ORIGINAL SCIENTIFIC PAPER

The Effect of Low Temperature Storage on the Lipid Composition of Baker's Yeast

Tamara Janković, Jelena Kažić, Marijana Matijašević, Ana Ruskaj, Gordana Čanadi Jurešić and Branka Blagović*

Summary

Production of frozen dough demands the usage of freezing/thawing resistant strains of baker's yeast that can adapt to low temperature and preserve fermentative performance, which is closely related to the lipid composition of their cells. In order to determine the effect of low temperature storage on the baker's yeast, we have analysed lipids of compressed commercial baker's yeast of Saccharomyces cerevisiae strain maintained at 4 °C (7 and 14 days) and -20 °C (7, 14 and 120 days). In the phospholipid composition of all groups the most abundant was phosphatidylcholine (42 – 56 % of total phospholipids), followed by phosphatidylinositol (21 – 28 %). Phosphatidylcholine to phosphatidylethanolamine ratio, one of the parameters of the adaptation capability to extracellular changes, was high (2.4 – 3.3). Sterol esters and ergosterol were the most abundant neutral lipids in all groups. Oleic and palmitoleic acids were by far the most abundant fatty acids in all groups (39 – 48 % and 33 – 38 % of total cell fatty acids, respectively). Compared to the fresh yeast, considerable differences were found in the composition of all three lipid classes in the samples maintained at both temperatures. The major changes were observed after 14 days of exposure to -20 °C.

Key words: *baker's yeast, low temperature storage, freezing, phospholipids, neutral lipids, fatty acids*

Abbreviations

CL, cardiolipin
FA, fatty acid
GC, gas chromatography
NL, neutral lipid
PL, phospholipid
PtdCho, phosphatidylcholine
PtdEtn, phosphatidylethanolamine
PtdIns, phosphatidylinositol
PtdOH, phosphatidic acid
PtdSer, phosphatidylserine
SE, steryl ester
SFA, saturated fatty acid
TAG, triacylglycerol
TL, total lipids
TPL, total phospholipids
UFA, unsaturated fatty acid

Introduction

The goals of the baker's yeast producers are to maximise growth and minimize fermentation, whereas the goals of bakers are 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 (Attfield 1997). When it is used for daily fresh bakery products it assumes storage at low temperature and when used for the production of frozen dough it demands freezing/thawing resistant strains (Murakami et al. 1996). Both ways of storage demand yeast capable not only to survive, but to adapt to stress evoked by low temperature and to preserve fermentative performance. The damages and tolerance mechanisms induced by freezing/thawing still are not fully understood, but several hypotheses have been proposed (Kronberg et al. 2008). The involvement of the proteins and trehalose in these mechanisms (and in stress response in general) has been recognized long time ago (Walker 2000), but there is increasing evidence of the importance of lipids (Kronberg et al. 2008; Rodriguez-Vargas et al. 2007; Steels et al. 1994; Swan and Watson 1999; Torok et al. 2014). In *S. cerevisiae*, low temperature stimulates the activity of genes that promote the synthesis of desaturase resulting in increased concentration of unsaturated FAs. The structure, fluidity, permeability and functionality of membranous systems depend strongly on the lipid composition. Sterol to phospholipid ratio, phospholipid composition and unsaturation of membrane lipids are of special importance (de Kroon et al. 2013; Gelinas et al. 1991; Rodriguez-Vargas et al. 2007). Among other, changes in membrane fluidity are the first signal for the cellular response to low temperature (cold shock response) (Aguilera et al. 2007). Lipids determine permeability and functionality of the membrane partially by influencing the conformation of membrane proteins (de Kruijff 2006). Many stresses, like heat, alcohol, organic acids, desiccation and freezing, can lead to decline in intracellular pH which has as a consequence that cells must use ATP to expel protons *via* plasma membrane H^+-ATPase (Attfield 1997), which is a transmembrane protein and as a such strongly influenced by its lipid environment.

Baker's yeast has become one of the best studied and most reliable model for biochemical, cell biological and molecular biological research of eukaryotic cells. The advantages of this microorganism, especially the ease of handling and the fact that the genome of *Saccharomyces cerevisiae* was the first eukaryotic genome that has been entirely sequenced, contributed to the success of this model system. In that context, industrial yeasts are also interesting, since they often undergo stre-

Corresponding author: branka.blagovic@medri.uniri.hr

ssful conditions similar to those in potentially important other purposes. Thus, for example, the mechanism of freeze-injury and tolerance important for the baker's yeast is interesting for frozen starters and culture collections, as well.

The aim of this study was to determine the effect of low temperature storage and freezing on the lipid composition of industrial strain of baker's yeast.

Materials and methods

Yeast strain

Commercial baker's yeast belonging to the *Saccharomyces cerevisiae* species, grown aerobically and harvested in the stationary phase was used throughout the study. It was the end product of industrial process in "KVASAC" d.o.o. factory in Savski Marof, Croatia and obtained in the form of compressed yeast (fresh cake).

Samples were divided into groups of three: the yeast kept at 4 °C for 7 and 14 days, the yeast frozen and kept at -20 °C for 7 and 14 days and the yeast frozen and kept at -20 °C for 120 days. Frozen yeast was thawed slowly at room temperature. For technical reasons we had two control groups: the yeast before cold storage and the yeast kept at 4 °C for 2-3 days.

Methods

Determination of dry biomass

Approximately 2 g of wet biomass was topped with 1 mL of ethanol and dried at 120 °C till constant weight.

Lipid analysis

Prior to any experiment the biomass was washed three times with cold distilled water and centrifuged at 1800 *g* for 5 min.

Extraction of lipids

To ensure efficient lipid extraction, the cells were transformed into spheroplasts. For their preparation we have used modified method originally developed for the isolation of mitochondria (Čanadi Jurešić and Blagović 2011, Zinser and Daum 1995), in which zymolyase (Seikagaku, Tokyo, Japan) is used for the digestion of the cell wall. Total lipids were extracted from the spheroplasts according to the method of (Folch et al. 1957) and determined gravimetrically.

Analysis of phospholipids

Total phospholipids were quantified spectrophotometrically as inorganic phosphorus by the method of (Broekhuyse 1968). 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 (Merck, Darmstadt, Germany). 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.

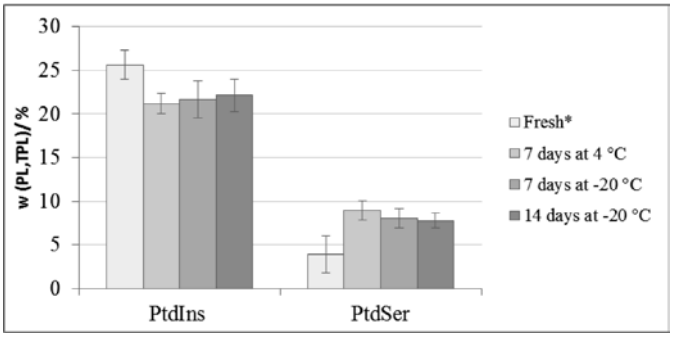
Analysis of neutral lipids

Neutral lipids were separated by two-step TLC on silica gel 60 plates 20 x 10 cm, 0.2 mm. Lipid extracts and standards were applied by sample applicator (Linomat V, CAMAG, Muttenz, Switzerland). The plates were developed by using light petroleum/diethylether/acetic acid (volume fractions 20:20:0.8) up to 1/3 of a plate as the first and light petroleum /diethylether (volume fractions 39.2:0.8) up to 2/3 of plate as the second solvent system. The bands were visualised by post chromatographic derivatization as follows: the plates were dipped with in 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. The lipid components were quantified by densitometry at 400 nm using BioRad GS-710 Calibrated Imaging Densitometer.

Fatty acid analysis

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). An SP-2330 capillary column (Supelco, USA), 30 m×0.32 mm×0.2 μm and helium as carrier gas with split injection (100:1) were used. Hydrogen was obtained with a Claind hydrogen generator. The analyses were carried out in programmed temperature mode from 140 to 220 ºC at 5 ºC/min and then isothermally for 25 min. The injector temperature was 300 ºC and the detector 350 ºC. Chromatography Software from Perkin-Elmer Nelson (Turbochrom 4) was used for data acquisition. The results were expressed as mass fractions of individual FA in total identified FAs. The degree of unsaturation, expressed as unsaturation index (UI) was calculated as follows:

UI = [w(monoenoic fatty acids) + 2 w(dienoic fatty acids) + 3 w(trienoic fatty acids)]/100.



*Control sample - lipid extraction after 2-3 days at 4 °C; **before freezing the yeast was kept for 2-3 days at 4 °C; the results are the mean of at least two independent experiments.

Figure 1. *PtdIns and PtdSer content in the fresh yeast, yeast kept for 7 days at 4 °C, 7 and 14 days at -20 °C (expressed as w% of total identified PLs).*

Results and discussion

The content of dry biomass was about 25% of the wet biomass in all groups of the baker's yeast and the total lipids

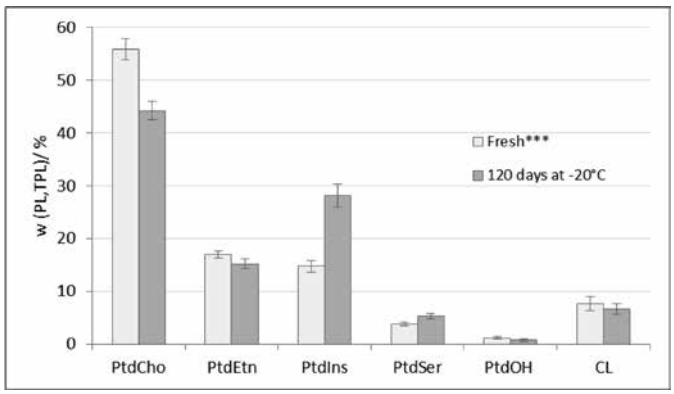
Table 1. *Phospholipid composition of fresh baker's yeast and the yeast kept at 4 and -20 °C for 7 and 14 days (expressed as w% of total identified PLs).*

	Baker's yeast				
	Fresh*	4 °C		-20 °C**	
		7 days	14 days	7 days	14 days
PtdCho	45.23 ± 2.13	43.65 ± 1.19	43.77 ± 2.12	42.16 ± 0.94	46.15 ± 0.53
PtdEtn	17.17 ± 1.98	16.51 ± 1.52	16.25 ± 2.13	17.50 ± 1.63	14.62 ± 0.90
PtdIns + PtdSer#	29.55 ± 1.14	30.16 ± 1.18	30.11 ± 2.28	29.72 ± 2.06	29.93 ± 1.43
PtdOH	1.21 ± 0.73	2.50 ± 1.10	2.11 ± 0.78	3.21 ± 1.17	2.21 ± 1.29
CL	6.84 ± 0.55	7.18 ± 0.78	7.77 ± 0.99	7.41 ± 0.45	7.08 ± 0.73

*Control sample - lipid extraction after 2-3 days at 4 °C; **before freezing the yeast was kept for 2-3 days at 4 °C;
in the samples kept for 14 days at 4 °C under applied experimental conditions PtdIns and PtdSer appeared as one spot; the results are the mean of at least two independent experiments.

accounted around 5.5% of its dry biomass which is in accordance with the literature (Sajbidor et al. 1994).

PtdCho was the main phospholipid in all analysed yeast groups and it was followed by PtdIns and PtdEtn (Table 1, Figure 1). For the samples kept for 14 days at 4 °C we can only assume that it was the same since under applied experimental conditions PtdIns and PtdSer appeared as one spot. Since the yeasts obtained in industrial process are harvested in the stationary phase usually the content of PtdIns is higher than the content of PtdEtn, which is opposite than in the yeasts harvested in the exponential phase (Janssen et al. 2000). Generally, the alterations in the PL compositions were relatively small apart for a few exceptions. They were marked at both temperatures regarding the content of PtdIns and PtdSer (Figure 1); the content of PtdIns decreased and that of PtdSer increased comparing with the fresh yeast. Figure 1 demonstrates their tight connection in the biosynthetic pathway where they compete for the same precursor, cytidine diphosphat diacylglycerol (CDP-DAG). As the result of their alterations, the ratio of PtdIns/PtdSer, which is one of the characteristic features of PL-composition, decreased markedly after the short storage of 7 and 14 days (Table 2). On the contrary, in the yeast kept for 120 day at -20 °C, the ratio increased (Table 2). In those samples PtdIns markedly increased, which did not affect directly the content of PtdSer, but of the PtdCho (the final product in this branch of PL-synthesis), whose content concomitantly decreased (Figure 2). PtdCho/PtdEtn ratio is one of the parameters of cell membrane adaptability to extracellular changes. In the analysed yeast it accounted between 2.4 and 3.3 (Table 2), which is very high comparing with the results of Murakami et al. (1996) and is typical for the yeasts resistant to stress in general. In their work, average PtdCho/PtdEtn- ratio for freeze-tolerant strains accounts for 1.4 and for freeze-sensitive for 1.2. The ratio in our yeast changed markedly upon storage at -20 C for 14 and 120 days (Table 2), but the difference was that after 14 days it increased and after 120 days it decreased, which remains to be clarified.



***Control sample - lipid extraction before cold storage; the results are the mean of at least two independent experiments.

Figure 2. *Phospholipid composition of fresh baker's yeast and the yeast kept at -20 °C for 120 days (expressed as w% of total identified PLs).*

The changes in frozen yeasts were less expressed in those samples that were kept before freezing for a few days at +4 °C, which can be regarded as a favourable pre-treatment. Decrease of PtdCho content and PtdCho/PtdEtn ratio in pre-treated yeast cells upon their exposure to low temperatures, followed by their increase after 14 days suggested the adaptation of the yeast cells to the new conditions (Table 2).

Table 2. *Ratio of PtdCho/PtdEtn in the fresh baker's yeast and the yeast kept at 4 and -20 °C.*

Mass ratio	Baker's yeast						
	Fresh*	4°C		-20°C**		Fresh***	-20°C
		7 days	14 days	7 days	14 days		120 days
PtdCho/PtdEtn	2.63 ± 0.43	2.64 ± 0.33	2.69± 0.55	2.41± 0.13	3.16 ± 0.23	3.30 ± 0.13	2.92 ± 0.33
PtdIns/PtdSer	6.55 ± 0.43	2.37 ± 0.22	-	2.67 ± 0.44	2.8 ± 0.32	3.94 ± 0.46	5.34 ± 0.17

*Control sample - lipid extraction after 2-3 days at 4 °C; **before freezing the yeast was kept for 2-3 days at 4 °C;
***control sample - lipid extraction before cold storage.

Fatty acids

FAs from 8 to 26 C-atoms were identified. The main were C16:0, C16:1, C18:0, C18:1 and C18:2, among which the highest percentage had 18:1 accounting between 43 and 49% and 16:1 accounting between 34 and 39% of identified acids (Table 3). Other identified FAs accounted for less than 1% each. Among them there were also the fatty acids with odd number of C-atoms like C15:0, C15:1, C17:0 and C17:1 and one very-long-chain FA, C26:0.

In all samples unsaturated fatty acids prevailed significantly, accounting for more than 80%. Consequently, the ratio UFAs/SFAs was high, but unsaturation index was relatively low because of the low content of PUFAs. According to Watson and co-workers (Steels et al. 1994; Swan and Watson 1999), yeast strains with such composition are less sensitive to heat and ethanol than strains with increased concentration of PUFAs. This may be attributable to decreased membrane fluidity and smaller cellular damage caused by oxygen-derived free radicals. Since there is similarity in the nature of damage or inhibition resulting from various stresses, which means that common mechanisms of protection and repair can provide for tolerance of a broad range of environmental insults (Attfield 1997), we can relate it to the low temperature effect and conclude that the analysed yeast is highly tolerant to low temperature.

This result also indicates that the growth media was poor or avoid of polyunsaturated fatty acids, since they cannot be synthesized in yeasts, but their origin could be only exogenous. Comparing the literature data for the fatty acid composition of the whole cells (and organelles, as well) in aerobically and anaerobically grown yeasts, it can be observed that in aerobic cells the content of UFAs is higher than in anaerobic, which

***Table 4**: The main FAs in the fresh baker's yeast and the yeast kept at -20 °C for 120 days expressed as w% of total identified FAs).*

	Baker's yeast	
Main FAs	**Fresh*** **	**120 days at -20 °C**
16:0	6.61 ± 0.31	6.78 ± 0.08
16:1	38.17 ± 1.49	35.76 ± 0.70
18:0	2.99 ± 2.44	5.41 ± 0.02
18:1	50.26 ± 1.56	49.07 ± 0.45
18:2	0.41 ±0.03	0.47 ± 0.04
SFAs, w/%	10.54 ± 2.39	13.28 ± 0.05
UFAs, w/%	89.46 ± 2.39	86.72 ± 0.05
UFAs/SFAs	9.01 ± 2.27	6.52 ± 0.02
Unsaturation index, UI	0.89 ± 0.03	0.86 ± 0.00
$C_{16,}$ w/%	44.78 ± 1.80	42.53 ± 0.55
$C_{18,}$ w/%	53.7 ± 0.90	54.95 ± 0.36
C_{16} / C_{18}	0.84 ± 0.04	0.77 ± 0.02
$C_{16:0}$ / $C_{16:1}$	0.17 ± 0.00	0.19 ± 0.00
$C_{18:0}$ / ($C_{18:1}$ + $C_{18:2}$)	0.06 ± 0.04	0.11 ± 0.00

***Control sample - lipid extraction before cold storage; the results are the mean of double analysis of at least two independent experiments.

***Table 3.** The main FAs in the fresh baker's yeast and the yeast kept at 4 and -20 °C for 7 and 14 days (expressed as w% of total identified FAs).*

Main FAs	Fresh*	Baker's yeast			
		4 ºC		-20 ºC**	
		7 days	14 days	7 days	14 days
16:0	7.02 ± 0.22	7.52 ± 0.58	7.19 ± 0.25	7.17 ± 0.02	8.19 ± 0.09
16:1	38.92 ± 1.43	39.15 ± 2.40	37.54 ± 1.59	37.89 ± 0.43	34.71 ± 0.33
18:0	5.61 ± 0.20	3.16 ± 2.05	5.82 ± 0.22	5.98 ± 0.13	6.39 ± 0.16
18:1	46.90 ± 0.88	48.75 ± 0.29	46.56 ± 0.80	48.24 ± 0.20	43.99 ± 0.14
18:2	0.32 ± 0.03	0.39 ± 0.23	0.33 ± 0.06	0.34 ± 0.31	4.63 ± 0.57
SFAs, w/%	13.50 ± 1.09	11.36 ± 2.36	14.21 ± 1.16	13.50 ± 0.33	15.83 ± 0.11
UFAs, w/%	86.50 ± 1.09	88.64 ± 2.36	85.89 ± 1.16	86.50 ± 0.33	84.07 ± 0.11
UFAs/SFAs	6.41 ± 0.78	7.80 ± 1.93	6.04 ± 0.82	6.41 ± 0.06	5.31 ± 0.04
Unsaturation index, UI	0.87 ± 0.00	0.89 ± 0.02	0.85 ± 0.01	0.87 ± 0.03	0.88 ± 0.00
$C_{16,}$ w/%	45.94 ± 0.44	46.68 ± 2.81	44.73 ± 0.65	45.06 ± 0.34	42.91 ± 0.29
$C_{18,}$ w/%	52.83 ± 0.99	52.30 ± 2.91	52.71 ± 0.91	54.56 ± 0.17	55.00 ± 0.27
C_{16} / C_{18}	0.87 ± 0.02	0.90 ± 0.10	0.85 ± 0.02	0.83 ± 0.00	0.78 ± 0.00
$C_{16:0}$ / $C_{16:1}$	0.18 ± 0.01	0.19 ± 0.00	0.19 ± 0.00	0.19 ± 0.01	0.24 ± 0.00
$C_{18:0}$ / ($C_{18:1}$ + $C_{18:2}$)	0.12 ± 0.01	0.06 ± 0.05	0.12 ± 0.02	0.12 ± 0.00	0.13 ± 0.00

*Control sample - lipid extraction after 2 days at 4 °C; **before freezing the yeast was kept for 2-3 days at 4 °C; the results are the mean of double analysis of at least two independent experiments.

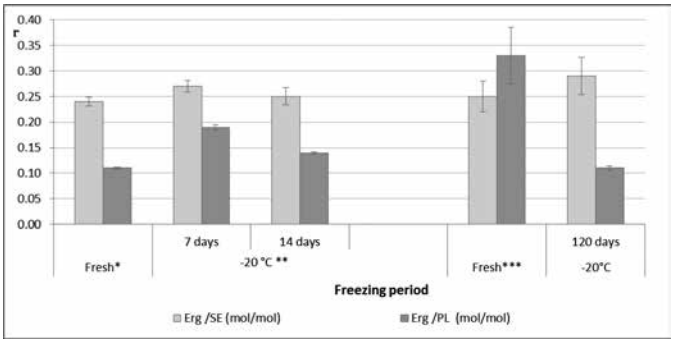
is in accordance with cognition that in the yeast double bond can be introduced only in the presence of molecular oxygen. In general, characteristic feature of the fatty acid composition is the correlation between UI and C16/C18-ratio: higher the UI, lower C16/C18 (Ahvenainen 1982; Blagovic et al. 2001; Blagovic et al. 2005). This can be explained as tendency of the yeast cells to compensate the increase of fluidity caused by unsaturated fatty acids with longer-chain acids. In this work, C18-acids prevailed slightly over C16-acids accounting between 54 and 55%, which decreased fluidity.

In the FA composition of the yeast analysed in this work, the alterations caused by low temperature storage were small, with slight tendency after 14 days toward decreased content of UFAs and of C16/C18- ratio, both of which lead to decreased membrane fluidity (Table 3). Similar alterations were noticed after 120 days in the frozen samples (Table 4). It is not easy to distinguish between the damages and tolerance mechanisms induced by freezing-thawing. Since freezing causes ice nucleation and dehydration of the cells (Kronberg et al. 2008), we can assume that cells respond by decreasing the membrane unsaturation thus making it more robust and resistant to water leakage.

The values of UI, varying between 0.8 and 0.9 in fresh yeasts, were similar to the values in the yeasts analysed by Murakami et al. (1996).

Neutral lipids

In the NL composition diacylglycerols, ergosterol, free fatty acids, squalene, SE and TAGs were identified. Ergosterol, squalene and SE were quantified (Table 5), while TAGs, present in the highest concentration, need additional analysis because of the big variations of the results.



*Control sample - lipid extraction after 2-3 days at 4 °C; **before freezing the yeast was kept for 2-3 days at 4 °C; ***control sample - lipid extraction before cold storage; the results are the mean of at least two independent experiments.

Figure 3. *Alterations in the molar ratios ergosterol/ sterol esters and ergosterol/ phospholipids in the yeast kept at -20 °C for 7, 14 and 120 days.*

Among quantified NLs, SE had the highest content accounting from 18 to 37% of TLs, and in the fresh yeast it was similar to the strain analyzed in our previous study (21.2 and 19%, respectively). On the contrary, Murakami et al. (1996) reported that in *S. cerevisiae* strains, both freeze-tolerant and freeze-sensitive, TAGs were by far the most abundant NLs accounting even for 90-98% of total NL content (apart from sterols and free fatty acids). Why are reserve lipids in some strains stored in the form of SE and TAGs, while in other strains almost exclusively in the form of TAGs remains to be clarified. In all groups, the content of ergosterol was rather low and also in the order of magnitude of the strain analysed in our previous work (Blagovic et al. 2005). According to Murakami et al. (1996), beside other parameters, it is the indicator of freeze-tolerant strains. The alterations of two main NL-classes, steryl esters and ergosterol, were marked and characterized by increased content after 7 days followed by decrease after 14 days, which is reverse to the alterations of PtdCho-content and PtdCho/PtdEtn-ratio. Characteristic parameters of the lipid composition, molar ratios Erg/SE and Erg/PL showed marked variations with freezing, which was especially pronounced for ergosterol/PL (Figure 3). In the case of the yeast kept for several days at 4 °C, the variations were less expressed which also can be regarded as favorable effect of pre-treatment. On the contrary, in the case of the group analyzed before cold storage, both, the high content of ergosterol and low content of PLs resulted in very high ergosterol/PL ratio, which should be checked additionally.

Conclusions

The results show that low temperature storage at +4 °C and -20 °C caused marked alterations in the lipid composition of analysed baker's yeast. The obtained results suggest that the analysed baker's yeast has high capability of adapting to low temperatures partially by changing the lipid composition, which among other makes it a freeze-tolerant strain and therefore suitable for the production of frozen dough. Short exposure to 4 °C prior to exposure to -20 °C resulted in less expressed alterations in the lipid composition which could be regarded

Table 5*: The main NLs (apart from TAGs) in the fresh baker's yeast and the yeast kept at 4 and -20 °C for 7, 14 and 120 days (w% in total lipids).*

w (NL/TL)/%	Baker's yeast						
	Fresh*	4 °C		-20 °C**		Fresh***	-20 °C
		7 days	14 days	7 days	14 days		120 days
Ergosterol	3.0 ± 0.4	5.2 ± 0.6	2.9 ± 0.5	4.6 ± 0.5	3.9 ± 0.9	5.5 ± 0.4	3.0 ± 0.5
Squalene	0.6 ± 0.1	1.7 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.5 ± 0.0
Steryl esters	21.2 ±1.2	33.7 ± 2.3	20.2 ± 2.4	28.3 ± 1.2	26.0 ± 3.7	37.1 ± 2.2	17.7 ± 0.7

*Control sample - lipid extraction after 2-3 days at 4 °C; **before freezing the yeast was kept for 2-3 days at 4 °C; ***control sample - lipid extraction before cold storage; the results are the mean of at least two independent experiments.

as a positive effect and, therefore, potentially used as a pre-treatment method for freezing the yeast. The method should be tested for suitability for other types of cells and other purposes.

Acknowledgements

This work was supported by the Croatian Ministry of Science and Technology (project 062-0621341-0061).

Literature

Aguilera J., Randez-Gil F., Prieto J. A. (2007) Cold response in Saccharomyces cerevisiae: new functions for old mechanisms. *FEMS Microbiology Review,* 31, 327-41.

Ahvenainen J. (1982) Lipid-Composition of Aerobically and Anaerobically Propagated Brewers Bottom Yeast. *Journal of the Institute of Brewing,* 88, 367-370.

Attfield P. V. (1997) Stress tolerance: The key to effective strains of industrial baker's yeast. *Nature Biotechnology,* 15, 1351-1357.

Blagovic B., Rupcic J., Mesaric M., Georgiu K., Maric V. (2001) Lipid composition of brewer's yeast. *Food Technology & Biotechnology,* 39, 175-181.

Blagovic B., Mesaric M., Maric V., Rupcic J. (2005) Characterization of lipid components in the whole cells and plasma membranes of baker's yeast. *Croatica Chemica Acta,* 78, 479-484.

Broekhuyse R. M. (1968) Phospholipids in tissues of the eye. I. Isolation, characterization and quantitative analysis by two-dimensional thin-layer chromatography of diacyl and vinyl-ether phospholipids. *Biochimica and Biophysica Acta,* 152, 307-15.

Čanadi Jurešić, G., Blagović, B. (2011) The influence of fermentation conditions and recycling on the phospholipid and fatty acid composition of the brewer's yeast plasma membranes. *Folia microbiologica*. 56, 215-224.

De Kroon A. I., Rijken P. J., De Smet C. H. (2013) Checks and balances in membrane phospholipid class and acyl chain homeostasis, the yeast perspective. *Progress in Lipid Research,* 52, 374-94.

De Kruijff B. (2006) Membranes, where lipids and proteins meet. *Chemistry and Physics of Lipids (Abstracts from 47th International Conference on the Bioscience of Lipids, Plenary Lectures),* 143, 41-42.

Folch J., Lees M., Sloane Stanley G. H. (1957) A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of Biological Chemistry,* 226, 497-509.

Gelinas P., Fiset G., Willemot C., Goulet J. (1991) Lipid-Content and Cryotolerance of Bakers-Yeast in Frozen Doughs. *Applied and Environmental Microbiology,* 57, 463-468.

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

Kronberg M. F., Nikel P. I., Cerrutti P., Galvagno M. A. (2008) Modelling the freezing response of baker's yeast pre-stressed cells: a statistical approach. *Journal of Applied Microbiology,* 104, 716-27.

Murakami Y., Yokoigawa K., Kawai F., Kawai H. (1996) Lipid composition of commercial bakers' yeasts having different freeze-tolerance in frozen dough. *Bioscience, biotechnology, and biochemistry,* 60, 1874-6.

Rodriguez-Vargas S., Sanchez-Garcia A., Martinez-Rivas J. M., Prieto J. A., Randez-Gil F. (2007) Fluidization of membrane lipids enhances the tolerance of Saccharomyces cerevisiae to freezing and salt stress. *Applied and Environmental Microbiology,* 73, 110-116.

Sajbidor J., Certik M., Grego J. (1994) Lipid Analysis of Bakers Yeast. *Journal of Chromatography A,* 665, 191-195.

Steels E. L., Learmonth R. P., Watson K. (1994) Stress tolerance and membrane lipid unsaturation in Saccharomyces cerevisiae grown aerobically or anaerobically. *Microbiology,* 140 (Pt 3), 569-76.

Swan T. M., Watson K. (1999) Stress tolerance in a yeast lipid mutant: membrane lipids influence tolerance to heat and ethanol independently of heat shock proteins and trehalose. *Canadian Journal of Microbiology,* 45, 472-9.

Torok Z., Crul T., Maresca B., Schutz G. J., Viana F., Dindia L., Piotto S., Brameshuber M., Balogh G., Peter M., Porta A., Trapani A., Gombos I., Glatz A., Gungor B., Peksel B., Vigh L., Jr., Csoboz B., Horvath I., Vijayan M. M., Hooper P. L., Harwood J. L., Vigh L. (2014) Plasma membranes as heat stress sensors: from lipid-controlled molecular switches to therapeutic applications. *Biochimica and Biophysica Acta,* 1838, 1594-618.

Zinser E., Daum G. (1995) Isolation and Biochemical-Characterization of Organelles from the Yeast, Saccharomyces-Cerevisiae. *Yeast,* 11, 493-536.