Characterization by Gas Chromatography/Mass Spectrometry of Sterols in *Saccharomyces cerevisiae* during Autolysis

Y. Le Fur,* G. Maume,‡ M. Feuillat,§ and B. F. Maume‡

Laboratoire de Biochimie Alimentaire, ENESAD, B.P. 1607, F-21036 Dijon Cedex, France

Yeast autolysis affects membrane stability and induces a release of vacuolar enzymes into the cell cytoplasm. Consecutively, it was important to study the evolution of sterol content in *Saccharomyces cerevisiae* for a fourteen day period of accelerated autolysis. Unesterified and esterified sterols were analyzed both in the biomass and in the autolysis medium. Ten sterols were identified by gas chromatography/mass spectrometry. A second group of six sterols was separated and partially characterized. Among the first group of 10 sterols, a dehydroergosterol was identified as ergosta-5,7,9(11),22-tetraen-3β-ol, not yet charaterized in *S. cerevisiae.* Yeast autolysis induced a decrease of esterified sterol content, especially first intermediates in the sequence of the ergosterol biosynthesis, as zymosterol. In contrast, the yeast autolysis resulted in the release of a low quantity of sterols into the medium. At the end of the fourteenth day of autolysis, 0.015% of the total sterol content of the initial biomass was found in the medium.

Keywords: *Yeast; autolysis; sterol; dehydroergosterol; gas chromatography/mass spectrometry; trimethylsilyl ether*

INTRODUCTION

Yeast autolysis can be defined as an enzymatic self-destruction by intracellular proteases and nucleases, which affects the cell membranes. Biochemical studies on yeast autolysis and ethanol tolerance have been carried out (Babayan et al., 1979; Feuillat and Charpentier, 1982; Alexandre et al., 1994; Sushma et al., 1994). Advances in the identification of the yeast lipid composition have shown that sterols, phospholipids, and triglycerides are the main compounds of the cell lipids content (Hunter and Rose, 1971; Sajbidor et al., 1994; Rencken et al., 1995; Murakami et al., 1996). The amount of total sterols in the yeasts represents 1−10% of total lipids and 0.03−4.6% of dry weight (Giovanelli et al., 1996). *Saccharomyces cerevisiae* yeasts are especially rich in sterols and were frequently used as biological material in the characterization of constitutive membrane sterols (Andreev et al., 1986; Nakanishi et al., 1987; Parks and Casey, 1995). More recently, the metabolic pathway was studied in yeasts in order to demonstrate the influences of antifungal agents or antibiotics on sterol biosynthesis (Sorres Hamdan and Casali, 1996; Sanati et al., 1997; Aubert et al., 1997; Kuchta et al., 1997). The method of combined gas chromatography/mass spectrometry (GC/MS) has permitted the identification of ergosterol and of several intermediates of its biosynthesis. Ergosterol, zymosterol, and lanosterol were found as main components (Behalova et al., 1994). Behalova et al. (1986) have already identified ergosta-5,7,22,24(28)-tetraen-β-ol. Yeast sterols were found unesterified or esterified by long-chain fatty acids. Studies on the biological role of sterols have demonstrated the influence of sterols on phospholipid−protein interaction, membrane fluidity, and membrane-bound enzyme activities (Nes et al., 1993). Sterols were also considered as architectural components in cellular membranes (Nes, 1987) essentially located in the plasma membrane (Hossack and Rose, 1976; Belrhiti et al., 1995). However, Hossack et al. (1977) and Zinser et al. (1993) have also reported the presence of sterols in lipid vesicles of the cell cytoplasm of *S. cerevisiae* and *Kluyveromyces fragilis*. In the present study, unesterified or esterified sterols from *S. cerevisiae* submitted to fourteen days of accelerated autolysis were separated and identified by GC/MS. A sterol which has not been described before in *S. cerevisiae* was identified as ergosta-5,7,9(11),22-tetraen-3β-ol. Quantitative variations of 10 individual yeast sterols present in the biomass during autolysis and released in the autolysis medium are described.

MATERIALS AND METHODS

Microorganisms. The yeast used in this study was a strain of *S. cerevisiae* utilized in winemaking; it was obtained in the form of active dried yeast (Uvaferme BC type, Damstar Ferment, Denmark). The yeast (625 g) was rehydrated in distilled water at 30 °C for 20 min by stirring in a 7 L Biolaffite incubator (Poissy, France). The rehydrated biomass was isolated by direct centrifugation at 10000*g* for 15 min.

Conditions of Accelerated Autolysis. Reactived yeast (1900 g) was suspended in 15 L of a medium containing

* To whom correspondence should be addressed. Tel: +33 3 80 77 27 92. Fax: +33 3 80 77 27 51. E-mail y.lefur@enesad.fr.

‡ Present address: Laboratoire de Biochimie des Interactions Cellulaires, Université de Bourgogne, BP 400, 21004 Dijon Cedex, France.

§ Present address: Laboratoire d'Oenologie, Institut Universitaire de la Vigne et du Vin, Université de Bourgogne, BP 138, 21004 Dijon Cedex, France.

10.1021/jf9806715 CCC: $18.00 © 1999 American Chemical Society
Published on Web 05/27/1999

ethanol (10%, v/v), tartaric acid (1 g/L), malic acid (0.1 g/L), K_2SO_4 (0.1 g/L), $MgSO_4$ (0.025 g/L), and sodium azide (0.1 g/L). Autolysis was carried out in a 20 L Biolaffite fermenter for 15 days. The temperature was kept at 40 °C and the pH at 3 in order to accelerate the yeast autolysis.

Sterol Extraction from the Supernatant and the Yeast Biomass. Autolysis homogenized samples were taken on the first, second, third, sixth, ninth, and fourteenth days of autolysis. The biomass was separated from the autolysis medium by centrifugation at 10000*g* for 15 min. After each sampling, the dry weight/moist weight ratio of the biomass was determined. The dry weight was determined after drying the washed yeasts in an oven at 100 °C for 48 h. A volume of supernatant (200 mL) was taken for sterol analysis. Lipids were extracted using 3 × 200 mL of chloroform/methanol (2:1, v/v). The organic phase was washed with 100 mL of distilled water and dried over anhydrous $MgSO_4$ at 5 °C for 12 h. The extract was filtered and vacuum evaporated to 4 mL. On the other hand, yeast cells were disrupted according to the experimental procedure previously described by Beaven et al. (1982). Lipids were extracted from the biomass using chloroform/methanol (2:1, v/v). The volume of the extract was 50 mL.

Derivatization and Gas Chromatography of Sterols. Part of the lipid extract (1 mL) was dried under a stream of dry nitrogen. Regarding the unesterified sterol analysis, the derivatization was directly carried out on the dried residue with BSTFA with 20% (v/v) of TMCS (100 μL) in a sand bath for 1 h at 65 °C. Regarding the analysis of total sterols, the lipid extract was saponified with 33% methanolic KOH (0.5 mL). The 5β-cholestan-3α-ol (epicoprostanol) (5 μg) was added before saponification in ethanol solution at 10 μg/mL (0.5 mL) as the first internal standard. Sterols were extracted with hexane (5 mL) containing 5a-cholestane (1 μg/mL). Trimethylsilyl (TMS) ethers were prepared from hexane extract (1 mL) by reaction with BSTFA/TMCS as previously described (Gambert et al., 1979; Cocito and Delphini, 1994). The Packard 427 (Delft, The Netherlands) gas chromatograph was equipped with a OV1 capillary column (25 m length, 0.32 mm id, 0.25 μm film thickness), a Ross injector, and a flame ionization detector. TMS derivatives were separated by programming the column temperature from 220 to 275 °C at 1 °C/min. The flow rate of the carrier gas (nitrogen) was 1 mL/min. The injector temperature was held at 240 °C and the detector at 290 °C. The methylene unit (MU) values of sterol were determined by simultaneous injection of C30 and C34 n-alcanes. Relative retention time (RRT) was also calculated using 5α-cholestane as the second internal standard.

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis. Briefly, the quadrupole mass spectrometer was a Ribermag R10−10C (Nermag, Rueil-Malmaison, France) coupled with a gas chromatograph equipped with a SE30 fused silica capillary column (30 m length, 0.32 mm id, 0.25 μm film thickness). Helium was used as the carrier gas. The column temperature was programmed at 2 °C/min from 220 °C. Ionization was obtained by electron impact: electron energy, 70 eV; and filament current, 0.19 mA. The source temperature was 250 °C.

RESULTS AND DISCUSSION

Yeast Sterol Identification by Gas Chromatography/Mass Spectrometry. Ten sterols of *S. cerevisiae* submitted to autolysis (compounds 1−10) were separated and identified as TMS ether by GC/MS (Figure 1). Relative retention times and MU values of each TMS ether are shown in Table 1. The values of their molecular ion, base peak, and abundant fragments of the mass spectra are given in Table 1. These data allowed the identification of the sterol structures by comparison to spectra of reference compounds or to data reported in the literature (Brooks et al., 1968; Djerassi, 1978; Andreev et al., 1986; Nakanishi et al., 1987). Ergosterol and some intermediates of its biosynthetic pathway were identified as indicated below.

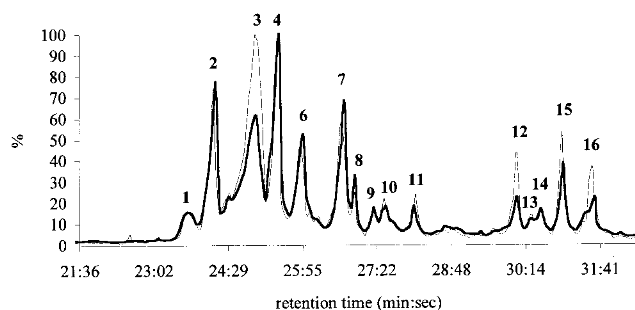

Figure 1. Gas chromatographic separation of the sterol fraction from *S. cerevisiae* after 3 days of autolysis. The chromatograms were obtained by coupled GC/MS. The chromatograph was equipped with a SE30 capillary column. The temperature was programmed at 2 °C/min from 220 to 275 °C. The first chromatogram is a total ionization current (bold line); the second is reconstructed gas chromatogram with the ion at *m/z* 129 characteristic of the sterol 5-ene-3-trimethylsilyloxy structure (thin line). The code of compounds 1−10 is given in Table 1, and compounds 11−16 are described in the text.

Compound 1 ($M^+ = 466$) is a new dehydroergosterol which had not been detected before in yeast cells during autolysis. The identification of this sterol is based on the study of its mass spectrum (Table 1). The main ion at *m/z* 251 (M-trimethylsilanol-side chain) is characteristic of a monounsaturated side chain of 125 amu. The mass spectrum of compound 1 is identical to that given previously for an authentic sample of ergosta-5,7,9(11),-22-tetraen-3β-ol as a TMS derivative. This sterol has been found at low concentrations in the ascomycete *Chaetomium cochliodes*. More recently, the same sterol has been identified in mycelia of *Coccidioides immitis* (Safe and Brewer, 1973; Heald et al., 1981). The presence of this sterol has been shown in the phycomycete *Mucor rouxii* where it was not considered to be an intermediate in the ergosterol biosynthesis but to be a dehydrogenated metabolite of ergosterol (Atherton et al., 1972). In this work the sterol was evidenced in the biomass of autolyzed *S. cerevisiae* yeasts. Furthermore recent experiments in this laboratory have also shown the presence of the sterol in *S. cerevisiae* during the period of exponential growth (results not shown).

Compounds 2 and 3 were identified as zymosterol and ergosterol, respectively, by comparison to GC and MS data of their TMS derivatives.

Compound 5 has a molecular ion at *m/z* 466 corresponding to the presence of four double bonds.This sterol can be identified as ergosta-5,7,22,24(28)-tetraen-3β-ol by the presence of two double bonds in the side chain (fragments at *m/z* 343 and 341 = M-side chain and M-side chain-2H, respectively) and by comparison to the already reported mass spectrum.

Compounds 4, 6, and 7 are isomeric sterols with a molecular ion at *m/z* 470 corresponding to the presence of two double bonds. Compounds 4 and compound 7 were identified as ergosta-7,22-dien-3β-ol and ergosta-7,24(28)-dien-3β-ol (episterol), respectively, by comparison to the data reported in the literature (Nakanishi et al., 1987; Steel and Henderson, 1972). Both were alreardy described in *S. cerevisiae* and in *Candida albicans* (Alais et al., 1979). Characteristic ions of compound 6 (Table 1) are at *m/z* 470 (M^+) and 343 (M-side chain). Among diunsaturated sterols reported in the literature, ergosta-8,22-dien-3β-ol (Goulston et al., 1975), ergosta-8,24(28)-dien-3β-ol (Nakanishi et al., 1987), and ergosta-

Table 1. Gas Chromatographic Characterization[a] and Mass Spectrometric Ions[b] of Sterols in *Saccharomyces cerevisiae* during Autolysis

			m/z			
code	RRT[c]	MU[d]	molecular ion (M^+)	base peak	principal ions in mass spectrum	identification
1	1, 76	31. 61 ± 0. 03[e]	466 (9)	251[w]	451[j] (3), 376[n] (41), 361[o] (8), 249[x] (18), 209[z] (12)	ergosta-5,7,9(11),22-tetraen-3β-ol[f]
2	1, 80	31.78 ± 0. 02	456 (100)	456	441[j] (71), 372[m] (10), 366[n] (16), 351[o] (50), 345[p] (13), 343[q] (23), 318[u] (9), 303[v] (8), 281[t] (6), 255[w] (15), 253[x] (14), 229[y] (32), 213[z] (47)	zymosterol[g]
3	1, 84	31. 91 ± 0. 03	468 (42)	363[o]	453[j] (5), 378[n] (32), 343[p] (29), 341[q] (7), 337[r] (100), 253[w] (48), 251[x] (18), 227[y] (10), 211[z] (33), 144 (47), 131 (72)	ergosterol[f,i]
4	1, 87	32. 03 ± 0. 02	470 (16)	255[w]	455[j] (13), 427[k] (6), 380[n] (8), 365[o] (13), 345[p] (31), 343[q] (98), 329[aa] (16), 318[u] (19), 303[v] (5), 253[x] (24), 229[y] (33), 213[z] (15)	ergosta-7,22-dien-3β-ol[f,i]
5	1, 90	32. 19 ± 0. 01	466 (100)	466	451[j] (8), 423[k] (3), 376[n] (9), 361[o] (5), 343[p] (11), 341[q] (19), 325[aa] (5), 253[w] (7), 251[x] (21), 245[s] (7), 211[z] (18), 147 (51)	ergosta-5,7,2224(28)tetraen-3β-ol[f]
6	1, 95	32. 40 ± 0. 04	470 (8)	343[q]	455[j] (7), 380[n] (2), 365[o] (6), 255[w] (6), 253[x] (6)	unidentified sterol[g]
7	2, 03	32. 72 ± 0. 03	470 (16)	343[q]	455[j] (34), 386[l] (71), 380[n] (9), 365[o] (21), 329[aa] (8), 318[u] (6), 303[v] (8), 281[t] (13), 255[w] (41), 253[x] (30), 229[y] (14), 213[z] (27)	episterol[f,i]
8	2, 05	32. 80 ± 0. 03	472 (57)	255[w]	457[j] (16), 382[n] (6), 367[o] (14), 345[p] (12), 343[q] (14), 303[v] (6), 229[y] (17), 213[z] (21)	ergosta-7-en-3β-ol[f,i]
9	2, 11	33. 05 ± 0. 01	470 (35)	339[r]	455[j] (27), 365[o] (31), 343[p] (8), 341[q] (36), 316[u] (16), 301[v] (15), 253[w] (20), 251[x] (52), 249[s] (67), 211[z] (18)	ergosta-5,7-dien-3β-ol[h]
10	2, 17	33. 27 ± 0. 02	498 (16)	393[o]	483[j] (15), 408[n] (3), 376 (48), 323[t] (5), 297[w] (5), 255[z] (11), 241 (22), 227 (12), 135 (26), 109 (68)	lanosterol[g]

[a] The compounds were analyzed as trimethylsilyl ethers on a 25 m × 0.32 mm OV1 capillary column, in a temperature programmed mode from 220 to 275 °C at 1 °C/min. [b] The compounds were analyzed by GC/MS with a SE30 capillary column programmed at 2 °C/min from 220 to 275 °C and a mass spectral source used in the electron impact mode at 70 eV. The number in parentheses was intensities in percent relative to the base peak. [c] Relative retention time toward 5α-cholestane (retention time: 9 min 55 s; RRT = 1) as the second internal standard determined on OV1 capillary column (see footnote a). [d] Methylen unit value. [e] Standard deviation (±); $n = 4$ (the procedure was repeated four times from the beginning). [f] In both the free and the esterified forms. [g] Exclusively in the esterified form. [h] Exclusively in the free form. [i] Sterol present in the biomass and in the autolysis medium; other sterols are only present in the biomass. [j] $[M-15]^+$. [k] $[M-43]^+$: $[M-C_{(25)}$ to $C_{(27)}]^+$. [l] $[M-84]^+$: $[M-C_{(23)}$ to $C_{(28)}-H]^+$. [m] $[M-84]^+$: $[M-C_{(23)}$ to $C_{(27)}-15]^+$. [n] $[M-90]^+$: $[M-(CH_3)_3-SiOH)]^+$. [o] $[M-90-15]^+$. [p] $[M\text{-side chain}]^+$. [q] $[M\text{-side chain-2H}]^+$. [r] $[M-131]^+$. [s] $[M-90-131]^+$. [t] $[M-90-15-C_{(23)}$ to $C_{(27)}-H]+$ or $[M-90-15-C_{(23)}$ to $C_{(28)}-H]^+$. [u] $[M\text{-side chain-}C_{(16)}$ to $C_{(17)})-H]^+$. [v] $[M\text{-side chain-42}]+$: $M\text{-side chain-}C_{(15)}$ to $C_{(17)})]^+$. [w] $[M\text{-side chain-90}]^+$. [x] $[M\text{-side chain-90}-2H]^+$. [y] $[M\text{-side chain-90}-26]^+$: $[M\text{-side chain-90-}C_{(16)}$ to $C_{(17)})]^+$. [aa] $[M-141]^+$: $[M-90-C_{(1)}$ to $C_{(4)})]^+$.

8,23-dien-3β-ol (Parks, 1978) have mass spectra not exactly identical to that of compound 6.

Compound 8 is a monounsaturated sterol with a typical spectrum of sterol containing a double bond at C7 with a preeminent M-side chain-90. It was identified as ergost-7-en-3β-ol.

The last sterol with a molecular ion at *m/z* 470 (compound 9) presents a spectrum very characteristic of 5,7-dien-sterol; the most abundant ion occurs at *m/z* ($M-131^+$), with a complementary fragment at *m/z* 131. This sterol presents a saturated side chain. It was identified as ergosta-5,7-dien-3β-ol. Finally, compound 10 was identified as lanotesterol, the first cyclic intermediate in the biosynthesis of ergosterol by yeasts.

At least six other compounds (11−16) have been separated by GC and characterized by GC/MS (Figure 1). All of these compounds showed a *m/z* 129 ion in their spectra, suggesting a 5-ene-3-hydroxy sterol structure as TMS ether. The GC/MS data determined for compound 11 were MU = 33.51 ± 0.03; RRT = 2.23; molecular ion at *m/z* 484; for compound 12 M^+ at *m/z* 482; for compound 13 MU = 34.30 ± 0.02; RRT = 2.43 molecular ion at *m/z* 482; for compound 14 M^+ at *m/z* 482; for compound 15 M^+ at *m/z* 500; and compound 16 M^+ at *m/z* 482. Structural studies are under investigation for precise identification of these compounds.

From the qualitative viewpoint, the composition of yeast sterols did not change much during autolysis. Compound 5 (ergosta-5,7,22,24(28)-tetraen-3β-ol) was found in the biomass and in the supernatant on the first day of autolysis only. Except for compound 5 and compound 10 (lanosterol), which disappeared in the biomass on the second and the fourteenth days, respectively, the sterols remained in yeasts during autolysis. In agreement with the works of Hunter and Rose (1971) and Hossack and Rose (1976), most sterols in autolyzed yeasts were in both the esterified and the unesterified forms. Among the first intermediates in the sequence of the ergosterol biosynthetic pathway, zymosterol (compound 2) and lanosterol (compound 10) as well as compound 6, an unidentified diunsatured ergosterol, were exclusively in the esterified form. On the contrary, ergosta-5,7-dien-3β-ol (compound 9) was found exclusively in the unesterified form.

Quantitative Analysis of Sterols in the Biomass during Autolysis. The unesterified sterol content of dried yeast used in our experiments varied between 1.4 and 1.8 mg per gram of dry weight during the fourteen day period of autolysis (Table 2). Conversely, the percentage of unesterified sterols to total isolated sterols increases from 20% on the first day to 39% on the fourteenth day. The total sterol content in the biomass decreases from 9.2 mg per gram of dry weight to 4.3 mg per gram of dry weight between the first and the fourteenth day of autolysis. This result shows that the loss of total sterol content in the biomass during autolysis was due to a decrease in esterified sterols, but the ratio between the esterified sterols and unesterified sterols (80/20) was not changed much until the last day (14th day) of autolysis (60/40). This phenomenon mainly occurred after 6 days of autolysis, and the effect of autolysis on the sterol content was stronger at the end

Table 2. Variation of Sterol Amount in the Biomass during Autolysis in Milligrams per Gram of Yeast Dry Weight[a]

	time of autolysis (days)				
	1	3	6	9	14
unesterified sterols	1.8 (20)[b] ± 0.1	1.6 (22) ± 0.2	1.7 (22) ± 0.1	1.4 (22) ± 0.1	1.7 (39) ± 0.3
esterified sterols[c]	7.4 (80) ± 0.2	5.8 (78) ± 0.2	6.2 (78) ± 0.1	5.1 (78) ± 0.2	2.6 (61) ± 0.2
total sterols	9.2 ± 0.3	7.4 ± 0.4	7.9 ± 0.2	6.5 ± 0.3	4.3 ± 0.5

[a] Moist weight of yeast samples was about 1.5 g. The mean value of the measured dry weight/moist weight ratios was 0.27 ± 0.01. [b] Values in parentheses correspond to the distribution between unesterified and esterified sterols in percent. [c] Values obtained by the difference between total sterols and unesterified sterols.

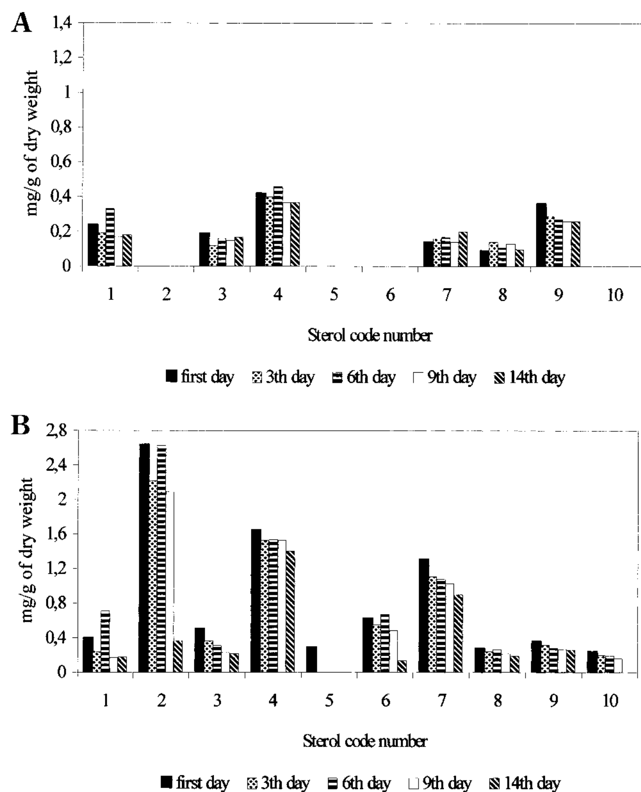

Figure 2. Evolution of individual sterol content during autolysis (milligram per gram of dried yeast) in form of both unesterified sterols (a) and total sterols (b). The code of compounds 1–10 is given in Table 1. The compounds 11–16 are described in the text.

of the period. This was obvious for sterols present in the esterified form only (Figure 2); the concentration of esterified zymosterol (compound 2) decreases rapidly between the sixth day and the fourteenth day of autolysis, from 0.3% dry weight to 0.04% dry weight. The concentration of the unidentified diunsaturated sterol (compound 6) decreases from 0.07% to 0.015% dry weight. Furthermore, the esterified part of ergosterol (compound 3) decreased. The unesterified fraction of ergosterol which represented around 37% of total ergosterol did not change. The ergosta-5,7-dien-3β-ol (compound 9) present eclusively in the unesterified form was kept constant in the biomass during autolysis (Figure 2), and this sterol was not released in the autolysis medium. The evolution of the unesterified and esterified yeast sterol during autolysis can be explained by their localization in the cell and therefore by their

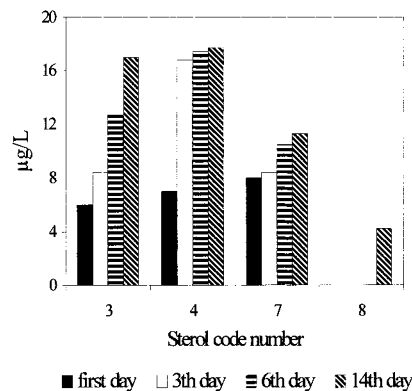

Figure 3. Individual sterols released in the medium during autolysis. Results are expressed in micrograms per liter. The code of compounds 3, 4, 7, and 8 is given in Table 1.

more or less strong interactions with other cellular components such as phospholipids and protein. Hossack et al. (1977) have suggested that esterified sterols may be preferentially concentrated in intracellular membranes rather than in plasma membranes. The loss in esterified sterol is consistent with the phenomenon of enzymatic alteration during autolysis as demonstrated by Babayan et al. (1979).

Quantitative Analysis of Relased Sterols in the Autolysis Medium. The presence of sterols in the supernatant during autolysis showed the release of yeast sterols into the hydro alcoholic medium which occurred in two stages. The first release occurred during the rehydration of active dried yeasts. This sterol transfer into the medium was swift and large and was observed simultaneously with a loss of viability of 30% of the cell population. Total sterol concentration in the redyration medium was 225 μg/L, which represents 0.14% of the initial total sterol content of the biomass. Sterols released during rehydration were the following: episterol (24% of the initial cell content of this sterol), zymosterol (14%), and the unidentified diunsaturated sterol (32%). Those present in low concentration in the rehydration medium include, among others, ergosta-7,-22-dien-3β-ol and ergosta-7-en-3β-ol. The second stage of release was consecutive to the autolysis (Figure 3) with a sterol concentration in the medium increasing progressively. The released sterols were the following: ergosterol, ergosta-7-en-3 β-ol, epsisterol, and ergosta-7,22-dien-3β-ol mainly in the esterified form (63%, 68%, 89%, and 75% of total sterols, respectively). This release of esterified sterols was quite low: 58 μg/L on the fourteenth day of autolysis. This value corresponded to a 0.015% release of the initial sterol content of the biomass.

The influence of the sterol forms in the yeasts is still not well-understood. It is not especially known whether the flatter shape of newly identified ergosta-5,7,9(11),-22-tetraen-3β-ol has an effect on the membrane fluidity during autolysis. Furthermore, it should be of interest to find out if one or some of the studied sterols have regulatory functions in the cells, as was previously suggested for esrgosteol (Hossack et al., 1977). Finally, as the yeast sterols were shown to be released in the medium during autolysis, their interaction with the microorganisms involved in the evolution of wine (lactic acid bacteria) may be an area for future investigations.

ABBREVIATIONS USED

amu, apparent mass unit; GC/MS, gas chromatography/mass spectrometry; MU, methylene unit; id, internal diameter; RRT, relative retention time; TMS, trimethylsilyl; zymosterol, cholesta-5,24-dien-3b-ol; episterol, ergosta-7,24(28)-dien-3b-ol; ergosterol, ergosta-5,7,22-trien-3b-ol; lanosterol, lanosta-8,24-dien-3b-ol.

ACKNOWLEDGMENT

This study was supported by the Direction de la Recherche du Ministère de l'Education Nationale and the Regional Cuncil of Bourgundy for the mass spectrometry instrumentation.

LITERATURE CITED

Alais, J.; Lablache-Combier, A.; Andrieu, S. Sterols de *Candida albicans. Bull. Soc. Fr. Mycol. Med.* **1979**, *8*, 11–16.

Alexandre, H.; Rousseaux I.; Charpentier C. Ethanol adaptation mechanisms in *Saccharomyces cerevisiae. Biotechnol. Appl. Biochem.* **1994**, *20*, 173–183.

Andreev, A. V.; Moseichuk, A. V.; V'yunov, K. A.; Mikhailova, N. P. Identification of the sterols of the yeasts *Saccharomyces cerevisiae* and *Candida guilliermondii* by the massspectometric method. *Khim. Prir. Soedin.* **1986**, *2*, 196–201.

Atherton, L.; Duncan, J. M.; Safe, S. Isolation and biosynthesis of ergosta-5,7,9(11),22-tetraen-3b-ol from *Mucor rouxii. J. Chem. Soc., Chem. Commum.* **1972**, 882–883.

Aubert, C.; Baumes, R.; Gunata, Z.; Lepoutre, J. P.; Cooper, J. F.; Bayonove, C. Effects of flusilazole, a sterol biosynthesis inhibitor fungicide, on the free and bound aroma fraction of Muscat of Alexandria wines. *J. Int. Sci. Vigne Vin* **1997**, *31*, 57–64.

Babayan, T. L.; Bezrukov, M. G.; Latov, V. K.; Belikov, V. M. Activity of amino acids as a function of their hydrophobicity in the process of the autolysis of the *Saccharomyces cerevisiae* yeast. *Curr. Microbiol.* **1979**, *2*, 301–304.

Beaven, M. J.; Charpentier, C.; Rose, A. H. Production and tolerance of ethanol in relation to phospholipid fatty-acyl composition in *Saccharomyces cerevisiae* NCYC 431. *J. Gen. Microbiol.* **1982**, *128*, 1447–1455.

Behalova, B.; Novotny, C.; Zajicek, J.; Paskova, J.; Latinak, J.; Kozeny, M. Level and composition of sterols of various strains of *Saccharomyces cerevisiae*. *Kvasny Prum.* **1986**, *32*, 194–196.

Behalova, B.; Blahova, M.; Behal, V. Regulation of sterol biosynthesis in *Saccharomyces cerevisiae*. *Folia Microbiol.* **1994**, *39*, 287–290.

Belrhiti, A.; Naji, B.; Bonaly, R. Membrane lipid and sterol distribution in *Saccharomyces bayanus. Process Biochem.* **1995**, *30*, 427–433.

Brooks, C. J. W.; Horning, E. C.; Young, J. S. Characterization of sterols by gas chromatography–mass spectrometry of the trimethylsilyl ethers. *Lipids* **1968**, *3*, 391–402.

Cocito, C.; Delphini, C. Simultaneous determination by GC of free and combined fatty acids and sterols in grape musts and yeasts as silanized compounds. *Food Chem.* **1994**, *50*, 297–305.

Djerassi, C. Recent advances in the mass spectrometry of steroids. *Pure Appl. Chem.* **1978**, *50*, 171–184.

Feuillat, M.; Charpentier, C. Autolysis of yeasts in Champagne. *Am. J. Enol. Vitic.* **1982**, *33*, 6–13

Gambert P.; Lallemant, C.; Archambault, A.; Maume B. F.; Padieu, P. Assesment of serum cholesterol by two methods: Gas–liquid chromatography on a capillary column and chemical ionization-mass fragmentography with isotopic dilution of [3, 4^{13}-C] cholesterol as internal standard. *J. Chromatogr.* **1979**, *162*, 1–6.

Giovanelli, G.; Peri, C.; Parravicini, E. Kinetics of grape juice fermentation under aerobic and anaerobic conditions. *Am. J. Enol. Vitic.* **1996**, *47*, 429–434.

Goulston, G.; Mercer, E. I.; Goad, L. J. The identification of 24-methylene-24,25-dihydrolanosterol and other possible ergosterol precursors in *Phycomyces blakesleeanus* and *Agaricus campestris*. *Phytochemistry* **1975**, *14*, 457–462.

Heald, S. L.; Jeffs, P. W.; Wheat, R. W. The identification of ergosterol and $D^{9(11)}$-dehydroergosterol from mycelia of *Coccidioides immitis* by reverse-phase high-performance liquid and gas chromatography and ultraviolet and mass spectrometry. *Exp. Mycol.* **1981**, *5*, 162–166.

Hossack, J. A.; Rose, A. H. Fragility of plasma membrane in *Saccharomyces cerevisiae* enriched with different sterols. *J. Bacteriol.* **1976**, *127*, 67–75.

Hossack, J. A.; Belk, D. M.; Rose, A. H. Environmentally induced changes in the neutral lipids and intracellular vesicles of *Saccharomyces cerevisiae* and *Kluyveromyces fragilis*. *Arch. Microbiol.* **1977**, *114*, 137–142.

Hunter, K.; Rose, A. H. Yeast lipids and membranes. In *The Yeasts*; Rose, A. H., Harrison, J. S., Eds.; Academic Press Inc.: New York, 1971; pp 211–231.

Kuchta, T.; Leka, C.; Kubinec, R.; Russell, N. J. Inhibitor of ergosterol biosynthesis is not accompanied by a change in fatty acid composition in *Saccharomyces cerevisiae* treated with an antifungal agent 6-amino-2-*n*-pentylthiobenzothiazole. *FEMS Microbiol. Lett.* **1997**, *150*, 43–47.

Murakami, Y.; Yokoigawa, K.; Kawai, F.; Kawai, H. Lipid composition of commercial bakers' yeasts having different freeze-tolerance in frozen dough. *Biosci. Biotechnol. Biochem.* **1996**, *60*, 1874–1876.

Nakanishi, S.; Nishino, T.; Nagai, J.; Katsuki, H. Characterization of nystatin-resistant mutants of *Saccharomyces cerevisiae* and preparation of sterol intermediates using the mutants. *J. Biochem.* **1987**, *101*, 535–544.

Nes, W. R. Structure–function relationships for sterols in *Saccharomyces cerevisiae*. *ACS Symp. Ser.* **1987**, *325*, 252–267.

Nes, W. D.; Janssen, G. G.; Crumley, F. G.; Kalinowska, M.; Akihisa, T. The structural requirements of sterols for membrane function in *Saccharomyces cerevisiae. Arch. Biochem. Biophys.* **1993**, *300*, 724–733.

Parks, L. W. Metabolism of sterols in yeast. *Crit. Rev. Microbiol.* **1978**, *6*, 301–342.

Parks, L. W.; Casey, W. M. Physiological implications of sterol biosynthesis in yeast. *Am. Rev. Microbiol.* **1995**, *49*, 95–116.

Rencken, I.; Fleming, V.; Meijering, I.; Axcell, B. Determination of selected sterols and fatty acids in yeast. *J. Chromatogr. Sci.* **1995**, *33*, 525–530.

Safe, S.; Brewer, D. Lipid composition of *Chaetomium cochliodes*: Effect of media. *Lipids* **1973**, *8*, 311–314.

Sajbidor, J.; Certik, M.; Grego, J.; Krupcik J. Lipid analysis of baker's yeast. *J. Chromatogr.* **1994**, *665*, 191–195.

Sanati, H.; Belanger, P.; Fratti, R.; Ghannoum, M. A new triazole, voriconazole (UK-109, 496), blocks sterol biosynthesis in *Candida albicans* and *Candida krusei. Antimicrob. Agents Chemother.* **1997**, *41*, 2492–2496.

Sorres Hamdan, J.; Casali, A. K. Effect of amphotericin B on the lipids of yeast cells of *Sporothrix schenckii. Mycopathologia* **1996**, *136*, 125–131.

Steel, G.; Henderson, W. Rapid method for detection and characterization of steroids. *Anal. Chem.* **1972**, *44*, 1302–1304.

Sushma, G.; Sukesh, C.; Balwant, S. Changes in the composition and peroxidation of yeast membrane lipids during ethanol stress. *Acta Microbiol. Immunol. Hung.* **1994**, *41*, 197–204.

Zinser, E.; Paltauf, F.; Daum, G. Sterol composition of yeast organelle membranes and subcellular distribution of enzymes involved in sterol metabolism. *J. Bacteriol.* **1993**, *175*, 2853–2858.

Received for review June 22, 1998. Revised manuscript received March 31, 1999. Accepted April 5, 1999.

JF9806715