the pH variation could mainly affect the compounds with polar functional groups: in fact, the percent diminutions of ethyl octanoate and β-ionone at pH 4.00, were approximately 36–37% higher than those recorded at pH 3.00, while this percentage rose to 50–60% for the other compounds.

It is probable that the extent of the pH variation carried out in this study (from 3.00 to 4.00) could have actually modified the particle charge of the cell wall proteins, and also affect the ability of the macromolecules to form hydrogen bonds; for this reason, the effect of the pH could be hypothetically explained by a possible increase of polar and particle charge interactions, probably

![Fig. 1. Percent diminution of the mean concentration of the volatile compounds in the model solution, after autolysate addition (450 mg/l) at the two different pH values; the diminutions connected to significant differences according to ANOVA analysis (p < 0.05) are marked with vertical arrows.](image)

![Fig. 2. Percent difference between the relative diminutions of the mean concentrations of the volatile compounds, as determined by the yeast walls at the two different pH values. $\Delta \%$ is calculated as reported in Eq. (1); it corresponds to the greater percentage of aroma which is fixed on the insoluble fraction of the autolysate at pH 4.00, with respect to pH 3.00.](image)

<table>
<thead>
<tr>
<th>3-Methyl-1-butanol addition</th>
<th>pH of the solution</th>
<th>Autolysate addition</th>
<th>Ethyl octanoate (mg/l) Mean ± SD</th>
<th>Linalool (mg/l) Mean ± SD</th>
<th>2-Phenylethanol (mg/l) Mean ± SD</th>
<th>β-ionone (mg/l) Mean ± SD</th>
<th>Octanoic acid (mg/l) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3.00</td>
<td>None</td>
<td>1.93±0.06a</td>
<td>0.43±0.03a</td>
<td>21.1±2.2a</td>
<td>1.10±0.03a</td>
<td>20.1±1.3a</td>
</tr>
<tr>
<td></td>
<td>4.00</td>
<td>450 mg/l</td>
<td>1.66±0.06a</td>
<td>0.41±0.01a</td>
<td>20.6±0.7a</td>
<td>1.02±0.08a</td>
<td>19.3±0.2a</td>
</tr>
<tr>
<td>100 mg/l</td>
<td>3.00</td>
<td>None</td>
<td>2.05±0.08b</td>
<td>0.46±0.01b</td>
<td>23.8±0.6a</td>
<td>1.31±0.05b</td>
<td>22.0±0.5b</td>
</tr>
<tr>
<td></td>
<td>4.00</td>
<td>450 mg/l</td>
<td>1.61±0.06a</td>
<td>0.42±0.00a</td>
<td>22.7±1.5a</td>
<td>0.99±0.02a</td>
<td>20.2±0.1a</td>
</tr>
</tbody>
</table>

Table 2

Effect of the yeast walls fraction of the autolysate on the fixation of the aroma compounds in model solution at different pH values (mean concentrations in mg/l ± standard deviations are reported); different letters represent means which are significantly different at p < 0.05; comparisons are related to the single pH value and 3-methyl-1-butanol addition.
connected with the protein and polysaccharide fractions of the cell walls added.

The observation that even the less polar compounds were affected by this effect confirms that the interaction between yeast walls and volatile compounds is a complex interaction, where several mechanisms can operate; the prevalence of one or another of them, probably not only depends on the macromolecular composition of the yeast walls, but also on the properties of the medium (e.g. pH), on the kind of volatile compounds and their concentration.

Besides these considerations, other factors could also be involved, as the presence of highly concentrated volatile compounds, which is a condition that is quite frequent in complex matrices such as wine. This hypothesis could be confirmed by the results obtained when adding 3-methyl-1-butanol to the model solutions, before autolysate addition: the presence of this component in the buffers (100 mg/l) reduced the amount of the five aromas fixed to the yeast walls. In fact, even if a certain reduction in their concentration was highlighted after the treatment, no significant differences were found by ANOVA analysis between treated and untreated samples (Table 2), with the only exception of ethyl octanoate at pH 3.00. On the basis of these results, we cannot exclude the possibility that concentrated volatile compounds (e.g. certain higher alcohols) could produce competitive effects for the aroma binding sites of the yeast cell walls, reducing in this way the elimination of the other volatile compounds after autolysate supplementation treatments.

3.2. Interactions between aroma compounds and autolysate colloids in model solution

The release of macromolecules and colloids from yeast autolysates occurs in a very short time when in a hydroalcoholic medium (Comuzzo et al., 2004); it is well known that these macromolecules are mainly polysaccharides and proteins.

As reported above, in this study, the interactions between volatile compounds and autolysate colloids were evaluated using SPME-GC. The results obtained for all the experimental variables considered (pH, temperature and addition of 3-methyl-1-butanol) are reported in Table 3.

The headspace concentrations of ethyl octanoate and linalool were poorly affected by the presence of yeast colloids and no significant differences were highlighted between test wines and autolysate-treated samples, independently of the combination of the experimental variables considered.

In contrast to that observed for the ester and the terpenol, 2-phenylethanol was subjected to more interesting modifications (Table 3). When 3-methyl-1-butanol was not added, at 20 °C the presence of yeast colloids determined a significant diminution of the headspace concentration of such alcohol, independently of the pH; this diminution seemed slightly higher at pH 3.00 with respect to pH 4.00. At 37 °C, the concentration of 2-phenylethanol significantly decreased only at pH 4.00.

According to this data it is possible to outline some practical hypotheses: in wines enriched with yeast colloids, the olfactory perception of the alcohol, at room temperature, could be reduced by the treatment, due to its interaction with colloidal substances themselves; nevertheless, during tasting, at the temperature of the mouth, the aroma could be released, and it could become perceptible at a retronasal level. This fact could explain the reason why the wines aged on the lees (more rich in colloids) are generally more complex from the sensory point of view, especially as regards the retronasal aroma perception.

The role of the pH in affecting these kind of interactions was also interesting: at the more acidic value the fixation of 2-phenylethanol seemed quantitatively higher, but the amount of aroma retained decreased more easily when the temperature rises; at pH 4.00, the interactions seemed quantitatively lower (lower binding capacity), but much stronger and they were reduced less by increasing the temperature (the difference between test and treated wine at 37 °C remains significant).

A similar behaviour with respect to that observed for 2-phenylethanol was highlighted also for β-ionone and octanoic acid. Even for such compounds, the autolysate treatment determined their retention and the consequent diminution of the absolute areas detected by performing SPME at 20 °C; this diminution was significant at pH 3.00 only. No significant diminutions were otherwise observed at 37 °C.

On the basis of these observations, we can confirm the conclusions drawn for 2-phenylethanol that the interactions of the aroma-colloids tendentially decrease when temperature increases. Nevertheless, with respect to that observed for the alcohol, the effect of the temperature in reducing aroma fixation seemed to be less affected by the pH, and no significant changes were observed at 37 °C, for neither of the two pH values considered.

---

**Table 3**

<table>
<thead>
<tr>
<th>3-Methyl-1-butanol addition</th>
<th>pH of the solution</th>
<th>SPME temperature</th>
<th>Autolysate addition</th>
<th>Ethyl octanoate (absolute area/1000) Mean ± SD</th>
<th>Linalool (absolute area/1000) Mean ± SD</th>
<th>2-Phenylethanol (absolute area/1000) Mean ± SD</th>
<th>β-ionone (absolute area/1000) Mean ± SD</th>
<th>Octanoic acid (absolute area/1000) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3.00</td>
<td>20 °C</td>
<td>None</td>
<td>16,41±4576a</td>
<td>398±41a</td>
<td>1195±27b</td>
<td>1178±134b</td>
<td>1998±194b</td>
</tr>
<tr>
<td></td>
<td>37 °C</td>
<td>450 mg/l</td>
<td>None</td>
<td>18,09±1781a</td>
<td>331±25a</td>
<td>906±51a</td>
<td>912±74a</td>
<td>1550±85a</td>
</tr>
<tr>
<td></td>
<td>4.00</td>
<td>37 °C</td>
<td>None</td>
<td>11,82±1671a</td>
<td>513±17a</td>
<td>2383±158a</td>
<td>2170±161a</td>
<td>5320±124a</td>
</tr>
<tr>
<td>100 mg/l</td>
<td>3.00</td>
<td>20 °C</td>
<td>None</td>
<td>13,13±1959a</td>
<td>533±25a</td>
<td>2216±150a</td>
<td>2293±118a</td>
<td>5032±395a</td>
</tr>
<tr>
<td></td>
<td>37 °C</td>
<td>450 mg/l</td>
<td>None</td>
<td>17,04±1623a</td>
<td>363±48a</td>
<td>1132±72b</td>
<td>1196±72a</td>
<td>1856±210a</td>
</tr>
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<td></td>
<td>100 mg/l</td>
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<td>13,34±23901a</td>
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<td>2217±166a</td>
<td>1505±200a</td>
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Summarising, a global quantification of the effects discussed above is shown in Fig. 3, where we have reported the percent diminutions of the mean concentrations of these three volatile compounds in the headspace of the model solutions (expressed in terms of absolute area), as determined by the added colloids (samples without 3-methyl-1-butanol addition). For instance, the figure can be read as follows: considering 2-phenylethanol, the autolysate treatment at pH 3.00 has determined a 24% diminution of its headspace concentration at 20 °C and a 7% diminution at 37 °C.

The data shown confirm what was already stated about the joint effects of temperature and pH: when pH increases, the interactions between yeast colloids and aroma compounds seem to be quantitatively lower (lower amount of aroma bound), but qualitatively stronger (stronger interaction with macromolecules), and, as a consequence, they show a lower diminution if the temperature rises; this effect seemed more important for the more polar compounds and it was particularly evident for 2-phenylethanol, less for β-ionone, while octanoic acid showed an intermediate trend.

In the presence of 3-methyl-1-butanol, practically all the volatile compounds tested in this study were not significantly affected by the treatment (Table 3): due to its higher concentration, 3-methyl-1-butanol could make a sort of competitive action for the aroma binding sites on yeast macromolecules, giving a tendential leveling of the effects of the treatment.

3.3. Global effects of the autolysate addition on a white wine

In order to relate the results obtained in the model solution with a more realistic situation, the effects of the autolysate treatment were also tested on a white table wine, which was either charged or not charged with the five standard compounds reported in Table 1.

In such a case, the effects of both yeast walls and the released colloids on the concentration of the volatile compounds in wine headspace were jointly evaluated by using SPME-GC, and performing SPME at both 20 and 37 °C; the results obtained are reported in Table 4.

No significant variations in the headspace concentrations were observed after autolysate addition for the white wine spiked with aroma compounds; this means that neither the yeast walls nor the released colloids have resulted, in the experimental conditions, in a detectable decrease of aroma volatility by fixation.
According to that observed in the model solution, this behaviour is quite obvious, for the presence of several possible interfering compounds in wine, as, for instance, 3-methyl-1-butanol itself (which was also detected in the samples). Furthermore, besides this and other highly concentrated volatile compounds, we should not forget that even certain non-volatile wine substances (e.g., polyphenols or proteins) could have affected the headspace concentration of the added aroma molecules, as well as their interaction with the autolysate.

On the other hand, as regards the wine samples not charged with aroma compounds (and so characterised by a less rich aromatic profile), some significant differences were highlighted, depending on SPME temperature. This observation particularly regards 2-phenylethanol and octanoic acid; ethyl octanoate did not show significant differences due to the treatment, while ß-ionone and linalool were not naturally present in the wine.

According to Table 4, at 37 °C, the headspace concentration of these two compounds remained unchanged after the treatment, while it shows a significant increase in the treated wines at 37 °C. On the basis of such consideration, it seemed that the addition of the same autolysate product could have an effect that, in wine, is completely opposite to what happened in model solution.

Speaking about yeast derivatives–aroma interactions, we had already observed a similar behaviour in wine (Comuzzo et al., 2006), and on the other hand, the increase of the volatility of certain aroma compounds in the presence of specific macromolecular fractions was even reported in other papers. Studying the binding capacity of several polysaccharides, Langouiroux and Crouzet (1994), reported a similar “salting out” effect on limonene and ethyl hexanoate water solutions, when two different dextrane fractions were added. Dufour and Bayonove (1999) found that the relative activity coefficient of 1-hexanol increases strikingly in the model system, when increasing amounts (0–30% weight) of a specific mannoprotein fraction were added; according to the data reported by the same authors, the effects of such additions on other volatiles, were less evident and varied depending on the level of macromolecules added. Finally, Lubbers, Voilley et al. (1994) found that the macromolecules released during the yeast fermentation of their model juice (they called them FERM extract) could have different effects, reducing (e.g. for ethyl hexanoate and octanal), increasing (e.g. for 3-methyl-1-butanol and ethyl octanoate) or leaving unmodified (e.g. for ethyl decanoate) the volatility of the different aroma compounds that they used for the experiments.

So, it is generally known that colloids (especially polysaccharides) can oppositely affect the perception of aroma substances, either reducing or increasing their volatility (Lubbers, 1993).

In relation to simple sugars, King (1983) explained this last effect, on the basis of the ability of the sugars themselves to reduce the stability of the volatile molecules in solution by sequestering a part of their solvation water, and so on the basis of a salting out effect; in certain circumstances, even polysaccharides from yeasts could act in a similar way, so that their addition can lead to an increase in the volatility of aroma substances.

Despite these considerations, it is quite difficult to explain the reason why, in this experiment, this trend was found only in wine and not in the model solution; it seems highly related to the temperature (the salting out effect occurred only if SPME was run at 37 °C), and also to aroma concentration (e.g. being not observable in the samples added with exogenous aromas). Furthermore, it could also be connected to other compositional variables; for instance, the kind of macromolecules released from the autolysate itself and their concentration, wine pH, alcoholic strength or any other factor which could differentiate wine with respect to model systems.

The question highlighted by this study is that the interaction between aroma molecules and yeast colloids is a phenomenon which is more complex than what has been considered since now. The papers published in the past have given a good knowledge about the opportunity of using such products to modulate wine aroma perception, but for the practical optimisation of their oenological use we need further investigations, not only performed in simplified matrices or model solutions, but also made in complex media and in real conditions.

Finally, these binding or salting out properties of yeast derivatives towards wine aromas should certainly be not neglected for the development of commercial products specifically designed for the oenological use.

References


