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ORIGINAL PAPER

Using nutritional and oxidative stress to increase content of health-beneficial fatty acids in oleaginous and non-oleaginous yeasts

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Yeast responses to stress conditions include an increase in lipid content and concomitant changes in content of saturated and unsaturated fatty acids. Some fatty acids are among the dietetically important fatty acids and new possibilities are sought for their biotechnological production in addition to those already exploited from marine organisms, nuts and other sources. The possibility of the production of palmitoleic and linoleic acids resulting from new approaches to traditional biotechnologically useful yeast species (*Kluyveromyces polysporus*, *Saccharomyces cerevisiae*, *Torulospora delbrueckii*) and species capable of high accumulation of lipids (*Rhodotorula glutinis*, *Trichosporon cutaneum*, *Candida* sp., *Yarrowia lipolytica*) was explored. The most promising was the combination of two stress factors: limitation of N-sources (C/N mass ratio of 70 : 1) and oxidative stress induced by zero- valent iron nanoparticles. These conditions were conducive to the production of palmitoleic acid commonly used in cosmetics and medicine and ω-6-linoleic acid, a precursor of thromboxanes, prostaglandins and leucotrienes. The yield of these two fatty acids in *T. cutaneum* was more than 500 mg g^{-1} (dry mass) and in *Candida* sp. more than 600 mg g^{-1} (dry mass).

Keywords: oleaginous yeasts, non-oleaginous yeasts, microbial lipids, unsaturated fatty acids, zero-valent iron

Introduction

The production of lipids by oleaginous yeasts is studied as a potential alternative to conventional vegetable or animal oils and fats. These lipids are a source of fatty acids suitable for humans as pharmaceuticals or nutraceuticals (Bellou et al., 2016). In particular, palmitoleic and linoleic acids are very important for human health. Palmitoleic acid (16 : 1) is an ω-7 mono-unsaturated fatty acid which is a part of the lipid bilayer in all human tissues. Palmitoleic acid has a positive effect on hyperglycaemia and hypertriglyceridemia and can prevent type-2 diabetes by suppressing the β-cell apoptosis induced by glucose or saturated fatty acids (Shinde et al., 2013). The source of palmitoleic acid can be animal fats, vegetable and marine oils (Shinde et al., 2013), but it can also be produced by some yeast strains such as *Candida krusei* or *Saccharomyces cerevisiae* (Kolouchová et al., 2015). Linoleic acid (18 : 2) is a poly-unsaturated ω-6 fatty acid which can be found in milk fat and in the tissues of ruminant animals. Studies have shown the capacity of linoleic acid to reduce body fat by stimulating lipolysis in adipocytes and fatty acid oxidation in both adipocytes and skeletal muscles (Blankson et al., 2000); linoleic acid also has a positive effect on immune system and suppressing cancer and cardiovascular diseases (Schmid et al., 2006). In addition, Alakhras et al. (2015) and Sayegh et al. (2015) detailed the anti-cancer and anti-microbial properties of γ-linoleic acid.

So-called oleaginous yeasts can accumulate lipids in amounts greater than 20 % of the dry biomass (Ageitos et al., 2011). Some studies have reported that the accumulation of lipids can be enhanced under the stress conditions often induced by one particular lim-

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iting element (nitrogen, phosphate, sulphur or magnesium) in the culture media (Wu et al., 2011; Sestric et al., 2014). When the limiting element is exhausted, the remaining carbon source is used for lipid synthesis instead of cell proliferation (Braunwald et al., 2013). Nitrogen limitation frequently influences yeast growth. The molar C/N ratio is, therefore, crucial in determining the oil content and biomass amount (Papanikolaou & Aggelis, 2011a). When microorganisms grow under conditions of nitrogen deficiency, or any other stress conditions limiting the growth, lipids are synthesised de novo as a more effective storage of energy than carbohydrates for rebuilding after stress (Rodolfi et al., 2009). In addition, the improvement in lipid accumulation can be seen as a response to the oxidative stress conditions (Murphy & Vance, 1999). Oxidative stress is defined as an increased level of reactive oxygen species (ROS) in cells when enzymatic and non-enzymatic defence mechanisms are no longer able to cleave ROS and repair the oxidative damages of cells (Gille & Sigler, 1995; Sigler et al., 1999; Lushchak, 2001). Although ROS are very damaging to biomolecules such as nucleic acids, proteins or lipids, they are important second messengers with a part in the mechanism of stress adaptation. Accordingly, low concentrations of ROS can support cell proliferation and adaptation to stress, whereas high concentrations cause serious cell damage (de la Torre-Ruiz et al., 2015). Bellou et al. (2014) showed that dissolved oxygen (0.1–1.5 mg L^{-1}) affects the physiology of *Yarrowia lipolytica*. At high concentrations, the activity of enzymes involved in lipid biosynthesis, such as ATP-citrate lyase and malic enzyme, was upregulated. By contrast, the activity of the enzymes implicated in glycerol assimilation was unchanged.

The level of ROS is regulated by cells' enzymatic and non-enzymatic mechanisms including changes in fatty acid representation. Polyunsaturated fatty acids (PUFA) are highly sensitive to ROS action and, due to their easy oxidation, are an important part of the antioxidative defence mechanism; their synthesis can thus be enhanced under oxidative stress conditions (Cipak et al., 2008). However, Fakas et al. (2008) showed that the peroxidation stability of lipids depended on their nature rather than on their polyunsaturated fatty acid content.

In the current study, zero-valent iron nanoparticles (nZVI) were chosen as a source of oxidative stress. The nZVI possess high chemical reactivity, hence have the capacity to induce the generation of reactive oxygen species and cause oxidative stress (Ševců et al., 2011). Kadar et al. (2012) and Pádrová et al. (2015) reported that trace concentrations of nZVI induced the enhancement of growth and lipid accumulation in microalgae and cyanobacteria strains. In addition, Pádrová et al. (2015) reported that, as a response to the action of nZVI and thus oxidative stress, the abundance of PUFA in all the microorganisms tested increased with increasing concentrations of nZVI.

Because the composition of the medium and the culture conditions significantly affect both the ability of cells to accumulate lipids and the fatty acid profile, the effect of nitrogen (nutrient) limitation in combination with oxidative stress on the production of dietary important palmitoleic and linoleic acids in oleaginous (*Rhodotorula glutinis*, *Trichosporon cutaneum*, *Candida* sp., *Yarrowia lipolytica*) and non-oleaginous (*Kluyveromyces polysporus*, *Saccharomyces cerevisiae*, *Torulospora delbrueckii*) yeast strains was examined.

Experimental

A suspension of zero-valent iron nanoparticles, Nanofer 25, was purchased from Nanoiron (Rajhrad, Czech Republic). Nanofer 25 is an aqueous suspension of uncoated iron nanoparticles. According to the manufacturer's information, Nanofer 25 is characterised by mean particle size of 50 nm, specific surface area > 25 m^2 g^{-1} and 85 mass % purity of iron in the solid phase.

The yeast strains under study were *Candida* sp. DBM 2163; *Kluyveromyces polysporus* DBM 2171 (CCY 30-5-10); *Rhodotorula glutinis* CCY 20-2-20; *Saccharomyces cerevisiae* DBM 2115; *Torulaspora delbrueckii* DBM 39; *Trichosporon cutaneum* CCY 30-5-10; *Yarrowia lipolytica* CCY 29-26-36 supplied by the Culture Collection of Yeast, Institute of Chemistry, Slovak Academy of Sciences, Bratislava (Slovakia) and by the Collection of Yeasts and Industrial Microorganisms of the University of Chemistry and Technology, Prague (Czech Republic). For long-term storage the stock cultures were maintained in 20 vol. % glycerol at –60 ℃. Malt extract agar (23 g L^{-1}, pH 7) was employed for short-term storage.

The yeast inocula were cultivated in 100 mL of YPD medium (g L^{-1}): peptone, 20; yeast extract, 10; glucose, 20; initial pH 6.0, and were autoclave-sterilised at 121 ℃ for 20 min. The cultivations were conducted in Erlenmeyer flasks on a rotary shaker at 150 min^{-1} at 30 ℃ to the late exponential growth phase.

For lipid content and biomass yield determination, the yeasts were cultivated in 250 mL of mineral medium on an orbital rotary shaker (150 min^{-1}) at 30 ℃ in triplicates. Statistical analysis was expressed as standard deviation (SD). The inoculation was at biomass optical density $OD_{600\ nm} = 0.2$. The composition of the mineral medium for the control population was (g L^{-1}): $(NH_4)_2SO_4$, 5; YNB (yeast nitrogen base without amino acids and ammonium sulphate, Himedia), 1.7; glucose 10. For determining the effect of nZVI, the mineral medium was amended by Nanofer 25 (13 mg L^{-1}) (Pádrová et al., 2016).

Nitrogen limitation was conducted in a mineral medium of the following composition (g L^{-1}):

$Na_2HPO_4 \cdot 12\ H_2O$, 5; $(NH_4)_2SO_4$ 0.81; KH_2PO_4, 7; trace element solution 1 mL ($MnCl_2 \cdot 4H_2O$, 20; $FeSO_4 \cdot 7H_2O$, 1; $NaMoO_4 \cdot 2H_2O$, 1; $CaCl_2 \cdot 2H_2O$, 20), pH 6.5, and was autoclave-sterilised at 121 ℃ for 20 min. Glucose was added as a carbon source to a concentration of 30 g L^{-1} and the nitrogen source was supplemented so as to achieve a C/N mass ratio of 70 : 1 (Kolouchová et al., 2015). In addition, the medium was amended with Nanofer 25 (13 mg L^{-1}) for evaluating the combined stress conditions.

The cultivations were carried out for 96 h (up to the early stationary phase). After cultivation, the cells were centrifuged (9000g, 10 min) and washed twice. The biomass was frozen at –75 ℃ and lyophilised.

The extraction and isolation of total lipid content from yeast suspension was based on the method detailed by Bligh and Dyer (1959). In summary, the yeast suspension was ground with glass beads under liquid nitrogen and the crushed yeasts underwent extraction.

Fatty acids methyl esters (FAMEs) were prepared after saponification and further esterification using BF_3/MeOH. The products were dissolved in hexane and analysed by gas chromatography-mass spectrometry (GC-MS). The GC-MS of FAMEs was performed on a Varian GC-MS system with a split/splitless injector (250 ℃) and a SP^{TM}-2380 Capillary GC column L × I.D. 60 m × 0.25 mm, 0.20 μm film thickness of the stationary phase. Helium was used as the carrier gas at 1.0 mL min^{-1}. The split/splitless injection port was maintained at 255 ℃. The split ratio was 1 : 90 (vol.). The injection volume was 1 μL. For FAMEs GC-MS analysis with the SP-2380 column, the temperature programme was as follows: 150 ℃ for 1 min, subsequently increasing at 20 ℃ min^{-1} to 180 ℃ and at 2 ℃ min^{-1} to 250 ℃, which was maintained for 1 min. FAMEs were identified according to their mass spectra (Vančura et al., 1987; Dembitsky et al., 1991) and using a mixture of chemical standards obtained from Sigma–Aldrich (Czech Republic). All experiments concerning the analysis of FAMEs and their derivatives were carried out by electron impact MS.

Results and discussion

Many studies have examined the possibility of using microbial cells as a source of lipids. Unlike algae, yeast are not capable of accumulating DHA but they can be used for the production of nutritionally important fatty acids (FA). This requires the use of such culture conditions as to positively affect the proportion of saturated and unsaturated fatty acids and increase the content of microbial lipids. To achieve optimal results, the cultivation parameters were based on previous studies (pH 6.0, 28 ℃ and 96 h) (Chen et al., 2013; Dey & Maiti, 2013) and made use of two stress factors that lead to lipid accumulation – nitrogen limitation (a C/N mass ratio of 70 : 1) and the action of nZVI (Nanofer 25–13 mg L^{-1}) separately and in combination. These two factors were selected on the basis of previous studies that appeared to be most effective for microbial lipid accumulation (Kolouchová et al., 2015; Pádrová et al., 2016). The yeast species under study included both oleaginous yeasts (*Candida*, *Trichosporon*, *Yarrowia* or *Rhodotorula*) (Dey & Maiti, 2013) and yeasts used in industry such as *S. cerevisiae*, *K. polysporus*, *T. delbrueckii* (Řezanka et al., 2013; Belda et al., 2015). Under the effect of nZVI, the proportion of unsaturated FAs in all seven strains was at least 79 %, at least 70 % at the C/N mass ratio of 70 : 1 and at least 79 % when a combination of both stress factors was used. Figs. 1a and 1b show the changes in the representation of saturated and unsaturated fatty acids.

The cultivation of all seven yeast species with Nanofer 25 (13 mg L^{-1}) led to a slight increase in the abundance of unsaturated fatty acids (15–30 % increase in oleaginous yeast strains *Candida* sp., *R. glutinis* and *Y. lipolytica*, 10 % in non-oleaginous yeasts *K. polysporus* and *S. cerevisiae* and 30 % in *T. delbrueckii*). The same phenomenon was reported by Pádrová et al. (2015) after treating micro-algal strains with 1.7–5.1 mg L^{-1} of Nanofer 25. An increase in unsaturated FAs compared with the control was also observed under N-limitation alone and in combination with Nanofer 25 (combined stress conditions) (Fig. 1b).

Effect of altered stress conditions on lipid content

Among the factors that affect the lipid content is an increased content of some elements such as Zn, Mg, Fe (Veen & Lang, 2004) or the type of nitrogen source (Kolouchová et al., 2015). A considerable increase in lipid content was observed after the addition of nZVI to the cells. The highest nZVI-induced increase, of more than 100 % (from 3.8 % to 8.3 %), was recorded for *S. cerevisiae*. The combined action of N-limitation and nZVI also led to increased lipid content compared with the control. This effect was the most apparent in non-oleaginous yeasts, among which *K. polysporus* recorded a 60 % increase in the content of cell lipids. However, the lipid content of the non-oleaginous yeast strains was below 10 % of cell dry mass. By contrast, the oleaginous yeast strains, in particular *T. cutaneum*, exhibited a high content of total lipids exceeding 50 % of dry cell mass.

From Tables 1 and 2 it can be concluded that the combination of stresses did not cause the same increase in the percentage of total lipids in all the species treated. This phenomenon can be explained by different species-dependent reactions under the same culture conditions (Papanikolaou & Aggelis, 2011a, 2011b).

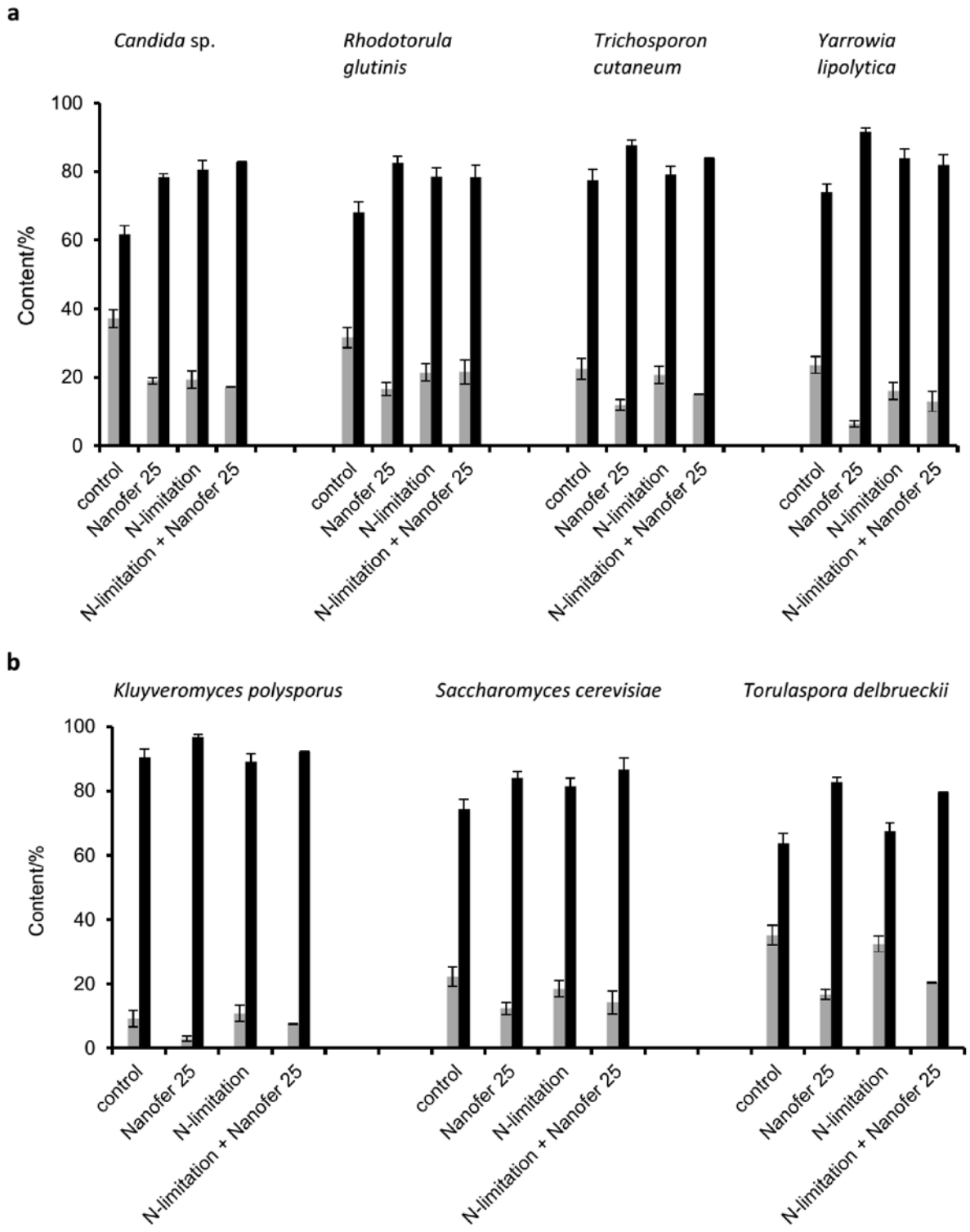

Fig. 1. Proportions of saturated (▒) and unsaturated (■) fatty acids produced by non-oleaginous (a) and oleaginous (b) yeasts. Culture conditions: N-limitation C/N mass ratio of 70 : 1), Nanofer 25 (13 mg L^{-1}), combined stress conditions (N-limitation – C/N mass ratio of 70 : 1, plus Nanofer 25 – 13 mg L^{-1}).

Fatty acid composition and profile

Sitepu et al. (2013) reported culture conditions as having a significant impact not only on total lipid content (%) but especially on the composition of fatty acids. This response of different yeasts varies between particular strains. Tables 1 and 2 show changes in the fatty acid profiles. According to the literature, the proportion of individual FA is highly strain- dependent and much less dependent on culture conditions

Table 1. Fatty acid profile (mass %) of four oleaginous yeast strains exposed to N-limitation (C/N mass ratio of 70 : 1), Nanofer 25 (13 mg L^{-1}) and N-limitation (C/N mass ratio of 70 : 1) plus Nanofer 25

Yeast	Conditions	16 : 0	16 : 1	18 : 0	18 : 1	18 : 2	20 : 0	Total
Candida sp.	Control	22.4	6.9	10.7	29.8	26.1	4.1	31.4
	Nanofer 25	16.9	9.8	1.6	37.2	34.0	0.5	35.9
	N-limitation	13.8	7.9	3.4	36.0	36.8	2.1	35.4
	N-limitation + Nanofer 25	15.1	12.3	0.7	39.2	31.4	1.3	35.4
Rhodotorula glutinis	Control	19.7	0.1	10.8	57.7	10.5	1.2	33.2
	Nanofer 25	12.3	1.1	4.3	65.9	16.4	0.0	34.2
	N-limitation	13.1	1.3	6.9	61.7	15.6	1.4	33.8
	N-limitation + Nanofer 25	13.6	0.1	7.1	65.3	13.0	0.9	35.5
Trichosporon cutaneum	Control	17.0	2.6	3.8	26.1	48.8	1.7	48.9
	Nanofer 25	10.5	0.8	0.8	30.1	56.8	1.0	58.5
	N-limitation	16.0	1.9	3.2	23.9	53.4	1.6	51.6
	N-limitation + Nanofer 25	13.1	2.0	2.6	27.4	54.5	0.4	53.5
Yarrowia lipolytica	Control	15.2	13.0	4.1	50.8	12.6	4.3	17.9
	Nanofer 25	3.5	7.3	2.0	64.4	21.9	0.9	17.4
	N-limitation	10.3	9.4	2.3	38.7	35.8	3.5	18.6
	N-limitation + Nanofer 25	8.1	14.1	4.0	50.6	22.3	0.9	19.2

For results including SD (for three separate determinations), see supplementary information Table 1S.

Table 2. Fatty acid profile (mass %) of three non-oleaginous yeast strains exposed to N-limitation (C/N mass ratio of 70 : 1), Nanofer 25 (13 mg L^{-1}) and N-limitation (C/N mass ratio of 70 : 1) plus Nanofer 25

Yeast	Conditions	16 : 0	16 : 1	18 : 0	18 : 1	18 : 2	20 : 0	Total
Kluyveromyces polysporus	Control	7.4	59.1	0.9	28.7	3.0	0.9	5.1
	Nanofer 25	1.9	57.7	0.6	34.0	5.3	0.5	7.3
	N-limitation	8.6	57.2	1.2	27.6	4.3	1.1	5.3
	N-limitation + Nanofer 25	5.8	59.7	0.9	27.5	5.0	0.8	8.1
Saccharomyces cerevisiae	Control	14.5	44.0	4.1	28.6	5.1	3.7	3.8
	Nanofer 25	10.3	48.8	1.9	30.4	7.9	0.2	8.3
	N-limitation	11.5	38.9	3.9	35.2	7.4	3.1	4.1
	N-limitation + Nanofer 25	11.0	49.5	1.8	31.1	6.1	1.5	4.3
Torulospora delbrueckii	Control	25.0	22.6	3.6	24.3	16.8	6.6	4.6
	Nanofer 25	11.3	23.4	1.9	33.6	25.8	3.5	6.3
	N-limitation	21.4	18.4	3.0	21.9	27.2	8.1	4.9
	N-limitation + Nanofer 25	18.1	24.2	2.1	30.7	24.7	0.2	5.7

For results including SD (for three separate determinations), see supplementary information Table 2S.

(Papanikolaou et al., 2004). The present data indicate a clear effect of stressful cultivation conditions, especially in oleaginous species, above all a significant decrease in the proportion of saturated FA, particularly palmitic acid. Non-oleaginous species retain a more balanced proportion of FA but nZVI exerts the same effect as in oleaginous yeast strains (Pádrová et al., 2016), a marked (more than 50 %) decrease in the content of palmitic acid in *K. polysporus* and *T. delbrueckii* (Table 2).

The major FA in oleaginous yeast strains are palmitic (16 : 0), oleic (18 : 1) and linoleic (18 : 2) acids, which constitute from 80 % to 98 % of total FA content. These data accord with the data given in the literature, which also reports on the possible technological use of these FA (Chen et al., 2013; Sitepu et al., 2013). A yeast species of especial interest in terms of nutrition is *T. cutaneum* which, under the action of nZVI, produced nearly 60 % of ω-6-linoleic acid. The most abundant in the non-oleaginous strains are palmitoleic and oleic acids which comprise more than 85 % of total FA content in *K. polysporus*, more than 70 % in *S. cerevisiae* and about 45 % in *T. delbrueckii*. *K. polysporus* has a high and almost constant content of palmitoleic acid, which accounts for nearly 60 % of FA. *S. cerevisiae* also has a high content of palmitoleic acid, approximately 40 %, which rises to 50 % under the combined action of the two stress factors. From this perspective, these strains are therefore suitable for the nutritional use of this FA. The wine yeast *T. delbruecki* is characterised by a relatively constant proportion of four FA : palmitic (16 : 0), palmitoleic

(16 : 1), oleic (18 : 1) and linoleic (18 : 2), each accounting for 20–25 %.

On reviewing these data, the application of stress factors in oleaginous yeast strains is seen to reduce the proportion of palmitic acid (16 : 0) and increase the content of linoleic acid (18 : 2). No significant changes in the relative proportions of individual FA were recorded in non-oleaginous strains.

Palmitoleic acid and linoleic acid yields

The medical and dietary importance of both palmitoleic and linoleic acids is indisputable. In the present study (Tables 1 and 2), oleaginous yeast was shown to possess a significantly higher amount of linoleic acid than palmitoleic acid. Sitepu et al. (2013) reported that cultivation under N-limitation causes an increase in the abundance of linoleic acid in oleaginous strains. Both N-limitation and Nanofer 25 (13 mg L^{-1}) treatment were found to result in an improvement of linoleic acid production in all the strains studied. However, cultivation under combined stress conditions caused a mild decrease in the abundance of linoleic acid but was conducive to affording the highest amount of palmitoleic acid compared with the control in all strains. That suggests the possibility of a simple modification of specific FAs production by means of artificial stress conditions.

The relevance of a microbial strain for producing dietary important palmitoleic (C16:1) and linoleic (C18:1) acids depends on several factors, namely a combination of a high cell biomass yield, the proportion of C16:1 and C18:1 fatty acids in the TAGs synthesised and a high cell specific yield of TAGs under the chosen culture conditions. These factors can be summarised in the yield of the fatty acids per dry matter (mg g^{-1} of dry mass). Figs. 2a, 2b document the overall proportions of palmitoleic and linoleic acid in each yeast strain together with the total lipid content.

The non-oleaginous yeast *K. polysporus* (Fig. 2b) performed the best in terms of palmitoleic and linoleic acid production (61.8 % of total FA). The maximal abundance (64.7 %) was achieved under the combined effect of N-limitation and Nanofer 25 (13 mg L^{-1}); their yield per dry mass increased by almost 50 % from 142 mg g^{-1} of dry mass to 216 mg g^{-1} of dry mass. Comparatively high proportions of palmitoleic and linoleic acid were exhibited in *S. cerevisiae* (55.6 %) and *T. delbrueckii* (48.9 %) after cultivation in N-limited plus Nanofer 25-amended media. The highest overall yields in the two species were again achieved under combined stress conditions: 106 mg g^{-1} of dry mass and 123 mg g^{-1} of dry mass, respectively. Nevertheless, unlike oleaginous yeasts, non-oleaginous species have no ability to accumulate high amounts of lipids in cytosol (Ageitos et al., 2011) and the overall yield of FAs per dry mass was significantly lower (Fig. 2b). However, these yeasts are among the biotechnologically important species cultivated in large amounts and their biomass remaining after the biotechnological process can thus serve as a comparatively low-cost source of FAs (Řezanka et al., 2013).

T. cutaneum (Fig. 2a) exhibited the highest proportion of palmitoleic and linoleic acid out of all the oleaginous and non-oleaginous yeasts studied, from 51.4 % in the control cultivation to 57.6 % under combined stress conditions (Table 1), with the proportion of palmitoleic acid being very low, in the range of 0.8–2.6 %. This FA composition is close to that reported for cows' milk, where linoleic acid constitutes the main component of FAs (Dhiman et al., 2000). The maximum percent ratio of palmitoleic and linoleic acids was recorded following the Nanofer 25 treatment which, however, reduced the growth by 33.7 %, resulting in the lowest yield of both FAs (286 mg g^{-1} of dry mass). By contrast, N-limitation boosted the FAs' production by 28.5 % but had no impact on the growth and the overall yield was the highest (517 mg g^{-1} of dry mass). Combined stress conditions increased the amount of both FAs in lipids by 30.2 % compared with the control and the production was 432 mg g^{-1} of dry mass.

R. glutinis (Fig. 2a), despite the high lipid content and growth, exhibited an insignificant (0.1–1.3 %) production of palmitoleic acid (Table 1). Linoleic acid represented from 10.5 % (control cultivation) up to 16.4 % (Nanofer 25 treatment). Stress conditions enhanced the production of these two FAs by 60–90 % compared with the control, i.e. to about 200 mg g^{-1} of dry mass.

Y. lipolytica (Fig. 2a) achieved the highest lipid and biomass accumulation under combined stress conditions. The maximal yield of FAs (193 mg g^{-1} of dry mass) was recorded under N-limitation. The proportion of palmitoleic and linoleic acids was 45.2 % in lipids. Combined stress conditions had no significant impact on the abundance of the two FAs, but the growth and lipid accumulation were the most increased and the overall yield was 184 mg g^{-1} of dry mass.

Candida sp. (Fig. 2a) was the best producer of palmitoleic and linoleic acids. The maximum yield (627 mg g^{-1} of dry mass) was achieved under combined stress conditions and N-limitation (609 mg g^{-1} of dry mass). Both culture conditions caused an increase in the FAs' abundance in lipids and in the growth. The composition of FAs is very similar to that of tree nuts and peanuts (Nishi et al., 2014).

The ability to accumulate lipids and their composition is influenced by changes in the amount of nitrogen or the phosphorus source and the quantity of Zn, Fe, Mn or Mg ions in the culture media (Chen et al., 2013), as well as the source of carbon (i.e. glucose, glycerol, waste substrates) (Huang et al., 2013; Chen et al., 2013; Juszczyk et al., 2013). Another possibility is to vary the culture conditions such as temperature,

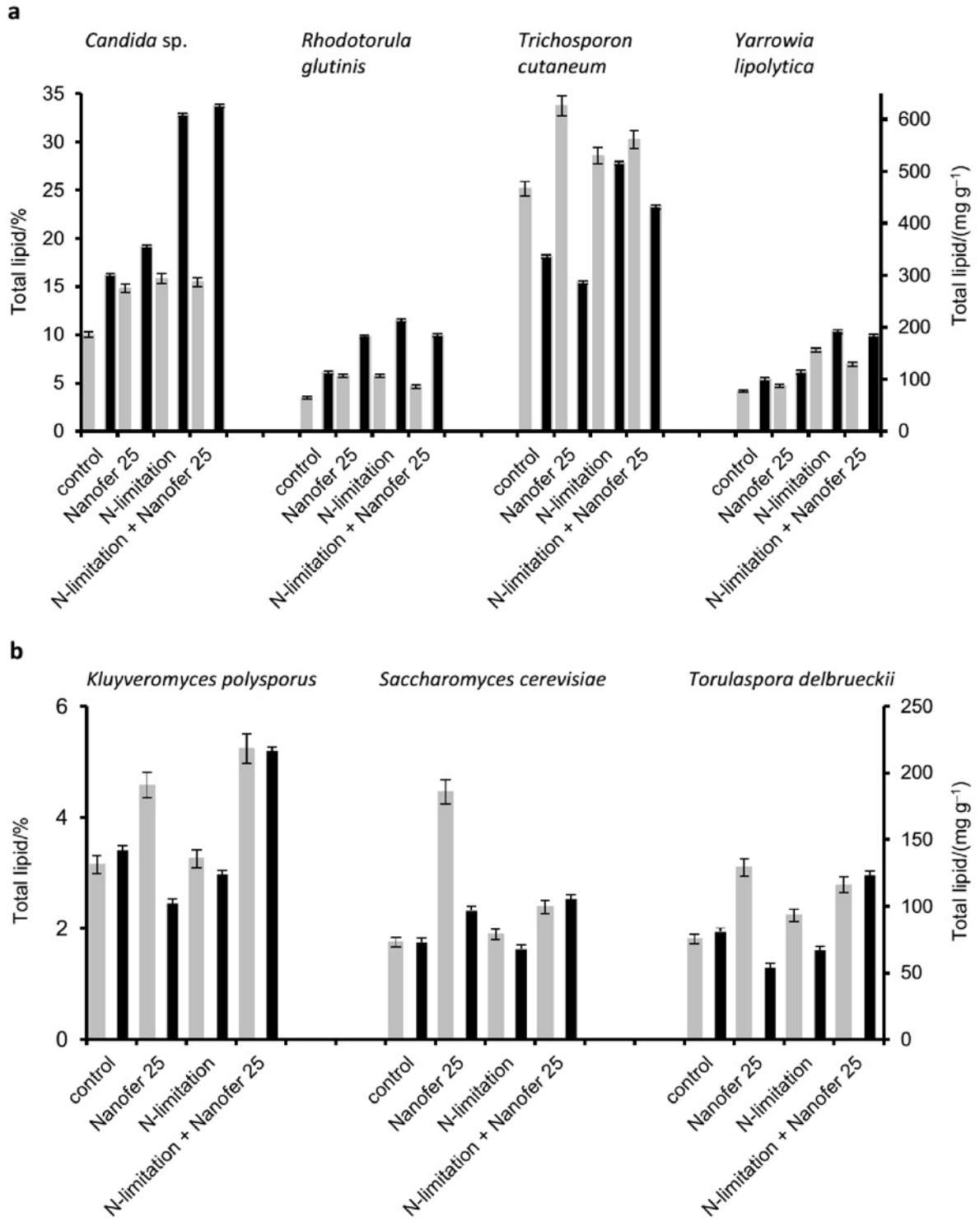

Fig. 2. Contents of palmitoleic and linoleic acid in total lipids (■) and yield of acids per dry mass (mg g^{-1}) (■) in oleaginous (a) and non-oleaginous (b) yeasts. Culture conditions: N-limitation (C/N mass ratio of 70 : 1), Nanofer 25 (13 mg L^{-1}), combined stress conditions (N-limitation – C/N mass ratio of 70 : 1, plus Nanofer 25 – 13 mg L^{-1}).

pH, length of cultivation (Ageitos et al., 2011) and artificial stress (nutritive or oxidative stress). In comparison with plant or animal sources, lipid production by microorganisms offers certain advantages including short duplication time (1–2 h), the possibility of large-scale fermentation and the absence of seasonal or climatic effects (Dey & Maiti, 2013; Enshaeieh et al., 2014). Because the composition of microbial lipids is similar to that found in conventional raw materials, a study of the culture conditions and an improvement

in quantitative and qualitative lipid production could lead to the use of certain microorganisms as a source of natural lipids (Ageitos et al., 2011).

Conclusions

In the present study, the best producer of nutritionally important fatty acids (palmitoleic and linoleic acid) was found to be *Candida* sp., which produced quantities of FAs (up to 600 mg g^{-1} of dry mass) similar to those produced in tree nuts and peanuts. An almost comparable yield was achieved by *T. cutaneum*. Both *Candida* sp. and *T. cutaneum* exhibited prevalent contents of ω-6-FAs and linoleic acid (80 % and 96 %, respectively). Non-oleaginous yeasts exhibited contents of these two acids in the opposite ratio; however, these yeasts, are widely used in biotechnology hence their waste biomass could be used as a raw material for FA production.

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Supplementary data

The supplementary data associated with this article (Using nutritional and oxidative stress to increase content of health-beneficial fatty acids in oleaginous and non-oleaginous yeasts) can be found in the online version of this paper (DOI: 10.1515/chempap-2016-0060).

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