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Lipid Composition of Brewer's Yeast

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Dedicated to the memory of Professor Vera Johanides

Summary

The lipid fraction of the *Saccharomyces uvarum* yeast obtained as a by-product of industrial beer production was analyzed in order to evaluate nutritive and potential pharmacological properties of the yeast biomass. Total lipids accounted for 4.4 % of dry biomass, 58 % of which were neutral lipids. Mono-, di- and triacylglycerols, squalene, lanosterol, ergosterol, steryl esters and free fatty acids were identified in the neutral lipid fraction. Squalene was by far the most abundant one making up 56 % of neutral lipids and 33 % of total lipids, respectively. The main phospholipid component was phosphatidylcholine. Saturated fatty acids predominated in the composition of total and neutral lipids, while in the polar lipid fraction the contents of saturated and unsaturated fatty acids were almost the same. In all three fractions palmitic acid was present in the highest amount. Fatty acid composition of phospholipid classes differed significantly. Although brewer's yeast does not belong to the so-called lipid yeasts, the high content of squalene gives reason for additional exploitation of this by-product of the brewing industry.

Key words: brewer's yeast, ergosterol, squalene, phospholipids, fatty acids

Introduction

Lipids are energy storage molecules and important structural components of all eukaryotic cell membranes. Relatively recent studies indicated that specific membrane lipids and their intermediates are involved in signalling pathways and have essential roles in the regulation of vital cellular processes (*1–3*). For this reason, considerable interest is focused now on the applications of these bioactive molecules in various fields concerning human health. Consequently, researches are concentrated on finding their new economical sources.

Yeasts, as well as other microorganisms, contain a variety of lipids, whose content and composition can be effected by growth conditions (*4–6*) and/or genetically, which makes them suitable for the production of highly specific lipids. In addition, they have few advantages over other microorganisms: they are eukaryotes, generally non-toxic and they have been traditionally used in the food industry. Industrial yeasts are becoming more and more significant nowadays as suppliers of enzymes,

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flavors, essences and proteins, and there is no reason why they could not serve as lipid sources as well.

Although by the new taxonomy and nomenclature of yeasts (*7*) many former species of brewing yeasts merged in the new definition of one species of *S. cerevisiae*, traditionally two types of the same species have been used in beer production. The first one is the ale type or the top fermenting yeast (temperature of fermentation: 20 °C). The second one is the lager type or the bottom fermenting yeast (formerly *S. uvarum*; temperature 8–15 °C). Both types are by-products of the beer industry. They are used for animal-feed mainly, but also partially end in the waste waters (*8*). Until now it has been shown that the brewery liquid waste is a good substrate for producing biomass, vitamins and enzymes. For economical, as well as ecological reasons, it would be useful to find some additional applications for surplus/waste brewer's yeast. Although the non–brewing yeasts of *Saccharomyces* strain are among the most intensively studied microorganisms, the lipid analysis of brewer's yeast obtained as a by-product of brewing industry has not been done yet. Therefore, for better evaluation of nutritive and potentially pharmacological properties of brewer's yeast a detailed analysis of lipid fraction was performed. The composition of neutral lipids and phospholipids was determined, as well as the composition of fatty acids in total cell lipids, polar and neutral fraction and main phospholipid classes. If found that the yeast contains significant amounts of biologically active lipids, it would be the basis for the elaboration of feasibility study of their economical isolation and, consequently, a better exploitation of the yeast biomass.

Material and Methods

Yeast strain

Saccharomyces uvarum (bottom-fermenting brewer's yeast) was used throughout this study. It was obtained as a by-product of beer fermentation in Zagreb brewery, Zagreb, Croatia.

Lipid analysis

Extraction of lipids

Prior to extraction, biomass was washed thoroughly twice with water and once with 0.1 % $NaHCO_3$ (in order to eliminate bitter components of hops), followed by centrifugation at 3000 × g for 5 min. Cells were resuspended in water and disrupted with glass beads in cell homogeniser. Total cell lipids were extracted from the wet biomass by the method of Folch *et al.* (*9*).

Separation and quantification of polar and neutral lipids

Polar and neutral lipid fractions were separated from total lipid extract by thin-layer chromatography (TLC). Chromatograms were developed on silica gel 60 plates (Merck, Darmstadt, Germany) 20 × 10 cm, 0.2 mm, using petrolether/diethyleter/acetic acid (70:30:2, by vol.). Chromatograms were developed up to 8 cm, just to allow the separation of polar and neutral lipids. At the edge of the plate, a small quantity of the sample was applied separately. That part of the chromatogram was cut off after development and the bands were visualised by spraying with 50 % sulphuric acid followed by heating for 30 min at 120 °C. Polar lipids remained at the start line, while neutral lipids were taken along the plate. The position of the bands on the preparative part of the plate was determined by comparison with their position on the small, visualised part of the plate. The bands were scraped off the plate and those of neutral lipids were pooled together. Lipids were recovered by dissolving in chloroform/methanol (2:1, by vol.) and determined gravimetrically after the removal of silica gel and solvents.

A sample of polar and neutral lipid fractions, obtained as described, was used for fatty acid analysis.

Analysis of neutral lipids

Neutral lipids were separated from a sample of total lipid extract by two-step TLC on silica gel 60 plates 20 x 10 cm, 0.2 mm. Lipid extracts and standards were applied by sample applicator (Linomat IV, CAMAG, Muttenz, Switzerland). The plates were developed by using petrolether/diethyleter/acetic acid (20:20:0.8, by vol.) up to 1/3 of a plate as the first and petrolether/diethyleter (39.2:0.8, by vol.) up to 2/3 of plate as the second solvent system. Ergosterol and steryl esters were quantified by direct densitometry at 275 nm using ergosterol as standard. For the determination of triacylglycerols and squalene, the bands were visualised by postchromatographic derivatization. With a chromatogram immersion device (CAMAG) plates were dipped for 4 s into developing reagent (0.63 g $MnCl_2 \cdot 4H_2O$, 60 mL H_2O, 60 mL methanol, 4 mL H_2SO_4 conc.), briefly dried and heated for 30 min at 100 °C. Lipid components were quantified by scanning at 400 nm.

Analysis of phospholipids

Total phospholipids were quantified spectrophotometrically as inorganic phosphorus by using ammonium molybdat and 8-anilino-1-naphthalenesulphonic acid (*10*). Single phospholipid classes were separated by two-dimensional thin-layer chromatography on silica gel 60 plates, 20 × 20 cm, 0.2 mm. Chloroform/methanol/ammonium hydroxide (65:35:5, by vol.) was used as the first solvent system and chloroform/acetone/methanol/acetic acid/water (50:20:10:10:5, by vol.) as the second one. Phospholipids were visualised by iodine staining, scraped off the plate and quantified the same way as total phospholipids.

In order to determine their fatty acid composition, main phospholipid classes were separated by thin-layer chromatography on silica gel 60 plates, 20 x 20 cm, 0.2 mm using chloroform/methanol/water (65:35:5, by vol.). The position of bands was determined by comparison with the standard mixture.

Analysis of fatty acids

Fatty acid composition of total lipids, polar and neutral lipid fractions, as well as that of main phospholipid classes were determined by gas-chromatography of the corresponding methyl esters. Methyl esters were obtained by acid methanolysis of lipid extracts with BF_3/

methanol (*11*). Hewlett Packard 5890 capillary gas chromatograph with flame ionisation detector was used; column HP5 (5 % diphenyl, 95 % dimethylpolysyloxan), temperature programmed from 200 °C to 280 °C at 10 °C/min. Fatty acids were identified by comparison with commercial fatty acid methyl ester standards (NuCheck, Inc., Elysian, Minnesota). The degree of unsaturation, expressed as unsaturation index according to Kates and Hagen (*12*), was calculated as follows:

Δ/mol = [% monoene + 2 (% diene) + 3 (% triene)] / 100.

Results

Lipid content and composition of main lipid classes

The content of total lipids was 4.4 % of dry cell biomass. The relative proportions of neutral and polar lipid fractions calculated from the yields of silica gel preparative thin-layer chromatography were 58 and 42 %, respectively.

The analysis of neutral lipid fraction by TLC revealed the presence of mono-, di- and triacylglycerols, squalene, lanosterol, ergosterol, steryl esters and free fatty acids. Triacylglycerols, squalene, ergosterol and steryl esters were quantified and they made up 51 % of total lipids. The main characteristic of neutral lipid fraction was an extremly high squalene content (33 % of total lipids, or 1.4 % of dry cell biomass). Triacylglycerols and ergosterol accounted each for less than 10 % of total lipids (Table 1).

Table 1. Mass fraction of the main classes of neutral lipids in total cell lipids and dry biomass of brewer's yeast; data represent the mean values of three independent experiments

	w(cell lipids) / %	*w*(dry biomass) / %
Triacylglycerols	8.6	0.38
Squalene	32.6	1.43
Ergosterol	9.5	0.42
Steryl esters	0.7	0.03
Total	51.4	2.26

Table 2. Phospholipid composition of brewer's yeast; data represent the mean values of three independent experiments

	w(total phospholipids) / %
N,N-dimethylphosphatidyl-ethanolamine	0.2
Phosphatidylethanolamine	21.5
Phosphatidylinositol	21.6
Phosphatidylcholine	33.1
Phosphatidylserine	5.2
Phosphatidic acid	6.0
Cardiolipin	2.6
Lysophospholipids	1.2
Unidentified	8.6

The amount of phospholipids was somewhat lower than that of neutral lipids (31 % of total cell lipids). Among them, phosphatidylcholine was predominant (33 % of total phospholipids). Other major components were phosphatidylethanolamine and phosphatidylinositol in almost the same amount (Table 2).

Fatty acid composition of total lipids, polar and neutral lipid fractions

Fatty acid (FA) composition of total lipids, polar and neutral fractions of total lipids and the principal features of their fatty acid profiles are presented in Table 3. The degree of unsaturation is expressed as unsaturation index as indicated in »Material and Methods«. The figures used in the expression represent mass fraction of mono-, di- and trienoic fatty acids, respectively, of total identified fatty acids.

The fatty acid composition of total lipids and neutral lipid fraction were characterized by a preponderance of saturated fatty acids. Palmitic acid (16:0) was the main one in very high concentration (44 % of total FAs of total lipids and 56 % of neutral lipids). Another major fatty acid was palmitoleic acid (16:1). Fatty acids of 16 carbon atoms accounted for 61 % of total FAs of cell lipids and for 72 % of neutral lipid fraction. Fatty acids of 18 carbon atoms were present in much lower concentration, among which stearic acid (18:0) predominated. In the fatty acid composition of polar lipids the ratio between saturated and unsaturated acids was close to 1. Palmitic, palmitoleic and oleic acids were the major constituent fatty acids in polar lipids; these three fatty acids made up 80 % of all acids.

Table 3. Fatty acid composition of total cell lipids, polar and neutral lipid fractions of brewer's yeast (expressed as mass fraction of identified fatty acids)

	w / %		
Fatty acid (FA)	Total lipids	Polar lipids	Neutral lipids
12:0	4.5	2.0	4.2
12:1	2.8	–	–
14:0	3.0	0.9	1.6
14:1	2.1	0.3	0.4
16:0	44.2	33.3	55.6
16:1	16.9	27.8	16.6
18:0	13.9	12.3	13.1
18:1	7.3	20.9	4.1
18:2	2.9	–	3.1
18:3	–	0.5	0.7
24:1	–	0.8	–
26:0	2.4	1.2	0.6
Saturated FA	68.0	49.7	75.1
Nonsaturated FA	32.0	50.3	24.9
Unsaturation index	0.35	0.51	0.29
C_{16} / C_{18}	2.5	1.8	3.5

Fatty acid composition of main phospholipid classes

Table 4 shows the constituent fatty acids of main phospholipid classes separated by one-dimensional TLC. Under conditions described in »Material and Methods« phosphatidylserine (PS) and phosphatidylinositol (PI), as well as phosphatidylethanolamine (PE) and cardiolipine (CL) comigrated. Therefore, the compositions of these combined classes (PS/PI, PE/CL) are presented in the table. Major fatty acids were palmitic (16:0), palmitoleic (16:1), stearic (18:0) and oleic (18:1) acid in all classes, but their amounts and ratios differed significantly.

Table 4. Fatty acid composition of the main phospholipid classes of brewer's yeast; PA, phosphatidic acid; PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; CL, cardiolipin (expressed as percentages of identified fatty acids)

	w / %			
Fatty acid (FA)	PA	PS / PI	PC	PE / CL
12:0	8.2	10.8	1.8	3.4
12:1	8.9	10.3	2.4	3.8
14:0	7.2	8.6	2.0	-
14:1	8.0	9.1	2.1	-
16:0	15.9	22.9	38.0	32.4
16:1	16.0	8.0	1.3	26.0
18:0	9.9	9.1	19.5	17.0
18:1	23.7	11.1	26.8	17.4
18:2	-	4.6	4.6	–
20:2	2.2	5.5	1.5	–
Saturated FA	41.2	51.4	61.3	52.8
Unsaturated FA	58.8	48.6	38.7	47.2
Unsaturation index	0.61	0.59	0.45	0.47
C_{16} / C_{18}	1.0	1.3	0.8	1.7

Discussion

There are only few data on the lipid composition of brewer's yeasts, but even less regarding *Saccharomyces uvarum* and practically none on *S. uvarum* obtained as a by-product in the industrial process. On the contrary, the yeast *S. cerevisiae* has been thoroughly studied since it serves as an experimental model organism to study biochemical, cell biological and molecular biological aspects of lipid synthesis. The content of total lipids in *S. cerevisiae* is ranging from 3.5 to 14.7 % depending on the growth stage and cultivation conditions (*4*,*5*,*13*,*14*). Vendramin-Pintar *et al.* (*15*) have analyzed lipids of two brewer's strains belonging to the *S. cerevisiae* species cultivated in the laboratory fermentor under aerobic conditions and found out that they contained 6.6 % lipids in the dry biomass. The content of total lipids in the *S. uvarum* yeast analyzed in this work was lower (4.4 %). It could be explained by decreased rate of lipid synthesis due to lowered ATP/ADP-ratio (*16*,*17*), since it was obtained as a by-product of anaerobic beer fermentation. On the other hand, the mass ratio of polar to neutral lipids was 0.74, which is similar to the data published by Vendramin-Pintar *et al.* (*15*).

Neutral lipids were major part of total lipids accounting for 58 % of total lipids, out of which triacylglycerols, squalene, ergosterol and steryl esters made up 51 %. A notable feature of neutral lipid composition was high squalene content not only in respect to the other components, but absolutely as well (33 % of total lipids). Triacylglycerols and ergosterol were present in rather low amount, each making up less than 10 % of total lipids (Table 1). High content of squalene is another consequence of the anaerobic growth conditions, since one of the essential steps in sterol synthesis is the oxygen-requiring conversion of squalene to squalene epoxide (*18*). In the absence of oxygen squalene is accumulated and for the same reason, the content of steryl esters, as sterol storage lipids, is very low. It has been proved that squalene has beneficial effects on the immune system and that is why it is used in the cancer therapy as well. The effect is related to its function as oxygen carrier. Its derivatives, squalane and squalamine, are successfully used in cosmetics for skin care and in the treatment of bacterial infections, respectively. For that purposes squalene is extracted from the olive oil or from the liver of deep sea sharks. Not only are both sources quite expensive, but the latter one is also unpopular for the ecological reasons. Therefore, the finding related to the high content of squalene in brewer's yeast is interesting from the standpoint of application in medicine and cosmetics.

There are no data in the literature on the content of ergosterol and steryl esters of brewer's yeast obtained in the process of industrial beer fermentation. Data relating to the yeasts grown under laboratory conditions vary a lot, for example for *S. cerevisiae* and *Zigosaccharomyces rouxii* the content of these components was from 0.5 to 5 %, depending on the strain, growth conditions and the extraction method used. Therefore, free sterols and steryl esters could be considered as the components of yeast cell whose content varies the most (*19*–*24*).

Triacylglycerols and steryl esters, serving as reserve lipid forms, are almost exclusively stored in the lipid particles of the yeast cell (*25*) and hence may also be used as their markers (*26*). Because of their high hydrophobicity they are not structural components of the membranes, while on the other hand, ergosterol is their essential component along with different classes of phospholipids and sphingolipids. Consequently, the content of ergosterol in total lipids reflected its content in the plasma membrane and mitochondria where it was also found to be very low (manuscript in preparation).

TLC of neutral lipid fraction revealed the presence of significant amounts (not quantified) of diacylglycerols and free fatty acids, which is in agreement with the published data (*19*,*27*). Diacylglycerols (DAG), generally derived from the degradation of phospholipids by catalysis of phospholipases type C, have few important functions, as it has been demonstrated so far. They are involved in cell signaling pathway and serve as the substrate for the synthesis of triacylglycerols (*28*). Free

fatty acids are also products of phospholipid breakdown in yeasts and according to Watanabe and Takakuwa (*29*) the content of these two lipid classes increases in post-fermentation period. On the other hand, Slaughter and Minabe (*13*) have reported the decrease in phospholipid content in that period, accompanied by the increase in the content of triacylglycerols.

Total phospholipids accounted for 31 % of total cell lipids which is at the low end of the range published for *S. cerevisiae* (31–49 %) and *T. delbrueckii* (34–50 %) strains by Murakami *et al.* (*27,30*). Phosphatidylcholine was the main one, followed by phosphatidylethanolamine and phosphatidylinositol in almost the same amount (Table 2). The results are partially in agreement with the literature (*27,31*) according to which phospholipid concentrations usually decrease in the following order: phosphatidylcholine > phosphatidylethanolamine > phosphatidylinositol > phosphatidylserine. Although phospholipids are indispensable as bulk components of yeast membranes, it is not yet clear, except for phosphatidylinositol, whether individual phospholipid classes are essential or not (*28*). Depending on growth conditions their ratio varies since phospholipids make an important part of the adaptation mechanism and reflects changes in extracellular environment. For that reason the concentration of cardiolipin, phospholipid of inner mitochondrial membrane, was found to be very low, since mitochondria are poorly developed in anaerobic conditions. Another feature of phospholipid composition of analyzed brewer's yeast was a relatively high content of phosphatidylserine, which was found to be a characteristic for yeasts with high ethanol tolerance (*32–34*). Relatively high percentage of phosphatidic acid (6 %) could imply intensified degradation processes of phospholipids (*13, 35,36*) since it is the key intermediate in the lipid metabolism (*37*).

Molar ratio of ergosterol to phospholipids was 0.60 in total lipids which is close to the values 0.67 and 0.5 obtained for freeze-sensitive strains of *S. cerevisiae* (*27*) and *Torulaspora delbrueckii* (*S. rosei*) (*30*), respectively. The values for freeze-resistant strains of the same yeasts range from 0.36 for *S. cerevisiae* to 0.4 for *T. delbrueckii* (*27,30*), respectively. These data prove the relationship between membrane composition and adaptation capability of the yeasts to temperature changes.

Fatty acids, with chain length ranging from C_{12} to C_{26}, were identified in the total lipids of brewer's yeast (Table 3). Saturated fatty acids prevailed accounting for 68 %. Palmitic acid (16:0) was the principal fatty acid making up 44 % and palmitoleic acid (16:1) was the second one (17 %) thus making the ratio between C_{16} and C_{18} rather high (2.54). Moreira da Silva *et al.* (*38*) reported on the fatty acid composition of a number of industrial strains, among them *S. uvarum*, and in all of them unsaturated fatty acids, palmitoleic and oleic acid, prevailed. Vendramin-Pintar *et al.* (*15*) had similar results for brewer's yeast of *S. cerevisiae* strain, while Šajbidor *et al.* (*39*) found in *S. uvarum* palmitoleic and palmitic acid as major fatty acid components. When the comparison of the results presented in this paper with previously published data is done, it should be pointed out that the later ones were obtained for the yeasts grown in aerobic conditions of the laboratory fermentor, and with the yeast extract as the source of unsaturated fatty acids. Yeast cells respond to environmental changes by the complex regulatory system. The regulation of the membrane fluidity and permeability by changing the fatty acid composition of membrane lipids has an important role (*40–43*). Therefore, the fatty acid composition depends strongly on the composition of growth medium and on the growth conditions (*44–47*). The brewer's yeast analyzed in this work was grown under anaerobic conditions that lead to the inhibition of desaturase system, since double bond is introduced into acyl-CoA by NADH- or NADPH-dependent oxydase in the presence of molecular oxygen (*28,48,49*). On the other hand, unsaturated fatty acids are necessary because they increase membrane fluidity thus increasing ethanol tolerance of the yeast cells (*41,50,51*). Regarding growth conditions and relating needs of the cell for adaptation, the differences between fatty acid composition of neutral and polar lipids were anticipated. In the composition of neutral lipids, which mainly serve as lipid storage molecules, saturated fatty acids prevailed making up even 75 %. In the polar lipids, mainly located in membranes and having a variety of functions, the contents of saturated and unsaturated fatty acids were nearly the same. Palmitic acid was the main fatty acid in both fractions, but its content was almost two-fold higher in neutral one. For that reason the ratio C_{16}/C_{18} was significantly lower in polar lipids, implying the need for longer fatty acids in membrane structures. Two very long chain fatty acids (VLCFAs) were identified in polar lipids, tetracosenoic (24:1) and hexacosanoic acid (26:0). VLCFAs are essential since they form a structurally important part of the ceramide moiety of sphingolipids and the lipid domain of glycosylphosphatidylinositol (GPI)-anchored proteins. It is interesting to note that hexacosanoic acid has recently attracted attention, due to findings proving its intriguing functions. It was observed that the strains, which survived without synthesising ceramide, produce novel C_{26} fatty acid-substituted inositol glycerophospholipids that structurally mimic sphingolipids. Also, it is suggested that its synthesis is directly or indirectly required for the stabilisation of nuclear membrane (*52–54*).

The fatty acid composition of different phospholipid classes differed significantly (Table 4). Palmitic acid was the main fatty acid in phosphatidylcholine and in two combined classes, PS/PI and PE/CL, while oleic acid was the most abundant in phosphatidic acid. It is interesting to note that in the fatty acid composition of PS/PI-class all identified fatty acids were present in relatively high amounts. In PE/CL-class only six fatty acids were detected, and in phosphatidylcholine three fatty acids (16:0, 18:0 and 18:1) accounted for 84 %.

Conclusion

Although the lipid content of the analyzed brewer's yeast was rather low, a high portion of squalene in the cell lipids could be the reason for additional exploitation of this by-product of the beer production.

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References

1. Y. Takai, A. Kishimoto, U. Kikkawa, T. Mori, Y. Nishizuka, *Biochem. Biophys. Res. Com. 91* (1979) 1218–1224.
2. S. I. Hakomori, *Annu. Rev. Biochem. 50* (1981) 733–764.
3. S. Spiegel, D. Foster, R. Kolesnick, *Curr. Opin. Cell Biol. 8* (1996) 159–167.
4. A. M. Ramsay, L. J. Douglas, *J. Gen. Microbiol. 110* (1979) 185–191.
5. J. B. M. Rattray: *Microbial Lipids*, Academic Press, London (1988).
6. J. Šajbidor, *Crit. Rev. Biotechnol. 17* (1997) 87–103.
7. N. J. W. Kreger – van Rij: *The Yeasts: A Taxonomic Study*, Elsevier, Amsterdam (1984).
8. D. Carfas, N. Millán, *Arch. Latinoamer. Nutr. 46* (1996) 67–70.
9. J. Folch, M. Lees, G. H. Sloane-Stanley, *J. Biol. Chem. 226* (1957) 497–509.
10. R. M. Broekhuyse, *Biochim. Biophys. Acta, 152* (1968) 307–315.
11. M. L. Vorbeck, L. R. Mattick, F. A. Lee, C. S. Pederson, *Anal. Chem. 33* (1961) 1512–1514.
12. M. Kates, P. O. Hagen, *Can. J. Biochem. 42* (1964) 481–488.
13. J. C. Slaughter, M. Minabe, *J. Sci. Food Agric. 65* (1994) 497–501.
14. J. Šajbidor, M. Čertik, J. Grego, *J. Chromatogr. A, 665* (1994) 191–195.
15. M. Vendramin-Pintar, K. Jernejc, A. Cimerman, *Food Biotechnol. 9* (1995) 207–215.
16. C. Abel, F. Linz, T. Scheper, K. Schügerl, *J. Biotechnol. 33* (1994) 183–193.
17. O'Connor-Cox, E. J. Lodolo, B. C. Axcell, *J. Inst. Brew. 102* (1996) 19–25.
18. L. Jahnke, H. P. Klein, *J. Bacteriol. 155* (1983) 488–492.
19. K. Hunter, A. H. Rose: *The Yeasts*, Academic Press, New York, Vol. 2 (1971).
20. B. Behalova, P. Hozak, M. Blahova, V. Šillinger, *Folia Microbiol. 37* (1992) 442–449.
21. B. Behalova, M. Blahova, V. Behal, *Folia Microbiol. 39* (1994) 287–290.
22. G. Maggiorotto, R. di Stefano, *Annali dell'Istituto sperimentale per l'enologia Asti, 23* (1992) 93–103.
23. M. Lamačka, J. Šajbidor, *Biotechnol. Tech. 11* (1997) 723–725.
24. K. Hosono, *J. Gen. Microbiol. 138* (1992) 91–96.
25. M. K. Clausen, K. Christiansen, P. K. Jensen, O. Behnke, *FEBS Lett. 43* (1974) 176–179.
26. E. Zinser, C. D. M. Sperka-Gottlieb, E.-V. Fasch, S. D. Kohlwein, F. Paltauf, G. Daum, *J. Bacteriol. 173* (1991) 2026–2034.
27. Y. Murakami, K. Yokoigawa, F. Kawai, H. Kawai, *Biosci. Biotech. Biochem. 60* (1996) 1874–1876.
28. G. Daum, N. D. Lees, M. Bard, R. Dickson, *Yeast, 14* (1998) 1471–1510.
29. Y. Watanabe, M. Takakuwa, *Agric. Biol. Chem. 47* (1983) 195–201.
30. Y. Murakami, K. Yokoigawa, H. Kawai, *Appl. Microbiol. Biotechnol. 44* (1995) 167–171.
31. D. R. Voelker, *Microb. Rev. 55* (1991) 543–560.
32. P. Mishra, R. Prasad, *J. Gen. Microbiol. 134* (1988) 3205–3211.
33. Z. Ciesarová, D. Šmogrovičová, *Chem. Listy, 90* (1996) 365–370.
34. Z. Ciesarová, D. Šmogrovičová, Z. Dömény, *Folia Microbiol. 41* (1996) 485–488.
35. M. Takakuwa, Y. Watanabe, *Agric. Biol. Chem. 45* (1981) 2167–2173.
36. F. Piton, M. Charpentier, D. Troton, *Am. J. Enol. Vitic. 39* (1988) 221–226.
37. K. Athenstaedt, G. Daum, *Eur. J. Biochem. 266* (1999) 1–16.
38. M. Moreira da Silva, M. Malfeito-Ferreira, V. Loureiro, *J. Inst. Brew. 100* (1994) 17–22.
39. J. Šajbidor, E. Breierová, A. Kocková-Kratohvilová, *FEMS Microbiol. Lett. 58* (1989) 195–198.
40. H. Cook: *Physiological regulation of membrane fluidity*, Academic Press, London (1988).
41. J. Šajbidor, J. Grego, *FEMS Microbiol. Lett. 93* (1992) 13– 16.
42. R. K. Khaware, A. Koul, R. Prasad, *Biochem. Mol. Biol. Int. 35* (1995) 875–880.
43. E. B. Cahoon, L. A. Mills, J. Shanklin, *J. Bacteriol. 178* (1996) 936–939.
44. P. Mishra, R. Prasad, *Appl. Environ. Microbiol. 30* (1989) 294–298.
45. J. Rupčić, B. Blagović, V. Marić, *J. Chromatogr. A, 755* (1996) 75–80.
46. J. Rupčić, M. Mesarić, V. Marić, *Appl. Microbiol. Biotechnol. 50* (1998) 583–588.
47. J. Rupčić, Č. Milin, V. Marić, *System. Appl. Microbiol. 22* (1999) 486–491.
48. A. G. Mitchell, C. E. Martin, *J. Biol. Chem. 270* (1995) 29766–29772.
49. L. C. Stewart, M. P. Yaffe, *J. Cell Biol. 115* (1991) 1249–1257.
50. C. J. Panchal, G. G. Stewart: *Current development in yeast research*. Pergamon Press, Toronto (1980).
51. R. Prasad, A. H. Rose, *Yeast, 2* (1986) 205–220.
52. A. G. Mitchell, C. E. Martin, *J. Biol. Chem. 272* (1997) 28281–28288.
53. R. Schneiter, S. D. Kohlwein, *Cell, 88* (1997) 431–434.
54. R. L. Lester, G. B. Wells, G. Oxford, R. C. Dickson, *J. Biol. Chem. 268* (1993) 845–856.

Sastav lipida pivskog kvasca

Sažetak

Lipidna frakcija pivskog kvasca *Saccharomyces uvarum*, dobivena kao nusproizvod u proizvodnji piva, analizirana je da bi se procijenila prehrambena i farmakološka vrijednost kvaščeve biomase. U suhoj biomasi bilo je 4,4 % ukupnih, od čega je 58 % neutralnih lipida. U frakciji neutralnih lipida identificirani su mono-, di- i triacilgliceroli, skvalen, lanosterol, ergosterol, sterolni esteri i slobodne masne kiseline, od čega je bilo najviše skvalena, tj. njegov je udjel iznosio 56 % od neutralnih, odnosno 33 % od ukupnih lipida. Glavni je fosfolipidni sastojak bio fosfatidilkolin. U sastavu ukupnih i neutralnih lipida prevladale su zasićene masne kiseline, dok je u polarnoj frakciji udjel zasićenih i nezasićenih masnih kiselina bio podjednak. U sve tri frakcije prevladavala je palmitinska kiselina. Bitno se razlikovao sastav masnih kiselina pojedinih fosfolipidnih frakcija. Iako pivski kvasac ne pripada u tzv. lipidne kvasce, velik udjel skvalena omogućava dodatno korištenje ovog nusproizvoda pivarske industrije.