JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Comparison of Components Released by Fermented or Active Dried Yeasts after Aging on Lees in a Model Wine

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Comparison of different components released during autolysis with fermented or active dried yeast, which has never been reported previously, is related in this paper. Three autolysates were elaborated with *Saccharomyces cerevisiae* in a model wine (pH 3.5) at 30 or 18 °C. Composition of the autolysate appears to depend on both the growth medium and the physiological state of the yeast. The autolysate obtained from active dried yeast presents a higher total nitrogen concentration (a factor of 1.5−1.9 for the fermented yeast autolysate), a greater proportion of free amino acids (42 vs 16−25%), the lowest proportion of oligopeptides (25 vs 31−37%) and polypeptides (27 vs 45−34%), and an absence of nucleosides. Distribution of amino acids in peptides and proteins is relatively homogeneous in the autolysates but shows significant differences in free amino acids. Parietal macromolecules (mannoproteins and glucans) are present in greater quantities in autolysates elaborated from fermented yeast. The influence of the different fractions of these autolysates (<0.5, 0.5−1, 1−10, and >10 kDa) on bacterial growth has been investigated.

KEYWORDS: Wine; yeast autolysis; nitrogen; nucleosides; sugars; *Oenococcus oeni*

INTRODUCTION

Autolysis has been defined as the hydrolysis of cellular components by hydrolytic yeast enzymes. Breakdown of cell membranes, release of hydrolytic enzymes, liberation of intracellular constituents, and hydrolysis of intracellular biopolymers into products of a low molecular weight are the main events that occur during this process (*1*−*4*). In wine production, yeast autolysis occurs in the case of sparkling wines (*5*, *6*) or in wines elaborated by the traditional practice of the "sur lies" method by contact between wine and lees during aging (*2*, *7*). The storage of wine in contact with yeast allows an enrichment in different components such as nitrogen compounds, volatile substances, lipids, and carbohydrates (*2*, *3*). Aging on lees enhances the wine's body and flavor (*2*, *8*), and yeast metabolites such as parietal macromolecules and peptides greatly enhance the growth of *Oenococcus oeni*, a bacterial species frequently conducting malolactic fermentation (*9*).

To investigate yeast autolysis, many scientific studies have described the changes in different components released into wine or into a model medium. All of these works reported mainly on the changes in free amino acids (*2*, *3*, *7*, *10*) and the yeast's macromolecule content released in the extracellular medium (*2*, *3*, *11*). Recently, liberation of peptides has been studied during this process (*4*, *12*, *13*). Depending on the author, the experimental methodologies can be very different, as composition of medium (presence or absence of ethanol, pH values), strain of yeast, effect of incubation temperature, inducers, and, most of all, changes in components released in the extracellular medium significantly affect the autolysis phenomenon. The yeast used is fermented yeast in wine, fermented yeast in a synthetic medium (with different concentrations in reducing sugars varying from 40 to 200 g/L), or active dried yeast only rehydrated before autolysis (*2*, *3*). Therefore, the obtained results are difficult to extrapolate in wine aging on lees. Thus, this study proposes to compare the composition of autolysates elaborated with a strain of yeast (*Saccharmocyes cerevisiae*) by using dried active yeast or fermented yeast obtained after alcoholic fermentation in a model synthetic must. Each autolysate was then fractioned into four molecular weight fractions: <0.5, 0.5−1, 1−10, and >10 kDa. The concentration and composition of free amino acids, oligopeptides, polypeptides, proteins, nucleotides, nucleosides, and parietal macromolecules (mannoproteins and glucans) were determined, and the influence of each fraction of these three autolysates was then tested on bacterial growth.

MATERIALS AND METHODS

Chemicals. Ethanol (99.9% purity), tartaric acid (99.5%), acetic acid (>99.8%), citric acid (99.5%), potassium sulfate (99%), trifluoroacetic acid, glucose, fructose, mannose, asparagines (>99%), magnesium sulfate (99%), monopotassium phosphate (99.5%), *myo*-inositol, manganese sulfate (>99%), ammonium chloride (>99%), biotin, thiamin, pyridoxine, panthothenic acid, nicotinic acid, and 3-aminobenzoic acid were obtained from Merck (Darmstadt, Germany). dl-Malic acid (>99%), EDTA, and standard nucleotides and nucleosides were obtained from Sigma (St. Louis, MO). Solutions and HLPC grade water

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10.1021/jf020135j CCC: $25.00
Published on Web 12/21/2002

were made up with ultrapure water, obtained from a Milli-Q system (Millipore, Bedford, MA). HPLC grade methanol, selenium catalyst, Folin−Ciocalteu reagent, and acetonitrile were acquired from Carlo Erba (Rodano, MI, Italy). All other reagents were of analytical grade unless otherwise specified.

Yeast and Bacteria Strains. A commercial active dry yeast, *Saccharomyces cerevisiae* (Levuline BRG) was provided by Oenofrance (Rueil-Malmaison, France). A commercial lactic acid bacterium, *Oenococcus oeni* (MicroenosB$_{16}$), was marketed by Laffort (Bordeaux, France) and kept in FT 80 liquid medium at 4 °C (*14*).

Alcoholic Fermentation. Yeast cells were rehydrated according to the manufacturer's instructions. After rehydration, yeast cells were harvested by centrifugation at 4600*g* for 15 min at 4 °C and washed three times with 0.9% NaCl. Then, the washed yeast cells were suspended in a model synthetic grape juice medium to a final concentration of 4×10^6 cells/mL. The model grape juice medium was composed as follows: glucose (85 g/L), fructose (85 g/L), tartaric acid (3 g/L), dl-malic acid (6 g/L), citric acid (0.3 g/L), asparagine (2 g/L), monopotassium phosphate (2 g/L), ammonium chloride (2 g/L), magnesium sulfate heptahydrate (0.2 g/L), manganese sulfate monohydrate (0.01 g/L), *myo*-inositol (0.3 g/L), biotin (0.04 mg/L), thiamin (1 mg/L), pyridoxine (1 mg/L), panthothenic acid (1 mg/L), nicotinic acid (1 mg/L), and 3-aminobenzoic acid (1 mg/L). The pH was adjusted to 3.5 with 5 M potassium hydroxide. After flash sterilization (105 °C for 1 min), 10 mL containing the 10× concentrated vitamins solution was added after it had been filtered through a sterilized 0.2 μm filter (Sartorius, Goettingen, Germany). The alcoholic fermentation was conduced in 6×3.3 L of medium in 5 L conical flasks at 30 °C, with only daily a gentle resuspension of the cells. Viable and total cells were determined by counting on a Malassez cell after coloration with methylene blue (*15*). Reducing sugar concentration was estimated by a colorimetric method using 3,5-dinitrosalicylic acid (*16*). At the end of fermentation, the yeast cells were harvested by centrifugation at 4600*g* for 15 min at 4 °C and washed three times with 0.9% NaCl to be available for autolysis.

Autolysis Conditions. Washed yeast cells obtained after alcoholic fermentation or after a simple rehydration were suspended in a model wine buffer in a 1 L bottle with 15 g of yeast dry weight. The model wine buffer contained ethanol (12%, v/v), DL-malic acid (3 g/L), acetic acid (0.1 g/L), potassium sulfate (0.1 g/L), and magnesium sulfate heptahydrate (0.025 g/L). The pH was adjusted to 3.5 with 5 M potassium hydroxide. Autolysis was performed without stirring, with rehydrated yeasts in favorable conditions: 30 °C for 2 weeks (autolysate designated LP) and with yeasts obtained after alcoholic fermentation in conditions simulating wine-making [18 °C for 8 weeks (autolysate designated OP)] or in favorable conditions [30 °C for 2 weeks (autolysate designated FP)].

Fractionation Procedure. At the end of autolysis, yeast cells autolyzed were separated from the autolysates by centrifugation (4600*g*, 15 min, 4 °C). The supernatant (2 L) was pooled and successively submitted to one tangential ultrafiltration in a pilot apparatus (Sartorius T 15-22) with Sartocom polysulfone membrane (10 kDa molecular weight cutoff) and two ultrafiltrations in a 180 mL Amicon cell (Millipore) with, respectively, 1 and 0.5 kDa molecular weight cutoff regenerated cellulose membranes (Millipore). The filtration temperature and the transmembrane pressure were, respectively, maintained at ∼15 °C and ∼200 kPa. At each step, an aliquot of retentate and permeate was kept for physicochemical measurements. All retentates and permeates were successively concentrated under reduced pressure at 25 °C with an RE-100 model rotary evaporator (Bibby Sterilin Limited, Stafforshire, U.K.) and by cryoconcentration until a concentration factor of 10 was reached. Each obtained fraction (>10, 1−10, 0.5−1, and <0.5 kDa) was divided in five parts and stocked at −18 °C until analysis.

Amino Acid Analysis. Amino acids were named by their symbols established by the IUPAC−IUB (1972). They were analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC), after precolumn derivatization with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC). The AQC reagent reacts rapidly with primary and secondary amino acids to yield highly stable derivatives that strongly fluoresce at 395 nm. The amino acid derivatization was made according to the specifications of the Waters AccQ-Tag method: 10 μL of sample, 20 μL of AQC reagent, and 70 μL of 0.2 M borate buffer, pH 8.8, were mixed in a small tube. This solution was heated for 10 min at 55 °C.

Separation was performed using an AccQ-Tag C_{18} column (150 × 3.9 mm i.d., 4 μm particle size) (Waters, Milford, MA), according conditions used by Desportes et al. (*17*). A standard solution of 0.1 mM amino acids was injected prior to each analysis group. α-Aminobutyric acid (1 mM), not detected in our samples, was used as internal standard. Total amino acid was determined after hydrolysis of samples in glass tubes with 6 M HCl, under vacuum, at 110 °C during 24 h. Due to the partial conversion of asparagine and glutamine into aspartic acid and glutamic acid, respectively, during hydrolysis, the data for asparagine plus aspartic acid and glutamine and glutamic acid are reported as Asx and Glx, respectively, in all of the hydrolyzed samples. Variation coefficients of the amino acids determination were <2%.

Determination of Nitrogen Compounds. Free amino nitrogen was estimated from the sum of the nitrogen of each amino acid determined individually by HPLC. Total amino nitrogen was determined in the same way as free amino acids, after hydrolysis. Peptide or protein nitrogen was considered as the difference between total amino nitrogen and free amino nitrogen.

Nucleotide and Nucleoside Analysis. Stock standard solutions of 1000 mg/L of nucleosides and monophosphate nucleotide mixtures were prepared in Milli-Q quality water (Millipore Corp., Bedford, MA) and stored at −20 °C. Analytical HPLC was performed with a Thermo Separation Products (Thermo-Quest, San Jose, CA) model P-1000 XR quaternary gradient liquid chromatography system, and detection was carried out with a Waters model 991 diode array detector. Data were recorded using version 6.22 of the detector software. An Interchrom (Interchim, Montluçon, France) stainless steel C_{18} analytical column (250 × 4.6 mm i.d., 5 μm Inertsil ODS-2 material, 150 Å porosity) protected by a guard column (15 × 4 mm i.d.) with the same phase (Macherey-Nagel, Düren, Germany) was used for reversed-phase ion-paired HPLC. Elution was carried out according to the procedure of Zhao and Fleet (*18*) as modified by Aussenac et al. (*19*). Variation coefficients of the nucleotide and nucleoside determination were <4%.

Monomeric Saccharide Analysis. An aliquot of each fraction (100 μL) was concentrated to dryness at 40 °C under a stream of nitrogen. Acid hydrolysis was performed using 2 M trifluoroacetic acid solution at 120 °C for 1.5 h (*20*). The sugars released by acid hydrolysis were converted into alditol acetates by reduction using sodium borohydride and acetylation with anhydride acetic acid in the perchloric acid (*21*). After extraction with chloroform, the organic phase was washed with distilled water and the alditol acetates were analyzed by GC. The GC apparatus (Chrompack 9001, Varian) was equipped with a fused silica capillary column (15 m × 0.32 mm i.d.; 0.2 μm bonded phase, Restek, Bellefonte, PA), a Rhoss injector (Lara-Spiral, Dijon France), and a flame ionization detector. The operating conditions were as follows: injector and detector temperatures, 265 °C; nitrogen carrier gas flow rate, 1.4 mL/min. Column temperature was raised from 175 to 240 °C at 8 °C/min, held for 1 min at 240 °C, and then increased to 265 °C at 8 °C/min. Each test was performed in triplicate, and the values given represent the average.

Bacterial Growth. A synthetic medium was used as a basal medium and consisted of glucose (2.5 g/L), fructose (2.5 g/L), arabinose (0.5 g/L), tartaric acid (3 g/L), DL-malic acid (6 g/L), citric acid (0.2 g/L), succinic acid (0.5 g/L), monopotassium phosphate (2 g/L), dipotassium phosphate (2 g/L), magnesium sulfate heptahydrate (0.2 g/L), manganese sulfate monohydrate (0.05 g/L), adenine (0.01 g/L), guanine (0.01 g/L), uracil (0.01 g/L), thymidine (0.01 g/L), xanthine (0.01 g/L), biotin (0.008 mg/L), thiamin (0.2 mg/L), riboflavin (0.2 mg/L), pyridoxine (0.2 mg/L), panthothenic acid (0.2 mg/L), nicotinic acid (0.2 mg/L), 3-aminobenzoic acid (0.2 mg/L), *myo*-inositol (0.02 g/L), folic acid (0.01 mg/L), cobalamine (0.0005 mg/L), and Tween 80 (1 mL/L). The pH was adjusted to 3.5 with 5 M potassium hydroxide. The nitrogen requirements were estimated in the medium by adding the different fractions obtained after fractionation one at a time in order to reach an added nitrogen final concentration of ∼50 mg/L. The pH values of all the media were adjusted to 3.5. The different media (15 mL) were sterilized after filtering through a sterilized 0.2 μm filter (Sartorius)

Table 1. Nitrogen Content (Milligrams of N per Gram of Dried Yeasts) in Fractions of Autolysates

fraction	total N	free amino N	peptide or protein[a] N
		Autolysate FP	
<0.5 kDa	9.5	3.0	6.5
0.5–1 kDa	*b*	*b*	*b*
1–10 kDa	9.5	0.1	9.4
>10 kDa	1.5	0.2	1.3
sum	20.5	3.3	17.1
		Autolysate OP	
<0.5 kDa	7.1	3.9	3.2
0.5–1 kDa	3.3	0.2	3.1
1–10 kDa	5.9	0.1	5.8
>10 kDa	0.6	0.1	0.5
sum	16.9	4.3	12.6
		Autolysate LP	
<0.5 kDa	14.9	11.7	3.2
0.5–1 kDa	5.3	0.4	4.8
1–10 kDa	8.8	0.1	8.7
>10 kDa	2.7	1.0	1.6
sum	31.6	13.2	18.3

[a] Proteins are compounds with a molecular mass >10 kDa, and peptides are compounds with a molecular mass <10 kDa. [b] Traces (nitrogen level <0.1 mg of N/g of dried yeast).

and distributed in 18 mL sterile screw-capped tubes. Cells were grown in FT 80 medium. In the middle of the exponential growth phase, cells were harvested by centrifugation (7000*g*, 20 min, 4 °C), washed twice with 0.9% NaCl, and used to inoculate the different media to a final concentration of 10^5 colony-forming units (cfu)/mL. Two control cell growths were prepared as follows: one medium without nitrogen requirements and one medium with all nitrogen requirements (FT80 medium). All cultures were incubated, unstirred, at 25 °C for 22 days. Growth was followed by measuring optical density at 600 nm using a Corning 253 spectrophotometer. Each test was performed in triplicate, and the values given represent the average.

RESULTS AND DISCUSSION

Amino, Peptide, and Protein Nitrogen. The values found for free amino, peptide or protein, and total nitrogen in the three autolysates studied are shown in **Table 1**. The major part of free amino acids (>90%) was present in the <0.5 kDa fraction. This corroborates our fractionation procedure. The total nitrogen content released during autolysis was significantly higher in the autolysate elaborated with active dried yeast (LP) than in the two autolysates elaborated with fermented yeasts (FP or OP). The lowest total nitrogen content was observed in the autolysate OP. This autolysate can be distinguished from the FP autolysate in that its temperature and autolysis time is different from that of the FP autolysate. This result is in accordance with those reported in the literature on autolysis and particulary with those reporting the negative influence of temperature (*2*, *3*). The release of nitrogen in the form of amino acids was due to two factors: first, a passive exorption of the internal content of the yeast and, second, a self-proteolysis process (*4*, *6*, *8*, *10*). Comparison among the three autolysates showed a great difference in the liberation of these components. The rise in amino acids represents approximatively 42% of the total nitrogen content released for the LP autolysate, 16% for the FP autolysate, and 25% for the OP autolysate. The largest enrichment in amino acids in the extracellular medium for the LP autolysate may be due to a greater amount of amino acids in the internal content of these yeast, cultivated for their production as active dried yeast in aerobic conditions. Moreover, yeast obtained after alcoholic fermentation (in the cases of FP and OP autolysates) gave the lowest amount of the intracellular pool

Table 2. Free Amino Acid Molar Distribution (Percent) of Fraction with a Molecular Mass <0.5 kDa in Different Autolysates

	autolysate				autolysate		
amino acid	OP	FP	LP	amino acid	OP	FP	LP
Ala	12.6	12.2	13.5	Leu	11.3	12.9	11.5
Arg	5.3	5.3	0.0	Lys	3.8	2.8	5.5
Asn	3.4	3.4	4.1	Met	2.7	3.4	2.9
Asp	6.8	7.3	5.5	Phe	6.0	7.0	5.4
Cys	0.2	0.3	0.2	Pro	4.9	4.3	3.6
GABA[a]	9.9	7.4	13.5	Ser	2.9	2.7	2.9
Gln	1.6	1.5	2.7	Thr	3.5	3.3	5.0
Glu	4.0	4.9	0.2	Trp	nd[b]	nd	nd
Gly	5.3	4.5	4.8	Tyr	2.7	2.8	0.1
His	0.6	1.0	0.7	Val	6.8	7.3	8.6
Ileu	5.7	5.8	9.4				

[a] GABA, γ-aminobutyric acid. [b] nd, not determined.

(*12*). As reported recently (*6*, *12*, *13*) the amount of peptides released during autolysis in the extracellular medium is important. Indeed, peptide concentration reached 15.8 mg of N/g of dried yeast for the FP autolysate, 12.1 mg of N/g of dried yeast for the OP autolysate, and 16.7 mg of N/g of dried yeast for the LP autolysate. Peptides of different sizes are present in the medium. According to Martinez-Rodriguez and Polo (*4*), who used *Saccharomyces bayanus*, 80% of the peptides released in the medium are of a small size (<0.7 kDa), whereas, in our case using the *S. cerevisiae* strain, only 40−50% are of a small size (<1 kDa). It is well-known that total nitrogen, protein, and peptide levels released during autolysis are strain dependent, but the quality of these nitrogen compounds (and mainly peptides) might be strain dependent as well. Proteins are also present in the extracellular medium and account for 3−6% of the total nitrogen content. These low values for proteins indicated that peptides were liberated into the medium by the action of both endo- and exocellular proteases (*4*, *6*, *8*, *10*, *12*). These results are original as we are the first to report on a comparison of the nitrogen composition among autolysates elaborated with fermented yeast and active dry yeast.

Free Amino Acid Composition. Values of free amino acid content in the fraction with a molecular weight <0.5 kDa for the three autolysates are noted in **Table 2**. To facilitate a qualitative comparison, amino acid levels are expressed as the percentage of molar distribution. Alanine (12.5% of total amino acids), leucine (≈12%), γ-aminobutyric acid (≈10%), and valine (≈8%) are the major amino acids for all autolysates. These results are in accordance with those studies concerning the release of free amino acids during autolysis in a model wine (*10*, *12*, *13*). Amino acids detected in the lowest concentration, such as histidine, glutamine, and cysteine, account for only 2−4% of total amino acids. The free amino acid composition of the autolysates studied here was different from those previously reported for French wines (*17*), Spanish wines (*6*, *22*, *23*), Japanese wines (*7*), and 62 Chardonnay varietal wines from around the world (*24*). This was especially true in the case of proline, the principal amino acid in berries, which can account for 50−70% of the total amino acid content in wine, whereas it represents only 4−5% in the studied autolysates. The most relevant differences observed during autolysis were not due to temperature itself but rather to the yeast physiological state, dried or fermented. Indeed, the free amino acid molar distributions were very similar for the two autolysates elaborated with fermented yeast (FP and OP), although it is fairly different in the autolysate obtained from active dried yeast (LP). In this last autolysate, we noted the absence of arginine, the quasi-

Table 3. Amino Acid Molar Distribution (Percent) in Peptides (Fractions <0.5, 0.5–1, and 1–10 kDa) and Proteins (Fraction >10 kDa) in the Three Autolysates Studied (OP, FP, and LP)

	<500 Da			500–1000 Da			1 000–10 000 Da			>10 000 Da		
amino acid	OP	FP	LP	OP	FP	LP	OP	FP	LP	OP	FP	LP
Ala	11.0	9.5	6.6	10.0	7.1	9.0	10.1	9.2	9.0	11.1	9.6	8.9
Arg	3.3	4.1	4.1	4.5	2.4	5.6	3.7	4.0	4.4	2.5	3.5	3.7
Asx	8.6	10.3	6.1	7.9	9.5	7.3	10.7	12.5	9.0	7.7	10.9	4.9
Cys	0.0	0.2	0.1	0.2	0.0	0.0	0.1	0.4	0.1	0.0	0.3	0.0
GABA[a]	1.8	0.8	0.0	0.0	4.8	0.0	0.0	0.0	0.0	0.3	0.1	0.0
Glx	14.0	11.6	14.2	10.7	12.7	10.7	10.9	11.0	10.7	9.6	10.5	11.7
Gly	11.8	8.3	14.0	16.0	11.9	14.1	13.4	9.7	14.5	10.7	8.9	18.5
His	1.0	1.5	1.8	2.2	1.6	2.8	2.4	2.5	2.9	1.3	2.1	2.2
Ileu	6.3	7.1	4.1	5.0	4.0	4.7	4.7	5.1	4.6	4.7	5.2	4.2
Leu	7.2	7.8	3.6	5.1	19.0	4.9	5.8	6.2	5.8	5.8	5.6	7.7
Lys	5.1	10.4	5.7	13.2	5.6	13.2	8.5	9.0	9.6	5.8	8.6	8.0
Met	0.0	0.0	0.0	0.0	0.0	0.0	0.1	1.0	0.0	0.0	0.0	0.0
Phe	2.0	4.2	1.7	1.7	0.0	1.9	1.6	2.2	1.9	1.8	2.3	1.2
Pro	4.9	2.1	14.4	3.7	1.6	5.0	6.9	4.8	6.3	5.4	6.0	7.1
Ser	5.7	5.3	6.4	5.7	7.9	6.3	6.6	7.0	6.9	12.4	8.6	7.2
Thr	6.6	6.7	8.2	6.1	4.0	7.1	6.4	6.7	6.9	12.5	9.0	8.2
Trp	nd[b]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Tyr	3.6	2.9	3.7	2.0	1.6	2.1	2.2	2.6	2.1	2.7	2.6	2.1
Val	7.2	7.2	5.3	5.9	6.3	5.3	5.8	6.1	5.3	5.9	6.1	4.4

[a] GABA, γ-aminobutyric acid. [b] nd, not determined.

absence of glutamic acid and tyrosine, and a higher proportion in γ-aminobutyric acid, isoleucine, and lysine (**Table 2**).

Amino Acid Composition of Peptides and Proteins. Table 3 shows the amino acid residue molar distribution in fractions >10, 1−10, 0.5−1, and <0.5 kDa. Whatever the autolysate, for each fraction the amino acid residue molar distribution is almost similar. These results corroborate with those previously reported on the composition of amino acids in different nitrogenous fractions in wine (*25*). Nitrogen components with a high molecular weight are first released in the extracellular medium during induced autolysis and then could be partially hydrolyzed in peptides of a lower molecular weight. The molar distributions of amino acids in peptides and proteins are very different from those of free amino acids. The major amino acids, present with a molar ratio >8% of the total amino acids, are glycine, glutamic acid and glutamine (Glx), alanine, asparagine and aspartic acid (Asx), and in some cases lysine. Glutamic acid and glutamine (Glx), glycine, lysine, serine, and threonine are present in greater proportions in peptides and proteins than in their free form, whereas leucine and phenylalanine are present in lower proportions. It should be also pointed out that γ-aminobutyric acid and methionine were not detected as amino acid residues, whereas they were found, respectively, from 7.4 to 13.5% and from 2.7 to 3.4% as free amino acids. Serine and threonine are some of the most represented amino acids in fractions corresponding to proteins (>10 kDa): 25, 18, and 15% for autolysates OP, FP, and LP, respectively. This could be explained by the presence in this fraction of partially hydrolyzed mannoproteins initially present in the yeast cell wall, because these two amino acids are involved in O-glycosylation in yeasts. Concerning the amino acid composition of peptides and proteins, few differences are noted for each fraction studied in the three autolysates. Nevertheless, in the proteic fraction, asparagine and aspartic acid proportions were lower in the autolysate obtained from active dried yeast than in the two other autolysates obtained from fermented yeast. The contrary was observed for glycine.

Nucleotide and Nucleoside Composition. Nucleotide and nucleoside data are reported in **Table 4**. For autolysate obtained from active dried yeast, neither nucleosides nor nucleotides were detected. Nucleotides were not found in autolysates obtained from fermented yeast, whereas nucleosides were present in low concentrations. This first indicates that RNA was rapidly degraded during autolysis and, second, that the physiological state of yeast influences this process more than temperature does. Indeed, the nucleoside contents in the two autolysates elaborated with fermented yeast were very similar: 12.5 mg/g of dried yeast for the autolysate kept at 18 °C and 10.1 mg/g of dried yeast for the one kept at 30 °C.

Table 4. Nucleotide and Nucleoside Content (Milligrams per Gram of Dried Yeasts) in the Fraction with a Molecular Mass <0.5 kDa of Autolysates

	autolysate		
	FP	OP	LP
Nucleosides			
cytidine	0.43	0.41	nd[a]
uridine	2.74	3.65	nd
guanisine	1.43	2.51	nd
adenosine	5.52	5.95	nd
Nucleotides			
cytidine monophosphate	nd	nd	nd
uridine monophosphate	nd	nd	nd
guanisine monophosphate	nd	nd	nd
adenosine monophosphate	nd	nd	nd

[a] nd, not detected.

Monosaccharide Composition. Amounts of monomeric sugars measured in the different fractions of the three autolysates are given in **Table 5**. For all autolysates, a major part of the mannose was found in the concentrated fraction >10 kDa, which probably corresponds to cell-wall polyosides (mannoproteins) liberated by *S. cerevisiae* during autolysis. Comparison of the monomeric sugar composition in this macromolecular fraction shows great differences among the autolysates. The highest levels of mannose and glucose were observed for the autolysates obtained from fermented yeasts (FP and OP). This could be explained by a slower degradation of the cell wall during autolysis with fermented yeast than with dried active yeast. The temperature during the autolytic process also has an important effect. The levels of monomeric glucose, depending on the presence of glucans, are significantly more important in the fractions >10 and 1−10 kDa for the autolysate kept at 18 °C (OP) than in the same fractions from autolysates kept at 30 °C

Table 5. Monosaccharide Composition in the Different Nitrogenous Fractions from Autolysates

fraction	mannose (mmol/L)	glucose (mmol/L)	galactose (mmol/L)	M/G[a]
		Autolysate FP		
<0.5 kDa	0.13 (0.01)[b]	0.98 (0.02)	0	0.1
0.5–1 kDa	0	0.06 (0.01)	0.03 (0.02)	
1–10 kDa	0.36 (0.01)	0.24 (0.01)	0.04 (0.03)	1.5
>10 kDa	5.17 (0.03)	0.58 (0.03)	0.04 (0.03)	8.9
		Autolysate OP		
<0.5 kDa	0.14 (0.01)	0.70 (0.02)	0.09 (0.01)	0.2
0.5–1 kDa	0	0.32 (0.01)	0.05 (0.03)	
1–10 kDa	0.41 (0.01)	2.21 (0.02)	0	0.2
>10 kDa	7.68 (0.07)	1.73 (0.01)	0	4.4
		Autolysate LP		
<0.5 kDa	0.06 (0.01)	0.07 (0.01)	0	0.9
0.5–1 kDa	0.02 (0.01)	0.06 (0.01)	0.02 (0.01)	0.3
1–10 kDa	0.21 (0.02)	0.11 (0.01)	0.06 (0.04)	1.9
>10 kDa	1.71 (0.02)	0.20 (0.01)	0.03 (0.02)	8.6

[a] Reported as mannose/glucose. [b] Numbers in parentheses correspond to standard deviation.

Table 6. Effect of Various Nitrogen Fractions (50 mg of N/L) on the Growth of *O. oeni* in Synthetic Medium

fraction	bacterial biomass	
	A_{600nm}	%[a]
	Autolysate FP	
<0.5 kDa	0.560 (0.025)[b]	62
0.5–1 kDa	nd[c]	
1–10 kDa	0.265 (0.005)	29
>10 kDa	0.085 (0.005)	9
	Autolysate OP	
<0.5 kDa	0.510 (0.010)	57
0.5–1 kDa	0.580 (0.030)	64
1–10 kDa	0.237 (0.013)	26
>10 kDa	0.157 (0.020)	17
	Autolysate LP	
<0.5 kDa	0.222 (0.007)	25
0.5–1 kDa	0.195 (0.010)	22
1–10 kDa	0.290 (0.005)	32
>10 kDa	0.140 (0.035)	16

[a] As percentage of the maximal biomass formed in a medium containing all nitrogen requirements. [b] Numbers in parentheses correspond to standard deviation. [c] nd, not determined.

(FP and LP). Yeast 1,3-β-D- and 1,6-β-D-glucanase activities are obviously lower at 18 °C than at 30 °C (*26*). The concerted action of all these autolysins therefore has an effect on the release of cell-wall glucans and D-glucose.

Influence of Nitrogen Fractions on Bacterial Growth of *O. oeni*. We tested each nitrogen fraction of the three autolysates separately at 50 mg of N/L on *O. oeni* in a synthetic medium. The results are illustrated in **Table 6**. As expected, no growth of *O. oeni* was observed in the control medium without nitrogen requirements. Although maximal growth was obtained in the basal medium with all nitrogen requirements (A_{600nm} reached 0.900 ± 0.050), each nitrogen fraction allowed growth of *O. oeni*. As expected, fractions containing components of a low molecular weight (<1 kDa) allowed higher bacterial development than fractions containing components of a high molecular weight (>1 kDa). The fact that free amino acids and peptide fractions of a molecular weight <1000 Da allow *O. oeni* to grow (*9*) is well-known. For fraction <0.5 kDa, levels of bacterial biomass appeared to be significantly lower in the autolysate elaborated from active dried yeast than in the autolysate elaborated from fermented yeast. This could be explained by the quasi-absence of glutamic acid and arginine in free amino acids and by the low concentration of isoleucine in peptides; *O.oeni* has an absolute requirement for this type of amino acid (*27*). Furthermore, our results show that this lactic acid bacteria is able to grow by metabolizing the nitrogenous macromolecular fraction (>10 kDa) and peptides (1−10 kDa) through the synthesis of proteases, which are normally expressed in starvation conditions (*28*); these would generate peptides and essential amino acids (29). For Leitao et al. (*30*), this proteolytic system appears to be closely related to the nutritional composition of the medium and seems to be strain dependent. Lactic acid bacteria have to grow in wines under quite unfavorable conditions, often in a medium greatly depleted of assimilable nitrogen compounds. The possible utilization by *O. oeni* of yeast proteins and peptides is therefore of great interest in winemaking.

At present, investigations are being carried to test the effect of autolysate fractions on wine and model wine flavors.

ACKNOWLEDGMENT

We thank D. V. Tomescu, B. Thibault, and J. Guerreau for assistance in autolysate fractionation and physicochemical analysis.

LITERATURE CITED

(1) Babayan, T. L.; Bezrukov, M. G. Autolysis in yeasts. *Acta Biotechnol*. **1985**, *2*, 129−136.
(2) Charpentier, C.; Feuillat, M. Yeast autolysis. In *Wine Microbiology and Biotechnology*; Fleet, G. H., Ed.; Harwood Academic Publishers: Chur, Switzerland, 1992; pp 225−242.
(3) Fornairon-Bonnefond, C.; Camarasa, C.; Moutonet, M.; Salmon, J.-M. New trends on yeast autolysis and wine aging on lees: a bibliographic review. *J. Int. Sci. Vigne Vin* **2001**, *35*, 57−78.
(4) Martinez-Rodriguez, A.; Polo, M. C. Characterization of the nitrogen compounds released during yeast autolysis in a model wine system. *J. Agric. Food Chem*. **2000**, *48*, 1081−1085.
(5) Feuillat, M.; Charpentier, C. Autolysis of yeasts in Champagne. *Am. J. Enol. Vitic*. **1982**, *33*, 6−13.
(6) Moreno-Arribas, V.; Pueyo, E.; Polo, C. Peptides in musts and wines. Changes during the manufacture of cavas (sparkling wines). *J. Agric. Food Chem*. **1996**, *44*, 3783−3788.
(7) Ari'izumi, K.; Susuki, Y.; Kato, I.; Yagi, Y.; Otsuka, K.; Sato, M. Winemaking from Koshu variety by the *sur lie* method: change in the content of nitrogen compounds. *Am. J. Enol. Vitic*. **1994**, *45*, 312−318.
(8) Leroy, M. J.; Charpentier, M.; Duteurtre, B.; Feuillat, M.; Charpentier, C. Yeast autolysis during Champagne aging. *Am. J. Enol. Vitic*. **1990**, *41*, 21−28.
(9) Divies, C.; Feuillat, M.; Cavin, J. F.; Guilloux-Benatier, M.; Guzzo, J. Bactéries lactiques en Œnologie. In *Œnologie: Fondements Scientifiques et Technologiques*; Flanzy, C., Ed.; Tec & Doc, Lavoisier Publisher: Paris, France, 1998; pp 498−533.
(10) Lurton, L.; Segain, J. P.; Feuillat, M. Etude de la protéolyse au cours de l'autolyse de levures en milieu acide. *Sci. Aliment*. **1989**, *9*, 111−124.
(11) Dupin, I. V. S.; McKinnon, B. M.; Ryan, C.; Boulay, M.; Markides, A. J.; Jones, G. P.; Williams, P. J.; Waters, E. J. *Saccharomyces cerevisiae* mannoproteins that protect wine from protein haze: their release during fermentation and lees contact and a proposal for their mechanism of action. *J. Agric. Food Chem*. **2000**, *48*, 3098−3105.
(12) Alexandre, H.; Heintz, D.; Chassagne, D.; Guilloux-Benatier, M.; Charpentier, M.; Feuillat, M. Protease A activity and nitrogen fractions released during alcoholic fermentation and autolysis in enological conditions. *J. Ind. Microbiol. Biotechnol*. **2001**, *26*, 235−240.

(13) Martinez-Rodriguez, A.; Carrascosa, A. V.; Polo, M. C. Release of nitrogen compounds to the extracellular medium by three strains of *Saccharomyces cerevisiae* during induced autolysis in a model wine system. *Int. J. Food Microbiol*. **2001**, *68*, 155−160.
(14) Guilloux-Benatier, M.; Le Fur, Y.; Feuillat, M. Influence of fatty acids on the growth of wine microorganisms *Saccharomyces cerevisiae* and *Oenococcus oeni*. *J. Ind. Microbiol. Biotechnol.* **1998**, *20*, 144−149.
(15) Fink, H.; Kuhles, R. Beiträge zur methylenblaufârbung der hefelzellmembran, II. Mitteilung eine verbesserte färbeflüssigkeit zur erkennung von toten hefezellen. *Hoppe Seiler's Z. Physiol. Chem.* **1993**, *218*, 65−66.
(16) Bruner, R. L. Determination of reducing value, 3,5-dinitrosalicylic acid method. *Chem. Anal.* **1964**, *19*, 67−71.
(17) Desportes, C.; Charpentier, M.; Duteurtre, B.; Maujean, A.; Duchiron, F. Liquid chromatographic fractionation of small peptides from wine. *J. Chromatogr. A* **2000**, *893*, 281−291.
(18) Zhao. J.; Fleet, G. H. Separation of twenty isomers of ribonucleotides and deoxyribonucleotides by reversed-phase ion pairing high-performance liquid chromatography. *J. Chromatogr. A* **1996**, *732*, 271−275.
(19) Aussenac, J.; Chassagne, D.; Claparols, C.; Charpentier, M.; Duteurtre, B.; Feuillat, M.; Charpentier, C. Purification method for the isolation of monophosphate nucleotides from Champagne wine and their identification by mass spectrometry. *J. Chromatogr. A* **2001**, *907*, 155−164.
(20) Albersteim, P.; Nevins, D. J.; English, P. D.; Karr, A. A method for the analysis of sugars in plant cell-wall polysaccharides by gas−liquid chromatography. *Carbohydr. Res*. **1967**, *5*, 340−345.
(21) Harris, P. J.; Henry, R. J.; Blakeney, A. B.; Stone, B. A. An improved procedure for the methylation analysis of oligosaccharides and polysaccharides. *Carbohydr. Res*. **1984**, *127*, 59−73.
(22) Acedo, M. L.; Pueyo, E.; Polo, M. C. Preliminary studies on peptides in wine by HPLC. *Am. J. Enol. Vitic*. **1994**, *45*, 167−172.
(23) Guitart A.; Orte, P. H.; Ferreira, V.; Pena, C.; Cacho, J. Some observations about the correlation between the amino acid content of musts and wines of the Chardonnay variety and their fermentation aromas. *Am. J. Enol. Vitic*. **1999**, *50*, 253−258.
(24) Carnevillier, V.; Schlich, P.; Guerreau, J.; Charpentier, C.; Feuillat, M. Characterization of the production regions of Chardonnay wines by analysis of free amino acids. *Vitis* **1999**, *38*, 37−42.
(25) Moreno-Arribas, V.; Pueyo, E.; Polo, C.; Martin-Alvarez, P. J. Changes in the amino acid composition of the different nitrogenous fractions during the aging of wine with yeasts. *J. Agric. Food Chem*. **1998**, *46*, 4042−4051.
(26) Guilloux-Benatier, M.; Puech, B.; Feuillat, M. Incidence d'une activité glucanasique exogène sur le devenir des macromolecules levuriennes du vin. *Vitis* **1999**, *38*, 133−137.
(27) Fourcassié, P.; Makaga-Kabinda-Massard, E.; Belarbi, A.; Maujean, A. Growth, D-glucose utilization and malolactic fermentartion by *Leuconostoc oenos* strains in 18 media deficient in one amino acid. *J. Appl. Bacteriol.* **1992**, *73*, 489−496.
(28) Rollan, G. C.; Farias, M. E.; Strasser de Saad, A. M.; Manca de Nadra, M. C. Exoprotease activity of *Leuconostoc oenos* in stress conditions. *J. Appl. Microbiol.* **1998**, *85*, 219−223.
(29) Manca de Nadra, M. C.; Farias, M. E.; Moreno-Arribas, M. V.; Pueyo, E.; Polo, M. C. Proteolytic activity of *Leuconostoc oenos*. Effect on proteins and polypeptides from white wine. *FEMS Microbiol. Lett.* **1997**, *150*, 135−139.
(30) Leitao, M. C.; Teixeira, H. C.; Barreto Crespo, M. T.; San Romao, M. V. Biogenic amines occurrence in wine. Amino acid decarboxylase and proteolytic activities expression by *Oenococcus oeni*. *J. Agric. Food Chem.* **2000**, *48*, 2780−2784.

Received for review February 1, 2002. Revised manuscript received October 28, 2002. Accepted October 28, 2002.

JF020135J