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RED FRUIT PRESERVATION TECHNOLOGIES BASED ON EUCALYPTUS GLOBULUS RESIDUES

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Abstract. The environmental interest of this proposal stems from the problem of reducing unused waste from eucalyptus plantations. as well as introducing a natural and more energy efficient "eco-friendly" preservative in the food sector. In short, the project aims to convert the residue of eucalyptus leaves into a value-added raw material that can be used as a food preservative. On the one hand, the validation of a preservative product based on eucalyptus essence for its use in the red fruit food industry was proposed. In particular, for the control of the fungus Colletotrichum acutatum at the post-harvest level, in which, unfortunately, the eucalyptus extract did not show a significant effect, giving values of growth speed of the fungus higher than the use of organic acids (lactic and citric). On the other hand, a technological innovation was proposed in the analysis of the performance of the extraction of essence of Eucalyptus globulus from the forests of Cantabria by different methods. The average yield of essential oil extraction was shown to be higher by the steam distillation method.

Keywords: forest residues, eucalyptus, essential oils, extraction, red fruits.

TECNOLOGÍAS DE CONSERVACIÓN DE FRUTOS ROJOS BASADAS EN RESIDUOS DE EUCALYPTUS GLOBULUS

Resumen. El interés ambiental de la presente propuesta parte de la problemática para reducir los residuos no aprovechados de las plantaciones de eucalipto. así como también introducir en el sector de la alimentación un conservante “eco-friendly” natural y más eficiente energéticamente. En definitiva, el proyecto pretende convertir el residuo de las hojas de eucalipto en una materia prima de valor añadido que pueda ser empleado como conservante alimentario. Por un lado, se planteó la validación de un producto conservante basado en esencia de eucalipto para su uso en la industria alimentaria de frutos rojos. En particular, para el control del hongo *Colletotrichum acutatum* a nivel de postcosecha, en el que

lamentablemente el extracto de eucalipto no mostró un efecto significativo dando valores de velocidad de crecimiento del hongo superiores al uso de ácidos orgánicos (láctico y cítrico). Por otro lado, se planteó una innovación tecnológica en el análisis del rendimiento de la extracción de esencia de *Eucalyptus globulus* de los bosques de Cantabria por diferentes métodos. El rendimiento promedio de extracción de aceite esencial demostró ser mayor por el método de destilación por arrastre de vapor.

Palabras clave: residuos forestales, eucalipto, aceites esenciales, extracción, frutos rojos.

Introduction

Eucalyptus is the botanical genus with the greatest diversity of species, all of great environmental value, of which 37 are of interest to the forestry industry and 15 are used for commercial purposes. Among the diversity of species are small shrubs to the tallest trees in the world (Eucalyptus regnans over 100 meters).

Eucalyptus is currently present in more than 90 countries, mostly in tropical and subtropical areas, although there are highly productive plantations in temperate areas of New Zealand, Chile, Argentina, Brazil, Uruguay, South Africa, the Iberian Peninsula, and the United States. It extends over more than 22 million hectares worldwide (0.53% of the world's forest area), although only 13 million hectares are of industrial interest. Plantations with industrial productivity represent 59% of eucalyptus forests. In Spain, eucalyptus plantations represent 3% of the forest area, providing an opportunity for natural and sustainable economic and social development.

As far as eucalyptus is concerned, in Cantabria alone, the felling of these plantations generates a total of 4,000 tons of waste per year. The high cost involved in removing from the forest the branches, stumps, and bark left over from the felling of eucalyptus trees has made it customary to burn these residues in situ, with the consequent risk of causing a forest fire. According to ENCE data, the leaves from which the eucalyptol is extracted are not used in eucalyptus felling: 22% of the cellulose material is used to obtain pulp, while the rest (roots, branches, bark, lignin) is used to obtain energy. But about 2% of the residual material tends to be wasted.

The main objective of this research is to generate knowledge applicable to the food industry for the utilization of *Eucalyptus globulus* residues. The research will evaluate the different alternatives for capturing forest residues generated by the Cantabrian timber industry in the logging of eucalyptus. Then, the industrial process for the extraction of essential oils from the forest residue will be evaluated and optimized. Finally, the product obtained will be characterized and validated for its use as a preservative in the food industry related to red fruits.

The by-product to be obtained from the *Eucalyptus globulus* leaf residues will be a eucalyptus essence to be applied as a preservative.

Methodology and Results

For the collection of forest residues, an exploration of the companies in the region that offer logging, pruning, and forest residue collection services was carried out, and qualitative information was also obtained regarding the possible willingness of these companies to participate in a circular economy initiative in which they would obtain remuneration for the type of residues they manage. Currently, some of these companies obtain profit from the use of these wastes as biomass for combustion, so the interest in

this alternative would be conditioned by the profitability and practicality that each procedure would offer in comparison.

During the temporary window in which the collection of this waste was carried out, it was not possible to formalize the business collaboration, so collection was carried out at privately owned sites, with prior authorization. However, it was established that the companies involved should comply with the following criteria: report continuous activity during the year, work with trees of the *Eucalyptus globulus species at* least twice a year, manage a volume of waste corresponding to forest plantations and not corresponding to residential areas or public sites.

The forest residues were obtained in Parbayón, municipality of Piélagos, with geographical location 43º 21'44"N, 3º54'09"W (Figure 1). This is an area whose main forest crop species is eucalyptus, specifically the *Eucalyptus globulus* species.

Figure 1. Geographical location of Eucalyptus (*Eucalyptus globulus*) residues

The forest residues were obtained "in situ" and then samples of about 5 kg were taken for each lot. A total of 3 batches were evaluated, corresponding to different days of forest residue collection, each of which was subjected to the same technical determinations in order to obtain an average result and statistical estimates of the dispersion of the data obtained.

The dates of the recruitment activity were: 07-28-2019, 08-04-2019, 08-18-2019.

Each batch had to be processed for analysis in the shortest possible time, as the essential oil found in the leaves decomposes rapidly once the leaves are separated from the tree.

The collected residues were subjected to a cleaning treatment to remove impurities that could alter the results of the study. The cleaning was carried out by immersing the eucalyptus leaves in water and making stirring movements to remove impurities (Figure 2), followed by a second washing with distilled water.

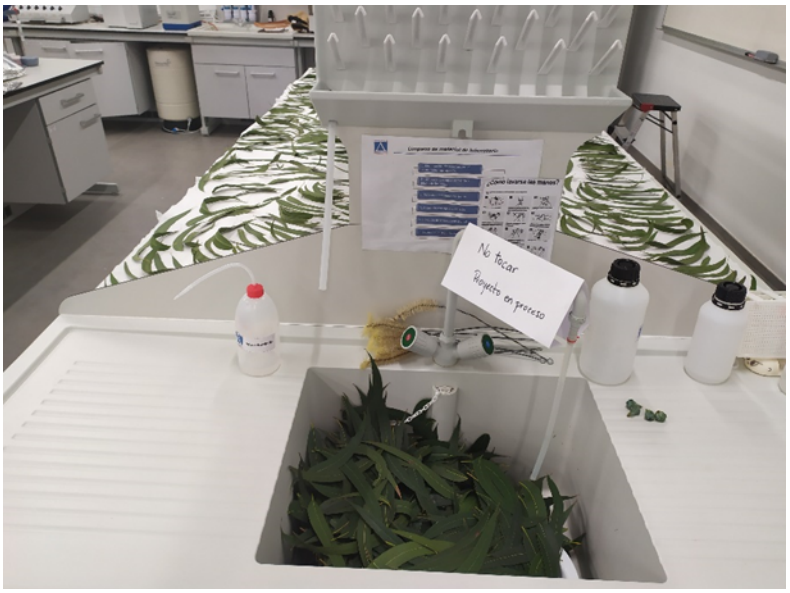

Figure 2. Washing of the leaves with water

The fresh leaves were then drained (Figure 3) and placed on absorbent paper to remove surface water (Figure 4), which corresponded to the initial drying of the leaves prior to the oven drying process.

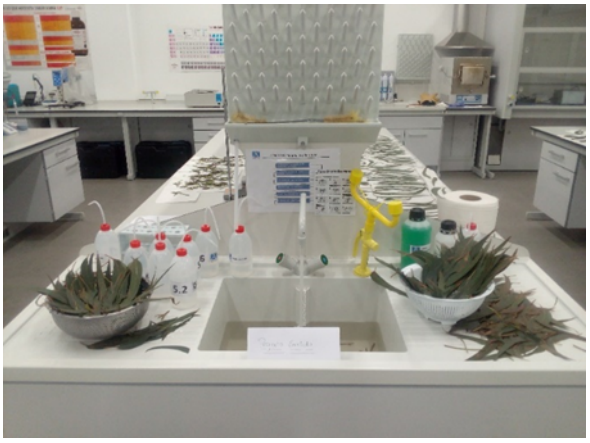

Figure 3. Washing and draining of the leaves

Figure 4. Initial drying of leaves

Drying was carried out following the optimal conditions determined by the work published by Moreno et al. 2010, corresponding to 20 hours at a temperature of 40 ºC. The samples were cut into small pieces of approximately 1 cm^2 (Figure 5) and placed in a Nahita drying oven model 631 (Figure 6).

Figure 5. Sample preparation prior to the drying process

Figure 6. Oven-dried samples subjected to the drying process

The final stage of the sample preparation phase consisted of grinding the leaves (Figure 7). This is a step that is commonly performed in essential oil extraction processes and has the objective of decreasing the particle size, which increases the contact surface between the sample and the extraction water, thus facilitating the transfer of heat and mass, all with the objective of achieving a better yield of the essential oil extraction operation.

Figure 7. Samples before and after the milling process

Essential oils

The biophysical phenomenon of essential oil extraction is described as the rupture of the histological structures of the plant (excretory glands), releasing the oil; and it is dispersed in the extraction fluid to be subsequently separated or isolated, usually by decantation (El Asbahani et al., 2015).

The extraction fluid can be in liquid or vapor form, either organic solvents or water. For food, only water can be used as the extraction solvent because organic solvents are toxic, and there is always a risk of finding traces in the essential oil extract (Kumar et al., 2011).

Hydrodistillation

Hydrodistillation is one of the oldest and simplest methods for the extraction of essential oils from plants (Meyer-Warnod et al., 1984). In this method (*Figure 8*), the plant material is immersed in boiling water, the vapors are condensed, and the liquid obtained is subsequently decanted to separate the essential oil from the aqueous phase of the condensate. The Clevenger apparatus is the one traditionally used for this type of extraction (Rassem et al., 2016).

This traditional technique has significant disadvantages compared to other conventional techniques, such as steam distillation. Among the main disadvantages are the chemical changes (hydrolysis, cyclization, among others) in the terpene molecules due to prolonged direct contact with boiling water and the loss of polar molecules of the essential oil when trapped in the aqueous phase of the boiling water (Peredo-Luna et al., 2009).

Steam distillation

Water vapor distillation (*Figure 9*) is carried out by injecting superheated water vapor directly into the plant sample, causing the breakdown of the plant structure and the release and evaporation of the volatile components. The volatile components are transported together with the water vapor to a condensation stage where a liquid mixture of two phases, aqueous and organic, is obtained, which is separated by decantation to obtain the pure essential oil extract (organic phase).

This method allows the separation of the volatile compounds from the non-volatile ones (selective vaporization) since the latter are not entrained by the steam. This method

also has the advantage that the physical interaction of the superheated water vapor does not produce significant chemical changes in the chemical species of the essential oil.

This technique has the advantage of allowing the use of lower temperatures to extract thermosensitive essential oils. This requires setting up a vacuum generation equipment to perform the extraction process at reduced pressures, and thus the evaporation of volatile compounds will occur at lower temperatures (Peredo-Luna et al., 2009).

Solvent extraction

Solvent extraction is also known as Soxhlet extraction as it is the most commonly used method. In this method, the sample, previously dried and ground, comes into contact with organic solvents (alcohol and chloroform among the most used), which are heated to accelerate the process of breaking down the plant structures and solubilization of the essence in the solvent.

One of the most important disadvantages of this method is that all the solvents that can be used are toxic (except ethanol, but this is not used because of its low yield), and therefore cannot be used to obtain essential oils to be added to food products. Another drawback is that the solvent also solubilizes and extracts other substances (creams, fats) and an impure extract is obtained at the end. It is also costly at the industrial level, and there is a risk of explosion and fire due to the flammable nature of organic solvents.

Thus, to obtain the pure essential oil extract, the extraction product must be filtered, and then temperature and pressure conditions are applied to induce volatilization of the solvent, which must also be recovered due to health and safety restrictions and for reuse (Ortuño, 2006).

Solvent extraction by alternative methods to Soxhlet have the disadvantage of requiring longer periods of time and the greater difficulty of separating the essential oil from the organic phase of the extract.

Evaluation of extraction performance by steam distillation method

The eucalyptus residues, once conditioned as described above, were subjected to the steam distillation process (Figure 8) at laboratory scale. For this purpose, samples of 500 g, previously weighed on a precision balance (Nahita 5062), were used to obtain experimental data on the weight of the material before extraction, which allowed calculating the yield.

The samples were contained in a flat-bottomed flask of 1000 ml capacity, and the flask for steam generation had a capacity of 2000 ml.

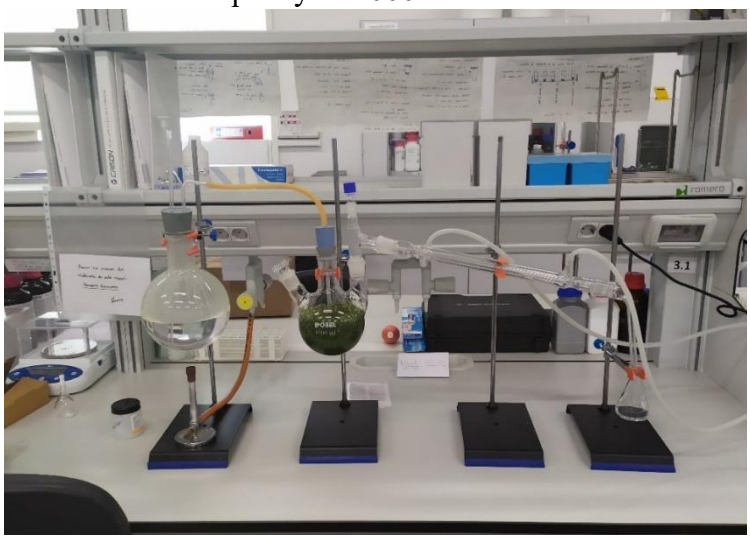

Figure 8. Vapor entrainment extraction method performed in the CITICAN laboratory, July-August 2019.

A conventional protocol for extraction was followed under optimal conditions obtained from previous research (Moreno *et al.,* 2010), which corresponds to 120 minutes of extraction time, with leaf extraction conditions of 42 ºC and 20 hours.

Three extraction cycles were performed for each batch of residues collected (three batches, collected at different times and in the same locality) and for each method to be compared. This extraction stage was carried out in approximately three weeks, not including the sample conditioning activities and the subsequent handling and storage of the extract. The extract obtained presented a light-yellow color, was stored at refrigerated temperature (4 °C), and in the absence of light (Figure 9).

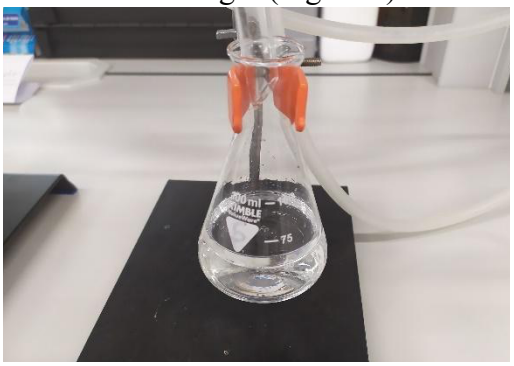

Figure 9. Extract obtained by the steam distillation method

Once the extract was obtained, it was separated from the aqueous fraction by decanting (Figure 10), using a separating funnel (emery 19/26, 100 ml).

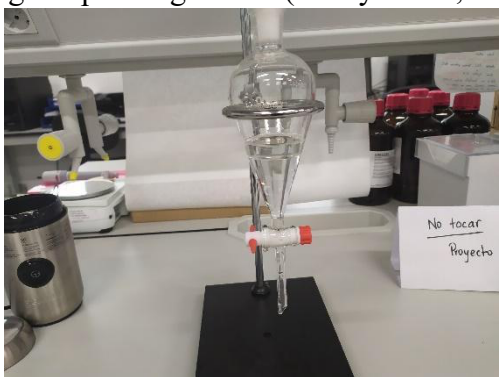

Figure 10. Separation of the extract from the aqueous fraction by decantation.

The essential oil extraction yield (*R*), in weight/weight ratio, was determined by the expression:

$$R\ (\%) = \frac{m_a}{m_e}.100$$

Where m_a is the mass of the extract obtained from the essential oil, and m_e is the mass of the leaves after the drying process, which was weighed before the steam distillation process to obtain the oil.

The average of the values obtained for the yield of this extraction method was 0.84%, with a standard deviation of 0.08% and a coefficient of variation of 9% (Table 1).

A wide range of essential oil extraction yield values using this method is evident in the literature, ranging from 0.35% to 1.30% (Sebei et al., 2015). There are multiple

factors that determine this variability in results, among these: tree location, soil type, tree age, drying method as a pretreatment of leaves, and season of the year (Aziz et al., 2018; Brooker et al., 2006). On the other hand, a coefficient of variation below 10% is usually considered acceptable in this type of research (Rao et al., 2014).

Table 1
Performance results of the steam stripping method

Lot	Yield (%)
1	0,85
2	0,91
3	0,76
Media	0,84
Standard deviation	0,08
CV (%)	9,0

Evaluation of extraction performance by hydrodistillation method

Similar to the previous procedure, the eucalyptus residues were conditioned as previously described before being subjected to the hydrodistillation process (Figure 11). In this case, 500 g samples were also used.

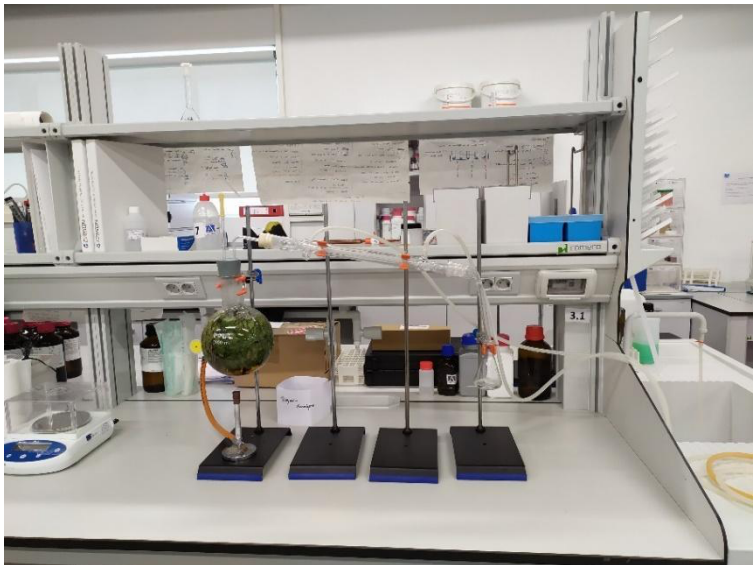

Figure 11. Hydrodistillation extraction method performed in the CITICAN laboratory, July-August 2019

The extraction protocol was followed using the same conditions as the comparison method, which were 120 minutes of extraction time and drying of the leaves at 42 ºC for 20 hours.

Similar to the previous method, three batches of leaves were collected in the same locality and three extraction cycles were performed for each batch. This extraction stage was carried out in approximately three weeks, not including the sample conditioning activities and the subsequent handling and storage of the extract. As in the previous method, the extract obtained was stored at refrigerated temperature (4 °C) and in the absence of light, before being subjected to physicochemical analysis. A slightly darker color was observed than the extract obtained by the previous method.

The extract was obtained by decanting separation of the aqueous fraction and the extract obtained was weighed for each extraction batch and, together with the initial mass data of the leaves, the yield was determined for each batch, according to equation 1.

The yield results by the hydrodistillation extraction method are presented in Table 2. An average of 0.78% was obtained with a standard deviation of 0.09% and a coefficient of variation of 10% (Table 2), the latter is considered acceptable in this type of research (Rao *et al.,* 2014).

Table 2
Performance results of hydrodistillation extraction method

Lot	Yield (%)
1	0,75
2	0,72
3	0,87
Media	0,78
Standard deviation	0,09
CV (%)	10,2

Comparison of essential oil extraction methods

As evidenced in the scientific literature, there is a great variability in the results of extraction yield (0.35% - 1.30%) that are not only due to the differences between the different extraction methods but also due to multiple factors related to the characteristics of the tree or leaves, without finding any correlation between at least one of these variables and the yield (Sebei et al., 2015; Brooker et al., 2006). To minimize the effect of these factors or variables, which can influence performance, the same temperature and time conditions were applied in each method for the leaf drying pretreatment, according to the optimal conditions determined by previous studies (Moreno et al., 2010). Likewise, the sample lots were obtained from the same location.

The characteristic of yield variability according to the location of the eucalyptus forest crop makes this study an original research contribution since, in the literature review, no other research work was found that studied the essential oil extraction yield for eucalyptus forest crops in this region of Spain. Thus, experimental data have been generated that may be useful in the future.

In the literature review, multiple references were found on the steam entrainment distillation extraction method, which is pointed out as the most viable procedure from a commercial exploitation point of view and with greater facilities to be scalable at an industrial level (Hesham et al., 2016; El Asbahani et al., 2015; Meyer-Warnod, 2004).

Although the average percentage yield was higher for the steam distillation extraction method, 0.85% ± 0.09%, with respect to the hydrodistillation method, 0.78% ± 0.10%; there are no statistically significant differences between the two values. On the other hand, hydrodistillation extraction is pointed out in multiple studies as a method that presents disadvantages such as chemical changes in the terpene molecules due to the prolonged direct contact with boiling water as well as the loss of polar molecules of the essential oil by being trapped in the aqueous phase of boiling water (Aziz et al.; 2018, Kumar et al.; 2011).

For the above reasons, it is considered that the steam distillation extraction method is the most appropriate for the purposes of this project. Therefore, it is recommended that

this method be chosen for the next phase of research, which consists of the physicochemical characterization of the extract.

Extraction process proposal

According to the evaluation of the different methods of extraction of the essential oil, the selected method was steam distillation. This proposal has been substantiated with the experimental results and with the knowledge generated in previous studies obtained from the scientific literature (Hesham et al., 2016; Meyer-Warnod, 2004).

On the other hand, the traditional hydrodistillation extraction method may present the disadvantage of producing chemical changes of the essential oil (Aziz et al., 2018); the organic solvent extraction method carries the risk of finding traces of the solvent in the essential oil extract, which is toxic if found in food (Kumar et al., 2014); while alternative methods represent disadvantages for laboratory scale-up to the industrial level, because they involve greater complexity in design and require higher installation and maintenance costs (El Asbahani et al., 2015; Stateva et al., 2011).

Physicochemical characterization of the essential oil

For the characterization of the essential oil obtained by the steam distillation extraction method, the procedures indicated in the Spanish technical standard UNE 84300 for the essential oil of *Eucalyptus globulus* from Spain (Spanish Association for Standardization and Certification [AENOR], 2006) were followed.

For the determination of the relative density of the essential oil at 20 °C, a clean and dry pycnometer (Figure 12) of 3 ml capacity was used, weighed empty on an analytical balance (Nahita 5062), then filled with the essential oil (which was in the bath at 20 °C), the excess sample was covered and cleaned, and the sample was weighed. This procedure was repeated for samples from the three batches of the study. The same procedure was performed with distilled water at 20 °C. The relative density of the essential oil was determined using the following equation.

$$\rho_{20°C} = \frac{(m_{a.e} - m_p)}{(m_a - m_p)}$$

Where ρ is the relative density at 20 °C, $m_{a.e}$ is the weight in grams of the pycnometer with the essential oil sample, m_p is the weight in grams of the pycnometer without the sample, and m_a is the weight in grams of the pycnometer with distilled water.

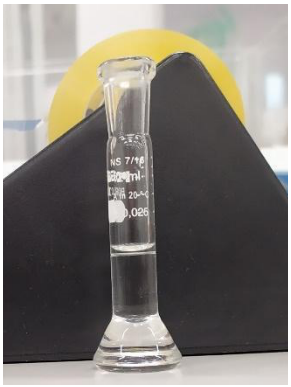

Figure 12. 3mL pycnometer for relative density determination.

This procedure was carried out following the guidelines of the corresponding Spanish standard for essential oils (AENOR, 2000), and values were obtained for each batch of essential oil extracted by steam distillation. The average value of relative density was 0.91 ±0.01, which is a value within the range indicated as acceptable by the Spanish standard, being between 0.905 and 0.925.

Table 3
Relative density obtained for the essential oil samples

Lot	Relative density
1	0,89
2	0,92
3	0,91
Media	0,91
Standard deviation	0,01
CV (%)	1,4

The refractive index is a property defined as the ratio between the velocities of light in two different media, calculated as the quotient of the sines of the angles of incidence (in the first medium) and the angle of refraction (in the second medium). The refractive index is a characteristic property of each essential oil and changes when it is mixed or diluted with other substances, so this is one of the parameters used to evaluate the purity of the essential oil (Ortuño, 2006).

A digital refractometer (Hanna instruments, model HI96801) was used, whose measurements are given in Brix units, and a conversion table corresponding to 20 °C was used.

Table 2 presents the refractive index values obtained for the samples of the different lots. On average, a refractive index value of 1.4601 was obtained, which is within the range of acceptable values, between 1.457 and 1.475, according to the Spanish standard UNE 84300 (AENOR, 2006).

Table 4
Refractive index of essential oil samples

Lot	Refractive Index
1	1.4601
2	1.4583
3	1.4597
Media	1.4594
Standard deviation	0.0009
CV (%)	0.0648

For the calculation of the ethanol index, the respective procedure according to the Spanish standard (AENOR, 2000) is also followed. In this procedure, ethanol-water mixtures of different volumetric concentrations (70 and 96%) are added to a known volume of essential oil (1ml). When a certain volume of the higher concentration alcohol mixture is added, a momentary turbidity is observed, which disappears by shaking. In a progressive way, the alcoholic mixture continues to be added, while the quantity added is measured with a burette.

The results obtained for the ethanol solubility of the essential oil samples showed complete solubility for the mixture with the highest alcohol concentration, 96%, while for the 70% mixture, a value of 7 parts of ethanol mixture was obtained to obtain a clear solution for each part (volume) of the essential oil.

Comparison with UNE 84300:2006 standard

The average value of relative density was 0.91 ±0.01 g/ml, while the standard indicates that values within the range of 0.905 and 0.925 are acceptable. The refractive index value obtained was 1.4594, which is within the range of acceptable values indicated by the standard, between 1.457 and 1.475. The result of solubility in ethanol, 7 volumes or soluble parts of ethanol at 70%, is also within the values indicated in the UNE 84300 standard (AENOR, 2006).

Preservative design

The objective of this phase of the research was to inhibit the growth of *Colletotrichum acutatum*. This fungus is responsible for a disease known as anthracnose and has a high incidence in the post-harvest life of all red fruits, causing great losses of commercial product and, therefore, economic losses for the companies.

This purpose is based on the low availability of authorized phytosanitary products available to producers in post-harvest stages, since this is the stage in which the product is acquired by the consumer for consumption.

To perform the test, the methodology proposed by EUCAST (European Committee on Antimicrobial Susceptibility Testing) was used, applied to a commercial eucalyptus extract and the one obtained in this project.

The principle of this methodology is to impregnate discs of laboratory quality paper in a solution of known concentration of the substance to be tested. This paper is placed on an agarified medium that stimulates the growth of the microorganism. This ensures that the agent that inhibits growth is the one that is inoculated on the disc.

If this substance has an antimicrobial property, it will inhibit the growth of the microorganism near it. On the other hand, if it does not have such a property, the microorganism will grow in that area. Since it is a paper disk, the substance diffuses homogeneously, creating a circumference called an inhibition halo, whose diameter is an estimate of the inhibitory power: the larger the diameter, the greater the inhibitory power (Figure 13).

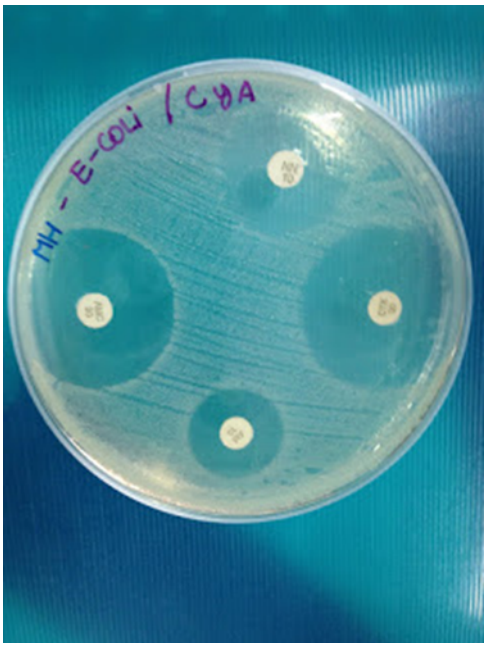

Figure 13. Diagram of inhibition halo

To carry out the tests, the anthracnose strain was acquired from the Spanish Type Culture Collection (CECT) but specifically its asexual or imperfect form, *Glomerella acutata*, which is the one certified by CECT, and which it also has the advantage of being easily reproducible under laboratory conditions.

After receiving the fungus, and having acquired the commercial eucalyptus extract, upon reading the label, it was observed that the product was based on an extract concentrate and also had among its ingredients some substance that could also present an inhibitory effect, so it was decided to compare the extract obtained in this project with other frequent agents in the control of pathogens in postharvest: lactic acid and citric acid (Feliziani et al., 2016; Romanazzi et al., 2009).

Once the assay was adjusted, the fungus was replicated in PDA (Potato-Dextrose-Agar) medium, and the paper discs were impregnated with 0.1-5% concentrations of eucalyptus extract, lactic acid, and citric acid. Figure 14 shows the paper discs, the replicated fungus slice, and the mycelium.

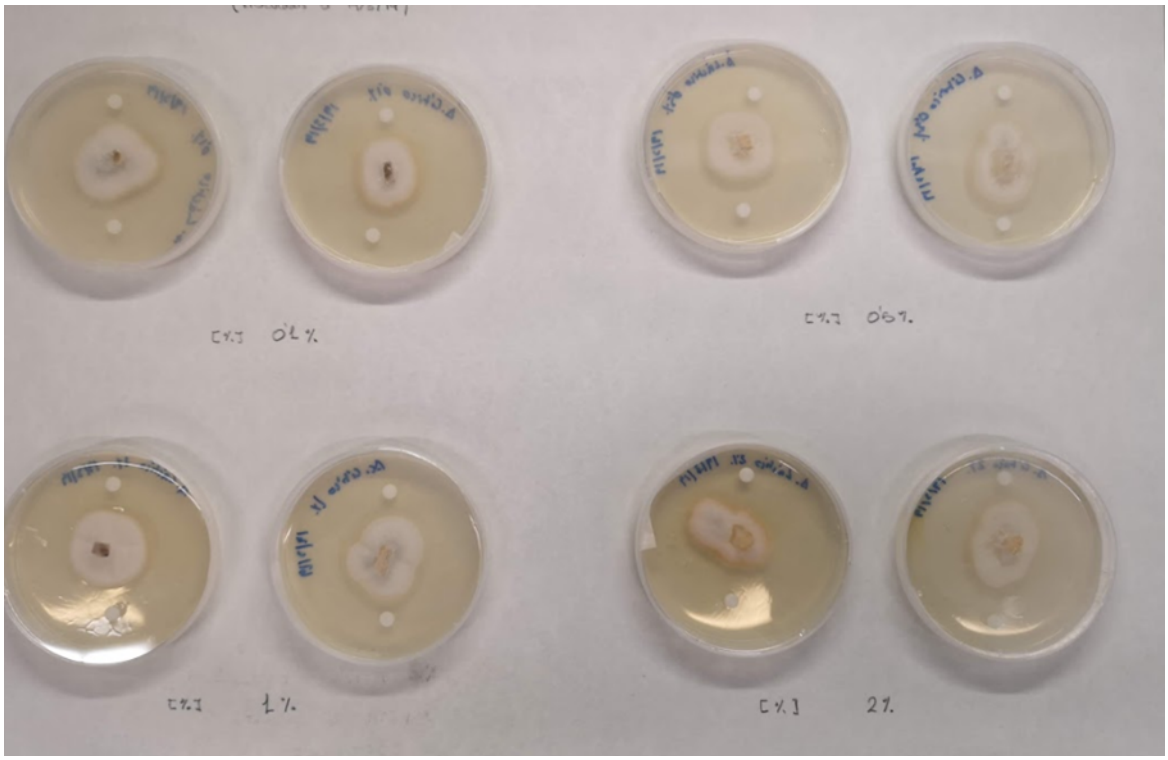

Figure 14. Piece of replicated fungus and mycelium.

The growth was monitored daily. As can be seen in the previous figure, the growth shape of the mycelium varied between circular and elliptical. In the first case, the diameter was measured and in the second case the largest diameter. It was observed that in a period of 12-15 days the Petri dish was completely covered by the mycelium of the fungus. It was also observed that the discs did not inhibit the development of the fungus, the mycelium growing on top of the discs.

This result forced to rethink the assay looking for an alternative methodology. It was decided to dilute the eucalyptus extract, citric acid, and lactic acid directly in the PDA medium at the estimated concentrations (0.1-5%) and replicate directly on the fungus. In this case the results were positive, clearly observing how the growth rate of the fungus was modified (Figure 15). The assay was maintained until the mycelium occupied the entire plate.

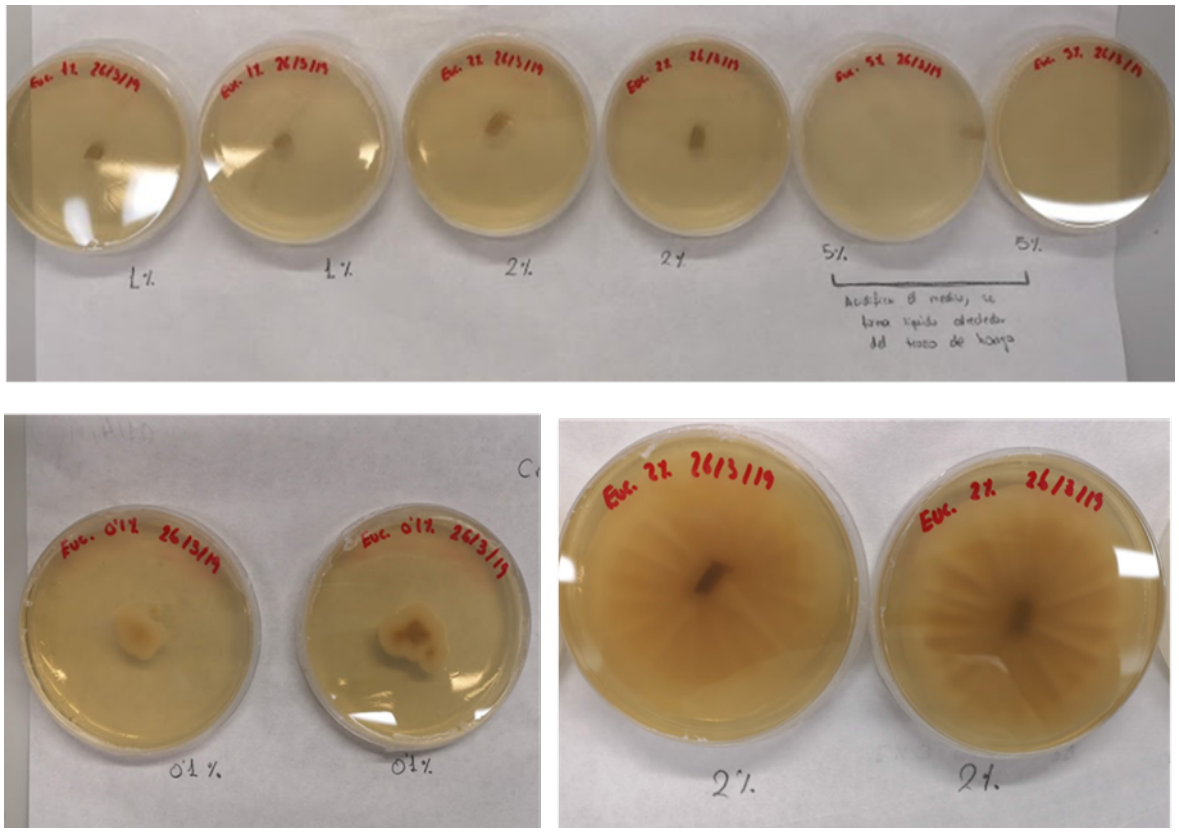

Figure 15. Growth rate of the fungus

The concentrations at which the three products were applied were: 0.1%, 0.5%, 1%, 2%, and 5%. In the case of citric and lactic acids, from a concentration of 2%, the fungus was inhibited, and specifically, from 1% the growth rate of lactic acid was much lower than that of citric acid. In the case of eucalyptus extract, the fungus continued to show intense growth activity at 2% and above.

The following figures show the graphs representing the growth rate of *G. acutata*. Each line is defined by an ascending section whose slope is the growth rate, expressed in mm/day, and a plateau or horizontal zone that corresponds to the time when the Petri dish was fully occupied.

In the case of the 0.1% concentration, the fungus grew more slowly with the eucalyptus extract than with the two acids. With the extract, it grew at an average rate of 4.7 mm/day, while for citric and lactic acids it was 6.7 mm/day and 7.1 mm/day, respectively. Despite having a lower rate, from the ninth day onwards, it filled the entire plate. This may be due to the fact that on the fifth and eighth day the fungus had a higher development activity that accelerated the process. However, no documentary references have been found to explain this phenomenon.

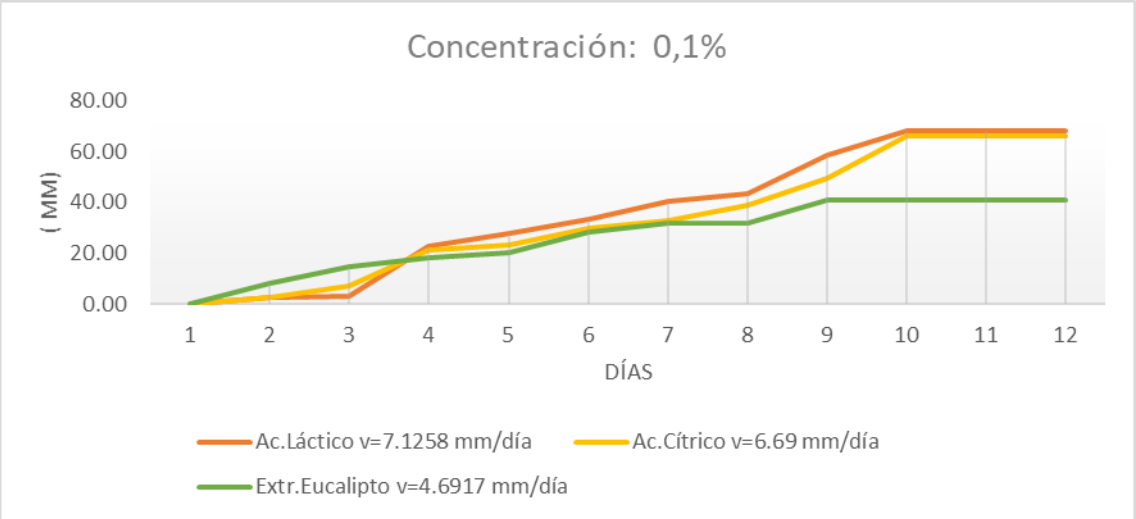

Figure 16. Growth rate of *G. acutata* for a concentration of 0.1% of the three control agents tested.

Discussion and conclusions

Although the average essential oil extraction yield was higher for the steam distillation method compared to the hydrodistillation method, there are no statistically significant differences between the two values. However, hydrodistillation extraction is pointed out in multiple studies as a method that presents disadvantages such as chemical changes in the terpene molecules due to the prolonged direct contact with boiling water, as well as the loss of polar molecules of the essential oil by being trapped in the aqueous phase of boiling water (Aziz et al., 2018; Kumar et al., 2011). Therefore, the steam distillation extraction method is considered to be the most suitable for the purposes of the present project.

Unfortunately, the eucalyptus extract showed no effect for the control of the fungus *Colletotrichum acutatum* at the postharvest level, giving higher values of fungal growth rate than the use of organic acids (lactic and citric).

Comparing the effect on color and composition in compounds with antioxidant activity and pH of the eucalyptus extract, significant differences were only observed in the application of eucalyptus extract in the case of pH and phenolic compounds, being in this case higher than the values found for the treatments with the two acids.

Sensorially, the extract-treated blueberries had the worst rating.

These results, a priori negative, invite further investigation on future occasions focused on:

- Evaluating the effect on different stages of development of *Colletotrichum acutatum.*
- Evaluating the control effect of the extract on bacteria affecting the crop such as the Rust (*Pucciniastrum vaccinii*).
- Evaluating the control effect of the extract on other fungi of interest for blueberry: *Aspergillus, Fusarium, Penicillium*, etc.
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References

Aris, A. & Morad, A. (2014). Effect of extraction time on degradation of bioactive compounds. *Journal Teknologi 67*(4), 63–66.

Asociación Española de Normalización y Certificación. 2006. Aceite esencial, crudo o rectificado, de *Eucalyptus globulus* de España (UNE 84300).

Asociación Española de Normalización y Certificación. 2002. Aceites esenciales. Determinación de la miscibilidad en etanol (UNE 84153).

Asociación Española de Normalización y Certificación. 2000. Aceites esenciales. Determinación de la densidad relativa a 20 °C. Método de referencia. (UNE 84156).

Asociación Española de Normalización y Certificación. 2000. Aceites esenciales. Determinación del índice de acidez. (UNE 84157).

Asociación Española de Normalización y Certificación. 2000. Aceites esenciales. Determinación del índice de refracción de los aceites esenciales. (UNE 84158).

Aziz, Z., Ahmad, A., Setapar, S., Karacucuk, A., Ganash, M., Kamal, M., & Ashraf, G. (2018). Essential oils: extraction techniques, pharmaceutical and therapeutic potential – A review. *Current Drug Metabolism, 19*, 11-20.

Bachir R. & Benali, M. (2012). Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against Eschericia coli and Staphylococcus aureus. *Asian Pacific Journal of Tropical Biomedicine 2*(9), 739-742.

Boom, E., Orozco, J., Alean, J., & Rojano, B. (2018). evaluación de la actividad antioxidante de aceites esenciales de eucaliptos cultivados en Colombia. *Información Tecnológica 29*(6), 57-66.

Brooker, M. & Kleinig, D. (2006). *Field Guide to Eucalyptus. South-Eastern Australia, Bloomings.* Melbourne.

Denev, P., Kratchanova, M., Ciz, M., Lojek, A., Vasicek, O., Nedelcheva, P., Blazheva, D., Toshkova, R., Gardeva, E., Yossifova, L., Hyrsl, P., & Vojtek, L. (2014). Biological activities of selected polyphenol-rich fruits related to immunity and gastrointestinal health. *Food Chem, 157*, 37–44.

El Asbahani, A., Miladi K., Badri, W., Sala, M., Aït Addi, E., & Casabianca, H., El Mousadik, A., Hartmann, D., Jilale, A., Renaud, F., & Elaissari, A. (2015) Essential oils: from extraction to encapsulation. International. *Journal of Pharmaceutics, 483*(12), 220-243.

EUCAST (European Committee on Antimicrobial Susceptibility Testing). http://www.eucast.org/organization/

Feliziani, E., Lichter, A., Smilanick, J., & Ippolito, A. (2016). Disinfecting agents for controling fruits and vegetables diseases after harvest. *Postharvest Biology and Technology, 122*, 53-69.

Hesham, R., Abdurahman, N., & Rosli, M. (2016). Techniques for extraction of essential oils from plants: A review. *Australian Journal of Basic and Applied Sciences 10*(16), 117-127.

Kang, H.-C., Park, Y.-H., & Go, S.J. (2003). Growth inhibition of a phytopathogenic fungus: colletotrichum species by acetic acid. *Microbiol. Res, 158*, 321–326.

Kumar, R. & Tripathi, Y. (2011). *Extraction technology of natural medicinal plants*. Forest Research Institute. Dehradun.

Li, D., Li, B., Ma, Y., Sun, X., Lin, Y., & Meng, X. (2017). Polyphenols, anthocyanins, and flavonoids contents and the antioxidant capacity of various cultivars of highbush and half-high blueberries. *Journal of Food Composition and Analysis, 62*, 84–93.

Marcel, S. (2018). Use of essential oils as new food preservatives (Case: Eucalyptus grandis and Eucalyptus crebra). *Journal of Plant Science and Phytopathology*, *2*, 083-090.

Meyer-Warnod, B. (1984). Natural essential oils: extraction processes and application to some major oils. *Perfume Flavorist, 9*, 93-104.

Moreno, J., López, G., & Siche, R. (2010). Modelación y optimización del proceso de extracción de aceite esencial de eucalipto (*Eucalyptus globulus*). *Scientia Agropecuaria 1*, 147–154.

Ochoa, K., Paredes, L., Bejaranao, D., & Silva, R. (2012). Extracción, caracterización y evaluación de la actividad antibacteriana del aceite esencial de Senecio graveolens. *Scientia Agropecuaria, 3*, 291-302.

Ortuño, M. F. (20069. *Manual Práctico de Aceites Esenciales.* Aiyana.

Rao, R., Syamasundar K., & Patel, R. (2014). Effect of method of distillation on the yield and chemical composition of Artemisa annua essential oil. *Journal of Essential Oil Research 26*(6), 486-491.

Romanazzi, G., Mlikota Gabler, F., Margosan, D., Mackey, B.E., & Smilanick, J.L. (2009). Effect of chitosan dissolved in different acids on its ability to control postharvest gray mold of table grape. *Phytopathology, 99*, 1028–1036.

Sebei, K., Sakoui, F., Larbi, M., & Boukhchina S. (2015). Chemical composition and antibacterial activities of seven eucalyptus species essential oils leaves. *Journal of Biological Research 48*(1), 70-83.

Stateva, R.P. & Sovová, H. (2011). Supercritical fluid extraction from vegetable materials. *Reviews in Chemical Engineering 27*, 79–156.

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