Influence of Bio-organic Additives on the Ability of Yeast to Provide Biotransformation of Pesticides in Apple Must

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Abstract. The problem of food safety is the most important state and scientific priority, aimed at preserving and improving the health of the population, the production of high quality and safe products. The deterioration of food quality is primarily due to the chemization of agriculture in order to increase production. The use of pesticides to control weeds, diseases and pests increases yields, but these substances accumulate in the soil, and through it in crops. In addition to the negative impact on human health, there is also a negative role of pesticides in the technological processes of food production, for example, in winemaking pesticides adversely affect the activity of fermentation and the physiological state of yeast. Industrial yeast used in the technological processes of alcoholic fermentation of grape or apple musts to wines consume organochlorine pesticides as a source of carbohydrate food and lower the level of their concentration depending on the initial content. The article presents results of studies of the influence of bio-organic additives on the ability of yeast to provide biotransformation of pesticides in apple must.

1. Introduction

Ensuring food safety is the most important task of modern production. The increasing world population has therefore put a tremendous amount of pressure on the existing agricultural systems, because the new area that can be increased for cultivation purposes is very limited. That’s why different pesticides (herbicides, insecticides, fungicides, nematicides, fertilizers and soil amendments) become more widely used than in the past [1, 2]. A distinctive feature of many pesticides is their high resistance to environmental factors and the ability to persist in the soil for 10 years or more and also pesticides accumulate in soil and plants [3, 4].

In addition, the most of the pesticides are non-specific and may kill the organisms that are harmless or useful for ecosystem. In general, it has been estimated that only about 0.1% of the pesticides reach the target organisms and the remaining bulk contaminates the surrounding environment. Pesticides with high persistence and toxicity are the most dangerous, first of all, it is organochlorine compounds. Consumption of food products, containing xenobiotics, poses a significant threat to human health and may be the cause of disorders in the nervous system and metabolism [5, 6].

In Russia, despite the legislative restrictions on the content of pesticides and other xenobiotics in food raw materials and products, the level of pesticide contamination remains quite high. Taking into account the increasing amount of foods containing xenobiotics, the international standard ISO 22000 has been developed. ISO 22000 contains requirements for the functioning of an effective food safety management system based on the principles of the HACCP system (Hazard Analysis and Critical Control Point) and on the implementation of programs of preconditions necessary for the production of safe food.
The basis for building a food safety system at the enterprise is the HACCP system, the most important principle of which is the analysis of dangerous factors, in particular chemical hazard - chemicals, the presence of which in food products can cause damage to the health of the consumer - pesticides, herbicides, fertilizers, insecticides, fungicides, antibiotics, growth promoters [7].

Grape and fruit wines may also contain pesticides, used in the cultivation of plants or coming from the environment. The content of some types of pesticides is significantly reduced during the process of wine production. All kinds of microorganisms, including industrial yeast of the genus *Saccharomyces* used in the technological processes of alcoholic fermentation of grape or apple musts to wines have the ability of biotransformation of pesticides. The highest sorption capacity had immobilized yeast. *Saccharomyces* yeast consume organochlorine pesticides as a source of carbohydrate food and lower the level of their concentration depending on the initial content of 60-100%. However, pesticides have a negative effect on the activity of fermentation and the physiological state of yeast and led to a decrease in the activity of yeast in 1.5-2 times [8, 9, 10].

2. Experimental section

The main purpose of the research is to study the ability of yeast to metabolize phosphorus- and chlorine-containing pesticides and the effect of yeast activation on the intensification of the fermentation process of apple must, containing pesticides.

In order to study the ability of yeast to degrade pesticides in the fermentation of apple must, model experiments were conducted. The influence of two widely used pesticides – Bayleton (1-(4-chlorophenoxoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butano, systemic fungicide belonging to theazole group highly effective against powdery mildew, rust, and various leaf spot diseases) and Phosphamide (O,O-dimethyl S-(methylcarbamoylmethyl) ester, organophosphorus insecticide with contact and systemic action, used to combat herbivorous mites, aphids and other sucking plant pests) on two industrial species of wine yeast *Saccharomyces cerevisiae* – K-17 and Vishnevaya 33 recommended for the production of fruit wines was investigated.

Five samples of apple must were fermented periodically at a temperature of 20 °C. The first sample does not contain pesticides (control) and the other samples contain 1.5 µg/dm³ of Phosphamide or 0.2 µg/dm³ of Bayleton, which is more than 3 times higher than maximum permissible concentration. Active biomass of yeast cultures in the amount of 2% of must volume or 10 million cells in 1 cm³ of must was used for must seeding.

The activity of fermentation and respiration was determined by gasometric method by the amount of carbon (IV) oxide and oxygen released during fermentation.

The mass concentration of pesticides was determined by high performance liquid chromatography on chromatograph " Shimadzu LC-4A " (Japan), equipped with spectrophotometric detector, linear flow rate measurement programmer and data processing system of CR-3A microcomputer.

The obtained results are shown in table 1.

| Sample                  | Fermentation time (days) | Pesticide residual content (% of original) | Activity |
|-------------------------|--------------------------|-------------------------------------------|----------|
|                         |                          |                                           | Fermentation (mkI/h Q CO₂) | Respiration (mkI/h Q O₂) |
| Apple must (control)    | 7                        | -                                         | 180      | 116 |
| Apple must + Phosphamide a | 12                     | 9.0                                       | 103      | 68  |
| Apple must + Bayleton    | 9                        | 23.0                                      | 112      | 82  |
| Apple must + Phosphamide b | 13                     | 10.8                                      | 98       | 66  |
| Apple must + Bayleton    | 10                       | 24.6                                      | 102      | 68  |

a *Saccharomyces cerevisiae*, K- 17  
b *Saccharomyces cerevisiae*, Vishnevaya 33

The obtained results showed that phosphorus- and chlorine-containing pesticides have an inhibitory effect on the physiological and biochemical properties of yeast. Fermentation and respiration activity of yeast significantly decreased compared to the control in the presence of pesticides in the fermented
environment, and, regardless of the yeast race, Phosphamid to a greater extent than Bayleton inhibited the processes of fermentation and respiration. The duration of fermentation of apple must containing pesticides, increased compared with the control of 1.5-2 times.

Activation of yeast *Saccharomyces cerevisiae* allows to intensify the process of fermentation of apple must containing pesticides, reduce its duration and actively carry out biodegradation of pesticides. Silicon compounds are known to play an important role in lipid metabolism in living organisms [11, 12, 13].

The use of a complex compound of silicon and carbohydrate can accelerate and simplify the process of diffusion of silicon into the yeast cell, which leads to the activation of nitrogen and carbohydrate metabolism in yeast [14].

In order to study the influence of three bio-organic additives (silicon digluconate, bio-organic supplements containing selenocysteine derivative and zinc respectively) on the fermentation activity and ability to metabolize pesticides of yeast, model experiments were conducted.

Bio-organic additives were introduced into apple must in different concentrations: an additive containing silicon digluconate in concentrations 0.5; 3.5; 7.0 ml/dm³, an additive containing selenocysteine derivative in concentrations 0.1; 0.5; 1.0 ml/dm³, an additive containing zinc in concentrations 0.5; 2.0; 5.0 ml/dm³.

Nine samples of apple must were prepared using yeast biomass *Saccharomyces cerevisiae* K - 17, activated by bio-organic additives. As a control was used apple must obtained with a yeast culture without additives.

Yeast was introduced at the rate of 2% per 1 cm³ of apple must. The accumulation of yeast biomass was carried out in stages, for several generations.

The samples were assigned the following numbering:
1. apple must prepared using a bio-organic additive containing 0.5 ml / dm³ silicon digluconate;
2. apple must prepared using a bio-organic additive containing 3.5 ml / dm³ silicon digluconate;
3. apple must prepared using a bio-organic additive containing 7.0 ml/dm³ silicon digluconate;
4. apple must prepared using a bio-organic additive containing 0.1 ml / dm³ selenocysteine derivative;
5. apple must prepared using a bio-organic additive containing 0.5 ml / dm³ selenocysteine derivative;
6. apple must prepared using a bio-organic additive containing 1 ml / dm³ selenocysteine derivative;
7. apple must made with bio-organic additive containing 0.5 ml/dm³ zinc;
8. apple must made with bio-organic additive containing 2 ml/dm³ zinc;
9. apple must made with bio-organic additive containing 5 ml/dm³ zinc.

Determination of the volume fraction of ethyl alcohol was carried out by distilling according to GOST 32095-2013 [15].

Determination of sugars was carried out by Bertrand method according to GOST 13192-73 [16].

Determination of titrating acids was carried out by direct titration according to GOST R 51621-00 [17].

The content of the extract was determined by the density of the aqueous solution.

The mass concentration of glycerin was determined on the liquid chromatograph "Stayer" with a refractometric detector.

The total content of anthocyanins was determined by pH-differential spectrophotometry at a wavelength of 510 nm and for fractional analysis of anthocyanins was used high performance liquid chromatography.

Determination of the content of organic acids was carried out on the liquid chromatograph "Stayer" with a spectrophotometric detector (column C18 250*4.6 mm, the volume of the injected sample is 20 µl) and by the method of high-performance liquid chromatography with column "Kromasil 5 - C18".

The chemical composition and content of volatile components were determined by gas chromatograph "Crystal 5000.1" with flame ionization detector (a capillary column with the stationary phase HP-FFAP, sample volume 1 µl).

3. Results section
The obtained results are shown in tables 2 - 4.
Table 2. The influence of activation of the yeast *Saccharomyces cerevisiae* biomass, K-17 on physicochemical characteristics of apple must

| Sample | Volume fraction of ethyl alcohol (%) | Mass concentration of sugars, in terms of invert sugar (g/dm³) | Mass concentration of titrating acids, in terms of malic acid (g/dm³) | Mass concentration of residual extract (g/dm³) | Mass concentration of glycerin (g/dm³) | Antioxidant activity, AE (mg/g) |
|--------|-------------------------------------|-------------------------------------------------|-----------------------------------------------------------------|-----------------------------------------|----------------------------------|-----------------------------|
| Control | 10.0 | 15.8 | 7.6 | 12.2 | 6.2 | 72 |
| 1       | 10.1 | 13.2 | 7.6 | 12.4 | 6.1 | 110 |
| 2       | 10.2 | 16.0 | 7.4 | 12.7 | 6.0 | 81 |
| 3       | 10.1 | 16.4 | 7.6 | 12.4 | 6.1 | 73 |
| 4       | 10.1 | 15.0 | 7.4 | 12.6 | 6.2 | 82 |
| 5       | 10.9 | 14.5 | 7.5 | 13.9 | 6.8 | 113 |
| 6       | 9.7  | 15.7 | 7.4 | 13.2 | 6.1 | 82 |
| 7       | 10.1 | 16.8 | 7.4 | 12.1 | 6.2 | 76 |
| 8       | 10.4 | 14.6 | 7.0 | 13.1 | 6.4 | 92 |
| 9       | 8.8  | 7.5  | 6.0 | 12.1 | 5.1 | 73 |

The alcohol content in samples 5 and 8 is higher compared to the control by 0.9% vol. and 0.4% vol. respectively. In addition, at the same time in the same samples there is an increase in antioxidant activity by 41 and 20 AE mg/g respectively.

The highest mass concentration of glycerin was observed in sample 5. The samples 6 and 9 contain the least amount of ethanol, titrating acids and glycerin in comparison with the control. Also in these samples were minimal residual extract and antioxidant activity.

Obviously, the optimal concentration of selenium and zinc necessary for the activation of yeast should not exceed the level of samples 5 and 8, that is 0.5 ml/dm³ selenocysteine derivative and 2 ml/dm³ zinc. A higher concentration of selenium and zinc leads to inhibition of yeast cell metabolism. In all experimental samples the content of organic acids decreases compared to the control by an average of 60 - 80%.

In addition, the mass concentration of citric acid decreased to zero in all samples, except sample 7, in which the additive containing zinc is introduced in a minimum concentration.

Table 3. The influence of activation of the yeast *Saccharomyces cerevisiae* biomass, K-17 on the composition of organic acids in apple must

| Acid     | Content of organic acids (g/ dm³) |
|----------|---------------------------------|
|          | Control | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Oxalic   | 0.319   | 0.006 | 0.008 | 0.007 | 0.011 | 0.008 | 0.012 | 0.015 | 0.014 | 0.014 |
| Wine     | 0.778   | 0.011 | 0.012 | 0.018 | 0.041 | 0.041 | 0.044 | 0.057 | 0.049 | 0.075 |
| Formic   | 2.963   | 0.290 | 0.202 | 0.241 | 0.315 | 0.303 | 0.294 | 0.435 | 0.284 | 0.362 |
| Malic    | 4.285   | 1.892 | 1.721 | 1.912 | 1.854 | 1.895 | 1.832 | 1.939 | 1.86 | 2.066 |
| Dairy    | 5.673   | 1.184 | 0.969 | 0.981 | 0.988 | 1.11 | 0.946 | 0.971 | 0.991 | 1.083 |
| Acetic   | 5.009   | 0.700 | 0.623 | 0.570 | 1.124 | 0.794 | 0.664 | 0.853 | 0.759 | 1.01 |
| Lemon    | 3.53    | -    | -    | -    | -    | -    | -    | 0.964 | -    | -    |
| Amber    | 6.386   | 0.387 | 0.706 | 0.406 | 0.360 | 0.438 | 0.343 | 0.375 | 0.41 | 0.357 |
The content of volatile compounds that give the wine undesirable shades in flavor and taste and tone of oxidation (ethyl acetate, acetaldehyde, isoamyl and isobutyl alcohols) decreased in samples 5 and 8 compared to the control. In the same samples there was an increase, compared to the control, the content of ethyl esters of fatty macromolecular acids, favorably affecting organoleptic parameters (ethyl acetate, ethyl caprylate, ethyl caprate). The largest increase of aroma compounds affecting the bouquet and taste of wine, observed in the sample 6.

| Table 4. The influence of activation of the yeast Saccharomyces cerevisiae biomass, K-17 on the residual sugar content in apple must |
|--------------------------------------------------|
| Sugar                        | Residual sugar content (g/ dm³) |
|                              | Control 1 2 3 4 5 6 7 8 9       |
| Fructose                     | 6.395 6.320 10.254 8.541 4.348 5.781 6.622 5.247 5.778 6.238 |
| Glucose                      | 1.832 1.738 2.067 1.736 1.439 1.360 1.429 1.343 1.315 1.426 |
| Sucrose                      | 2.592 2.863 3.301 2.695 2.002 2.191 2.987 2.853 2.783 3.235 |
| Total sugar content          | 17.638 17.794 21.975 19.417 13.876 15.346 17.116 15.603 16.049 16.982 |

In order to study the effect of activated yeast biomass on organoleptic characteristics of wine materials, a working tasting was conducted. As a result of which sample 5 was recognized as the best: the wine has mild harmonious taste, the aroma is clean, with pronounced tones of apples, 9.5 points.

Thus, the best results were obtained for sample 5, so for further studies was chosen the option of yeast activation with a bio-organic additive containing 0.5 ml/dm³ selenocysteine derivative, which corresponded to the variant of the experiment 5.

The ability of activated yeast to degrade pesticides in the fermentation of apple must was studied according to the previously described method.

The obtained results are shown in table 5.

| Table 5. The influence of activation of the yeast Saccharomyces cerevisiae biomass, K-17 on the ability to metabolize phosphorus- and chlorine-containing pesticides in the fermentation of apple must |
|--------------------------------------------------|
| Sample                                | Fermentation time (days) | Pesticide residual content (% of original) | Fermentation (mkl/h Q CO₂) | Respiration (mkl/h Q O₂) |
| Apple must (control)                  | 7                        | -                                         | 180                         | 116                        |
| Apple must + Phosphamidé³             | 12                       | 9.0                                       | 103                         | 68                         |
| Apple must + Bayleton³                 | 9                        | 23.0                                      | 112                         | 82                         |
| Apple must + Phosphamidé²             | 11                       | 6.8                                       | 119                         | 77                         |
| Apple must + Bayleton²                 | 8                        | 18.5                                      | 126                         | 81                         |

³ Saccharomyces cerevisiae, K-17 activated with a bio-organic additive containing 0.5 ml/dm³ selenocysteine derivative

4. Discussion section

Thus, activation of yeast Saccharomyces cerevisiae, K-17 allows to intensify the process of fermentation of apple must containing pesticides, reduce its duration and actively carry out biodegradation of pesticides. In general, the activation of yeast Saccharomyces cerevisiae, K - 17 by using an organic additive containing selenocysteine derivative, can improve their ability to metabolize phosphorus- and chlorine-containing pesticides, as well as improve the biochemical composition of wine samples.
5. Conclusions
Thus, the use of bio-organic additives in the production of wine materials (silicon digluconate, bio-organic supplements containing selenocysteine derivative and zinc respectively) in optimal for yeast race *Saccharomyces cerevisiae*, K-17 concentrations, allows to optimize the biochemical composition and organoleptic characteristics of wine samples and increase the ability of yeast to metabolize phosphorus- and chlorine- containing pesticides by an average of 22.5%.

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