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Lipid composition of mitochondria of aerobically grown baker's and brewer's yeast

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ABSTRACT

The mitochondria of baker's yeast (obtained as the end-product of industrial production) and bottom-fermenting brewer's yeast (obtained at the end of propagation in industrial beer production) were analysed in this work. It was determined that the main phospholipid of baker's yeast mitochondria was phosphatidylcholine, accounting for more than 47% of total phospholipids, followed by phosphatidylethanolamine, phosphatidylinositol and cardiolipin. In the neutral lipid composition, ergosterol was the main component, accounting for 0.039 mg/mg proteins. The fatty acid composition was characterised by high predominance of monounsaturated fatty acids (79%). In the mitochondria of brewer's yeast, the phospholipid classes were almost evenly represented accounting between 23 and 16%. Ergosterol was the main neutral lipid accounting for 0.016 mg/mg protein, but significant amount of squalene was also determined (0.007 mg/mg proteins) despite the aerobic conditions. Fatty acid composition was characterised by small predominance of unsaturated acids (55%) and high predominance of C16-acids (69%). The results led to the conclusion that estimation of the lipid composition of industrial yeasts based on the results obtained in laboratory conditions is uncertain and that the analysis of the yeast grown in specific conditions should be performed.

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KEYWORDS: baker's yeast, brewer's yeast, lipids, squalene, mitochondria, *Saccharomyces cerevisiae*, aerobic conditions

ABBREVIATIONS

PtdCho	:	phosphatidylcholine
PtdEtn	:	phosphatidylethanolamine
PtdIns	:	phosphatidylinositol
PtdSer	:	phosphatidylserine
PtdOH	:	phosphatidic acid
CL	:	cardiolipin
PLs	:	phospholipids
Erg	:	ergosterol
FAs	:	fatty acids
SE	:	steryl esters
TAGs	:	triacylglycerols

INTRODUCTION

During production processes and applications, industrial yeasts are exposed to various stressors. These stressors may occur simultaneously or sequentially and moreover with high fluctuations [1]. To survive, they must activate adaptive responses, such as changes in the composition of cell components, including lipids.

The goal of baker's yeast producers is to maximise growth and minimize fermentation, whereas the goal of bakers is opposite. Therefore, baker's yeast is required to exhibit efficient respiratory metabolism during its production and to leaven bread efficiently by producing considerable quantities of carbon dioxide (mostly *via* ethanolic fermentation of various sugars in dough), plus

desirable flavour and aroma from by-products of secondary metabolism [2]. It is grown on an entirely different medium compared with the one in which it is destined to "work", to grow and metabolize. Besides, baker's yeast is being increasingly used for the production of frozen dough, for which it must have high freeze-thaw resistance [3]. The mechanisms of freeze-injury and tolerance to freezing and thawing are important for other areas of applied microbiology such as frozen starters and culture collections. The degree of membrane damage or resistance to stressors is strongly related to the membrane lipid composition. For the bottom-fermenting brewer's yeast oxygen depletion and ethanol toxicity are of special importance [4]. The yeast required for industrial-scale fermentation is propagated in wort under aerobic conditions, but within a fermentation vessel initial oxygen is rapidly depleted within the first few hours after pitching, leading to an anaerobic environment and accumulation of CO_2. Besides, under normal fermentation conditions final ethanol concentration is 3-6%.

Membrane lipids play an essential role in the biophysical characteristics of cell membranes; they determine permeability and fluidity, enable proper functioning of membrane-attached proteins and participate in the cell signalling. The ability of yeast cells to alter sterol to phospholipid ratio, PL composition and degree of unsaturation in the membranes is an important factor in adaptation to environmental conditions [5-7].

The mitochondria are engaged in many cellular 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, well-developed mitochondria are of vital importance for the cells [8]. They are dynamic organelles whose functional integrity requires a coordinated supply of proteins and lipids adjusted to meet physiological and functional demands [7, 9]. Their lipid composition is of great importance since they contain double membranous system, outer and inner membrane, of which the inner one has a creasy structure shaped by lipids.

There are a lot of data on the lipids of standard laboratory strains of *Saccharomyces cerevisiae* and its organelles since this species serves as an experimental model organism in biochemical, cell biological and molecular biological studies of the synthesis and transport of cell components, including lipids [10]. Phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol are considered as bulk phospholipids of *S. cerevisiae*, which correspond to the mitochondria, as well. Some phospholipids are characteristic for certain organelles, for example cardiolipin (CL) for the mitochondria where, among others, it is the key determinant in the maintenance of mtDNA stability and segregation [11, 12]. Ergosterol is the main and essential class of neutral lipids present in all membranous systems of *S. cerevisiae*, which modulates membrane fluidity and permeability. It is highly enriched in the plasma membrane (PM), while triacylglycerols and steryl esters, representing reserve lipid molecules, are mainly located in the lipid particles. Squalene, another neutral lipid, is a triterpenic hydrocarbon formed in the first part of ergosterol biosynthetic pathway, the so-called mevalonate pathway, and further converted to squalene epoxide by squalene epoxidase Erg1p [13]. This step requires oxygen, making ergosterol synthesis strictly aerobic. Compared to other subcellular fractions, specific characteristic of mitochondria also include low phospholipid to protein and sterol to protein ratios [14]. Taking into consideration the fatty acid (FA) composition of *S. cerevisiae*, the major FAs are palmitic (16:0), palmitoleic ($16:1\Delta^9$), stearic (18:0) and oleic ($18:1\Delta^9$) acids. Since the only FA desaturase in *Saccharomyces* is the Δ-9 desaturase (Ole1), these yeasts do not contain polyunsaturated FAs unless they are added exogenously.

Very little information is available on industrial yeasts, while it would be very useful from the viewpoint of application to know the lipid composition of the whole cells and the organelles, since the resistance, viability and functionality of the cells are largely determined by their lipid composition [3]. The addition of supplements in the growth medium could be used to modulate the lipid composition and thus to obtain yeasts with improved properties. In our previous papers, we published data on the lipid composition of the whole cells and PMs of baker's yeast analysed in this study [15]. Regarding brewer's yeast,

we published data on the lipid composition of the mitochondria obtained at the end of fermentation, which is - contrary to propagation - highly anaerobic in the case of the bottom-fermenting brewer's yeast [16-20]. Published data show significant differences between these two industrial strains and the laboratory strains (literature data) which led us to investigate the lipid composition of their cell powerhouses, mitochondria, since they are not only capable of surviving extreme conditions, but also 'work' in such conditions. Additionally, we aimed to investigate whether the mitochondrial lipid composition of brewer's yeast grown in favourable conditions (propagation process) was more like the composition obtained in laboratory strain or it would retain the composition adjusted to unfavourable conditions. Therefore, we analysed the lipid composition of the mitochondria of baker's and brewer's yeast, both aerobically grown. Obtained results represent a contribution to the present knowledge of the cell composition of industrial yeasts and could be valuable not only for the manipulation of industrial processes and development of new industrial strains, but also for the research of mitochondrial structure and function in general.

MATERIALS AND METHODS

Yeast strains

Baker's yeast belonging to the species *Saccharomyces cerevisiae* was used throughout the study. It was obtained as compressed yeast at the end of the industrial process in "KVASAC" factory, Savski Marof, Croatia. It was grown under aerobic conditions and harvested in the stationary phase. The second yeast was the bottom-fermenting brewer's yeast or lager brewing strain of the species *S. cerevisiae* from the Collection of Microorganisms of the University of Weinstephan, Munich, Germany, which was kindly provided from the local brewery. Briefly, starter culture or the so-called 'zero generation culture' analysed in this study is obtained as follows: the laboratory-grown culture is transferred into the yeast propagator containing fresh malt wort and continuously aerated. The yeast is harvested in the stationary phase.

Prior to any experiment, biomass was washed three times with cold distilled water and centrifuged at 1800 g for 5 min. In the case of brewer's yeast, biomass was washed the third time with 0.1% $NaHCO_3$ in order to eliminate bitter components of hops.

Isolation and characterisation of the mitochondria

The mitochondria were isolated by differential centrifugation after enzymatic disruption of the cell wall using zymoliase [21]. The quality of the isolated mitochondria was tested by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Relative enrichment of porin, protein of the outer mitochondrial membrane, was determined by immunoblotting. The protein content was determined by the method of Lowry using bovine serum albumin as the standard. Proteins were precipitated with trichloracetic acid (10% final concentration) and solubilized in 0.2% SDS-0.1 M NaOH prior to the determination. SDS-PAGE was carried out by the method of Laemmli [22]. The samples were dissociated at 37 °C. Western blot analysis was carried out after the separation of proteins on SDS-10% polyacrylamide gels and transferred to nitrocellulose sheets (Hybond-C, Amersham) by standard procedures [23]. 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, raised in rabbits, were gift of G. Daum Institute of Biochemistry, Graz.

Extraction of lipids

Total lipids were extracted from the mitochondria by the Folch method.

Lipid analysis

Chromatographic methods used for the lipid analysis are the standard ones for the determination of basic lipid composition and accepted by *Saccharomyces cerevisiae* – EUROFAN 2 group.

Analysis of phospholipids

Total phospholipids were quantified spectrophotometrically as inorganic phosphorus by the method of Broekhuyse [24]. Individual phospholipid classes were separated by two-dimensional thin-layer chromatography of total lipid extract on silica gel 60 plates, 20 x 20 cm,

0.2 mm. Chloroform/methanol/ammonium hydroxide (volume fractions 65:35:5) was used as the first solvent system and chloroform/acetone/methanol/ acetic acid/water (volume fractions 50:20:10:10:5) as the second one. Phospholipids were visualised by iodine staining, scraped off the plate and quantified the same way as total phospholipids.

Analysis of neutral lipids

Neutral lipids were separated by two-step TLC on silica gel 60 plates 20 x 10 cm, 0.2 mm. Lipid extracts and standards (ergosterol, squalene and triolein) were applied by sample applicator (Linomat IV, CAMAG, Muttenz, Switzerland). The plates were developed by using petrolether/ diethylether/acetic acid (volume fractions 20:20:0.8) up to 1/3 of a plate as the first and petrolether/ diethylether (volume fractions 39.2:0.8) up to 2/3 of plate as the second solvent system. Ergosterol and ergosteryl esters were quantified by direct densitometry at 275 nm using ergosterol as the standard. For the determination of acylglycerols and squalene, the bands were visualised 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 conc.) for 4 s, briefly dried and heated for 30 min at 100 °C. Bands corresponding to triacylglycerols and squalene were identified and quantified by comparison with standards upon scanning at 400 nm.

Analysis of fatty acids

FA composition was determined by GC analysis of the corresponding methyl esters obtained by acid methanolysis of lipid extracts with BF_3–methanol [25]. GC analyses of FA methyl esters were done using an Auto System XL from Perkin-Elmer with flame ionization detector. An SP-2330 capillary column (30 m × 0.32 mm × 0.2 μm, Supelco, USA) and helium as carrier gas with split injection (100:1) were used. Hydrogen was obtained using a Claind hydrogen generator. The analyses were carried out in programmed temperature mode from 140 °C to 220 °C at 5 K/min and then isothermally for 25 min. The injector temperature was 300 °C and that of the detector 350 °C. For data acquisition, Chromatography Software from Perkin-Elmer Nelson (Turbochrom 4) was used. The results were expressed as mass fractions of an individual FA in total identified FAs.

RESULTS AND DISCUSSION

Phospholipids

The proportion of total phospholipids *vs.* proteins was 0.37 mg/mg in the baker's yeast and 0.022 mg/mg in the brewer's yeast mitochondria. The main phospholipid in the baker's yeast was phosphatidylcholine accounting for more than 46% of total phospholipids (Table 1), followed by phosphatidylethanolamine, phosphatidylinositol and cardiolipin.

The composition of mitochondria of both yeasts, especially of the brewer's yeast, differed markedly from the composition of the mitochondria of standard laboratory *S. cerevisiae* strains [21, 26, 27]. In the baker's yeast, the content of PtdCho was markedly higher and that of PtdEtn lower, which made the PtdCho/PtdEtn ratio also markedly higher (2.4 compared to 1.5 for the laboratory strain) [11]. Partially, it is the consequence of different growth phase, since the analysed industrial yeast was harvested in the stationary phase, while the laboratory strain, as they usually are, was harvested in the exponential phase. According to Janssen *et al.* [28], PtdCho content increases at the expense of PtdEtn upon entering the stationary phase. The difference between the mitochondria of the analysed baker's yeast and standard laboratory yeast strain was even more pronounced in the PtdIns/PtdSer ratio which accounted for 8.9 and 5.0, respectively (Table 1). This result can also be explained as another feature of the yeast in the stationary phase since it was found that PtdIns content increases upon entering the stationary phase irrespective of the composition of growth media [28]. Comparing PL composition of the mitochondria with that of the PM of the same baker's yeast [15], it was found that the mitochondria were characterized by markedly higher content of PtdCho, PtdEtn and CL at the expense of PtdIns and PtdOH. The resulting ratio of PtdCho/PtdEtn in the mitochondria was lower (2.4 *vs.* 2.9), while PtdIns/PtdSer was higher (9.0 *vs.* 8.0) than in the PM. If we take into consideration the shapes of PtdCho and PtdEtn, we can suppose the reason for

Table 1. Phospholipid composition of mitochondria: comparison of the results for the commercial baker's yeast and the bottom-fermenting brewer's yeast of the zero generation both grown aerobically and harvested in the stationary phase.

% Total mitochondrial phospholipids		
Phospholipid	**Baker's yeast**	**Brewer's yeast**
PtdCho	46.5 ± 3.8	22.8 ± 2.2
PtdEtn	19.3 ± 4.8	17.6 ± 2.1
PtdIns	16.1 ± 2.2	17.8 ± 1.8
PtdSer	1.8 ± 1.1	7.2 ± 0.5
PtdOH	1.4 ± 0.1	18.4 ± 3.2
CL	14.9 ± 1.8	16.2 ± 1.7
PtdCho/PtdEtn	2.4 ± 1.5	1.3 ± 0.1
PtdIns/PtdSer	8.9 ± 3.3	2.5 ± 0.6

Results represent mean value of double analysis of at least three independent experiments.

such differences. PtdCho with its large head group and cylindrical shape is the main bilayer forming phospholipid, which makes it ideally suited to preserve membrane integrity, while PtdEtn with its small head group and cone-like shape is a non-bilayer forming PL [29] whose concentration increases in the membranes of great curvature like mitochondria. Besides, it was hypothesized that by changing the PtdCho/PtdEtn ratio the yeast cells adjust membrane properties to environmental conditions. High content of CL (14.9%), a PL enriched and synthesised in the mitochondria, was in agreement with the literature for the laboratory strains and is a feature of the well-developed mitochondria in conditions of good oxygen supply. The concentration of PtdSer in the baker's yeast mitochondria was somewhat lower than in the laboratory strain (1.8 and 3.0, respectively), while in the PM it was almost 10 times lower (3.4% and 33.6%, respectively) [15]. The reason for such a low content of PtdSer in the PM of baker's yeast is because of the high content of PtdIns, which competes for the same precursor.

In the brewer's yeast mitochondria phospholipids were almost evenly represented, except for the markedly lower content of PtdSer, which is a typical plasma membrane phospholipid (Table 1). Both, PtdCho/PtdEtn and PtdIns/PtdSer ratios were slightly higher than in the PM of the same yeast (1.3 and 1.1, respectively and 2.5 and 2.3, respectively) [18]. In comparisson to baker's yeast, brewer's yeast mitochondria were characterized by markedly lower concentrations of PtdCho and higher concentrations of PtdSer and PtdOH (Table 1), which made the differences in PL-ratios, especially PtdIns/PtdSer, significant. The reason for such alterations remains to be clarified.

Neutral lipids

With respect to the neutral lipid composition of the baker's yeast mitochondria (Table 2), ergosterol had the highest proportion, which is in accordance with the highly aerobic conditions prevailing during industrial process of baker's yeast production. Triacylglycerols and steryl esters accounted for half of the ergosterol content, which is rather high for a membranous system. Since we analysed the crude mitochondria, this finding could be a result of the contamination with other organelles (mainly lipid particles), but the enrichment factor of 6.1 for porin was in agreement with the data published for highly purified mitochondria indicating good quality of the isolates. Besides, the activity of steryl ester synthase and hydrolase is determined in the mitochondria of *S. cerevisiae*,

Table 2. Neutral lipid composition of the mitochondria of commercial baker's yeast and bottom-fermenting brewer's yeast both grown aerobically and harvested in the stationary phase.

Mass ratio of lipid component to proteins in the mitochondria (mg/mg)		
	Baker's yeast	**Brewer's yeast**
Ergosterol	0.039 ± 0.005	0.016 ± 0.003
Squalene	ND	0.007 ± 0.001
Steryl esters	0.020 ± 0.002	BLQ
Triacylglycerols	0.018 ± 0.004	BLQ

Results represent mean value of double analysis of at least three independent experiments; ND, not detected; BLQ, below limit of quantification.

as well, and therefore their presence is expected. Squalene was not detected since it is converted into squalene epoxide and finally into ergosterol under aerobic conditions [13].

In the mitochondria of brewer's yeast, ergosterol was also the main neutral lipid. According to the literature, exceptions in the *Regnum Fungi* are only in the family *Pythiaceae*, which is unique in so far that its members do not synthesize detectable amounts of sterols and therefore are called non-sterol producing fungi [30]. The second exception is the bottom-fermenting brewer's yeast of the *S. cerevisiae* species which is a sterol-producing yeast, but as adaptation to anaerobic conditions it can constitute membranes with squalene, as well [16, 17]. When it is harvested at the end of beer fermentation, which is anaerobic, it contains significant amounts of squalene in the mitochondria and PM [17]. Surprisingly, in this study we have found that its mitochondria contained relatively high content of squalene when harvested at the end of highly aerobic propagation process (Tables 2 and 3). It can be assumed that during thousands of years of usage in beer fermentations, the bottom-fermenting brewer's yeast has acquired the ability to satisfactorily organize its membranes using squalene both in anaerobic and aerobic conditions. Inspired by a similar finding in bacteria, Hauss *et al.* [31] have determined the incorporation of squalene in the midplane of lipid bilayer in artificial membranes. On the other hand, working with deletion strain of *S. cerevisiae* Spanova & Daum [13] have proven the accumulation of squalene in all intracellular membranes.

For the mitochondria of baker's yeast analysed in this work, the ratio of ergosterol to steryl esters was almost 6 times smaller than the ratio of ergosterol to ergosteryl esters in the laboratory strain (3.2 and 20, respectively) [26]. However, it is in accordance with the finding that the mitochondrial and other intracellular membranes contain a smaller percentage of ergosterol than the PM on account of its precursors, generally present as one third in the form of esters [10]. Besides, the yeast analysed in this study was harvested in the stationary phase, which is characterised by higher content of reserve lipids. Both, ergosterol to protein ratio and phospholipid to protein ratio were approximately four times higher than in the laboratory strain [11] and consequently the ratio of ergosterol to phospholipid was almost the same. The reason for high lipid or low protein content in the mitochondria of baker's yeast remains to be clarified.

Comparing the ratio of ergosterol and phospholipids in baker's and brewer's yeast, markedly bigger similarity can be noticed between the mitochondria than between the PMs [15, 17]. It is probably because different industrial yeasts undergo different changes in the conditions of technological processes and the PMs are the first to experience it. Sterol to phospholipid (S/PL) ratio generally correlates with the freeze-thaw resistance and according to Murakami *et al.* [3] in the whole cells of baker's yeasts it amounts to 0.27-0.50 mol/mol for the freeze-resistant strains, while 0.57-0.76 for the sensitive strains. In the whole cells of our baker's yeast strain [15] it accounted for 0.24 which corresponds to the resistant strains.

Table 3. Mass and molar ratios of phospholipids, ergosterol and steryl esters in the mitochondria of baker's yeast and bottom-fermenting brewer's yeast both grown aerobically and harvested in the stationary phase.

	Baker's yeast	**Brewer's yeast**
Erg/proteins (mg/mg)	0.039 ± 0.004	0.016 ± 0.003
PLs/proteins (mg/mg)	0.37 ± 0.08	0.22 ± 0.02
Erg/PLs (mg/mg)	0.11 ± 0.01	0.073 ± 0.004
Erg/PLs (mol/mol)	0.21 ± 0.02	0.14 ± 0.006
Erg/SE (mol/mol)	3.2 ± 0.4	

Results represent mean value of double analysis of at least three independent experiments.

Table 4. Fatty acid composition and the main features of the mitochondria of baker's yeast and bottom-fermenting brewer's yeast, both grown aerobically and harvested in the stationary phase.

Mitochondria		
	Baker's yeast	**Brewer's yeast**
Fatty acid (FA)		
10:0	ND	0.1 ± 0.0
12:0	5.3 ± 2.5	0.2 ± 0.0
14:0	5.7 ± 0.1	1.1 ± 0.1
16:0	5.2 ± 0.7	37.2 ± 0.5
16:1	37.0 ± 0.3	31.6 ± 0.3
18:0	4.2 ± 0.1	6.9 ± 0.3
18:1	42.1 ± 3.1	18.9 ± 0.2
18:2	ND	3.2 ± 0.2
24:1	ND	0.8 ± 0.0
26:0	0.5 ± 0.0	ND
Saturated FA	20.9 ± 1.8	45.5 ± 2.2
Unsaturated FA	79.1 ± 1.8	54.5 ± 2.2
Unsaturation index	0.79 ± 0.11	0.59 ± 0.04
$\mathbf{C_{16}/C_{18}}$	0.91 ± 0.67	2.37 ± 0.34
$\mathbf{C_{\leq 16}/C_{18}}$	1.15 ± 0.23	2.42 ± 0.45
$\mathbf{C_{16:0}/C_{16:1}}$	0.14 ± 0.02	1.18 ± 0.12
$\mathbf{C_{18:0}/C_{18:1}}$	0.10 ± 0.00	0.37 ± 0.02

Results represent mean value of double analysis of at least three independent experiments; ND, not detected.

In the mitochondria, it was similar as in the whole cells (0.21), while in the PM it accounted for 2.7 [15]. Just for comparison, in the standard laboratory strain it accounts for 0.20 in the mitochondria and 3.31 in the PM [26]. Markedly higher S/PL ratio in the PM could be explained by the need of the cell to be protected by a more condensed cell membrane with decreased permeability, which can be achieved by sterol incorporation. Steryl esters and triacylglycerols were identified in the brewer's yeast, as well, but their concentration was too low to be determined. The same was with diacylglycerols and free fatty acids in both yeasts.

Fatty acids

The fatty acid composition of the baker's yeast mitochondria was very simple, containing only 7 FAs, which was similar to the brewer's yeast, but they differed markedly in percentages (Table 4).

In the baker's yeast, monounsaturated acids predominated significantly: oleic acid (18:1) accounted for 42% and palmitoleic (16:1) for 37% which corresponds to 79% of the yeast in standard growth conditions [10]. Very long chain fatty acid, C26:0, which was identified in a rather high concentration in the PM [17], was identified in the mitochondria as well, but its concentration was very low (12.4% and 0.5%, respectively). According to some authors FA profile has no influence on *S. cerevisiae* freeze-tolerance, while according to others it has [32]. However, there is consent about the importance of the unsaturation of the whole cells and of the unsaturation and FA profile of the PM – higher the degree of unsaturation, higher freeze-tolerance [3, 33]. Accordingly, the analysed yeast belongs to the freeze-tolerant ones [15]. There are no published data about the correlation between freeze-tolerance and mitochondrial FA composition. However, in the mitochondria of the analysed baker's yeast, the FA composition, unsaturation index and C16/C18 ratio were similar to the values of the whole cells, which suggests direct correlation. FA composition of the mitochondria differed markedly from that of the PMs, but had similar unsaturation index and C16/C18 ratio. In the case of this yeast, it is not clear if the unsaturation index solely is important for the freeze- and stress-tolerance in general, or if the specific FA pattern of the organelles is also involved. In the brewer's yeast mitochondria, unsaturated acids also prevailed, accounting for 55%. The unsaturation index was rather low (0.59), which was - like the low content of ergosterol - quite unusual for aerobic conditions. Another characteristic feature was high predominance of C16 acids (69% of total fatty acids).

CONCLUSION

It can be concluded that estimation of the lipid composition of industrial yeasts based on the results obtained in laboratory conditions even for the same strain is very uncertain and that the analysis of the yeast grown in specific conditions should be performed. Another important conclusion is that the bottom-fermenting brewer's yeast, adapted to unfavourable conditions during beer fermentations, retains similar cell composition in the first step of recycling process (propagation) although it is conducted in favourable conditions.

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CONFLICT OF INTEREST STATEMENT

Hereby we declare that there is no conflict of interest regarding our manuscript.

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