Chapter

Using Native Plants of the Northeast of Mexico for Developing Active Antimicrobial Food Packaging Films

Cecilia Rojas de Gante, Judith A. Rocha and Carlos P. Sáenz Collins

Abstract

The development of active food packaging is addressed using polyolefins such as LDPE and PVOH, as well as biopolymers from flour (sorghum and corn) and by-products of the food industry. Bacteriocins (nisin, natamycin), plant extracts such as oregano and thyme, as well as native plants of the northeast region of Mexico (*Larrea tridentata*, *Schinus molle*, *Cordia boissieri*, *Leucophyllum frutescens*), and essential oils of oregano and thyme as antimicrobial agents have been studied. The effect exerted by the process of incorporation of the antimicrobial agent (casting, extrusion) on the barrier and mechanical properties of the package as well as the antimicrobial activity of the containers (broad spectrum or selective activity) has been observed and the establishment of methods for their traceability.

Keywords: sorghum, maize, flour, nisin, thyme, oregano, *Larrea tridentata*, *Schinus molle*, *Cordia boissieri*, *Leucophyllum frutescens*, *Listeria monocytogenes*, *Staphylococcus aureus*

1. Introduction

Since the last decade and a half (2000 to date), the main forces that have unleashed the greatest developments in the packaging of food are the great concern of society for the care of their integral health including its nutritional status through foods with less or no presence of additives but in convenient presentations that facilitate their preparation, heating, and intake as well as foods with therapeutic action. A consumer who is very concerned about the safety of food, where food packaging and storage systems do not represent or have physical, biological, or even toxicological risks, nor for the protection of the environment.

All of the previous demand constantly forces the change on the nature of the food packaging and consequently on the materials of which it is composed [1]. Therefore, new materials are being developed to comply with the above. First, packages that contain in their formulation substances that migrate from the container to the food exert a positive action avoiding deterioration reactions likewise increase the sensory quality through the positive migration of substances or have a therapeutic effect. In this category are the so-called active packaging [1]. Second, in relation

to the protection of the environment: the development of biodegradable packaging using, for example, biomaterials obtained from agri-food sources [1].

An active packaging is defined as the one that produces a change in the state of the packaged food to prolong its shelf life, improve its safety and quality, and provide a barrier between the food and its environment [1]. The mechanisms of action in active packages can be acting as emitting systems or as sequestering systems for substances. In the emitting systems, compounds or additives generally recognized as safe (GRAS), such as antioxidants and antimicrobial agents, are released into the food through the walls of the package. Sequestering systems remove undesirable compounds such as oxygen, H_2O, ethylene, CO_2, and impurities, among others [2]. Great diversity of active packaging is being developed in order to control the emission or absorption of substances and thus modify the environment of the product or directly the product. Thus, active packaging has substances or systems that absorb oxygen, ethylene, CO_2 and humidity; others absorb or release desired aromas [2–4]. Other active packages contain active enzyme systems and antimicrobial substances or systems. All these active containers seek the elimination of microbial growth, the extension of the useful life, and/or the increase of organoleptic qualities of the product [5].

The proposal of our line of research is based on obtaining a series of products (active antimicrobial and active biodegradable packages according to a defined food or conservation need), as well as the processes for their elaboration. Most developments use materials obtained only from starches or proteins. With our development, a single product has a biopolymer matrix that includes both biopolymers (starches and proteins) in a single stage. By starting from a matrix that includes both biopolymers (starches and proteins and sometimes antimicrobials or antioxidants), unit operations are eliminated, which reduces costs of equipment and energy, consequently operating costs. The technological impact of the developments of the research line will be reflected in the conservation of food (fresh or dehydrated) through the use of antimicrobial/antioxidant active packaging that contribute to preserve the environment when they are discarded since they are potentially biodegradable.

From the scientific point of view, this solves a couple of problems at the same time, the first concerning the toxicological risk of the abuse of additives in the formulation and conservation of food and the second discarding the ecological and environmental problems generated by food packaging. Our developments will have, on the one hand, low environmental impact due to the development of biodegradable products from nature-friendly processes. On the other hand, they will have a high economic impact since currently in the country there are no companies dedicated to the development of biopolymer containers, creation of own technologies, and high added value to products of low commercial value.

2. Antimicrobial active packaging developed at the Tecnológico de Monterrey

2.1 Biopolymer active packaging

Our first works focused on the use of starches from several varieties of sorghum (high-production cereal in northeast of Mexico) whose different proportion in amylose and amylopectin plays an important role in the water vapor barrier of the containers reinforcing them with prolamines (kafirin and zein) to increase their impermeability [6] and use of antifungal agents such as the sorbates and benzoates of Na and K. The inclusion of broad-spectrum antimicrobial additives in plastic polymers and/or biopolymers through the proprietary technology generated at the Tecnológico de Monterrey, for example, enabled active packaging to be obtained on a laboratory scale

that reduced biological risks by manual or semi-manual packaging (risks of contamination with pathogens such as *Listeria monocytogenes* and *E. coli* O157: H7) [7–9]. On the other hand, there is great interest in the development and research of biopolymers obtained from agricultural sources. The matrices most commonly used to obtain this type of biopolymers are starch, proteins, and other polysaccharides. Some examples are corn zein, gluten and wheat gliadin, soy proteins, sorghum kafirin, cactus mucilage, and different types of starch (corn, potato, banana, tapioca, pea, waxy starch, and high amylose content, among others) [10–13]. In works carried out by our research group, it has been shown that it is possible to incorporate natural antimicrobial agents into films that could be used as active packaging. For example, Schause succeeded in establishing both the dry extraction conditions of starches and proteins from cereals such as sorghum (*Sorghum bicolor* Moench) as well as the casting process to obtain a film from sorghum flour and incorporate nisin as an antimicrobial active compound [8]. Nisin is a bacteriocin produced by some strains of *Lactococcus lactis* and *Streptococcus lactis* that has a broad antimicrobial power against Gram-positive bacteria. Nisin and lysozyme are used as a food preservative in dairy products as an inhibitor of *Clostridium tyrobutyricum*, *Clostridium butyricum*, *Clostridium saccharobutyricum* (causes swelling in cheese production), and pathogens like *Clostridium botulinum*, *Clostridium sporogenes* (which is used as a surrogate for *C. botulinum*), and *L. monocytogenes* [14]. The bactericidal action of nisin occurs in the cytoplasmic membrane, causing cell damage due to proton loss and damage to the integrity of the cell membrane [14]. Gram-negative bacteria have an outer membrane that protects the cytoplasmic membrane, so the bactericidal action of nisin is limited and the development of Gram-negative bacteria such as *E. coli* O157: H7 and *Salmonella* would not be inhibited.

Subsequently, Ríos-Licea conducted a search of natural substances of broad spectrum, so he analyzed the antimicrobial activity of aqueous extracts of known plants. Ríos-Licea also succeeded in developing antimicrobial films by incorporating natural extracts of garlic and oregano into the same biopolymer matrix of sorghum flour using the method established by Schause [15]. However, it was necessary to incorporate high concentrations of natural extracts, due to the low potency of the antimicrobial activity of the commercial product tested.

Tinoco-Pérez studied a variety of corn rich in anthocyanins (blue corn) by applying the process of dry milling and establishing the process to obtain active films in antioxidants (anthocyanins) from flour of this cereal [16]. Two biopolymers present in corn with a filmogenic capacity are starch and zein, the first being the most abundant in this grain [16]. There are a significant amount of reports published on films made from corn starch and zein; the effect of different additives, copolymers, and processes on the performance of films for different applications has been evaluated. In 2009, Mexico produced 29.4 million tons of corn using 38.5% of its total cultivated area. The production of this grain has shown an increase in its average annual growth rate of 2.1% in the period from 1994 to 2008. Of total corn production in 2008, 92% was white corn, 7% was blue corn, and 1% was of other varieties. Basically, white corn is destined for national consumption, yellow for export, and the rest of the varieties are commonly produced for self-consumption of rural populations. Among the 1% of the varieties not defined is the blue corn (*Zea mays* amylacea) [17, 18]. Blue corn (*Zea mays* amylacea) is a type of corn rich in anthocyanins (responsible for its pigmentation) and floury endosperm. It is cultivated in areas of dry climate and demands minimal care. Despite its nutraceutical potential, blue corn is only produced by rural communities for self-consumption due to its devalued commercial value, since the urbanized areas consume mainly white and yellow corn products. Among the few current uses of blue corn is the extraction of anthocyanins for use as natural food coloring and antioxidants [16, 19].

Among the processes studied to obtain films from corn fractions are casting, different types of extrusion (double screw/flat die, single screw/flat die, and extrusion/calendering, among others), stretching of zein resins, and pressing by heat [10, 20–22]. The effects of various additives and chemical treatments, for example, plasticizers, hydrophobic agents, copolymers, and the use of chemically modified starches on the structural, molecular, thermal, mechanical, and barrier performance characteristics, have been studied extensively [22–25].

In the case of sorghum, the cultivation of this cereal is less demanding in agronomic terms than corn (water and nutrients) [26, 27]. Sorghum is the fifth most important grain in the world, being the United States the country with the highest production in the world, followed by India and Nigeria. For the year 2010, Mexico contributed with 10.5% of the total world production, equivalent to 6,250,000 metric tons [15, 16]. In Mexico, sorghum is the second most important grain in production after corn; during the period 1996–2006, sorghum production contributed with 22% of the total production of cereals [26].

In Mexico, this cereal is destined mainly for livestock feed and secondarily for human food and obtaining inputs such as starch, alcohol, glucose, acetone, and butanol. One of the great advantages of sorghum is that it has the capacity to adapt to arid and semiarid climatic conditions and to be resistant to drought for long periods [26]. In previous works, it was able to demonstrate that antimicrobial active films can be obtained from corn and sorghum flour [8, 15, 16].

The biopolymers obtained in this way through a technique and process patented by Tecnológico de Monterrey as PCT [28] have the advantage of being biodegradable because their chemical structure is primarily based on proteins and starches. Additionally, they have the possibility of forming films with plasticity (custom flexibility) and of being formulated also tailored to the requirements of the product to be packaged. Additionally, they can be heat sealed to form bags of different dimensions or not to be sealed and act as "active" pads or pads in combination with other packaging. In addition to the advantages in terms of sustainability, the interest in using these sources to produce biopolymers lies in adding value to agricultural products [8, 29].

It is important to note that for any application of the said technology, it will be necessary to make an adaptation of the formulations and the process to satisfy the specific protection requirements for each food to be packaged. For what it is proposed to demonstrate in this work, the film-like packages obtained by adapting the formulations and process of the said published patent work to preserve and keep refrigerated for 30–45 days a commercial presentation in slices of semi-matured cheese [30].

The biopolymeric antimicrobial films described in WO2010/024657 A1 from cereals are limited to the packaging of dry foods or as pads for adsorption of exudates and emission of antimicrobial agents for fresh meat and cheese products [28]. Because of its sensitivity to water and low mechanical resistance to contain products with intermediate moisture, the biopolymeric matrix was reformulated to improve both parameters [30]. The results of refrigerated shelf stability of the cheese in terms of the control capacity exercised by the antimicrobials used in this study (nisin and natamycin) through the active packaging against fungi and yeast were effective throughout storage compared to vacuum packaging (control). The results of the microbial kinetics throughout the refrigerated storage for the fungi and yeast count showed the effectiveness of the active packaging. The development of fungi and yeasts remained controlled, showing the effectiveness of this emerging food preservation technology [30].

The plasticizing effects of two different polyols (glycerol and sorbitol) on the mechanical, thermal, and microstructural properties of flour films were studied by Valderrama and Rojas, and the results showed that films plasticized with sorbitol had better mechanical properties and less affinity for water than those plasticized with

glycerol. The attenuated total reflectance-Fourier-transform infrared (ATR-FTIR) spectra of blue corn flour plasticizer with sorbitol showed the presence of the additional band at 1745 cm^{-1} characteristic of the carbonyl peak, which confirms the chemical linkages between sorbitol and a polymeric matrix. The effect of the plasticizer on the glass transition temperature (Tg) showed that Tg decreased as the plasticizer content increased. Plasticized glycerol films showed lower Tg values than those with sorbitol. Observations by scanning electron microscopy (SEM) showed that it was necessary to add plasticizer to maintain film integrity. The sorbitol-plasticized flour films revealed better adhesion between phases, and these films showed a compact structure [31].

Finally, bioplastics were produced through thermoplastic processing using different cereals derived raw materials, namely, blue maize flour (BM), white sorghum flour (WS), maize starch, and the maize prolamin (zein). The overall performance of the bioplastics was investigated emphasizing on the study of the effect of different process strategies on the compatibilization of the starch and prolamin using mixtures of urea and formamide (UF) and maleated starch (MS) as compatibilizing agents [32, 33]. Results suggest that two competing phenomena, thermoplasticization and degradation, occurred simultaneously during the thermoplastic process. Fourier-transform infrared (FTIR) spectroscopy analysis evidenced the chemical changes induced by these phenomena. Moreover, chemical modification had also a major effect on the properties of the produced materials. WS films made with chemically modified flour increased their tensile strength in 29%, as compared to their native counterparts. Thermogravimetric analysis and FTIR analysis showed that the chemical interaction between starch and zein occurred more extensively in films made with formamide than those made with maleated starch [32, 33].

2.2 Plastic active packaging

In Valderrama's work, natural aqueous extracts are exchanged for essential oils because they have a higher concentration of antimicrobial active substances. It analyzed essential oils of oregano, thyme, tea tree, and mint, which have greater antimicrobial activity than the natural extracts used by Ríos-Licea [15]. In particular, the effect of incorporating two essential oils such as oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) on polyolefin materials such as low-density polyethylene (LDPE) and polypropylene (PP) was studied.

The mechanical, barrier, and antimicrobial properties of the packaging were evaluated against *Salmonella typhimurium*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7. The results demonstrate that films developed by extrusion incorporating 4% (w/w) of essential oils had a higher inhibitory effect than those obtained using the ionizing treatment. The packaging developed by extrusion containing 1% (w/w) showed a positive inhibitory effect, while those obtained by the ionizing treatment had no inhibitory effect against any of the test microorganisms. The incorporation of essential oils on the LDPE films generated a plasticizer effect, whereas the ones obtained by means of ionizing treatment did significantly affect the barrier properties of the films Valderrama and Rojas [9].

A simple and rapid Fourier-transform infrared (FTIR) spectroscopy method was developed by Valderrama and Rojas to determine the main essential oil components (carvacrol, thymol, and p-cymene) in the antimicrobial LDPE films incorporated with oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) essential oils. The ATR-FTIR spectroscopy with chemometrics, using the PLS-first derivative spectra, could predict the active compounds content accurate to an r2 >0.99 and a standard error of prediction (SEP) of <0.7. The developed method was successfully applied to predict the concentration of active compounds: carvacrol, thymol, and p-cymene in oregano and thyme essential oils with results compared to those of the GC-MS

method. The described nondestructive method can be applied to make the traceability of active compounds of essential oils in antimicrobial food packaging [34].

The work of Rocha is described below, who worked with the same essential oils of oregano and thyme that Valderrama used to obtain active plastic containers. This was due to the fact that they presented greater antimicrobial activity than the aqueous extracts of oregano and garlic from previous studies in our research group [9, 15]. It also proposed the use of a polymeric film for the preparation of the active container with essential oils, in order to present an alternative to vacuum cheese packaging. For this project, polyvinyl alcohol (PVOH) has been chosen for the preparation of the packaging due to its unique characteristics: permeability, biodegradability, and its facility to form films by the casting method. The purpose of this work is to propose an alternative, a packaging that is not dependent on complex plastic structures that requires vacuum packaging for provide the high barrier. The main challenge of the present project is the incorporation of essential oils that are lipophilic to a hydrophilic PVOH matrix, which is why it was suggested encapsulating them in cyclodextrins.

3. Control of the development of *Listeria monocytogenes* in fresh cheese during shelf life at refrigeration by means of an antimicrobial PVOH film (pad) with microcapsules of active compounds of oregano and thyme

The presence of *Listeria* spp. has been found in cheeses from developed countries such as the United States, Sweden, France, Germany, Italy, Brazil, and Japan. Hence, there is an urgent need to find alternative conservation systems, which allow to contribute to the inhibition of this type of pathogen. Listeriosis infections represent only 0.02% of cases of diseases in the USA; however, this bacterium is responsible for 25% of deaths in outbreaks related to food [35, 36]. Especially worrisome is the fact that it can survive pasteurization and be able to develop even in refrigeration temperatures. Hence, the importance of finding new technologies for their control and/or elimination, in particular, considering that the use of antimicrobial compounds in dairy products is restricted, that there is great interest in using natural compounds for this purpose but, above all, that these substances are added to the containers and not to the food, which can be achieved through the active packaging, is the objective of the present study. The development of *L. monocytogenes* has a particular health interest, because it is responsible for a fifth of the deaths related to foodborne diseases, especially considering that it survives the pasteurization process and develops even in refrigeration. It is important to highlight the tendency to decrease and, in some cases, eliminate preservatives in food. In dairy products, the direct use of antimicrobial agents is specifically forbidden, so the use of natural additives is becoming an alternative. If these are combined with the primary function of packaging to maintain sanitary safety and minimize the toxicological impacts of food, we are getting an active packaging. An active container that inhibits its development in fresh cheese during its storage in refrigeration can help to reduce the incidence of outbreaks and deaths due to this bacterium. The main goal was to develop an active packaging system that allows to control the development of pathogenic bacteria, in particular *Listeria monocytogenes* in refrigerated fresh cheeses, using natural antibacterial agents. As specific objectives: select and establish the conditions of incorporation of essential oils in a hydrophilic polymer, polyvinyl alcohol (PVOH), studying three methods of incorporation and formation of films; determine the antimicrobial activity of the films obtained against *Salmonella typhimurium*, *Listeria monocytogenes*, and *Escherichia coli* O157: H7 as target microorganisms; and, finally, check the effectiveness of the film and packaging system in the inhibition of the development of *L. monocytogenes* in fresh goat cheese, during 29 days of refrigerated storage.

3.1 Methods

3.1.1 Selection of substances, materials, microorganisms, and cheese

The selected essential oils were oregano (OEO) and thyme (TEO) (Primavera Life) for their potent antimicrobial activity and their availability in the national market. The antimicrobial activity of these inclusion complexes such as films using *Salmonella typhimurium*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 as target microorganisms was determined. Polyvinyl alcohol (PVOH) soluble in hot water, 87–90% hydrolyzed, with a molecular weight of 30,000–70,000 (Sigma-Aldrich), and glycerol (DEQ) were used for the production of active film. To obtain the inclusion complexes of the essential oils, crystalline α-cyclodextrin and β-cyclodextrin (Sigma-Aldrich) were used. The plastic films for the cheese packaging were LDPE film made by extrusion by Valderrama [9] and multilayer film Zublon® 5CR (Zubex Industrial S.A. de C.V.). For the activation of the microorganisms, the following selective broths were used: UVM-modified Listeria Enrichment Broth for *L. monocytogenes* (Becton Dickinson, DIFCO, México), Brilliant green bile lactose broth (BRILA broth) for *E.coli* O157: H7 (Merck KGaA, Germany), and Brain Heart Infusion broth (BHI broth) for *S. typhimurium* (Merck KGaA, Germany). For the plate count and antimicrobial activity tests, the following were used: Oxford Agar for *L. monocytogenes* (Becton Dickinson, DIFCO, México), SS agar for *Salmonella* and *Shigella* (Merck KGaA, Germany), Modified EC Broth and Bacto Agar for *E. coli* (Becton Dickinson, DIFCO, México). Oxford Agar with Oxford selective supplement (Becton Dickinson, DIFCO, México) was used for the counting of *L. monocytogenes* in the fresh cheese packaged experiment. For the goat cheese, fresh goat cheese, CAPRICO brand Cabrero cheese, was obtained in 400 g presentations directly with the company CAPRICO (manufacturing lot JL09210PN) located in Linares, N.L.

3.1.2 Process for developing PVOH films with active microcapsules of essential oils

The PVOH films were elaborated adapting the method used by Schause [8] and Ríos-Licea [15], previously studying three methods of incorporation of the essential oils in it: dispersion, emulsification, and formation of inclusion complexes with α and β cyclodextrins (CD) and the following variables: amount of PVOH, type and amount of cyclodextrin, coprecipitation strategy, solvent (water, ethanol), and concentration of EO. The encapsulation with β-CD is being the one selected for the incorporation of EO in the PVOH film. A PVOH film without inclusion complex was made as a control. With the resulting films, pads of 8 × 4 cm were made to be used in the active packaging system.

3.1.3 Thermal stability of essential oils and confirmation of inclusion complex formation by differential thermal analysis (DTA)

In order to determine the degradation temperature of the essential oils and establish if these would be affected during the film making process, the thermal stability of the same and their active compounds were evaluated. Firstly, a thermal evaluation of the essential oils of oregano and thyme was carried out, as well as its main active components with carvacrol, thymol, and p-cymene standards. Next, the β-CD and the inclusion complexes of OEO and TEO to confirm the formation of such complexes and not only a physical mixture. Finally, a thermal evaluation of the PVOH films with the inclusion complexes of CD:EO of oregano and thyme was made to determine the optimal storage temperature of the active films. The thermal evaluation was performed with a home DTA validated by Martínez and collaborators [37]. For each substance, at least two runs of food matrix were performed to verify the repeatability of the analysis.

3.1.4 Microbiological evaluation and disk diffusion method

To determine the antimicrobial activity of PVOH films, the disk diffusion method was applied (Kirby-Bauer method). After preparing and inoculating the agar with 10^6 CFU of each microorganism, samples of the films were cut in the form of 6 mm diameter disks and deposited on the agar, evaluating both the rough and smooth side of the films [7, 38, 39]. After 24 hours of incubation at 37 ± 1°C in inverted position, the inhibition halo was measured with a digital micrometer (Mitutoyo Digimatic 2,931,051 m, 0.001 mm sensitivity).

3.1.5 Control study of L. monocytogenes in fresh goat cheese using an active packaging system

The packaging system consisted of a pad of PVOH with EO microcapsules of oregano and thyme in a LDPE bag. First, 7 × 7 cm bags with LDPE film of 0.023 ± 0.003 mm thickness obtained by extrusion by Valderrama [9] were made, which were obtained by sealing two films on three sides with a vacuum packing machine Torrey brand. In the same way, bags were obtained with the multilayer film (Zublon® 5CR from Zubex Industrial). Second, in aseptic conditions, portions of cheese of 3 cm × 3 cm and 10 ± 0.5 g of weight were cut and exposed to UV treatment for 15 min on each side, a methodology adapted from Suppakul [40] for the purpose to reduce the interference of microorganisms typical of cheese in the study. Then, the samples were packed in the bags of the four treatments to be analyzed and inoculated with 100 mL of *Listeria monocytogenes* at a concentration of 5×10^3 CFU/mL. Finally, the bags were heat sealed in a packaging machine (TORREY) and stored in a refrigerator (Torrey Model VRD42) at 4 ± 1°C for up to 29 days.

The four treatments evaluated were (1) multilayer bag for vacuum packaging as control, (2) LDPE bag with PVOH "pad" without essential oils, (3) LDPE bag with PVOH "pad" with inclusion complex of β-CD:OEO, essential oil at a concentration of 25% in the film, and (4) LDPE bag with PVOH "pad" with inclusion complex of β-CD:TEO, essential oil at a concentration of 25% in the film.

The inhibition kinetics of *L. monocytogenes* was determined in the cheese samples packaged at 0, 1, 3, 5, 7, 15, 22, 26, and 29 days at refrigerated conditions. The analysis of the microbial count proceeded according to NOM110-SSA1-1994, making decimal dilutions and plate count [41]. The LabVIEW software for differential thermal analysis was used.

3.2 Results

3.2.1 PVOH films process with microcapsules of essential oils of oregano and thyme

It was possible to produce PVOH films with the inclusion complexes of oregano and thyme in all the experimental conditions. Films with 1, 4, and 15% EO were prepared with molar ratio 1:10, which they are shown in **Figure 1**. Films made with 1% EO were those most similar to the PVOH control film. Continuous, elastic, and transparent films were obtained. The higher the concentration of the essential oil in the film, the more presence of the inclusion complex affects the transparency of the film, with the PVOH matrix being observed as white, as can be seen in the film at 15% EO (**Figure 1**). It should be noted, however, that although the inclusion complex is observed in the film, no migration of this or the essential oil to the touch is perceived, which is why it has been well incorporated into the PVOH matrix. The films also presented less transparency when approaching the β-CD:EO ratio at 1:1

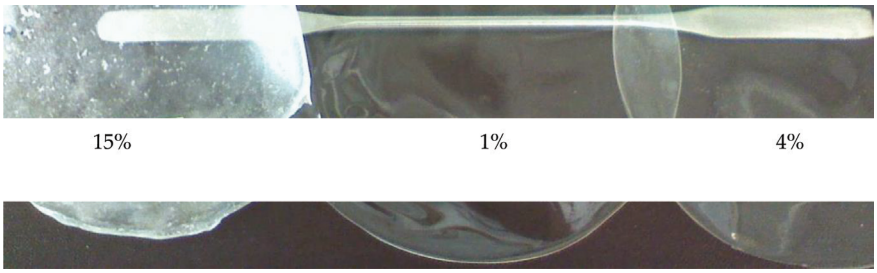


Figure 1.
Appearance of PVOH films with different concentrations of inclusion complex. Films elaborated at concentrations of 1, 4, and 15% of EO by process A (molar ratio β-CD: AO 1:10).

molar proportions; this is because a greater amount of inclusion complex tends to saturate the film. The films whose inclusion complex was dissolved in 30% ethanol also showed greater transparency than those in which it was prepared in water; this is because the inclusion complex in the 30% ethanol solution was better solubilized.

3.2.2 Thermal stability of essential oils and confirmation of inclusion complex formation by DTA

Firstly, the thermal stability of β-cyclodextrin and the essential oils of oregano and thyme, as well as its components (carvacrol, thymol, and p-cymene), was evaluated to confirm the formation of inclusion complexes. The thermograms of the carvacrol, thymol, and p-cymene standards are shown in **Figure 2** and **3**. Here it is shown that these three components are stable up to a temperature of 182°C, which refers to the boiling temperature of the p-cymene. The melting point of thymol shown in **Figure 2** is in agreement with that obtained by Ponce, which reports the melting point of thymol at 50°C [42]. The boiling point of p-cymene matches with the one reported by the supplier (178–180°C Sigma-Aldrich). Carvacrol was analyzed by broadening the study temperatures, as shown in **Figure 3**. This compound has an interesting behavior, since it has a crystallization temperature of −20°C followed by a melting point of 2°C and a point of boiling of 240° C. Sigma-Aldrich reports its melting point at 3–4°C and its boiling point at 236–237°C, which also coincides with that reported by Dahmane, which reports the boiling point of carvacrol at 237.7°C [43]. The closeness of the crystallization and fusion transitions does not allow the existence of a solid state of this intermediate substance at the reported temperatures. A similar behavior is reported by Ponce for cinnamaldehyde [42]. According to the obtained in **Figure 5**, it was successful to form an inclusion complex, since otherwise the signal of the boiling point would have been shown at 214°C of the essential oils (**Figure 4**).

The thermogram obtained by DTA of the β-CD:EO inclusion complexes of oregano and thyme (**Figure 5**) shows that they are stable at temperatures below 115°C, so there is no risk of degradation of the active compounds if the inclusion complex dissolves PVOH in situ in a solution with inclusion complex at 8°C (process B of PVOH film making with inclusion complexes). **Figure 6** shows the thermogram of the PVOH films with the inclusion complexes of oregano and thyme. The film made with essential oil of thyme had a little moisture on its surface, so we can see a couple of peaks at 0 and 100°C corresponding to the melting and boiling point of water, respectively. PVOH control films and those with inclusion complexes of essential oil of oregano and thyme are stable up to a temperature of 110°C, which indicates that they can be stored in shelves at room temperature without problem.

The PVOH control film has a peak at 150°C, which refers to the point of fusion of unplasticized PVOH. The last peak corresponds to the degradation of PVOH at 230°C, which is supported by Holland and Hay [44].

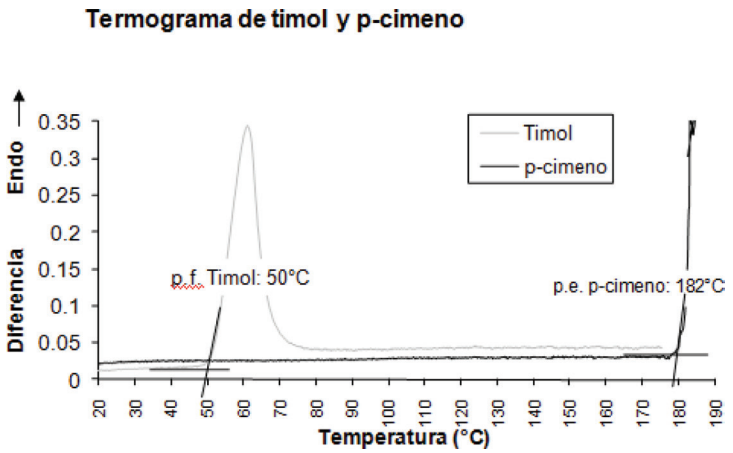


Figure 2.
Thermogram of thymol and p-cymene.

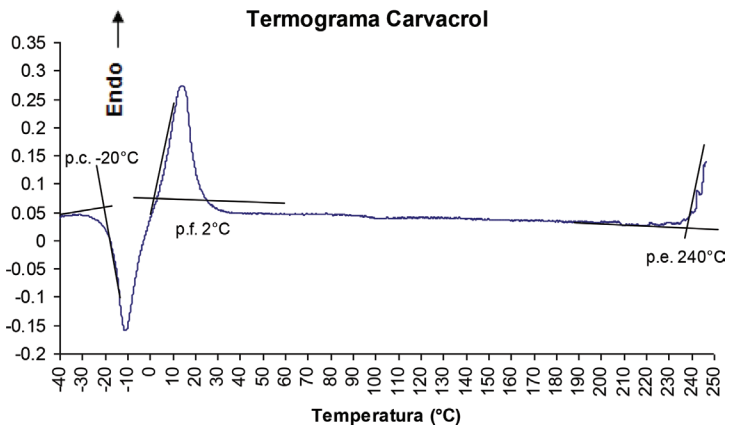


Figure 3.
Carvacrol thermogram.

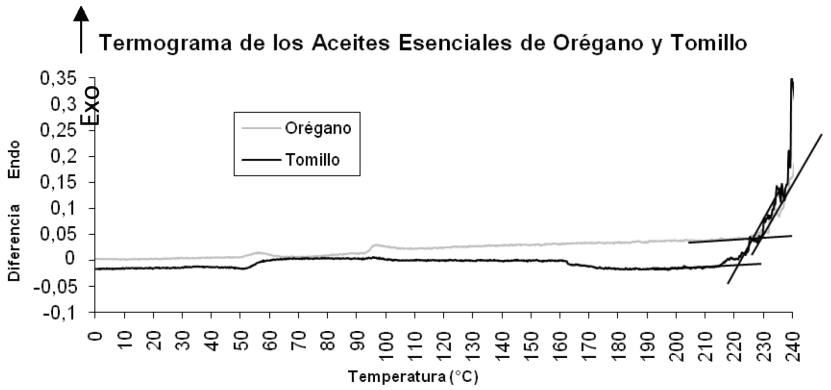


Figure 4.
Thermogram of thyme and oregano essential oils.

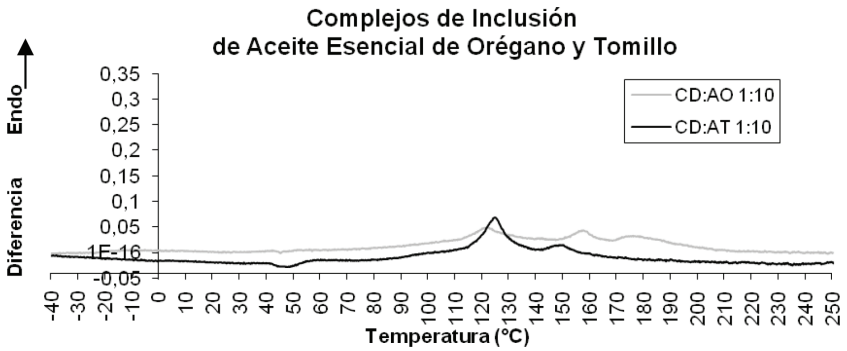


Figure 5.
Thermogram of the inclusion complexes of OEO and TEO with β–CD.

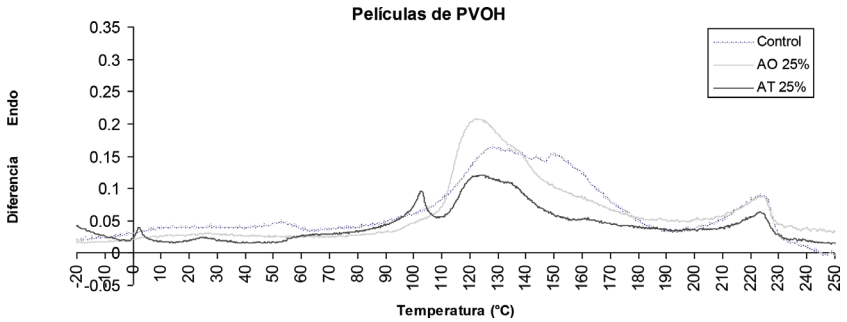
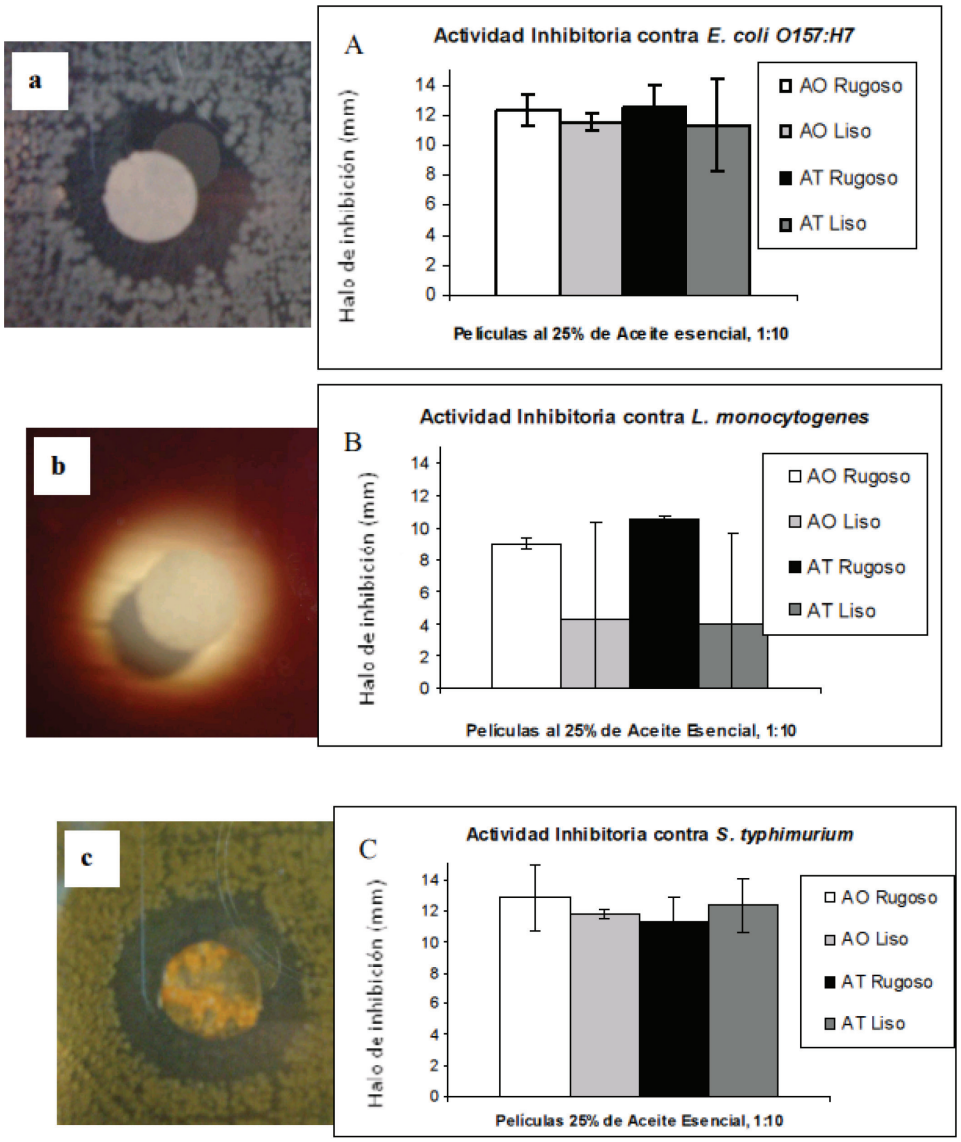


Figure 6.
Thermogram of PVOH films with inclusion complexes of OEO and TEO.

3.2.3 Antimicrobial activity of the active films in vitro against L. monocytogenes, S. typhimurium, and E. coli O157: H7

The films made with a concentration of 25% essential oil of oregano and thyme presented broad-spectrum antimicrobial activity by inhibiting the growth against the Gram-positive and Gram-negative microorganisms evaluated. The antimicrobial activity and the inhibition halo against *E. coli* O157: H7, *L. monocytogenes*, and *S. typhimurium* is shown in **Figure 7**. In this test the antimicrobial activity of both films was very similar against *E. coli* O157: H7 and *S. typhimurium*. The opposite occurred against *L. monocytogenes*, where the films with 25% of TEO showed higher antimicrobial activity than the films with 25% of OEO. The halos of inhibition against *E. coli* O157: H7 and *S. typhimurium* shown in **Figure 7(A)** and **(C)** suggest that the rough side of the film shows a slightly higher antimicrobial activity against microorganisms, a capacity that was confirmed in the analysis against *L. monocytogenes* (**Figure 7(B)**). The treatment of the film with the highest antimicrobial activity was that elaborated at 25% of essential oil, with the inclusion complex in molar ratio β-CD:EO 1:10 and evaluated by its rough side. In experimental designs not reported in this work, we could verify that both, the concentration of the essential oil (1, 4, 8, 15%) and the molar ratio of the inclusion complex β-CD:EO (1:2 and 1:5), are factors that influence the antimicrobial activity, as well as the speed of diffusion of the antimicrobial through the walls of the microcapsule [45].

The antimicrobial activity of the films is mainly due to the phenol group of carvacrol and p-cymene. The concentration of these compounds in the essential oils of oregano and thyme used for the production of films is shown in **Table 1**. The



Películas de PVOH elaboradas con 25% de Aceite de Orégano (AO) y de Tomillo (AT) con relación molar 1:10 (β-CD:AE) frente a *E. coli* O157:H7 (A), *L. monocytogenes* (B) y *S. typhimurium* (C) y sus halos de inhibición (a),(b) y (c) respectivamente.

Figure 7.
Antimicrobial activity of PVOH fims with inclusion complexes against pathogenic bacteria. 7a against E. coli, 7b against L. monocytogenes, and 7c against S. typhimurium.

phenol group is essential for bacterial inhibition, since it destabilizes the cytoplasmic membrane and also functions as a proton exchanger which reduces the pH gradient in the membrane and causes cell collapse and death [46–48]. The destabilization of the membrane occurs because carvacrol and thymol have affinity for lipids and accumulates in the bilayer between fatty acid chains, which causes changes in the conformation of the membrane. This mechanism of action does not present p-cymene; however, it has been found to have a synergy with phenols, expanding the membrane and destabilizing it [46]. The position of the hydroxyl group in the phenolic compounds does not seem to influence the degree of antimicrobial activity so that the activity of carvacrol and thymol is similar.

Concentration*	Oregano Essential Oil	Thyme essential oil
Phenolic compounds (carvacrol and thymol)	77.7%	54.05%
Terpene compounds (p-cymene and other compounds)	20.07%	41.34%

*Concentrations determined from the GC analysis of the supplier´s quality certificates

Table 1.
Concentration of phenolic compounds in the OEO and TEO.

3.2.4 Analysis of the control of L. monocytogenes in fresh cheese packed during storage in a refrigerated rack

The antimicrobial activity of the PVOH films with inclusion complexes of oregano and thyme in a model with fresh cheese inoculated with *L. monocytogenes* and stored in a rack refrigerated at 4°C was evaluated. As seen in **Figure 8**, the pathogenic microorganism shows inhibition when it is packed with the films developed with the antimicrobial agents, since the fresh cheese develops fewer colonies than that packaged with PVOH control or with the multilayer film under vacuum. The concentration of *L. monocytogenes* gradually decreased in the cheeses packaged with the active pads as shown in **Figure 8**. After 15 days of storage, the cheese packed with the films with oregano and thyme no longer had a microbial count; this performance was better than in the cheeses packaged with the vacuum multilayer film (red line) that did present a microbial account. This fact would have been interpreted as that the films developed with inclusion complexes of oregano and thyme presented bactericidal activity against *L. monocytogenes* in cheese, in refrigerated shelf after 15 days of exposure. Unfortunately, the films do not have bactericidal activity since colonies were detected on days 22 and 26 of storage. For this reason, it is concluded that the active films have a bacteriostatic control against *L. monocytogenes*. It is also observed in **Figure 8**, that the vacuum multilayer film showed greater control of the microbial load of the inoculated cheese, than the package with PVOH film without essential oils. This is due to the fact that in vacuum packaging with multilayer film, the oxygen available in the head space is reduced, together with the gas impermeability of the film, preventing microorganisms from developing. *L. monocytogenes* in particular is

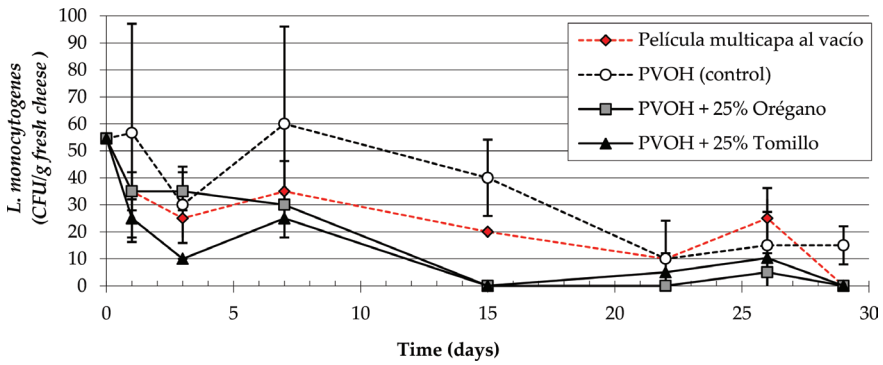


Figure 8.
Antimicrobial activity of PVOH films with inclusion complexes against fresh cheese inoculated with L. monocytogenes in refrigerated storage. PVOH films with 25% of OEO and TEO incorporated as inclusion complex. PVOH and multilayer vacuum packages were used as controls.

an aerobic bacterium, which is why the vacuum-packed product has control over its development during the time of storage.

3.3 Conclusions

Active PVOH films were obtained with essential oils of oregano and thyme, which showed broad-spectrum antibacterial activity by inhibiting pathogenic Gram-positive and Gram-negative bacteria specifically against *L. monocytogenes*, *E. coli* O157: H7, and *S. typhimurium*. The best conditions for the production of active films were 25% essential oil and elaboration of inclusion complex with a relation of 1:10 β-CD:EO. The active pad elaborated in the aforementioned conditions presented bacteriostatic activity against *L. monocytogenes* in cheese inoculated, packaged, and stored at 4°C for 29 days. The proposed packaging system ("pad" of the developed active film and a low-density polyethylene bag) can be an alternative to vacuum packaging using a multilayer film for cheeses. The experimental results showed that they provide a shelf life equivalent to vacuum packaging. In addition to the control of microbial activity, the proposed system is more accessible to small cheese producers as no special packaging technology is required other than a heat sealer machine. Likewise, the proposed packaging system can help reduce the incidence of outbreaks of diseases transmitted by foods contaminated with *Listeria monocytogenes*.

4. Antimicrobial agents from plants of the northeast of Mexico

As Mexico is a country that stands out for its floristic richness and taking into account the extensive knowledge of medicinal plants that since the pre-Columbian Era conserve Mexicans, mainly those of rural communities, it was natural that we were interested in the study of incorporation of some of them as active substances to be included in polymer matrices for food packaging.

4.1 *Larrea tridentata* plant as an alternative source for obtaining antimicrobial extracts

The flora of arid zones represents a great potential for the wealth based on its biological specialization, since it is the product of thousands of years of physiological adaptation for its survival [50]. The governor plant (*L. tridentata*) typically develops under conditions of these zones. The *Larrea* genus includes five species of evergreen shrubs distributed throughout the Americas; its name was given in honor of the Spanish cleric Juan Antonio Hernández Larrea who was dean of the Zaragoza Chapter and bishop of Valladolid. This plant is commonly known as the governor, due to its dominance in the large areas of the arid zones of northern Mexico, but it is also known as guamis, sonora, tasajo, jarilla, creosote, and hediondilla due to its characteristic smell, mainly after the rain. In the Seri language, it is called "haaxat," and in the English language it has the common names of "creosote bush" and "greasewood" [49, 50].

The governor plant has a wide range of adaptation in elevation since it is located in the Valley of Death in California located 86 m below sea level, to more than 2500 m in the sierras of northern Mexico. Its growth is good in dry plains and plateaus, also around hills and slopes, and in several types of soils except clayey, saline, or granitic [50]. The lifetime of this plant is negatively correlated with disturbance and soil compaction, being intolerant to soils with high phosphorus content [49]. In Mexico, the distribution of the governor plant is in part of the Sonoran Desert,

which includes the states of Baja California, Baja California Sur and Sonora, and in the Chihuahuan Desert, which includes the states of Chihuahua, Durango, Coahuila, Nuevo León, Zacatecas, and San Luis Potosí [50]. The infusion of the whole *L. tridentata* plant especially of the branches is used in urinary tract disorders to undo the kidney stones. The firing of branches, root, and bark is used to treat discomforts such as kidney pain and bladder inflammation. The decoction of the leaves is suggested in vaginal washes in gynecological problems such as female sterility. The infusion of branches, root, and bark is used in baths to treat hemorrhoids, fever, malaria, pimples, bumps, good healing, and rheumatism. And the infusion of the leaves is used as a remedy for gallstones, rheumatism, dermatitis, hepatitis, antiseptic, gastric discomforts, venereal diseases, and tuberculosis, in addition to having antiamoebic activity [49, 51, 52].

Among the uses that the governor plant has traditionally had, it stands out in its use as an antioxidant that was given to it in the United States since 1943; although in the decade of the 1990s, it was suspended by the US Food and Drug Administration (FDA) due to the strong interaction of nordihydroguaiaretic acid (NDGA) comment on results that were not found in the extracts studied, with several enzymatic processes. NDGA inhibits enzymatic activity, in addition to inhibiting the signaling pathway of lipoxygenase in which arachidonic acid generates leukotrienes and other oxygenated products [53–55].

4.2 *Cordia boissieri* plant as an alternative source for obtaining antimicrobial extracts

The anacahuita plant (*Cordia boissieri*) is the official flower of the state of Nuevo León, México. The genus *Cordia* gets its name in honor of the sixteenth-century German botanist Valerius Cordus and the *boissieri* species gets its name in honor of the nineteenth-century French botanist Boissier [56]. This plant is commonly known by the names of anacahuita, Mexican olive, Texas olive, wild olive, trompillo, and rasca viejo [56]. *C. boissieri* is a shrub or small tree up to 5 m high, with ovate leaves, 15–20 cm long and velvety surface. The flowers are white, grouped from 5 to 8, with the yellow center, up to 45 mm in length. The fruit is ovoid from 25 to 30 mm, brownish-green to purple, fleshy, sweet, and contains 1–4 seeds [56]. This plant species is native to North America. It is mainly distributed in Mexico, in the states of Nuevo León, Coahuila, Tamaulipas, San Luis Potosí, and Veracruz, and in the State of Texas in the United States. There are reports that the fruits of the *C. boissieri* plant are used as a remedy for coughs and colds. Traditionally the leaves of the plant are used to treat rheumatism and bronchial problems. Also in traditional medicine, the flowers are used in the treatment of diseases of bacterial origin [51, 56, 57].

4.3 *Leucophyllum frutescens* plant as an alternative source for obtaining antimicrobial extracts

The ash plant (*Leucophyllum frutescens*) is an evergreen shrub. This plant is commonly known, in Mexico, with the name of ash and in the United States with the names of Texas ranger, Texas sage, silverleaf, and barometer bush, because the flowering is triggered by moisture [58]. It is a grey bush of 1.5–2 m in height. The silver-grey and green leaves are covered with silver hair. The violet to purple flowers are bell-shaped or funnel with five lobes and two lips and reach to measure 2.5 cm in length, which appear intermittently from spring to autumn. The fruit has the shape of a small capsule [58]. This plant is native to northern Mexico and the Southwestern United States. It is a species that is part of the medium and high bushes that develop

preferably in *lomeríos* of capricious soils [59]. Reports were found that in traditional medicine the leaves of *Lecophyllum frutescens* are used in the treatment of diseases caused by bacteria [51]. No work has been found on the compounds present in *Leucophyllum frutescens* to which an antibacterial action against *S. aureus* can be attributed.

4.4 *Schinus molle* plant as an alternative source for obtaining antimicrobial extracts

The plant pirul (*Schinus molle*) is a perennial tree native to South America and naturalized in Mexico by Viceroy Antonio de Mendoza in the sixteenth century [60]. This plant is commonly known by the names of pirul in Mexico, aguaribay in Argentina, anacahuita in Uruguay, molle in Peru and, false pepper in Colombia [60]. It is a tree from 4 to 15 m high. The leaves are compound, alternate, 15–30 cm long, hung, with milky sap, and yellowish green. Its flowers are axillary panicles in the terminal leaves, 10–15 cm long, yellowish in color. The fruits are drupes in hanging clusters, each fruit 5–9 mm in diameter, pink or red [60]. *S. molle* is distributed, in Mexico, by the states of Aguascalientes, Chiapas, Coahuila, Federal District, Durango, Guanajuato, Guerrero, Hidalgo, State of Mexico, Jalisco, Michoacán, Morelos, Nuevo León, Oaxaca, Puebla, Querétaro, San Luis Potosí, Sinaloa, Tlaxcala, Veracruz, and Zacatecas. It is also naturalized in California, the Canary Islands, and China [61, 62]. It has been reported that the leaves of the *Schinus molle* plant serve to remedy respiratory diseases and for the treatment of skin wounds. For its part, the resin is also used to treat oral conditions [51, 60]. No works have been found on the compounds present in the leaves of the *Schinus molle* plant to which an antibacterial action against *S. aureus* can be attributed in an alcoholic extract.

4.5 Inhibition of *Staphylococcus aureus* with extracts of anacahuita (*Cordia boissieri*), governor (*Larrea tridentata*), ash (*Leucophyllum frutescens*), and pirul (*Schinus molle*) with potential application in active packaging

4.5.1 Introduction

Staphylococcus aureus is recognized as one of the main pathogenic agents for humans [63]. This microorganism is a natural inhabitant of the man's skin without causing damage to it, but when the skin's defenses diminish, it can cause a disease [64]. *S. aureus* produces abscesses and superficial lesions of the skin and causes impetigo, septicemia, and fevers, besides producing infections in the nervous system, endocarditis, and osteomyelitis [63]. It also has an extraordinary ability to develop resistance to antimicrobials and has the potential to cause viable infections to be fatal. It is responsible for 32–47% of infections in the skin and subcutaneous tissue [65]. Annually *S. aureus* causes around 100,000 deaths in hospitalized patients in the USA [66]. Several research groups have focused their studies on the antimicrobial activity of various natural extracts. Medicinal plants are considered a potential source of new drugs because of their phytochemical content and their little toxic effect [67]. Molina-Salinas et al. reported that the methanolic extracts of *Cordia boissieri* and *Leucophyllum frutescens* show inhibitory activity against *Streptococcus pneumoniae* and *Mycobacterium tuberculosis*, respectively, and that the hexanic extract of *Schinus molle* exhibits inhibitory activity against *S. aureus* [68]. For its part, Tello-Baca reported that the aqueous extract of *Larrea tridentata* has inhibitory activity against *Escherichia coli* and *S. aureus* [69].

The objective of the present investigation was to evaluate if the alcoholic extracts of the plants of anacahuita (*Cordia boissieri*), governor (*Larrea tridentata*), ash

(*Leucophyllum frutescens*), and pirul (*Schinus molle*) exhibit antimicrobial activity against *S. aureus* and select those with potential use in the formulation of active food packaging.

4.5.2 Materials and methods

Vegetal material: The first variable of this research was the origin of the plant material. The plants were obtained in two ways: collection and purchase. The collection was carried out in the municipality of García, Nuevo León, and Mexico, and the purchase was made at the San Judas Hierbería in Monterrey, Nuevo León, Mexico.

Preparation of the extracts: The flowers of *C. boissieri* and the leaves of *L. frutescens*, *L. tridentata*, and *S. molle* were used to prepare the extracts. The plants were subjected to a fine grind in a porcelain mortar. The samples were passed through a sieve with 1 mm mesh. The second variable is the nature of the solvent (ethanol and methanol, at 70% v/v). The extracts were prepared at 6% (w/v). The extraction of the active compounds was carried out by soaking for 15 min at 35°C on a stirring and heating plate (PMC). The solutions were left to stand for 48 h at room temperature in hermetically sealed containers protected from light. The extracts were filtered on Panama flax cloth to remove large particles. Subsequently, the samples were centrifuged at 7000 rpm for 10 min. Finally, a filtration in a Kitasato flask with Whatman paper No. 4 was carried out. The obtained extracts were stored at 4°C in glass containers, hermetically sealed and covered against light.

Evaluation of inhibitory activity: The inhibitory activity was evaluated by the disk diffusion method in Trypticase Soy Agar (Becton Dickinson) (NCCLS, 2003). The tested extracts were purchased *C. boissieri* extracted with ethanol (ACE), purchased *C. boissieri* extracted with methanol (ACM), collected *C. boissieri* extracted with ethanol (ARE), collected *C. boissieri* extracted with methanol (ARM), purchased *L. frutescens* extracted with ethanol (CCE), purchased *L. frutescens* extracted with methanol (CCM), *L. frutescens* collected extracted with ethanol (CRE), *L. frutescens* collected extracted with methanol (CRM), purchased *L. tridentata* extracted with ethanol (GCE), purchased *L. tridentata* extracted with methanol (GCM), *L. tridentata* harvested extracted with ethanol (GRE), *L. tridentata* harvested extracted with methanol (GRM), purchased *S. molle* extracted with ethanol (PCE), purchased *S. molle* extracted with methanol (PCM), *S. molle* collected extracted with ethanol (PRE), and collected *S. molle* extracted with methanol (PRM). *S. aureus* (ATCC 6538) was used at a concentration of 10^8 CFU/ml. Plates were incubated at 37°C for 24 and 48 h. Negative controls were used for ethanol and methanol, as the extraction solvent, and as positive controls, kanamycin (50 mg/ml) and chloramphenicol (34 mg/ ml), because they are broad-spectrum antibiotics. The tests were done in triplicate. The statistical analysis, to select the best extract of each of the plants, was carried out using the Kruskal-Wallis test.

Minimum inhibitory concentration: The tube dilution method was used to determine the minimum inhibitory concentration of the selected extracts (NCCLS, 2000). Five concentrations of each extract (100, 200, 300, 400, and 500 μl) were placed in tubes with 5 ml of Trypticase Soy liquid medium (Becton Dickinson) with 500 μl of *S. aureus* (10^8 CFU/ml). The tubes were incubated at 37°C for 24 h. The tests were performed in triplicate. To determine if the inhibitory activity of the extracts is bactericidal or bacteriostatic, two tests were performed: (a) *S. aureus* (10^8 CFU/ml) in liquid medium of Trypticase Soybean (Becton Dickinson) was placed in 50/50 ratio with each one of the extracts was reseeded on Trypticase Soy Agar (Becton Dickinson) by the swab technique and incubated 24 h at 37°C and (b) a sample of bacterial cells from the inhibition halo formed was reseeded on Trypticase Soy Agar with striatum (Becton Dickinson) by each of the extracts and incubated at 37°C for 24 h. The tests were performed in triplicate. To select the two plants with the greatest inhibition, a statistical analysis was performed using the Kruskal-Wallis test.

Extract	Diameter of inhibition halo (mm)	
	24 h	48 h
ACE	8.33 ± 1.53	11.66 ± 2.89
ACM	10.00 ± 1.00	8.00 ± 1.00
ARE	11.00 ± 1.73	12.00 ± 2.00
ARM	11.67 ± 2.87	12.00 ± 1.00
CCE	7.33 ± 0.58	7.67 ± 1.15
CCM	9.33 ± 1.15	8.33 ± 2.31
CRE	10.67 ± 2.52	10.67 ± 1.52
CRM	8.00 ± 2.00	10.00 ± 2.00
GCE	10.00 ± 2.00	16.33 ± 1.15
GCM	14.33 ± 1.15	18.33 ± 2.89
GRE	17.00 ± 1.00	18.00 ± 1.00
GRM	11.33 ± 1.15	14.67 ± 0.58
PCE	10.00 ± 3.00	11.33 ± 3.79
PCM	15.67 ± 2.08	16.00 ± 1.73
PRE	16.67 ± 1.53	18.00 ± 2.65
PRM	16.67 ± 1.53	16.00 ± 3.00
Ethanol	6.50 ± 0.70	6.00 ± 0.50
Methanol	6.50 ± 0.70	6.00 ± 0.50
Kanamycin	7.30 ± 0.20	7.10 ± 0.10
Chloramphenicol	0	0

Table 2.
Diameters of the inhibition halo against S. aureus of the alcoholic extracts of C. boissieri, L. frutescens, L. tridentata, and S. molle plants.

Characterization of the extracts: The two selected extracts were analyzed by gas chromatography (Agilent 6890)/mass spectrometry (Agilent 5973) (GC/MS). An HP-5MS column (30 m × 0.25 mm × 0.25 mm) was used for the separation of the components. Helium as a carrier gas has a flow rate of 15 ml/min. The injection temperature was 270°C in "split" mode. The temperature of the column, after a period of 1 min at 80°C, was increased to 320°C at a rate of 15°C min^{-1}, and maintained at

this temperature for 20 min. The mass spectrum had an ionization energy of 70 eV, a temperature of the ionization source of 230°C, and a quadrupole temperature of 150°C. The compounds were characterized with respect to the "Wiley7n.1" database.

4.5.3 Results

According to the obtained results in **Table 2**, we can observe that all alcoholic extracts of the plants *C. boissieri*, *L. frutescens*, *L. tridentata*, and *S. molle* have inhibitory activity against *S. aureus*. The tested extracts showed an increase in the diameter of the inhibition halo after 24–48 h (except for ACM and CCM extracts); this may be due to the fact that increasing the contact time increases the diffusion of the active compounds toward the middle. From the *C. boissieri* plant extracts, ACE (48 h), MCA (24 h), ARE (24 and 48 h), and MRA (24 and 48 h) showed a significantly higher inhibition than controls ($p < 0.05$), being the ARM extract (24 and 48 h) the one that showed the highest inhibitory activity against *S. aureus*.

Of the extracts of *L. frutescens*, CCM (48 h), CRE (24 and 48 h), and CRM (48 h) showed a significantly higher inhibitory activity than the controls ($p < 0.05$). CRE (24 and 48 h) was the extract with the highest inhibition against *S. aureus*. All evaluated extracts of *L. tridentata* showed a significantly higher inhibition than that of the controls ($p < 0.05$), presenting GRE (24 and 48 h) as the extract with greater inhibition against *S. aureus* and with a lower variance. In extracts of *S. molle*, PCM (24 and 48 h), PRE (24 and 48 h), and PRM (24 and 48 h) showed a significantly higher inhibition than that of the controls ($p < 0.05$), finding that PRE (24 and 48 h) is the extract with greater inhibitory activity against *S. aureus*.

Table 3 shows the minimum inhibitory concentration of the extract with the highest inhibition of each of the plants studied. The extract of the plant *L. tridentata* had the lowest minimum inhibitory concentration on *S. aureus* with 20 μl/ml, followed by the extract of *S. molle* with 80 μl/ml. Extracts of *C. boissieri* and *L. frutescens* had the highest minimum inhibitory concentration with 100 μl/ml each. It was also determined that the extracts of *L. tridentata* and *S. molle* have inhibitory activity against *S. aureus* of the bactericidal type and that the extracts of *C. boissieri* and *L. frutescens* show inhibitory activity of bacteriostatic type. **Figure 9** shows the comparison of the inhibition diameters of the extracts with greater inhibition of each of the plants studied. The GRE (24 and 48 h) and PRE (24 and 48 h) extracts have a significantly higher inhibitory activity than the ARM extracts (24 and 48 h) and CRE (24 and 48 h) ($p < 0.05$).

From GC/MS of *L. tridentata* extract (GRE), 12 compounds were identified, being 9,12-octadecanoic acid and 3,4′, 5,6,7-pentahydroxyflavone at 20.36 and 32.44 min, respectively, compounds they identified with greater certainty. Of the *S. molle* extract (PRE), 10 compounds were identified by GC/MS, with α-pinene compounds being

Extract	Minimum inhibitory concentration (μl/ml)
ARM	100
CRE	20
GRE	100
PRE	80

Table 3.
Minimum inhibitory concentration of the extracts of C. boissieri, L. frutescens, L. tridentata, and S. molle plants with the highest inhibition against S. aureus.

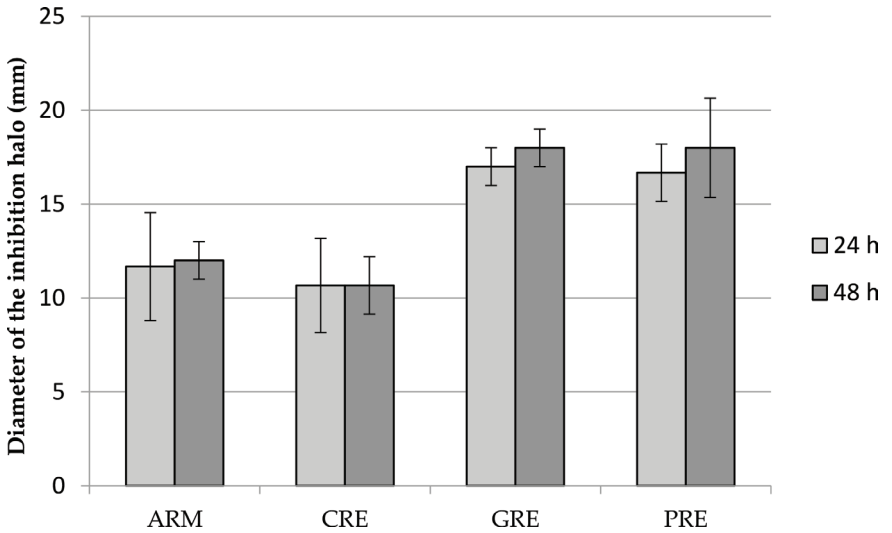


Figure 9.
Diameter of the inhibition halo of the extracts with greater inhibition against S. aureus of each of the plants C. boissieri, L. frutescens, L. tridentata, and S. molle. ARM, C. boissieri collected extracted with methanol; CRE, L. frutescens harvested extracted with ethanol; GRE, L. tridentata harvested extracted with ethanol; and PRE, S. molle harvested extracted with ethanol.

identified with greater certainty at 5.07 min, camphene at 5.30 min, p-mentha-1.5-comes at 6.12 min, p-mentha-1 (7), 2-diene at 6.51 min, 3 (15), 6-caryophylladiene at 12.15 min, 1 (10), 4 (15), 5-germacratriene a 12.92 min, 1 (10), 4-candinadiene at 13.39 min, 1.3-elemandien-11-ol at 13.71 min, and 4.9-cadinadiene at 16.27 min. The presence of 9, 12-octadecanoic acid and 3, 4 ', 5, 6, 7-pentahydroxyflavone was identified in the ethanolic extract of *L. tridentata* harvested and α-pinene; camphene; p-mentha-1, 5-diene; p-mentha-1 (7), 2-diene; 3 (15), 6-caryophyldiene; 1 (10), 4 (15), 5-germacratriene; 1 (10), 4-candinadiene; 1, 3-elemandien-11-ol; and 4, 9candinadiene in the ethanol extract of *S. molle* collected.

4.5.4 Discussion

For the four plants evaluated, there is greater inhibition in the extracts formulated with the harvested plants than with the purchased plants, since in the purchased plants, the storage time and the management that has been given are not known. In the extracts of the plants *L. frutescens*, *L. tridentata*, and *S. molle*, the ethanol was the solvent with which greater diameters were obtained in the inhibition zone. In the extracts of the *C. boissieri* plant, the solvent that allowed greater inhibition was methanol. The best inhibition results were obtained from the extracts of *L. tridentata* and *S. molle* plants. These plants represent a great antimicrobial potential; because as a plant that develops in arid conditions, it has a richness based on its biological specialization, since it is the product of thousands of years of physiological adaptation for its survival [50]. The inhibitory activity of *L. tridentata* can be attributed to the interaction of compounds present in the extracts, among which the 3,4′-5,6,7-pentahydroxyflavone exhibits an important role because the flavonoids are compounds with recognized antimicrobial activity [70]. The results obtained indicate that the four extracts exhibit antimicrobial activity, whether bactericidal or bacteriostatic. The activity observed was always greater than that of the controls (70% ethanol (E), kanamycin at a concentration of 50 mg/ml (K) and chloramphenicol at a concentration of 34 mg/ml (F)). Of the four extracts analyzed, that corresponding to *L. tridentata* showed the highest antimicrobial activity and the

lowest minimum inhibitory concentration. The extract corresponding to *C. boissieri* showed the lowest antimicrobial activity and the highest minimum inhibitory concentration in relation to the other extracts.

The presence of 9, 12-octadecanoic acid and 3, 4 ', 5, 6, 7-pentahydroxyflavone was identified in the ethanolic extract of *L. tridentata* harvested and α-pinene; camphene; p-mentha-1, 5-diene; p-mentha-1 (7), 2-diene; 3 (15), 6-caryophyldiene; 1 (10), 4 (15), 5-germacratriene; 1 (10), 4-candinadiene; 1, 3-elemandien-11-ol; and 4, 9 candinadiene in the ethanol extract of *S. molle* collected.

In a previous work, Sáenz-Collins demonstrated that it was possible to obtain active antimicrobial PVOH biofilms against *S. aureus*, with potential use as dressings due to their biocompatibility. The extracts have no effect on the formation of biofilms. It was found that the higher the concentration of the extract in the biofilm, the greater the inhibition against *S. aureus*. Also, it was demonstrated that the alcoholic extracts had antimicrobial activity against Gram-negative bacteria as *Salmonella* and *E. coli* [71]. The drying temperature of the biofilm shows a diminishing effect on the antimicrobial activity; however, this remains present. It was demonstrated that the alcoholic extracts based on methanol and ethanol of *L. tridentata* show antimicrobial activity against *S. aureus* and that the ethanolic extract is more active. Sáenz-Collins also verified, through gas chromatography coupled to a mass spectrometer, that all the extracts of the governor plant possess an important amount of compounds with potential antimicrobial activity such as 4-vinylguaiacol, 4-hydroxybenzoic acid, and norisoguaiacin [71]. Although these plants can be purchased in some traditional local markets for medicinal use, their potential as a source of natural antimicrobial agents for use in active food packaging must be further investigated.

4.5.5 Conclusions

In the present work, it was demonstrated that the alcoholic extracts (ethanolic and methanolic) of the plants *C. boissieri*, *L. tridentata*, *L. frutescens*, and *S. molle* have inhibitory activity against *S. aureus*. The ethanolic extracts of the harvested plants show a greater halo of inhibition, being *L. tridentata* and *S. molle* the plants that present the best results. The results obtained indicate that the four extracts exhibit antimicrobial activity, whether bactericidal or bacteriostatic. Of the four extracts analyzed, that corresponding to *L. tridentata* showed the highest antimicrobial activity and the lowest minimum inhibitory concentration. The extract corresponding to *C. boissieri* showed the lowest antimicrobial activity and the highest minimum inhibitory concentration in relation to the other extracts. It was demonstrated that the antimicrobial activity of *L. tridentata* and *S. molle* is bactericidal and have potential use in active food packaging.

Author details

Cecilia Rojas de Gante*, Judith A. Rocha and Carlos P. Sáenz Collins
Departamento de Bioingeniería, Tecnológico de Monterrey, Campus Ciudad de México, Ciudad de México, Mexico

*Address all correspondence to: crd@itesm.mx

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

[1] Brody AL. Active packaging becomes more active. Food Technology. 2005;**12**:82-84

[2] Han JH. Antimicrobial food packaging. Food Technology. 2000;**54**(3):56-65

[3] Suppakul P, Miltz J, Sonneveld K, Bigger SW. Active packaging technologies with an emphasis on antimicrobial packaging and its applications. Journal of Food Science. 2003;**68**(2):408-420

[4] Vermeiren L, Devlieghere F, van Beest M, de Kruijf N, Debevere J. Developments in the active pakaging of foods. Trends in Food Science & Technology. 1999;**10**(3):77-86

[5] Lopez-Rubio A, Almenar E, Hernandez-Munoz P, Lagaron JM, Catala R, Gavara R. Overview of active polymer-based packaging technologies for food applications. Food Reviews International. 2004;**20**(4):357-387

[6] Alvarez García LY. Películas flexibles a base de sorgo (*Sorghum bicolor* Moench) como alternativa a envases plásticos y evaluación de sus propiedades barrera y mecánica [ITESM Tesis de Maestría]. Monterrey, N.L.: Centro de Biotecnología ITESM; 1999

[7] Schause AP. Envases biopoliméricos activos en nisina obtenidos a partir de almidón y harina de sorgo (*Sorghum bicolor* Moench) [Tesis de Maestría]. Monterrey N.L., México: Centro de Biotecnología ITESM; 2002

[8] Schause A, Rojas de Gante C. Active edible films from sorghum starch and flour with nisin. In: Worldpak 2002. Improving the Quality of Life through Packaging Innovation. Proceedings of the 13th IAPRI Conference on Packaging. Vol. I, Book 2. East Lansing (USA); 2002. pp. 819-825

[9] Valderrama-Solano AC, Rojas de Gante C. Two different processes to obtain antimicrobial packaging containing natural oils. Food and Bioprocess Technology. 2012;**5**:2522-2528

[10] Wang Y, Padua GW. Tensile properties of extruded zeinsheets and extrusion blown films. Macromolecular Materials and Engineering. 2003;**288**(11):886-893

[11] Corradini E, Felix AJ, Da Silva AA, Marcondes JA, Caparelli LH. Preparation and characterization of thermoplastic starch/zein blends. Materials Research. 2007;**10**(3):227-231

[12] Di Maio E, Mali R, Iannace S. Investigation of thermoplasticity of zein and kafirin proteins: Mixing process and mechanical properties. Journal of Polymers and the Environment. 2010;**18**(4):626-633

[13] Espino-Díaz M, Ornelas-Paz J, Martinez-Tellez MA, Santillán C, Barbosa-Cánovas GV, ZamudioFlores PB, et al. Development and characterization of edible films based on mucilage of *Opuntiaficus-indica* (L.). Journal of Food Science. 2010;**75**(6):E347-E352

[14] Bower CK, McGuire J, Daeschel MA. Influences of the antimicrobial activity of surface adsorbed nisin. Journal of Industrial Microbiology. 1995;**15**:227-233

[15] Ríos-Licea J. Envases biopoliméricos activos en sorbato de potasio y extractos naturales de orégano y ajo obtenidos a partir de harina de sorgo (*Sorghum bicolor* Moench) y evaluación de su actividad antimicrobiana frente a S.typhimurium, L.monocytogenes y *E. coli* [Tesis de Maestría]. Monterrey N.L., México: Centro de Biotecnología ITESM; 2008

[16] Tinoco-Pérez B. Cuantificación de antocianinas en envases biopolimericos activos con potencial acción antioxidante a partir de harina de maíz azul (*Zea mais* amilacea) [Tesis de Maestría]. Monterrey, N.L.: Tecnológico de Monterrey; 2007

[17] SAGARPA. Producción de Maíz en México [Internet]. 2009. Available from: http://www.sagarpa.gob.mx/agricultura

[18] SIAP. Cierre de la producción agrícola por cultivo [Internet]. 2009. Available from: http://www.siap.gob.mx/index.php?option=com_wrapper&view=wrapper&Itemid=350

[19] De la Rosa-Millán J. Análisis fisicoquímico, estructural y molecular de almidones de diferentes variedades de maíz azul [Tesis Maestría en Ciencias en desarrollo de productos bióticos]. Yautepec (México): Instituto Politécnico Nacional, Dependencia; 2009. pp. 1-12

[20] Dawson PL, Hirt DE, Rieck JR, Acton JC, Sotthibandhu A. Nisin release from films is affected by both protein type and film-forming method. Food Research International. 2003;**36**(9-10):959-968

[21] Pushpadass HA, Marx DB, Hanna AM. Effects of extrusion temperature and plasticizers on the physical and functional properties of starch films. Starch-Starke. 2008;**60**(10):527-538

[22] Wang Y, Rakotonirainy AM, Padua GW. Thermal behavior of zein-based biodegradable films. Starch-Starke. 2003;**55**(1):25-29

[23] López OV, Zaritzky NE, García MA. Physicochemical characterization of chemically modified corn starches related to rheological behavior, retrogradation and film forming capacity. Journal of Food Engineering. 2010;**100**(1):160-168

[24] Zeng M, Huang Y, Lu L, Fan L, Lourdin D. Effects of filler-matrix morphology on mechanical properties of corn starch-zein thermomoulded films. Carbohydrate Polymers. 2011;**84**(1):323-328

[25] Yang Q, Lue A, Qi H, Sun Y, Zhang X, Zhang L. Properties and bioapplications of blended cellulose and corn protein films. Macromolecular Bioscience. 2009;**9**(9):849-856

[26] Financiera Rural. Monografía del Sorgo [Internet]. Available from: http://www.financierarural.gob.mx/informacionsectorrural/Documents/Monografias/MonografiaSorgo%28jun11%29.pdf

[27] U.S. Grains Council. Sorghum [Internet]. 2010. Available from: http://www.grains.org/index. php?option=com_content&view=article&id=74&Itemid=120

[28] Rojas de Gante C, Rios-Licea J, Tinoco-Pérez B. Method for producing biofilms from cereal grains, biofilms obtained said method and use thereof for producing receptacles for containing and preserving foods. Patent No. WO2010/024657 A1. 2010

[29] Parra D, Tadini C, Ponce P, Lugao A. Mechanical properties and water vapor transmission in some blends of cassava starch edible films. Carbohydrate Polymers. 2004;**58**(4):475478

[30] Cecilia Rojas-de G, Grissel Trujillo-de S, Carlos Patricio Sáenz C, Andrea Valderrama S. Desempeño de una película de maíz azul en el envasado de un queso de humedad intermedia. Revista de Biotecnologia en el Sector Agropecuario y Agroindustrial Ed. Especial No. 2. 2013:49-58

[31] Valderrama Solano AC, Rojas de Gante C. Development of biodegradable films based on blue corn flour with potential applications in food packaging.

Effects of plasticizers on mechanical, thermal, and microstructural properties of flour films. Journal of Cereal Science. 2014;**60**:60-66

[32] Trujillo-de Santiago G, Rojas-de Gante C, García-Lara S, Verdolotti L, Di Maio E, Iannace S. Strategies to produce thermoplastic starch–zein blends: Effect on phase compatibilization. Journal of Polymers and the Environment. 2015;**22**(4):508-524

[33] Trujillo-de Santiago G, Rojas-de Gante C, García-Lara S, Verdolotti L, Di Maio E, Iannace S. Thermoplastic processing of blue maize and white sorghum flours to produce Bioplastics. Journal of Polymers and the Environment. 2015;**23**(1):72-82

[34] Valderrama ACS, Rojas de GC. Traceability of active compounds of essential oils in antimicrobial food packaging using a chemometric method by ATR-FTIR. American Journal of Analytical Chemistry. 2017;(8):726-741

[35] MacDonald PDM, Whitwam RE, Boggs JD, MacCormack JN, Anderson KL, Reardon JW, et al. Outbreak of listeriosis among Mexican immigrants as a result of consumption of illicitly produces Mexican-style cheese. Clinical Infectious Diseases. 2005;**40**:677-682

[36] Indu MN, Hatha AAM, Abirosh C, Harsha U, Vivekanandan G. Antimicrobial activity of some of the South-Indian spices against serotypes of *Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and *Aeromonas hydrophila*. Brazilian Journal of Microbiology. 2006;**37**:153-158

[37] Martínez LM, Videa M, Mederos F, Mesquita J. Constructing a high-sensitivity, computerinterfaced, differential thermal analysis device for teaching and research. Journal of Chemical Education. 2007;**84**(7):1222-1223

[38] Rojas JJ, García AM, López AJ. Evaluación de dos metodologías para determinar la actividad antimicrobiana de plantas medicinales. BLACMA. 2005;**4**(2):28

[39] Pasterán F, Galas M. Manual de procedimientos, Sensibilidad a los antimicrobianos en Salmonella, Shigella y *E. coli*. Centro Regional de Referencia WHO- Global Salm. Surv. Para América del Sur. 2008

[40] Suppakul P, Sonneveld K, Bigger SW, Miltz J. Efficacy of polyethylene-based antimicrobial films containing principal consituents of basil. LWT Food Science and Technology. 2008;**41**:779-788

[41] Norma Oficial Mexicana NOM-110-SSA1-1994 – Bienes y servicios. Preparación y dilución demuestras de alimentos para su análisis microbiológico

[42] Ponce-Cevallos P, Buera M, Elizalde B. Encapsulation of cinnamon and thyme essential oils components (cinnamaldehyde and thymol) in β-cyclodextrin: Effect of interactions with water on complex stability. Journal of Food Engineering. 2010;**99**(1):70-75

[43] Dahmane M, Athman F, Sebih S, Guermouche MH, Bayle JP, Boudah S. Effect of the chain length on the thermal and analytical properties of laterally biforked nematogens. Journal of Chromatography A. 2010;**1217**(42):6562-6568

[44] Holland BJ, Hay JN. The thermal degradation of poly (vinyl alcohol). Polymer. 2001;**42**:67756783

[45] Rocha J. Desarrollo de películas de alcohol polivinílico (PVOH) con aceites esenciales naturales, para ser usados como "pads" (almohadillas) activos antimicrobianos en el envasado de quesos de cabra [Tesis de Maestría]. Monterrey N.L., México: Centro de Biotecnología ITESM; 2011

[46] Ultee A, Bennik MHJ, Moezelaar R. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Applied and Environmental Microbiology. 2002;**68**(4):1561-1568

[47] Burt S. Essential oils: Their antibacterial properties and potential applications in foods, a review. International Journal of Food Microbiology. 2004;**94**:223-253

[48] Lambert RJW, Skandamis PN, Coote PJ, Nychas GJE. Study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Journal of Applied Microbiology. 2001;**91**:453-462

[49] Vázquez-Yanes C, Batis-Muñoz AI, Alcocer-Silva MI, Gual-Díaz M, Sánchez-Dirzo C. Árboles y arbustos potencialmente valiosos para la restauración ecológica y la reforestación. Reporte técnico del proyecto J084. CONABIO - Instituto de Ecología, UNAM; 1999

[50] Lira-Saldivar RH. Estado actual del conocimiento sobre las propiedades biocidas de la Gobernadora [*Larrea tridentata* (D.C.) Coville]. Revista Mexicana de Fitopatología. 2003;**21**(2):214-222

[51] Adame J, Adame H. Plantas curativas del noreste mexicano. Monterrey: Ediciones Castillo; 2000

[52] García-Llamas F. La gran enciclopedia de las plantas curativas. México: Editorial Diana; 2005

[53] Bronfman M. Nuevos Factores de Transcripción Nuclear. Santiago: Pontificia Universidad Católica de Chile; 2002

[54] Bruneton J. Farmacognosia: fitoquímica, plantas medicinales. Zaragoza: Acribia; 2001

[55] FDA/CFSAN. Illnesses and Injuries Associated With the Use of Selected Dietary Supplements. Miami: Dockets Management Branch Food and Drug Administration; 1993

[56] Alvarado MA, Foroughbakhch R, Jurado E, Rocha A. Caracterización morfológica y nutricional del fruto de anacahuita (*Cordia boissieri*) en dos localidades del Noreste de México. Phyton, International Journal of Experimental Botany. 2004;**73**:85-90

[57] Hernández-Magaña R, Gally-Jorda M. Plantas medicinales. México: Árbol Editorial; 1981

[58] Irish M. Trees and Shrubs for the Southwest. China: Congrees; 2008

[59] Villegas-Durán G, Bolaños-Medina A, Miranda-Sánchez JA, García-Aldape J, Galván-García OM. Flora nectarífera y polinífera en el estado de Tamaulipas. México: SAGARPA; 2003

[60] Hernández-Vázquez J, Sánchez-González S, González-Pacheco C, Luna-Peñaloza SR, Durán-Carmona V, Toledo-Ocampo A, Rodríguez-Velasco A, González-Lorenzo JR. Programa ambiental de la Universidad Autónoma de la Ciudad de México. 2011. Recuperado de: http://desarrollo.uacm.edu.mx.html

[61] Rzedowski GC, Calderón J. Flora del Bajío y regiones adyacentes. Pátzcuaro: Instituto de Ecología-Centro regional del Bajío; 1999

[62] Villaseñor JL, Espinosa FJ. Catálogo de malezas de México. México: UNAM; 1998

[63] Ruocco E, Donnarumma G, Baroni A, Tufano MA. Bacterial and viral skin diseases. Dermatologic Clinics. 2007;**25**(4):663-676

[64] Richardson AR, Libby SJ, Fang FC. A nitric oxide-inducible lactate dehydrogenase enables *Staphylococcus aureus* to resist innate immunity. Science. 2008;**319**(5870):1672-1676

[65] Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN. Survey of infections due to Staphylococcus species: Frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY antimicrobial surveillance program, 1999. Clinical Infectious Diseases. 2001;**32**(2):S114-S132

[66] Demain A, Sánchez S. Microbial drug discovery: 80 years of progress. The Journal of Antibiotics. 2009;**62**:5-16

[67] Beg AZ, Ahmad I. Effect of *Plumbago zeylanica* extract and certain curing agents on multidrug resistant bacteria of clinical origin. World Journal of Microbiology and Biotechnology. 2000;**16**(8-9):841-844

[68] Molina-Salinas GM, Pérez-López A, Becerril-Montes P, Salazar-Aranda R, Said-Fernández S, Waksman-de Torres N. Evaluation of the flora of Northern Mexico for in vitro antimicrobial and antituberculosis activity. Journal of Ethnopharmacology. 2007;**109**(3):435-441

[69] Tello-Baca R. Actividad antibacteriana de plants con actividad hemaglutinante [QFB dissertation]. Morelia, México: Universidad Michoacana de San Nicolás de Hidalgo; 2009

[70] Cushnie T, Lamb AJ. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents. 2005;**26**:343-356

[71] Sáenz-Collins CP. Aprovechamiento de la planta gobernadora (*Larrea tridentata*) para la obtención de un agente activo antimicrobiano frente a *Staphylococcus aureus* con aplicación en biopelículas de alcohol polivinílico para uso médico en forma de apósitos [Tesis] (Maestría en Ciencias). 2009