The Efficiency of Application Nanosilver in Technological Processes of Making Red Wine

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Abstract: The efficiency of using nanostructured silver in technological processes of making red wine has been studied with the purpose of substituting sulfur dioxide. For preparation of research objects, we used dry wine material of red grape variety “Saperavi”. After completion of malolactic fermentation, the second racking the wine material off the lees and its treatment with antiseptics were carried out. Sulfur dioxide (Kadifit) and different doses of nanostructured silver were used. Microbiological investigation was performed on the presence of lactic bacteria after completion of malolactic fermentation and racking off the lees. The investigation of phenolic compounds was conducted at the following stages of technological processes: (1) after completion of alcoholic fermentation and racking off the lees; (2) after completion of malolactic fermentation; (3) after the second racking off the lees and treatment with antiseptics. By means of the HPLC analysis, the amounts of catechins, phenolcarboxylic acids and flavonols were determined. It has been established that after conduction of malolactic fermentation in the process of racking the wine material off the lees and storage, the application of 0.6 mg/L of nano-silver for blocking of lactic-acid bacteria and inhibition of oxidation of phenolic compounds has the same effect as sulfiting with 50 mg/L of Kadifit.

Key words: Nano-silver, red wine, malolactic fermentation, phenolic compounds, sulfur dioxide.

1. Introduction

Biologically active substances such as polyphenols, organic acids, etc., play a crucial role in the assessment of wine quality. Their oxidation and development of microbiological processes deteriorate the wine quality. Sulphur dioxide is the most universal means that is recommended for wines to preserve their quality in the storage process. Along with the desirable properties, the SO₂ has some of the undesirable properties associated with a wide range of severe effects (and sometimes fatal) on people suffering from asthma and allergies. Therefore, the use of sulphur dioxide has recently come under review due to health concerns. Hence, the EU regulations now require that wines must prominently display on the

label the presence of total sulphites in excess of 10 mg/L. On January 1, 1987, the FDA passed a law requiring domestic wines, beers and spirits containing more than 10 parts per million of sulphites to bear a “contains sulphites” warning label.

The overview of numerous scientific literature published in recent years shows that a lot of efforts have been applied to solve the problem of sulphites reduction or elimination in wine processing, which is considered as a primary objective in oenology. The Orwine Project Meeting concluded that actual research does not offer any product to replace sulphur with the same conservation capacities [1]. Similar objective has been pursued by a group of scientists from Greece [2].

An alternative product described as being of “biological origin and containing mainly a black radish

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(*Raphanus niger*) extract and ascorbic acid” was tested for its potential to reduce or replace the sulphur dioxide content of wine. The result was described as following: while the new additive may not lead to removal and replacement of sulphur dioxide, it can lead to partial substitution of contentious dioxide.

Antimicrobial enzymes (e.g., chitinases, endoglucanases, etc.) and peptides (zymocins and bacteriocins other than nisin) are currently being investigated and considered as possible biopreservatives. It was found that these bacteriocins had no effect on yeasts and acetic acid bacteria normally associated with wine and were stable for a sufficient period of time in the simulated wine environment [3].

In order to address the wine consumers’ call for healthier wines of higher quality, SunBio, a commercialization initiative of the Institute for Wine Biotechnology, was launched in 2004. SunBio is fully funded by Cape Biotech, the Biotechnology Regional Innovation Centre (BRIC) established by the Department of Science and Technology. No product was found to substitute SO₂ [4].

Lysozyme is another component which has recently been introduced to the wine industry, it offers another means of controlling the malolactic fermentation in wine. The studies showed that there were no significant changes in the chemical and organoleptic features of wine after the addition of lysozyme [5].

Hundreds of studies conducted over the past 90 years at top medical universities in Europe and America and other countries have confirmed microbe-fighting (in many cases stronger than antibiotics) power of colloidal silver. Moreover, silver, unlike antibiotics, does not damage probiotic or friendly digestive bacteria [6-12]. The Merck Index identifies the following medicinal uses of silver: antiseptic particularly for mucous membranes and infectious sinusitis [13].

The safe use of silver as an orally consumed preventive agent was demonstrated and supported by

reports from the EPA and the United States Department of Health and Human Services, Federal Commission of Medicine and Nutrition, Drinking Water Criteria Document for Silver, Toxicological Profile on Silver 1990 [14]. Colloidal silver is an active compound of more than hundred reputedly made and labelled FDA approved dietary supplements [15, 16]. As the best antiseptic, it has been successfully used in the developed countries [17]. Currently, the researchers consider silver as a strong immune-boosting microelement. At the concentration of 0.05-0.1 mg/L, colloidal silver has a rejuvenating effect on blood production by increasing the haemoglobin amount [18, 19]. The investigations conducted in US medical clinics and biotech labs evidence that colloidal silver blocks and destroys viruses of AIDS, Bird Flu, Hepatitis B and C [7, 20, 21].

Innovations are continually hitting the market, proving that nanoscience and nanotechnology are new frontiers of this century. They will have a big impact on the food and beverage industry. The first nanofood contact material (a silicon dioxide coating) has already been approved by the European Food Safety Authority (EFSA).

Hence, the application of a natural antiseptic—a product of nanotechnology, colloidal silver instead of sulfurous anhydride in technological processes of wine-making and storage is a topical issue.

In the technology of red wine making the sulfurous anhydride is used in pre-treatment of the raw pulp prior to alcoholic fermentation, removal of the wine materials from the lees in dry fermented wine materials after malolactic fermentation and storage processes.

The purpose of the work is to study the efficiency of application nanostructured silver substituting sulfurous anhydride to inhibit the oxidation of biologically active substances and microbiological processes in red wines.

2. Materials and Methods

For preparation of the research objects dry wine material from red grape variety, Saperavi was used.

The mentioned wine material was prepared by dry fermentation of the pulp processed prior to alcoholic fermentation with the application of 0.4 mg/L nanostructured silver. Before conducting this experiment, it was established that the application of the aforementioned dose of nanosilver (0.4 mg/L) for processing of the pulp prior to alcoholic fermentation has the effect identical to the use of 50 mg/L of Kadifit on enochemical indices of red wines [22].

After the first racking off the yeast lees, malolactic fermentation was conducted in wine material. Lactic bacteria were used "Extraflora" Souche *Oenococcus oeni* (Institut Oenologique de Champagne Strain *Oenococcus oeni*. Direct inoculation bacteria for malolactic fermentation on red wines).

The second racking of wine material off the lees was conducted just after completion of malolactic fermentation and its treatment with antiseptics. There was used sulfurous anhydride (Kadifit) and various doses of nanostructured silver. For the treatment with nanosilver, we used colloidal silver with 500 ppm concentration produced in the US (the company "Natural Path Silver Wing"), also nanosilver generator patented by Bibiluri [23].

The following variants of wine materials have been prepared:

- (1) wine material + 0.5 mg/L nano-silver (from generator);
- (2) wine material + 0.6 mg/L nano-silver (from generator);
- (3) wine material + 0.8 mg/L nano-silver (from generator);
- (4) wine material + 50 mg/L Kadifit;
- (5) wine material + 0.6 mg/L nano-silver (of the US company);
- (6) wine material + 0.8 mg/L nano-silver (of the US company);
- (7) wine material not treated with antiseptics.

In the above wine, samples microbiological study of lactic-acid bacteria was performed. The investigation of phenolic compounds was conducted at different

stages of technological processes, namely: (1) after completion of alcoholic fermentation and racking off the yeast lees; (2) after conduction of malolactic fermentation; (3) after second racking and treatment with antiseptics.

For investigation of lactic-acid bacteria, cabbage medium was used as nutrient medium. The inoculation of bacteria from wine materials was performed on liquid and solid nutrient media [24].

In research objects, the determination of catechins, phenolcarbonic acids, flavonols and vanillin aldehyde was conducted using the HPLC method, on the apparatus Pro Star of the firm Varian with UV detector. Separation of components was performed on chromatographic column with reversed-phase sorbent Microsorb 100-S C18 (250 × 4.6 × 5.0 mm). Elution was performed in gradient mode at the rate of mobile phase feed equal to 1 mL/min. The following solutions were used: solution A—water/phosphoric acid (in the ratio of 99.5/0.5); solution B—acetonitrile/water/phosphoric acid (in the ratio of 50/49.5/0.5). The wine samples were filtered through membrane filter (pore diameter 0.22 µm). The solvents and commercial standards used during the analysis were purchased from Sigma-Aldrich (Germany). The detection was performed at wavelengths: 280 nm (gallic, chlorogenic, vanillic, caffeic, *p*-coumaric, syringic, *t*-cinnamic acids, (+)-catechin, (-)-epicatechin and vanillin aldehyde) and 360 nm (quercetin, quercetin-3-β-D-glucosid, kaempferol and ellagic acids). Identification was conducted by comparison of retention time of standard substances and defined components as well as by using the method of standard substances addition known in special literature [25, 26].

3. Results and Discussion

The obtained data are given in the Table 1 and illustrated in Figs. 1-3.

After completion, the process of malolactic fermentation, test objects racked off the lees were investigated on the presence of lactic-acid bacteria.

Fig. 1 Lactic-acid bacteria in wine material untreated with antiseptics.

Fig. 2 Lactic-acid bacteria in wine material treated with 0.5 mg/L of nanosilver.

Fig. 3 Lactic-acid bacteria from suspension "extraflores".

Analysis of the obtained results has shown that bacterial growth proceeds without delay in wine sample not treated with antiseptics. However, the use of 0.5 mg/L of nanostructural silver in the treated sample considerably limits growth of the lactic-acid

bacteria (Figs. 1-3). 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.

During malolactic fermentation, as a result of catechins oxidation, their amounts are reduced of (+)-catechin by 2.2% (110.77 mg/L vs. 108.33 mg/L) and (-)-epicatechin by 1% (74.27 mg/L vs. 73.54 mg/L). In this process, the amount of chlorogenic (1.51 mg/L vs. 1.12 mg/L), syringic (18.7 mg/L vs. 18.44 mg/L) and cinnamic (2.92 mg/L vs. 2.60 mg/L) acids is insignificantly reduced. At the same time, the amount of quercetin increases 19.8% (5.96 mg/L vs. 7.14 mg/L) and comparatively slight increase in the amount of caffeic (20.76 mg/L vs. 21.98 mg/L) and ellagic (4.03 mg/L vs. 4.14 mg/L) acids is marked.

Growth in the amount of these components must be explained by the hydrolysis of their acylated forms during malolactic fermentation. The tendency of increasing phenol carbonic acids in the process of malolactic fermentation was fixed by other researchers, too [24].

After malolactic fermentation, the oxidation of phenolic compounds and accordingly their reduction also occur in the process of the second racking of wine materials off the lees. This process with less intensive in wine material which was subjected to the technological process of the second racking off the lees with the use of 0.6 mg/L nanosilver from generator. Total amount of monophenols in it is higher by 3.8% than in the sample treated with Kadifit (290.98 mg/L vs. 301.96 mg/L) and higher by 9% than in the sample treated with the use of the same dose of the company "Natural Path Silver Wing" US colloidal silver (277 mg/L vs. 301.96 mg/L).

Variation of the total amounts of monophenols in the samples occurs at the expense of reduction in catechines and flavanols. In samples treated with Kadifit, the amount of (+)-catechins decreases by 8.7% (108.33 mg/L vs. 98.93 mg/L); of (-)-epicatechins by 17.6% (73.54 mg/L vs. 60.59 mg/L); total amount of flavanols by 21.8% (42.16 mg/L vs. 32.98 mg/L).

Table 1 Phenolic components in Saperavi wine materials on various stages of technological processes.

Phenolic components (mg/L)	After racking off the yeast lees	After malolactic fermentation	After the second racking off the lees and treatment with antiseptics		
			0.6 mg/L nano-silver (US company)	50 mg/L Kadifit	0.6 mg/L nano-silver (from 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

In samples treated with the application of nano-silver (from generator), the amount of (+)-catechins decreases by 3.8% (108.33 mg/L vs. 104.24 mg/L); of (-)-epicatechins by 9.6% (73.54 mg/L vs. 66.47 mg/L); total amount of flavonols 18.2% (42.16 mg/L vs. 34.48 mg/L). Oxidation of quercetin occurs especially actively (7.14 mg/L vs. 2.35 mg/L).

4. Conclusions

During malolactic fermentation, the amount of (+)-catechin and (-)-epicatechin is reduced by 1%-2.2%. In this process, the amount of chlorogenic, syringic and cinnamic acids is insignificantly reduced. At the same time, the amount of quercetin increases by 19.8%; a slight increase in the amount of caffeic and ellagic acids is marked. The oxidation of phenolic compounds and accordingly the reduction of their amounts occur after malolactic fermentation, in the process of the second racking the wine materials off the lees. The reduction of phenolic compounds is less intensive in wine material which was subjected to the technological process of the second racking off the lees with the use of 0.6 mg/L nanosilver. Application of this dose in the above-mentioned technological processes for blocking the growth of lactic-acid bacteria and inhibition of the oxidation of phenolic compounds has the same effect as sulfiteization with 50 mg/L Kadifit.

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