Promjene udjela i sastava fosfatidilkolina i fosfatidiletanolamina tijekom vrenja pivskoga kvasca Saccharomyces cerevisiae

Čanadi Jurešić, Gordana; Blagović, Branka; Rupčić, Jasminka

Source / Izvornik: **Food Technology and Biotechnology, 2009, 47, 246 - 252**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:184:670764

ISSN 1330-9862
(FTB-2187)

original scientific paper

Alterations in Phosphatidylcholine and Phosphatidylethanolamine Content During Fermentative Metabolism in *Saccharomyces cerevisiae* Brewer's Yeast

Gordana Čanadi Jurešić, Branka Blagović and Jasminka Rupčić*

Department of Chemistry and Biochemistry, School of Medicine, University of Rijeka, Braće Branchetta 20, HR-51000 Rijeka, Croatia

Received: November 10, 2008
Accepted: March 9, 2009

Summary

During beer production and serial recycling, brewer's yeasts are exposed to various stress factors that, overpowering the cellular defence mechanisms, can impair yeast growth and fermentation performance. It is well known that yeast cells acclimatize to stress conditions in part by changing the lipid composition of their membranes. The main focus of this study is the effect of stressful fermentation conditions on two phospholipid species, phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEtn), in *Saccharomyces cerevisiae* bottom-fermenting brewer's yeast. For this purpose the content and fatty acid profile of these major classes of phospholipids have been compared, as well as their ratio in the whole cells of the starter culture, non-stressed yeast population, and the first three recycled yeast generations. The stressed yeast generations showed an increased mass fraction of PtdCho and a decreased mass fraction of PtdEtn, which led to an increased PtdCho/PtdEtn ratio in the recycled cells as compared to the non-stressed yeast culture. The most pronounced variation of PtdCho/PtdEtn ratio was found in the second yeast generation, yielding a 78 % increase with respect to the starter culture. Variations in the content of both, PtdCho and PtdEtn, were accompanied by a higher mass fraction of unsaturated fatty acids in both phospholipid species (palmitoleic acid in PtdCho, and palmitoleic and oleic in PtdEtn) and by the increased ratio of C_{16}/C_{18} acids in PtdCho. The results suggest that both phospholipid species, including their fatty acids, are highly involved in the adaptation of brewer's yeast to stressful fermentation conditions.

Key words: brewer's yeast, *Saccharomyces cerevisiae*, phospholipids, phosphatidylcholine, phosphatidylethanolamine, fatty acids, stress tolerance, recycling

Introduction

Phospholipids (PL) are major components of cellular membranes and play a prominent role in defining the properties of unicellular organisms such as *Saccharomyces cerevisiae* yeast. Both the phospholipid class composition, defined by the nature of the polar head group, and the composition of the fatty acyl chains of the phospholipid species determine the physical properties of membranes, and are subject to adaptation in response to environmental changes (*1–3*). These physical properties include membrane thickness, intrinsic curvature and fluidity (*3*), which affect membrane permeability and influence the activity of membrane-associated enzymes (*4*), transport mechanisms (*5,6*), and, in the case of fermentative yeast, can affect the viability, fermentation activity and tolerance to ethanol (*7,8*). PtdCho, PtdEtn, phosphatidylinosytol, phosphatidylserine and cardiolipin are the major phospholipids found in the cellular membranes of *S. cerevisiae* (*9*). PtdCho is the main bilayer-forming phos-

*Corresponding author; Phone: ++385 51 651 134; Fax: ++385 51 651 135; E-mail: branka.blagovic@medri.hr

This paper was presented at the Congress of Croatian Society of Biochemistry and Molecular Biology in Osijek, Croatia, September 17–20, 2008

pholipid species because of its large head group and its cylindrical shape, which makes it ideally suited for preserving membrane integrity. In contrast, PtdEtn with its small head group and its cone-like shape is a phospholipid that forms nonbilayer structures (*10*,*11*). The shortening and increased saturation of the PtdEtn acyl chain were shown to decrease the nonbilayer propensity of PtdEtn (*12*). It was suggested that by changing the PtdCho/PtdEtn ratio the yeast cells adjust membrane properties according to the environmental conditions (*11*,*12*).

S. cerevisiae commonly undergoes stress conditions in its natural environment or during various biotechnological applications. The yeast has developed the ability to cope with a variety of stressors (*e.g.* temperature shifts, oxygen deprivation or high ethanol level) using different mechanisms, including changing the lipid profile of its membranes and consequently their physical properties such as fluidity and permeability (*2*,*13–15*). For example, when *S. cerevisiae* cells are subjected to temperature shifts they adapt by increasing the level of unsaturated fatty acids in membranes (*16*). Cells grown under anaerobiosis have a lower level of unsaturated fatty acids than cells grown aerobically, but the anaerobically grown cells exhibit higher membrane fluidity (*17*). Furthermore, aerobic cells with membranes enriched in palmitoleic and oleic acids exhibit higher resistance to both heat and oxidative stress, while in the case of anaerobically grown cells, the most stress resistant ones have membranes enriched in saturated fatty acids (*18*). An increase in the mass fraction of ergosterol and unsaturated fatty acid levels in membrane lipids increases ethanol tolerance (*19*,*20*). Chi and Arneborg (*21*) found that a more ethanol-tolerant strain of *S. cerevisiae* had a higher ergosterol/phospholipid ratio and a higher mass fraction of PtdCho.

Whereas the impact of several stress factors on lipid composition of *S. cerevisiae* under laboratory growth conditions is known rather well, as mentioned above, alternations in the lipid composition induced by the conditions during industrial processes such as beer fermentation are still obscure. Indeed, when brewing strains of *S. cerevisiae* convert the malt wort into beer, they are, in fact, simultaneously and sequentially exposed to various stress factors during fermentation and repeated fermentation cycles including nutrient and oxygen limitation, high ethanol levels at the end of fermentations, pH/temperature fluctuations, and osmotic stress (*22*). For beer production, brewer's yeast must be able to respond to these stress conditions without significant viability loss (*23*). In addition, when yeast is repeatedly used for a series of primary fermentations (recycling in the brewery), the yeast cells are continually transferred from the growth cycle to the stationary phase and back to the growth cycle.

The aim of this study is to determine the effect of the accumulation of several stress factors during fermentation cycle and recycling of yeast cells on phospholipid composition, particularly PtdCho and PtdEtn species, and to clarify if their ratio is involved in the stress tolerance. To address this question, non-stressed starter yeast culture and three recycled yeast generations exposed to stress during beer production in batch fermentation have been used.

Materials and Methods

Yeast strain

The bottom-fermenting brewer's yeast or lager beer yeast of the species *S. cerevisiae* deposited in the Collection of Microorganisms of the University of Weinstephan, Munich, Germany was kindly provided from the brewery BUP, Buzet, Croatia. Aerobically propagated starter culture (designated the zero yeast generation) and cultures of the first three recycled yeast generations (the 1st, 2nd and 3rd) grown in anaerobic fermentations were analysed.

Lipid extraction

The biomass was washed three times with 0.1 % $NaHCO_3$ in order to eliminate bitter components of hops. To ensure efficient lipid extraction, yeast cells were transformed into spheroplasts by digestion of the cell wall using Zymolyase® (Seikagaku, Tokyo, Japan).

For the preparation of spheroplasts, modified method originally developed for the isolation of mitochondria (*24*) was used. Total lipids were extracted from the spheroplasts according to the method of Folch *et al.* (*25*) and determined gravimetrically.

Phospholipid analysis

Total phospholipids (TPLs) were determined in total lipid extract spectrophotometrically as inorganic phosphorus according to the method of Broekhuyse (*26*). Samples of total lipid extracts were digested with the mixture of H_2SO_4 and $HClO_4$ in a volume ratio of 9:1 for 30 min at 180 °C under reflux in a thermo block. The intensity of blue colour developed by ammonium molybdate and 8-anilino-1-naphthalenesulphonic acid was measured at 830 nm. KH_2PO_4 was used as standard, and the concentration of TPLs was calculated by multiplying mass concentration of phosphorus by 25. PL classes were separated by two-dimensional thin layer chromatography on silica gel 60 plates, 20×20 cm, 0.2 mm. Chloroform/methanol/ammonia (in a volume ratio of 13:7:1) was used as the first solvent system and chloroform/acetone/methanol/acetic acid/water (in a volume ratio of 10:4:2:2:1) as the second one. PLs were visualised by iodine staining, scraped off the plate and quantified the same way as TPLs, using factor 24.2, while for phosphatidylinositol factor 27.1 was used.

Fatty acid analysis

Fatty acid (FA) composition was determined by GC analysis of the corresponding methyl esters obtained by acid methanolysis of lipid extracts with BF_3/methanol. GC analyses of FA methyl esters were carried out using an Auto System XL from Perkin-Elmer with flame ionization detector (FID), capillary column SP-2330 (30 m ×0.32 mm×0.2 μm), Supelco, USA, and helium with split injection (100:1) as a carrier gas. The analyses were carried out in programmed temperature mode from 140 to 220 °C at 5 °C/min and then isothermally for 25 min. The injector temperature was 300 °C and 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 individ-

ual FA in total FAs. The degree of unsaturation is expressed as unsaturation index (UI) calculated as follows:

$$UI=(w(\text{monoenoic fatty acids})+2w(\text{dienoic fatty acids}))/100 \quad /1/$$

Statistical analysis

The data were analysed using software Statistica, v. 7.0. Statistical significance was calculated by Mann-Whitney *U* test. Data were expressed as mean±standard deviation. The results were considered statistically significant at $p<0.05$.

Results and Discussion

Phospholipid content and composition

Cell phospholipids accounted for 34–37 % of total cell lipids, which is in accordance with the data published for brewer's yeasts (*27,28*). The main phospholipid constituents of all analysed yeast generations were PtdCho and PtdEtn, accounting for 32–44 and 22–28 mass percentage of total PLs, respectively (Fig. 1), followed by anionic phospholipids phosphatidylinositol, phosphatidylserine, cardiolipin and phosphatidic acid (data not shown).

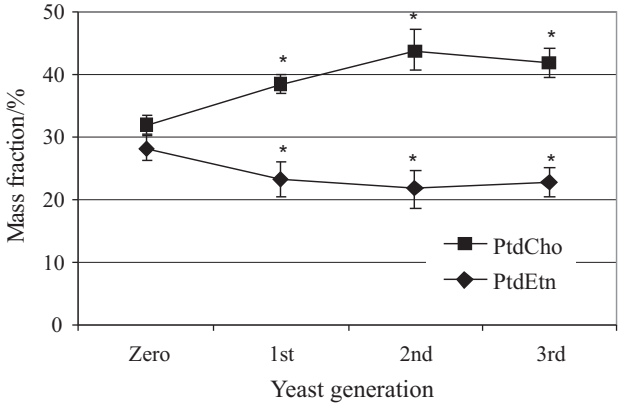

Fig. 1. The mass fractions of PtdCho and PtdEtn in the starter culture and first three recycled yeast generations. The results represent the mean value of at least three independent experiments performed in duplicate; error bars represent standard deviation; *significantly different from the zero generation (Mann-Whitney *U* test, $p<0.05$)

Considerable variations in the PtdCho and PtdEtn content were observed comparing recycled yeast generations to the referent, non-stressed yeast population (Fig. 1). The mass fraction of PtdCho markedly increased in recycled yeast generations, accounting for more than 40 % of the total PLs in the 2nd and 3rd yeast generations, while the mass fraction of PtdEtn significantly decreased in the recycled generations. The most prominent variations of both phospholipid species were between the zero and first recycled generation. Furthermore, the level of PtdCho showed a slight decrease in the 3rd generation, while the PtdEtn level, which was reversed, showed a slight concomitant increase.

PL composition of yeast generations individually was in agreement with the literature data for laboratory strains of *S. cerevisiae* (*29*) regarding the order of the main PL classes. However, our results differed significantly from the data published for two industrial strains of brewer's yeast grown aerobically in the laboratory conditions and harvested in the exponential phase (*27*). As for PtdCho and PtdEtn, Vendramin-Pintar *et al.* (*27*) found that the amount of PtdEtn is as high as the amount of PtdCho. Significant differences between the mass fractions of PtdCho and PtdEtn found in our study can be attributed to the different yeast strain and growth conditions, but also to the different growth phase (*30*). According to some authors, an increased concentration of PtdCho correlates with an increased ethanol tolerance (*21,27*), which supports our results regarding recycled yeast populations exposed to high ethanol levels. Mass fraction profile of PtdCho had a characteristic arch shape with the value in the 3rd generation shifting towards the value in the zero generation, which was aerobically propagated. The profile of PtdEtn followed the same shape, only reversed. Taking into consideration that the 3rd generation is preferred in many breweries, such characteristic shift could be attributed to the adaptation to stressful conditions, which the brewer's yeast has developed with recycling. Because of their highly interconnected metabolic pathways, the reversed profiles suggest the involvement of both species in the same adaptive mechanism, possibly the one hypothesised by de Kruijff (*11*). According to de Kruijff, the cells adjust their membrane properties to environmental conditions, among other mechanisms, by changing the ratio of PtdCho/PtdEtn. PtdCho is a major bilayer-forming lipid because of its large head group that gives a cylindrical shape to its molecule. On the other hand, PtdEtn characterized by a small head group and its cone-like shape is a lipid forming nonbilayer structures. An increase in the synthesis of PtdCho results in a more stable bilayer, while its decrease results in the loose packing found in disordered membranes. In addition, due to the different molecular shape, PtdCho and PtdEtn have a different influence on the conformation of transmembrane proteins and consequently on the membrane organization and function (*11,12*).

Literature data for the PtdCho/PtdEtn ratio in the whole cells of *S. cerevisiae* differ quite a lot depending on the strain, growth phase and growth media (*21,27, 28,30,31*). In the aerobically propagated starter culture analysed in our study, the ratio of PtdCho/PtdEtn was only slightly higher than in the brewer's yeasts analyzed by Chi and Arneborg (*21*) and Vendramin-Pintar *et al.* (*27*) (1.1 *vs.* 0.9 and 1.0, respectively). However, it was significantly higher in all recycled generations (Fig. 2), which is in agreement with our previously reported results for recycled brewer's yeast of another industrial strain (*28*). High PtdCho/PtdEtn ratio indicates the formation of a compact, more stable membrane bilayer induced by changes in extracellular conditions during beer fermentation. Partially, it was due to the growth in a medium containing ethanol, since such alterations have been recognized as one of the ways in which yeast cells enhance ethanol tolerance. According to Chi and

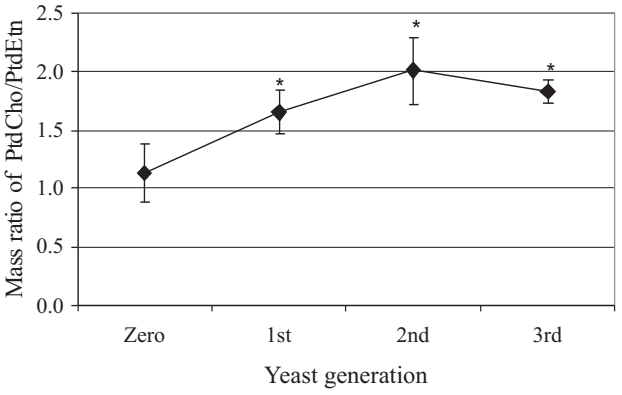

Fig. 2. The mass ratio of PtdCho/PtdEtn in the starter culture and the first three recycled generations. The results represent the mean value of at least three independent experiments performed in duplicate; error bars represent standard deviation; *significantly different from the zero generation (Mann-Whitney *U* test, $p<0.05$)

Arneborg (*21*), the more ethanol-tolerant strain contains a higher mass fraction of PtdCho and a lower mass fraction of PtdEtn than the less ethanol-tolerant strain, resulting in PtdCho/PtdEtn ratio of 1.5 and 0.9, respectively. Observing the changes in PtdCho/PtdEtn ratio related to the growth phase, Homann *et al*. (*32*) found only small variations. On the contrary, using different growth media and taking into account more time points, Janssen *et al*. (*30*) found significant changes in PL composition during the transition to the stationary phase, including an increased PtdCho level at the expense of the PtdEtn level, which is consistent with our results. The variations of PtdCho/PtdEtn ratio with recycling also form the characteristic arch-shaped profile. It is worth mentioning that some other parameters of the total lipid composition, and even more parameters of the plasma membrane lipid composition of recycled brewer's yeast have similar profile (data not shown).

Fatty acid composition of PtdCho and PtdEtn

In the FA composition of PtdCho and PtdEtn 18 FAs were identified, ranging from C_{12} to C_{26}. Mass fractions of those accounting for more than 0.5 % are presented in Tables 1 and 2. Characteristic features of their FA compositions are presented in Table 3.

Fatty acid compositions of PtdCho and PtdEtn were characterised by a high preponderance of C_{16} acids (68–78 and 76–79 %, respectively), whose mass fractions, at the same time, differed the most between generations. Palmitic acid was the main fatty acid in the starter yeast culture, and palmitoleic ($C_{16:1}$) in all recycled generations. The increase of $C_{16:1}$ concentration in recycled generations of both species was considerable, changing from 28 % in the starter culture to 43 % in PtdCho of the 3rd generation, and 33 to 43 % in PtdEtn, respectively. Consequently, unsaturated fatty acids (UFAs) prevailed in all recycled generations of both species. Observed changes were the most expressed between the zero and 1st recycled generation (Table 3). It is worth mentioning that in total fatty acids (TFAs) as well, C_{16} acids predominate in all generations and UFAs in all recycled generations (data not shown). Since the FA composition of membrane lipids is responsible for the regulation of membrane fluidity and permeability, alterations play an important role in stress tolerance. Therefore, the FA profile depends strongly on the composition of the growth medium and cultivation conditions (*1*,*33*,*34*). Since the synthesis of UFAs in *S. cerevisiae* is strictly aerobic (*29*), as well as the synthesis of ergosterol, another essential membrane component, oxygen deprivation has serious consequences on the cell membranes. Under such unfavourable growth conditions, viability and fermentation performance depend on the yeast's adaptive capability. The results of our FA analysis, relating the increased UFAs in the recycled yeast generations that were harvested at the end of anaerobic beer fermentations, seem contradictory, but they can be explained in the following way. Generally, bottom fermenting brewer's yeasts have

Table 1. Fatty acid composition of PtdCho in the zero and first three recycled generations of brewer's yeast

Fatty acid	Mass fraction of fatty acids/%			
	Yeast generation			
	Zero	1st	2nd	3rd
12:0	0.71±0.09	(0.46±0.12)*	(0.40±0.17)*	(0.37±0.03)*
14:0	1.02±0.40	0.90±0.10	0.84±0.15	0.85±0.09
16:0	40.01±1.35	35.55±3.98	(34.87±2.14)*	(34.95±0.85)*
16:1	28.29±2.90	(38.52±5.01)*	(40.50±4.22)*	(42.58±1.48)*
17:1	0.37±0.15	0.58±0.06	0.54±0.24	(0.70±0.08)*
18:0	10.30±0.76	(8.51±0.33)*	8.52±1.11	(8.24±0.29)*
18:1	10.52±0.49	(8.65±0.42)*	9.10±1.17	(8.77±0.29)*
18:2	2.36±0.66	2.18±0.88	1.63±0.49	(0.77±0.33)*
24:1	3.79±1.30	3.00±1.02	2.19±1.37	(1.88±0.20)*
26:1	0.96±0.16	(0.30±0.07)*	(0.28±0.10)*	(0.22±0.10)*
Others	1.67±0.08	1.35±0.05	1.13±0.03	0.67±0.02

Mean±SD, calculated on the basis of peak areas; the results represent the mean value of at least three independent experiments performed in duplicate; *significantly different from the zero generation (Mann-Whitney *U* test, $p<0.05$)

Table 2. Fatty acid composition of PtdEtn in the zero and first three recycled generations of brewer's yeast

Fatty acid	Mass fraction of fatty acid/%			
	Yeast generation			
	Zero	1st	2nd	3rd
12:0	0.23±0.04	(0.61±0.01)*	(0.08±0.02)*	(0.43±0.15)*
14:0	0.73±0.12	0.64±0.14	0.60±0.15	0.57±0.07
16:0	43.04±2.36	(34.66±0.97)*	(34.14±4.76)*	(35.13±0.87)*
16:1	32.76±2.62	(42.19±2.04)*	(42.66±4.78)*	(43.47±0.29)*
17:1	0.26±0.22	0.32±0.01	0.21±0.13	(0.59±0.09)*
18:0	5.53±1.13	(3.89±0.36)*	3.99±0.93	(3.65±0.42)*
18:1	9.70±1.62	10.81±1.79	11.36±1.32	11.38±0.51
18:2	2.33±1.16	2.77±0.94	2.67±1.00	0.95±0.33
24:1	3.13±1.55	2.69±1.22	2.98±0.77	3.03±0.46
26:1	1.39±0.42	(0.21±0.09)*	(0.24±0.00)*	(0.30±0.05)*
Others	0.90±0.08	1.21±0.09	1.07±0.05	0.50±0.02

Mean±SD, calculated on the basis of peak areas; the results represent the mean value of at least three independent experiments performed in duplicate; *significantly different from the zero generation (Mann-Whitney *U* test, $p<0.05$)

Table 3. Distribution and the relative ratios of some characteristic fatty acid groups of PtdCho and PtdEtn of brewer's yeast in the starter culture and the first three recycled generations

	PtdCho				PtdEtn			
	Yeast generation				Yeast generation			
	Zero	1st	2nd	3rd	Zero	1st	2nd	3rd
Saturated	52.62±0.32	45.83±0.54	45.03±0.43	44.71±0.15	49.90±0.44	40.23±0.20	39.19±0.67	39.90±0.17
Unsaturated	46.87±0.66	53.74±0.93	54.78±0.95	55.20±0.31	49.86±0.87	59.29±0.78	60.41±0.99	59.83±0.21
SFAs/UFAs	1.12	0.85	0.82	0.81	1.0	0.68	0.65	0.67
Unsaturation index	0.50	0.56	0.57	0.56	0.52	0.62	0.63	0.61
C_{16}	68.30±2.12	74.06±4.50	75.38±3.18	77.54±1.17	75.80±2.49	76.85±1.51	76.80±4.77	78.59±0.58
C_{18}	23.38±0.51	19.37±0.54	19.30±0.71	17.78±0.23	17.64±1.00	17.51±1.02	18.12±0.81	15.99±0.31
C_{16}/C_{18}	2.92	3.82	3.91	4.36	4.30	4.39	4.24	4.92
$C_{16:0}/C_{16:1}$	1.41	0.92	0.86	0.82	1.31	0.82	0.80	0.81
$C_{18:0}/C_{18:X}$	0.79	0.81	0.79	0.86	0.46	0.29	0.28	0.30

X – degree of unsaturation; mean±SD calculated on the basis of peak areas, the results represent the mean value of at least three independent experiments performed in duplicate; *significantly different from the zero generation (Mann-Whitney *U* test, $p<0.05$)

three options: (*i*) to synthesize the excess of UFAs in the phase of aerobic propagation and at the beginning of each fermentation cycle, when oxygen is still available, and to store them in the form of reserve lipids; (*ii*) to activate transport mechanisms and use exogenous UFAs if they are present in the wort; and (*iii*) to induce hypoxic genes encoding the key enzyme of UFA synthesis, OLE1 (*35*). In the case of brewer's yeast analysed in this work, all three pathways were possible since the FA analysis of wort revealed significant concentrations of palmitoleic and oleic acids, as well as some minor UFAs, like $C_{17:1}$ and $C_{18:2}$ (data not shown). Regarding the reserve lipid forms of FAs, Ferreira *et al*. (*35*) found that the excess saturated FAs are not stored mainly within triacylglycerols and sterylesters, but rather within specific phospholipids with a marked preference for phosphatidylinositol. A rather high unsaturation index determined in our study for PtdEtn and PtdCho (Table 3) gives us the reason to speculate that these two PL species serve also as depots for UFAs.

Ethanol produced during fermentation in brewing process is toxic for the producing organism as well, and thus represents another stress factor strongly influencing the FA composition of the membranes. The studies dealing with the alterations in the FA composition caused by ethanol exposure differ largely depending on the yeast strain, ethanol concentration and supplementation of FAs in the growth media (*20,21,34,36–39*). According to most authors, the content of UFAs in *S. cerevisiae* increases in the presence of ethanol, thus increasing membrane fluidity. According to some authors, in PtdCho and PtdEtn, the content of oleic and palmitic acid increases, which is not the case with the yeast analysed in this study since in PtdCho only palmitoleic acid increased and in PtdEtn palmitoleic and slightly oleic. De-

spite the anaerobic conditions, which govern during fermentation, the fact that UFAs prevailed in the recycled generations shows that the influence of ethanol can be considered as more significant, overcoming the effect of oxygen depletion.

The conditions of induced unsaturation and, therefore, increased membrane fluidity and permeability as a diffusion barrier could cause alterations in the functioning of membrane proteins. This refers more to PtdEtn because of its small polar head, since unsaturated fatty acids accentuate its conical shape, which leads to its increased nonbilayer propensity (*3*,*10*). Concomitant to unsaturation of both species, the increase in the PtdCho content found in our study can be regarded as counterbalancing, as a response of the yeast cells in the sense of stabilizing membrane by 'filling up', condensing its surface and providing proper conditions for protein functioning. This mechanism is even more important in the conditions of ergosterol depletion, which is the case with bottom-fermenting brewer's yeasts (*28*). Reversed profiles of PtdCho and PtdEtn suggested that PtdCho was synthesised *via* PtdEtn-methylation (*3*).

Besides fatty acyl unsaturation, chain length also influences membrane properties. Thomas *et al.* (*40*) hypothesised that the increase in the relative content of C_{16} acids increases ethanol tolerance. Accordingly, a generally high C_{16} content in all generations of the analysed yeast indicates that it is a strain with a high ethanol tolerance. The changes in the chain length of PtdCho occurred mostly by the exchange between C_{16} and C_{18} FAs, *i.e.* C_{16} increased on the account of C_{18} resulting in a significant increase in C_{16}/C_{18} ratio from 2.9 to 4.4. In PtdEtn the observed changes occurred mainly within C_{16} and C_{18}, resulting in small variations of C_{16}/C_{18} ratio (4.2–4.9). The increment of the C_{16} content in PtdCho of the recycled generations (up to 10 % of TFAs comparing the zero and 3rd generation) was considerable and may be regarded as an indicator of an increased ethanol tolerance gradually developed with recycling.

Conclusions

PtdCho and PtdEtn mass fractions, as well as their fatty acid compositions, altered markedly in the stressed recycled generations. The alterations were mostly expressed in the first recycled generation compared to the starter culture. These changes can be attributed to the stress response caused by the changes in environmental conditions to which the yeast was subjected during beer fermentation. Relatively small differences in the mass fractions and fatty acid compositions of PtdCho and PtdEtn between the recycled generations point out that the yeast cells accommodated gradually to the unfavourable fermentation conditions. Taking into consideration that the 3rd generation is preferred in many breweries, the characteristic arch-shaped profile of several analysed parameters could also be attributed to the brewer's yeast adaptation developed with recycling. It can be concluded that the brewer's yeast is capable of adapting to stress conditions partially by modifying the PtdCho/PtdEtn ratio and the unsaturation degree of their FAs, which indicates that it is suitable for multiple recycling.

Acknowledgements

This work was supported by the Croatian Ministry of Science, Education and Sports (technological project TP-01/0062–64). We wish to thank Mrs Katica Georgiú for technical assistance. We are deeply grateful to Professor Günther Daum and his co-workers from the Institute of Biochemistry in Graz for sharing unselfishly with us their knowledge and experience.

References

1. M.E. van der Rest, A.H. Kamminga, A. Nakano, Y. Anraku, B. Poolman, W.N. Konings, The plasma membrane of *Saccharomyces cerevisiae*: Structure, function, and biogenesis, *Microbiol. Rev. 59* (1995) 304–322.
2. J. Šajbidor, Effect of some environmental factors on the content and composition of microbial membrane lipids, *Crit. Rev. Biotechnol. 17* (1997) 87–103.
3. A.I.P.M. de Kroon, Metabolism of phosphatidylcholine and its implications for lipid acyl chain composition in *Saccharomyces cerevisiae*, *Biochim. Biophys. Acta, 1771* (2007) 343–352.
4. H. Alexandre, B. Mathieu, C. Charpentier, Alteration in membrane fluidity and lipid composition, and modulation of H^+-ATPase activity in *Saccharomyces cerevisiae* caused by decanoic acid, *Microbiology, 142* (1996) 469–475.
5. R. Prasad, A.H. Rose, Involvement of lipids in solute transport in yeasts, *Yeast, 2* (1986) 205–220.
6. G. Szolderits, A. Hermetter, F. Paltauf, G. Daum, Membrane properties modulate the activity of a phosphatidylinositol transfer protein from the yeast, *Saccharomyces cerevisiae*, *Biochim. Biophys. Acta, 27* (1989) 301–309.
7. C. Ivorra, J.E. Pérez-Ortin, M. del Olmo, An inverse correlation between stress resistance and stuck fermentations in wine yeasts: A molecular study, *Biotechnol. Bioeng. 64* (1999) 668–708.
8. I. Mannazzu, D. Angelozzi, S. Belviso, M. Budroni, G.A. Farris, P. Goffrini, T. Lodi, M. Marzona, L. Bardi, Behaviour of *Saccharomyces cerevisiae* wine strains during adaptation to unfavourable conditions of fermentation on synthetic medium: Cell lipid composition, membrane integrity, viability and fermentative activity, *Int. J. Food Microbiol. 121* (2008) 84–91.
9. G. Daum, N.D. Lees, M. Bard, R. Dickson, Biochemistry, cell biology and molecular biology of lipids of *Saccharomyces cerevisiae*, *Yeast*, *14* (1998) 1471–1510.
10. P.R. Cullis, B. de Kruijff, Lipid polymorphism and the functional roles of lipids in biological membranes, *BBA – Biomembranes, 559* (1979) 399–420.
11. B. de Kruijff, Membranes, where lipids and proteins meet, *Chem. Phys. Lipids*, *143* (2006) 41–42.
12. H.A. Boumann, J. Gubbens, M.C. Koorengevel, C.S. Oh, C.E. Martin, A.J.R. Heck, J. Patton-Vogt, S.A. Henry, B. de Kruijff, A.I.P.M. de Kroon, Depletion of phosphatidylcholine in yeast induces shortening and increased saturation of the lipid acyl chains: Evidence for regulation of intrinsic membrane curvature in a eukaryote, *Mol. Biol. Cell*, *17* (2006) 1006–1017.
13. R.P. Jones, P.F. Greenfield, Ethanol and the fluidity of the yeast plasma membrane, *Yeast, 3* (1987) 223–232.
14. N.J. Russell, Adaptive modifications in membranes of halotolerant and halophilic microorganisms, *J. Bioenerg. Biomembr. 21* (1989) 93–113.
15. T.W. Jeffries, Y.S. Jin, Ethanol and thermotolerance in the bioconversion of xylose by yeasts, *Adv. Appl. Microbiol. 47* (2000) 221–268.

16. M.E. Guerzoni, M. Ferruzzi, M. Sinigaglia, G.C. Criscuoli, Increased cellular fatty acid desaturation as a possible key factor in thermotolerance in *Saccharomyces cerevisiae*, *Can. J. Microbiol. 43* (1997) 569–576.

17. J.C. Mauricio, C. Millán, J.M. Ortega, Influence of oxygen on the biosynthesis of cellular fatty acids, sterols and phospholipids during alcoholic fermentation by *Saccharomyces cerevisiae* and *Torulaspora delbrueckii*, *World J. Microbiol. Biotechnol. 14* (1998) 405–410.

18. E.L. Steels, R.P. Learmonth, K. Watson, Stress tolerance and membrane lipid unsaturation in *Saccharomyces cerevisiae* grown aerobically or anaerobically, *Microbiology, 140* (1994) 569–576.

19. H. Alexandre, I. Rousseaux, C. Charpentier, Relationship between ethanol tolerance, lipid composition and plasma membrane fluidity in *Saccharomyces cerevisiae* and *Kloeckera apiculata*, *FEMS Microbiol. Lett. 124* (1994) 17–22.

20. J. Šajbidor, Z. Ciesarová, D. Šmogrovičová, Influence of ethanol on the lipid content and fatty acid composition of *Saccharomyces cerevisiae*, *Folia Microbiol. 40* (1995) 508–510.

21. Z. Chi, N. Arneborg, Relationship between lipid composition, frequency of ethanol-induced respiratory deficient mutants, and ethanol tolerance in *Saccharomyces cerevisae*, *J. Appl. Microbiol. 86* (1999) 1047–1052.

22. K.A. Smart, S. Whisker, Effect of serial repitching on the fermentation properties and condition of brewing yeast, *J. Am. Soc. Brew. Chem. 54* (1995) 41–44.

23. P.V. Attfield, Stress tolerance: The key to effective strains of industrial baker's yeast, *Nat. Biotechnol. 15* (1997) 1351–1357.

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

25. J. Folch, M. Lees, G.H. Sloane Stanley, A simple method for the isolation and purification of total lipides from animal tissues, *J. Biol. Chem. 226* (1957) 497–509.

26. R.M. Broekhuyse, Phospholipids in tissues of the eye. Isolation, characterization and quantitative analysis by two--dimensional thin-layer chromatography of diacyl and vinyl-ether phospholipids, *Biochim. Biophys. Acta, 152* (1968) 307–315.

27. M. Vendramin-Pintar, K. Jernejc, A. Cimerman, A comparative study of lipid composition of baker's and brewer's yeasts, *Food Biotechnol. 9* (1995) 207–215.

28. B. Blagović, J. Rupčić, M. Mesarić, K. Georgiú, V. Marić, Lipid composition of brewer's yeast, *Food Technol. Biotechnol. 39* (2001) 175–181.

29. F. Paltauf, S. Kohlwein, S.A. Henry: Regulation and Compartmentalization of Lipid Synthesis in Yeast. In: *The Molecular and Cellular Biology of the Yeast Saccharomyces cerevisiae: Gene Expression*, E.W. Jones (Ed.), Cold Spring Harbor Laboratory Press, New York, USA (1992) pp. 415–500.

30. M.J.F.W. Janssen, M.C. Koorengevel, B. de Kruijff, A.I.P.M. de Kroon, The phosphatidylcholine to phosphatidylethanolamine ratio of *Saccharomyces cerevisiae* varies with the growth phase, *Yeast, 16* (2000) 641–650.

31. G. Daum, G. Tuller, T. Nemec, C. Hrastnik, G. Balliano, L. Cattel, P. Milla, F. Rocco, A. Conzelmann, C. Vionnet, D.E. Kelly, S. Kelly, E. Schweizer, H.J. Schüller, U. Hojad, E. Greiner, K. Finger, Systematic analysis of yeast strains with possible defects in lipid metabolism, *Yeast, 15* (1999) 601–614.

32. M.J. Homann, M.A. Poole, P.M. Gaynor, C.T. Ho, G.M. Carman, Effect of growth phase on phospholipid biosynthesis in *Saccharomyces cerevisiae*, *J. Bacteriol. 169* (1987) 533–539.

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

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

35. T. Ferreira, M. Régnacq, P. Alimardani, C. Moreau-Vauzelle, T. Bergès, Lipid dynamics in yeast under haem-induced unsaturated fatty acid and/or sterol depletion, *Biochem. J. 378* (2004) 899–908.

36. H.J. Heipieper, S. Isken, M. Saliola, Ethanol tolerance and membrane fatty acid adaptation in *adh* multiple and null mutants of *Kluyveromyces lactis*, *Res. Microbiol. 151* (2000) 777–784.

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

38. H. Mizoguchi, Acquisition of ethanol tolerance by *Saccharomyces cerevisiae* in the sake brewing process and the tolerance determinants, *Seibutsu-kogaku Kaishi, 76* (1998) 122–130.

39. K.M. You, C.L. Rosenfield, D.C. Knipple, Ethanol tolerance in the yeast *Saccharomyces cerevisiae* is dependent on cellular oleic acid content, *Appl. Environ. Microbiol. 69* (2003) 1499–1503.

40. D.S. Thomas, J.A. Hossack, A.H. Rose, Plasma-membrane lipid composition and ethanol tolerance in *Saccharomyces cerevisiae*, *Arch. Microbiol. 117* (1978) 239–245.