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THE INHIBITION OF WINE MICROORGANISMS BY SILVER NANOPARTICLES

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ABSTRACT

The presented work aimed to study the inhibition using nanoparticles produced by the green synthesis in selected acetic acid and lactic acid bacteria, which are related to viticulture. The degree of ability to eliminate silver particles produced by green syntheses was determined using the plate method on Petri dishes. This is done using two different approaches - the method of direct application of the solution to the surface of the inoculated medium (determination of inhibition zones) and the method of application using nanoparticles to the inoculated medium. *Gluconobacter oxydans* (CCM 3618) and A*cetobacter aceti* (CCM 3620T) were studied from acet acetic bacteria. The lactic acid bacteria were *Lactobacillus brevis* (CCM 1815) and *Pediococcus damnosus* (CCM 2465). The application of silver nanoparticles was always in concentrations of 0, 0.0625, 0.125, 0.25, 0.5, and 1 $g.L^{-1}$. All applied concentrations of silver nanoparticles showed an inhibitory effect on the monitored microorganisms. Silver particles could be used in wine technology for their antibacterial effects, mainly to inhibit microorganisms during vinification, as a substitute for sulfur dioxide.

Keywords: acetic acid bacteria; lactic acid bacteria; inhibition; silver particles; green synthesis

INTRODUCTION

Silver nanoparticles, or silver-based materials and preparations, are already well known for their proven antimicrobial activity and are currently commonly used in practice across various branches of human activity. Silver is effective in treating burns, severe chronic osteomyelitis, urinary tract infections, and central venous catheter infections **(Feng et al., 2000)**.

In recent years, they have also been the subject of research in viticulture, in connection with their possible use in the inhibition of undesirable microorganisms, and thus as a means of reducing the need for sulfur dioxide **(Moreno-Arribas and Sualdea, 2016)**.

Traditionally, silver nanoparticles have only been prepared by physical and chemical methods. Chemical synthesis often leads to toxic effects caused by the rest of the chemicals on the surface of the nanoparticles, which significantly limits their use for medical and food purposes **(Parashar, Saxena and Srivastava, 2009)**. In recent years, silver nanoparticles have also been prepared using so-called green synthesis. The production of nanoparticles using green synthesis is a new, cheap, fast, and gentle method for synthetic production. Green synthesis can be used to modify the surface of the obtained silver nanoparticles by biomolecules from the extracts used **(Forough and Farhadi, 2010)**.

From the ranks of bacteria, in wine technology, we encounter mainly bacteria of acetic and lactic fermentation. Acetic acit bacteria, in contrast to lactic acid bacteria, are classified in enology primarily as undesirable representatives of microorganisms. However, lactic acid bacteria can also cause many wine diseases. Representatives of both mentioned groups of bacteria occur not only on grapes but also in wine-growing areas and on all wine-growing equipment, from where they can spread and subsequently contaminate the wine. It is therefore important to keep the winery clean at all times. The use of silver particles in winemaking technology could be one of the options for sanitation in the cellar, but also a possible use in the actual production of wine.

Izquierdo-Cañas et al. (2012) and **Ebelashvili et al. (2014)** focused on the possible influence of nanosilver in terms of significant analytical parameters of the resulting wine. They equally concluded that red wines treated with the tested silver particles and wines prepared with SO_2 did not show significant differences in this respect. The same was true of the white wines observed in the work of the first-mentioned **Izquierdo-Cañas et al. (2012)**.

The aim of this work is the production of nanoparticles by green synthesis using grape seed extract and the study of the inhibitory effect of these nanoparticles on bacterial cultures of acetic acid and lactic acid bacteria.

Scientific hypothesis

Silver particles have antimicrobial properties, they are usually prepared by a synthetic route. The production of nanoparticles by green synthesis is a possible gentle alternative to the production of nanoparticles by chemical synthesis. A grape seed extract has high levels of antioxidant components. Its incorporation into the process of production of silver nanoparticles should increase the antimicrobial effects of silver nanoparticles. The possible use of nanoparticles prepared in this way is in the food industry, as they show lower toxicity. In viticulture, silver-based particles could be used to eliminate unwanted acetic acid and lactic acid bacteria.

MATERIAL AND METHODOLOGY

Production of grape seed extract

Samples

This extract was made from seeds of the Cerason variety. (Merlot × Seibel 13 666).

Chemicals

75% Ethyl alcohol (Lach-Ner, s.r.o, Czech Republic) was used for extraction.

Instruments

Grinder (IKA MF 10 basic, Merci, Czech Republic).

Centrifuge (Hettich MIKRO 220, Hettich, Czech Republic).

Lyophilizer (Heto PowerDry PL 3000, Trigon-plus, Czech Republic).

For vacuum evaporation was used IKA RV 10 digital V-C (IKA Werke, Staufen im Breisgau, Germany).

Laboratory Methods

The antioxidant activity was determined by the DPPH method, the content of polyphenolic compounds was determined by the Folin-Ciocalte method, and selected antioxidant components were determined by HPLC-UV/VIS.

The antioxidant activity value was 12.436 $g.L^{-1}$ gallic acid equivalent and the value of total polyphenolic compounds 8.568 $g.L^{-1}$ gallic acid equivalent. The values of the individual components are shown in Table 1 below.

Table 1 Values of the contents of individual antioxidant components.

Compound	Value ($mg.L^{-1}$)
Gallic acid	19.9 ±0.435
Caffeic acid	1.8 ±0.124
Koutaric acid	0.431 ±0.00602
Ferulic acid	0.26 ±0.0163
Fertaric acid	0.41 ±0.0135
Catechin	0.393 ±0.0126
Epicatechin	136.9 ±1.569
Trans-piceid	0.786 ±0.0568
Trans-piceatannol	0.0238 ±0.00165
Rutin	1.62 ±0.040
QuercetinGlukoside	0.428 ±0.0172
Quercitrin	0.135 ±0.00986
Myricetin	0.155 ±0.00650

Description of the Experiment

The seeds have undergone sorting, purification, subsequent drying, and grinding. Next, these seeds were dried in an oven at 55 °C for 12 hours. After drying and cooling to room temperature, the seeds were ground with a laboratory grinder. The seeds were then extracted in 75% ethanol for 120 hours in the following ratio: 10 parts of ethanol were added to 1 part of ground seeds. After complete extraction, the resulting sample was divided into 25 mL tubes. Subsequently, centrifugation was performed at 4 °C and RCF 25 000 x g. Subsequently, the sample was transferred to a distillation flask of a vacuum evaporator. In this step, the excess ethanol was evaporated and evaporated in vacuo at 90 °C. Such an extract was then transferred to Petri dishes and lyophilized at -55 °C.

Production of nanoparticles using green synthesis

Chemicals

Distilled water.

99.8% Silver nitrate (ETC GmbH, Germany).

99.9% Methanol Honeywell, France)

Biological Material

From the acetic fermentation bacteria, one representative of the acetic fermentation from the genus *Gluconobacter* and one from the genus *Acetobacter* was selected for experimental work. The first species was *Gluconobacter oxydans* CCM 3618 and the second *Acetobacter aceti* CCM 3620^{T}.

In the case of lactic acid bacteria, there was one heterofermentative representative of the genus *Lactobacillus* and one homofermentative representative of the genus *Pediococcus*. The first bacterium was *Lactobacillus brevis* CCM 1815 and the second *Pediococcus damnosus* CCM 2465.

All the mentioned species of bacteria come from the Czech Collection of Microorganisms, Faculty of Science, Masaryk University in Brno.

Instruments

Magnetic stirrer with heating (STAR LAB, United Kingdom).

Centrifuge Hettich MIKRO 220, (Hettich, Czech Republic).

Description of the Experiment

The next step was to homogenize the seeds extract to a fine powder in a mortar. The material thus prepared was used to prepare the extract itself. This material was then mixed with distilled water at 80 °C. This extraction was carried out with stirring for 24 hours. A 0.1 M solution of silver nitrate was added to the filtered and cooled extract in a ratio of 1:1. Subsequently, the solution prepared in this way was stirred on a magnetic stirrer with heating at room temperature (22 °C) for 24 hours. During the formation of the silver particles, the color gradually changed from a solution from bright red to the resulting gray-green solution.

These particles were precipitated in a 1:1 ratio (precipitant and AgNPs solution) with the aid of an organic solvent - methanol. The solution was precipitated for 10 minutes with stirring. The next step was centrifugation for 30 minutes, 14,000 g and at 5 °C. After centrifugation, the tubes were removed from the apparatus, in which the nanoparticles were already visibly precipitated and deposited. Next, the supernatant was pipetted very carefully and the open tubes were dried at 60 °C for 24 hours. Already dried and cooled silver particles were weighed and diluted with distilled water to a weight concentration of 1 $g.L^{-1}$. The tube with the resulting suspension had to be homogenized in ultrasound

three times for 15 minutes at room temperature because this homogenization increases the surface area of the produced particles and thus also increases the antibacterial activity of the product.

Application of test substance to the inoculated nutrient medium

Chemicals

Homogenized silver particles.
Distilled water.
Mueller-Hinton agar (HiMedia Laboratories, USA).

Description of the Experiment

10 μL of a solution of homogenized silver particles and 10 μL of distilled water as control were applied to the inoculated medium. Five different concentrations (the sixth was a control variant with distilled water) of silver particles were applied to one petri dish, namely 0, 0.0625, 0.125, 0.25, 0.5, and 1 $g.L^{-1}$. After 48 hours, the Petri dishes were removed from the incubators and the resulting inhibition zones were measured separately, to the nearest 1 mm. All experiments were performed in three independent replicates, from which the average inhibition zones were then calculated.

Application of the test substance directly to the inoculum

Chemicals

Homogenized silver particles.
Distilled water.
Mueller-Hinton agar (HiMedia Laboratories, USA).

Description of the Experiment

By applying the solution directly to the inoculum, the inhibitory properties of various homogenized concentrations of silver particles were tested, with concentrations of 0, 0.0625, 0.125, 0.25, 0.5, and 1 $g.L^{-1}$. First, a suspension of a broth mixture (250 μL) with bacteria with a degree of turbidity of 1 McF and a concentration of silver particles (50 μL) was prepared. After the preparation of these suspensions, this inoculum was applied to the surface of the culture medium. The sixth suspension was a control, replacing 50 μL of the homogenized particle solution with 50 μL of distilled water. The inoculated Petri dishes were again left in the laminar box for 10 minutes to allow the inoculum to be completely absorbed into the culture medium. Subsequently, these samples were transferred to an incubator for 48 hours. After 48 hours, the percentage of the inhibited area was determined, when the entire area of the Petri dish was divided into identically large divisions. Subsequently, the sections on which bacterial growth was inhibited were counted, and the ratio of inhibition to total area was determined.

Sample preparation:

Inoculation of microorganisms

Chemicals

Nutrient Broth No. 1 (SIGMA ALDRICH, Germany).

Description of the Experiment

The bacterial culture in the form of gelatin disks was first transferred with a sterile needle to an Erlenmeyer flask together with sterile Nutrient Broth No. 1, all flasks being covered with a special laboratory foil – PARAFILM® M (SIGMA ALDRICH, Germany). The flasks covered in this way were placed in a shaker incubator. This cultivation was carried out for 24 hours at temperatures. Subsequently, the bacterial suspension was diluted with sterile Nutrient Broth No. 1 with a degree of turbidity of 1 McF. Next, 100 μL of the bacterial suspension prepared in this way was pipetted and spread with a sterile glass loop on the prepared culture medium in a disposable petri dish. After 10 minutes, when all the inoculum was absorbed into the culture medium, subsequent application of silver particles with concentrations of 0, 0.0625, 0.125, 0.25, 0.5, and 1 $g.L^{-1}$ took place.

Number of samples analyzed:

During the course of the research, a total of four species of wine bacteria were analyzed for susceptibility to the test substance.

Number of repeated analyses:

A total of two methods were selected for this research to test the sensitivity of the selected bacteria to the test substance. These were the Application of the test substance to the inoculated nutrient medium and the Application of the test substance directly to the inoculum

Number of experiment replication:

Each method for testing the sensitivity of a given bacteria to the test substance was repeated three times. From the results, averages were calculated and used for this research. The results were also statistically processed.

Statistical Analysis

An analysis of variance was performed (single–factor ANOVA), which showed significant differences in antibacterial effects between given concentrations on selected species of microorganisms. Statistical results are significant at $p = 0.05$. Subsequently, a post-hoc test (Tukey HSD test) was performed for each concentration. All statistical evaluation is available from the author.

RESULTS AND DISCUSSION

The standard diffusion disc method was first and foremost tested for testing silver particles, but this method of application appeared to be highly inappropriate as the particles were not released from the disk into the agar. After that, the solution was tested directly into the medium, unfortunately, the automatic pipette failed to exert a strong enough pressure to inject the test substance into the rigid agar. The syringe with the needle was able to inject the solution into the medium, but soon after the needle was ed, all the test substances flowed uncontrollably to the surface of the agar. Also, injection with a needle did not allow a sufficiently accurate measurement of the applied volume. Finally, two methods were chosen: A) the method of direct application of the solution to the surface of the inoculated medium (determination of inhibition zones) and B) the method of application of the solution to the inoculated medium.

This chapter is therefore divided into two parts. Thanks to two different methods, it was possible to better interpret the results of the microorganisms studied.

Inhibition of bacterial growth by application of test substance to the inoculated nutrient medium

This method is characterized by sufficient diffusion of the sample with agar. Many optimizations were carried out, with the greatest stability after several tests, due to the highest surface tension, a drop of 10 μL. The applied

concentrations and sizes of inhibition zones are given in table 2 below, the average size of the zone of inhibition being rounded to one decimal place.

In Figure 1 we can see the inhibition zones produced by different concentrations of silver particles, where 0 $g.L^{-1}$ is marked as F, 0.0625 $g.L^{-1}$ as E, 0.125 $g.L^{-1}$ as D, 0.25 $g.L^{-1}$ as C, 0.5 $g.L^{-1}$ as B, and 1 $g.L^{-1}$ as A. The same concentrations were applied to all bacteria. The zones were used to demonstrate the inhibitory capabilities of the silver particles tested against all the bacteria studied, as even a concentration of 0.125 $g.L^{-1}$ was always able to limit the growth of bacteria.

At lower concentrations (less than 0.25 $g.L^{-1}$), approximately half of the experiments experienced a slight increase in resistant colonies within the range of the applied substance. In lactic bacteria, in cases of testing of silver particles diluted to a mass concentration of 0.0625 $g.L^{-1}$, almost no inhibition occurred and, if so, the inhibited area has always been increased by resistant colonies. From this point of view, we can say that the concentration of 0.0625 $g.L^{-1}$ appears insufficient to limit the growth of lactic acid bacteria. Acetic bacteria at a concession of 0.0625 $g.L^{-1}$ had already been colony-wide, but the inhibition zone values were embroidered (2.7 mm for *G. oxydans* and 3.7 mm for *A. aceti).*

In Figure 2 we can see the size of the inhibition zones formed after application of the homogenized silver particles, which were diluted to a mass concentration of 1 $g.L^{-1}$ measured in representatives of the acetic acid species *Acetobacter aceti*. In this bacterium, the size of the inhibition zones ranges from 16.2 mm to 19.2 mm. In contrast, the smallest growth restriction was observed in *Glukonobacter oxydans*. In this bacterium, the size of the measured inhibition zones ranged from 10.5 mm to 18.2 mm.

In Figure 3 we can see the sizes of the inhibition zones formed after the application of the homogenized silver particles, which were diluted to a mass concentration of 0.5 $g.L^{-1}$. vinegar bacteria of the species *Acetobacter aceti*. In this bacterium, the size of the inhibition zones ranged from 12.3 mm to 17.5 mm. In contrast, the smallest growth restriction was observed in *Glukonobacter oxydans*. In this bacterium, the size of the measured inhibition zones ranged from 7.5 mm to 13.1 mm.

Figure 4 shows the sizes of the inhibition zones formed after the application of the homogenized silver particles, which were diluted to a mass concentration of 0.25 $g.L^{-1}$. representatives of acetic bacteria of the species *Acetobacter aceti*. In this bacterium, the size of the inhibition zones ranged from 7.8 mm to 10.8 mm. On the contrary, the smallest growth restriction was recorded this time in the lactic acid bacterium *Lactobacillus brevis*. In this bacterium, the size of the inhibition zones ranged from 5.9 mm to 8.8 mm.

In Figure 5 we can see the sizes of the inhibition zones formed after the application of homogenized silver particles, which were diluted to a mass concentration of 0.125 $g.L^{-1}$. All representatives of lactic and acetic fermentation bacteria showed some sensitivity to this lower concentration of the applied substance, while the largest inhibited areas were also measured in the representative of acetic bacteria of the species *Acetobacter aceti*. In this bacterium, the sizes of the inhibition zones ranged from 4.9 mm to 7.8 mm. On the contrary, the smallest growth restriction was recorded in this case in the lactic acid bacterium *Pediococcus damnosus*. In this bacterium, the sizes of the inhibition zones ranged from 3.2 mm to 6.1 mm.

Figure 6 shows the sizes of the inhibition zones formed after the application of homogenized silver particles, which were diluted to a mass concentration of 0.0625 $g.L^{-1}$. In some cases, all representatives of lactic and acetic fermentation bacteria showed some sensitivity to this lower concentration of the applied substance, while the largest inhibited areas were also measured in the representative of acetic bacteria of the species *Acetobacter aceti*. In this bacterium, the sizes of the inhibition zones ranged from 2.2 mm to 5.1 mm. On the contrary, the smallest growth restriction was recorded this time in the lactic acid bacterium *Lactobacillus brevis*. However, there was no inhibition of lactic acid bacteria in about half of the testing. These were the *Lactobacillus brevis* and *Pediococcus damnosus* species. In these bacteria, the sizes of the inhibition zones ranged from 0 mm to 6.3 mm.

Distilled water was used as a control and showed no inhibitory properties in either case.

Growth inhibition by applying nanoparticles directly to the inoculum

The antimicrobial properties of silver particles against both lactic and acetic fermentation bacteria have also been confirmed by this method. The results of the application of different concentrations of homogenized silver nanoparticles are given in Table 3 below.

In Figure 7 we can see the inhibitory effects of different concentrations of the tested silver particles against individual bacteria by applying the test substance directly into the inoculum. *L. brevis* exhibits a high sensitivity to high concentrations of the applied substance in this method. At the highest concentration (1 $g.L^{-1}$), the inhibited area was 95%. When this concentration was reduced to 0.5 $g.L^{-1}$, there was a slight increase in lactic acid bacteria, which is also true for a concentration of 0.25 $g.L^{-1}$. Although *P. damnosus* exhibits some sensitivity to the test substance, this method of application of silver particles directly into the inoculate in the bacterium does not show as much inhibition as in *L. brevis*. At a high concentration of the applied substance (1 $g.L^{-1}$), growth was limited (44%), but this was only about half the inhibition of previous bacteria. When these particles were diluted to 0.125 $g.L^{-1}$, the inhibited area was only around 10%. The concentration of 0.0625 $g.L^{-1}$ no longer showed any ability to limit the growth of these lactic acid bacteria. *G. oxydans* show considerable sensitivity to high concentrations of the applied substance in this method. At the highest concentration (1 $g.L^{-1}$), the inhibited area was 92 %. By reducing this concentration to 0.5 $g.L^{-1}$, the number of colonies increased proportionally to 46%. At further dilution, the inhibited area decreased considerably, with a concentration of 0.625 $g.L^{-1}$ only to a small extent (15%) inhibited. *A. aceti* also exhibits considerable sensitivity to high concentrations of the applied substance in this method. At the highest concentration (1 $g.L^{-1}$), the inhibited area was 94%. When this concentration was reduced to 0.5 $g.L^{-1}$, there was no significant increase in acetic fermentation bacteria, such as *G. oxydans.*

Table 2 Size of inhibition zones (expressed in mm) resulting from different mass concentrations of silver nanoparticles in the *L. brevis*, *P. damnosus*, *G. oxydans*, and *A. aceti* bacteria studied.

Concentration (g.L^{-1})	*L. Brevis*	*P. damnosus*	*G. oxydans*	*A. aceti*
1	15.3 ±1.3	13 ±1	14.3 ±1.7	17.7 ±0.7
0.5	10.7 ±1.3	12 ±1	10.3 ±1.3	13 ±1
0.25	7.3 ±0.7	7.7 ±1.3	8 ±1	9.3 ±0.7
0.125	5 ±1	4.7 ±0.7	5.7 ±0.7	6.3 ±0.7
0.0625	2 ±2	2 ±2	2.7 ±0.7	3.7 ±0.7
0 (Dest. H_20)	0 ±0	0 ±0	0 ±0	0 ±0

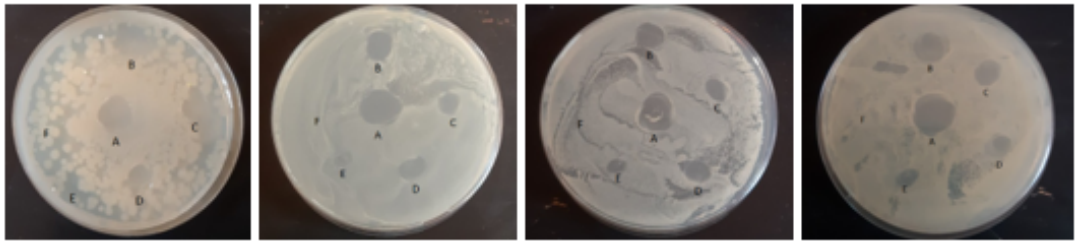

Figure 1 Photographs of inhibition of micro-organisms by silver nanoparticles at concentrations 0 (F), 0.0625 (E), 0.125 (D), 0.25 (C), 0.5 (B), and 1 g.L^{-1} (A) in micro-organisms (sorted from left to right) *L. brevis*, *P. damnosus*, *G. oxydans* and *A. aceti*.

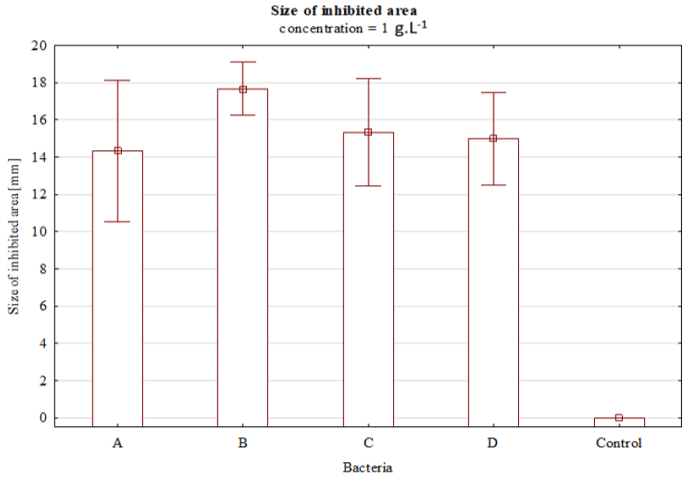

Figure 2 Statistical expression of the measured values of the test substance diluted to 1 g.L^{-1}.

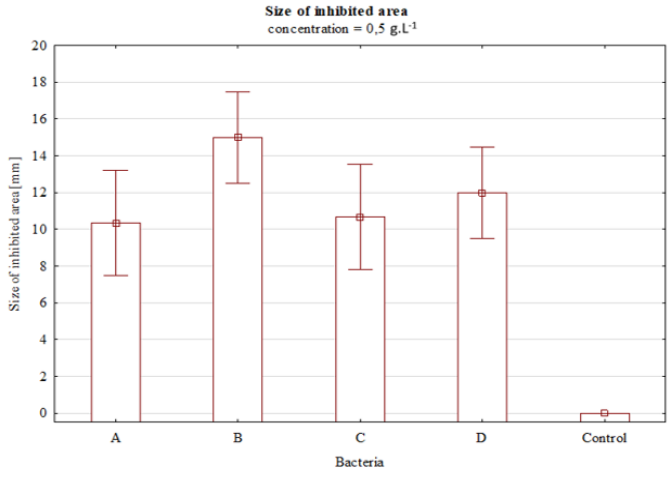

Figure 3 Statistical expression of the measured values of the test substance diluted to 0.5 g.L^{-1}.

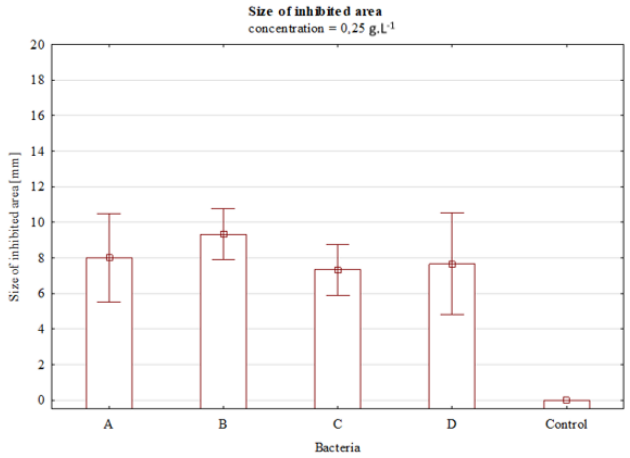

Figure 4 Statistical expression of the measured values of the test substance diluted to 0.25 g.L^{-1}.

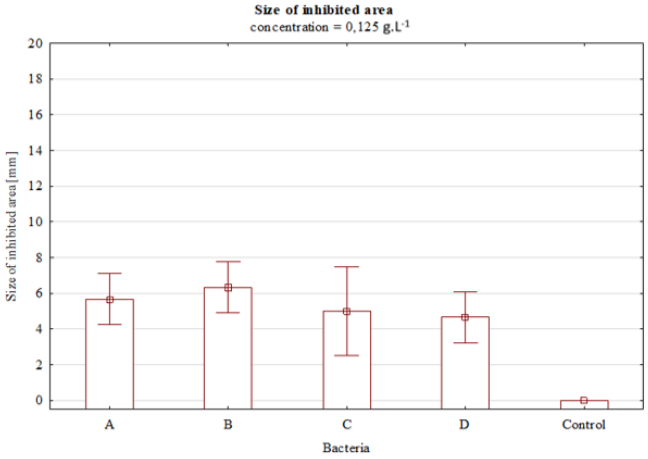

Figure 5 Statistical expression of the measured values of the test substance diluted to 0.125 g.L^{-1}.

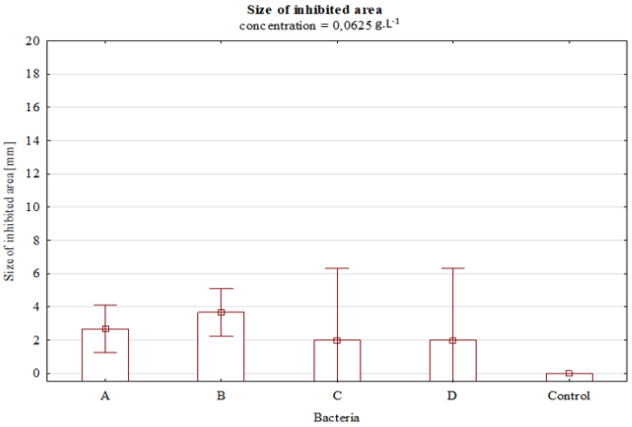

Figure 6 Statistical expression of the measured values of the test substance diluted to 0.0625 g.L^{-1}.

Table 3 Size of inhibited area (expressed in %) concentrations of silver particles in the *L. brevis*, *P. damnosus*, *G. oxydans*, and *A. aceti* bacteria studied.

Concentration (g.L^{-1})	***L. brevis***	***P. damnosus***	***G. oxydans***	***A. aceti***
1	95%	44%	92%	94%
0.5	74%	28%	46%	89%
0.25	61%	18%	41%	43%
0.125	41%	12%	32%	40%
0.0625	8%	0%	15%	10%
0 (Dest. H_20)	0%	0%	0%	0%

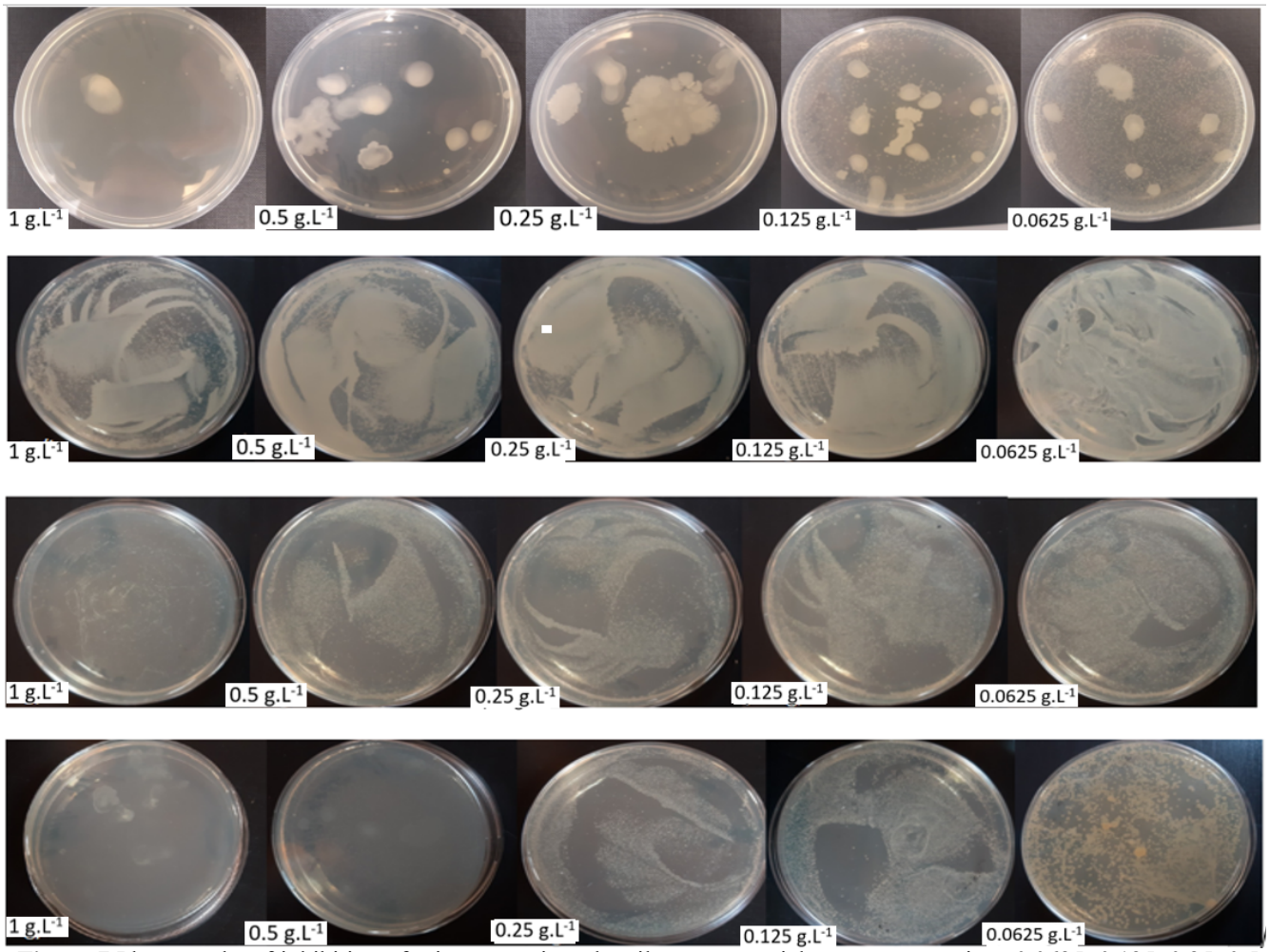

Figure 7 Photographs of inhibition of micro-organisms by silver nanoparticles at concentrations 0.0625, 0.125, 0.25, 0.5, and 1 g.L^{-1} in micro-organisms (sorted from top to bottom) *L. brevis*, *P. damnosus*, *G. oxydans*, and *A. aceti*.

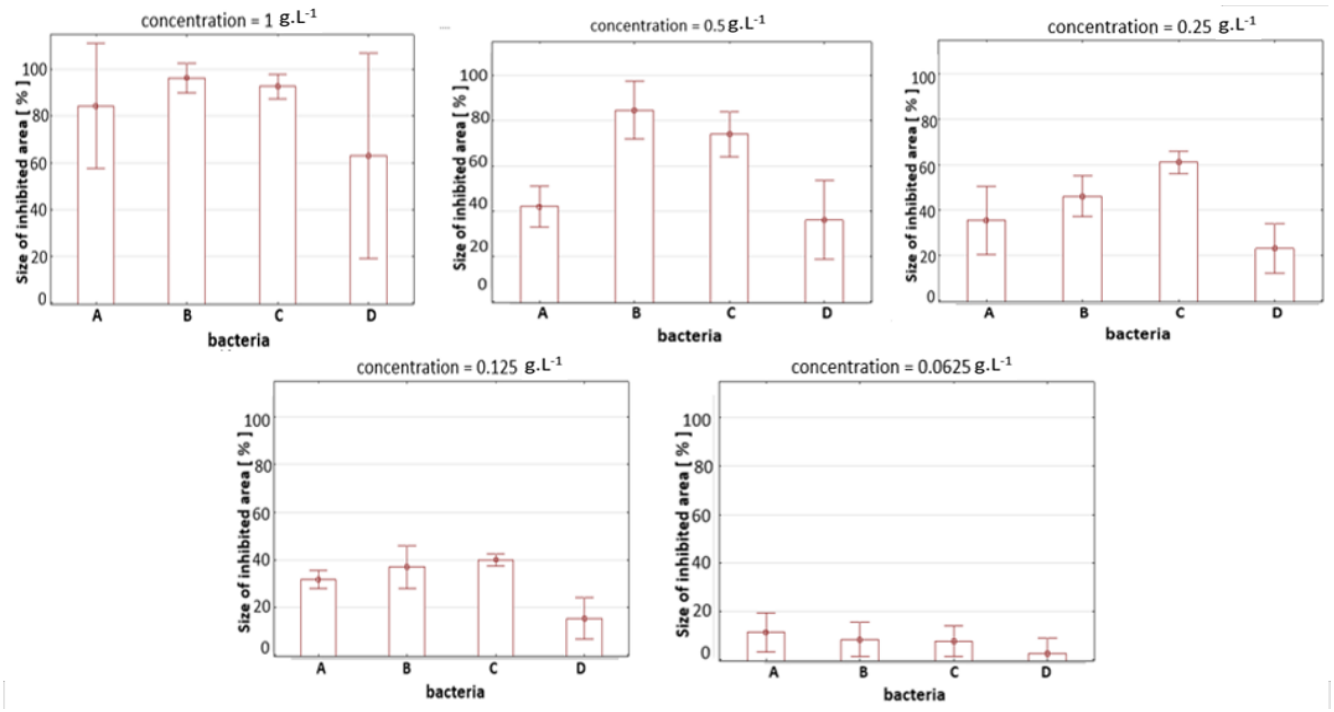

Figure 8 Growth inhibition by applying nanoparticles directly to the inoculum. The following graphs show how decreasing concentrations of silver particles affect the growth of test bacteria. A= *Gluconobacter oxydans*, B= *Acetobacter aceti*, C = *Lactobacillus brevis*, D = *Pediococcus damnosus.*

However, with further dilution, the inhibited area decreased considerably. At a concentration of 0.25 $g.L^{-1}$, the size of this area was only 43%. When the applied substance was diluted to 0.0625 $g.L^{-1}$, growth was limited only slightly.

In Figure 8 we can see the inhibitory abilities of homogenized silver particles in all bacteria tested. In this method, the acetic acid species *Acetobacter aceti* showed the highest sensitivity to the test substance, and, conversely, the growth of the lactic acid bacterium *Lactobacillus brevis* was least restricted in this way.

Distilled water was used as a control in all experiments and showed no inhibitory properties against the monitored bacterial strains.

The antimicrobial effects of silver have been known for thousands of years. In ancient times, silver containers were used to store food. Silver coins were inserted into the milk to extend the shelf life of the product. Even the use of silver cutlery was supposed to lead to disease prevention. During the 15th century, bowls of silver powder appeared on the tables of privileged families, and a small amount of ingested powder was supposed to lead to a lower probability of infection with various diseases, which led to the coloration of the blood turning gray-blue. This is the source of the so-called blue blood. Today, silver, due to its properties, is used in many fields **(Večeřová, 2016)**.

Antibacterial activity of silver particles has been shown in several types of research, but it is difficult to compare their results, since there are not yet standard protocols for evaluating the antimicrobial activity of silver particles, and there is also the problem that scientists use different methods to determine the antimicrobial activity of silver particles **(Zarei, Jamnejad and Khajehali, 2014)**.

A very important aspect that determines the degree of antibacterial properties is the resulting size of the particles produced, where we attribute better inhibitory properties to smaller units of particles **(Espinosa-Cristóbal et al., 2009)**.

The antimicrobial action of silver particles, specifically in the form of a commercially available 1% colloidal silver preparation, was also tested by **Garde-Cerdán et al. (2014)**. They investigated the effects of this substance both during vinification and subsequently during wine storage. They then concluded the experiment by also confirming the sufficient antibacterial effects of silver. Wines treated with the product did not show any of the possible signs of undesirable microbial activity **(Garde-Cerdán et al., 2014; Moreno-Arribas and Sualdea, 2016)**.

García-Ruíz et al. (2015) researched the sensitivity of different types of acetic and milk bacteria to silver nanoparticles. In this research, antibacterial abilities were tested on two types of acetic bacteria and a total of eighteen strains of *Lactobacillus plantarum, Lactobacillus casei, Pediococcus pentosaceus, and Oenococcus oeni.* Of the acetic bacteria, it was *a* species of *Acetobacter aceti and Gluconobacter oxydans*. Identical representatives of acetic bacteria were chosen for research in this work as well. The results of the research demonstrated the good inhibitory abilities of silver nanoparticles to selected representatives of acetic and milk bacteria. The effect of silver nanoparticles was to some extent comparable to the antimicrobial effect of a commonly used device, potassium dissimite.

Silver particles can largely limit the growth of bacteria in wine, which could certainly lead to an overall reduction in the sulfur dioxide dosage in wine production. However, the facts about silver particles used in this way in the actual production of wine are very important. Through these conditions, we can mention the study of **Ebelashvili et al. (2014)** which carried out research involving precisely testing the inhibitory properties of silver particles against acet acetic and lactic aceti bacteria in the production of red wine. According to its findings, the rate of growth limitation in both acetic and lactic acid bacteria was substantially comparable to that of sulfur dioxide. This work also provided foran appropriate dose of silver particles at different stages of red wine production, with a dose of 0.4 $mg.L^{-1}$ silver particles are recommended in this study before the start of alcohol fermentation and a dose of 0.6 $mg.L^{-1}$ should be administered after fermentation **(Ebelashvili et al., 2014)**.

From earlier research, we can also mention the extensive work of Izquierdo-Cañas and Co. from 2012, which also examined the possibilities of replacing sulfur dioxide with silver particles in the production of red and white wines. The results of this work largely coincide with the previous work of **Ebelashvili et al. (2014)**. In both types of research, they concluded that in the case of red wines, there were no significant differences in the wine into which silver particles were applied compared to wines treated with the classical method using sulfur dioxide, both from an organoleptic and analytical point of view. There was a slight difference in results for white wines, as wines treated only with silver particles had higher wine oxidation, which is understandable because sulfur dioxide acts as an antioxidant in wine **(Izquierdo-Cañas et al., 2012; Ebelashvili et al., 2014)**.

Gil-Sánchez et al. (2016) also found in their work that silver nanoparticles have strong inhibitory properties against lactic acid bacteria. Their experiment was conducted in 50 ml samples of Airén wine variety. All samples treated with the tested silver nanoparticles showed complete inhibition of the original lactic acid bacteria populations, while the control (untreated sample) contained the same amount of bacteria as at the start of the experiment. It is also important to note to mention that the applied nanoparticles were able to inhibit the growth of the bacteria studied even slightly more effectively and faster than sulphur dioxide.

From a laboratory point of view, we can compare the results of this work with the **El-Fadly et al. (2016)** study, which concluded that G – bacteria, as acetic bacteria, show greater sensitivity to silver particles than G + bacteria, or lactic acid bacteria. This finding is to some extent consistent with the results of this thesis, as it was found through the disc diffusion method that silver particles were able to inhibit acetic bacteria to a greater extent than lactic acid bacteria.

But there are also studies **(Devi and Bhimba, 2014)** that show the opposite effect, i.e. a stronger inhibitory effect against gram-positive bacteria, or at least identical or not so different **(Shoo-Hwan et al., 2011)**. Thus, the findings are consistent with the fact that the exact mechanism of inhibitory effects against specific genera, species, or strains of bacteria is still not fully understood and very likely varies within different bacterial groups.

Through the above-mentioned results of two types of research, we could conclude that the treatment of wines with silver particles is more suitable for red wines since these wines are not so much susceptible to higher oxygen content.

Many studies have shown the antimicrobial activity of silver particles against various species of pathogens such as bacteria, viruses, fungi, yeast, etc. **(Panyala, Peña-Méndez and Havel, 2008)**.

A study by **Yoon et al. (2008)** shows the bactericidal effects of silver particles on two representatives of bacteria. These were *Escherichia coli* and *Bacillus subtilis*. These bacteria also showed some sensitivity to silver particles. In this study, it was found that G^+ bacteria Bacillus subtilis had a higher sensitivity to silver particles than G^- Escherichia coli. These results are mild contradict the results in this presented work. On the contrary, we found a greater sensitivity of G^- acetic acid bacteria to silver particles than in G^+ lactic acid bacteria **(Yoon et al., 2008)**. These data suggest that it is still not the exact mechanism of the antibacterial effect of silver particles against various species of bacteria.

An interesting finding regarding the use of antimicrobial effects comes from the study of **El-Rafie et al. (2010)**, where silver particles were applied to various types of fabrics. Was determined the efficacy and durability of the inhibitory effects of silver particles against *Staphylococcus aureus* and *Escherichia coli*. In this study, a high reduction in the number of both bacteria was found on fabrics treated with silver particles compared to ordinary fabrics. The high ability to reduce the growth of both bacteria was demonstrated even after several works cycles **(El-Rafie et al., 2010)**.

The issue is the consumption of silver particles in the wine itself. It has been shown that the topical and total use of colloidal silver can lead to adverse effects in this respect in the form of allergic reactions in sensitive individuals, or even irreversible grey-blue discoloration of the skin and eyes (so-called argyria) due to the deposition of silver and its salts in the body's tissues. Therefore, the internal use of colloidal silver has been banned within the EU since 1. January 2010. However, due to the low concentrations required to inhibit bacteria, it is currently the subject of intensive research to remove this barrier **(Večeřová, 2016)**. The possible future application of silver in viticulture naturally depends on it.

CONCLUSION

The results show that silver particles can eliminate to a sufficient extent both genera of the bacteria studied. The plot method found that acetic fermentation bacteria showed slightly higher sensitivity to the silver particles tested than dairy fermentation bacteria. However, even a low concentration of silver particles (0.125 $g.L^{-1}$) has always been able to limit the growth of all kinds of bacteria tested in the case of the plate method. Silver particles could be used in wine technology to inhibit microorganisms during vinification. Further use of these particles could be the surface treatment of materials in wine technology, which would simplify sanitary work during wine production.

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