Factors Affecting on the Concentration of Aromatic Alcohol 2-Phenylethanol in Alcoholic Fermentation of Grape Juice

Paata Vashakidze and Marine Bezhuashvili

***Abstract*— It's been studied the concentration varies of 2-Phenylethanol under influence of some factors. The study was conducted using the grape juice of Rkatsiteli, a Georgian white wine grape variety. The influencing factors are selected: Nitrogen nutrition of wine yeast, strains of wine yeasts, pH of grape juice and fermentation temperature. In the process of alcoholic fermentation as nitrogenous nutrition source for yeasts have been used Diammonium phosphate (DAP) and alternative nitrogenous source (ANS). Alcoholic fermentation was carried out with natural microflora and following wine yeast strains: *Saccharomyces vini-Kakhuri 42* and Saccharomyces *vini- Rkatsiteli 61*. The pH of grape juice was 3, 0; 3, 8; the alcoholic fermentation was carried out in the following temperature intervals: 22-23^0 C; 27-28^0 C.**

The obtained results about the accumulation of aromatic alcohol 2-phenylethanol are important in terms of regulating the total composition of higher alcohols in Georgian wines in order to improve wine quality.

***Keywords*— Alcoholic Fermentation, 2- Phenylethanol, DAP, ANS.**

I. Introduction

Alcoholic fermentation of grape juice is one of the important stages of wine technology. In addition to its main products - ethyl alcohol and CO_2, secondary metabolites accumulate in the fermented area, which have a significant impact on the quality indicators of wine. The components obtained as a result of numerous biochemical transformations of various classes of compounds present in the fermented grape juice are localized in the wine material, pass through the period of formation of the wine material and significantly determine the organoleptic characteristics and chemical composition of the wine, the combination of which is reflected in the quality of the wine. Higher alcohols occupy an important place among the secondary metabolites of alcoholic fermentation of grape juice. The main part of the higher alcohols of wine is formed from free amino acids in the process of alcoholic fermentation according to the Ehrlich scheme. This conversion takes place on the basis of assimilation of free amino acids by yeasts [1]. Both aliphatic amino acids (leucine, isoleucine, valine, etc.) and aromatic amino acids (phenylalanine, tyrosine and tryptophan) are assimilated by yeasts. Higher alcohols are formed from aliphatic amino acids - isoamyl, amyl, isobutyl, isopropyl, hexyl, etc. Aromatic higher alcohols are formed from aromatic amino acids: 2-phenylethanol, tyrosol and tryptofol. Higher alcohols formed from aliphatic amino acids are characterized by a strongly pronounced pungent smell and have a great influence on the aroma of wine. Their excess amount is unpleasantly felt in the wine and therefore it is considered undesirable for the wine. These higher alcohols are known as fusel oils [2] Among aromatic higher alcohols, 2-phenylethanol, which is characterized by the scent of a rose, is important for the aroma of wine. Tyrosol and tryptofol are associated with "buffer" [3]. The interaction of triptofol with SO_2 in wine and the effect of the obtained product on the wine aroma, which is related to the bitterness of the wine, have been determined [4]. Along with rose-scented 2-phenylethanol, its aromatic ester 2- Phenethyl acetate is important for the pleasant aroma of wine. It adds fruity and floral aroma to wine [5, 6]. In order to make the wine with the above-mentioned pleasant aroma, it is necessary to have a high concentration of the precursor free amino acid - phenylalanine in the grape juice and the correct selection of yeast strains.

As a result of experiments carried out by scientists in this direction, wine and sake with an enhanced floral aroma were obtained [7-9]. Australian scientists have investigated the alcoholic yeast of Chardonnay grapes with their new 6 yeast strains and determined aromatic alcohols: 2-phenylethanol, tyrosol and tryptofol. A sulfur compound of tryptofol is also defined. These studies confirmed the superiority of the used strains and the improvement of wine aroma [10]. As we mentioned, the presence of a large amount of higher alcohols in the form of "fusel oils" in the wine has a negative effect on the aroma of the wine. In order to improve the quality of wine, it is advisable to regulate the quantity of higher alcohols. Reducing the concentration of "fusel oils" in wine

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and maintaining free amino acids, we implemented by adding an alternative nitrogen source of yeasts to the alcoholic fermentation of grape juice. For this, the influence of various factors was studied and as a result optimal conditions were determined [11-13]. The aim of the present study is to present the variation concentration of aromatic alcohol 2-phenylethanol in the alcoholic fermentation of grape juice under the influence of some factors.

II. Objects And Methods

As research objects We used dry European-type white wines made from Rkatsiteli grape juice, a white-grape wine grape variety common in Eastern Georgia.

To prepare them, we carried out alcoholic fermentation by adding DAP (control option) and adding ANS (test option) to grape juice. Alcoholic fermentation of Rkatsiteli grape juice was also carried out with 2 strains of wine yeast Saccharomyces vini-Kakhuri 42 and Saccharomyces vini-Rkatsiteli 61. under conditions of variable pH (3.0; 3.8) in the following temperature intervals: 22-23^0 C; 27-28^0 C.

We extracted 2-phenylethanol from the research objects together with other higher alcohols and volatile aromatic compounds with a pentane-diethyl ether mixture (2:1), then processed it according to the method and analyzed it on a gas chromatograph. We have prepared 4 wine research objects:

Sample I - control - a free flowing grape juice + 200 mg/l DAP.
Sample II - a free flowing grape juice + 200 mg/l ANS.
Sample III - a free flowing grape juice + 300mg/l ANS.
Sample IV - a free flowing grape juice + 100 mg/l ANS

Trial Options (Grape juice + 2, 5% Sacch. Vini Kakhuri 42)

1. Control - Grape juice + DAP (100 mg/l); Fermentation 22-23 °C, pH 3.8;
2. Grape juice + ANS(100 mg/l); Fermentation 22-23°C, pH 3.8;
3. Grape juice + DAP(100 mg/l); Fermentation 22-23°C, pH 3.0;
4. Grape juice + ANS(100 mg/l); Fermentation 22-23°C, pH 3.0;
5. Grape juice + DAP(100 mg/l); Fermentation 27-28°C, pH 3.8;
6. Grape juice + ANS(100 mg/l); Fermentation 27-28 °C, pH 3.8

Trial Options (Grape juice +2,5% Sach. vini Rkatsiteli 61)
1. Control-grape juice + DAP(100mg/l) Fermentation 22-23C, pH3.8;
2. Grape juice + ANS(100mg/l); Fermentation 22-23°C, pH 3.8;
3. Grape juice + DAP(100 mg/l); Fermentation 22-23°C, pH 3.0;
4. Grape juice + ANS(100mg/l); Fermentation 22-23°C, pH 3.0;
5. Grape juice + DAP(100mg/l); Fermentation 27-28°C, pH 3.8;
6. Grape juice + ANS(100mg/l); Fermentation 27-28 °C, pH 3.8;

III. Results and discussion

The results of the experiment are shown in the tables 1, 2.

TABLE I. Concentration (mg/l) of 2-phenylethanol in European-type wine materials made from Rkatsiteli

Samples	2-phenylethanol
I - control - a free flowing grape juice + 200 mg/l DAP	67,2
II-a free flowing grape juice + 200 mg/l ANS	41,0
III -a free flowing grape juice + 300mg/l ANS	21,8
IV - a free flowing grape juice + 100 mg/l ANS	30,0

TABLE II. Variation of 2-phenylethanol concentration (mg/l) according to alcoholic fermentation conditions of Rkatsiteli grape juice

Trial Options (Grape juice + 2, 5% Sacch. Vini Kakhuri 42)

samples	2-phenylethanol
Control - Grape juice + DAP (100 mg/l); Fermentation 22-23 °C, pH 3.8	69,0
Grape juice + ANS (100 mg/l); Fermentation 22-23°C, pH3.8	37,3
Grape juice + DAP (100 mg/l); Fermentation 22-23°C, pH 3.0	42,5
Grape juice + ANS (100 mg/l); Fermentation 22-23°C, pH 3.0	32,2
Grape juice + DAP (100 mg/l); Fermentation 27-28°C, pH 3.8	21,7
Grape juice + ANS (100 mg/l); Fermentation 27-28 °C, pH 3.8	19,2

Trial Options (Grape juice +2,5% Sach. vini Rkatsiteli 61)

samples	2-phenylethanol
Control-grape juice + DAP(100mg/l) Fermentation 22-23 C, pH 3.8	67,0
Grape juice + ANS(100mg/l); Fermentation 22-23°C, pH 3.8	34,0
Grape juice + DAP(100 mg/l); Fermentation 22-23°C, pH 3.0	40,0
Grape juice + ANS (100mg/l); Fermentation 22-23°C, pH 3.0	29,2
Grape juice + DAP(100mg/l); Fermentation 27-28°C, pH 3.8	20,3
Grape juice + ANS(100mg/l); Fermentation 27-28 °C, pH 3.8	16,5

The concentration of 2-phenylethanol in European-type wine materials varies according to the concentration of ANS. Specifically, when using a high amount of ANS - 300mg/l, a small concentration of 2-phenylethanol - 21 mg/l was observed in the wine material. As for when using DAP and

ANS with equal concentration - 200 mg/l, the use of ANS leads to a significantly lower concentration of 2-phenylethanol in the corresponding wine material. This indicates the non-intense assimilation of amino acid - phenylalanine by yeast in alcoholic fermentation (tab. 1). Using the wine yeast strains Sacch. Vini Kakhuri 42 and Sach. vini Rkatsiteli 61 in the Rkatsiteli grape juice fermented according different trial options, the process occurs with same regularity. Specifically, in the same temperature interval of 22-23 °C and pH 3.8, the use of DAP resulted in a higher concentration of 2-phenylethanol compared to the ANS variant. At the same pH and in the high temperature interval of 27-28°C, the same regularity is maintained, but with lower concentrations of 2-phenylethanol (tab.2)

IV. Conclusion

The results of the experiment reflect the influence of a number of factors on the concentration of the highest aromatic alcohol 2-phenylethanol in alcoholic fermentation of grape juice. These results should be considered together with other higher alcohols in order to regulate their totality with optimal technology. This is the basis of high-quality wine production.

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About PhD student Paata Vashakidze:

It`s very important to publish this kind of topic article because it covers one of my PhD research goals. I`m going to defense my thesis regarding “Improving Quality of European Wine by Regulation of Transformation Free Amino Acids” in this running year.