CONCENTRATIONS PROFILES OF AROMA COMPOUNDS DURING WINEMAKING

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Abstract

One selected autochthonous and one commercial Saccharomyces cerevisiae strains were used as starters for fermentation process. Two fermentative medium were used: real grape must variety Gewürztraminer and medium. The ammonium and amino acid composition of synthetic medium was similar to must. The only sugar in the synthetic medium was glucose, which was present at the same concentration of 225 g/l as total reduced sugars in real must. At the same testing conditions, each strain of S. cerevisiae produced individual metabolites in different concentrations and combinations which significantly influenced resulting wine flavor. This paper deals with experimental comparison of selected aromatic compounds (alcohols and esters) that directly affect the secondary aroma of produced wines. We looking for the tool to produce an original and attractive product in form of suitable autochthonous strain Saccharomyces cerevisiae.

Key words: *Aroma compounds, wine aroma profile, yeast strain, alcoholic fermentation*

1. INTRODUCTION

Wine is a complex mixture of many compounds originated in various stages of winemaking process. One of most important aspects of wine is its flavoring. Wine flavor can be classified into three groups: primary (varietal), secondary (fermentative) and tertiary (wine ageing) aroma. With modern analytical methods and equipment more than 800 aroma compounds [1] such as alcohols, esters, organic acids, aldehydes, ketones, terpenes etc. have been identified in wines with a wide concentration range varying between hundreds of mg/l to the μg/l levels. Their combinations form the character of wine and enable the differentiation of one wine from another.

Secondary aroma of wine originates from yeast and bacterial metabolism during alcoholic and malolactic fermentation and contains various organic substances. Among the compounds influencing wine aroma, higher alcohols and esters are the most abundant. Redundant concentrations of higher alcohols lead to strong, pungent flavor and taste. However, its optimal concentrations give the wine fruity character [2]. At low concentrations, most esters have pleasant fruity aromas, but (likewise the higher alcohols) excessive concentrations are unwelcome and smell like organic solutions [3]. Production of esters and higher alcohols depends on many factors including aeration, concentration of fatty acids, higher alcohols and their precursors. Important role by production of esters plays the species and strain of used yeast.

Gewürztraminer is typical variety of north wine producing regions of Europe. In 16-th century it has been spread down from French Alsace through German and Austria to middle and east Europe. Later it was exported to America and Australia where it is grown until today [4]. Traminer has a great variety of flavors which is considerably influenced by diversity of natural locality like soil and climate conditions and used winemaking technology. Gewürztraminer offers full bodied wines with excellent bouquet, high content of extractive compounds and appropriate concentration of ethanol.

The aim of the present contribution was to evaluate influence of one autochthonous strain Saccharomyces cerevisiae on secondary aroma of Gewürztraminer wine and to compare properties of these strain with commercial culture. To investigate tine concentrations profiles of particular volatile organic compounds responsible for the sensory profile of wine and to achieve following comparison of yeast strains, we have used liquid-liquid extraction of samples followed by GC-TOF-MS method.

2. MATERIALS AND METHODS

2.1. Yeast strains

In experiment, two yeast strains of *Saccharomyces cerevisiae* were used. *S. cerevisiae* with operating name TC-2 was autochthonous culture isolated from natural source (vine) and is a part of collection of microorganisms of Faculty of Chemical and Food Technology (Slovak University of Technology, Bratislava, Slovakia). *S. cerevisiae* Oenoferm belle arome F3 is a commercial preparation of dry wine yeast produced by company Erbslöh Geisenheim, Germany. Both strains are characterized by high production of esters and are suitable for production of white wines with expressive fruity aroma.

2.2. Preparation of inoculum

Yeast starters for fermentation were prepared from a yeast strain culture grown aerobically for 24 h in a 100 ml of liquid medium (20 g/l glucose, 10 g/l yeast extract (Merck); pH=6,5) in a 500 ml cultivation flask, on an orbital shaker (2 Hz) at 28°C. After cultivation, concentration of the yeast biomass was determined by counting in a Bürker chamber. The calculated volume of biomass was withdrawn and centrifuged (10 min, 1370×*g*). Separated biomass was washed with MilliQ deionised water, centrifuged again and finally added to fermenting medium to achieve the starting concentration of biomass 10^6 cells/ml.

2.3. Experimental fermentation of grape must



Fig. 1. Experimental layout

For the experimental fermentation grapes of vine variety Gewürztraminer of vintage 2016 were used. Grapes originated from production of Slovak company Vino Natural Domin&Kušický, s.r.o. Veľký Krtíš. It is localized in the south of Middle Slovak vineyard region, locality Dolné Plachtince Destemmed and crushed grapes were macerated for 4 hours and subsequently pressed. Clarification of pressed grape juice was performed statically using bentonite and must gelatine (preparation Mostgelatine, Erbslőh Geisenheim, Germany) (dose 1 g/l). After 12 h clarified juice was treated by gaseous SO_2 (10 mg/l), filled into 50 l glass flasks and inoculated by autochthonous yeast strain TC-2 in form of liquid yeast starter. Starting concentration of biomass in grape juice was 10^6 cells/ml. The main alcoholic fermentation had proceeded for 2 weeks at the temperature 17°C. Layout of experimental fermentation is depicted in Fig.1.

2.4. Experimental fermentation of synthetic (YD) medium

For experimental fermentation YD medium of following final composition was used: 225 g/l glucose, 8 g/l yeast extract (Merck), pH=3,5. Fermentation took place in 5 litres fermenter: 4 l of operating volume in fermenter BIOFLO® 415 (New Brunswick, USA). Before fermentation, 900 g of glucose was dissolved in 3500 ml of MilliQ deionized water and sterilized directly in fermenter.

After sterilization, sterile water solution of yeast extract (32 g in 500 ml) was added and pH was adjusted by L-tartaric acid (Sigma Aldrich). Medium was aseptically inoculated and the main alcoholic fermentation had proceeded for 2 weeks at controlled temperature 17°C, without stirring.

2.5. Sampling

At regular intervals (every 24 h), samples of fermenting medium were taken and analyzed. Before sampling, content of fermenter was shortly stirred to homogenize the yeast biomass. After determination of biomass concentration samples were centrifuged and analyzed in terms of the other analytical parameters.

2.6. Chemical analysis

Basic oenological parameters as well as detailed volatile profile of all wines have been determined. Concentrations of reducing sugars have been analyzed by Schoorl method, concentration of ethanol and extract pycnometrically (OIV-MA-AS312-01A, OIV-MA-AS2-03B). Total volatile acids (expressed as acetic acid) have been separated from the sample by steam distillation and then as well as total acids (expressed as the tartaric acid) determined by acid-base titration with 0,1 mol/l KOH (OIV-MA-AS313-01, OIV-MA-AS313-02).

Profiles of volatile organic compounds were analyzed by comprehensive two-dimensional gas chromatography using method presented in [5]. By the analysis, benzophenone was used as the internal standard (20 µl of methanol solution of benzophenone with concentration 16 mg/l was added to 6 ml of sample). For bioengineering modeling relative concentrations c_{rel} in µg/l of individual VOCs were used. Values of relative concentrations were calculated based on following formula and expressed as equivalents of internal standard benzophenone.

$$c_{rel} = 53{,}3\frac{A_{xi}}{A_{is}}$$

where A_{xi} is the chromatographic peak area of identified VOC, A_{is} is the peak area of internal standard and 53.3 µg/l means real concentration of benzophenone in sample.

3. RESULTS

Samples of fermentation broth were analyzed to obtain time dependent concentrations of basic analytical parameters: reduced sugars, ethanol, assimilate nitrogen. For fermentation we used above mentioned two yeast strains: autochthonous strain TC-2 and commercial strain Oenoferm belle arome F3 (OBA). In both cases were used clarified Gewürztraminer must. Results are depicted on Fig.2 for OBA yeast strain and on Fig.3 for autochthonous TC-2 yeast strain.

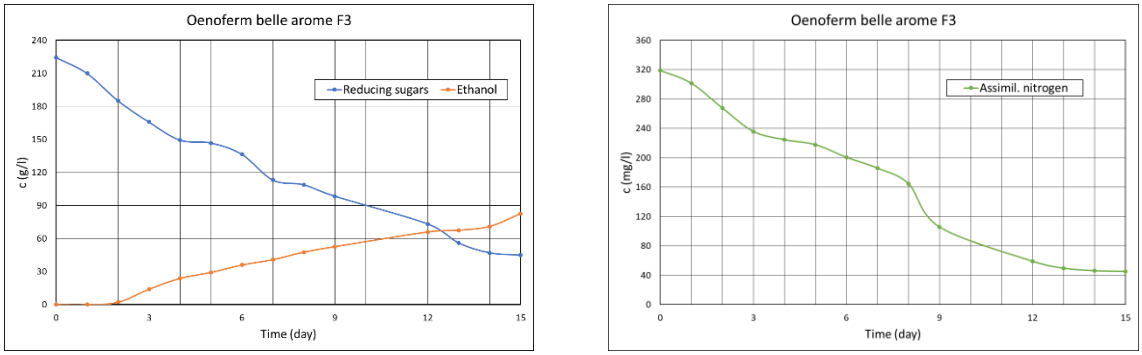


Fig. 2. Profiles of reducing sugars, ethanol and assimilable nitrogen by fermentation with strain OBA.

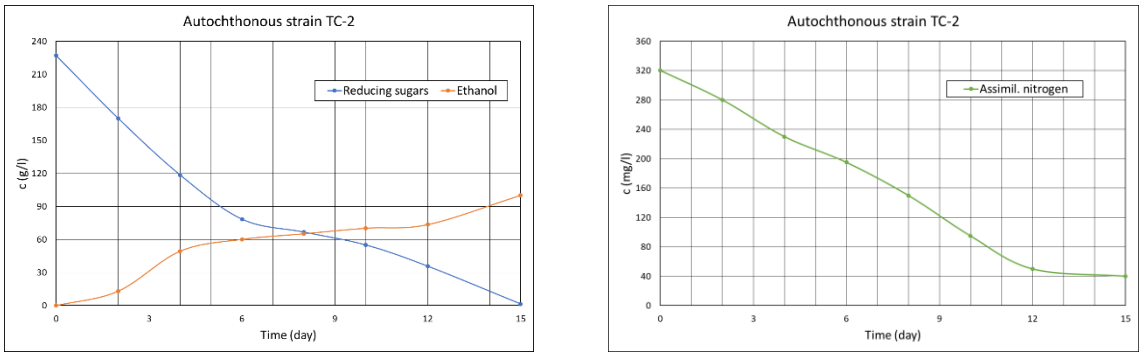


Fig. 3. Profiles of reducing sugars, ethanol and assimilable nitrogen by fermentation with strain TC-2.

From these pictures it can be seen, that during 15 days fermentation very similar concentration profiles of basic parameters was achieved, no significant differences occurred.

Besides this, we used gas chromatography and mass spectrometry for evaluation the influence of yeast strain and fermented media on volatile organic compounds (VOC) profile. In particular samples was identified approximately 250 VOCs creating of aroma profile. Five majority volatile organic compounds (isoamyl alcohol, 2-phenylethanol, 2,3-butanediol, ethyl oktanoate, 2-phenylethyl acetate) were monitored from point of view its time concentration changes.

3.1. Higher alcohols

Isoamyl alcohol, 2-phenylethanol and 2,3-butanediol are higher alcohols, which are typical products of yeast metabolism during alcoholic fermentation. Affirmative acknowledgement of this conclusion is that above mentioned compounds had practically zero concentrations in must and during fermentation concentrations were growing.

Majority element of this group is **isoamyl alcohol** (3-methyl butanol). It features with fermentative, excitative, similar to banana flavor. Production of isoamyl alcohol inside cell of yeast is connected with sugars metabolism. Changes of isoamyl alcohol concentration during fermentation in both synthetic (YD) medium and grape must each with two strains of *S. cerevisiae* Oenoferm belle arome F3 and autochthonous strain TC-2 are visible in Fig.4.

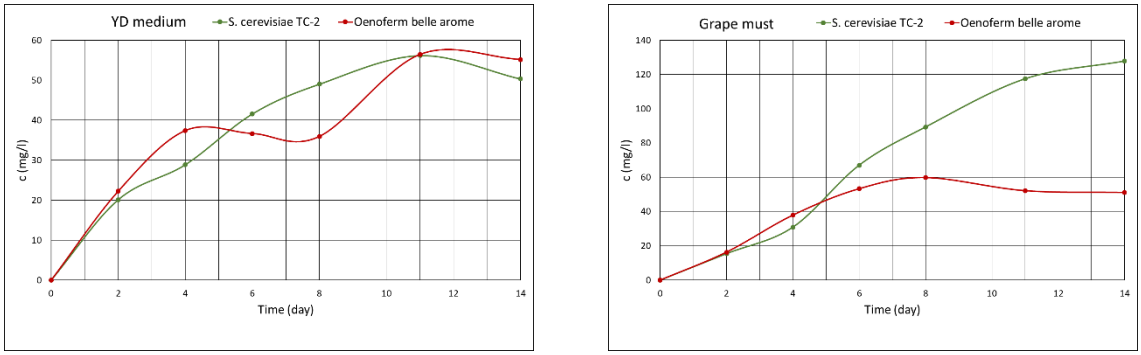


Fig. 4. Production of isoamyl alcohol during fermentation of YD medium and grape must.

As can be seen from Fig.4 in YD medium production of isoamyl alcohol by strain OBA is more intensive up to 4. day, then up to 8. day concentration is stagnant. After 8. day of fermentation production of isoamyl alcohol again rises to the 11. day and then again is stagnant. Final concentration reaches 55 mg/l. Situation with autochthonous strain TC-2 in YD medium is a little different. Production of isoamyl alcohol continually rises from the beginning of fermentation to 11. day. After this point production decreases and final concentration is about 50 mg/l.

Another situation is in fermentation of grape must. In this case both strains produce isoamyl alcohol with similar speed up to 5. day of fermentation. From this point TC-2 produces isoamyl alcohol more quickly in comparison with OBA. Concentration profiles given by OBA is similar in both media, but TC-2 strain produces isoamyl alcohol more intensive in real must environment. Significant difference is in final concentration of isoamyl alcohol in real grape must: TC-2 approximately 130 mg/l versus OBA 55 mg/l.

Next important higher alcohol produced by yeast is **2-phenyl ethanol**. This compound has characteristic flavor of sweet rose and in wines facilitates flower aromas. Production of 2-phenylethanol has similarly rising trend as isoamyl alcohol. Concentration profiles of 2-phenylethanol during fermentation are depicted in Fig.5.

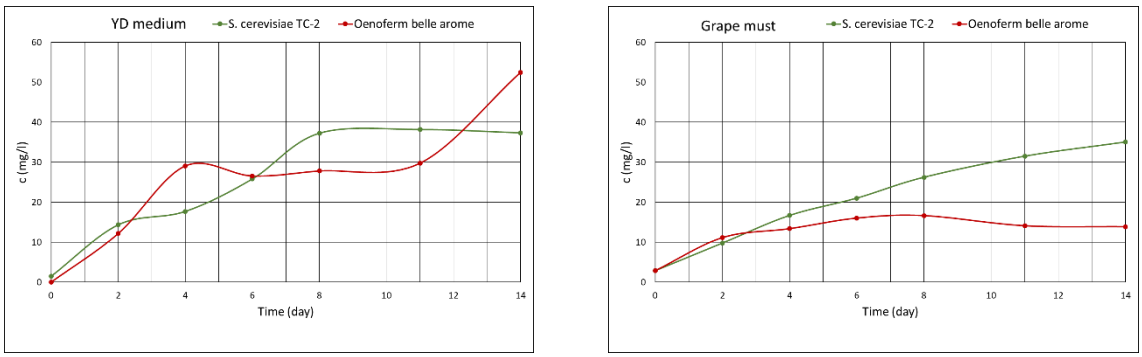


Fig. 5. Production of 2-phenylathanol alcohol during fermentation of YD medium and grape must.

From Fig.5 one can see similarity of profiles both yeast strains in both media. But for aroma profile of wine is most important final concentration of appropriate aromatic substance. In case of 2-phenylethanol strain OBA produces more substance in YD medium as in grape must (52 versus 14 mg/l). Autochthonous strain TC-2 produces approximately the same final concentration in both media. But in grape must TC-2 produces finally more 2-phenylathanol in comparison to OBA (35 versus 14 mg/l).

Dibasic alcohol **2,3-butanediol** was not identify in grape must before fermentation. 2,3-butanediol has buttery, creamily and bitter-sweet aroma, but due to higher perception threshold (600 mg/l) is not sensory registered. Measured concentration profiles of 2,3-butanediol are presented in Fig.6.

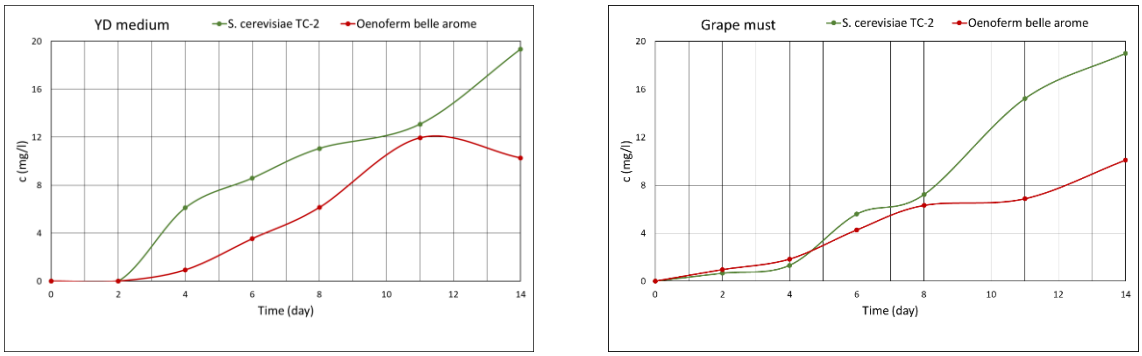


Fig. 6. Production of 2,3-butanediol during fermentation of YD medium and grape must.

From Fig.6 it is possible to see similar concentration profiles in both media with approximately the same concentrations of 2,3-butanediol after 14 days of fermentation. But concentrations are very small deeply under the perception threshold of this substance.

3.2. Esters

Esters are most numerous group of volatile organic compounds in wine. Esters can be produced by enzymatic or chemical way. Enzymatic production depends on yeast strain used by fermentation. Chemical way attendant upon thermodynamic equilibrium between esterification and hydrolysis. Ethyl oktanoate and 2-phenylethyl acetate are two investigated esters in our experiments. Further identified esters had very low concentrations in comparison with detection limit.

Ethyl oktanoate has flavor of tropical fruit, pineapple, apple and wines gives fresh fruity aroma. This ester reaches highest concentrations in measured profiles among esters taken into account. Concentration profiles versus time in fermented media are presented in Fig.7.

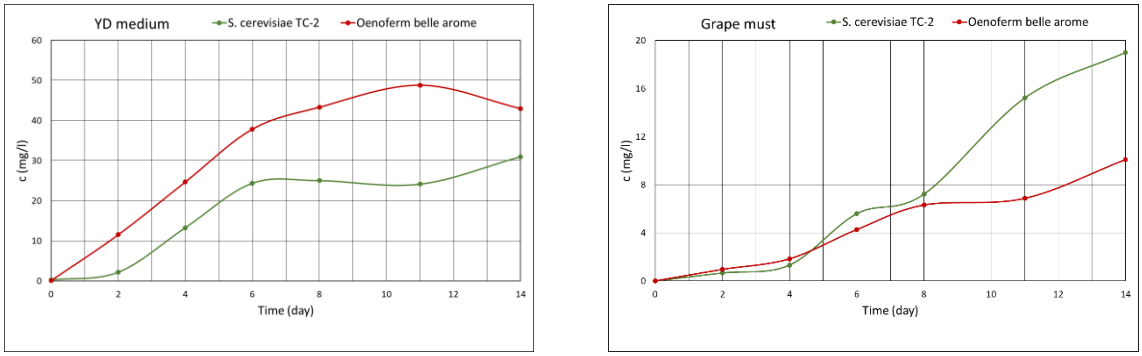


Fig. 7. Production of ethyl oktanoate during fermentation of YD medium and grape must.

From Fig.7 can be seen, that in YD medium production of ethyl oktanoate is always higher by commercial yeast OBA, but in real grape must it is oppositely. By fermentation of real grape must autochthonous yeast strain TC-2 produced final concentration double by comparison with strain OBA. From this point of view yeast strain TC-2 is more suitable to use, because ethyl oktanoate has agreable flavor eligible for wine consumers.

2-phenylethyl acetate has a light anisic, tropical fruity, sweet rose odor. Its concentrations in fermented media are ten times lower compare to ethyl octanoate. Measured time concentration profiles of 2-phenylethyl acetate are presented in Fig.8.

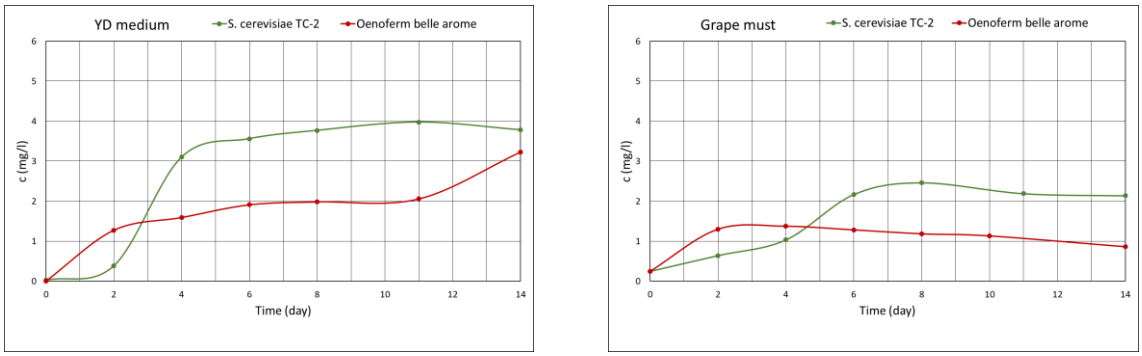


Fig. 8. Production of 2-phenylethyl acetate during fermentation of YD medium and grape must.

From Fig. 8 is clear similar behavior of both yeast strains in both fermentation broths. Starting production quickly rises up to 4. day and after this point production rate is stabilized. Final concentration of above mentioned ester is higher in both media for autochthonous strain TC-2. Interesting is, that final concentration in YD media is two times higher by comparison to grape must. In previous experiments it was contrarily.

4. CONCLUSION

Presence of higher alcohols and esters is very important for wine aroma profile. These substances are produced mainly by metabolism of yeast and therefore are responsible for secondary aroma of wine. From this point of view very considerable aspect is selection of adequate strain of yeast for alcohol fermentation by winemaking. Metabolic activity of each strain *Saccharomyces cerevisiae* is unique, therefore compounds produced by yeasts are in different concentrations in wines.

Analyzing results of our experiments it is possible to say, that in all cases higher concentrations of examined volatile organic compounds were achieved with autochthonous strain yeast TC-2 when fermentation broth was grape must. In YD broth higher alcohols and esters were produced too, but its concentrations were lower. In this medium absents grape substratum with many precursor substances and enzymes essential for production and interaction with other compounds in fermentation broth and therefore aroma profile was weaker.

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