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Higher Alcohols of Wine-Transformation Regulation of Intermediate Products in Alcoholic Fermentation

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***Abstract* – The materials of European type of wine made by Georgian native varieties of Rkatsiteli have been studied. Alcoholic fermentation of grape juice is carried out by addition of nitrogenous substances, with Diammonium phosphate (DAP) and alternative nitrogen substance (ANS). In prepared wine materials have been studied the correlation of higher alcohols and residual sugar concentrations on the following factors: The concentration of alternative nitrogen substance, temperature of alcoholic fermentation and titratable acidity, for the experiment have been used wine materials fermented by the natural microflora, strains of cultural yeast Sacch. Vini - kakhuri 42, Sacch. Vini - Rkatsiteli 61 and dry yeast B 2000. Significant positive effects of alternative nitrogenous substance (ANS) have been identified with a significantly reduced concentration of higher alcohols (fusel oils) produced in the fermentation of grape juice proceeded by natural microflora, pure yeast cultures and dry yeast.**

***Keywords* – White Wine, Alcoholic Fermentation, Amino Acids, Higher Alcohols, ANS.**

I. Introduction

Alcoholic fermentation of grape juice is one of the important processes in high quality wine making technology. Numerous biochemical transformations in alcoholic fermentation along with the production of basic products lead to the formation of plenty of compounds as a co-products and intermediates, which are localized in wine material and significantly defines the quality of wine. From these conversions, it is important transformation of higher alcohols by free amino acids.

Higher alcohols are identified by a strong, pungent smell and taste and can have a significant effect on the sensorial quality and character of wine and brandy [1, 2, 3]. Higher alcohols are composed of aliphatic and aromatic alcohols [4].

Additionally there are other volatile compounds formed during yeast fermentation like higher alcohols, medium chain fatty acids and their corresponding esters, which also play an important role in the overall aroma of the young white wine. All of these compounds are affected by several fermentation factors like nutrition and fermentation temperature [5, 6]. Higher alcohols are characterized with a particularly strong odor, with a high concentration negatively affecting the bouquet and aroma of the wine. Higher alcohols are also known as fusel alcohols, and the word of fusel refers to a fraction smelling very strong and bad, which is obtained from the distillation of the fermented products [7]. The process of alcoholic fermentation depends on the vital activity of the yeast, which raises by adding a nitrogen nutrition source in the fermentation process.

The formation of higher alcohols by free amino acids is the result of the action. During the fermentation process, the higher alcohols like the 2-methylpropan-1-ol, 2-methylbutan-1-ol, 3-ethylbutan-1-ol, 2-phenylethan-1-ol and propan-1-ol, are usually metabolized by yeast either from the grape amino acids via the Ehrlich pathway or directly from sugars. To be specific, 65% of HA derived from amino acids and 35% from sugars under the enological conditions [8]. Nowadays, various nitrogen source are used in the production of wine as food for yeast, which provides a quantitative preservation of free amino acids in the ferment sphere and in wine too. As mentioned above, the increased specific smell of higher alcohols has a negative impact on the aroma of wine. In the higher alcohols as a dominant are shown Isoamyl alcohols and their formation and impact on the bouquet of wine is an actual issue for scientific researches. Isoamyl alcohol is the major higher alcohol found in wines (more than 50%) and its concentration has been reported in the range of 90 to 292 mg/l. [9].

Isoamyl alcohol, active amyl alcohol and isobutanol are also known as branched-chain alcohols because they are the degradation products of the branched-chain amino acids, leucine, isoleucine and valine [10]. Hence it follow that, free amino-acids are characterized with high nutritional value and higher alcohols with specific odors, improving the quality of wine by preservation quantities of free amino acids and reducing the concentration of higher alcohols (fusel oils) at the same time is a topical issue.

There are several researches about the combined effects of temperature and nitrogen nutrition on aromatic composition of wine, but most of them studied above volatile compounds released by yeast. They affirm that nitrogen addition is the most influence factor on the production of desirable fermentative aromas and the effect of temperature is linking to the volatilization or retention effect on the concentration of them [6, 11]. Winemakers routinely use additions of ammonium salts to musts in order to reduce the risk of problematic fermentations. It is well known that these salts and a-amino acids are the main sources of yeast assimilable nitrogen (YAN), which have the capacity to improve biomass synthesis, increase the fermentation rate, and prevent H_2S production [12]. Nitrogen supplementation also regulates the formation of many volatile and non-volatile compounds that can contribute to the improvement of the final flavor of the wines [13, 14].

At present, there are two solutions available for decreasing the content of HA, the first is to regulate the factors influencing the formation of HA during the fermentation so as to control the formation to a lower level, and the second is to remove or degrade the HA occurring in wines by means of physical or chemical techniques [15, 16, 17].

Alcoholic fermentation at low temperature (12-20-C) is nowadays a common practice in white winemaking, aimed to improve aromatic profiles by a high formation and retention of aromas. Furthermore, low temperature can improve the quality of wine in terms of volatile and total acidity, pH, alcohol and glycerol production [18]. However, low temperature has a strong influence on fermentation kinetics by increasing the probabilities of sluggish or even stuck fermentations, due to the negative effect on yeast growth and to the diminution on fermentation rate [19]. On the other hand, low temperature increased the unsaturated degree of yeast membrane fatty acids making yeast more ethanol tolerant [20]. Low temperature displayed slow fermentations and the nitrogen salts were hardly consumed by yeast in this process condition [21], due to the results of wine sample, indicates that decreasing of higher alcohols could be definitely affected by several factors, such as tartaric acid and ions in wine, which might be attributed to the free radicals originated from ultrasound cavitation and its subsequent reactions. All the results which has done due to this side, mostly took place in China. As a conclusion the research may help to understand the effects of ultrasound irradiation on improving the sensory properties of wine by decreasing the higher alcohols [22].

Regarding of the active issue, the aim of the research was to test the alternative nitrogenous substance during the process of making European type wine materials. To study its impact on vital activity of natural microflora, pure culture of yeasts and dry yeast used in winemaking production - In point of higher alcohols produced in wine materials and residual sugar concentration.

II. Objects and Methods

A. *Objects*

The objects of the study were European type of wine materials from local Rkatsiteli white grape varieties distributed in Eastern Georgia (Kakheti). The harvest period was technical maturity during the 2015-2016.

B. *Methods*

From Rkatsiteli grape, we prepared wine materials with classic technology in semi-factoring conditions. We have prepared several varieties of wine materials (alcoholic fermentation of grape juice by natural microflora) according to the concentration of nitrogen source used in alcoholic fermentation. As a control, we used European-made Rkatsiteli wine material, in which we added diammonium phosphate (DAP) as a nitrogenous substance in alcoholic fermentation of grape juice. Experimental wine options are as follows:

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.

To prepared European Type wine materials we used cultural yeasts from collection of Agricultural University of Georgia: Sacch. Vini - kakhuri 42, Sacch. Vini - Rkatsiteli 61. Wine materials where made with different temperature and acid (pH) conditions.

Trial Options (Grape juice + 2, 5% Sacch. Vinikakhuri 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, pH3.8;

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;

One of the experimental wine sample was dry wine material made by Rkatsiteli grape fermented with dry yeast - B 2000.

In wine materials, higher alcohols were analyzed using gas chromatography after separated volatile compounds by pre - treatment of objects with mixture of Pentane - ethyl ethers. Residual sugars (glucose, fructose) were determined by liquid chromatography.

C. *Results and Discussion*

The following tables show the results of the research.

It should be mentioned, that using ANS in the conditions of spontaneous fermentation, to determining maximal efficiency of yeast vital activity, especially, we chose Rkatsiteli grape with 25% sugar content.

As the results of the experiment showed, during fermentation process with natural microflora, was revealed ability fermentation of grape juice till semi-dry condition. Residual quantity of glucose and fructose were different from each other.

In control sample residual fructose concentration more exceeds than residual glucose concentration, accordingly, consists - 12,08g/l and 2,29g/l.

Quantity of residual fructose fixed in another research samples, but, moreover with different characteristics than control samples have. This difference is due to the different concentrations of ANS used in alcoholic fermentation.

The results of the experiment confirmed that with the addition of ANS in all variants compared to the control, the alcoholic fermentation was completed 48 h earlier.

Taking course, of alcoholic fermentation of grape juice with 25% sugar content to semi-dry wine conditioning (residual sugars according to 2nd and 3rd samples 8.90g/l -13.27g/l and control - 14.37g/l, respectively) indicates on vital activity of wine yeast and stimulating effect of ANS.

From the received wine materials, considering the organoleptic properties and reduced higher alcohols (fusel oils) we obtained 100g/l as the optimal concentration of ANS.

The amount of accumulating higher alcohols, during alcoholic fermentation, produced by two different yeast strain, with different temperature regime and acidity, reveals that, ANS used by us has considerable efficiency then its control variant - (DAP), which is attest by tables mentioned above, according to research samples are illustrated quantity of date of formed higher alcohols.

Table 1. Residual sugar content (g/l) in Rkatsiteli wine (initial grape juice sugar content 25%, the juice was fermented by the natural microflora, with the addition of nitrogenous substances).

Wine material	Glucose	Fructose	Total	%
Sample 1 - Control	2.2935	12.0815	14.375	1.44
Sample 2	2.335	10.9405	13.2755	1.33
Sample 3	2.9465	14.506	17.4525	1.74
Sample 4	2.142	6.7585	8.9005	0.89

Source: Ph.d research, P. Vashakidze, M. Bezhuashvili, 2015-2016.

It is interesting the variability of the total amount of higher alcohols according to the concentration of used nitrogen. The higher alcohols in the analysis materials are mainly in the form of isoamylol, isobutanol, 1-propanol, hexanol. The total amount of higher alcohols in all test wines is significantly reduced compared to the control wine sample (Table 2). Specifically, this reduction is reflected in the following data: Sample I - Control - 387 mg/l; Sample II - 230.77 mg/l; Sample III - 123.1 mg/l; Sample IV - 164.85 mg/l.

Table 2. Higher alcohol content (mg/l) in Rkatsiteli wine materials (25% grape juice, natural microflora juice added with nitrogenous substances).

Wine materials	Isoamyl	Isobutanol	1-Propanol	Hexanol
Sample 1 – Control	357.6	28.0	1.44	N/A
Sample2	213.1	17.2	0.47	5.0
Sample 3	116.0	7.1	N/A	N/A
Sample 4	155.0	9.24	N/A	0.61

Source: Ph.d research, P. Vashakidze, M. Bezhuashvili, 2015-2016.

As a result of the experiment with higher alcohols, according to residual sugars concentration in wine materials, also with regard to organoleptic properties of wine materials added to alcoholic fermentation of grape juice, the optimal appearance of concentration of ANS was 100 mg/l. Taking into consideration the obtained results, we continued our studies to determine the effect of ANS on the activity of cultured yeast vital activity, using the optimum concentration determined by considering the influence of alcohol fermentation temperature and acidity factors.

Table 3. Concentration of residual sugars (g/l) in grape juice fermented by cultured yeasts strains - Sacch. Vinikakhuri 42 (Initial grape juice sugar content 25%).

Kakhuri 42	Glucose	Fructose
1	2.28	11.19
2	1.25	1.91
3	1.61	3.45
4	1.61	3.03
5	3.2	16.4
6	3.14	14.06

Source: Ph.d research, P. Vashakidze, M. Bezhuashvili, 2015-2016.

Table 4. Concentration of residual sugars (g/l) in grape juice fermented by cultured yeasts strains - Sacch. Vini Rkatsiteli 61 (Initial grape juice sugar content 25%).

Rkatsiteli 61	Glucose	Fructose
1	0.5785	0.2418
2	0.5511	0.1647
3	0.8811	1.0596
4	1.0256	0.5978

Rkatsiteli 61	Glucose	Fructose
5	0.5602	0.1204
6	0.6002	0.1119

Source: Ph.d research, P. Vashakidze, M. Bezhuashvili, 2015-2016.

As shown by the experiment, the high vital activity of both strains of culture yeast is reflected in the low concentration of residual sugars by dry conditioning of the wine. This fact is explained by the added of ANS to alcohol fermentation by the activating effect on yeasts.

Table 5. Isoamylalcohols content (mg/l) in grape juice fermented by cultural yeasts**.**

Kakhuri 42	Isoamyl	Rkatsiteli 61	Isoamyl
1	250.65	1	119.2
2	129.9	2	101.25
3	236.4	3	79.3
4	84.6	4	61.1
5	44.4	5	55.5
6	42.54	6	44.7

Source: Ph.d research, P. Vashakidze, M. Bezhuashvili, 2015-2016.

It should be mentioned, that concentration of residual sugars in wine material fermented by the dry yeast B - 2000 consists: glucose - 0.77 g/l; Fructose - 4.0 g/l and Isoamyl alcohol 236 mg/l.

III. Conclusion

Based on conducted researches it is established the efficiency of ANS in alcoholic fermentation of grape juice. This is reflected by the significantly reduced higher alcohols (fusel oils) compared to the control (DAP) with high sugar content (25%) in spontaneous fermentation of grape juice with cultural yeast strains and dry yeast B - 2000. According to this, the optimal concentration of ANS revealed 100mg/l; the optimal fermentation temperature is 22-23 ° C and pH of the grape juice varies from 3.0 to 3.8.

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