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Biotechnology

Effect of Nanostructured Silver on Biologically Active Substances and Microbiological Processes of Dry Red Wine

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ABSTRACT. In the present study we investigated the effect of various doses of nanostructured silver on the content of polyphenols, organic acids, main conditional indices, lactic acid bacteria and acetic acid bacteria in biotechnological processes of making dry red wines. Research material was prepared from the red grape variety "Saperavi". Sulfur dioxide – Kadifit, 50 mg/l concentration and various doses of nanostructured silver (0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 0.8 mg/l) were used during the first year of making red wine material for different biotechnological stages: 1) processing of destemmed grape pulp prior to alcoholic fermentation; 2) after malolactic fermentation; 3) at the second and 4) third racking off the lees. Malolactic fermentation of the wine material was conducted after termination of alcoholic fermentation and racking off the yeast lees using lactobacteria of the strain "Extraflore" of *Oenococcus oeni* as starters. Content of catechins, phenolcarbonic acids, flavanols and organic acids was investigated by means of the HPLC analysis. The main conditional indices were determined using standard international methods. Efficiency of application of nanostructured silver in the wine samples infested with lactic acid bacteria and acetic acid bacteria was evaluated. Nanostructured silver was found in biotechnological processes of making dry red wines to have the effect similar to that of sulfur dioxide. The optimum doses of using nanostructured silver 0.4 mg/l - prior to alcoholic fermentation for the processing of grape pulp; 0.6 mg/l – in the processes of the second and third racking off the lees, for the oxidation of biologically active substances and inhibition of growth of lactic- and acetic acid bacteria were identified.

Key words: *biologically active substances, red wine, nanostructured silver, sulfur dioxide.*

Sulfur dioxide (SO_2), as the most universal antiseptic, is used for processing all types of wine being an aid in preserving their qualitative indices in the process of storage. Along with desirable properties SO_2 has some adverse properties associated with a wide range of toxic effects, sometimes even fatal, especially for the people suffering from asthma or allergy.

Reduction of sulphites content in wine or their substitution with another harmless antiseptic is considered to be a primary objective of oenology. However, there are no specific tools that could completely replace this controversial additive. Such a concern becomes particularly important for organic wine.

Various physical methods (ultraviolet and infrared rays, ultrasound), chemical preservatives (ascorbic acid, sorbic acid, diethyl ester of pyrocarbonic acid) and antibiotics (actidione, pimaricin) were tested [1].

Antiseptic in wine making despite its nontoxic nature should not effect useful human microflora, taste and bouquet of the wine. Colloidal nanostructured silver is proposed to be such an antiseptic.

Colloidal silver is a product of nanotechnology obtained by electrolytic method. Solution of colloidal silver consists of microscopic (average size up to 10 nm) particles of chemically pure silver, which are in suspended state in water solution. Numerous scientific researches published in recent years show that colloidal silver has a wide range of antibacterial action. It kills about a thousand pathogenic microorganisms (bacteria, fungi). According to the research data, silver ion accumulates on the surface of microorganism cell, causes inhibition of its enzyme(s) resulting in the death of a microbe. Studies conducted at top medical universities of Europe, US and other countries have confirmed that colloidal silver often reveals much stronger antimicrobial effect than antibiotics. Moreover, in contrast to antibiotics, silver does not damage probiotics of microflora of friendly bacteria involved in food digestion [2-7]. Colloidal silver is allowed to be used as natural antibiotic by the U.S. Food and Drug Administration (FDA) [2].

Colloidal nanostructured silver was reputed to be successful against more than 650 diseases. Investigations conducted in medical clinics and Biotech Labs of the US evidence that colloidal nanostructured silver blocks and destroys viruses of AIDS, Bird Flu, Hepatitis B and C [4-6].

Currently, the researchers consider silver as a microelement strengthening immune system essential for normal functioning of human internal organs. Colloidal silver at 0.05-0.1 mg/l concentration has a rejuvenating effect on blood, increases haemoglobin amount. Silver is a constituent element of human brain, endocrine glands, liver, kidneys and bones. A person's daily ration on colloidal silver makes 88 mkg [3].

Colloidal silver, as a biologically active compound, is approved by the U.S. FDA and is used in more than a hundred reputable and labelled dietary supplements [5, 7].

Sulfur dioxide is known to be applied in technological processes of making dry red wines for inhibition of oxidation of the biologically active substances and suppression of the development of microorganisms. In particular, sulfur dioxide is used for processing the destemmed grape pulp before alcoholic fermentation; for racking wine material off the lees after malolactic fermentation and for the of wine storage.

The purpose of this paper is to study the effect of application of different doses of natural antiseptic, nanostructured silver on the content of polyphenols, organic acids, main conditional indices and microflora in the above described biotechnological processes of making dry red wines; to determine the efficiency of application of nanostructured silver and establish its optimum doses; to substitute sulfur dioxide with natural antiseptic – nanostructured silver.

Materials and Methods

Red grape variety Saperavi was used for preparation of research material.

Sulfur dioxide (Kadifit 50 mg) and various doses of nanostructured silver (0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 0.8 mg/l) were used at different stages of biotechnological processes carried out during the first year of dry red wine making.

For the treatment of raw wine materials we used colloidal nanostructured silver at concentration of 500 ppm produced by the US company "Natural Path

Silver Wing" and, also a nanosilver generator, patented by N. Bibiluri "Georgian Patent U 1187 GE U 2005" [8].

Prior to alcoholic fermentation, Saperavi grape pulp was treated with colloidal silver and Kadifit. The test samples were prepared by means of treating the pulp with different doses of colloidal silver: I. 0.2 mg/l; II. 0.3 mg/l; III. 0.4 mg/l.

The control sample was treated with 50 mg/l Kadifit. Shortly after completion of alcoholic fermentation the wine material was racked off the gross yeast lees.

After the first racking off the yeast lees malolactic fermentation of wine material was conducted using the lactic acid bacteria of the strain "Extraflore" of *Oenococcus oeni* (Institut Oenoloqique de Champagne) as starters.

The processes of the second and third racking off the lees were conducted using Kadifit and various doses of nanostructured silver.

The following samples of wine materials have were prepared: 1. Wine material+50 mg/l Kadifit; 2. Wine material+0.5 mg/l nanostructured silver (from the generator); 3. Wine material+0.6 mg/l nanostructured silver (from the generator); 4. Wine material+0.8 mg/l nanostructured silver (from the generator); 5. Wine material+0.6 mg/l nanostructured silver (of the US company); 6. Wine material+0.8 mg/l nanostructured silver (of the US company); 7. Wine material untreated with antiseptics.

Saperavi wine material was infested with acetic acid bacteria. The research samples from the infested wine materials were prepared similar to those infested with lactic acid bacteria.

Study of catechins, phenolcarbonic acids, flavanols, vanillin aldehyde and organic acids was performed using the method of chromatography on the apparatus of Varian Pro Star HPLC system with UV detector. Separation of components was performed on the chromatographic column with reversed-phase sorbent Microsorb 100-S C18 (250mm x 4.6 mm x 5.0 mm). Elution was performed in a gradient mode at the rate of mobile phase feed equal to 1 ml/min. The following solutions were used: for phenolic components - solution A: water/phosphoric acid (at a ratio of 99.5/0.5); solution B: acetonitrile/water/phosphoric acid (at a ratio of 50/49.5/0.5); for organic acids - solution A: 0.1%H3PO4+1% methanol; solution B: methanol [8, 10]. The wine samples were filtered through the membrane filter (pore diameter 0.22 μm). The solvents and commercial standards used during the analysis were purchased from the company Sigma-Aldrich (Germany). Detection was performed at the following wavelengths: 280 nm (gallic, chlorogenic, vanillic, caffeic, p-coumaric, syringic, t-cinnamic acids, (+)- catechin, (-)-epicatechin, and vanillin aldehyde); 360 nm (quercetin, quercetin-3-β-D-glucoside, kaempherol and ellagic acids) and 215 nm (organic acids). The values of retention time of standard substances and studied components were compared and the method of adding standard substances known in special literature was used [9-11]. The main conditional indices in the sample were investigated using standard international methods.

Results and Discussion

The obtained data are presented in the Tables 1-4 and illustrated in Figs. 1-3.

A detailed analysis of chemical characteristics of red wine grape pulp processing with various doses of Kadifit and nanostructured silver has shown that test samples do not differ from each other by the content of alcohol and titrable acidity and the difference between indices of volatile acidity and pH is small.

Total amounts of tartaric, malic and lactic acids were identical in the samples treated with 0.4 mg/l nanostructured silver and Kadifit. The same identity was also demonstrated in quantitative content of monophenols [12].

Results of investigation presented in Table 1 demonstrate that treatment of the pulp of grape variety

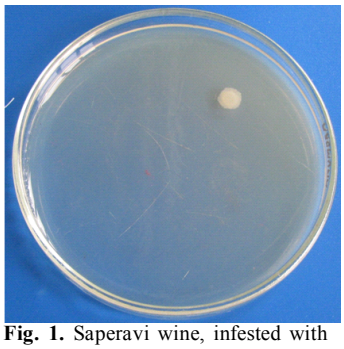

Fig. 1. Saperavi wine, infested with lactic acid bacteria, treated with 0.5 mg/l of nanostructured silver

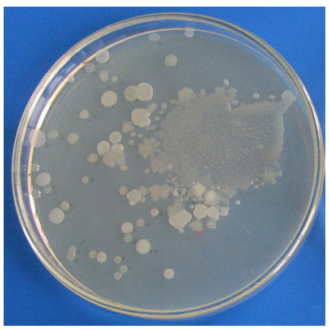

Fig. 2. Saperavi wine, infested with lactic acid bacteria, untreated with antiseptics

Fig. 3. Saperavi wine, infested with acetic acid bacteria, untreated with antiseptics

Saperavi with colloidal silver at 0.4 mg/l concentration prior to alcoholic fermentation has the same effect on oenological characteristics of red wine as application of sulfur dioxide (Kadifit 50 mg).

During malolactic fermentation the amount of catechins is reduced as a result of their oxidation (Table 2): by 2.2% of (+)-catechin and by 1% of (-)epicatechin. Amounts of chlorogenic, syringic and cinnamic acids insignificantly decrease in this process. At the same time, the amount of quercetin increases by 19.8% and comparatively slight increase takes place in the amount of caffeic and ellagic acids. Growth in the amount of these components must be explained by the hydrolysis of their acylated forms in the course of malolactic fermentation. The tendency to the increase of phenolcarboxylic acids in the process of malolactic fermentation was reported by other researchers too [1].

After malolactic fermentation the oxidation of phenolic compounds and, accordingly, reduction of their amount also occurs in the process of the second racking of wine material off the lees (Table 2). This process was less intensive in wine material subjected to the technological process of the second racking off the lees using 0.6 mg/l nanostructured silver from the generator. Total amount of monophenols in it is by 3.8% higher than in the sample treated with Kadifit and by 9% higher than in the sample treated with the same dose of colloidal silver produced by the US company "Natural Path Silver Wing".

Reduction of the total amounts of monophenols in wine samples occurs at the expense of reduction in catechines and flavanols. In samples treated with Kadifit, the amount of (+)-catechin decreases by 8.7%; of (-)-epicatechins - by 17.6%; total amount of flavanols - by 21.8%.

In samples, treated with the nanostructured sil-

Table 1. The effect of grape pulp processing with Kadifit and different doses of nanostructured silver on oenological characteristics of red wine

Oenological characteristics	Test sample I	Test sample II	Test sample III	Control
	Treatment with 0.2 mg/l colloidal silver	Treatment with 0.3 mg/l colloidal silver	Treatment with 0.4 mg/l colloidal silver	Treatment with 50 mg/l Kadifit
Alcohol, %	12. 5	12.5	12.6	12.6
Titrable acidity, g/l	8.12	8.12	8.12	8.12
Volatile acidity, g/l	0.31	0.18	0.3	0.3
pH	3.56	3.55	3.55	3.56
Total amount of organic acids (tartaric, malic and lactic acids)	7.0714	7.3466	7.4418	7.4303

Table 2. Content of phenolic components in Saperavi wine materials at different stages of technological processes of wine making

Phenolic components, mg/l	After racking off the yeast lees	After malolactic fermentation	Treatment with antiseptics after the second racking off the lees		
			0.6 mg/l nano-structured silver (of the US company)	50 mg/l Kadifit	0.6 mg/l nano-structured silver (from the generator)
(+)-catechin	110.77	108.33	92.47	98.93	104.24
(-)-epicatechin	74.27	73.54	53.31	60.59	66.47
Chlorogenic acid	1.51	1.12	0.26	0.63	0.28
Caffeic acid	20.76	21.98	19.56	20.68	20.22
Syringic acid	18.70	18.44	16.30	14.12	16.79
Cinnamic acid	2.92	2.60	1.05	1.26	1.62
Ellagic acid	4.03	4.14	3.46	3.76	3.72
Quercetin glucoside	34.39	34.85	30.21	32.08	32.11
Quercetin	5.96	7.14	0.46	0.90	2.35
Kaempferol	0.12	0.17	0.00	0.00	0.02
Total of phenolics	329.38	328.15	277	290.98	301.96

ver (from the generator) the amount of (+)-catechins decreases by 3.8%, of (-)-epicatechins - by 9.6%; total amount of flavanols - by 18.2%. Quercetin is especially actively oxidized.

After completion of the process of malolactic fermentation the test objects racked off the lees were investigated on the presence of lactic acid bacteria. The analysis of the obtained results (Fig. 1, 2) shows that bacterial growth in the wine samples untreated with antiseptics proceeds without delay. However, the use of 0.5 mg/l of nanostructured silver in the treated sample considerably limits the growth of the lactic acid bacteria. Growth of lactic acid bacteria is blocked: 1) in wine material treated with 0.6 mg/l nanostructural silver; 2) in wine material treated with 50 mg/l Kadifit.

Results of microbiological study are in a good agreement with the results of investigation of oenological characteristics (Table 3).

Similarity of the results is well manifested in samples treated with 50 mg/l Kadifit and 0.6 mg/l nanostructured silver from the generator. Due to similar inhibition of lactic acid bacteria index of titrable acidity in them is 7.431 g/l and 7.54 g/l correspondingly; the amount of malic acid – 2.7593 g/l and 2.7510 g/l; pH index is 3.69. In the wine material, infested with lactic acid bacteria, but untreated with antiseptics, malolactic fermentation takes place. As a result

Table 3. Chemical characteristics of Saperavi wine material infested with lactic acid bacteria

Chemical characteristics	Infested wine material untreated with antiseptics	Different treatments with antiseptics of wine material, infested with lactic acid bacteria			
		50 mg/l Kadifit	0.6 mg/l nanostructured silver (from the generator	0.6 mg/l nanostructured silver (US company)	0.8 mg/l nanostructured silver (US company)
Malic acid, g/l	0.8621	2.7593	2.7510		
Lactic acid, g/l	3.7323	2.0290	2.1013		
Titrable acidity, g/l	7.15	7.431	7.54	7.38	7.43
pH	3.77	3.69	3.69	3.72	3.7

Table 4. Oenochemical characteristics of wine materials after the third racking off the lees

Oenological characteristics	Wine material treated with 50 mg/l Kadifit	Wine material treated with 0.6mg/l nanostructured silver
Titrable acidity, g/l	7.1	7.09
pH	3.75	3.76
(+)-catechin, mg/l	69.36	76.28
(–)-epicatechin , mg/l	60.45	63.96
Vanillic acid, mg/l	3.71	2.70
Caffeic acid, mg/l	6.80	6.53
Syringic acid, mg/l	13.73	15.01
p-coumaric acid, mg/l	0.14	0.20

of decarboxylation of malic acid the amount of lactic acid is increased and titrable acidity is reduced (Table 3).

Similar effect was obtained in the study of Saperavi sample infested with acetic acid bacteria (Fig. 3). In Saperavi wine material treated with 0.6 mg/l nanostructured silver inhibition of acetic acid bacteria proceeds in the same way as in case of application of 50 mg/l Kadifit.

Investigations carried out after the third racking wine materials off the yeast lees revealed identity of oenological characteristics of wine materials treated with 0.6 mg/l nanostructured silver and 50 mg/l Kadifit.

Conclusions

Application of nanostructured silver at different stages of biotechnological processes carried out during the first year of making dry red wine material has the same effect on oenochemical characteristics and microflora of wine as treatment with sulphur dioxide.

The following optimum doses of application of nanostructured silver have been established: 0.4 mg/l - for processing the grape pulp prior to alcoholic fermentation; and 0.6 mg/l - for the processes of the second and third racking off the lees; for inhibition of oxidation of biologically active substances and blocking the growth of lacto- and acetic acid bacteria.

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ბიოტექნოლოგია

ნანოსტრუქტურული ვერცხლის გამოყენების გავლენა მშრალი წითელი ღვინის ბიოლოგიურად აქტიურ ნივთიერებებზე და მიკრობიოლოგიურ პროცესებზე

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სტატიაში გამოკვლეულია მშრალი წითელი ღვინოების დამზადების ბიოტექნოლოგიურ პროცესებში ბუნებრივი ანტისეპტიკის, ნანოსტრუქტურული ვერცხლის, სხვადასხვა დოზის გამოყენების გავლენა პოლიფენოლებზე, ორგანულ მჟავებზე, ძირითად კონდიციურ მახასიათებლებზე, რძემჟავა და ძმარმჟავა ბაქტერიებზე. კვლევის ობიექტების დასამზადებლად გამოყენებული იყო ყურძნის წითელი ჯიში „საფერავი“. გოგირდის დიოქსიდი (კადიფიტი 50მგ/ლ) და ნანოსტრუქტურული ვერცხლის სხვადასხვა დოზა (0,2; 0,3; 0,4; 0,5; 0,6; 0,7; 0,8 მგ/ლ) გამოვიყენეთ მშრალი წითელი ღვინომასალის დამზადების პირველ წელს ჩატარებული ბიოტექნოლოგიური პროცესების შემდეგ ეტაპებზე: 1) დურდოს დამუშავება ალკოჰოლური დუღილის წინ; 2) ვაშლ-რძემჟავა დუღილის ჩატარების შემდეგ; 3) ლექიდან მეორე; 4) მესამე გადაღება. ალკოჰოლური დუღილის დასრულებისა და ღვინომასალის საფუვრის ლექიდან გადაღების შემდეგ ჩატარდა ვაშლ–რძემჟავა დუღილი. გამოყენებული იყო რძემჟავა ბაქტერიების **Oenococcus oeni** შტამი **“Extraflore”**. კატექინების, ფენოლკარბონმჟავების, ფლავონოლების და ორგანული მჟავების გამოკვლევა ჩატარდა მაღალეფექტური სითხური ქრომატოგრაფიის მეთოდით; ძირითადი კონდიციური მახასიათებლებისა – საერთაშორისო სტანდარტული მეთოდებით. გამოკვლეულია ნანოსტრუქტურული ვერცხლის გამოყენების ეფექტურობა რძემჟავა და ძმარმჟავა ბაქტერიებით დაინფიცირებულ ღვინის ნიმუშებში. დადგენილია, რომ წითელი ღვინის დამზადების ბიოტექნოლოგიურ პროცესებში ნანოსტრუქტურული ვერცხლის გამოყენებას გოგირდის დიოქსიდის იდენტური ეფექტი აქვს. ნანოვერცხლის გამოყენების ოპტიმალური დოზებია: ალკოჰოლური დუღილის წინ დურდოს დამუშავებისათვის 0,4 მგ/ლ; ლექიდან მეორე და მესამე გადაღებისას ბიოლოგიურად აქტიური ნივთიერებების დაჟანგვის შეფერხებისა და რძემჟავა და ძმარმჟავა ბაქტერიების ინჰიბირებისათვის 0,6 მგ/ლ.

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