Utilization of waste yeast biomass from brewery industry for the production of nutritional fatty acids

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Abstract Unsaturated fatty acids (FAs) are accepted as significant in human nutrition and therefore new possibilities are looked for in biotechnological production. One of these sources may be yeast biomass, a waste by-product of brewery and winemaking that is generated regularly in large volumes. The possibility of utilization of this resource for the production of dietetically beneficial FAs (e.g. palmitoleic acids) was investigated.

Two strains of biotechnologically important yeast strains and two samples of waste yeast biomass from brewery industry were studied for their FA content. Because many factors affect the yeast cells growth and the lipid content and composition, we have studied a range of cultivation conditions in the laboratory strains. The analysis of FA profile showed that biotechnological yeasts might be viable source of the nutritional FAs, as their content in *S. cerevisiae* was not impacted by the cultivation conditions. Our data suggest that, like *Kluyveromyces polysporus*, *Saccharomyces cerevisiae* can be used for production of palmitoleic acid in a wide range of conditions (unlimited or limited). Data from the waste yeast biomass confirmed the high content of palmitoleic acid but also showed the importance of the fermentation strain type that is employed in the process.

Keywords: palmitoleic acid; waste biomass; yeasts

1. Introduction

Yeast normally do not produce essential fatty acids (ω- a ω-6) but they might be employed in production of pharmacologically or nutritionally important unstaurated fatty acids, such as palmitoleic acid (Ratledge, 1993). Palmitoleic acid is known to possess many attributes that can be utilized in both health- and diet- related concerns. It has been shown to improve hyperglycemia and to prevent beta-cell apoptosis induced by glucose or saturated fatty acids. These benefits are correlated with the observed effect of total and LDL cholesterol reduction and the lower risk of new-onset diabetes mellitus. In cosmetics, e.g. in skin care, palmitoleic acid has been presented as antioxidant with a potential application in skin cells regeneration. Palmitoleic acid applications can help in the treatment of burns and in reducing dermatitis and eczema effects. Consequently, the demand for palmitoleic acid is increasing. Nowadays, main sources are macadamia nut oil (*Macadamia integrifolia*), sea buckthorn oil (*Hippophae rhamnoids*) and mink oil. Palmitoleic acid content of sea buckthorn oil is approximately 40 %, macadamia nuts oil consist of 12 % to 22 % of palmitoleic acid. Mink oil contains about 15 % of palmitoleic acid. Alternative sources of palmitoleic acid could provide a new supply source, but the conditions of a high concentration of palmitoleic acid, simple isolation methods, low cost and availability year round must be met (Shinde *et al*., 2013). The isolation of palmitoleic acid mainly relies on plant sources but there have been efforts to extend the possibilities of isolating this FA from microbial biomass.

Our work focuses on the possibility of using yeast biomass for the production of palmitoleic acid with the aim of using waste biomass from industry. To study the influence of cultivation conditions, which are known to significantly affect the ability of cells to accumulate lipids (Braunwald *et al*., 2013), we monitored total FA composition and the content of unsaturated acids (with focus on palmitoleic acid) under various N sources and P limiting conditions.

We examined two important biotechnological yeast strains *Kluyveromyces polysporus* and *Saccharomyces cerevisiae* that have different capacities to accumulate lipids and have different uses in biotechnology and two types of waste yeast biomass from breweries, employing top and bottom fermentation.

2. Material and methods

2.1 Microorganisms

The model yeast strains used in the present study were *Kluyveromyces polysporus* DBM 2171 and *Saccharomyces cerevisiae* DBM 2115 supplied by Collection of Yeasts and Industrial Microorganisms (DBM) of University of Chemistry and Technology, Prague. For long term storage the stock cultures were maintained in 20 % glycerol at -60 °C. Top-fermenting brewer´s yeast (type 1) and bottom-fermenting brewer´s yeast (type 2) from breweries in the Czech Republic were also studied (obtained as a waste biomass from the breweries).

2.2 Cultivation conditions

The yeast strains precultures were grown in YPD medium (20 g/L peptone, 10 g/L yeast extract, 20 g/L glucose, initial pH 6.0) in Erlenmeyer flasks at 150 rpm at 28°C to the late exponential growth phase (26 h).

For lipid production, 200 ml of mineral medium (in 500 ml Erlenmeyer flasks) was inoculated to a final OD_{600} 0.2 and incubated at 150 rpm and 28 °C until the early stationary phase of growth. The biomass for analysis was harvested by centrifugation (9 000 *g*, 10 min). The mineral medium composition was: KH_2PO_4 (3.5 g/L); Na_2HPO_4 (2 g/L); $MgSO_4.7\ H_2O$ (1.5 g/L); yeast extract (1.5) and trace element solution 1 mL/L (composition: $MnCl_2{\cdot}4H_2O$ (20 mg/L); $FeSO_4{\cdot}7H_2O$ (1 mg/L); $NaMoO_4{\cdot}2H_2O$ (1 mg/L); $CaCl_2{\cdot}2H_2O$ (20 mg/L)), pH 6.0. Glucose (30 g/L) was added as carbon source. The N sources and P limiting conditions were added and modified according to the experiment (see Table 1). The dry cell weight was determined after drying the biomass in the stationary phase of growth to a constant weight at 110 °C. All experiments were performed at least in triplicate.

2.4 Lipid extraction

The yeast biomass was lyophilized after harvesting and mixed with 2 mL of 0.1 M Na_2CO_3. The mixture was overlaid with liquid nitrogen and ground with ballotini glass beads (diameter 0.2 mm). After 3 cycles, final volume 50 mL of 0.1 M Na_2CO_3 was added. The solution was extracted with a chloroform-methanol mixture. The sample was centrifuged and the lower phase was evaporated to dryness.

2.5 FAME analysis

The extracted lipids (~5 mg) were saponified in 10% KOH-MeOH at room temperature overnight. The FA fraction was partitioned between diethyl-ether and alkali solution (pH 9) to remove neutral and basic components. The aqueous phase containing FAs was acidified to pH 2 and extracted with hexane. The FA fraction was methylated using BF_3/MeOH (14% solution of BF_3 from Sigma-Aldrich) to form fatty acid methyl esters (FAME).

The FAME (~1 mg) were dissolved in dimethyl disulfide (0.2 mL) and a solution of iodine in diethyl ether (3 mg in 0.05 mL) was added. The mixture was stirred for 24 hrs, then hexane (5 mL) was added and the mixture was washed with dilute sodium thiosulfate solution, dried over anhydrous sodium sulfate and evaporated. The products were dissolved in hexane and analyzed by GC-MS.

Gas chromatography–mass spectrometry of FAME was performed on a GC–MS system (Varian 450-GC, Varian BV, Netherlands), Varian 240-MS ion trap detector with electron ionization (EI), and CombiPal autosampler (CTC, USA) equipped with split/splitless injector. A SP-2380 column (Supelco) was used for separation (100 m, 0.25 mm ID, 0.20 μm film thickness). The temperature program started at 60 ˚C and was held for 1 min in splitless mode. Then the splitter was opened and the oven was heated to 160 ˚C at a rate of 25 ˚C min^{-1}. The second temperature ramp was up to 220 ˚C at a rate of 1.0 ˚C min^{-1}, this temperature being maintained for 10 min. The solvent delay time was set to 8 min. The transfer line temperature was set to 280 ˚C. Mass spectra were recorded at 3 scans s^{-1} under electron ionization at 70 eV, mass range m/z 50-600. FAMEs (fatty acid methyl esters) were identified according to their mass spectra and using a mixture of chemical standards obtained from Sigma-Aldrich.

3. Results and Discussion

The suitability of a microbial strain for producing palmitoleic acid or another fatty acid is given by several factors, it is a combination of a biomass high yield, high proportion of the target FA in total FAs, and the possibility of obtaining a high lipid content under specific cultutivation conditions. The ability of cells to accumulate lipids is influenced by changes in the C/N or C/P ratio, i.e. the amount (and type) of nitrogen or phosphorous source in the medium. The lipid content and FA composition is known to be species and even strain-specific (Zhu a kol., 2008). For the utilization of microbial biomass two strategies are proposed – a) strain and cultivation conditions are modified to promote maximum lipid accumulation of a certain type and b) utilization of waste biomass from industrial fermentation processes (da Rosa a kol., 2014, Papanikolaou a Aggelis, 2010, Yu a kol., 2011). We investigated the effect of variation of nitrogen sources and C/N and C/P ratio on lipid content and composition of the FAs. Cell growth and accumulation of lipids were also monitored in the cultivations. The YPD medium was used as control, as it represents a rich source of all types of nutrients (the initial C/N ratio in the medium is 3). Table 1 displays the determined proportion of fatty acids, total lipids and biomass dry weight in *K. polysporus* and *S. cerevisiae* at different C/N and C/P ratios and N sources and the fatty acid composition of biomass of two brewery yeasts. In addition to the C/N ratio, several different N sources were studied – ammonium ions, nitrate and urea.

Figure 1 shows the comparison of the contents of saturated, monounsaturated and polyunsaturated FAs in the total lipids. The use of different N sources did not have a significant effect on the saturation of fatty acid in both laboratory yeast strains, except for ammonium ions. The change of C/N ratio in *S. cerevisiae* from 30 to 70 (with ammonium ions) led to higher content of polyunsaturated FAs, effect which was not observed when nitrate or urea was used at C/N 70. In all strains, the monounsaturated FAs formed the majority of total FAs. In the waste biomass from brewery yeasts, the highest amount of polyunsaturated and saturated FAs was found in the bottom-fermenting yeast (type 2), the top-fermenting yeast strain showed higher proportion of monounsaturated FAs. From these FAs, palmitoleic acid formed the majority (Table 1).

Table 1. Proportion of fatty acids and total lipids in *K. polysporus*, *S. cerevisiae* at different C/N and C/P ratios and N sources and in two brewery yeast strains (Po – palmitoleic; P – palmitic; L – linoleic; O – oleic; S – stearic; A – arachidic acid).

Yeast		**Nitrogen source**	**Dry weight (g/l)**	**Total lipids %**	**16:1 Po**	**16:0 P**	**18:2 L**	**18:1 O**	**18:0 S**	**20:0 A**
Kluyveromyces	complex medium	YPD	4.5	5.1	59.2	7.5	2.7	29.1	1.0	0.5
polysporus	C/N 30 (C/P lim.)	NH_4^+	2.6	9.3	66.7	14.6	1.3	16.7	0.7	0.0
	C/N 70 (C/P 6)	NH_4^+	1.0	5.3	57.2	8.6	4.3	27.6	1.2	1.1
	C/N 70 (C/P 6)	NO_3^-	1.0	6.1	58.0	9.2	1.5	28.1	1.5	1.7
	C/N 70 (C/P 6)	urea	2.3	4.8	55.3	9.6	1.1	28.9	1.8	3.3
Saccharomyces	complex medium	YPD	5.2	4.5	39.3	11.8	3.6	39.5	4.3	1.5
cerevisiae	C/N 30 (C/P lim.)	NH_4^+	4.8	2.9	56.1	11.2	3.0	27.9	1.8	0.0
	C/N 70 (C/P 6)	NH_4^+	6.3	4.5	38.9	11.5	7.4	35.2	3.9	3.1
	C/N 70 (C/P 6)	NO_3^-	5.3	4.9	38.5	14.1	1.0	39.1	4.0	3.3
	C/N 70 (C/P 6)	urea	6.1	4.2	35.5	12.4	0.9	38.7	5.7	6.8
brewery yeast	„type 1“	-	-	0.9	41.2	9.2	2.7	11.6	12.3	0.0
brewery yeast	„type 2“	-	-	2.3	40.8	22.0	6.0	11.6	12.3	0.0

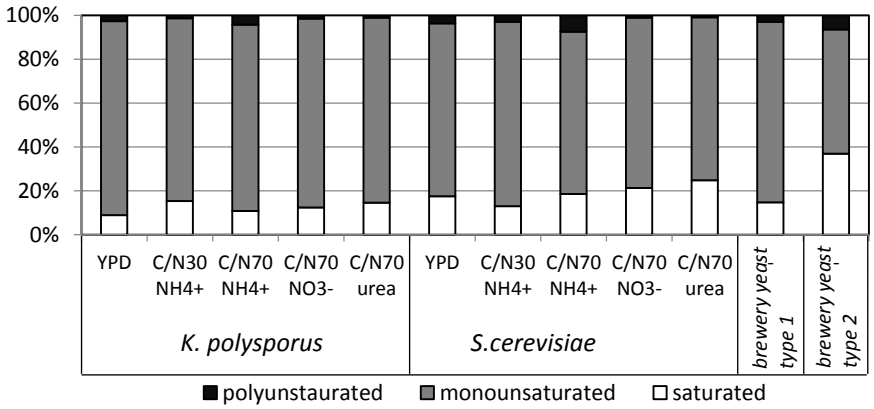

Figure 1.The proportion of saturated, monounsaturated and polyunsaturated FAs in total lipids of *K. polysporus*, *S. cerevisiae* at different C/N and C/P ratios and N sources and in two brewery yeast strains.

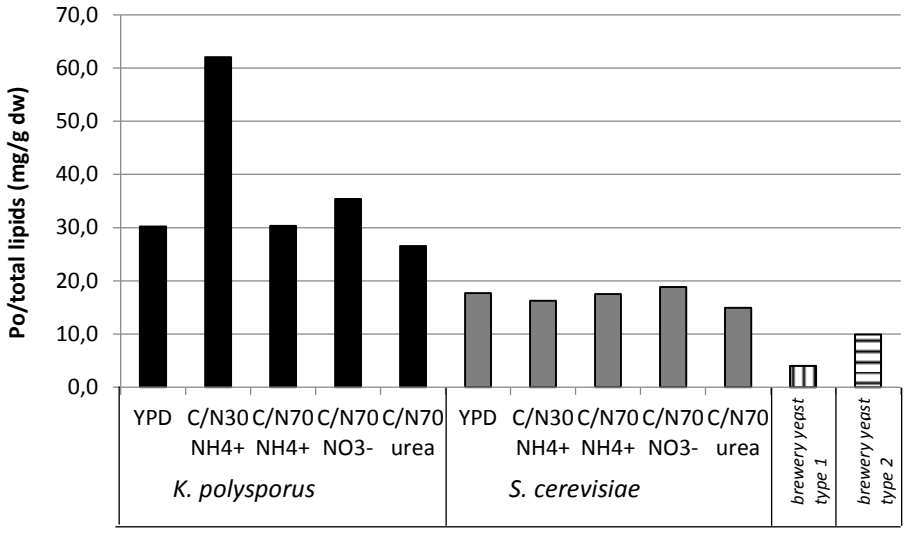

Figure 2. Content of palmitoleic acid (Po) in dry biomass (mg/g dw) in *K. polysporus*, *S. cerevisiae* at different C/N and C/P ratios and N sources and two brewery yeast.

When the content of palmitoleic acid is considered in relation to the content of total lipids in dry biomass, the highest amount (50 mg/g dw) is observed in *K. polysporus* at C/N 30 (ammonium ions). The waste brewery biomass contains lower amounts of palmitoleic acid per dry weight, the top-fermenting yeast 10 mg/g dw, therefore the main advantage of this source lies in its availability as waste by-product. The laboratory *S. cerevisiae* strain showed that the cultivation conditions did not influence the content of palmitoleic acid in dry weight.

The microbial biomass from brewery industry poses an interesting source of nutritionally interesting FAs. In our work, we have demonstrated the high content of palmitoleic acid in biotechnologically important yeasts, in all studied strains, the palmitoleic acid formed the most abundant fatty acid.

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