# Antimicrobial properties of PLA membranes loaded with pink pepper (Schinus terebinthifolius Raddi) essential oil applied in simulated cream cheese packaging

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# Abstract

Ultrafine fiber membranes of polylactic acid (PLA) 8% (w/v) loaded with pink pepper essential oil (PPEO) in 10, 20 and 30% (v/v) were produced and evaluated for antimicrobial potential against the bacteria *Escherichia coli*, *Salmonella enteritidis*, *Listeria monocytogenes* and *Staphylococcus aureus*. The membranes were applied in simulated cream cheese packaging and characterized by morphological, thermal, structural, antimicrobial and wettability analysis. The addition of PPEO reduced the diameter of fibers and increased the initial degradation temperature in relation to pure PPEO. The ultrafine membranes had hydrophobic character. The PPEO presented myrcene as major component and had antimicrobial action for *S. aureus* and *L. monocytogenes*. The membranes applied to the cream cheese packaging showed inhibitory effect on the 21st day of storage, for *L. monocytogenes*. For *S. aureus*, the membranes inhibited the growth of the colonies on days 14 and 21, with reductions of 30 and 62%, respectively.


# Highlights

- Protective thermal effect of PLA on PPEO.
- Membranes of ultrafine fibers showed hydrophobicity.
- Potential action of PPEO volatile compounds in reducing bacteria.
- Antimicrobial effect of membranes in simulated cream cheese packaging.

# 1. Introduction

Microbial contamination of food can occur during different stages of the food chain, from primary production to consumption, both by deteriorating and pathogenic microorganisms. This fact raises concerns about foodborne diseases and also influences the sensory aspects of food, such as visual appearance, taste and odor. Because of this, the field of food science and technology has been betting on antimicrobial packaging systems to minimize food waste and consequently increase consumer safety (Thakali & Macrae, 2021). The presence and development of these microorganisms in food, such as *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Aspergillus niger*, *Penicillium sp*, among others, depends on intrinsic factors, such as pH and water activity, and extrinsic factors, such as temperature, relative humidity and presence of gases (López-Pedemonte, Roig-Sagués, De Lamo, Hernández-Herrero, & Guamis, 2007).

Of the many synthetic and natural antimicrobial agents that can be used in food packaging, essential oils, which are extracted from the bark, seeds, flowers, fruits, roots and leaves of plants, are gaining increasing importance, mainly because they contain antioxidant and antimicrobials that help preserve the product (Pandey, Kumar, Singh, Tripathi, & Bajpai, 2017). The composition of essential oils is multiple and consists mostly of terpenes or their derivatives. As for obtaining, the oils can be acquired through extraction techniques, such as hydrodistillation, hydrodiffusion and using solvents (Aziz et al., 2018). Hydrodistillation is the simplest process, where the materials are immersed in water inside a container

where the mixture is heated, with the main advantage of extracting plant material below 100 ºC (El Asbahani et al., 2015). Also, to apply essential oils in packaging, one must pay attention to some prerequisites, as well as knowing the properties of the oils, the minimum inhibitory concentrations, the target microorganisms, the mechanisms of action and possible interactions with the matrix to feed (Hyldgaard, Mygind, & Meyer, 2012).

*Schinus terebinthifolius* Raddi (Anacardiaceae) is a species native to Brazil, known as *aroeira-vermelha*, widely distributed in the southern region (Souza, Arthur, & Nogueira, 2012). Literature reports confirmed antimicrobial action (Gomes, Procópio, Napoleão, Coelho, & Paiva, 2013; Uliana et al., 2016; Dannenberg, Funck, Mattei, Silva, & Fiorentini, 2016; Dannenberg et al., 2017; Romani, Hernández, & Martins, 2018), as well as anti-inflammatory (Medeiros et al., 2007), antifungal (Khan, 2013), anti-tumor (Queires, Crépin, Vachero, De La Taille, & Rodrigues, 2013) and insecticide (Santos et al., 2009) of the essential oil extracted from its fruit, called pink pepper. Thus, there is great potential for the use of pink pepper essential oil (PPEO), as it is a fruit available in large quantities in the region where this study was developed. However, there is still no research on the use of PPEO in the development of ultrafine fibers via electrospinning for subsequent application in food packaging.

The application of essential oils in active packaging can be used in the form of films, coatings and sachets. However, Carpena, Nuñez-Estevez, Soria-Lopez, Garcia-Oliveira, and Prieto (2021) reported that it must also be considered that they are chemically unstable and easily oxidized, being sensitive to light, oxygen and changes in temperature. In this sense, nanoencapsulation techniques can be used to improve the stability of essential oils and increase their physical-chemical capacities, thus allowing their use in food packaging (Rehman et al., 2020). Among these techniques, there is the use of electrospinning, as it has the advantage of not using high temperatures during its processing (Bhushani & Anandharamakrishnan, 2014). Furthermore, in the area of packaging development, there is a growing concern with the environment, which has stimulated research on biodegradable, renewable and compatible polymers with other synthetic polymers, such as poly (lactic acid) (PLA) (Moreira, Terra, Costa, & De Morais, 2018). PLA is widely used in packaging and has also been cited as a raw material in the production of nanofiber membranes with biomedical potential (Valente et al., 2016), tissue engineering (Xu, Shen, Yan, & Gao, 2017), controlled drug release (Preis et al., 2020) and food packaging (Altan et al., 2018).

Therefore, the objectives of this work were to develop ultrafine PLA fibers loaded with different concentrations of PPEO using the electrospinning technique, and to evaluate their morphological, thermal, structural, antimicrobial and wettability characteristics. In order to evaluate the action of the ultrafine fiber membranes produced with PPEO as a preservative in food, they were applied in cream cheese packaging, replacing the aluminum seal present in the original packaging with an aluminum seal containing the ultrafine fiber membrane. Choi, Lee, Lee, Kim, & Yoon (2016) reported that depending on the origin of the raw material and processing, there is a frequent incidence of pathogenic bacteria in this product, such as *L. monocytogenes*, *S. aureus* and *E. coli.* Because of this, its use for preliminary evaluations in the field of dairy foods is justified.

# 2. Material And Methods

## 2.1 Materials

The PLA used contained the characteristic of high molecular weight and was in the form of pellets (PLA IngeoTM, 4032D). Pink pepper fruits were collected from trees present within the campus of the Federal University of Pelotas (UFPel), city of Capão do Leão-RS, Brazil, with location coordinates 31°48'0459" latitude and 52°24'5532" longitude. The botanical identification was performed by means of comparison, considering the similarities with the specimen 25.131 from the herbarium of the Department of Botany at UFPel, being recognized as *Schinus terebinthifolius* Raddi. For the study of the application of the developed ultrafine fibers membranes, packages of cream cheese (Temper Cheese) (150 g) from different batches of only one brand were purchased in the local market in the city of Bagé-RS-Brazil.

## 2.2 Extraction of PPEO

Essential oil was extracted by hydrodistillation using a Clevenger apparatus as described by Dannenberg et al. (2016), with some modifications. The fruits were collected, cleaned with water, and frozen to -20°C (Consul CVU30, Brazil). Approximately 200 g of the fruits were triturated in a blender (Oster Classic 4126, Brazil) with 1 L of distilled water and translocated to a 2 L flask, which was coupled to the Clevenger apparatus and heated for 2 h using a heating mantle. Subsequently it was dehydrated by filtration with anhydrous sodium sulfate ($Na_2SO_4$ - Synth®). PPEO was stored in an ultrafreezer (CL200-86V, Brazil) at -80 ± 2°C in a parafilm-sealed amber glass vial until analysis.

## 2.3 Composition of PPEO

The composition of PPEO was analyzed by gas chromatography coupled to mass spectrometry (GC-MS) (Shimadzu QP2010 Ultra, Japan), following a method described by Juliani et al. (2008), with some adaptations. A capillary column model RTx5-MS (60 m x 0.25 mm, 0.25 µm) (Restek, USA) was used and the parameters defined for GC-MS were as follows: injector temperature, 250°C; column temperature, 60°C; helium gas flow rate, 1.08 mL/min; GC oven, 60°C for 3 min followed by a ramp of 3°C/min to 280°C, maintained at this temperature for 10 min; temperature of the MS ion source, 280°C; interface temperature, 300°C; linear velocity of 35.9 cm/s. The PPEO components were identified using the AOC 20-i mass spectra library from the National Institute of Standards and Technology (NIST).

## 2.4 Preparation of polymeric solutions

Previous tests with different contents of PLA and essential oil indicated that only concentrations between 10 and 30% of PPEO were viable for fiber formation. The polymeric solutions were prepared using 8% PLA, [8 g PLA dissolved in 100 mL of chloroform:acetone mixture (3:1)] and subjected to stirring (Fisatom, model 752/6, Brazil) for 3 h, until the total dissolution of the pellets, as defined by means of preliminary tests. Afterwards, concentrations of 10, 20 or 30% (v/v, on a dry basis) of PPEO were added and a further stirring was carried out for another 3 h. The PLA only solution was used as a control.

## 2.5 Electrospinning process

The production of ultrafine fibers was performed by electrospinning technique and the optimal conditions for the formation of ultrafine fibers were obtained through preliminary tests, based on results found in a study by Fontes et al. (2021). The polymeric solutions were poured in a 3 mL syringe with a metal needle of 0.7 mm diameter, being deposited in a aluminum foil coupled to the metal collector.

During the electrospinning process, the flow rate was controlled at 0.5 mL/h by an infusion pump (KD Scientific, Model 100, Holliston, England); the voltage used was 20 kV on the positive electrode and 1 kV on the negative electrode, being monitored by a power supply (INSTOR, INSES-HV30, Brazil) and the horizontal distance between the tip of the syringe needle and the collector was set at 30 cm. Ambient temperature was maintained at 23 ± 2°C by air conditioning and humidity was regulated to 45 ± 2% with a dehumidifier.

## 2.6 Apparent viscosity and conductivity of the polymeric solutions

The apparent viscosity of polymer solutions was analyzed using a digital viscometer (Model DV – II, USA) and the electrical conductivity assessed by a conductivity meter (MSTECNOPON, model mCA 150P, Brazil), as reported by Fontes et al. (2021).

## 2.7 Morphology and size distribution of the ultrafine fiber membranes

The ultrafine fibers morphology was analyzed by a scanning electron microscope (SEM) (Jeol JSM-6610 LV, Japan). The samples were sputter coated with gold and analyzed using a voltage acceleration of 10 kV, according to Fonseca et al. (2020). Afterwards, the size distribution and diameter of the nanofibers was determined by calculating the average of 50 measurements obtained from different areas of the images.

## 2.8 Structural characterization of the ultrafine fiber membranes

The functional chemical groups present in the ultrafine fibers were evaluated using a Fourier transform infrared (FTIR) spectrometer (IR Prestige-21, Shimadzu, Japan) equipped with an attenuated total reflection (ATR) accessory, according to Silva et al. (2018). The spectra were recorded between 4000 and 500 $cm^{-1}$ with a 4 $cm^{-1}$ spectral resolution, at room temperature (25 ± 2°C).

## 2.9 Thermal stability of the ultrafine fiber membranes

The thermal stability of the ultrafine fibers and its constituents (PLA and PPEO) was determined by a thermogravimetric analyzer (TGA, TA-60WS, Shimadzu, Japan), according to the method described by Bruni et al. (2020). The samples, about 5 mg, were heated in platinum capsules with heating rate of 10°C/min in a range of 30–600°C and nitrogen flow of 50 mL/min. An empty platinum capsule was used as the reference.

# 2.10 Wettability of the ultrafine fiber membranes

The measurement of surface wettability was performed according to that described by Fombuena, Balart, Boronat, Sánchez-Nácher, & Garcia-Sanoguera (2013), using a drop of water into the surface of the ultrafine fibers membrane from PLA/PPEO and the image was obtained by microscope (Digital Blue, QX5, USA). The Surftens 3.0 software was used to evaluate five measurements of each image using five different points arranged around the water drop.

### 2.11 Antimicrobial activity

The antimicrobial activity of PPEO and ultrafine fibers membranes were assessed against four bacteria relevant to food. The gram-positive bacteria tested were *Listeria monocytogenes* ATCC 7644 and *Staphylococcus aureus* ATCC 12598. The gram negatives were *Salmonella enteritidis* ATCC 13076 and *Escherichia coli* ATCC 11230.

### 2.11.1 Disk diffusion of PPEO and membrane of the ultrafine fiber

The efficacy of PPEO against microorganisms was assessed using the disk-diffusion technique (CLSI, 2015a). The bacterial cultures were diluted in peptone water (0.1%) producing a concentration of $10^4$ CFU/mL, from the McFarland scale. This inoculum was spread with sterile swabs on the surface of the Petri dishes containing Mueller-Hinton Agar. Sterile paper discs were placed on the plate and 10 µL of PPEO was added to each. Then, the Petri dishes were incubated at 37 ° C. After 24 hours, the presence or absence of inhibition halos was verified with a digital pachymeter. To evaluate the efficiency of ultrafine fiber membranes the same procedure was used, just replacing the filter paper discs with circular samples of the fibers (2.4 ± 0.1 cm in diameter, ≈ 3 mg), which were sterilized under ultraviolet light for 15 min on each side.

### 2.11.2 Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of PPEO was evaluated by the broth microdilution technique (CLSI, 2015b). The PPEO was diluted in Brain Heart Infusion broth (BHI) supplemented with 3% tween 80. The bacteria were inoculated to reach initial concentrations of $10^4$ CFU/mL in each well. The microtiter plates were incubated at 37°C for 24 hours, and the readings were performed with a plate reader (Robonik® Readwel plate) at a wavelength of 625 nm, considering the MIC as the largest dilution where there was no visible cell growth (Ojeda-Sana, Van-Baren, Elechosa, Juárez, & Moreno, 2013).

### 2.11.3 Minimum bactericidal concentration (MBC)

To detect the minimum bactericidal concentration (MBC), 10 µL aliquots from each well where there was no visible growth with the naked eye in the MIC test were inoculated into plates containing BHI medium. The concentration where there was no growth in this new medium was considered the MBC.

### 2.11.4 Antimicrobial activity in micro-atmosphere

The antimicrobial activity in micro-atmosphere was performed based on the technique described by Ghabraie, Vu, Tata, Salmieri, & Lacroix (2016). An aliquot of 0.1 mL of bacterial cell suspension (McFarland scale $10^8$ CFU/mL adjusted to $10^4$ CFU/mL) was spread on the surface of Petri plates containing BHI agar (15 ml – 6 mm layer). Three sterile filter paper discs were placed on the lid of each plate, to which 15 µL of pure PPEO or 15 µL of oil were added at a concentration of 30%. The control contained sterile filter paper discs impregnated with sterile distilled water. The plates were sealed with parafilm, inverted and incubated at 37º C for 24 h. The antimicrobial action was expressed as a percentage of CFU reduction after treatment with PPEO, in relation to the control.

### 2.11.5 Application of the ultrafine fiber membranes in simulated cream cheese packaging

Due to the promising results found in the antimicrobial analysis by micro-atmosphere, tests were carried out for the microbiological evaluation of the effect of PPEO volatilization on cream cheese containing *L. monocytogenes* or *S. aureus*, by the direct plating method (APHA, 2001). From the cream cheese packaging purchased from the local market, 30 g of sample were removed from each one, being transferred to test tubes with thread, previously sterilized. Afterwards, $10^2$ CFU/g of the bacteria were inoculated separately.

In the control, no inoculation occurred and the seal used was only aluminum foil, with the lid closing later. In the positive control, inoculation occurred and the seal was also only aluminum foil. In the experiments, inoculation occurred and the seal used was aluminum foil containing the ultrafine fibers from the PLA/PPEO 30% treatment. All tubes were subjected to refrigeration (5 ± 1 ºC). The process was monitored over time, with an aliquot of 5 g being removed one day after inoculation, and after 7, 14 and 21 days. At each point removed, the sample was inserted into sterile homogenization bags and diluted with peptone water, being submitted to the sample homogenizer (MA440, Marconi).

Then, the method of direct plating on the surface of the serial dilutions ($10^{-1}$ to $10^{-3}$) previously prepared was used. 0.1 ml of each dilution was inoculated on the surface of the solidified medium in the Petri dishes. For *L. monocytogenes* the medium used was Oxford modified (MOX) and for *S. aureus*, the supplemented Baird Parker medium. Then, with the aid of a drigalsky handle, the inoculum was carefully spread over its entire surface, until there was complete absorption. The plates were incubated inverted in an oven at 35 ± 2 ºC for 26 ± 2 h, and the result expressed by the number of colony forming units per gram of sample.

### 2.12 Statistical analysis

Analytical determinations were performed in triplicate, except for the TGA and FTIR analysis. The results were evaluated by analysis of variance (ANOVA) and the averages were compared by Tukey's test at 5% significance level.

# 3. Results And Discussion

### 3.1 Composition of PPEO

The extraction yield of PPEO was 6.7% on a wet basis. The PPEO presented 25 constituents, being myrcene (28.2%) the major compound, followed by α-pinene (20.2%), germacrene D (15.3%), and limonene (10.5%) (Table 1). Dannenberg et al. (2019) detected predominance of β-myrcene (41.0%), followed by β-cubebene (12.2%) and limonene (8.9%). Santos et al. (2009) also evaluated PPEO and obtained 20.4% of myrcene, 17% of limonene, and 10.8% of germacrene D. In contrast, Ennigrou, Hosni, Casabianca, Vulliet, & Smiti (2011) found predominance of germacrene (27.1%), α-felandrene (22.1%), and β-cubebene (10.0%). Such variations in data of studies regarding the composition and quantity of PPEO phenolic compounds are expected owing to differences in soil, rainfall periods, seasonality, plant age, and extraction methods (Sadeh et al., 2019).

According to Guimarães et al. (2019), studies that generally investigate the antimicrobial activity of essential oils are incomplete, as they cannot identify which compounds act with greater influence or whether it is a synergism between the components. Therefore, it is possible that compounds in smaller amounts also contribute to the activity.

### 3.2 Viscosity and conductivity of polymeric solutions

The apparent viscosity and the electrical conductivity of the PLA polymeric solutions with different concentrations of PPEO are shown in Table 3. The incorporation of PPEO reduced the viscosity of the polymeric solution, decreasing as the concentration of PPEO increased. Silva et al. (2018) also reported that the addition of ginger essential oil (*Zingiber officinale*) decreased the viscosity of the polymeric solution containing soy protein isolate, polyethylene oxide and zein. Similarly, Teilaghi, Movaffagh, & Bayat (2020) found the same behavior in zein solutions added with 5, 10 and 15% essential oil of cumin seed (*Nigella sativa*). Pelissari et al. (2012) reported that the viscosity of a solution is associated with the interactions between the component molecules and depends on the concentrations and nature of the solutes and reagents used. Thus, the presence of PPEO possibly interferes with the interactions and bonds inherent between the molecules of the PLA solution, weakening the bonds among its constituent components and resulting in a lower viscosity of the solution.

The presence of the essential oil also reduced the electrical conductivity of the solutions significantly, in all concentrations. Rafiq, Hussain, Abid, Nazir, & Masood (2018) evaluated the effect of incorporating lavender essential oil (*Lavandula officinalis*), cloves (*Eugenia caryophyllus*) and cinnamon (*Cinnamomum cassia*) on the formation of PVA nanofibers and sodium alginate, finding that their presence influenced the decrease in electrical conductivity. In another study, Mori et al. (2015) observed that the addition of candeia essential oil (*Eremanthus erythropappus*) to develop PLA nanofibers showed a performance similar to the aforementioned studies, also presenting reduction in conductivity with the increase in oil concentration.

As well as viscosity, electrical conductivity is affected by different factors, such as the ionic strength of the medium and the type of polymers and solvent (Ghorani & Tuker, 2015). Thus, for a constant solvent

system in the electrospinning process, changes in the mass ratio of the polymer and bioactive mixtures present or in the type of polymers are the main factors for the changes in electrical conductivity.

### 3.3 Morphology of the ultrafine fiber membranes

The ultrafine fibers containing only PLA (control) exhibited bead-free morphology, with an average diameter of 426 nm. The addition of PPEO provided a reduction in the diameter of ultrafine fibers in treatments containing 10, 20 and 30% of PPEO in relation to the control, with values of 239, 226 and 167 nm, respectively, as shown in Figure 4. Similar behavior was found in a study by Unalan et al. (2019), who developed polycaprolactone nanofibers loaded with peppermint essential oil (*Mentha piperita*) by the electrospinning technique, showing that the addition of the oil led to a slight decrease in the diameter of the fibers.

The presence of PPEO in the formulation promoted the formation of beads and lumps, probably due to insufficient evaporation of the solvent used. Mori et al. (2015) reported that in mixtures of PLA and essential oil of candeia (*Eremanthus erythropappus*), the addition of the oil influenced the increase in the diameter of the nanofibers and a reduction in the amount of beads. Still, Scaffaro, Maio, & Lopresti (2018) observed that the presence of carvacrol in the functional PLA membranes affected the morphology of the nanofibers, leading to an increase in their diameters.

The presence of beads (Figures 4c, 4e and 4g) is generally negatively associated with the formation of the material. However, it is assumed that they can be not so negative, since their structures can hold some percentage of the bioactive compound present in the structure and be gradually released into the environment in which it is in contact. In nanofibers composed of polyethylene oxide and soy protein, Silva et al. (2018) found that the addition of ginger essential oil also increased their diameter. According to Bhardwaj & Kundu (2010), solutions that have low conductivities result in insufficient elongation of the jet to be electrified by electrical forces and lead to the production of nanofibers with larger diameters. However, it was observed that in the present study, the behavior proved to be opposite to the data from most of the literature. This fact can be explained by the inconstancy of the stretching of the solution, sometimes causing it to occur until the needle is clogged, influencing the heterogeneity of the formed material. Also, the solution was sometimes deposited in the collector in the form of fibers and sometimes in the form of beads. According to Haider, Haider, & Kang (2018), the presence of beads is attributed to the influence of gravitational force and another important factor that can cause these distortions in the fiber structure is the surface charge density, since any change in this parameter can also affect the morphology of the nanofiber.

### 3.4 Thermal properties of the ultrafine fiber membranes

The initial (TDi) and final (TDf) decomposition temperatures and the percentage of mass loss are shown in Table 4. The PPEO presented two stages of decomposition, one close to 82.3 ºC, indicating 58.4% mass loss and the other close to 151.4 ºC, showing 27.1% mass loss. These degradation peaks can be attributed to the evaporation of volatile compounds. The PLA showed a decomposition stage at 360.7 ºC

and approximately 90% of mass loss. The degradation temperature of pure PLA around 300 ºC was reported by Thangaraju, Srinivasan, Kumar, Sehgal, & Rajiv (2012), being characteristic of this polymer. The incorporation of the oil provided less thermal stability in the treatments with 10, 20 and 30%, indicating mass losses from 106 ºC, in comparison with fibers produced with pure PLA (Figure 5).

Furthermore, it was observed that in ultrafine fibers, the PLA protected the PPEO because the TDis presented were from 131.8, 120.2 and 106.2 to 10, 20 and 30% of PPEO, compared to the TDi of 44.9 ºC of the pure PPEO. Thus, it is emphasized that this material can be applied in food packaging that will not be subjected to processes that require temperatures above 100 ºC.

### 3.5 FTIR of the ultrafine fiber membranes

The chemical interactions between the PLA and the PPEO were investigated by the FTIR, and the spectrum is shown in Figure 6. The characteristic absorptions of the PLA are three strong bands due to the vibrations of the C-CO-O-C group, that is, the band derived from the stretch of the C=O in 1747 $cm^{-1}$, the band coming from the asymmetrical stretching of the CO in approximately 1195 $cm^{-1}$ and, in 1110 $cm^{-1}$, coming from the symmetrical stretching C-O-C. The lack of an intense band in the 3500-3000 $cm^{-1}$ region (stretching of the O-H group) is indicative of the absence of PLA hydrolysis by-products (Palmieri, Pierpaoli, Riderelli, & Ruello, 2020).

For pure PPEO, the spectrum showed a characteristic band around 750 $cm^{-1}$ related to the aromatic C-H bond. Also, bands between 1400 and 1500 $cm^{-1}$ correspond to C=C bonds from aromatic rings characteristic of the oil (Mukherji & Prabhune, 2014). Bands that appear between 2750 and 3000 $cm^{-1}$ are probably related to O-H bonds of terpenoid compounds (Boughendjioua & Djeddi, 2017).

The bands around 900 $cm^{-1}$ are related to monoterpenic compounds in the oil, and those around 2943 $cm^{-1}$ are attributed to C-H bonds of methyls and methylenes (Oréfice, Vasconcelos, & Moraes, 2004). The peaks were more accentuated in pure PPEO when compared to the lower intensities shown in the treatments with 10% (almost imperceptible), 20 and 30%. Thus, it can be inferred that a certain loss of PPEO probably occurred during the electrospinning process, through volatilization.

### 3.6 Wettability of the ultrafine fiber membranes

The wettability character of ultrafine fiber membranes was determined by the angles of contact with water that were measured, as shown in Figure 7. Regardless of the composition, all treatments had a contact angle greater than 90º, implying the hydrophobic character of the membranes of ultrafine fibers formed. This performance was expected due to the fact that the PLA has a hydrophobic character (Sun et al., 2020). As essential oils are composed of highly hydrophobic molecules (Dhifi, Bellili, Jazi, Bahloul, & Mnif, 2016), it was expected that the presence of PPEO would increase water repulsion. However, there was no significant increase in this aspect when adding the PPEO in the different concentrations.

### 3.7 Antimicrobial activity by disk-diffusion, MIC, MBC and in micro-atmosphere

The results referring to the inhibition halos, MIC and MBC of the PPEO are shown in Table 2. For the membrane, only the inhibition halo was used (Table 2). The lowest MIC value observed was for *S. aureus*, with 256.9 mg/mL. For *E. coli*, the PPEO did not indicate an antimicrobial effect. As for the inhibition halos, it was observed that the effect of the PPEO did not show any significant difference between *L. monocytogenes* and *S. aureus*, with halos of 11.5 ± 1.1 and 13.2 ± 1.7 mm, respectively. In agreement with the MIC and MBC assay, *E. coli* showed resistance to the PPEO.

As for the ultrafine fiber membrane, the diameters of the halos for *L. monocytogenes* and *S. aureus* were smaller compared to pure PPEO, and for *S. enteritidis* there was no inhibition. It is noteworthy that this behavior is probably due to the lower concentration of PPEO (30%) used in the manufacture of fiber membrane.

Dannenberg et al. (2019) developed investigations about the essential oil of pink pepper and found that the MIC values for *S. aureus* (ATCC 6538) and *L. monocytogenes* (ATCC 7644) were 0.68 and 1.36 mg/mL, respectively, whereas the MBC was 2.72 mg/mL for both. On the other hand, Santos et al. (2020) tested different concentrations of the essential oil of pink pepper fruits to inhibit strains of *E. coli* (ATCC 25922), *S. enteritidis* (ATCC 13076), *L. monocytogenes* (ATCC 19117) and *S. aureus* (ATCC 25923), verifying inhibition only in the last, with an MIC of 5 µg/mL. In comparison to our study, these values are well below, a fact that can be justified by the time of harvest of the fruits, climate, soil situation, precipitations and different types of strain used.

Gram-positive and Gram-negative bacteria have distinct cytological structures, a fact that corroborates the greater resistance of Gram-negative bacteria and greater sensitivity of Gram-positive bacteria in relation to the action of essential oils. The Gram-positive cell wall is composed of approximately 90 to 95% peptidoglycan, which is bound to proteins and teioic acid (Nazzaro, Fratianni, De Martino, Coppola, & De Feo, 2013). In addition, it allows hydrophobic molecules to easily cross and act on both the cell wall and the cytoplasm. The phenolic compounds present in oils, for example, are considered one of the most responsible for the antimicrobial action against Gram-positive bacteria, but their effect depends on the amount of the compound: at low concentrations, they can interfere with enzymes involved in energy production, while at high concentrations they can denature proteins (Tiwari et al., 2009). However, there are also studies that prove the antimicrobial activity of essential oils acting against gram-negative bacteria, such as *Cinnamomum camphora* essential oil, reducing the development of *E. coli* (Wu et al., 2019) and essential oil of oregano and lemongrass acting against *Salmonella enteritidis* present in refrigerated steaks (Oliveira, Soares & Piccoli, 2013).

The micro-atmosphere test is based on the action of volatile compounds in the essential oil, which can significantly inhibit the growth of some bacteria. The reductions in microbial load in this assay are shown in Figure 1, only for Gram-positive bacteria, since Gram-negative bacteria did not show positive results for the antimicrobial action in the disk-diffusion assay of ultrafine fibers. It was possible to observe that the pure oil (100%) indicated reductions of around 90% for both bacteria. On the other hand, the

concentration of 30% of PPEO showed a reduction of around 40% for *L. monocytogenes* and 50% for *S. aureus.*

In a similar study using pink pepper essential oil, Dannenberg et al. (2017) found that in the micro-atmosphere, the reduction was 100% in the development of *S. aureus* and *L. monocytogenes*, and 16 and 15% for *E. coli* and *S. typhimurium*. Antunes et al. (2017) developed nanofibers with eucalyptus essential oil and observed that at concentrations of 0.25, 0.38 and 0.63 μL/cm³ there was total inhibition of the growth of viable cells of *S. aureus* and *L. monocytogenes*. Silva et al. (2018) evaluated the application of nanofibers with polyethylene oxide, isolated soy protein and ginger essential oil, noting that the last influenced the reduction of approximately 43% in the count of *L. monocytogenes*, using concentrations of 0.2 and 0.3 μL/cm³.

According to Trombetta et al. (2005), Gram-positive bacteria are more susceptible to the vapor phase that contains terpenes. This fact can be observed in the present study, since the PPEO presented a greater amount of myrcene, which is considered a monoterpene. However, some exceptions have also been reported in the literature, indicating that there is no apparent association or positive correlation between the nature of the bacterial wall and the degree of inhibition of microbial strains (Saida et al., 2020).

The components of PPEO may have acted in synergism to affect the activity. However, the mechanisms of action are complex, requiring further investigation of the raw material and substrate on which they will act. According to Saad, Muller, & Lobstein (2013), the mechanisms of action of the oils will depend on their chemical composition. The location of one or more functional groups can influence its antimicrobial activity. As an example, thymol and carvacrol have similar antimicrobial effects, but have different mechanisms of action against Gram-positive and Gram-negative bacteria. Reyes-Jurado et al. (2020) reported that in the vapor phase, the oil disperses freely: it has a particular impact against microorganisms due to its surface action, making them more susceptible to volatiles.

In this way, the volatile antimicrobial capacity of PPEO, without requiring direct contact with food, promotes investigations for the development of packaging systems that can control the spread of pathogenic and deteriorating bacteria.

### 3.7.1 Antimicrobial action of the ultrafine fiber membranes on cream cheese

For the evaluation of the effect of the developed ultrafine fiber membrane, the treatment with the concentration containing 30% PPEO was chosen because it showed better results in the antimicrobial evaluations against Gram-positive bacteria, although it also indicated inhibition against *S. enteritidis*. The analysis for the verification and quantification of colony forming units was carried out one day after the beginning of the experiment. However, the results were not expressed because there was not enough growth of both bacteria.

For *L. monocytogenes*, it was observed that the presence of the ultrafine fiber membrane in the period of 7 days did not indicate growth inhibition, in relation to the positive control. The same behavior was

observed in the 14-day period. However, in 21 days a significant reduction in colony count was noticed, around 26%, as shown in Figure 2. For *S. aureus*, the presence of the ultrafine fiber membrane in the period of 7 days indicated a reduction in cell content, but it was not significant. On the other hand, in 14 days there was a significant reduction of approximately 30%. Analogous behavior was identified after 21 days, with an even greater significant reduction, around 62%, as shown in Figure 3.

Considering that the expiration date indicated on the evaluated food is 5 days after opening the package, the results obtained for both bacteria showed that until the end of the period, the presence of fibers in the package was not relevant. However, if the fibers were inserted into the packaging lid at the time of filling, soon after the product was manufactured, there would probably be a positive effect, as the volatile compounds would be trapped in the hermetically sealed packaging, as there was a relevant result for a longer period, 21 days. The results also served to show the behavior profile of the product during a longer storage period, suggesting that PPEO has been gradually released.

Dannenberg et al. (2017) studied the effect of the presence of pink pepper essential oil in cellulose acetate films produced by the casting technique and applied to cheeses. It was observed that the release of the oil is related to the affinity between the nonpolar compounds of the oil and the evaluated food. Silva et al. (2018) produced nanofibers containing ginger essential oil and applied it to slices of Minas cheese, verifying that the presence of the material indicated a significant reduction in *L. monocytogenes* colonies on days 3 and 9 of storage. The latter presented about 17% reduction in relation to the positive control.

Therefore, it is assumed that at first, the volatiles of the PPEO came into contact at least with the surface of the cream cheese layer. As the storage time passed, the retention of PPEO inside the package was prolonged, causing these compounds to act more actively. As a result, the data shown in this study stimulates further investigation on foods that have a longer shelf life, as the PPEO has been shown to be effective in reducing cell counts on the 21st day.

The antimicrobial activity of essential oils is commonly assessed using methods of direct contact between pathogen and microbial agent, through diffusion and dilution methods. However, the role of essential oils in the vapor phase as antimicrobial agents is increasing in importance. Tyagi & Malik (2010) suggested that essential oils in the vapor phase have a greater degree of antimicrobial activity, since the active compounds are highly volatile and can quickly disperse in the environment. According to Kloucek et al. (2012), each constituent present in the oil has a different volatility, therefore, when the oil is introduced into a closed microenvironment, the volatiles begin to disperse at different rates in the vapor phase within the space in question, according to the degree of volatility, until they reach equilibrium.

Thus, it was observed that the ultrafine fiber membrane showed a good result, contributing to microbial reduction when compared to the positive control. In addition, the release of compounds from essential oils to the food through volatilization did not require direct contact, allowing the reduction of undesirable sensory characteristics that may occur in the food.

# 4. Conclusion

Ultrafine fiber membranes from PLA and PPEO were successfully obtained and showed antimicrobial action. The PPEO influenced the reduction of the conductivity and viscosity of the polymeric solutions, affecting the fiber morphology, with the presence of beads in the treatments in which it was included. The pure PPEO starts its thermal degradation in 44.9 ºC; thus, the PLA had the effect of protecting the essential oil, since the ultrafine fibers of all treatments with PPEO had the first peak of degradation temperature between 106 and 131 ºC. The ultrafine fiber membrane showed hydrophobic surface.

As for antimicrobial activity, *L. monocytogenes* had MIC of 513.8 mg/mL and MBC of 642.3 mg/mL, inhibition halos of 11.5 mm against pure PPEO and 5.6 mm against ultrafine fiber membrane with 30% of PPEO. *S. aureus* had MIC of 256.9 mg/mL and MBC of 385.38 mg/mL, inhibition halos of 13.2 mm for action of pure PPEO and 7.9 mm for action of ultrafine fiber membrane with 30% PPEO. *E. coli* was not sensitive to the action of the PPEO. *S. enteritidis* had MIC and MBC of 770.7 mg/mL, 9.6 mm inhibition halo for the action of pure PPEO and absence of sensitivity to ultrafine fibers. In micro-atmosphere analysis, it was observed that pure PPEO provided a 90% reduction in the microbial load of *L. monocytogenes* and *S. aureus*. The PPEO in membrane with concentration of 30% provided a reduction of 40% for *L. monocytogenes* and 50% for *S. aureus*.

The ultrafine fibers applied to the cream cheese packaging showed an inhibitory effect only on the 21st day of storage, for *L. monocytogenes*. For *S. aureus*, the fiber membrane inhibited the growth of the colonies on the 14th and 21st day, with reductions of 30 and 62%, respectively. Microbial inhibition data promoted by the membrane containing the PPEO showed that a slow release occurred, possibly due to the hydrophobic characteristics of PLA. Thus, for future work, we suggest the use of blends with hydrophilic polymers together with PLA, to ensure a faster release of the essential oil in cream cheese packaging.

# Declarations


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### Author's contribution

**MRVF:** Conceptualization, investigation, writing – original draft preparation, methodology, laboratory practice, data curation, visualization.

**CRC:** Resources, laboratory practice, conceptualization, writing – reviewing and editing.

**CCM:** Project administration, supervision, formal analysis, validation, writing – reviewing and editing.

**ERZ:** Project administration, supervision, writing – reviewing and editing.

**ARGD:** Project administration, supervision, writing – reviewing and editing.

All authors read and approved the final manuscript.

### Ethics approval and consent to participate

The study in question was not submitted/evaluated by the ethics committee because tests on animals and humans were not performed.

### Conflict of interest

On behalf of all the authors of the manuscript entitled "**Antimicrobial properties of PLA membranes loaded with pink pepper (*Schinus terebinthifolius* Raddi) essential oil applied in simulated cream cheese packaging"**, I confirm that we have no conflict of interest.

### Availability of data and materials

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# Tables

Table 1. Chemical composition of PPEO.

| Compound | Retention time (min) | Peak area (%) |
|---|---|---|
| 4-Carene | 4.1 | 0.1 |
| α-pinene | 4.2 | 20.2 |
| p-Menth-8-en-2-ol acetate | 4.6 | 0.1 |
| (+)-Sabinene | 5.2 | 1.5 |
| (-)-β-Pinene | 5.3 | 3.0 |
| Myrcene | 5.8 | 28.2 |
| Limonene | 6.9 | 10.5 |
| δ-Elemene | 19.4 | 0.7 |
| α-Copaene | 20.9 | 1.1 |
| ι-Gurjunene | 22.3 | 0.6 |
| β-Caryophyllen | 22.6 | 2.7 |
| α-Caryophyllene | 24.0 | 0.2 |
| Germacrene D | 25.1 | 15.3 |
| (+)-Ledene | 25.7 | 1.6 |
| α-Muurolene | 26.0 | 0.8 |
| Y-Muurolene | 26.5 | 0.6 |
| δ-Cadinene | 26.9 | 5.6 |
| Di-epi-α-cedrene | 27.1 | 0.4 |
| Germacrene B | 28.0 | 0.3 |
| Germacrene D-4-ol | 28.8 | 0.8 |
| 14-Methylcholest-8-ene-3,6-diol | 29.9 | 0.3 |
| δ-Cadinol | 31.3 | 1.4 |
| τ-Muurolol | 31.5 | 0.1 |
| α-Cadinol | 31.8 | 1.4 |
| 5β,7βH,10α-Eudesm-11-en-1α-ol | 33.0 | 1.6 |

Table 2. MIC and MBC of PPEO, and inhibition halos from pure PPEO and ultrafine fiber membrane containing 30% PPEO.

| Bacteria | MIC (mg/mL) | MBC (mg/mL) | Halos (mm) | |
|---|---|---|---|---|
| | PPEO | PPEO | PPEO | Membrane |
| *L. monocytogenes* | 513.8 | 642.3 | 11.5 ± 1.1[a] | 5.6 ± 1.2[b] |
| *S. aureus* | 256.9 | 385.38 | 13.2 ± 1.7[a] | 7.9 ± 1.2[a] |
| *E.coli* | - | - | 0.0 ± 0.0[c] | 0.0 ± 0.0[c] |
| *S. enteritidis* | 770.7 | 770.7 | 9.6 ± 0.9[b] | 0.0 ± 0.0[c] |

Different lower case letters in the same column represent a significant difference between means by the Tukey test at 5% significance.

Table 3. Apparent viscosity and electrical conductivity of polymeric solutions with PLA and different concentrations of PPEO.

| PPEO (%) | Apparent viscosity (mPa/s) | Electrical conductivity (µS/cm) |
|---|---|---|
| 0 | 128.8 ± 1.6[a] | 0.62 ± 0.01[a] |
| 10 | 100.1 ± 0.2[b] | 0.38 ± 0.01[b] |
| 20 | 69.9 ± 0.3[c] | 0.34 ± 0.02[c] |
| 30 | 60.3 ± 0.6[d] | 0.28 ± 0.01[d] |

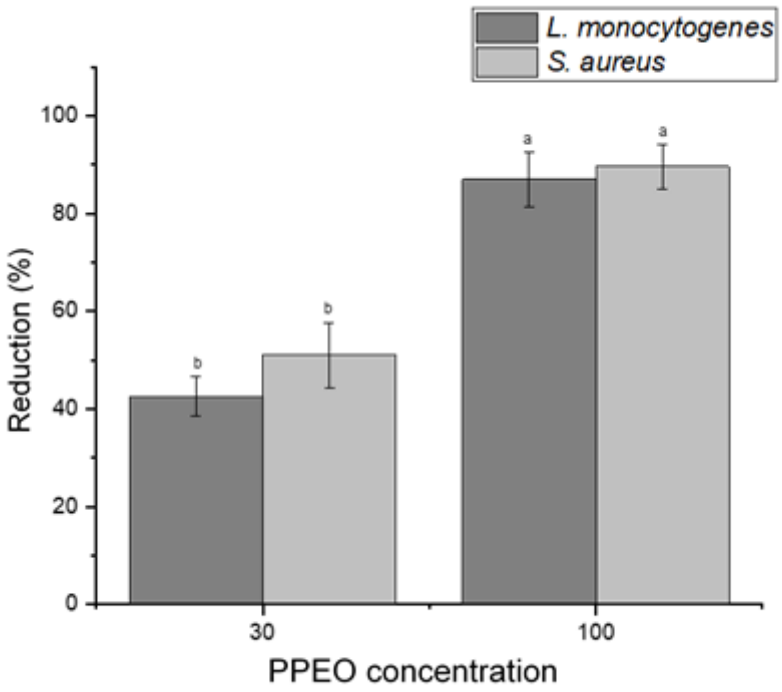
Different lower case letters in the same column represent a significant difference between means by the Tukey test at 5% significance.

Table 4. Profiles of temperature and mass loss of the isolated constituents and of the membrane of ultrafine fibers evaluated by TGA.

| Sample | TDi (ºC) | TDf (ºC) | T (ºC) | Weight loss (%) |
|---|---|---|---|---|
| Individual components | | | | |
| PPEO | 44.9 | 120.5 | 82.3 | 58.4 |
| | 119.1 | 181.2 | 151.4 | 27.1 |
| PLA | 305.4 | 382.6 | 360.7 | 89.8 |
| Membrane of ultrafine fibers | | | | |
| PPEO (%) | | | | |
| 10 | 131.8 | 164.5 | 148.6 | 2.2 |
| | 282.1 | 376.5 | 355.6 | 77.,7 |
| 20 | 120.2 | 173.2 | 154.5 | 11.5 |
| | 321.0 | 382.3 | 362.8 | 56.3 |
| 30 | 106.2 | 172.3 | 145.9 | 13.7 |
| | 301.6 | 376.2 | 356.1 | 62.4 |

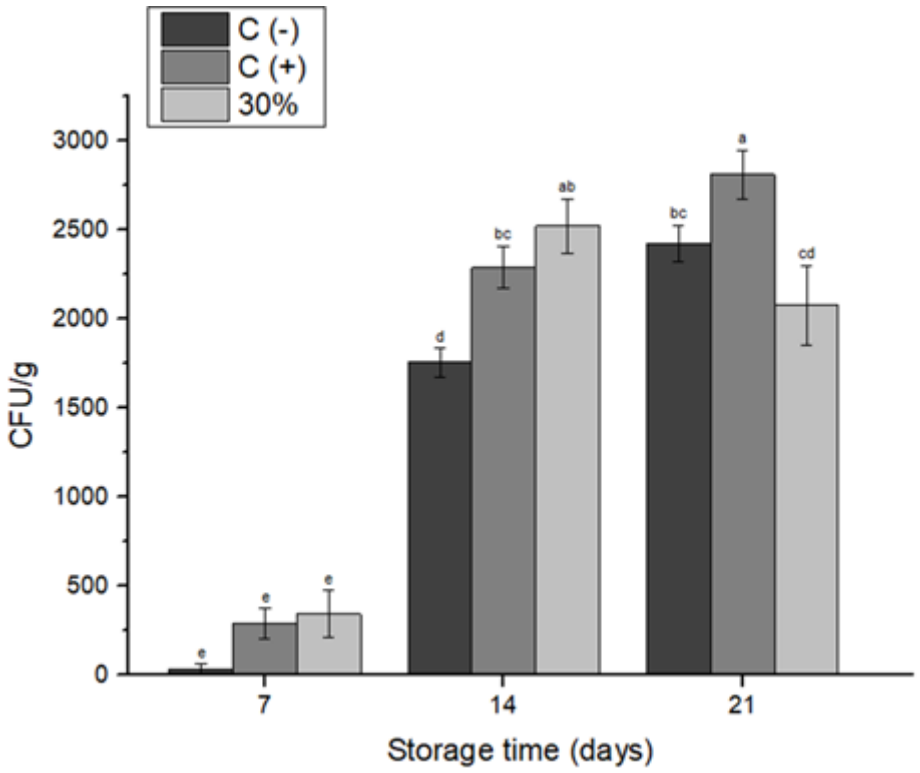
Tdi = Initial decomposition temperature; Tdf = Final decomposition temperature; T = Temperature where the greatest loss of mass occurred; PPEO = Pink pepper essential oil; PLA = poly lactic acid.

# Figures



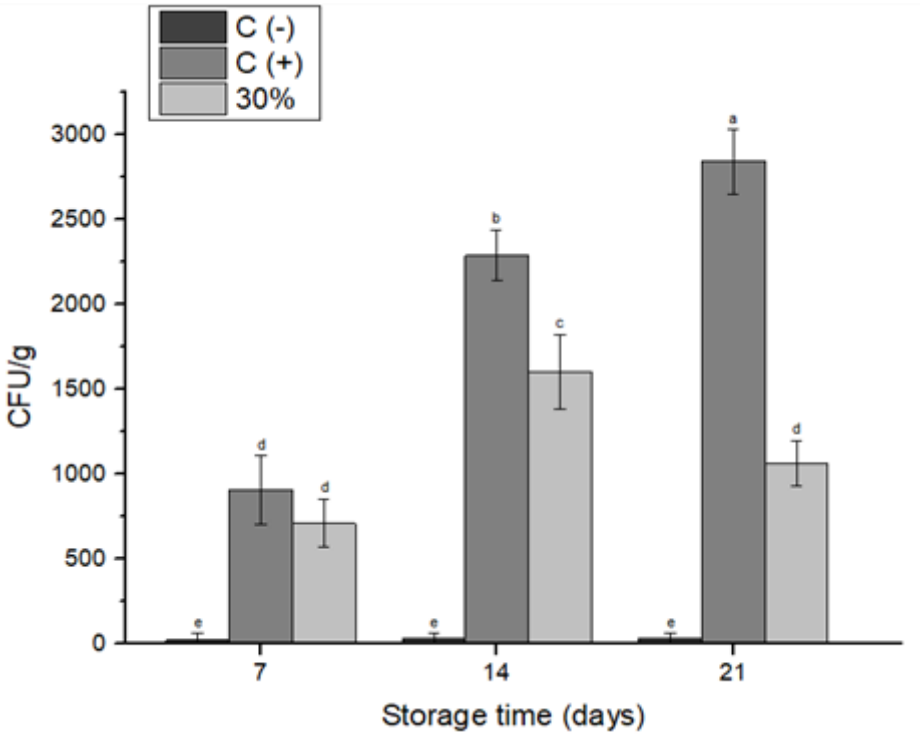
## Figure 1

Antimicrobial activity of PPEO by action of volatiles by means of the micro-atmosphere assay on the growth of *L. monocytogenes* and *S. aureus*.



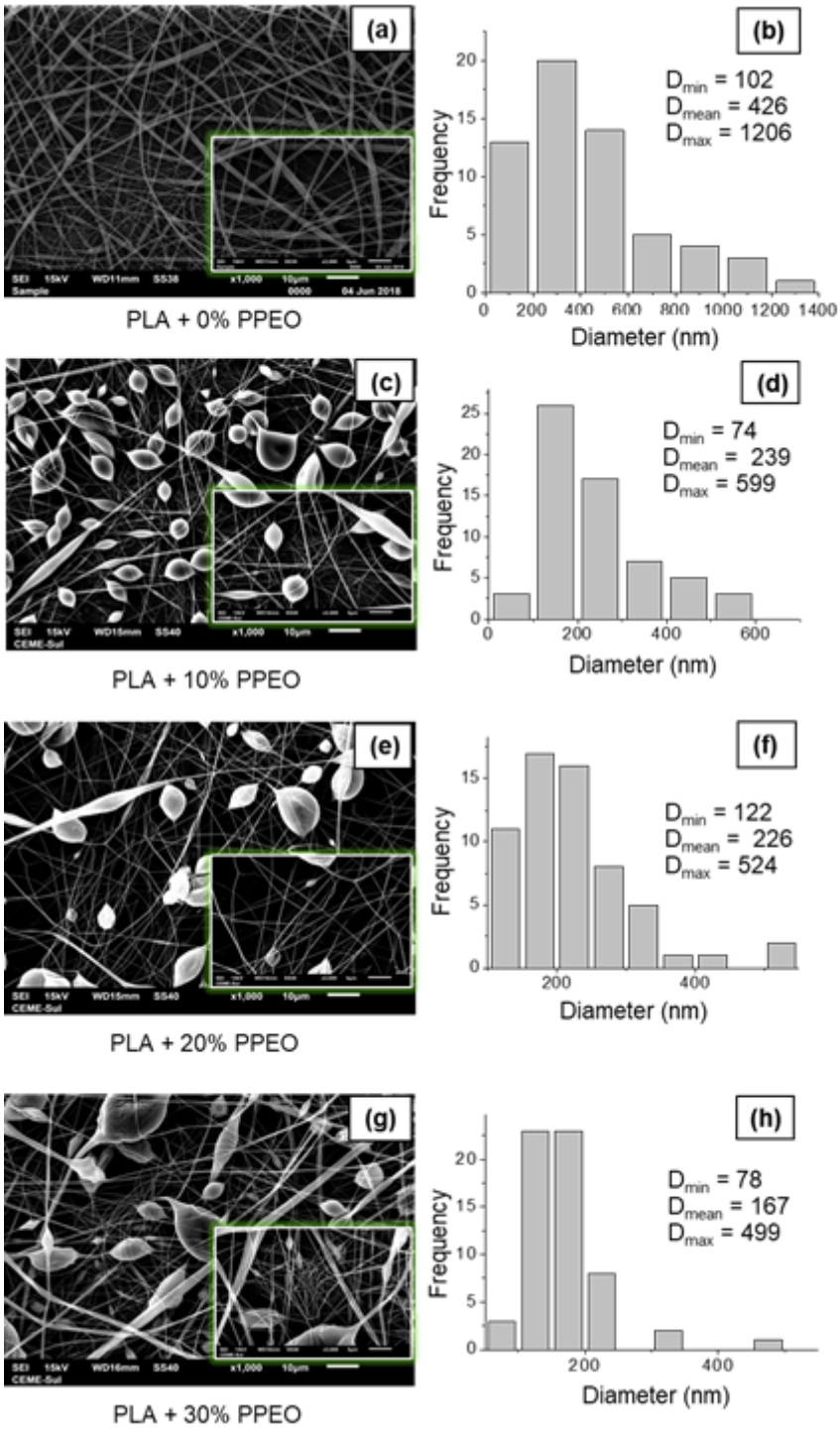

## Figure 2

Evaluation of the effect of applying the membrane of ultrafine fibers containing 30% PPEO and the storage time of cream cheese in the presence of *L. monocytogenes*. C (-) = Negative control and C (+) = Positive control.



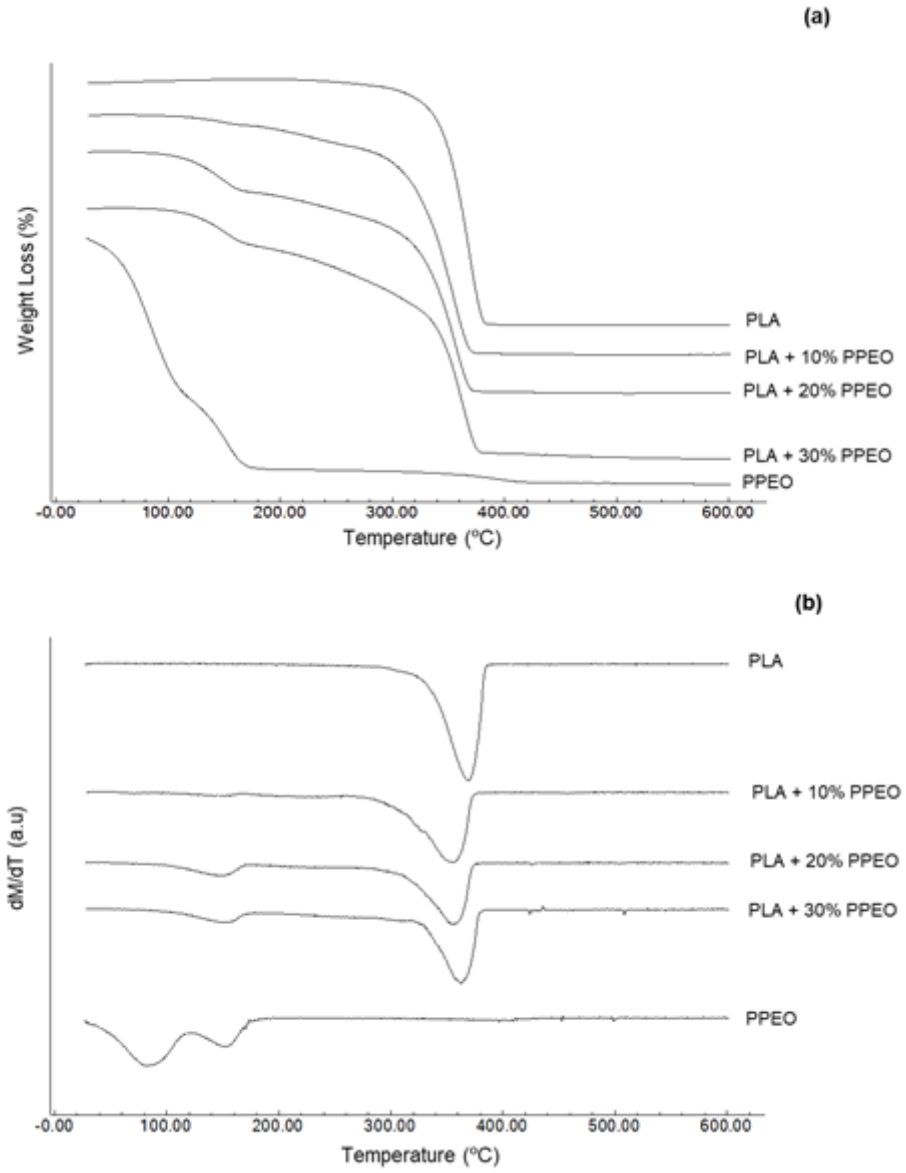

## Figure 3

Evaluation of the effect of applying the membrane of ultrafine fibers containing 30% PPEO and the storage time of cream cheese in the presence of *S. aureus*. C (-) = Negative control and C (+) = Positive control.



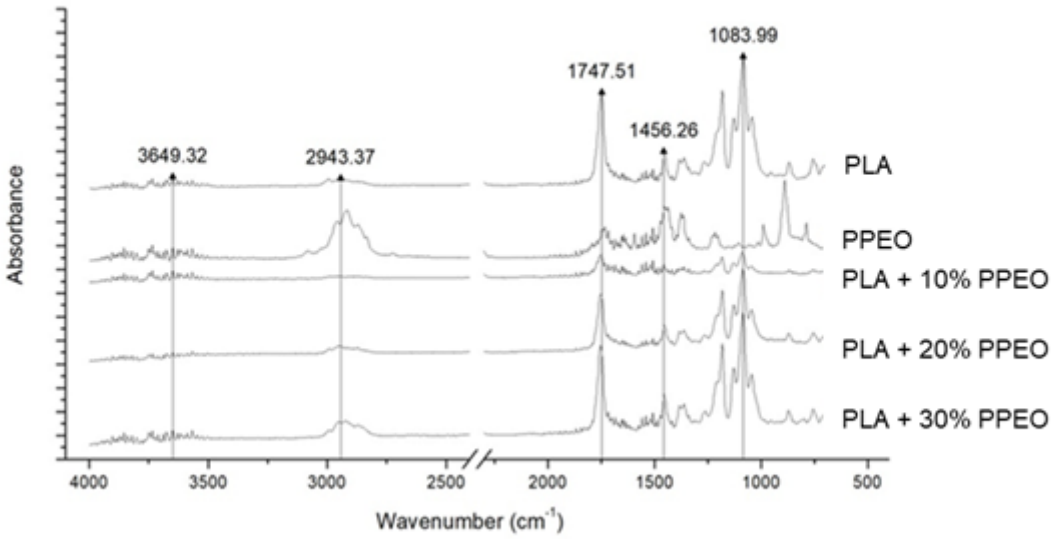

## Figure 4

Morphology and frequency distribution of ultrafine fibers with different concentrations of PPEO.



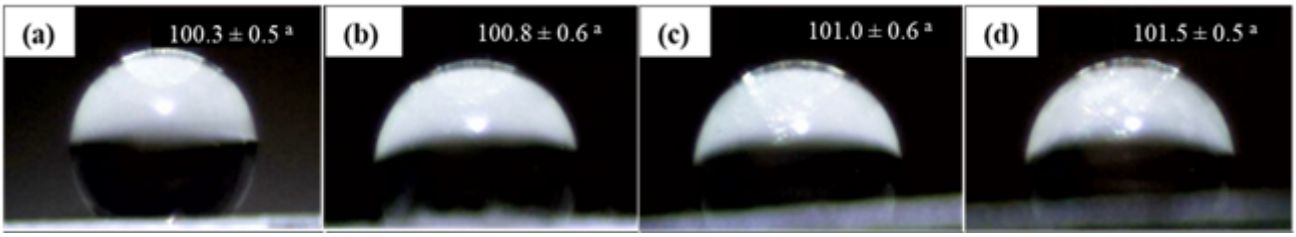

## Figure 5

Curves of the thermogravimetric analysis (TGA) (a) and the first derivative (b) of the isolated constituents and of the ultrafine fibers with different concentrations of PPEO.




## Figure 6

FTIR-ATR spectrum of isolated constituents and ultrafine fibers with different concentrations of PPEO.




## Figure 7

Wettability test to evaluate the contact angles with water in treatments with (a) 10%, (b) 20% and (c) 30% PPEO.