JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Effect of Different Lysis Treatments on the Characteristics of Yeast Derivatives for Winemaking

Piergiorgio Comuzzo,* Lara Tat, Anna Liessi, Laura Brotto, Franco Battistutta, and Roberto Zironi

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

ABSTRACT: The effects of three preparation techniques on the oenological properties of a yeast autolysate were investigated: enzymatic autolysis, thermolysis, and the combination of a slow freezing−defrosting and mechanical disruption were carried out on a commercial formulation of active dry yeasts (*Saccharomyces cerevisiae*). The powders obtained by freeze drying, were characterized: volatile compounds were analyzed by SPME-GC with mass spectrometric (MS) and olfactometric detection (O); the release of colloids in winelike solution was studied by SDS-PAGE and size exclusion chromatography (SEC). Finally, the effects of the powders addition on the aroma composition of a white wine were investigated by SPME-GC-MS, SPME-GC-O, and sensory evaluation. The products obtained were quite different from each other. In particular, enzymatic autolysis led to higher contents of nonglycosilated soluble proteins in the powders and determined a higher retention of wine aroma compounds. On the contrary, thermal autolysate was richer in glycoproteins, and it was able to increase the wine aroma intensity; nevertheless, in the wines treated with such preparation, a slight yeastlike olfactory note was perceived.

KEYWORDS: *inactive dry yeasts, thermolysis, enzymatic autolysis, mechanical disruption, wine volatile compounds, yeast colloids*

INTRODUCTION

Yeast derivatives (YD) are commonly used in winemaking for several purposes. They can be classified in four types, depending on the manufacturing process:[1] inactive yeasts (obtained by thermal inactivation of the yeasts and drying), yeast autolysates (thermal inactivation followed by an incubation allowing enzymatic activities and cell wall degradation), yeast hulls or walls (yeast walls without cytoplasmic content), and yeast extracts (the soluble part of the autolysates, after elimination of the cell walls).

YDs are often, generically and quite incorrectly, identified with the term of "mannoproteins"; as a matter of fact, despite their important content in mannans,[2,3] YDs contain even other constituents of yeast cell structures. Mannoproteins are yeast-derived products in all respects, but as compared with extracts and autolysates, they undergo additional purification treatments.[4]

The production process of YDs for food industry has been summarized by several authors:[3,5−7] briefly, it involves the propagation of yeasts in sugar-containing media (e.g., molasses, glucose syrup) and aerating conditions; yeasts are then harvested, washed, and then subjected to the lysis process.[6] Autolysis is the most frequently used cell disruption method: basically, the cell is degraded by its own enzymes, in conditions of controlled temperature and pH; it is possible to speed up the process by adding degradation enzymes (e.g., proteases, β-glucanases). Thermolysis (e.g., boiling of yeasts), plasmolysis (osmotic shock for the cells by adding sodium chloride), or acidic hydrolysis are less used. After the lytic process, spray-drying or drum-drying are used to obtain a paste or a powder.[3]

The ability of YDs to affect wine flavor and aroma is related to four main properties: the ability of yeast walls to bind aroma molecules,[8] their characteristic of flavor enhancers,[7,9] the ability of yeast macromolecules and colloids to affect the volatility of wine aroma,[10−13] and the release of volatile compounds into the wine.[14,15]

As concerns this last aspect, we have to remind that the use of these preparations in winemaking derives from the food industry, where they are utilized as flavoring and aromatizing agents.[5,9,16] This problem was already highlighted by Charpentier and Feuillat:[2] they reported that the YDs for food industry have undergone excessive proteolysis and may give rise to off-flavors when added to wines. In our experience, the macromolecules released by YD can have some positive effects on wine aroma, increasing the volatility of some esters,[14] but even the risk of releasing off-flavors cannot be neglected.[15]

Unfortunately, despite the large use of YDs in winemaking, most of the commercial products are generic and not specifically designed for the wine sector. As far as we know, at this moment, the only published manuscript that has tackled the problem from the scientific point of view is that written by Pozo-Bayón and co-workers,[17] who have developed a procedure based on the application of supercritical carbon dioxide extraction, to remove odorant volatile compounds from a YD preparation. Authors were able to reduce to approximately 70% the volatile compounds that may be released into the wines, without affecting the nonvolatile fraction of the deodorized preparations (nitrogen compounds and neutral polysaccharides). Nevertheless, the use of supercritical carbon dioxide is generally considered an expensive technology (particularly for its start-up costs), and it is still not easily available.

On the basis of these considerations, the aim of this work was to start studying the production process of yeast autolysates, to

Received: November 15, 2011
Revised: February 7, 2012
Accepted: February 26, 2012
Published: March 19, 2012

dx.doi.org/10.1021/jf204669f | J. Agric. Food Chem. 2012, 60, 3211−3222

suggest simple and economically sustainable manufacturing practices, for obtaining formulations with suitable characteristics for winemaking. As a first approach, the goal was to assess the effect of the lysis treatment on the oenological characteristics of the products. For this purpose, three yeast autolysates were self-prepared, starting from a commercial preparation of *Saccharomyces cerevisiae* wine yeasts and using different lysis methods. The powders obtained by freeze-drying were characterized for several parameters, concerning the release of soluble proteins and glucidic colloids in winelike solution and the composition of volatile compounds in the headspace (both factors that can modify the wine aromatic profile). Finally, the effects of the addition of the three autolysates on the volatile fraction of a white wine were investigated from the analytical and the sensory point of view.

MATERIALS AND METHODS

Lysis Treatments and Autolysate Samples Preparation. Three lysis treatments were tested: enzymatic autolysis (E), thermolysis (T), and the combination of slow freezing−defrosting and mechanical disruption (M). Each treatment was performed in triplicate. A commercial *S. cerevisiae* active dry yeasts formulation (ADY), Actiflore F33, purchased from Laffort Œnologie (Bordeaux, France), was used for the trials; the preparation was selected for its high polysaccharide production, as indicated by the supplier.

Enzymatic autolysis was carried out by a modification of the method reported by Moine Ledoux:[18] 25 g of active dry yeasts was suspended in 250 mL of distilled water; after 20 min (to allow the rehydration of the cells), 50 g of glass spheres with a mean diameter of 3 mm was added, and the yeast suspension was subjected to four cycles of alternating Vortex mixing (5 min) and immersion in an ice bath (5 min, 0 °C). Enzymatic autolysis was performed by adding 500 mg of a β-glucanase preparation (Glucanex - Novo Nordisk, Bagsværd, Denmark) and storing the suspension at 37 °C for 24 h.

Thermal autolysate was produced by a modification of the method of Peat, Whelan, and Edwards,[19] as reported by Moine-Ledoux:[18] 25 g of active dry yeasts was suspended in 100 mL of distilled water, and 5 mL of 20 mM citrate buffer (pH 7.00) was added; nitrogen was bubbled inside the suspension to eliminate the dissolved oxygen, and the bottle was thermally treated in autoclave for 2 h at 121 °C.

Mechanical disruption was based on a combination of a slow freezing (followed by defrosting) and mechanical treatment: 10 g of active dry yeast preparation was suspended in 80 mL of distilled water, and after 20 min (to allow the rehydration of the cells) 20 g of glass spheres (mean diameter, 3 mm) was added. The sample was subjected to four cycles of alternating Vortex mixing (5 min) and immersion in an ice bath (5 min, 0 °C). The yeast suspension was then frozen at −18 °C, and after one night, it was defrosted by four cycles of repeated heating in boiling water (1 min), dipping in ice bath (few seconds), and Vortex mixing (5 min) to determine the mechanical breaking of the cells, without overheating.

To minimize the effects of the drying process on the aromatizing characteristics of the powders, the three autolysates obtained as reported above were freeze-dried using a pilot plant model Mini Fast 1700 (Edwards Alto Vuoto, Milan, Italy). The liquid suspensions were arranged in a thin layer (approximately 1 cm) in food-grade aluminum trays, frozen at −18 °C, and then put into the freeze-drying plant. After freeze-drying, the samples were ground in a ceramic mortar and stored in sealed vials, at 0/+4 °C, until analysis.

For the characterization of the volatile compounds in the headspace of the autolysate preparations, 2.00 g of powder was introduced in a 50 mL glass vial and closed with a PTFE/silicone septum; vials were analyzed by solid-phase microextraction and gas chromatography (SPME-GC) just after their preparation. To evaluate and characterize the soluble proteins and glucidic colloids released in winelike solution, 1.00 g of autolysate powder was suspended in 100 mL of hydroalcoholic-tartaric buffer: ethanol 12% v/v in 0.03 M tartaric acid, buffered at pH 3.20 with a 4 M NaOH solution (all reagents were from Carlo Erba Reagents, Milan, Italy); after they were stirred for 30 min, the suspensions were centrifuged (4000 rpm for 10 min) to eliminate the insoluble particles (yeast walls), and the limpid autolysate solutions (LAS) were subjected to the determinations reported below.

Size Exclusion Chromatography (SEC). The LAS obtained after centrifugation (see previous section) were filtered on 0.20 μm pore size nylon membranes (Albet-Hahnemühle, Barcelona, Spain), and 20 μL was directly injected in the liquid chromatograph. The high-performance liquid chromatography (HPLC) system was a Jasco model 880 PU pump (Jasco Co. Ltd., Tokyo, Japan), equipped with a 7125 NS Rheodyne manual injection valve and with two different detectors: a refractive index detector model RID-10A (Shimadzu, Kyoto, Japan) was used for the detection of polysaccharides and glucidic colloids (e.g., glycoproteins), while a UV−vis detector model 875-UV (Jasco Co. Ltd.) was set at a wavelength of 280 nm for the detection of nonglycosylated proteins. The HPLC separation was performed in isocratic mode, on a 5 μm particle size, 300 mm × 7.8 mm i.d. TSK-GEL G2000SW$_{XL}$ SEC column (Tosoh Bioscience, Tokyo, Japan), coupled with a 40 mm × 6.0 mm i.d. precolumn packed with the same stationary phase. To simulate as much as possible the conditions of alcoholic strength and pH that can be found in wine, the elution was performed using the same hydroalcoholic-tartaric buffer (0.03 M tartaric acid, pH 3.20, ethanol 12% v/v) used for sample preparation; the flow rate was 0.60 mL/min. The molecular weight calibration curve was obtained using different standard proteins (molecular weights are reported in brackets): thyroglobulin (660.0 kDa), bovine IgG (156.0 kDa), bovine serum albumin (67.0 kDa), egg albumin (43.0 kDa), horseradish peroxidase (40.2 kDa), β-lactoglobulin (35.0 kDa), myoglobin (16.9 kDa), ribonuclease A (13.7 kDa), and cytochrome C (12.4 kDa).

Polyacrylamide Gel Electrophoresis under Denaturing Conditions (SDS-PAGE). The samples prepared for SEC were also characterized by polyacrylamide gel electrophoresis under denaturing conditions; 100 μL of the 0.20 μm filtered solution prepared for SEC analysis was loaded on a Vivaspin 500 ultrafiltration tube, with a molecular weight cutoff of 5 kDa (Sartorius Stedim Biotech, Aubagne, France) and centrifuged at 12000 rpm for 8 min. After centrifugation, the original volume was restored by redissolving the retentate in 100 μL of a sample loading buffer prepared as follows: 25 μL of 2-mercaptoethanol was added to 475 μL of a solution obtained by mixing 4.8 mL of distilled water, 1.2 mL of 0.5 M TRIS-HCl solution (pH 6.80), 1.0 mL of electrophoresis grade glycerol, 2.0 mL of 10% sodium dodecyl sulfate (SDS), and 0.5 mL of 0.1% bromophenol blue (all of the reagents were from Sigma-Aldrich, St. Louis, MO). The samples were then heated for 5 min at 100 °C, and 25 μL was directly loaded in the polyacrylamide gel wells; this corresponds to an amount of approx 20−30 μg of protein per well, depending on sample protein content (as quantified by Lowry method). At the same time, a comparative trial was performed for each sample, simply suspending 10 g/L of the autolysate powders in the sample loading buffer described above; these samples were then subjected to the same treatments of those extracted in winelike solution, except for the ultrafiltration step.

SDS-PAGE was carried out on 4−20% linear gradient Ready Gel TRIS-HCl precast gels (10 wells, 30 μL) from Bio-Rad Laboratories Inc. (Hercules, CA): the cathode buffer was an aqueous solution of TRIS (3 g/L), glycine (14.4 g/L), and SDS (1 g/L); the anode buffer was prepared as the cathode one but without SDS (all the reagents were from Sigma-Aldrich); the pH of both buffers was 8.3. A Mini Protean II electrophoresis cell and a PowerPac 300 power supply (both from Bio-Rad Laboratories Inc.) were used for the separation; the electrophoretic run was performed at 26 mA (constant amperage) for 140 min. For each run, two gels were loaded in the electrophoresis cell, and each of them was subjected to a different staining method: nonglycosylated proteins were stained by Coomassie Brillant Blue R-250, while for the glycosylated fractions, the second gel slab was treated with Fuchsin-Schiff reagent; both staining reagents were from Sigma-Aldrich. The molecular weight of the different fractions was evaluated by a commercially available molecular weight standard mix

(Broad Range SDS-PAGE standard - Bio-Rad Laboratories Inc.), which was prepared and loaded on each gel slab according to the supplier instructions; myosin (200.0 kDa), β-galactosidase (116.3 kDa), phosphorilase B (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa), trypsin inhibitor (21.5 kDa), lysozyme (14.4 kDa), and aprotinin (6.5 kDa) were present in the mix.

Soluble Proteins (Lowry Method) and Glucidic Colloids (Ethanol Precipitation). The results obtained by SDS-PAGE and SEC were compared with those obtained by some rapid methods normally used for the detection of soluble proteins and glucidic colloids (e.g., glycoproteins). Soluble proteins were analyzed, on LAS, by the Lowry method, as reported by Regenstein and Regenstein,[22] while the determination of soluble glucidic colloids was performed by ethanol precipitation as described by Usseglio-Tomasset and Castino.[23] This last method was based on the precipitation of glucidic colloids from the autolysate solutions (LAS), by the addition of 5 volumes of 96% ethanol v/v (Carlo Erba Ragents, Milan, Italy); colloids were recovered by filtration on a 0.45 μm pore size nylon membrane (Albet-Hahnemühle, Barcelona, Spain) and then determined by weighing. Results were given in mg of soluble colloids per g of autolysate powder.

Wine Samples Preparation. A white Chardonnay wine (2006, DOC Isonzo del Friuli, Italy) was used for the trials, preparing, at the same time, the samples for both chemical and sensory evaluation; 200 mg of the autolysate powders was directly weighed in 1 L glass bottles; the wine was then drawn from a 15 L bulk, pumping it by a nitrogen flow to avoid oxidations. Bottles were closed with a crown cap closure, manually homogenized, and then stored at 20 °C for 24 h; after this time, each sample was racked under nitrogen flow and fractionated as follows: 25 mL was introduced in a 50 mL PTFE/silicone sealed glass vial and used for the analytical determinations; a 750 mL crown-capped bottle was filled with the rest of the sample for sensory evaluation; both vials and bottles were stored at 20 °C until analysis. A reference test sample was prepared without autolysate addition. All of the trials were replicated three times.

SPME-GC-MS and SPME-GC-O Analyses. The vials containing the autolysate powders and the treated wines were analyzed by SPME-GC, using both a mass spectrometric (MS) and an olfactometric detector (O); the methods used for SPME extraction, GC-MS, and GC-O analyses are reported elsewhere.[14] The identification of the 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 library Wiley 5; linear retention indexes were also calculated from the retention times of *n*-alkanes, and orders of elution were compared with those available in literature.[5,14,24−32] As regards GC-MS, chromatograms were registered in scan mode; the absolute areas were directly used for data elaboration.

Sensory Evaluation. An attribute difference test was carried out on the treated wines by a panel of 11 judges (6 males and 5 females), whose ages ranged from 25 to 45 years; all of the judges were oenologists with at least 1 year of practical winemaking experience. The choice of oenologists was for describing the sensory perceptions just according to an oenological terminology; this is particularly important, because winemakers are the ultimate users of YDs in the wineries. Wine samples (test wines and treated samples, all in triplicate) were given one by one to the panel following a randomized balanced design, and the judges had to evaluate each sample for different attributes, on the basis of a 0−9 scale (0, attribute not perceived; 9, maximum intensity for that attribute). The attributes were selected on the basis of the characteristic olfactory notes described for YDs,[6] on the basis of the results of previous experiences,[14] and according to what the suppliers generally declare, promoting their yeast-derived products for winemaking: aroma intensity (intended as fruity/positive notes), yeast extract, broth, reduction odor, retronasal persistence, body, viscosity, and global impression.

Statistical Analysis. As concerns analytical determinations, principal component analysis (PCA) and one-way analysis of variance (ANOVA) were carried out on the absolute areas of the odor-active compounds, as detected by SPME-GC-O and SPME-GC-MS, in both wines and autolysate samples; both statistical evaluations were performed using the specific software Statistica for Windows (StatSoft, Tulsa, OK), Version 8.0. As regards ANOVA, significant differences were assessed by Tukey honest significant difference test (HSD test); results were considered significant at $p < 0.01$. ANOVA was also performed on the data obtained by Lowry method and glucidic colloids determination (ethanol precipitation).

The scores given by the judges during sensory evaluation were analyzed by main effects ANOVA and least significant difference test (LSD test), considering as factors judges and samples; results were considered significant at $p < 0.05$. Moreover, to assess the accordance of each judge with the rest of the panel, a correlation analysis was carried out between the scores given by each judge and the means of the whole group. In such a case, the specific software Senstools for Windows (OP&P, Utrecht, The Netherlands), Version 2.3, was used for the elaborations.

RESULTS AND DISCUSSION

Characterization of the Colloids Released by the Autolysates in Wine-Like Solution. Table 1 reports the levels of soluble proteins and colloids, released in winelike solution by the three autolysate products and by the active dry yeast formulation used for their preparation (ADY).

Table 1. Levels of Soluble Proteins and Colloids Detected in the Three Autolysate Products and in the Active Dry Yeast Preparation Used for the Trials (ADY)[a]

	mg/g	
sample code	soluble proteins[b]	soluble colloids[c]
ADY	38 ± 13	ND[d]
T	87 ± 3 b	213 ± 41 a
M	75 ± 3 a	120 ± 29 a
E	131 ± 6 c	185 ± 27 a

[a]Data are reported in mg/g of powder. Different letters represent means that are significantly different at $p < 0.05$ (ANOVA and Tukey HSD test); comparisons are related to the three autolysate products only: T, thermal autolysate; M, mechanical autolysate; and E, enzymatic autolysate. [b]Lowry method as reported by Regenstein and Regenstein.[22] [c]Determined by ethanol precipitation.[23] [d]Not detectable.

In YDs, proteins originate from the cell wall and from the cytoplasm, and they are released after cell degradation. Therefore, the levels of soluble proteins detected in the autolysates are higher with respect to those released by the ADY formulation. In particular, thermolysis and mechanical disruption determined an amount approximately double, while after enzymatic treatment, soluble proteins became about three times higher than in ADY. According to this behavior, enzymatic autolysis seemed the most intense treatment, leading to a higher degradation of cell structures and, consequently, to higher levels of soluble proteins. The significantly lower amounts of such macromolecules, released by the thermal autolysate, could depend on a minor degree of cell degradation, but it could also be due to a denaturation of proteins, determined by the prolonged heating. Mechanical disruption showed the lowest release of proteins in model wine, being apparently the less intense treatment.

Soluble glucidic colloids (e.g., polysaccharides and glycoproteins), not detectable in ADY formulation, also increased after the applied treatments. Even if not significantly from the statistical point of view (due to the quite wide standard

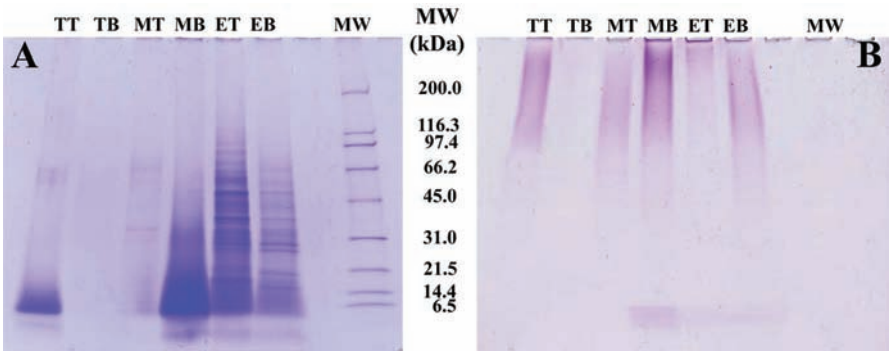

Figure 1. SDS-PAGE separation of nonglycosylated (A) and glycosylated proteins (B), released by YDs in winelike medium and in sample loading buffer; the gels are stained with Coomassie Brillant Blue R-250 (A) and Fuchsin-Schiff reagent (B). TT, thermal autolysate in hydroalcoholic-tartaric buffer; TB, thermal autolysate in sample loading buffer; MT, mechanical autolysate in hydroalcoholic-tartaric buffer; MB, mechanical autolysate in sample loading buffer; ET, enzymatic autolysate in hydroalcoholic-tartaric buffer; EB, enzymatic autolysate in sample loading buffer; and MW, molecular weight standard.

deviation), thermolysis determined the highest mean level of these macromolecules; enzymatic treatment also led to a good (even if lower) release, while mechanical autolysate showed the lowest average value, confirming the lesser intensity of such treatment.

The macromolecules released in winelike solution were characterized by SDS-PAGE; the results are reported in Figure 1. On the two gel slabs, the lanes of the samples obtained by suspending the autolysates in hydroalcoholic-tartaric buffer (TT, MT, and ET) and in sample loading buffer (TB, MB, and EB) are reported. The latter extraction method should have to simulate the optimal solubilization conditions for yeast macromolecules, being the reducing buffer used, a good solvent for proteins. Nevertheless, contrary to what expected, it is possible to note that the extraction in winelike buffer gave generally more intense lanes (with the exception of mechanical autolysate). This lead us to suppose that the wine environment can be a very suitable medium for allowing a good and relatively fast extraction of soluble macromolecules from YDs. For this reason, only the lanes related to the samples prepared in winelike solution (TT, MT, and ET) will be considered in the subsequent discussion.

Looking at Figure 1, nonglycosylated soluble proteins (Figure 1A) appeared to be more abundant in enzymatic autolysate (lane ET), where the major number of bands was highlighted; the apparent molecular weight distribution ranged from less than 6.5 to approx 100 kDa. Thermal (lane TT) and, particularly, mechanical autolysate (MT) seemed less rich in nonglycosylated proteins: they both gave light-colored lanes by Coomassie Brillant Blue staining; the product obtained by thermolysis (TT) is characterized by a relatively wide, scarcely focused low molecular weight band. This behavior, the higher intensity of the bands obtained for the enzymatic product, seems to confirm the results of Table 1, where enzymatic autolysate was the most rich in soluble proteins.

Figure 1B reports the lanes obtained by staining the gels with Fuchsin-Schiff reagent; contrarily to what was observed for Coomassie staining, thermal autolysate (TT) gave more intense lanes, with respect to the mechanical treatment (MT) and, more particularly, with respect to the enzymatic preparation (ET); this could be connected to a higher glycoprotein content in the first product but also to their higher glycosylation degree. Regarding this last aspect (glycosylation degree), some other considerations can be done, looking at the shape of the lanes. Contrary to what happened for Coomassie staining, glycoproteins appear in Figure 1B as wide and unfocused lanes, without well-defined bands; this difficulty in SDS-PAGE separation of glycoproteins has already been reported,[33] and it could be connected just with the high glycosylation degree of these macromolecules. In fact, sodium dodecyl sulfate reacts with the protein moiety of the macromolecule; if this portion is small, the SDS will be hardly bound to the glycoprotein, and the macromolecule will migrate with difficulty when the electric field is applied to the gel. Looking at Figure 1B, the three products seem characterized by glycoproteins, which have, apparently, a high molecular weight but, more probably, which are marked by a high glycosylation degree; this fact could have limited a lot their electrophoretic mobility in the applied SDS-PAGE conditions. These fractions are not stained by Coomassie Brillant Blue (Figure 1A), confirming that, probably, their protein moiety is a minority, and this is in agreement with what reported in literature as concerns glycoproteins (mannoproteins in particular) from *S. cerevisiae*.[2,18,34,35] As already highlighted, such soluble glycoproteins were more abundant in the thermal autolysate, while mechanical and enzymatic treatments released lower amounts. This fact can confirm quite well the data reported for soluble glucidic colloids in Table 1: the average value detected for thermal autolysate was the highest.

Besides these highly glycosylated protein fractions, enzymatic autolysate appeared to be characterized also by a further not well focused band with a low apparent molecular weight (average value next to 6.5 kDa); this fraction was detectable also by Coomassie Blue staining, denoting a probable lower glycosylation degree. The presence of this further fraction in the lane ET could be related to the apparent major intensity of enzymatic autolysis, with respect to the other two cell disruption methods; this can have determined a higher degradation of cell structures and macromolecules.

The results of SEC separation of the macromolecules released in winelike buffer can partially confirm these observations. In Figure 2, the chromatograms obtained by UV detection are reported. Six protein fractions were detected in the three products: one of them (fraction 4; estimated mean molecular weight, 18.9 kDa) was present only in the enzymatic product; this fraction, together with fraction 2 (estimated mean molecular weight, 31.4 kDa), gave a signal also by refractive index detection, suggesting the presence of glycosylated

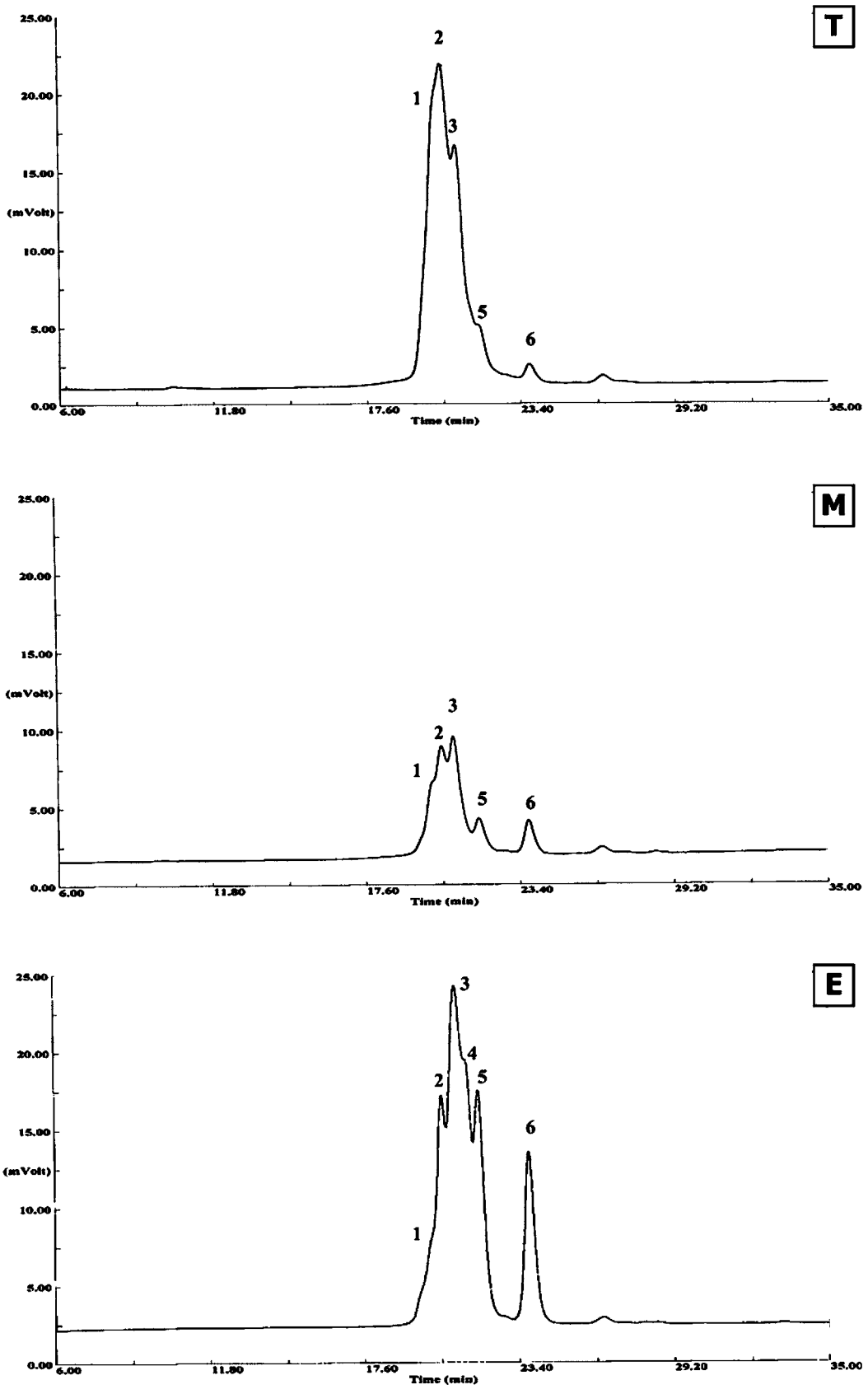

Figure 2. Separation by SEC and UV detection (λ 280 nm) of the macromolecules released by the autolysates in winelike solution. T, thermal autolysate; M, mechanical autolysate; and E, enzymatic autolysate. The estimated mean molecular weights of the six fractions (in kDa) are as follows: 36.6, fraction 1; 31.4, fraction 2; 23.8, fraction 3; 18.9, fraction 4; 15.7, fraction 5; and 5.9, fraction 6. Fractions 2 and 4 were also detectable by a refractive index detector.

Table 2. Odor-Active Compounds Detected by SPME-GC-MS and SPME-GC-O in the Headspace of the Three Autolysate Products[a]

				absolute area/1000		
				thermal autolysate (T)	mechanical autolysate (M)	enzymatic autolysate (E)
compound	RI[b]	RI lit.[c]	IM[d]	mean ± SD		
3-methylbutanal	893	937[e]	MS, IR, S	4330 ± 522 a	1631 ± 2826 a	0[p] ± 0 a
ethyl acetate	893	885[i]	MS, IR, S	6226 ± 333 a	77736 ± 10824 b	118499 ± 17509 b
ethyl 2-methylpropanoate	982	955[o]	MS, IR	0 ± 0 a	25638 ± 7588 a	22160 ± 18766 a
unknown	1036			75 ± 19 a	69 ± 7 a	34 ± 13 a
ethyl 2-methylbutanoate	1057	1060[g]	MS, IR	0 ± 0 a	4224 ± 755 b	1408 ± 438 a
ethyl 3-methylbutanoate	1076	1082[g]	MS, IR	0 ± 0 a	15717 ± 2213 b	3743 ± 991 a
hexanal	1082	1079[h]	MS, IR, S	653 ± 105 a	928 ± 238 a	580 ± 206 a
p-xylene	1135	1128[f]	MS, IR	67 ± 12 a	68 ± 8 a	44 ± 15 a
ethyl pentanoate	1135	1133[o]	MS, IR	0 ± 0 a	253 ± 31 c	78 ± 17 b
limonene	1186	1206[e]	MS, RI, S	650 ± 146 a	469 ± 104 a	319 ± 45 a
2 and 3-methylbutanol	1207	1219[i]	MS, IR, S	51158 ± 2530 a	342597 ± 107243 b	462865 ± 27562 b
ethyl hexanoate	1236	1238[i]	MS, IR, S	0 ± 0 a	17286 ± 2318 b	4560 ± 274 a
1,2,4-trimethylbenzene	1238	1246[f]	MS, IR	282 ± 25 b	239 ± 40 ab	137 ± 19 a
unknown	1243			93480 ± 11099 b	55459 ± 9371 a	34266 ± 1599 a
2-heptenal	1324	1299[o]	MS, IR	11 ± 2 a	211 ± 32 b	294 ± 18 c
2,6-dimethylpyrazine	1324	1325[e]	MS, IR	51 ± 3 a	126 ± 22 a	302 ± 55 b
6-methyl-5-hepten-2-one	1343	1336[f]	MS, IR	84 ± 11 a	206 ± 160 a	157 ± 13 a
2,3-dimethylpyrazine	1345	1330[l]	MS, IR	94 ± 10 a	167 ± 22 b	162 ± 15 b
2-nonanone	1392	1388[o]	MS, IR	114 ± 26 a	64 ± 17 a	52 ± 4 a
a benzene	1392		MST	52 ± 5 b	37 ± 6 ab	21 ± 3 a
nonanal	1394	1382[l]	MS, IR	517 ± 97 a	533 ± 222 a	72 ± 7 a
2,3,5-trimethylpyrazine	1397	1387[e]	MS, IR	61 ± 9 a	111 ± 16 ab	132 ± 18 b
unknown	1398			68 ± 8 a	43 ± 6 a	247 ± 12 b
ethyl 2-hydroxybutanoate	1400		MS	0 ± 0 a	0 ± 0 a	486 ± 25 b
a methylcyclohexanol	1404		MS	4 ± 1 a	195 ± 26 b	146 ± 10 b
3-octanol	1405	1395[m]	MS, IR	18 ± 1 a	2 ± 1 a	59 ± 12 b
2-octanol	1425	1332[o]	MS	11 ± 2 a	197 ± 46 b	174 ± 17 b
3-methyl-3-cyclohexen-1-one	1426	1417[f]	MS, IR	456 ± 52 a	1741 ± 200 c	1023 ± 37 b
acetic acid	1440	1446[f]	MS, IR, S	13680 ± 6305 a	75667 ± 9387 b	272026 ± 12857 c
2-furaldehyde	1465	1449[i]	MS, IR, S	30 ± 3 a	47 ± 5 a	103 ± 14 b
propanoic acid	1490	1528[j]	MS, IR, S	0 ± 0 a	0 ± 0 a	17 ± 2 b
2-ethyl-1-hexanol	1493	1477[k]	MS, IR, S	1724 ± 214 c	890 ± 157 b	178 ± 8 a
2-decanone	1493	1492[n]	MS, IR	74 ± 17 b	29 ± 7 a	20 ± 1 a
unknown	1494			11 ± 3 a	6 ± 1 a	5 ± 1 a
2-methylpropanoic acid	1571	1574[j]	MS, IR, S	46253 ± 3607 a	128209 ± 16750 b	256166 ± 25718 c
γ-valerolactone	1601	1617[l]	MS, IR	819 ± 43 a	3382 ± 340 b	4293 ± 471 b
γ-butyrolactone	1612	1634[f]	MS, IR, S	519 ± 13 a	1136 ± 110 b	1736 ± 156 c
unknown	1614			107 ± 17 a	61 ± 37 a	25 ± 3 a
butanoic acid	1631	1638[f]	MS, IR, S	1224 ± 99 a	5307 ± 532 a	16034 ± 2242 b
3-methylbutanoic acid	1667	1677[f]	MS, IR, S	35608 ± 3146 a	133633 ± 20394 a	376323 ± 54303 b
unknown	1713			3996 ± 679 b	2443 ± 215 a	1967 ± 85 a
2-methylpentanoic acid	1742	1773[f]	MS, IR	261 ± 62 a	336 ± 43 a	823 ± 139 b
2-butenoic acid	1774	1808[f]	MS, IR	6 ± 3 a	0 ± 0 a	34 ± 4 b
unknown	1779			11 ± 3 a	9 ± 2 a	15 ± 6 a
geranyl acetone	1851	1888[f]	MS, IR	43 ± 10 a	34 ± 8 a	33 ± 19 a
guaiacol	1854	1840[e]	MS, RI, S	7 ± 1 b	0 ± 0 a	3 ± 1 a
2-phenylethanol	1917	1859[e]	MS, RI, S	904 ± 294 a	4521 ± 966 a	16102 ± 3224 b
2-pyrrolidinone	2025	2045[f]	MS, IR	83 ± 18 a	160 ± 44 a	345 ± 63 b
pantolactone	2028	2050[f]	MS, IR	25 ± 2 ab	15 ± 1 a	31 ± 7 b
unknown	2302			5 ± 1 a	13 ± 3 ab	28 ± 7 b

[a]Means and standard deviations of the absolute areas detected for three repetitions are reported. Different letters represent means that are significantly different at $p < 0.01$ (ANOVA and Tukey HSD test). [b]Retention index. [c]Retention index from the literature. [d]Identification method: S, comparison of mass spectra and retention time with those of standard compounds; RI, comparison of order of elution according to different authors. [e]Ames and MacLeod.[5] [f]Comuzzo et al.[14] [g]Culleré et al.[29] [h]Baek and Cadwallader.[26] [i]Lopez et al.[27] [j]Münch et al.[25] [k]Girard et al.[28] [l]Jennings and Shibamoto.[24] [m]Pennarun et al.[30] [n]Lei and Boatright.[31] [o]Flavornet database;[32] MS, comparison of mass spectra with those reported in Wiley 5 mass spectrum library; MST, tentative identification by mass spectrum. [p]Not detected.

Table 3. Odor-Active Compounds and Olfactory Descriptions, Detected by SPME-GC-O in the Headspace of the Autolysate Powders*

Compound	RI [a]	Thermal autolysate (T)	Mechanical autolysate (M)	Enzymatic autolysate (E)
3-methylbutanal	893			
ethyl acetate	893		sweet, alcoholic, cheese	
ethyl 2-methylpropanoate	982			pungent, solvent
unknown	1036	yeast, fruity		
ethyl 2-methylbutanoate	1057		fruit candy, tropical fruits	tropical fruits, pineapple
ethyl 3-methylbutanoate	1076		cheese, tropical fruits	tropical fruits, sweet
hexanal	1082			
p-xylene	1135		tropical fruits	alcohol, pungent, sweet, tropical fruits
ethyl pentanoate	1135			
limonene	1186		sweet	tropical fruits
2 and 3-methylbutanol	1207	cheese	broth, glutamate	cooked vegetables, broth, cheese, mould
ethyl hexanoate	1236			
1,2,4-trimethylbenzene	1238		burnt	tropical fruits
unknown	1243			
2-heptenal	1324			pungent
2,6-dimethylpyrazine	1324			
6-methyl-5-hepten-2-one	1343	potato, cooked vegetables, broth	smoked cheese	
2,3-dimethylpyrazine	1345			
2-nonanone	1392			
a benzene	1392			
nonanal	1394		pungent, paint, pear	
2,3,5-trimethylpyrazine	1397			
unknown	1398			
ethyl 2-hydroxybutanoate	1400			mushroom, earth, cooked vegetables
a methylcyclohexanol	1404			
3-octanol	1405			
2-octanol	1425		sweet, candy, fruits	
3-methyl-3-cyclohexen-1-one	1426			mushroom, earth
acetic acid	1440		vinegar, pungent	vinegar, paint
2-furaldehyde	1465	broth	boiled potato, cooked vegetables	pungent, paint, broth
propanoic acid	1490			
2-ethyl-1-hexanol	1493	paint, solvent	pungent, cooked vegetables, paint	pungent, paint, herbaceous
2-decanone	1493			
unknown	1494			
2-methylpropanoic acid	1571	cheese	pungent, rotten	cheese, mould
γ-valerolactone	1601	sweet		
γ-butyrolactone	1612			
unknown	1614	burnt		
butanoic acid	1631		cheese, mould	cheese, pungent
3-methylbutanoic acid	1667	cheese, cooked vegetables	cheese	goat cheese, pungent
unknown	1713		cheese	
2-methylpentanoic acid	1742			burnt
2-butenoic acid	1774			cheese
unknown	1779	fruit candy		
geranyl acetone	1851	fruit candy		
guaiacol	1854	unpleasant	medicine	
2-phenylethanol	1917	caramel, rose	rose	rose
2-pyrrolidinone	2025	yeast, bread, sweet, milk candy	cotton candy, candy, malt	malt
pantolactone	2028			
unknown	2302	burnt		

*Each description includes all of the odors detected in the three repetitions prepared for that product. Vertical bars mark the odors perceived in a given chromatographic zone. [a]Retention index.

moieties in the macromolecular structures of peaks 2 and 4. Looking at the three chromatograms, it is possible to observe that the intensities of the detected peaks are higher in thermal (T) and enzymatic products (E), confirming the data reported in Table 1, as concerns soluble proteins. Enzymatic autolysate, in particular, gave the most intense peaks, especially as concerns

fraction 3 and the fractions with the lowest molecular weight (fractions 5 and 6); these fractions did not give any signal by refractive index detection, suggesting their nonglycosylated nature. These observations seem in agreement with what observed by SDS-PAGE, regarding the higher nonglycosylated protein content of enzymatic preparation (Figure 1A).

On the basis of what reported, glycoproteins were presumably eluted in fractions 2 and 4; the former seemed most abundant in thermal autolysate, giving a partial confirmation to the data of Table 1, as well as to what observed in SDS-PAGE separation of glycoproteins (Figure 1B); the latter could be basically connected to the unfocused low molecular weight band detected by Fuchsin staining in the enzymatic autolysate and reported in Figure 1B. We have to underline that the molecular weight range estimated by SEC is narrower respect to that reported in Figure 1 for SDS-PAGE; this could be due to interactions between the stationary phase of the column and the macromolecules, which could occur in the acidic environment of the mobile phase (pH 3.20) and which could have affected the estimation of the apparent molecular weights of the six fractions.

In conclusion, thermolysis seems the disruption method that gave the highest content of soluble glucidic colloids and glycoproteins in the autolysate powders: the data obtained by SDS-PAGE and SEC suggested that these macromolecules have generally a quite high molecular weight, if compared with other macromolecular fractions released in winelike medium, but more probably, they are characterized by a high glycosylation level, which reduces their electrophoretic mobility. On the other hand, enzymatic product was the richest in non-glycosylated and low molecular weight fractions, even if glycoproteins were also found in this preparation. Mechanical disruption was the less intense treatment and gave the lowest release of macromolecules in model wine. The basic indices reported in Table 1 (soluble proteins and colloids) were generally in agreement with SDS-PAGE and SEC results; for this reason, they could be used as control parameters for a rapid evaluation of yeast cell disruption treatments.

Characterization of the Volatile Fraction of the Autolysate Powders. The results of SPME-GC-MS analysis of the three autolysate preparations are reported in Table 2. Forty volatile compounds were tentatively identified, within the odor-active regions perceived in the SPME-GC-O chromatograms. ANOVA analysis marked significant differences for many of them.

As one can observe, as a general rule, mechanical and particularly enzymatic treatment led to higher amounts of volatile compounds in the headspace of the powders, while thermolysis seems to have the lowest impact on the overall aroma concentration. The sum of the absolute areas reported in the table can confirm this statement: it corresponds to approximately 264 ± 20 millions for thermal product, 902 ± 181 millions for mechanical, and 1598 ± 99 millions for enzymatic autolysate.

The headspace of this last product appeared mostly characterized by significantly higher levels of short chain fatty acids (e.g., acetic, butanoic, 2-methylpropanoic, and 3-methylbutanoic); in our past experience, these compounds resulted to be some of the most representative volatiles in the chromatographic profile of YD products, and their concentration in wines rose progressively after the addition of increasing amounts of a yeast extract.[14] Besides fatty acids, even other compounds, normally detected in YDs, were present in more relevant amounts in the enzymatic preparation: 2,3- and 2,6-dimethylpyrazine, 2,3,5-trimethylpyrazine, and 2-furaldehyde were already reported as components of yeast extracts;[5] their occurrence in such products is related to the Maillard reaction.[7] The major concentrations found in enzymatic autolysate could be justified considering the higher intensity of degradation, highlighted for this product (see the previous section); this fact could have determined higher levels of precursors (e.g., sugars and low molecular weight nitrogen compounds) and, as a consequence, more relevant amounts of these volatile molecules in the preparation. In contrast, for higher temperature treatments (e.g., thermolysis), such volatile compounds (e.g., 2-furaldehyde) might be subjected even to other mechanisms involved in the Maillard reaction (e.g., melanoidins formation), so that their final concentration will be lower. Anyway, we have to underline that all of such molecules (e.g., pyrazines) gave quite low analytical responses in the applied instrumental conditions. Nevertheless, some of them (e.g., trimethylpyrazine) demonstrated to be released in the wines treated with inactive dry yeast preparations.[15]

The product obtained by mechanical disruption (M) appeared characterized by significantly higher levels of some ethyl esters, such as ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, and ethyl hexanoate. Their presence is quite unclear: they could be already present in the cell (e.g., consequently to yeast growth) and released after the lytic treatment, but they could also be produced by residual enzymatic activities, during the rehydration of the ADY formulation used for the preparation.

Finally, the headspace of the thermal autolysate was characterized by a significantly higher presence of some unknown compounds (e.g., those detected for retention index values of 1243 and 1713), as well as by a more relevant concentration of molecules as 2-decanone, 2-ethyl-1-hexanol, and guaiacol. Ethyl acetate was detected at the lowest level in this product.

These differences in the headspace composition of the three autolysates reflect also the results of GC-O analysis (Table 3): the formulation obtained by thermolysis was the one where the lowest number of olfactory notes was highlighted, while wider olfactory regions, characterized by pungent, vegetal, and cheeselike notes, were detected in the headspace of both enzymatic and mechanically treated products.

Summarizing, thermolysis led to the production of a less odorous formulation; this could be a favorable aspect, as regards the management of the lytic treatments, for the production of autolysates specifically oriented to winemaking.

Effect of the Autolysates Addition on the Volatile Fraction of a White Wine. The effects of the three autolysate preparations on the aromatic fraction of a white wine were investigated from the analytical and the sensory point of view. As regards the former aspect, the differences observed among the analyzed samples were generally less evident than those highlighted on the powders: ANOVA analysis did not mark significant behaviors, for the largest part of the 77 odor-active compounds, which were tentatively identified (SPME-GC-MS and SPME-GC-O) in the headspace of the treated wines. Looking at the global effect of the treatment, the sum of the absolute areas detected for the untreated wine (reference test) was 1120 ± 182 millions; this value slightly increased to 1176 ± 60 and to 1176 ± 248 millions for thermal and mechanical autolysate respectively, while it decreased to 1042 ± 208 millions for the sample treated with enzymatic product. So, at a

first approach, it seems that the autolysates addition has not changed a lot the volatile profile of the wine; nevertheless, it could be interesting to better examine these behaviors.

Figure 3 reports the results of PCA, performed on the mean values of the absolute areas, detected for the three repetitions

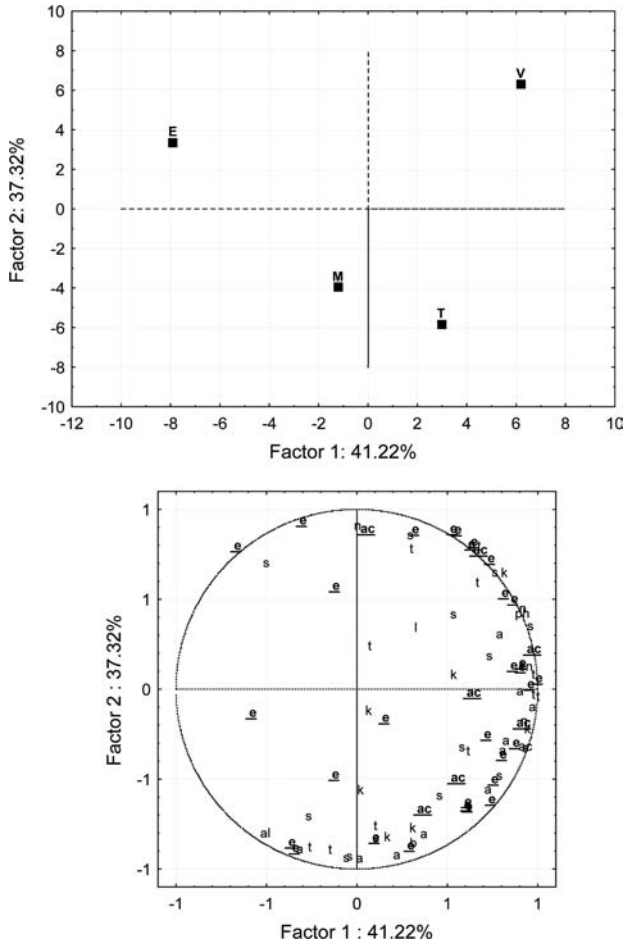

Figure 3. Results of PCA, carried out on the mean values of the absolute areas detected by SPME-GC-MS, in the headspace of the analyzed wines. V, test wine, no autolysate added; T, wine treated with thermal autolysate; M, wine treated with mechanical autolysate; and E, wine treated with enzymatic autolysate. Lower case letters identified different classes of volatile compounds: e, esters; ac, organic acids; a, alcohols; al, aldehydes; b, benzenes; k, ketones; l, lactones; n, norisoprenoids; ph, phenols; t, terpenes; and s, unknown compounds.

carried out for each experiment. The aroma compounds are grouped, indicating them with lower case letters, on the basis of their chemical class. As one can observe, the wines treated with the enzymatic preparation (E) were those characterized by the lowest level of volatile compounds, which, contrarily, seemed more concentrated in the sectors of the graph related to samples V (reference wine), T (wine treated with thermal autolysate), and M (mechanical autolysate addition). In particular, the aroma composition of these three wines appeared characterized by the presence of some esters, while certain carboxylic acids (e.g., butanoic and 3-methylbutanoic acid) were detected in more relevant mean concentrations in the samples treated with thermal autolysate. In our past experience,[14] these two classes of volatile compounds were the most affected by YD addition, among wine aroma substances. The lower mean concentration of volatile compounds detected in the samples treated with enzymatic preparation was also evident by SPME-GC-O analysis (Table 4); contrarily to the other samples (V, T, and M), those wines (E) were characterized by the lack of three specific olfactory zones in the GC-O chromatograms, in particular, the regions (reported in Table 4) defined by the following retention index intervals: 1265−1381, 1605−1669, and 2113-2197.

The results of sensory evaluation also confirmed the lower aroma impact of the wines treated with enzymatic autolysate. Among the attributes tested, only aroma intensity (fruity/positive notes) and yeast extract were detected as statistically relevant by the judges (Table 5): no significant differences were highlighted for any of the other attributes. As concerns aroma intensity, this attribute was perceived with the lowest scores in the wines treated with enzymatic autolysate (E), while thermal (T) and mechanical (M) products determined the most intense aroma perception; it was detected as significantly higher with respect to the evaluation reported for sample E. As regards the attribute yeast extract, thermal autolysate determined the most intense perception for this attribute; this means that the characteristic odor of the powders was recognized in the wine by the judges, even if the low values of the scores suggest a barely perceptible impact of the autolysate samples. The perception of the attribute yeast extract could be connected to the presence of free fatty acids in the wines: some of these compounds (e.g., butanoic, 3-methylbutanoic, and hexanoic acid) seemed to characterize the samples treated with product T (see Figure 3), and it has been suggested that they could be released from the powders into the wine.[14] Anyway, in the case of such experiment, this is quite strange, because the autolysate obtained by thermolysis showed the lowest content in volatile compounds and free fatty acids too, with respect to the other two preparations (Table 2); moreover, hexanoic acid was even not detected in the powder, while it characterized the wines treated with product T. For these reasons, the higher concentration of short-chain fatty acids, found in the wines treated with the thermal product, might not be connected with their release from the powders, but more probably, it could be determined by a salting out effect, promoted just by the macromolecules released by the thermal autolysate. Besides free fatty acids, no other volatile substance, previously detected in the powders (see Table 2) and described as typical aroma compound of YDs (e.g., pyrazines), was found in detectable amounts in the wines.

It is interesting to note that the previous hypothesis, the salting out effect promoted by thermal autolysate addition, could also justify the higher wine aroma intensity reported in Table 5 for the wines treated with product T, and it might be explained by considering the macromolecular composition of the colloids released in winelike solution by this preparation. Glycoproteins seemed the most relevant soluble macromolecular fraction of thermal autolysate (Figure 1): polysaccharides and mannoproteins have been reported as enhancers of the volatility of some aroma compounds,[20,21] so the behaviors observed could be traced back just to these macromolecules. On the other hand, the lower aroma perception, observed for the wines treated with enzymatic autolysate, could be explained considering the retentive effects of the yeast walls of the product added (the insoluble fraction of the autolysate) but also on the basis of the higher level of nonglycosylated soluble proteins detected in such preparation (Figure 1); in fact, it is well-known that proteins can retain aroma compounds, reducing their volatility.[36,37]

Table 4. Olfactory Descriptions (SPME-GC-O) of Some of the Odor-Active Compounds Detected in the Headspace of the Chardonnay Wine Treated and Not (Reference Test) with the Three Autolysate Preparations*

Compound	RI [a]	RI *lit* [b]	IM [c]	Sample [i]			
				V	T	M	E
3-methylbutyl butanoate	1265	1259 [d]	MS,IR	tropical fruits		fruity	
hexyl acetate	1271	1275 [e]	MS,IR,S	sweet, medicine			
a terpene	1322		MST	pungent, methane, truffle			
3-methyl-1-pentanol	1328	1297 [d]	MS,IR				
ethyl heptanoate	1341	1321 [d]	MS,IR,S				
ethyl 2-hexenoate	1347	1346 [d]	MS,IR	tea	fresh		
ethyl lactate	1349	1358 [h]	MS,IR,S				
1-hexanol	1354	1359 [e]	MS,IR,S	tropical fruits			
a benzene	1381		MST			sweet, flowers, pear	
2-nonanone	1381	1388 [h]	MS,IR				
3-hexenyl butanoate	1605	1526 [h]	MS		asparagus		
γ-butyrolactone	1612	1634 [e]	MS,IR,S	pungent, paint	flowers	fruits	
ethyl 2-furoate	1613	1599 [d]	MS,IR		semolina, baby food		
butanoic acid	1631	1612 [g]	MS,IR,S				
3-methylbutyl octanoate	1648	1654 [f]	MS,IR				
citronellyl propionate	1664		MS			broth	
3-methylbutanoic acid	1669	1651 [g]	MS,IR,S	cheese	cheese		broth
unknown	2113				burnt	candy	
a furanone	2140		MST	candy, sweet	candy		
a furanone	2156		MST	undefined	burnt	candy, sweet	
2-hydroxy-5-methylacetophenone	2192		MS	medicine	medicine		
unknown	2197					undefined	

*Each description includes all of the odors detected in the three repetitions prepared for that product. Vertical bars mark the odors perceived in a given chromatographic zone. [a]Retention index. [b]Retention index from the literature. [c]Identification method: S, comparison of mass spectra and retention time with those of standard compounds; RI, comparison of order of elution according to different authors. [d]Jennings and Shibamoto.[24] [e]Baek and Cadwallader.[26] [f]Lopez et al.[27] [g]Münch et al.[25] [h]Flavornet database;[32] MS, comparison of mass spectra with those reported in Wiley 5 mass spectrum library; MST, tentative identification by mass spectrum. [i]V, reference test wine, no autolysate added; T, wine treated with thermal autolysate; M, wine treated with mechanical autolysate; and E, wine treated with enzymatic autolysate.

Table 5. Results of Sensory Evaluation of the Treated Wines for the Attributes "Aroma Intensity" and "Yeast Extract"[a]

wine sample[b]	mean ± SD
score for aroma intensity	
V	5.3 ± 2.7 ab
T	5.9 ± 1.9 b
M	5.8 ± 0.9 b
E	4.8 ± 2.9 a
score for yeast extract	
V	1.8 ± 0.9 a
T	2.7 ± 2.7 b
M	2.2 ± 1.5 ab
E	2.3 ± 2.1 ab

[a]Different letters represent means which are significantly different at $p < 0.05$ (Main Effects ANOVA and LSD Test). [b]V, test wine, no autolysate added; T, wine treated with thermal autolysate; M, wine treated with mechanical autolysate; and E, wine treated with enzymatic autolysate.

Summarizing, the use of different cell disruption methods for the production of yeast autolysates can determine very different results as concerns the composition of the formulations and their effects on wine. As reported in the introduction, the release of volatile compounds from the powders and the salting out/retentive effects of yeast macromolecules on wine aroma compounds are two of the most important modifications determined by the addition of YDs in wine.

In this experiment, enzymatic autolysis led to products with a higher content of volatile compounds, which could have potentially a most relevant impact on wine sensory characteristics; this fact could be related to the higher degradation of cell structures, supposed for this preparation. On the other hand, mechanical and particularly thermal autolysate showed a lower level of aroma compounds in the powders, and this represents undoubtedly a positive factor for their oenological use.

The lytic treatments also affected the composition of the three autolysates as regards soluble macromolecules. In particular, thermal and mechanical products were mainly characterized by glycosylated proteins; these macromolecules could be able to increase the volatility of certain aroma compounds, and this could justify the more intense aroma, perceived in the wines treated with such preparations. Contrarily, nonglycosylated soluble proteins were detected in more relevant amounts in enzymatic autolysate; their retentive capacity, together with that of yeast walls, could have determined the lower aroma intensity perceived in the wines treated with this formulation.

Finally, no clear analytical evidence was found (in the experimental conditions) regarding the release of volatile compounds from the powders into the wines, even if the samples treated with thermal autolysate were characterized by a perceptible yeast extract olfactory note. This behavior could be related to the presence of short-chain fatty acids: these compounds could be released from the powders into the wine, but the fact that they were present also in the untreated

samples and the observation that thermal product was the less characterized by their presence suggest that, more probably, wine carboxylic acids could be salted out by the macromolecules released by the preparation.

In conclusion, thermolysis seemed the most promising lytic technique for the production of YDs specifically designed for winemaking, due to the characteristics of the powders (higher glycoprotein content and lower concentration of volatile compounds) and to the positive effects of the thermal autolysate in increasing wine aroma intensity. Moreover, the technique is very simple and easily accessible. Nevertheless, the question related to the perception in wine of sensory notes referable to the powders remains unclear; as regards the production of yeast autolysates specific for winemaking, further investigations should not neglect this aspect, as well as the optimization of thermolysis treatments, considering different temperatures and conditions, on the basis of a logic of energy saving and simplification, which could make this technology sustainable in several producing situations.

AUTHOR INFORMATION

Corresponding Author

*Tel: +39 0432 55 8376. Fax: +39 0432 55 8130. E-mail: piergiorgio.comuzzo@uniud.it.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are grateful to Prof. Marco Zancani (University of Udine) and to Dr. Simone Vincenzi (University of Padova) for their suggestions regarding SDS-PAGE separation.

REFERENCES

(1) Pozo-Bayón, M. Á.; Andújar-Ortiz, I.; Moreno-Arribas, M. V. Scientific evidences beyond the application of inactive dry yeast preparations in winemaking. *Food. Res. Int.* **2009**, *42*, 754−761.

(2) Charpentier, C.; Feuillat, M. Yeast autolysis. In *Wine Microbiology and Biotechnology*; Fleet, G. H., Ed.; Harwood Academic Publishers: Chur, Switzerland, 1993; pp 225−242.

(3) Sommer, R. Yeast extracts: Production, properties and components. *Food Aust.* **1998**, *50*, 181−183.

(4) Feuillat, M.; Charpentier, C.; Nguyen Van Long, T. Les mannoprotéines de levures: Un adjuvant œnologique possible. *Bull. O.I.V.* **1998**, *813−814*, 944−967.

(5) Ames, J. M.; McLeod, G. M. Volatile components of a yeast extract composition. *J. Food Sci.* **1985**, *50*, 125−135.

(6) Ames, J. M.; Elmore, J. S. Aroma components of yeast extracts. *Flavour Fragrance J.* **1992**, *7*, 89−103.

(7) Nagodawithana, T. Yeast-derived flavors and flavor enhancers and their probable mode of action. *Food Technol.* **1992**, *46*, 138−144.

(8) Lubbers, S.; Charpentier, C.; Feuillat, M.; Voilley, A. Influence of yeasts walls on the behavior of aroma compounds in a model wine. *Am. J. Enol. Vitic.* **1994**, *45*, 29−33.

(9) Dziezak, J. D. Yeasts and yeast derivatives: Applications. *Food Technol.* **1987**, *41*, 122−125.

(10) Lubbers, S.; Voilley, A.; Feuillat, M.; Charpentier, C. Influence of mannoproteins from yeast on the aroma intensity of a model wine. *Lebensm.-Wiss. Technol.* **1994**, *27*, 108−114.

(11) Chalier, P.; Angot, B.; Delteil, D.; Doco, T.; Gunata, Z. Interactions between aroma compounds and whole mannoprotein isolated from *Saccharomyces cerevisiae* strains. *Food Chem.* **2007**, *100*, 22−30.

(12) Pozo-Bayon, M. A.; Andujar-Ortiz, I.; Alcaide-Hidalgo, J. M.; Martin-Alvarez, P. J.; Moreno-Arribas, M. V. Characterization of Commercial Inactive Dry Yeast Preparations for Enological Use Based on Their Ability To Release Soluble Compounds and Their Behavior toward Aroma Compounds in Model Wines. *J. Agric. Food Chem.* **2009**, *57*, 10784−10792.

(13) Comuzzo, P.; Tat, L.; Fenzi, D.; Brotto, L.; Battistutta, F.; Zironi, R. Interactions between yeast autolysates and volatile compounds in wine and model solution. *Food Chem.* **2011**, *127*, 473−480.

(14) Comuzzo, P.; Tat, L.; Tonizzo, A.; Battistutta, F. Yeast derivatives (extracts and autolysates) in winemaking: Release of volatile compounds and effects on wine aroma volatility. *Food Chem.* **2006**, *99*, 217−230.

(15) Pozo-Bayon, M. A.; Andujar-Ortiz, I.; Moreno-Arribas, M. V. Volatile profile and potential of inactive dry yeast-based winemaking additives to modify the volatile composition of wines. *J. Sci. Food Agric.* **2009**, *89*, 1665−1673.

(16) Münch, P.; Schieberle, P. Quantitative studies on the formation of key odorants in thermally treated yeast extracts using stable isotope dilution assays. *J. Agric. Food Chem.* **1998**, *46*, 4695−4701.

(17) Pozo-Bayón, M. A.; Andújar-Ortiz, I.; Mendiola, J. A.; Ibáñez, E.; Moreno-Arribas, M. V. Application of supercritical CO_2 extraction for the elimination of odorant volatile compounds from winemaking inactive dry yeast preparation. *J. Agric. Food Chem.* **2010**, *58*, 3772−3778.

(18) Moine Ledoux, V. Recherches sur le rôle des mannoprotéines de levure vis à vis de la stabilisation protéique et tartrique des vins. Thése de Doctorat, Université de Bordeaux II, 1996.

(19) Peat, S.; Whelan, W. J.; Edwards, T. E. Polysaccharide of baker's yeast. Part 4. Mannan. *J. Chem. Soc. (London)* **1961**, *1*, 28−35.

(20) Langourieux, S.; Crouzet, J. Study of aroma compounds-polysaccharides interactions by dynamic exponential dilution. *Lebensm.-Wiss. Technol.* **1994**, *27*, 544−548.

(21) Dufour, C.; Bayonove, C. L. Influence of wine structurally different polysaccharides on the volatility of aroma substances in a model system. *J. Agric. Food Chem.* **1999**, *47*, 671−677.

(22) Regenstein, J. M.; Regenstein, C. E. Food protein chemistry. In *Food Science and Technology. A Series of Monographs*; Schweigert, B. S., Hawthorn, J., Eds.; Academic Press: New York, 1984.

(23) Usseglio-Tomasset, L.; Castino, M. I colloidi solubili di natura glucidica dei mosti e dei vini. Parte I. *Riv. Vitic. Enol.* **1975**, *28*, 374−391.

(24) Jennings, W.; Shibamoto, T. *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography*; Academic Press: New York, 1980.

(25) Münch, P.; Hofmann, T.; Schieberle, P. Comparison of key odorants generated by thermal treatment of commercial and self-prepared yeast extracts: Influence of the amino acid composition on odorant formation. *J. Agric. Food Chem.* **1997**, *45*, 1338−1344.

(26) Baek, H. H.; Cadwallader, K. R. Contribution of free and glycosidically bound volatile compounds to the aroma of muscadine grape juice. *J. Food Sci.* **1999**, *64*, 441−444.

(27) Lopez, R.; Ferreira, V.; Hernandez, P.; Cacho, J. Identification of impact odorants of young red wines made with Merlot, Cabernet Sauvignon and Grenache grape varieties: A comparative study. *J. Sci. Food Agric.* **1999**, *79*, 1461−1467.

(28) Girard, B.; Fukumoto, L.; Mazza, G.; Delaquis, P.; Ewert, B. Volatile terpene constituents in maturing Gewurztraminer grapes from British Columbia. *Am. J. Enol. Vitic.* **2002**, *53*, 99−109.

(29) Culleré, L.; Escudero, A.; Cacho, J.; Ferreira, V. Gas chromatography-olfactometry and chemical quantitative study of aroma of six premium quality Spanish aged red wines. *J. Agric. Food Chem.* **2004**, *52*, 1653−1660.

(30) Pennarun, A.-L.; Prost, C.; Demaimay, M. Identification and origin of the character-impact compounds of raw oyster *Crassostrea gigas*. *J. Sci. Food Agric.* **2002**, *82*, 1652−1660.

(31) Lei, Q.; Boatright, W. L. Compounds Contributing to the Odor of Aqueous Slurries of Soy Protein Concentrate. *J. Food Sci.* **2001**, *66*, 1306−1310.

(32) Flavornet Kovats retention indexes, http://www.flavornet.org/flavornet.html, 2011.

(33) Durchschlag, H.; Jaenicke, R. SDS-Electrophoresis of glycoproteins. *Chim. Oggi* **1997**, *15*, 15−24.
(34) Moine Ledoux, V.; Dubourdieu, D. An invertase fragment responsible for improving the protein stability of dry white wines. *J. Sci. Food Agric.* **1999**, *79*, 537−543.
(35) Waters, E. J.; Wallace, W.; Tate, M. E.; Williams, P. J. Isolation and partial characterization of a natural haze protective factor from wine. *J. Agric. Food Chem.* **1993**, *41*, 724−730.
(36) Kinsella, J. E. Flavor perception and binding. *Int. News Fats, Oils Relat. Mater.* **1990**, *1*, 215−226.
(37) Voilley, A.; Beghin, V.; Charpentier, C.; Peyron, D. Interaction between aroma substances and macromolecules in a model wine. *Lebensm.-Wiss. Technol.* **1991**, *24*, 469−472.