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Lipid Analysis of the Plasma Membrane and Mitochondria of Brewer's Yeast

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ABSTRACT. The plasma membrane and mitochondria of bottom fermenting brewer's yeast obtained as a by-product of industrial beer production were isolated and the lipid fraction was analyzed. The phospholipid content accounted for 78 mg/g protein in the plasma membrane and 59 mg/g protein in the mitochondria. Major phospholipids in both preparations were phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine but their proportions differed significantly. In the plasma membrane phosphatidylinositol, and in the mitochondria phosphatidylcholine were present in the highest concentration (37 and 30 %, respectively). The main classes of neutral lipids (triacylglycerols, ergosterol, squalene and steryl esters) were twice more abundant in the plasma membrane than in the mitochondria (61 and 33 mg/g protein, respectively). A characteristic of the neutral lipid composition of both organelles was the low content of ergosterol (12 and 7 mg/g protein, respectively) and a high content of squalene (25 and 22 mg/g protein). The main feature of the fatty acid composition of both organelles was the preponderance of saturated fatty acids (78 and 79 %, respectively), among which palmitic acid was the principal one. The most expressed characteristics of lipid fractions of the analyzed plasma membranes and mitochondria, high concentration of squalene and preponderance of saturated fatty acids are the consequences of anaerobic growth conditions. The lack of oxygen had possibly the strongest effect on the lipid composition of the plasma membranes and mitochondria of bottom fermenting brewer's yeast.

Abbreviations

BSA	bovine serum albumin	CL	cardiolipin
DMPtdEtn	dimethylphosphatidylethanolamine	FA	fatty acid(s)
Lyso-PL	lysophospholipids	MT	mitochondria
PA	phosphatidic acid	PL	phospholipid(s)
PM	plasma membrane(s)	PtdCho	phosphatidylcholine
PtdEtn	phosphatidylethanolamine	PtdIns	phosphatidylinositol
PtdSer	phosphatidylserine	SDS-PAGE	sodium dodecylsulfate polyacrylamide gel electrophoresis
TFA	total FA		
TPL	total PL	VLCFA	very long-chain FA

Lipids are the essential structural components of eukaryotic cell membranes, which regulate their permeability and fluidity. Besides, it has been shown that specific membrane lipids are involved in cell signaling and regulation of different metabolic processes. The PM separates internal organelles and intracellular components from the external medium. As the site of primary contact of the cell with the environment, it has an important role in maintaining the viability and functionality of the cell, which is largely determined by its lipid composition (van den Rest *et al*. 1995; Šajbidor 1997; Löffler *et al*. 2000; Krasowska *et al*. 2002; Flegelová *et al*. 2003). The MT separate and fulfil different cell processes, one of the most important being oxidative degradation of nutrients for the production of energy. Since the cell growth and normal functioning depend on the generation of metabolic energy, MT are paid increased attention (Capaldi 2000).

Knowledge about the structure and function of the yeast membranes is important for the manipulation of metabolic processes inside the cell. Therefore, data on their lipid composition are important from the viewpoint of application, among others in brewing industry (O'Connor-Cox *et al*. 1996; Mizoguchi 1998; Hammond 2000). Lipid composition is rigidly maintained during exponential growth under favorable conditions; however, it changes markedly in response to extracellular changes (availability of exogenous precursors, nutrient deprivation, ethanol exposure, osmotic stress, oxygen depletion, high or low tempera-

ture *etc*.) (Mishra and Prasad 1988; Casey *et al*. 1993; Šajbidor *et al*. 1995; Murakami *et al*. 1996; Šajbidor 1997; Rupčić *et al*. 1998; Tuller *et al*. 1999; Heipieper *et al*. 2000).

There are numerous data on the lipids of the aerobic baker's yeast, *Saccharomyces cerevisiae* and its organelles since this species serves as an experimental model organism to study biochemical, cell biological and molecular biological aspects of the synthesis and transport of cell components, including lipids (Daum and Vance 1997; Schneiter and Kohlwein 1997; Achleitner *et al*. 1999; Daum *et al*. 1999; Janssen *et al*. 1999; Marx *et al*. 1999; Grant *et al*. 2001; Pichler *et al*. 2001; Athenstaedt and Daum 2002; Sorger and Daum 2003). In general, PtdCho, PtdEtn and PtdIns are considered as bulk PL of aerobically grown *S. cerevisiae*, while some PL classes are characteristic of certain organelles, such as PtdSer of the PM and CL of the MT (Paton and Lester 1991; Paltauf *et al*. 1992; Tuller *et al*. 1999). Ergosterol is the main and essential class of neutral lipids present in the membranous systems of *S. cerevisiae.* As in other yeasts, it is highly enriched in the PM (Zinser *et al*. 1993) while acylglycerols and steryl esters, representing reserve lipid molecules, are mainly located in the lipid particles of cells. Squalene and other ergosterol precursors may be also present in very small amounts in the PM and MT. Considering the FA composition of *S. cerevisiae*, the major FA are palmitic (16:0), palmitoleic ($16:1\Delta^9$), stearic (18:0) and oleic ($18:1\Delta^9$) acids. Since the only fatty acyl desaturase in *Saccharomyces* spp. is Δ-9 desaturase (acyl-CoA 9-desaturase; EC 1.14.19.1), the yeast does not contain oligo-unsaturated FA unless they are added exogenously (Paltauf *et al*. 1992).

In contrast, data on the lipid composition of anaerobically grown brewer's yeast *S. cerevisiae* and its organelles are rare. In this paper we present the results of a lipid analysis of the PM and MT of bottom-fermenting brewer's yeast *S. cerevisiae* (formerly *S. uvarum*). The composition of their neutral lipids, PL and TFA was determined. The results represent the first comparative analysis of the lipid composition of the organelles isolated from industrial yeast obtained as a by-product of anaerobic beer production. They could be useful for yeast recycling in beer fermentation or for the use of surplus yeast for different purposes, *viz*. food additive, pharmaceuticals, cosmetics, feed-stuff.

MATERIAL AND METHODS

Yeast strain. The strain of bottom-fermenting brewer's yeast *Saccharomyces uvarum* 21 from the collection of microorganisms of *Faculty of Food Technology and Biotechnology* in Zagreb was used. It belongs to the species *S. cerevisiae* (formerly *S. uvarum*). It was obtained as a by-product of beer fermentation in *Zagreb Brewery*, Zagreb (Croatia). The yeast cells were grown anaerobically and harvested in the stationary phase.

Isolation of organelles. Prior to any experiment, the biomass was washed 3× and centrifuged (3000 ***g***, 5 min). Crude PM were isolated after mechanical disruption of the cell wall by glass beads (0.3–0.5 mm) in a cell homogenizer. Highly purified PM were prepared using a combined method of differential and density gradient centrifugation (Zinser and Daum 1995). The MT were isolated by differential centrifugation after enzymic disruption of the cell wall using Zymolyase® (Zinser and Daum 1995)

Characterization of yeast organelles. The quality of the isolated organelles was tested routinely by SDS-PAGE. Relative enrichment of specific markers, namely ATPase for the PM and porin for the MT, was determined by immunoblotting. The protein content was determined by the Lowry method using BSA as standard. Proteins were precipitated with CCl_3COOH (10 % final concentration) and solubilized in 0.2 % SDS–0.1 mol/L NaOH prior to the analysis. SDS-PAGE was carried out by the method of Laemmli (1970). The samples were dissociated at 37 °C. Western blot analysis was carried out after the separation of proteins on SDS–10 % polyacrylamide gels and transfer to nitrocellulose sheets (Hybond-C; *Amersham*) by standard procedures (Haid and Suissa 1983). Proteins were detected by the enzyme-linked immunosorbent assay method with rabbit antibodies against the respective antigens and peroxidase-conjugated goat anti-rabbit secondary antibodies. Antibodies against porin, protein of the outer mitochondrial membrane, raised in rabbits were a gift of G. Daum, Graz (Austria).

Lipids were extracted from the isolated PM and MT according to Folch *et al*. (1957).

Analysis of phospholipids. TPL were quantified by the method of Broekhuyse (1968). Individual PL classes were separated by 2D-TLC on silica gel 60 plates (200 × 200 × 0.2 mm). $CHCl_3$–MeOH–NH_4OH (13 : 7 : 1, *V*/*V*/*V*) was used as the first solvent system and $CHCl_3$–Me_2CO–MeOH–CH_3COOH–H_2O (10 : 4 : 2 : 2 : 1, *V*/*V*/*V*/*V*/*V*) as the second one. PL were visualized by iodine staining, scraped off the plate and quantified in the same way as TPL.

Analysis of neutral lipids. Neutral lipids were separated by 2-step TLC on silica gel 60 plates (200 × 100 × 0.2 mm). Lipid extracts and standards were applied by a sample applicator (Linomat IV; *Camag*, Switzerland). The plates were developed by using light petroleum–diethyl ether–acetic acid (20 : 20 : 0.8,

V/*V*/*V*) up to ⅓ of a plate as the first, and light petroleum–diethyl ether (39.2 : 0.8, *V*/*V*) up to ⅔ of plate as the second solvent system. Ergosterol and ergosteryl esters were quantified by direct densitometry at 275 nm using ergosterol as standard. For the determination of triacylglycerols and squalene, the bands were visualized by post-chromatographic derivatization. The plates were dipped with a chromatogram immersion device (*Camag*) into the developing reagent (0.63 g $MnCl_2 \cdot 4H_2O$, 60 mL H_2O, 60 mL methanol, 4 mL H_2SO_4) for 4 s, briefly dried and heated for ½ h at 100 °C. The lipid components were quantified by scanning at 400 nm.

Analysis of fatty acids. The FA composition of the PM and MT was determined by gas chromatography of the corresponding methyl esters; they were obtained by acid methanolysis of lipid extracts with BF_3/MeOH (Vorbeck *et al*. 1961). *Hewlett-Packard* 5890 capillary gas chromatograph with flame ionization detector was used; column HP5 (5 % diphenyl-, 95 % dimethylpolysiloxane), programmed temperature 200–280 °C at 10 K/min. FA were identified by comparison with commercial FA methyl-ester standards (*NuCheck Inc*., USA).

RESULTS AND DISCUSSION

Prior to the lipid analysis, an analysis of the quality of the isolated organelles was done. The results obtained by Western blotting, and expressed as relative enrichment of the specific markers over the corresponding homogenate, were 5.5 for the PM-ATPase and 4.1 for the mitochondrial porin. The relative enrichment factor of porin was in good correlation with the data of Zinser *et al*. (1991, 1993) and Gaigg *et al*. (1995); those of the PM-ATPase differed significantly (Zinser *et al*. 1993; Zinser and Daum 1995).

There are several possible reasons for the low enrichment factor of the PM-ATPase. One of them is that the yeast cells analyzed here were harvested in the stationary phase while the published data refer to laboratory grown yeasts harvested in the exponential growth phase. PM-H^+-ATPase can account for almost 50 % of the PM protein content but only in the exponentially growing cells while it accounts for ≈25 % of that in cells in the stationary phase (Zinser and Daum 1995; van den Rest *et al*. 1995). Besides, ethanol, similarly to heat stress, causes changes in the PM protein composition, reducing the levels of PM-H^+-ATPase protein and inducing the PM-associated Hsp30 (Piper 1995). The negative result of immunodetection of mitochondrial porin in the PM proved that they were not cross-contaminated with the MT.

The PL composition of the PM and MT is presented in Tables I and II. The PL content expressed *vs*. protein was significantly higher in the PM than in the MT (78 and 59 μg/mg, respectively). The major PL in both organelles were PtdCho, PtdEtn and PtdIns; however, their relative proportions differed significantly. In the PM PtdIns was present at the highest concentration (37 % of total PL) while the contents of PtdCho and PtdEtn were twice lower. In the MT PtdCho was the main constituent but it accounted for only a few percent more than PtdIns (30 and 26 %, respectively). The high content of PtdIns corresponds to the observation of Janssen *et al*. (2000) that, irrespective of the medium, PtdIns levels increase upon entering the stationary phase. The concentration of PtdSer was low in both organelles, which is in accordance with the literature for MT but much lower for the PM, where it usually accounts for 20–34 % (Table II). On the other hand, it is in accordance with the high concentration of PtdIns since both phosphatidylinositol synthase (EC 2.7.8.11) and phosphatidylserine synthase (EC 2.7.8.8) compete for cytidine diphospho-diacylglycerol as the common precursor. It is noteworthy that the content of PtdSer in the PM, although low compared with the literature, was twice higher than in the PM of analyzed baker's yeast which was also harvested in the stationary phase (Blagović, *in press* 2005). This is in agreement with the observation that a higher concentration of PtdSer increases the ethanol tolerance (Mishra and Prasad 1988; Ciesarová and Šmogrovičová 1996; Ciesarová *et al*. 1996).

Table I. Comparison of phospholipid composition of the plasma membrane and mitochondria of brewer's yeast (% of total phospholipids, *M*/*M*)

Component	Plasma membrane	Mitochondria
PtdCho	18.7	30.3
PtdEtn	16.6	20.9
PtdIns	36.6	25.6
PtdSer	5.0	2.8
CL	6.2	9.7
PA	13.4	8.4
DMPtdEtn	0.94	0.63
Lyso-PL	2.5	1.2

The presence of mono-, di- and triacylglycerols, squalene, ergosterol, steryl esters and free FA was detected in both organelles. The major components, triacylglycerols, squalene, ergosterol and steryl esters were quantified (Table III). The main characteristic of both neutral fractions was a high content of squalene: 25 mg/g proteins in the PM and 22 mg/g proteins in the MT, which was 2–3×, respectively, more than the content of ergosterol. Such a high content of squalene is in agreement with the lipid composition of the

whole cells of the same yeast (Blagović *et al*. 2001). It is a consequence of anaerobic growth conditions, since one of the essential steps in sterol synthesis is the oxygen-requiring conversion of squalene to squalene epoxide (Jahnke and Klein 1983). In the absence of oxygen, squalene accumulates in the cell. The presence of squalene in the membranous systems of the yeast cells may be explained as a way of gradual accommodation of the yeast to unfavorable anaerobic conditions during beer fermentation since the analyzed yeast was recycled (normal practice in the brewing industry).

Table II. Comparison of the phospholipid composition (PL to TPL; %, *M*/*M*) of the plasma membrane and mitochondria between brewing strain of *S. cerevisiae* analyzed here (stationary phase cells, anaerobic conditions) and laboratory strains (exponential phase cells, aerobic conditions)

PtdCho	PtdEtn	Ptdlns	PtdSer	CL	PA	Others	Reference
Plasma membrane							
18.9	16.5	36.7	4.8	6.3	13.3	3.5	*a*
24.6	20.2	40.0	5.5	6.1	3.6	–	*b*
16.8	20.3	17.7	33.6	0.2	3.9	6.9	*c*
11.3	24.6	27.2	32.2	–	3.3	1.4	*d*
21.0	18.3	13.3	23.1	4.9	7.5	11.9	*d*
10.0	41.8	7.9	20.3	–	3.5	16.5	*e*
Mitochondria							
30.1	21.1	25.7	2.9	9.7	8.5	2.0	*a*
40.2	26.5	14.6	3.0	13.3	2.4	–	*c*
33.4	22.7	20.6	3.3	7.2	1.7	5.0	*d*
39.8	26.4	10.9	2.3	15.7	1.7	3.2	*d*

[a]Our results; [b]Paton and Lester 1991; [c]Zinser and Daum 1995; [d]Tuller *et al.* 1999 (cells grown on glucose and lactate); [e]van den Hazel 1999.

Table III. Composition of the neutral lipid fraction of the plasma membrane and mitochondria of brewer's yeast (mass ratio of the component to proteins, μg/mg)

Component	Plasma membrane	Mitochondria
Triacylglycerols	2	2
Squalene	25	22
Ergosterol	12	7
Steryl esters	22	2
Total	61	33

Mass and molar ratios of PL, ergosterol and steryl esters are presented in Table IV. There were notable differences between the two organelles: the ratios of PL and ergosterol to proteins were significantly higher in the PM. However, the ratio of ergosterol to steryl esters showed the largest difference, being almost 6× higher in the MT. Both in the PM and MT, the ratio of total PL to proteins, as well as that of ergosterol to proteins was significantly lower in comparison with the published data relating to aerobically grown yeasts harvested in the exponential growth phase (Zinser and Daum 1995; Schneiter *et al*. 1999; van den Hazel *et al*. 1999). On the other hand, the molar ratio of ergosterol and PL was in the range of published data (van den Rest *et al*. 1995), which is, however, still a matter of controversy.

The FA composition of the PM and MT and the principal features of their FA profiles are in Table V. The degree of unsaturation (mono-, di-, tri) is expressed as unsaturation index (IU; Šajbidor 1997):

$$IU = (\%_{mono} + 2 \times \%_{di} + 3 \times \%_{tri})/100\,,$$

where the values represent the mass percentage of FA in the total (identified) FA.

FA $C_{12–26}$ were identified in the PM and those ranging from C_{12} to C_{18} in the MT. The main feature of the composition of both organelles was the preponderance of saturated FA (78 and 79 %, respectively), among which palmitic acid was the principal one, making up 41 and 43 % of the TFA, respectively. It is well known that yeast cells respond to environmental changes by a complex regulatory system. Regulation of membrane fluidity and permeability by changing the FA composition of membrane lipids has an important role. Therefore, the FA composition depends strongly on the composition of the growth medium and on the cultivation conditions (Mishra and Prasad 1989; Suutari *et al*. 1990; Šajbidor and Grego 1992; Khaware *et al*. 1995; Cahoon *et al*. 1996; Rupčić *et al*. 1996; Šajbidor 1997; Rupčić *et al*. 1998). Preponderance

of saturated FA is a consequence of anaerobic growth conditions, since double bond is introduced into acyl-CoA by an NADH- or NADPH-dependent oxidase (acyl-CoA desaturase) in the presence of molecular oxygen.

Table IV. Mass and molar ratios of phospholipids, ergosterol, steryl esters and proteins in the plasma membrane and mitochondria of brewer's yeast

Ratio	Plasma membrane		Mitochondria	
	μg/mg	mol/mol	μg/mg	mol/mol
Phospholipids to proteins	78		59	
Ergosterol to proteins	12		7	
Ergosterol to phospholipids	150	0.30	120	0.23
Ergosterol to steryl esters	550	0.91	3500	5.85

Table V. FA composition of the total lipids of the plasma membrane and mitochondria of brewer's yeast

FA	Plasma membrane	Mitochondria
	Total FA, % (*M*/*M*)	
12:0	12.9	12.9
14:0	1.6	17.3
14:1	1.6	–
16:0	41.1	42.5
16:1	11.7	16.5
18:0	20.4	5.9
18:1	6.7	4.9
18:2	2.5	–
26:0	1.5	–
Saturated FA	77.5	78.7
Unsaturated FA	22.5	21.4
Unsaturation index	0.25	0.21
C_{16}/C_{18}	1.78	5.46
$C_{\leq 16}/C_{18}$	2.20	8.30

Ethanol is also one of the effects with strong influence on membrane composition. Reports dealing with the alterations of the FA composition caused by ethanol exposure refer to the whole cell and differ quite a lot, depending on the yeast strain, ethanol concentration and supplementation of FA in the growth media (Šajbidor and Grego 1992; Mizoguchi and Hara 1997; Mizoguchi 1998; Heipieper *et al*. 2000). According to some authors the content of unsaturated FA in yeast cells increases in the presence of ethanol, thus increasing membrane fluidity and hence ethanol tolerance. According to the others, the presence of ethanol increases the content of palmitic and oleic acid in major membrane PL (PtdCho and PtdEtn) and palmitoleic in steryl esters and triacylglycerols. Heipieper *et al*. (2000) found a direct correlation between nonlethal ethanol concentration and a decrease of the unsaturation index for *Kluyveromyces lactis*.

Beside saturated FA, the FA with chain length shorter than 18 C atoms prevailed significantly in both organelles, which was much more expressed for the MT. Since membrane-bound elongation systems are localized in the endoplasmic reticulum or MT (Paltauf *et al*. 1992), the prevalence of shorter FA might be attributed to poorly developed MT, which is also a consequence of anaerobic conditions (O'Connor-Cox *et al*. 1996). In the PM a VLCFA, hexacosanoic acid ($C_{26:0}$) was identified. It has been found that VLCFAs are essential since they form a structurally important part of the ceramide moiety of sphingolipids and the lipid domain of glycosyl-PtdIns-anchored proteins. Hexacosanoic acid has attracted attention due to the findings that the strains, which survived without synthesizing ceramide, produce C_{26}-FA-substituted *myo*-inositolglycero-PL that structurally mimic sphingolipids (Schneiter and Kohlwein 1997).

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REFERENCES

ACHLEITNER G., GAIGG B., KRASSER A., KAINERSDORFER E., KOHLWEIN S.D., PERKTOLD A., ZELLNIG G., DAUM G.: Association between the endoplasmic reticulum and mitochondria of yeast facilitates interorganelle transport of phospholipids through membrane contact. *Eur.J.Biochem*. **264**, 545–553 (1999).

ATHENSTAEDT K., DAUM G.: Biosynthesis of phosphatidic acid in the yeast *Saccharomyces cerevisiae*, pp. 17–28 in H. Dipak, K.D. Salil (Eds): *Lipids: Glycerolipid Metabolizing Enzymes*. Research Signpost, Kerala (India) 2002.

BLAGOVIĆ B., RUPČIĆ J., MESARIĆ M., GEORGIÚ K., MARIĆ V.: Lipid composition of brewer's yeast. *Food Technol.Biotechnol*. **39**, 175–181 (2001).

BROEKHUYSE R.M.: Phospholipids in tissues of the eye. *Biochim.Biophys.Acta* **152**, 307–315 (1968).

CAHOON E.B., MILLS L.A., SHANKLIN J.: Modification of the fatty acid composition of *Escherichia coli* by coexpression of plant acyl–acyl carrier protein desaturase and ferredoxin. *J.Bacteriol.* **178**, 936–939 (1996).

CAPALDI R.A.: The changing face of mitochondrial research. *Trends Biochem.Sci.* **25**, 212–214 (2000).

CASEY W.M., ROLPH C.E., TOMEO M.E., PARKS L.W.: Effects of unsaturated fatty acid supplementation on phospholipid and triacylglycerol biosynthesis in *Saccharomyces cerevisiae*. *Biochem.Biophys.Res.Com*. **193**, 1297–1303 (1993).

CIESAROVÁ Z., ŠMOGROVIČOVÁ D.: A study of ethanol tolerance in yeasts. (In Slovak) *Chem.Listy* **90**, 365–370 (1996).

CIESAROVÁ Z., ŠMOGROVIČOVÁ D., DÖMÉNY Z.: Enhancement of yeast ethanol tolerance by calcium and magnesium. *Folia Microbiol.* **41**, 485–488 (1996).

DAUM G., VANCE J.E.: Imports of lipids into mitochondria. *Prog.Lipid Res*. **36**, 103–130 (1997).

DAUM G., LEES N.D., BARD M., DICKSON R.: Biochemistry, cell biology and molecular biology of lipids of *Saccharomyces cerevisiae*. *Yeast* **14**, 1471–1510 (1998).

DAUM G., TULLER G., NEMEC T., HRASTNIK C., BALLIANO G., CATTEL L., MILLA P., ROCCO F., CONZELMANN A., VIONNET C., KELLY E.D., KELLY S., SCHWEIZER E., SCHÜLLER H.-J., HOJAD U., GREINER E., FINGER K.: Systematic analysis of yeast strains with possible defects in lipid metabolism. *Yeast* **15**, 601–614 (1999).

FLEGELOVÁ H., CHALOUPKA R., NOVOTNÁ D., MALÁČ J., GÁŠKOVÁ D., SIGLER K., JANDEROVÁ B.: Changes in plasma membrane fluidity lower the sensitivity of *S. cerevisiae* to killer toxin K1. *Folia Microbiol.* **48**, 761–766 (2003).

FOLCH J., LEES M., SLOANE-STANLEY G.H.: A simple method for the isolation and purification of total lipids from animal tissues. *J.Biol.Chem.* **226**, 497–509 (1957).

GAIGG B., SIMBENI R., HRASTNIK C., PALTAUF F., DAUM G.: Characterization of a microsomal subfraction associated with mitochondria of the yeast, *Saccharomyces cerevisiae. Biochim.Biophys.Acta* **1234**, 214–220 (1995).

GRANT A.M., HANSON P.K., MALONE L., NICHOLS J.W.: NBD-labeled phosphatidylcholine and phosphatidylethanolamine are internalized by transbilayer transport across the yeast plasma membrane. *Traffic* **2**, 37–50 (2001).

HAID A., SUISSA M.: Immunochemical identification of membrane proteins after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Meth.Enzymol.* **96**, 192–205 (1983).

HAMMOND J.R.M.: Yeast growth and nutrition, pp. 77–84 in K. Smart (Ed.): *Brewing Yeast Fermentation Performance*. Blackwell Science, Oxford (UK) 2000.

VAN DEN HAZEL H.B., PICHLER H., DO VALLE MATTA M.A., LEITNER E., GOFFEAU A., DAUM G.: *PDR16* and *PDR17*, two homologous genes of *Saccharomyces cerevisiae*, affect lipid biosynthesis and resistance to multiple drugs. *J.Biol.Chem*. **274**, 1934–1941 (1999).

HEIPIEPER H.J., ISKEN S., SALIOLA M.: Ethanol tolerance and membrane fatty acid adaptation in *adh* multiple and null mutants of *Kluyveromyces lactis*. *Res.Microbiol*. **151**, 777–784 (2000).

JAHNKE L., KLEIN H.P.: Oxygen requirement for formation and activity of the squalene epoxidase in *Saccharomyces cerevisiae*. *J.Bacteriol*. **155**, 488–492 (1983).

JANSSEN M.J.F.W., KOORENGEVEL M.C., DE KRUIJFF B., DE KROON A.I.P.M.: Transbilayer movement of phosphatidylcholine in the mitochondrial outer membrane of *Saccharomyces cerevisiae* is rapid and bidirectional. *Biochim.Biophys.Acta* **1421**, 64–76 (1999).

JANSSEN M.J.F.W., KOORENGEVEL M.C., DE KRUIJFF B., DE KROON A.I.P.M.: The phosphatidylcholine to phosphatidylethanolamine ratio of *Saccharomyces cerevisiae* varies with the growth phase. *Yeast* **16**, 641–650 (2000).

KHAWARE R.K., KOUL A., PRASAD R.: High membrane fluidity is related to NaCl stress in *Candida membranaefaciens*. *Biochem.Mol. Biol.Internat.* **35**, 875–880 (1995).

KRASOWSKA A., CHMIELEWSKA L., GAPA D,. PRESCHA A., VÁCHOVÁ L., SIGLER K.: Viability and formation of conjugated dienes in plasma membrane lipids of *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Rhodotorula glutinis* and *Candida albicans* exposed to hydrophilic, amphiphilic and hydrophobic pro-oxidants. *Folia Microbiol.* **47**, 145–151 (2002).

LAEMMLI U.K.: Cleavage of structural protein during the assembly of the head of the bacteriophage T4. *Nature* 227, 680–685 (1970).

LÖFFLER J., EINSELE H., HEBART H., SCUMACHER U., HRASTNIK C., DAUM G.: Phospholipid and sterol analysis of plasma membranes of azole-resistant *Candida albicans* strains. *FEMS Microbiol.Lett*. **185**, 59–63 (2000).

MARX U., POLAKOWSKI T., POMORSKI T., LANG C., NELSON H., NELSON N., HERRMANN A.: Rapid transbilayer movement of fluorescent phospholipid analogues in the plasma membrane of endocytosis-deficient yeast cells does not require the Drs2 protein. *Eur.J.Biochem*. **263**, 254–263 (1999).

MISHRA P., PRASAD R.: Role of phospholipid head groups in ethanol tolerance of *Saccharomyces cerevisiae*. *J.Gen.Microbiol*. **134**, 3205–3211 (1988).

MISHRA P., PRASAD R.: Relationship between ethanol tolerance and fatty acyl composition of *Saccharomyces cerevisiae*. *Appl.Environ. Microbiol.* **30**, 294–298 (1989).

MIZOGUCHI H.: Acquisition of ethanol tolerance by *Saccharomyces cerevisiae* in the sake brewing process and the tolerance determinants. *Seibutsu-Kogaku* **76**, 122–130 (1998).

MIZOGUCHI H., HARA S.: Ethanol-induced alterations in lipid composition of *Saccharomyces cerevisiae* in the presence of exogenous fatty acids. *J.Ferment.Bioeng*. **83**, 12–16 (1997).

MURAKAMI Y., YOKOIGAWA K., KAWAI F., KAWAI H.: Lipid composition of commercial baker's yeasts having different freeze-tolerance in frozen dough. *Biosci.Biotech.Biochem*. **60**, 1874–1876 (1996).

O'CONNOR-COX E.S.C., LODOLO E.J., AXCELL B.C.: Mitochondrial relevance to yeast fermentative performance: a review. *J.Inst.Brew*. **102**, 19–25 (1996).

PALTAUF F., KOHLWEIN S., HENRY S.A.: Regulation and compartmentalization of lipid synthesis in yeast, pp. 415–500 in *The Molecular and Cellular Biology of the Yeast* Saccharomyces cerevisiae*: Gene Expression.* Cold Spring Harbor Laboratory Press, New York 1992.

PATTON J.L., LESTER R.L.: The phosphoinositol sphingolipids of *Saccharomyces cerevisiae* are highly localized in the plasma membrane. *J.Bacteriol*. **173**, 3101–3108 (1991).

PICHLER H., GAIGG B., HRASTNIK C., ACHLEITNER G., KOHLWEIN S.D., ZELLNIG G., PERKTOLD A., DAUM G.: A subfraction of the yeast endoplasmic reticulum associates with the plasma membrane and has a high capacity to synthesize lipids. *Eur.J.Biochem*. **268**, 2351–2361 (2001).

PIPER P.W.: The heat shock and ethanol stress responses of yeast exhibit extensive similarity and functional overlap. *FEMS Microbiol. Lett*. **134**, 121–127 (1995).

VAN DEN REST M.E., KAMMINGA A.H., NAKANO A., ANRAKU Y., POOLMAN B., KONINGS W.N.: The plasma membrane of *Saccharomyces cerevisiae*: structure, function, and biogenesis. *Microbiol.Rev*. **59**, 304–322 (1995).

RUPČIĆ J., BLAGOVIĆ B., MARIĆ V.: Cell lipids of the *Candida lipolytica* yeast grown on methanol. *J.Chromatogr. A* **755**, 75–80 (1996).

RUPČIĆ J., MESARIĆ M., RUPČIĆ J., MESARIĆ M., MARIĆ V.: The influence of carbon source on the level and composition of ceramides of the *Candida lipolytica* yeast. *Appl.Microbiol.Biotechnol*. **50**, 583–588 (1998).

ŠAJBIDOR J.: Effect of some environmental factors on the content and composition of microbial membrane lipids. *Crit.Rev.Biotechnol.* **17**, 87–103 (1997).

ŠAJBIDOR J., GREGO J.: Fatty acid alterations in *Saccharomyces cerevisiae* exposed to ethanol stress. *FEMS Microbiol.Lett*. **93**, 13–16 (1992).

ŠAJBIDOR J., CIESAROVÁ Z., ŠMOGROVIČOVÁ D.: Influence of ethanol on the lipid content and fatty acid composition of *Saccharomyces cerevisiae*. *Folia Microbiol*. **40**, 508–510 (1995).

SCHNEITER R., KOHLWEIN S.D.: Organelle structure, function, and inheritance in yeast: a role for fatty acid synthesis? *Cell* **88**, 431–434 (1997).

SCHNEITER R., BRÜGGER B., SANDHOFF R., ZELLNIG G., LEBER A., LAMPL M., ATHENSTAEDT, HRASTNIK C., EDER S., DAUM G., PALTAUF F., WIELAND F.T., KOHLWEIN S.D.: Electrospray ionization tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals acyl chain-based sorting/remodeling of distinct molecular species and route to the plasma membrane. *J.Cell Biol.* **146**, 741–754 (1999).

SORGER D., DAUM G.: Triacylglycerol biosynthesis in yeast. *Appl.Microbiol.Biotechnol*. **61**, 289–299 (2003).

SUUTARI M., LIUKKONEN K., LAAKSO S.: Temperature adaptation in yeasts: the role of fatty acids. *J.Gen.Microbiol*. **136**, 1469–1474 (1990).

TULLER G., NEMEC T., HRASTNIK C., DAUM G.: Lipid composition of subcellular membranes of an FY1679-derived haploid yeast wild-type strain grown on different carbon sources. *Yeast* **15**, 1555–1564 (1999).

VORBECK M.L., MATTICK L.R., LEE F.A., PEDERSON C.S.: Preparation of methyl esters of fatty acids for gas–liquid chromatography. *Anal.Chem*. **33**, 1512–1514 (1961).

ZINSER E., DAUM G.: Isolation and biochemical characterization of organelles from the yeast *Saccharomyces cerevisiae. Yeast* **11**, 493–536 (1995).

ZINSER E., SPERKA-GOTTLIEB C.D.M., FASCH E.-V., KOHLWEIN S.D., PALTAUF F., DAUM G.: Phospholipid synthesis and lipid composition of subcellular membranes in the unicellular eukaryote *Saccharomyces cerevisiae*. *J.Bacteriol*. **173**, 2026–2034 (1991).

ZINSER E., PALTAUF F., DAUM G.: Sterol composition of yeast organelle membranes and subcellular distribution of enzymes involved in sterol metabolism. *J.Bacteriol*. **175**, 2853–2858 (1993).