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Preparation of synthesized silver nanoparticles from extract of rosemary leaves (*Rosmarinus officinalis L.*) and its used as a perservative

Elaboración de nanopartículas de plata sintetizadas a partir de extracto de hojas de romero (*Rosmarinus officinalis L.*) y su uso como conservante

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Abstract

Nanoparticles are materials that measure from 1 to 100 nm of length. Currently, the antimicrobial property of silver nanoparticles is used by industries for the manufacture of beauty products and medicines. Nanoparticles can be synthesized from plants, algae or microorganisms, and they can also be obtained from combustion products. In this study, extracts of rosemary leaves (*Rosmarinus officinalis* L.) were used for the synthesis of silver nanoparticles (NPs-Ag) in order to produce an antimicrobial compound to be used as a fruit preservative. NPs-Ag were characterized qualitatively and quantitatively by phytochemical analysis and UV-VIS spectroscopy, showing an absorption in the range of 389-418 nm, which corresponds to their surface plasmon resonance. Furthermore, Scanning Electronic Microscopy was used to determine the size and morphology of the NPs-Ag, observing a spherical shape of 10 nm of diameter. Two bacterial strains were used in the antimicrobial tests, the Gram-negative (*Escherichia coli*) and the Gram-positive (*Staphylococcus aureus*) to verify the antimicrobial activity of the NPs-Ag. For *E. coli*, a better antibacterial activity was obtained with an inhibition halo of 3.21 mm. Subsequently, the NPs-Ag were used in apples to determine their use as a preservative, using beeswax smeared on the surface of the fruit as control, observing that synthesized NPs-Ag prolonged the maturation time of the fruits.

Keywords: Aqueous extract, silver nanoparticle, spectroscopy, rosemary.

Resumen

Las nanopartículas son materiales que pueden llegar a medir entre 1 a 100 nm de longitud, y en la actualidad la propiedad antimicrobiana de las nanopartículas de plata es aprovechada por las industrias para la fabricación de productos de belleza y medicamentos. Las nanopartículas pueden ser sintetizadas a partir de plantas, algas o microorganismos, y también pueden ser obtenidas como productos de combustión. En este estudio se utilizaron extractos de las hojas de romero (*Rosmarinus officinalis* L.) para la síntesis de nanopartículas de plata (NPs-Ag) con la finalidad de producir un compuesto antimicrobiano para usarse como conservante de frutas. Las NPs-Ag se caracterizaron cualitativa y cuantitativamente mediante análisis fitoquímicos y espectroscopia UV-VIS, presentando una absorción en el rango de 389-418 nm, que corresponde a la resonancia de su plasmón superficial. Además, se empleó la microscopía electrónica de barrido para determinar el tamaño y morfología de las NPs-Ag, observándose una forma esférica de 10 nm de diámetro. Se emplearon dos cepas bacterianas en los ensayos antimicrobianos realizados, la gramnegativa (*Escherichia coli*) y la grampositiva (*Staphylococcus aureus*) para comprobar la actividad antimicrobiana de las NPs-Ag. Para *E. coli* se obtuvo una mejor actividad antibacteriana con un halo de inhibición de 3,21 mm. Posteriormente se usaron las NPs-Ag en manzanas para determinar su uso como conservante, usando la cera de abeja como control untada en la superficie de las frutas, observándose que las nanopartículas sintetizadas alargaron el tiempo de maduración de la frutas.

Palabras clave: Espectroscopia, extracto acuoso, nanopartícula de plata, romero

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1 Introduction

Nanoparticles are considered materials with an approximate length from 1 to 100 nm (Vázquez and Blandón, 2014). Today the antimicrobial properties of Ag-NPs and other metals such as gold (Au), platinum (Pt) and palladium (Pd) are exploited by industries for ellaborating beauty products such as soap, shampoo, detergent, toothpastes, medicines, among other applications within medicine, electronics, polymers and ceramics (Anandalakshmi and Venugobal, 2017; Vera et al., 2017). Many research has been carried out on the use of nanotechnology in the food industry, and the use and application of nanoparticles has been investigated in the area of food safety and quality, packaging and development of new foods. Nanoparticles have also been used to prevent microbial contamination in packaged foods, improve the physical properties of foods, and increase the bioavailability of their components (Pardo de Santayana, 2018; Thiruvengadam et al., 2018; Ávalos et al., 2013). Currently there is no legislation regulating nanotechnology and nanomaterials in food; for this reason, they are included in the European Chemicals Regulation (REACH). The European Food Safety Authority in its publication on guidance for assessing risks posed by the use of nanotechnology and nanoscience in food recommends the need for the development, validation of methodologies and improvements in research about toxicity and genotoxicity of nanoparticles (Ávalos et al., 2013).

There are various types of metal nanoparticle synthesis such as chemical synthesis using redox reaction (reducing agent), electrochemical method, photochemical reduction and ultraviolet irradiation, and all of these use chemical reagents, consume energy and cause environmental pollution because a large amount of chemical waste is generated, having water, soil or air as its final destination (Vera et al., 2017; Chandran et al., 2006; Rodríguez et al., 2013; Torres, 2017).

Nanoparticles are currently being synthesized by green synthesis which produces less pollutant wastes compared to traditional methods. Nanoparticles are obtained by natural sources through this synthesis by changing the chemical compounds to natural extracts of plants, algae or microorganisms, being this method effective, easy and non-toxic, becoming a synthesis technique of ecological and eco-friendly nanoparticles (Flores, 2014). This non-conventional technique provides the possibility of obtaining nanoparticles of noble metals in aqueous solutions at low temperatures, using plant extracts that act as a reducing agent found in high concentrations in addition to containing natural stabilizers (Belmares et al., 2015). For this reason, to obtain nanoparticles from the green synthesis, plants are mostly used with respect to other natural sources such as algae, bacteria, yeasts, among others, because they are less toxic (Vera et al., 2017).

Nanoparticles of silver have been synthesized from the peels of some fruits or vegetables such as banana, garlic, aloe vera, basil, matico (*Buddleja globosa*), coriander and rosemary, which have bioactive components with naturally antimicrobial content. According to their properties, they are able to produce a reduction in silver (Madrid, 2017).

Rosemary (*Rosmarinus officinalis* L.) belongs to the Lamiaceae family; it is native to the Mediterranean region of Europe and is known as an aromatic and medicinal plant. This plant presents secondary metabolites and essential oils such as flavonoids, terpenes, phenolic acids, among others, and is also used as seasoning and flavoring (Purca, 2013). Rosemary is used in the food, pharmaceutical, cosmetic and other industries. According to Briones (2017), the most important by-products of rosemary are aqueous extracts and essential oils. In addition, Avila-Sosa et al. (2011) expressed that plant extracts extracted from the rosemary leaves possess antioxidant and antimicrobial compounds and properties that were used in ancient times as ancestral medicine.

The aim of this study was to extract and synthesize silver nanoparticles from the extract of rosemary leaves and to evaluate their antimicrobial activity in a traditional fruit for its conservation.

2 Materials and Methods

2.1 Obtaining of the natural extract of rosemary leaves

The following steps were followed to obtain the natural extract of the rosemary plant that favors

the reduction process of silver nitrate and the formation of nanoparticles: first the rosemary plant was harvested, then the leaves that showed the best condition were selected. These were washed and disinfected with a liquid chlorine dioxide solution at 10 ppm for 10 minutes following Garmendia and Vero (2006). The reduction of the size of the leaves was then carried out using a mortar, following the methodology of Martinez et al. (2013). The extract obtained was let to boil (100 °C), boiling 100 gr of leaves with 100 ml of water for 20 to 25 minutes with constant agitation.

The sample was then filtered on paper (Whatman, 0,45 μm), and 100 gr of additional leaves were added to the broth to concentrate it to a volume of 50 ml, repeating the above extraction procedure. Finally, the final extract was filtered, allowed to cool at room temperature and then stored in a glass container. To preserve the extract, the extract was refrigerated at a temperature of 4 °C for 6 days.

The secondary metabolites were then determined by qualitative and quantitative analysis, using three types of extract with different solvents each: distiled water (EA), ethanol (EE) and acidulated water (EAC), in order to use the extract that presented the best results by its compounds and chemical properties for its use as nanoparticle. Each of these extracts had phytochemical analyses to check whether it contained functional groups with reducing chemical properties.

The latter was determined by phytochemical screening, using a successive extraction with solvents of increasing polarity, starting the analysis on the ethereal extract, followed by the alcoholic extract and finally the aqueous extract. This technique allowed identifying the secondary metabolites, using appropriate reagents (Table 1) that resulted in colored reactions or precipitation of the secondary metabolites (Amaguaña, 2018; Santorum, 2017).

Table 1. Phytochemical analysis of the aqueous, ethanolic and acidified extract of *Rosmarinus officinalis L.* used reagents and expected results.

Phyto-chemical essays	Reactive or treatment	Positive results
Saponins	Agitate	Presence of foam for 2min
Reducing compounds	Fehling Reactive	Red color and presence of reducing disaccharide sugar
Phenolic compounds	3 drops of ferric chloride solution at 5%.	Red color and presence of phenolic compounds. Dark green or blue and presence of tanins
Flavonoids	1 ml of HCl+ 1 ml H_2O + 2ml of alcohol	Red to Brown and presence of flavonoids
Alkaloids	1 drop of hydrochloric acid + 3 drops of Dragendorff reactive	Opalescence (+), defined turbidity observed (++), pellet observed (+++).
Resins	Distilled water	Pellet
Terpenoids	1 ml de chloroform + 1ml of carbon dioxide + 2 to 3 drops of concentrated H_2SO_4	Pink, green and dark green indicate positive results

2.2 Synthesis of silver nanoparticles

Silver nitrate (CAS number: 7761-88-8) with a purity of (95%) was used as a metallic precursor. For the preparation of aqueous silver nitrate ($AgNO_3$) 0.034 grams of $AgNO_3$ (1 mM) were dissolved in 200 ml of distilled water to prepare the stock solution. The rosemary leaf extract was then used in volumes of 5ml, 10ml, 20ml, 30ml, 40ml and 50ml to check the appropriate concentration, keeping the silver nitrate solution constant (5 ml). The pH of each of the prepared solutions was adjusted with sodium hydroxide (NaOH) at 0.1 N to obtain a basic pH between 8 and 10, measured with a pH meter (Thermo Scientific ™Orion Star ™A211). The temperature at which these solutions were maintained was 65 °C in constant agitation. This solution was stored at 4 °C Subsequently, the Ag-NPs was characterized by UV-VIS spectroscopy in a spectral absorption range between 350-800 nm, corresponding to the resonance of the surface plasmon of metal na-

noparticles, using a spectrophotometer (Genesys 10 UV scanning). To determine the size and shape of the Ag-NPs obtained, these were observed with a scanning electron microscope (Sánchez, 2017).

2.3 Effectivity of synthetized silver nanoparticles

The antimicrobial effectiveness of Ag-NPs was measured using antimicrobial disks against the Gram-negative *E. coli* bacteria and the Gram-positive *S. aureus*. For this purpose, the inhibition halo size (mm) corresponding to the area where antimicrobial effectiveness was evidenced was considered (Bauer et al., 1966). In Petri dishes with Mueller-Hinton culture medium, a cell concentration of $1{,}5 \times 10^8$ UFC/ml of the Gram-positive and Gram-negative bacteria used was planted. Before inoculation, Petri dishes were divided into three quadrants where the pure extract, the silver nitrate solution and the synthetized nanoparticles were placed in each of them. The Antimicrobial Susceptibility Disks were then placed and the plates were incubated at 37 °C for 24 hours. Finally, bacterial inhibition halos were measured using a vernier.

2.4 Synthetized silver nanoparticles used in a fruit

To determine whether the extract of synthetized Ag-NPs can be used as a preservative, the surface of a seasonal fruit (red apple) was covered and the results were compared with bee wax. Twelve red apples (*Red delicious*) bought in the market were used, which were washed and disinfected with water containing 10 ppm of chlorine for 5 minutes. Subsequently, two apples were placed in preservative boxes that were divided into two cells. The apples of preservation box 1 were the control (white), the two apples of preservation box 2 received the nanoparticle solution with a 5:5 ratio. The two apples of preservation box 3 received the nanoparticle solution with a 5:10 ratio, and the two apples of preservation box 4 received beeswax. The two apples in preservation box 5 received beeswax plus the nanoparticle solution with a 5:5 ratio and the apples placed in preservation box 6 received beeswax and a nanoparticle solution with a 5:10 ratio (Figure 1). This study was conducted for 30 days, during this time the weight, color and maturity of the fruit were monitored.

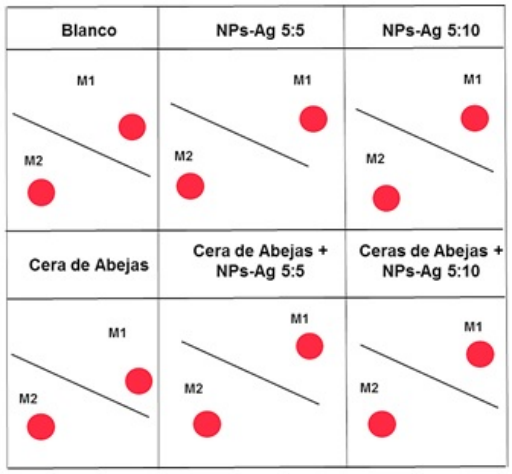

Figure 1. Graphical representation of the trial of the Ag-NPs used as a preservative (Ag-NPs= Silver Nanoparticles, M1= Apple 1 and M2= Apple 2).

3 Results and Discussion

3.1 Phytochemical characterization of rosemary leaf extract

The results obtained from the phytochemical analyzes of aqueous, acidulated and ethanolic extracts are shown in Table 2. The phytochemical characterization showed the presence of the metabolites present in the chemical composition of this plant, identifying 2844.0 mg/kg of terpenes and 24% of phenols. The presence of saponins was not evident because there was no foam formation in the sample, nor were resins observed by the aqueous extract, but there were phenolic compounds and terpenoids, as well as reducing sugar, flavonoids and alkaloids, which are considered the chemical compounds of the rosemary plant with reducing properties. This analysis was carried out to corroborate that rosemary is a plant rich in active principles, which have been widely studied. These chemical compounds act in almost all organs of the human body by possessing high percentages of essential oils whose active ingredients are flavonoids, phenolic acids, triterpenic acid and triterpenic alcohols. Rosemary leaves have a high content of Romarisnic acids and their derived rosalmarin is also present in carnosic acid, which is characterized by being unstable (Avila-Sosa et al., 2011).

As indicated by Salguero and Pilaquinga (2017), when silver nanoparticles are synthesized through the use of plant extracts, the power of the phytochemical and ethnopharmacological properties of plants is exploited. In this case, rosemary has numerous beneficial properties, along with the bactericidal property of silver nanoparticles that contributes to increasing its usefulness in the biomedical and microbiological area, without generating environmental pollution. The above may contribute to the fact that the synthesized Ag-NPs from rosemary leaf can be useful for fruit conservation.

Table 2. Phytochemical analysis of the aqueous, ethanolic and acidified extract of *Rosmarinus officinalis L.* leaves

Phytochemical essays	Aqueous extract	Ethanolic extract	Acidified extract
Saponins	Negative	Negative	Negative
Reducing compouns (Fehling reactive)	Positive	Positive	Positive
Phenolic compounds	Positive	Positive	Negative
Flavonoids	Positive	Positive	Negative
Alkaloids: (Dragendorff trial)	(+++) (++)	(+++) (+)	(+)
Resins	Negative	Negative	Negative
Terpenoids	Positive	Positive	Positive

(+) Opalescence is observed, (++) defined turbidity is observed, (+++) precipitate is observed

3.2 Synthesis of silver nanoparticles

Rosemary leaf extract was combined with silver nitrate ($AgNO_3$) for the formation of metal nanoparticles by green routes, resulting in a dark brown solution with intense spicy odor at a pH 6 and at a temperature of 65 °C, as shown in Figure 2. Out of the prepared concentrations (5:5, 5:10, 5:20, 5:30 and 5:40), the best concentration of Ag-NPs was obtained at day 3, which was when the solutions turned from yellow to dark brown. These prepared Ag-NPs solutions were characterized by UV-VIS spectroscopy and those within the manometric range were those with the ratios of 5:5, 5:10 and 5:20, as shown in Table 3. The ratios of 5:5 and 5:10 showed the best behavior because the nanosilver solution showed better stability in less time, and because its wavelengths were between the ranges of 412-418 nm. The ratio 5:5 showed a wavelength of 418 nm at day 1 and 405 nm at day 5, while in the ratio 5:10 the wavelength was 409 at day 1 and 412 at day 5.

The results obtained agree with those reported by Vera et al. (2017), who used the aqueous rosemary extract technique and the oxonitrate silver agent in their research on the synthesis of metal nanoparticles by green routes (AgNO) for the formation of Ag-NPs, estimating a 30-minute reaction time, resulting in a yellow color and confirming the formation of nanoparticles and their application as an effective antimicrobial agent.

Similarly, Salguero and Pilaquinga (2017) synthesized and characterized silver nanoparticles prepared with aqueous cilantro extract coated with Drago's blood, observing a color change from yellow to orange when 10 ml of $AgNO_3$ (10 nM) at 60 °C was mixed with 2.2 ml of the cilantro extract.

In the same way, Cardeño and Londoño (2014), used the garlic extract (*Allium sativum*) as a reducing agent, and then added an aqueous solution of $AgNO_3$ at a temperature of 50 to 60 °C, a method that allowed observing the reduction of silver ions for 30 minutes by changing the color of the solution

from gold to yellow. The authors performed UV-visible measurements by using the surface plasmon resonance method to demonstrate the presence of the Ag-NPs, finding the best peaks in the prepared solutions between the bands of 400 and 470 nm, these wavelengths are similar to those reported in the present study.

Figure 2. Dark Brown colouring of Ag-NPs

As in the study carried out by Amaguaña (2018), who synthesized silver nanoparticles from the leaves of sensitiva (*Mimosa albida*), the presence of silver nanoparticles was evidenced when the coloring of the solution turned from greenish yellow to brown when the silver nitrate solution was added to it, mentioning that this brown coloring is observed due to the resonance of the superficial plasmons, which is characterized by the formation of nanoparticles.

Bijanzadeh et al. (2012) in their study on the synthesis of silver nanoparticles using chemical methods and characterizing them by UV-VIS spectroscopy observed the plasmon surface for the Ag-NPs absorption bands at wavelengths between 395 and 425 nm. Santorum (2017), in the study on the synthesis of Ag-NPs in (*Piper adumcun*) mentioned that the presence of Ag-NPs is observed during peak absorption at 400nm, and also noted that the size and shape of nanoparticles caused the absorption band to move to larger wavelengths. All these results agree with those reported in this study, where plasma resonance values between 405 and 418 were obtained for the proportions of Ag-NPs synthesized with a 5:5 and 5:10 ratio (Table 3).

Table 3. Characterization of the best proportions of Ag-NPs by UV-VIS spectroscopy

Volume ratio (ml)	Day	Wave lenght (λ)	Absorbance			Absorption factors (L/mol.cm)	Concentration (mg/l)		
5:5*	1	418	2.328	2.326	2.329	16.98	34.21	34.27	34.25
	3	405	2.362	2.363	2.362	14.12	34.72	34.75	34.76
5:10*	1	409	2.374	2.373	2.376	12.32	34.61	34.67	34.65
	3	412	2.312	2.317	2.318	14.54	34.50	34.52	34.52
5:20	1	404	2.368	2.367	2.367	16.38	34.78	34.77	34.77
	3	399	2.416	2.417	2.418	15.03	34.84	34.84	34.83

* Better synthesized ratio

Figure 3 shows that silver nanoparticles on day 1 (pH 10) showed a peak absorbance at a wavelength of 418 nm with a 5:5 ratio, on day 1 (pH 10) a peak of maximum wavelength absorbance higher than 409 nm was observed with a 5:10 ratio. On day 3, nanoparticles showed a peak of maximum wavelength absorbance higher than 405 nm with a 5:5 ratio (pH 10) and a peak of maximum wavelength absorbance higher than 412 nm was presented with a 5:10 ratio (pH 10). All absorbance peaks obtained are typical of Ag-NPs.

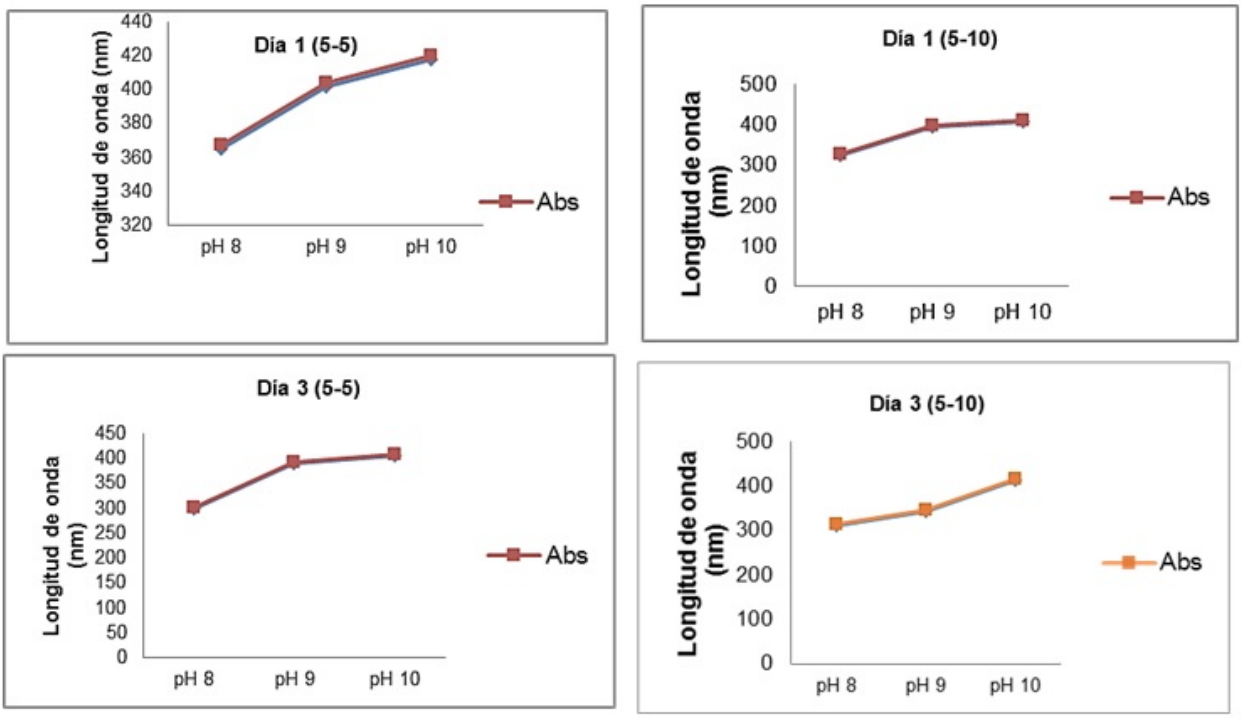

Figure 3. Graphical representation of spectrophotometric analysis during days 1 and 3 in 5-5 and 5-10 ratios

Silver nanoparticles of 5:5 and 5:10 ratios at 3 and 5 days (Figure 4) were observed on the scanning electronic microscope as bright, rounded, white spots. Ag-NPs was more detected after 3 days with a size of 10 nm in a 5:5 ratio and in smaller quantity after 5 days with a size of 10 nm in a 5:10 ratio. As Ávalos et al. (2013) the form of Ag-NPs influences antimicrobial activity. In addition, truncated triangle forms have been found to be more effective than spherical and elongated shapes, which by having more faces tend to be more active against microorganisms.

3.3 Antimicrobial effectiveness of synthesized Ag-NPs

The antimicrobial activity of silver nanoparticles for *E.coli* and *S.aureus*. bacteria was analyzed. As indicated in the methodology, petri dishes were divided into three quadrants before being inoculated with the bacteria mentioned above: one of the quadrants was placed with the pure extract, another with the silver nitrate solution, and the other with the synthesized nanoparticles. The results obtained for *E. coli* were a halo with a 2.88 mm diameter for the extract, a halo with a 1.55 mm diameter for the quadrant whose content was $AgNO_3$, a halo with a 3.21 mm diameter for Ag-NPs. For *S. aureus*, a halo of 2.12 mm, 1.30 mm and 2.18 mm was obtained for the extract, solution of $AgNO_3$ and Ag-NPs, res-

pectively. *E. coli* bacteria presented halos with large diameter compared with *S. aureus bacteria* (Figure 5). Observing a greater antibacterial efficacy against this mmicroorganism

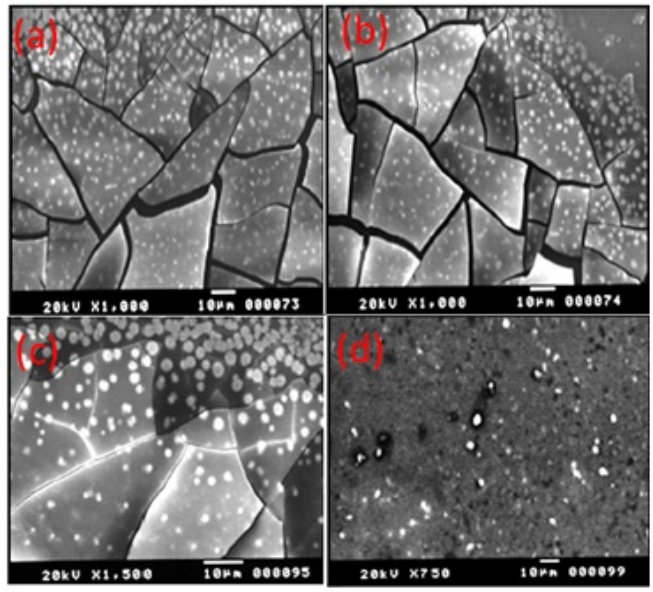

Figure 4. Observation of silver nanoparticles through the scanning electron microscope ratios of 5:5 (3 days (a)), (5 days (b)) and 5:10 (3 days (c)) and (5 days (d))

Monge (2009) said that research carried out to analyze the antimicrobial activity of silver nanoparticles synthesized for *Escherichia coli* and *Staphylococcus aureus* have reported that nanoparticles inhibit more efficiently *E. coli* bacteria. *E. coli* is a Gram-negative bacterium with a cell wall layer consisting of phospholipids and lipopolysaccharides, a cytoplasmic membrane and a thin layer of peptidoglycan, unlike Gram-positive bacteria such as *S. aureus*, which has a thick layer of peptidoglycan and plasma membrane that prevents synthesized nanoparticles from entering easily into the membrane (Villamizar and Monroy, 2015; Cruz-Monterrosa et al., 2017).In addition, Fernández (2017) also mentions that the antibacterial activity of the Ag-NPs is associated with the structural difference in the cell wall of the two bacteria. The above explains why bacterial inhibition was more efficient against *E. coli* compared to *S. aureus*. It is also important to state that bioactive compounds in rosemary leaf extract affect the cell membrane of bacteria, and cytotoxic activity directly affects the mitotic phase of Gram-positive and Gram-negative bacteria. Microorganisms such as *E. coli*, *Listeria monocitogenes*, *Salmonella spp.* and *S. aureus* are sensitive to rosemary extract components, where compounds such as caffeic acid, rosmarinic acid, carnosol, carnosolic acid and flavonoids prevail (Avila-Sosa et al., 2011; Centeno et al., 2010).

Other studies state that silver is oligodynamic because it has the ability to produce a bactericidal effect at low concentrations, as it is reactive to substances such as proteins, enzymes, DNA, RNA, among others (Monge, 2009; Nair and Laurencin, 2007). In addition, Fernández (2017) indicates that the antimicrobial activity of Ag-NPs is due to the action of silver ions that act by interfering the cellular respiration, and once inside the cell they alter their enzyme system by inhibiting their metabolism, causing the microorganism to lose all capacity to grow and reproduce, hence its death. According to Anandalakshmi and Venugobal (2017), the bactericidal action of silver nanoparticles against Gram-negative bacteria occurs when they have a size between 1 and 10 nm. In the current study, the best synthesized Ag-NPs had a size of 10nm as mentioned above.

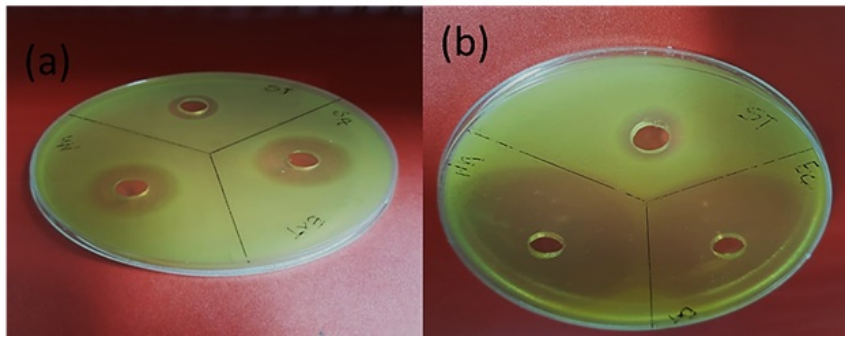

Figure 5. Antimicrobial effectiveness of silver nanoparticles against microorganisms (a) *S. aureus* and (b) *E. coli*

3.4 Aplication of silver nanoparticles in apple

The test carried out to check the effectiveness of synthesized Ag-NPs used as a preservative in red apples with bee wax, since this is normally used as a preservative because it inhibits microbial growth in fruits, showed a positive result. This is because Ag-NPs, like bee wax, delayed fruit ripening, preserving its color and initial weight from day 1 until day 30. As shown in Figure 6, apples that did not contain any preservatives changed their color, lost weight, and showed decomposition after 30 days of testing. Some studies show that nanoparticles could be applied to protect food by incorporating them into their packaging, although this possibility is still being investigated because there is no legislation on the application of Ag-NPs in food. Nanoparticles could be applied to a fruit coating or used for the manufacture of active packaging materials that protect food against pathogens (Ávalos et al., 2013; Aguilar, 2009).

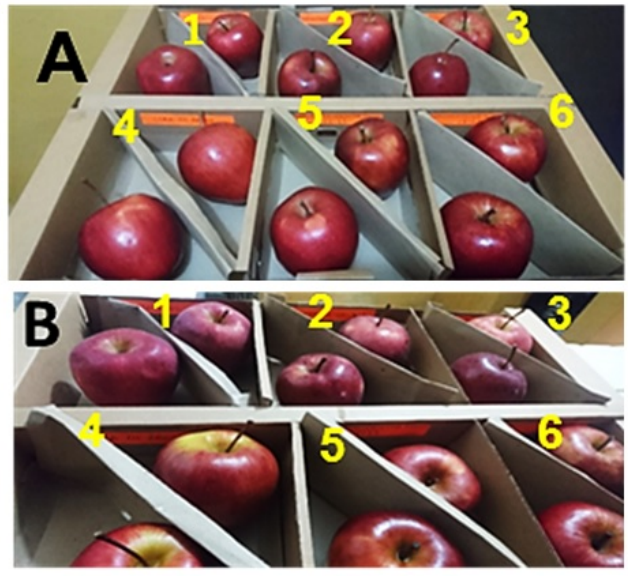

Figure 6. A. Preservative boxes with apples at the beginning of the test and B. Preservative box at 30 days (1 control, 2 Ag-NPs at 5:5, 3 Ag-NPs at 5:10, 4 bee wax, 5 bee wax and Ag-NPs at 5:5 and 6 bee wax and Ag-NPs at 5:10)

An et al. (2008), evaluated the effects of applying Ag-NPs coating on post-harvested asparagus. The coating reduced weight loss, maintained firmness, reduced chlorophyll loss and ascorbic acid formation, slowed color changes and inhibited microbial growth, increasing the post-harvest life of asparagus for 10 days.

Aguilar (2009) synthesized Ag-NPs by chemical reduction to evaluate the antifungal activity *in vitro* and *in situ* against the fungus *Colletrotrichum gloesporioides*, a fungus that causes the anthracnose of papaya. *In vitro* tests showed a fungal effect of 90% inhibition, although papayas coated with the film containing silver nanoparticles had dark sections on the surface, affecting the appearance of the fruit.

In the study conducted by Villamizar and Monroy (2015), synthesized Ag-NPs of *Aspergilus flavus* was used for the preservation of tree tomatoes and golden berry. Fruits were packed in polyethylene bags containing Ag-NPs, and presented reduced growth of yeasts and Gram-positive and Gram-negative bacteria. In addition, Li et al. (2009) combined silver nanoparticles into a packaging containing titanium dioxide and kaolin to preserve chinese dates, demonstrating that the materials containing the packaging maintained longer the quality of the fruits than the fruits packaged in the standard container.

4 Conclusions

Silver nanoparticles were synthesized from the aqueous extract of rosemary leaves, and the qualitative and quantitative characterization of the Ag-NPs obtained by phytochemical analysis showed the presence of secondary metabolites such as phenolic acids, flavonoids, terpenoids, and reducing compounds. The color change of the rosemary leaf extract solution from yellow to dark Brown, and spicy and intense odor evidenced the formation of Ag-NPs. The characterization by UV-VIS spectrophotometry showed that ratios 5:5 and 5:10 were the best because they presented wavelengths between 212-418 nm and increased stability over time. Analysis by scanning electron microscopy showed spherical nanoparticles of 10 nm diameter. Ag-NPs showed greater bacterial inhibition against *E. coli* Gram-negative compared with bacterial inhibition presented against *S. aureus*. Gram-positive. On the other hand, Ag-NPs synthesized from rosemary leaf used as preservative in apples slowed the maturation process, maintained the initial weight, and prevented microbial contamination and fruit decomposition for 30 days.

References

Aguilar, M. (2009). Síntesis y caracterización de nanoparticulas de plata: efectos sobre *Colletotrichum gloesporioides*. Tesis de maestría, Instituto Politécnico Nacional. Online: https://bit.ly/3eN2QF9.

Amaguaña, P. (2018). Síntesis y caracterización de nanopartículas de plata usando como reductor químico el extracto acuoso de las hojas de sensitiva (*Mimosa albida*). Tesis de grado, Pontificia Universidad Católica del Ecuador. Online: https://bit.ly/3awSRCy.

An, J., Zhang, M., Wang, S., and Tang, J. (2008). Physical, chemical and microbiological changes in stored green asparagus spears as affected by coating of silver nanoparticles-PVP. *Lebensmittel Wissenshaft and Technologie*, 41(6):1100–1107. Online: https://bit.ly/3auhXC5.

Anandalakshmi, K. and Venugobal, J. (2017). Green synthesis and characterization of silver nanoparticles using *Vitex negundo* (karu nochchi) leaf extract and its antibacterial activity. *Medicinal Chemistry*, 07(07):218–225. Online: https://bit.ly/3xddb5u.

Ávalos, A., Haza, A., and Morales, P. (2013). Nanopartículas de plata: aplicaciones y riesgos tóxicos para la salud humana y el medio ambiente. *Revista Complutense de Ciencias Veterinarias*, 7(2):1–23. Online: https://bit.ly/3aW88gu.

Avila-Sosa, R., Navarro-Cruz, A. R., Vera-López, O., Dávila-Márquez, R. M., Melgoza-Palma, N., and Meza-Pluma, R. (2011). Romero (*Rosmarinus officinalis* l.): una revisión de sus usos no culinarios. *Ciencia y mar*, 15(43):23–36. Online: https://bit.ly/3h3MGcW.

Bauer, W., Kirby, W., Sherris, J., and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45(4):493–496. Online: https://bit.ly/3veidNr.

Belmares, S., Torres, E., Coparán, J., Arriaga, J., and Elizondo, N. (2015). Síntesis y caracterización de nanopartículas de oro, plata y fierro por el método de fisicoquímica verde. In *Tercer Foro de Divulgación Científica y Tecnológica*, Online: https://bit.ly/338D6NT.

Bijanzadeh, A., Vakili, M., and Khordad, R. (2012). A study of the surface plasmon absorption band for nanoparticles. *International Journal of Physical Sciences*, 7(12):1943–1948. Online: https://bit.ly/3dEGqqg.

Briones, A. (2017). Diseño y funcionalidad en sistemas in vivo denanocápsulas líquidas de aceite de romero (*Rosmarinus officinalis L.*). Tesis de grado, Universidad Veracruzana. Online: https://bit.ly/2RKwFy2.

Cardeño, L. and Londoño, M. (2014). Síntesis verde de nanopartículas de plata mediante el uso de ajo *Allium sativus*. *Revista Soluciones de Postgrado*, 6(12):129–140. Online: https://bit.ly/33cRFzS.

Centeno, S., Calvo, M., Adelantado, C., and Figueroa, S. (2010). Antifungal activity of extracts of *Rosmarinus officinalis* and *Thymus vulgaris* against *Aspergillus flavus* and *A. ochraceus*. *Pakistan Journal of Biological Sciences*, 13(9):452–455. Online: https://bit.ly/3dFe0fH.

Chandran, S., Chaudhary, M., Pasricha, R., Ahmad, A., and Sastry, M. (2006). Synthesis of gold nanotriangles and silver nanoparticles using aloe vera plant extract. *Biotechnology Progress*, 22(2):577–583. Online: https://bit.ly/2QPMIug.

Cruz-Monterrosa, R., Reséndiz, V., Landa, P., Jiménez, J., Díaz, M., Rayasr, A., and García, J. (2017). Nanotecnología en la industria alimentaria. nanopartículas usadas en la conservación de la carne. *Agro Productividad*, 10(10):39–46. Online: https://bit.ly/2QnKKkD.

Fernández, B. (2017). Estudio de las aplicaciones biomédicas de las nanopartículas de plata. Tesis de grado, Universidad de Sevilla. Online: https://bit.ly/3sCKStO.

Flores, C. (2014). *Nanopartículas de plata con potenciales aplicaciones en materiales implantables: síntesis, caracterización fisicoquímica y actividad bactericida*. Tesis doctoral, Universidad Nacional de la Plata. Online: https://bit.ly/2QGvgbV.

Garmendia, G. and Vero, S. (2006). Métodos para la desinfección de frutas y hortalizas. *Horticultura*, 197:18–27. Online: https://bit.ly/3xeBJLs.

Li, H., Li, F., Wang, L., Sheng, J., Xin, Z., Zhao, L., Xiao, H., Zheng, Y., and Hu, Q. (2009). Effect of nano-packing on preservation quality of chinese jujube (*Ziziphus jujuba Mill.* var. *inermis (Bunge) Rehd*). *Food Chemistry*, 114(2):547–552. Online: https://bit.ly/3sHOf2F.

Madrid, A. (2017). Síntesis y caracterización de nanopartículas de plata a partir de varios extractos pigmentados de dos plantas para su aplicación en celdas solares híbridas. Tesis de grado, ESPE. Online: https://bit.ly/3aZwHcl.

Martinez, M., Zuñiga, E., and Sanchez, A. (2013). Método de síntesis de nanopartículas de plata adaptable a laboratorios de docencia relacionado con la nanotecnología. *Mundo Nano. Revista Interdisciplinaria en Nanociencias y Nanotecnología*, 6(10. Online: https://bit.ly/3ncvpiY).

Monge, M. (2009). Nanopartículas de plata: métodos de síntesis en disolución y propiedades bactericidas. *Anales de la Real Sociedad Española de Química*, 105(1):33–41.Online: https://bit.ly/3aZgMdZ.

Nair, L. and Laurencin, C. (2007). Silver nanoparticles: Synthesis and therapeutic applications. *Journal of Biomedical Nanotechnology*, 3(4):301–316. Online: https://bit.ly/3sCZd9C.

Pardo de Santayana, M. (2018). Nanotecnología y alimentación.

Purca, P. (2013). Efectividad antibacteriana "*in vitro*"del extracto de *Rosmarinus officinalis* (romero) sobre flora salival.

Rodríguez, M., Bello, L., Yee, H., and González, R. (2013). Mechanical and barrier properties of film elaborated with rice and banana flour reinforced with nanoparticles: study with response surface. *Revista Mexicana de Ingeniería Química*, 12(1):165–176. Online: https://bit.ly/3aUW8f5.

Salguero, S. and Pilaquinga, F. (2017). Síntesis y caracterización de nanopartículas de plata preparadas con extracto acuoso de cilantro (*Coriandrum sativum*) y recubiertas con látex de sangre de drago (*Croton lechleri*). Tesis de maestría, Pontificia Universidad Católica. Online: https://bit.ly/32EIoAx.

Sánchez, M. (2017). Nanopartículas de plata: Preparación, caracterización y propiedades con aplicación en inocuidad de los alimentos. Tesis de maestría, Universidad Nacional de Educación a distancia. Online: https://bit.ly/3tWdpMx.

Santorum, N. (2017). Síntesis y caracterización de nanopartículas de plata empleando el extracto de las hojas de matico (*Piper adumcun*) como un agente reductor.

Thiruvengadam, M., Rajakumar, G., and Chung, I. (2018). Nanotechnology: current uses and future applications in the food industry. *3 Biotech*, 8(74):1–13. Online: https://bit.ly/33dyV3y.

Torres, I. (2017). Síntesis verde y caracterización de nanopartículas de oro y plata mediante el uso de algunas plantas endémicas del ecuador.

Vázquez, M. and Blandón, L. (2014). Comportamiento antimicrobiano de nanopartículas de plata sintetizadas electroquímicamente. *Cuaderno Activa*, 6(1):99–107. Online: https://bit.ly/3xgdaxN.

Vera, G., Farías, L., and Castañeda, A. (2017). Síntesis de nanopartículas metálicas por rutas verdes. *Journal of Bioprocess and Chemical Technology. Revista Científica de la Universidad Autónoma de Coahuila*, 9(19):15–20. Online: https://bit.ly/2S0ZEhv.

Villamizar, G. and Monroy, P. (2015). Uso de nanopartículas de plata en el control de microorganismos patógenos presentes en alimentos. *@limentech, Ciencia y Tecnología Alimentaria*, 13(1):54–59. Online: https://bit.ly/3nHIXDs.