



## Comparative Analysis of Fatty Acid Composition in Some *Saccharomyces cerevisiae* Strains

Eslanda A. Khalilova<sup>1</sup>, Svetlana Ts. Kotenko<sup>1</sup>, Eivira A. Islammagomedova<sup>1</sup>  
and Dinara A. Aliverdieva<sup>1\*</sup>

<sup>1</sup>Department of Biochemistry and Biotechnology, Caspian Institute of Biological Resources of the Russian Academy of Sciences, Gadjeva, 45, Makhachkala, 367000, Russia.

### Authors' contributions

This work was carried out in collaboration between all authors. Author EAK designed the study, wrote the protocol, wrote the first draft of the manuscript and managed the literature searches. Authors STK and EAI performed the statistical analysis of the study. Author DAA supervised the study. All authors read and approved the final manuscript.

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### ABSTRACT

**Aims:** Comparative study of fatty acid composition of biomass in alcohol and wine *S. cerevisiae* strains, Champagne wine and ethanol, produced by yeast.

**Place and Duration of Study:** The study was undertaken in the Laboratory of Biochemistry and Biotechnology, Caspian Institute of Biological Resources, Russian Academy of Sciences, Russia. The duration of the study was the period of June 2015 to March 2016.

**Methodology:** Yeast strains were identified biochemically and by ITS rDNA gene sequencing. Capillary electrophoresis and gas-liquid chromatography were used for analyzing composition and content of carboxylic acids in the yeast biomass, Champagne wine and ethanol produced by yeast.

**Results:** Comparative analysis of fatty acid composition in biochemically active yeasts *S. cerevisiae* Y-503, *S. cerevisiae* Y-3980 and *S. cerevisiae* Litto – Levure CHA showed that the distinctive feature of alcohol Y-503 strain from wine strains was the lack of 16 fatty acids, the predominance of eicosapolyene acids (up to 58.1% in biomass and 56.6% in ethanol) and almost half the content of saturated fatty acids. All strains were found to be predominated with palmitic

\*Corresponding author: E-mail: [aliverdieva\\_d@mail.ru](mailto:aliverdieva_d@mail.ru);

(16:0) and linoleic (18:2 $\omega$ -6) acids that is typical for yeast *Saccharomyces*. This pattern was observed in Champagne wine and ethanol.

**Conclusion:** The results of comparative analysis of fatty acid composition of new and traditionally used commercial yeast strains may be useful in the selection of strains in order to manufacture high quality products in winemaking and alcohol industry.

**Keywords:** Yeast; *S. cerevisiae* Y-503; *S. cerevisiae* Litto Levure CHA; *S. cerevisiae* Y-3980; Champagne wine; ethanol; fatty acids.

## 1. INTRODUCTION

Today microbial lipid production has received applied interest [1-4]. Due to high growth rate and biomass yield, fast lipid accumulation, yeasts exhibit advantages over bacteria and another microorganisms [5,6] for subsequent lipid isolation and processing in biofuel production [1]. All types of microorganisms produce lipids but they differ from each another by the capacity to produce and accumulate lipids. Many oleaginous microorganisms are capable of producing lipids even at a level greater than 20% of their dry cell weight [7]. Moreover a systematic lipid biosynthesis engineering approach centered on diacylglycerol acyltransferase gene overexpression in *Yarrowia lipolytica* for increased lipid yield has been developed [8]. *S. cerevisiae* as nonoleaginous microorganism do not accumulate lipids in high quantity, but being traditional organism exploitable in food industry it is also of current interest [9]. The ability of yeast to produce or accumulate fatty acids is dependent on nutrient medium composition, carbon source, pH and many other factors (cultivation time, temperature, etc.) [10]. Numerous studies have reported fatty acid composition of yeasts [1,11,12], the interrelation between the lipid composition and the ability of *S. cerevisiae* strains to adapt to cultivation conditions [13], the possibility of utilizing various yeast species as potential producers of dietetically important major fatty acids [11]. Fatty acids along with carbonyl and sulphur compounds are known to play a role in the sensory quality of wine [14,15]. A number of commercial *S. cerevisiae* strains are used around the world in winemaking and alcohol industry. It is of applied interest to investigate new native and selected yeast strains that are responsible for the production of wines with peculiar flavours [9]. The aim of this study was evaluation of fatty acid composition of biomass in newly selected in our laboratory alcohol and wine *S. cerevisiae* strains versus traditionally used commercial wine strain, Champagne wine and ethanol, produced by yeast. New *S. cerevisiae*

strains have successfully passed laboratory and industrial testing and were recommended for industrial use.

## 2. MATERIALS AND METHODS

We used *S. cerevisiae* Y-503, *S. cerevisiae* Y-3980, strains engineered in the Caspian Institute of Biological Resources, Dagestan Scientific Center of Russian Academy of Sciences (a collection of Biochemistry and Biotechnology Laboratory, Russian National Collection of Industrial microorganisms of Federal State Unitary Enterprise «State Scientific Research Institute of Genetics») and industrial *S. cerevisiae* Litto Levure CHA strain.

### 2.1 Molecular and Genetic Studies

The Y-503 strain was shown to belong to the *S. cerevisiae* taxon by UP-PCR method in the group of S. Bulat (Laboratory of Eucaryote Genetics, Department of Molecular and Radiation Biophysics, Petersburg Nuclear Physics Institute, Russian Academy of Sciences). The Y-503 strain is heterozygous tetraploid. Species identification of Y-3980 strain was carried out by analyzing the nucleotide sequences of ITS1-5.8S-ITS2 region of rDNA. The amplified DNA fragment was sequenced in the scientific and manufacturing company «Syntol» (Moscow). Species identification was carried out by comparing the nucleotide sequences obtained with the data of genetic bank of NCBI ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) and CBS ([www.cbs.knaw.nl](http://www.cbs.knaw.nl)).

### 2.2 Cultivation

To cultivate *S. cerevisiae* Y-503, culture media with the following composition were used (g / l): Control medium: molasses - 488.74, (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> - 1.53, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> - 4.6, H<sub>2</sub>O - water, pH 4.5; Development medium №1: molasses - 488.74, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> - 2.58, H<sub>2</sub>O - geothermal water from well №36 of Makhachkala field (chloride - sulphate - hydrocarbonate sodium), pH 4.5. In the development media

molasses was diluted with geothermal water of different composition and in the control medium molasses was diluted with water till hydrocarbon content amounted to 20.0 g/100 cm<sup>3</sup> (in each variant). To optimize *S. cerevisiae* cultivation conditions the selection of media composition was made. The yeast was cultivated in 3000 ml flasks (working volume 1500 ml) for 120 h at 30°C, pH 4.5 in the laboratory using the depth method in a batch mode under anaerobic conditions. Cell cultures grown in nutrient media that were identical to control or development media were used as inoculum. To obtain inoculum (with hydrocarbon content 20 g/100 cm<sup>3</sup>) the cells were sequentially adapted to the media containing hydrocarbon: 7.9 – 10.8 – 12.4 – 20.0 g/100 cm<sup>3</sup>. The inoculum was cultivated for 5 days at temperature 30±1°C in a batch mode under anaerobic conditions. The content of inoculum was 10% of culture medium volume. 80% struktol – oily substance – (0.1 ml/1.5 l of medium) was used as defoamer. After 120 hours of cultivation and separation of the yeast by centrifugation (5000 g, 15 min) in a stationary laboratory centrifuge CLS - 344.2, the nutrient medium was named «fermented substrate».

The yeasts *S. cerevisiae* Y-3980 and *S. cerevisiae* Litto – Levure CHA were cultivated in the medium of the following wine materials (%): Pinot – 40, Riesling – 20, Aligote – 20, Chardonnay – 20. Fermentation mixture contained 2.5% of carbohydrate, 11.2% of alcohol, 0.3 g/dm<sup>3</sup> of phenolic substances, 17.8 g/dm<sup>3</sup> of reductones as well as 9.1 g/dm<sup>3</sup> of titratable and 0.17 g/dm<sup>3</sup> of volatile acidity. The inoculum (cultivated for 48 hours in deep culture of the grape must) was 3% of the medium volume. Champagnization of wine materials was carried out in a batch mode in the acratophore (Champagne – fermenting tank) at temperature 9 – 10°C, pressure 0.4 – 0.5 MPa, pH 3.3-3.5 for 20 days. The concentration of ethanol in alcohol stripper obtained by distilling the fermented substrate was determined using standard procedure.

### 2.3 Analysis of Low-molecular Organic Compounds

The content study of free low-molecular organic acids in biomass, aqueous-alcoholic solutions and Champagne wine was carried out by the capillary electrophoresis device "Capel-105" (Russia). The method of direct interesterification with methanol solution of sodium methoxide was

used to obtain methyl esters of fatty acids from triglycerides.

### 2.4 Fractionation of Methyl Esters of Fatty Acids

Fractionation of methyl esters of fatty acids (biomass, aqueous-alcoholic solutions, Champagne wine) and determination of impurities in ethanol were carried out with the gas chromatography using TRACE GC 2000 gas chromatograph (CE Instruments, Italy) with a flame ionization detector (PID) on a capillary gas column HP-FFAP (SGE Analytical Science, Australia) (50 m x 0.32 mm x 0.52 mm) with 10% diethylene glycol-succinate (Chromlab, Russia). The column temperature was 185°C; the evaporator temperature - 230°C. Nitrogen was used as the carrier gas (1.8-2.7 dm<sup>3</sup>/h). Acids were identified according to the period of retention of individual fractions using standard solutions of certain fatty acids. The content of methyl esters of fatty acids was obtained by the methods of statistical analysis and presented as mean values with standard deviation.

## 3. RESULTS AND DISCUSSION

### 3.1 The Study of Yeast Biomass

It is well known that volatile fatty acids being components of various aromatic compounds produced by *S. cerevisiae* can affect the oenological characteristics of sparkling wine [16]. The data in the Table 1 shows that the distinctive feature of alcohol *S. cerevisiae* Y-503 strain from wine *S. cerevisiae* Y-3980 and *S. cerevisiae* Litto Levure CHA strains is the lack of 16 fatty acids: butyric (4:0), caprylic (8:0), undecylic (11:0), tridecylic (13:0), myristic (14:0), myristoleic (14:1ω-9), pentadecenic (15:1), palmitoleic (16:1ω-7), margaric (17:0), stearic (18:0), elaidic (18:1ω-9), oleic (18:1ω-9), linoeladic (18:2), arachidic (22:0), behenic (22:0), cervonic (22:6 ω-3).

It is shown that the content of palmitic (16:0) and linoleic (18:2ω-6) acids is predominated in the biomass of all strains that is typical for the yeast *Saccharomyces*. The study of the biomass of wine strains revealed the increased content of saturated acids (1.64 and 1.78 times) as compared to the alcohol *S. cerevisiae* Y-503 strain (see Table 1). However, the saturated capric (10:0), and lauric (12:0) fatty acids (that are contained in all species of yeasts and

important for the quality of Champagne wine) in wine yeast were higher 1.55/2.61 and 1.57/2.34 times, respectively, on average than in alcohol Y-503 strain.

The total content of unsaturated fatty acids in the biomass of *S. cerevisiae* Y-3980 and *S. cerevisiae* Litto Levure CHA, on the contrary, was 2.3 times less than in *S. cerevisiae* Y-503. All studied strains are able to synthesize eicosapolyene acids, and their total content in the biomass of *S. cerevisiae* Y-503 reaches 58.10% and in wine strains – 26.27% on average of the total acid amount. Accordingly, the maximum values of linoleic (18:2  $\omega$ -6), arachidonic (20:4 $\omega$ -6), and dihomo- $\gamma$ -linolenic (20:3 $\omega$ -6) acids (well known as precursors of the extensive group of physiologically and pharmacologically active compounds) were observed in the alcohol strain. Some fatty acids in the biomass of all studied strains were found in a small amount, less than 1% (see Table 1).

### 3.2 The Study of Champagne Wine and Ethanol

Analyzing the data of the fatty acid pool of Champagne wine and ethanol we identified specific characteristics in the composition of the fatty acids depending on the strains used. Many researchers demonstrated the value of the fatty acids in the biosynthesis of aroma-producing complex in sparkling wine and the formation of sensory properties including fruit and herbal aroma, sunflower tones, foamy and antioxidant properties [9,16,17].

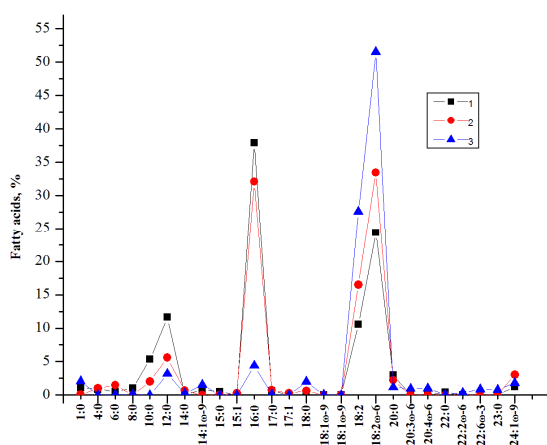
In all variants we found formic (1:0), pentadecenic (15:1), linoleic (18:2 $\omega$ -6) and dihomo- $\gamma$ -linolenic (20:3 $\omega$ -6) acids absent in the biomass of wine and alcohol strains. Palmitic (16:0) and lauric (12:0) acids are obligatory for all kinds of yeasts, ethanol and wine. Significant differences were found in the amount of saturated and unsaturated

**Table 1. Fatty acids content of the *S. cerevisiae* biomass**

<b>Fatty acids</b>	<b><i>S. cerevisiae</i> Y-503</b>	<b><i>S. cerevisiae</i> Litto – Levure CHA</b>	<b><i>S. cerevisiae</i> Y-3980</b>
butyric (4:0)	-	2.6	3.60
caproic (6:0)	1.95	1.45	0.80
caprylic (8:0)	-	4.50	6.04
capric (10:0)	5.22	13.65	8.07
undecylic(11:0)	-	7.10	3.75
lauric (12:0)	4.77	14.00	7.48
tridecylic (13:0)	-	0.90	3.76
myristic (14:0)	-	9.30	2.20
myristoleic (14:1 $\omega$ -9)	-	4.86	5.25
pentadecenic (15:1)	-	0.75	2.77
palmitic (16:0)	27.76	5.70	22.75
palmitoleic (16:1 $\omega$ -7)	-	5.25	2.85
margaric (17:0)	-	0.80	0.92
margaroleic (17:1)	2.20	2.35	0.93
stearic (18:0)	-	1.10	3.84
elaidic (18:1 $\omega$ -9)	-	4.00	-
oleic (18:1 $\omega$ -9)	-	4.55	14.65
linoeladic (18:2)	-	0.21	0.91
linoleic (18:2 $\omega$ -6)	26.14	2.78	6.88
arachidic (20:0)	-	5.15	1.28
dihomo- $\gamma$ -linolenic (20:3 $\omega$ -6)	11.75	-	-
arachidonic (20:4 $\omega$ -6)	20.21	-	-
behenic (22:0)	-	7.55	1.25
cervonic (22:6 $\omega$ -3)	-	1.45	0.020
including unsaturated polyene	58.10	25.45	31.49
saturated	41.90	74.55	68.51
unsaturated fatty acids /saturated fatty acids	1.39	0.34	0.46

Notes: The values in the table are means of three independent determinations

acids (including eicosapolyene acids) in Champagne wine and ethanol (Fig. 1). Palmitic (16:0) and linoleic (18:2  $\omega$ -6) acids predominated in wine and yeasts (up to 37.88 and 33.44% of the total amount of fatty acids, respectively). But ethanol and biomass of the alcohol strain were predominated with linoleic (18:2  $\omega$ -6) and linoeladic (18:2) acids (51.51 and 27.57%). Short-chain butyric (4:0), caproic (6:0) and capric (10:0) fatty acids that ensuring the formation of colonization resistance in a living organism and affect the formation of foam in sparkling wine [18,19] are characterized by their complete absence or low content in ethanol.



**Fig. 1. Fatty acids content in Champagne wines and ethanol, produced by different *S. cerevisiae* strains 1 - Champagne wine (*S. cerevisiae* Litto-Levure CHA); 2 - Champagne wine (*S. cerevisiae* Y-3980); 3 – Ethanol (*S. cerevisiae* Y-503)**

Plots are representative of three independent determinations

It was found out that ethanol produced by *S. cerevisiae* Y-503 strain contained significantly less amount of saturated fatty acids (3.3 - 4.4 times) compared to Champagne wine. It was also detected that ethanol was predominated with unsaturated fatty acids (85.71%) compared to wine (37.02: 53.27%). According to the data shown in Fig. 1, the content of unsaturated fatty acids in ethanol is higher due to the predominance of essential, linoleic (18:2 $\omega$ -6) and linoeladic (18:2) (1.8 and 2.1 times on average) compared to wine. Along with this, the amount of eicosapolyene acids in wine is 1.6 and 2.1 times lower. It was found out that ethanol contained in a little amount polyene acids of the following groups  $\omega$ -3,  $\omega$ -6,  $\omega$ -9: linoleic (18:2 $\omega$ -6), oleic (18:1 $\omega$ -9), dihomo- $\gamma$ -linolenic (20:3 $\omega$ -3),

arachidonic (20:4 $\omega$ -6), docosadienoic (22:2 $\omega$ -6), cervonic (22:6 $\omega$ -3) that were absent in Champagne wine. Linoleic (18:2 $\omega$ -6) known as vitamin F and nervonic (24:1 $\omega$ -9) acids were found in all studied variants.

#### 4. CONCLUSION

According to our studies, the amount of fatty acids in Champagne wine and ethanol corresponds to the standard physiological and biochemical indicators of product quality [17,20]. Comparative analysis of fatty acid composition of biochemically active *S. cerevisiae* Y-503, *S. cerevisiae* Y-3980 and *S. cerevisiae* Litto Levure CHA strains showed that the distinctive characteristic of alcohol Y-503 strain was the absence of 16 fatty acids compared to wine strains. Biomass of Y-503 strain was characterized by the predominance of eicosapolyene acids and almost half the content of saturated fatty acids compared to wine strains. Accordingly, this pattern was detected in Champagne wine and ethanol. Comparing the new *S. cerevisiae* Y-3980 wine strain with commercial *S. cerevisiae* Litto Levure CHA have led to the conclusion that the new strain may be recommended to wine industry. Future more extensive studies are required in particular to select new alcohol and wine strains with proper characteristics. The results of the study may be useful for selection of natural strains in order to obtain high quality products of alcohol industry and winemaking with peculiar characteristics, highlighting regional specificities.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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