World Applied Sciences Journal 32 (10): 2092-2101, 2014
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2014.32.10.86248

Antimicrobial Activity of Edible Methyl Cellulose Films Enriched with Essential Oils Against Three Common Foodborne Pathogens

[1]Hamdy A. Shaaban, [2]Khaled F. Mahmoud, [2]Mona A. Ibrahim and [1]Gamil Ibrahim

[1]Chemistry of Flavour and Aroma NRC, ElBouhouth st., Dokki, Cairo, Egypt
[2]Food Science and Technology Departments, NRC, ElBouhouth st., Dokki, Cairo, Egypt

Abstract: Essential oils are effective antimicrobials on some important pathogenic bacteria and can be added to packaging materials due to their absorbance on various surfaces. In this study, the aim is to determinate of antimicrobial effects of methyl cellulose films containing various proportions of clove, marjoram, cinnamon, coriander and cumin oils (0.5%, 1% and 1.5% v/v) against three foodborne pathogens. Commercial MC (viscosity = 4,000 cP), Polyethylene glycol and tween 80 were used to conduct research. Essential oils; Clove (*Syzgium aromaticum*), cumin (*Cuminum cyminum*), marjoram (*Origanum majorana*), cinnamon (*Cinnamomum zeylanicum*) and coriander (*Coriandrum sativum*) were extracted by hydro-distillation. The antibacterial effects of clove, coriander, marjoram, cinnamon and cumin essential oils were studied against three important food pathogens; *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* by application of agar diffusion method. Also, antimicrobial effectiveness of films were studied by using tryptone soy agar with 3% NaCl (Sigma Aldrich, Germany) as a model solid food system (TSA-NaCl). The results revealed that the intensity of antimicrobial efficacy of Eos was in the following order: marjoram > clove > cinnamon > coriander > cumin. In the next step, the three most potent essential oils were subsequently incorporated into methyl cellulose films. The antibacterial effectiveness of the prepared films against *E. coli*, *S. aureus* and *L. monocytogenes* was studied during 12 days. The antibacterial activity of the essential oils was maintained when incorporated into the methyl cellulose film. The nature and amount of the essential oils play an important role in the film's antimicrobial activity. In all film matrices, marjoram showed the highest antimicrobial activity. Films with 1.5% marjoram decreased the numbers of *L. monocytogenes*, *E. coli* and *S. aureus* populations with respect to the control up to 6.33, 4.52 and 5.80 log, respectively. In Conclusion: The edible MC films with EOs incorporated were more effective against gram-positive bacteria (*S. aureus* and *L. monocytogenes*) than gram-negative bacteria (*E. coli*). Furthermore, complete growth inhibition was observed for the films containing the highest concentration of marjoram essential oil in each of the three pathogens utilized in this study.

Key words: Antimicrobial Activity · Methyl Cellulose · Edible Film · Food Packing

INTRODUCTION

In recent years, due to the increased interest in minimally processed foods depending on consumer demand, use of new technologies and approaches started in the packaging industry. The active packaging technique is the most prominent in these technologies. It can be considered an emerging technology that could have a significant impact on the shelf life extension and food safety. The Active packaging includes engagement of the various active components through the packaging material. The active component, antimicrobial becomes active in various architectures such as the synthetic polymer and the edible film and coatings [1].

Nowadays, there is a widespread use of traditional packaging material made of synthetic structure. These synthetic materials are safe, convenient and economical but they are not biodegradable, so they are one of the factors of environmental pollution. For this reason, researchers have focused on the use of proteins, lipids and polysaccharide polymers that may be biologically degradable and easily consumed with food

Corresponding Author: Hamdy A. Shaaban, Chemistry of Flavour & Aroma NRC, ElBouhouth st., Dokki, Cairo, Egypt.

and offer more environmental alternatives as packaging materials. In this context, the use of edible films and coatings containing antimicrobial components is increasingly widespread and adopted [2]. Cellulose is the most abundant renewable resource and its derivatives have excellent film-forming properties. Cellulose derivative based edible films are very efficient barriers to oxygen and aroma compound barriers [3]. Cellulose derivative is an MC (methyl cellulose), is of interest to researchers because they are able to form a continuous matrix. MC is cellulose that exhibits thermal gelation, forms excellent films and is used in pharmaceutical and food industries [4]. MC has been combined with lipids [5- 8] and polysaccharides [7, 9] to improve edible films that can serve as effective barriers to hydrocarbon, oxygen and water vapor. Polyethylene glycols are effective plasticizers for MC films [10, 11].

Essential oils and their components are commonly used as flavoring in the food industry. Also, they have antimicrobial and antioxidant properties. There are some studies explaining compounds responsible for the major antimicrobial and antioxidant effects of essential oils. For example, thymol, eugenol and carvacrol are the main components responsible for the antimicrobial activity of basil and thyme oils [12, 13]. Similarly, Lambert *et al*. [14] reported that essential oils containing a high percentage of phenolic components, such as carvacrol, thymol and eugenol, present stronger antibacterial properties against foodborne pathogens. These compounds are able to disintegrate the outer membrane of Gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of cytoplasmic membrane to ATP. According to Smith-Palmer *et al*. [15], Gram positive bacteria are slightly more sensitive to essential oils than Gram negative bacteria. However, not all studies on essential oils concluded than Gram positive bacteria are more susceptible to their activity [14]. The chemical composition of essential oils varies according to various factors such as the geographical origin and harvesting period. This variation could be enough to explain the variability in the degree of susceptibility of Gram negative and Gram positive bacteria [12]. Essential oils which their antimicrobial activity has been proven by many studies may show antimicrobial activity taking place within the food packaging materials such as methyl cellulose edible films [16]. Use of antimicrobial agents such as essential oils in edible food packaging can control the microbial population and target specific microorganisms to provide higher safety and quality products [1]. The major advantage of this technology is that the diffusion rate of the antimicrobial agents can be slowed down. Thus, edible films can extend the shelf life of products by keeping high concentrations of active components on the product surface [12].

The present study had two goals. First, it tested the antimicrobial properties of six selected essential oils against three common pathogenic foodborne bacteria including *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes*, to select the most effective EOs in the enrichment of methyl cellulose based films by agar diffusion method. Second, this study assigned the effect of essential oil incorporation in the antimicrobial properties of methyl cellulose films to evaluate the effectiveness of prepared films against the three above-mentioned foodborne pathogens evaluated at 10°C during a storage period of 12 days.

MATERIALS AND METHODS

Materials: Commercial MC (viscosity = 4,000 cP) was purchased from Sigma Aldrich. Polyethylene glycol and tween 80 were acquired from Merck. Essential oils; Clove (*Syzgium aromaticum*), cumin (*Cuminum cyminum*), marjoram (*Origanum majorana*), cinnamon (*Cinnamomum zeylanicum*) and coriander (*Coriandrum sativum*) were extracted by hydro-distillation from the dried samples by the Clevenger type apparatus and the obtained oils were stored in a dark container at 4°C until used.

Bacterial Strain and Maintenance: Stock culture of *Listeria monocytogenes* (ATCC 35152), *Staphylococcus aureus* (ATCC 43300) and *Escherichia coli* (ATCC 27325) were obtained from the culture collections of the Microbiological Dept. National Research Center (NRC), Dokki, Giza, Egypt. All strains were stored in brain heart infusion (BHI) broth supplemented with 30% glycerol at –20°C until used. Subculturing was carried out every 30 days to maintain bacterial viability. The thawed culture (0.1 ml) was transferred to 10 ml of BHI broth and grown in a shaker incubator at 37°C for 24 h. A second transfer of 0.1ml of culture into 10ml of BHI broth was grown in a shaker incubator at 37°C for 24 h to the end of the exponential phase of growth. Subsequently, these appropriately diluted cultures were used for the inoculation of agar plates in order to obtain a target inoculum of 10^2 CFU/ml.

Antimicrobial Activity of Essential Oils: The antibacterial properties of the five mentioned essential oils were studied using the agar diffusion method [17]. 30 µl of the essential oils were poured into an agar well (5 mm diameter), previously created with a sterile core bore on the agar, after their plates had been seeded with 0.1 ml of inoculums by swab containing approximately 10^6 CFU/ml of the indicated bacteria. All the strains were cultured in tryptone soy agar. The plates were incubated at 37°C for 48 h in the suitable incubation chamber. After incubation, the microbial growth was observed and the degree of inhibition was expressed as follows: (–) no inhibition, weak activity (zone of inhibition≤12mm), moderate activity (zone of inhibition≤20mm) and strong activity (zone of inhibition≥20mm [18, 19].

Preparation of Antimicrobial Methyl Cellulose Films: The MC films were prepared by the method of Turhan and sahbaz [4]. 3% MC was dissolved into the ratio of 2:1 distilled water and ethanol and rotary shaking was undertaken concurrently for 30 min. As the edible MC film was brittle, 33% of Polyethylene glycol (PEG 400) was added to the edible film solution. Then Tween 80, at a level of 0.25 g/g of essential oil, was added as an emulsifier to aid essential oil dissolution in the MC film-forming solution, After 30 min of stirring, essential oils Clove, marjoram and cinnamon at 0.5, 1 and 1.5% v/v concentration were added to the MC film-forming solution. The solution was homogenized with a homogenizer at room temperature for 2 min at 7000 rpm [20]. The solution was kept overnight at 4°C in order to remove all bubbles. Fourteen grams of every solution were cast on the glass plates then dried in room temperature. Dried films were peeled from the plates and stored in a desiccator at 25–27°C and 50±2% relative humidity until evaluation.

Antimicrobial Effectiveness of Films: The methodology was adapted from [21]. Tryptone Soy Agar (TSA) with 3% NaCl (SigmaeAldrich, Germany) was used as a model solid food system (TSA-NaCl). Aliquots (20 g) of TSA-NaCl were poured into Petri dishes (9 cm diameter). After the culture medium solidified, aliquots (0.1 ml) of the properly diluted culture were inoculated on the surface of TSA-NaCl. Then, different test films (containing or without antimicrobial substances) of the same diameter as the Petri dishes were placed on the inoculated surface. Inoculated uncoated TSA-NaCl was used as control. Plates were then covered with parafilm to avoid dehydration and stored at 10°C for 12 days. Instead of using 4°C, a typical refrigerated storage temperature, a relative high temperature was used so that the efficacy of antimicrobial film against mentioned pathogens could be determined in a relatively shorter period of time. Microbial counts on TSA-NaCl plates were examined immediately following inoculation and periodically during the storage period. To this end, the agar was removed aseptically from the Petri dishes and placed in a sterile glass container. 100 ml of 0.9% NaCl solution was added to each container and homogenized for 3 min, which resulted in a very homogeneous system. Serial dilutions were made and then poured onto TSA. Plates were incubated at 37°C for 48 h before colonies were counted. All tests were done in triplicate.

Statistical Analysis: The statistical analysis of the data was performed through SPSS version 16.0. Quantitative data were represented in form of mean ± standard deviation (SD). Analysis of Variance (ANOVA) was used in the analysis of the results. Duncan's multiple range test was used to determine any significant differences in mean log CFU/cm^2 among treatments at a 95% confidence interval. The *p*-value of the test was ≤ 0.05.

RESULTS

Antimicrobial Activity of the Essential Oils: The qualitative antimicrobial activity of the essential oils is shown in Table 1. Marjoram essential oil presented the highest inhibitory effect. Cumin essential oil was least effective against the three studied pathogens. The intensity of antimicrobial efficacy was in the following order: marjoram > clove > cinnamon > coriander > cumin. Among the three tested pathogens, *E. coli* were the most resistant. Based on inhibition zone test results, marjoram, clove and cinnamon Eos were selected for further study.

Antimicrobial Effectiveness of Films

***Listeria monocytogenes*:** Fig. 1 shows the growth curves of *L. monocytogenes* on control TSA-NaCl plates and TSA-NaCl plates covered with EOs enriched films. The counts of *L. monocytogenes* on the control TSA-NaCl plates increased substantially from the initial inoculation level of 2–7.86 log CFU/cm^2 by the end of the experiment. Similarly, Kristo *et al*. [22], reported that *L. monocytogenes* grew approximately 8 log on TSA-NaCl plates when stored at 10°C for 12 days. No significant differences ($p < 0.05$) were observed between growths of *L. monocytogenes* on control TSA-NaCl plates and plates

Table 1: Antibacterial activity of the EOs against the three tested bacterial strains

	Inhibition zone diameters (mm)				
Microorganisms / Essential oil	Marjoram	Clove	Cinnamon	Coriander	Cumin
Listeria monocytogenes	35	25	20	16	15
Staphylococcus aureus	40	28	21	18	17
Escherichia coli	19	17	15	12	10

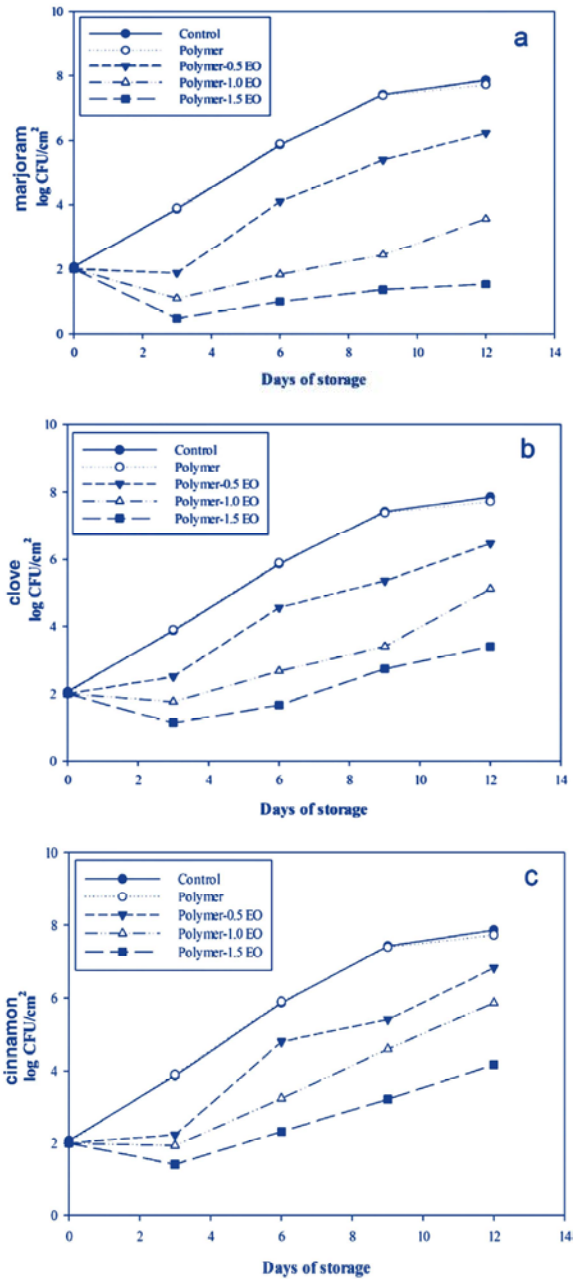

Fig. 1: Antibacterial activity of different films containing marjoram (a), clove (b) and cinnamon (c) against *Listeria monocytogenes* on TSA-NaCL medium stored at10 °C.

coated with the methyl cellulose film during the storage period. The absence of antimicrobial activity of pure methyl cellulose films has been reported in other studies [23, 24]. Results indicated that the methyl cellulose films containing EOs were effective in reducing the growth of *L. monocytogenes*. In this study, the methyl cellulose composite containing marjoram essential oil demonstrated the lowest *L. monocytogenes* population ($p < 0.05$) in comparison with other active methyl cellulose composites (containing clove and cinnamon essential oils at the same concentration) on inoculated TSA-NaCl plates. Lis-Balchin and Deans [25], reported the potent antimicrobial activity of marjoram against the *L. monocytogenes* strain. With the highest concentration of marjoram (1.5%), a nearly total inhibition of the pathogen growth occurred during the storage period. When 1% of the marjoram essential oil was incorporated into the film matrix, methyl cellulose composite films displayed less antimicrobial activity, so that total inhibition of pathogen growth occurred during the first 7 days of the storage period. Subsequently, the *L. monocytogenes* population increased up to 3.6 log UFC/cm^2 at the end of the storage period. As can be seen in Fig. 1a, following a storage period of 12 days, films with 1% marjoram reduced microbial growth with respect to the control to 4.3 log. As expected, the antimicrobial effect was less marked with 0.5% marjoram, where a microbial reduction of approximately 1.63 log, as compared to the control plates, was observed during the storage period. Fig. 1b showed that methyl cellulose films containing clove essential oil had less effect in controlling *L. monocytogenes* growth than methyl cellulose films containing marjoram essential oil. At the highest concentration of clove (1.5%), a nearly total inhibition of the pathogen growth occurred during the first 7 days of the storage period and this film reduced the population of *L. monocytogenes* more than 4.2 log UFC/cm^2 compared with the control plates. In the presence of 1% clove, a complete inhibition of microbial growth was observed during the first 3 days of the storage period. Similar to 0.5% marjoram, the 0.5% cinnamon essential oil was not sufficient to control the microbial growth for the entire storage period. Methyl cellulose films containing cinnamon essential oil showed

a less marked antimicrobial activity than methyl cellulose containing marjoram and clove (Fig. 1c). The highest level of cinnamon oil (1 and 1.5%) led to a complete inhibition of *L. monocytogenes* growth for the first 3 days; then the *L. monocytogenes* population increased to 5.86 and 4.16 log CFU/cm^2 at the end of the storage period, respectively.

In the presence of 0.5% cinnamon, *L. monocytogenes* population increased to 6.83 log CFU/cm^2 at the end of the storage period. These results are in agreement with those of (26), when analyzing the anti-listerial activities of chitosan film containing tea tree essential oil against *L. monocytogenes* in TSA-NaCl plates. Their results demonstrated that the incorporation of tea tree essential oil into chitosan matrix (2% w/w) improves the antilisterial effect of chitosan.

***Staphylococcus aureus*:** Fig. 2 shows the growth curves of *S. aureus* on control TSA-NaCl plates and on TSA-NaCl plates covered with the antimicrobial methyl cellulose film stored for 12 days at 10°C. No significant differences $p < 0.05$) were observed between growths of *S. aureus* on control TSA-NaCl plates and on plates covered with the EOs-free film during storage. These findings indicate that the methyl cellulose film had no inhibitory effect on the growth of *S. aureus* (similar to *L. monocytogenes* and *E. coli*). Regardless of the type of essential oil, incorporating this agent in the film matrix increased the antimicrobial activity of film and the application of EO-enriched films resulted in a significant inhibition of *S. aureus* counts after 12 days of storage at 10°C (more than 3 log UFC/cm^2). During the whole storage period, films containing marjoram were more effective ($p < 0.05$) than films containing clove or cinnamon in reducing the growth of *S. aureus* (Fig. 2a). After 12 days of storage, the *S. aureus* population reached a value of 7.36 log UFC/cm^2 in the control samples, while the use of films enriched with the highest concentration of marjoram maintained the population of *S. aureus* under the initial inoculation level. In addition, the use of films containing 1% marjoram resulted in a significant reduction of the *S. aureus* population during the entire storage period ($p < 0.05$) and a total inhibition of the pathogen growth occurred during the first 9 days of the storage period.

As can be seen in the Fig. 2c, although methyl cellulose films containing cinnamon showed less antimicrobial activity in comparison to other active methyl cellulose, the highest level of cinnamon essential oil (1.5%) led to a total inhibition of *S. aureus* growth for 6

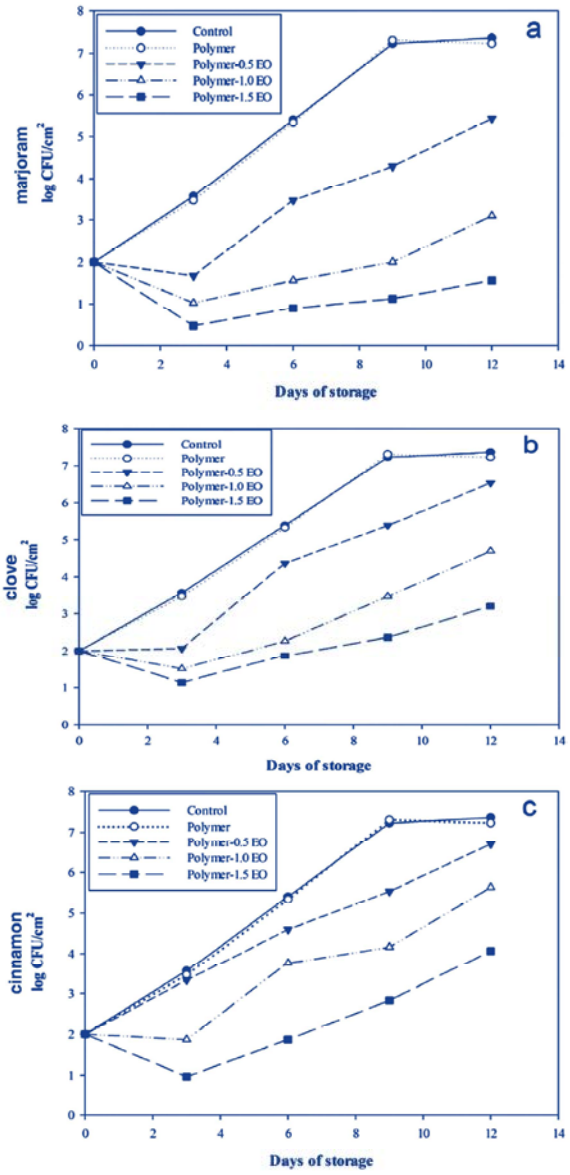

Fig. 2: Antibacterial activity of different films containing marjoram (a), clove (b) and cinnamon (c) against *Staphylococcus aureus* on TSA-NaCL medium stored at10°C.

days, reaching a reduction of approximately 3.3 log at the end of the storage period. The lower cinnamon concentration levels (0.5) showed less marked antimicrobial activity so that a pathogen reduction, as compared to the control plates, reached about 0.67 and 1.73 log, respectively, at the end of the storage period.

***Escherichia coli*:** Fig. 3 shows the growth curves of *E. coli* on control TSA-NaCl plates and on TSA-NaCl plates covered with the EOs-enriched films. The counts of

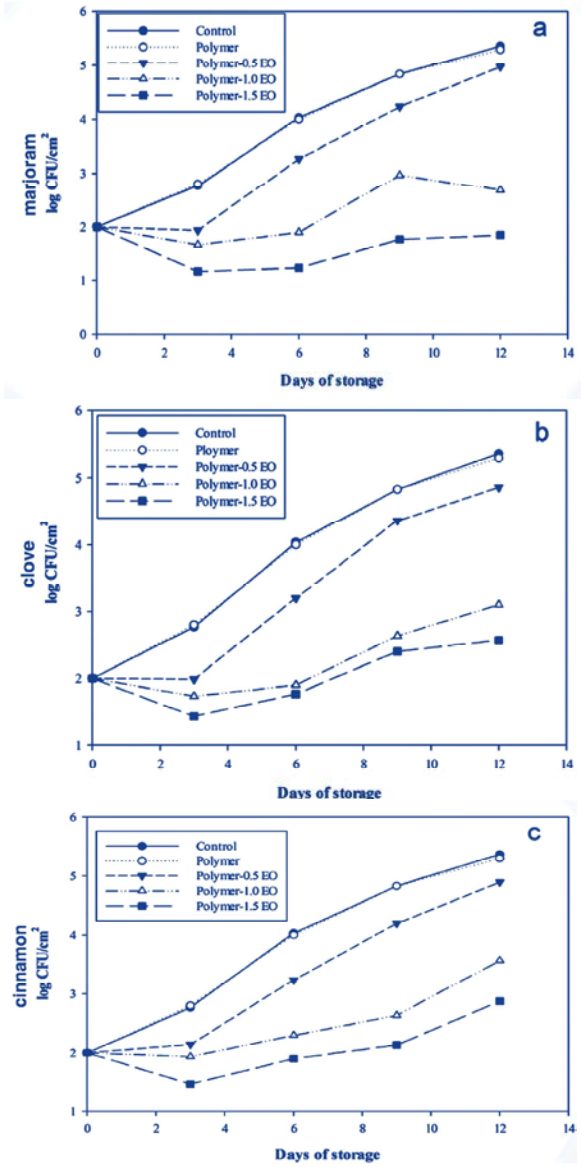

Fig. 3: Antibacterial activity of different films containing marjoram (a), clove (b) and cinnamon (c) against *Escherichia coli* on TSA-NaCL medium stored at10 °C.

E. coli in the control TSA-NaCl plates increased substantially from the initial inoculation level of 2 to 5.36 log CFU/cm^2 by the end of the experiment. This pathogen was less affected by the active films than other microorganisms. As can be seen from the growth curve, no significant differences were observed among the growths of *E. coli* on the control TSA-NaCl plates and on plates covered with the methyl cellulose film during the storage period. Similar to data obtained for the *L. monocytogenes*, marjoram was the most effective antimicrobial to control the growth of *E. coli* and in the highest concentration of marjoram (1.5%), a total inhibition of pathogen growth occurred during the storage period (Fig. 3a). In the presence of 1% marjoram, a complete inhibition of microbial growth was observed during the first 6 days of the storage period and this film reduced the population of *E. coli* more than 2.68 log CFU/cm^2 compared with the control TSA-NaCl plates at the end of the study period. Clove incorporated into methyl cellulose films reduced pathogen growth. Microbial growth was totally inhibited by the highest concentration of clove (1.5%) during the first 6 days of the study period although the antimicrobial effectiveness decreased afterwards. However, at the end of the storage period, a pathogen reduction, as compared to the control plates, of about 2.26 and 2.8 log was observed for the 1% and 1.5% concentrations of clove, respectively (Fig. 3b).

At the end of the 12-day storage at 10°C, *E. coli* reached populations of 4.86 log UFC/cm^2 on TSA-NaCl plates covered with films containing 0.5% clove. Methyl cellulose films containing cinnamon essential oil (Fig. 3c) showed less marked antimicrobial activity than methyl cellulose film containing marjoram and clove. However, the incorporation of cinnamon in methyl cellulose films also resulted in significantly lower ($p < 0.05$) *E. coli* counts in comparison to the control TSA-NaCl plates. The reduction in pathogen growth was consequent to increasing the cinnamon concentration of the film. Cinnamon at the highest concentration (1.5%) totally inhibited pathogen growth for the first 6 days of storage. Furthermore, at the end of the storage period, *E. coli* counts on TSA-NaCl plates covered with film containing 1% and 1.5% cinnamon were at 1.8 and 2.49 log CFU/cm^2, respectively, considerably lower than that of the control TSA-NaCl plates (5.36 log CFU/cm^2). 0.5% cinnamon was not sufficient for controlling the growth of *E. coli* and the population of pathogens increased to 4.9 CFU/cm^2 at the end of the study period. However, this value was lower than population of *E. coli* on the control TSA-NaCl plates.

DISCUSSION

Results obtained showed that of the 3 essential oils tested in this study, marjoram, clove and cinnamon exhibited strong antibacterial activities and among the three tested pathogens, *E. coli* were the most resistant. Gómez-Estaca *et al*. [27] showed that clove, Lavender, thyme and rosemary had strong and consistent inhibitory effects against various pathogens and spoilage

microorganisms. In addition Winward *et al*. [28] reported that cinnamon essential oil had a good inhibitory effect on pathogenic bacteria such as *E. coli* and *S. aureus*. The lower antimicrobial activity against *E. coli* can be attributed to the fact that Gram-negative bacteria are in general more resistant due to the external lipopolysaccharide wall surrounding the peptidoglycan cell wall [29]. The mechanism of antimicrobial activity of essential oils is related with the attack on the phospholipid present in cell membranes, which causes increased permeability and leakage of cytoplasm, or in their interaction with enzymes located on the cell wall [27]. Thus, the resistance of Gram negative bacteria to the essential oils likely lies in the protective role of their cell wall lipopolysaccharides or outer membrane proteins. Moreover, the antimicrobial activity of EOs depends on the type of spice or herb, the chemical composition and the content of extracts and essential oils [30]. Chemical composition of EOs is complex and strongly dependent on the variety of plant, the part of the plant considered (e.g., seed vs. leaves), origin, time of harvest, the harvesting season and processing, as well as storage conditions [29, 31]. The major components in EOs are phenolic substances, which are thought to be responsible for the antimicrobial properties. In this regard, the major components in marjoram, cinnamon and clove are Terpinen-4-ol, Cinnamaldehyde and Eugenol, respectively. Sublethal concentrations of Eugenol and Cinnamaldehyde have been found to inhibit production of amylase and proteases by *Bacillus cereus*. Cell wall deterioration and a high degree of cell lysis were also noted [32]. In the next step of study, in order to form the active composite films, marjoram , cinnamon and clove essential oils were incorporated into methyl cellulose films at several concentrations (0.5, 1.0 and 1.5% w/v) and antibacterial activity of them was evaluated against *L. monocytogenes*, *E. coli* and *S. aureus*. When antimicrobial agents such EOs are incorporated into food packaging films, these materials diffuse through agar gel and result in a clear zone around the film discs [33]. In this regards, when EOs were incorporated into the film matrix, the antimicrobial activity of Eos was maintained but EOs showed less antibacterial activity in film forming solution in comparison with pure essential oil. The causes that would explain this result could be the lower amount of EOs in the film solution in comparison with the well test for pure EOs as well as partial loss of volatile compounds during film preparation [26, 33]. Fisher and Phillips noted that essential oils contain around 85-99% volatile and 1-15% non-volatile components [34]. According to Sánchez-González, *et al.* [35] various factors affect the antimicrobial action of film, such as the nature of EOs, type of bacteria, characteristics of the film matrix, method and manufacturing conditions of films. Like the disk test result, the antimicrobial activity of methyl cellulose films varied distinctively depending on the nature and amount of the essential oils and microorganisms tested. In this sense, a composite film containing marjoram was most effective in controlling the growth of the pathogens. The reasons for the generally higher efficacy of marjoram over the other investigated EOs are mainly attributed to marjoram's high content of phenolic compounds as well as good interaction between the constituents of the marjoram with the polymer matrix [30, 36]. As above mentioned, concerning the type of bacteria, given their extra protective outer membrane, gram-negative bacteria usually are considerably more resistant to antibacterial agents than their gram-positive counterparts. So the active composite films showed lower antimicrobial activity against *E. coli* in comparison with the gram-positive bacteria (*L. monocytogenes, S. aureus*). Several authors observed in vitro studies that the incorporation of different essential oil into methyl cellulose films improved its antimicrobial properties. Benavides, *et al*. [23] showed this effect in oregano methyl cellulose against several strains as *E. coli*, *L. monocytogenes*, *Salmonella enteritidis* and *S. aureus*. The combination of essential oil with methyl cellulose is also effective to control the growth of pathogens in food products (*in vivo*). In this sense, Oussalah, *et al.*[37] observed a good inhibition of *Salmonella Typhimurium* and *E. coli* O157:H7 on whole beef muscle coated with methyl cellulose films containing 1% (w/v) of Spanish oregano, Chinese cinnamon, or savory essential oils. Another factor that affects the antimicrobial activity of active films is polymer structure. Some studies have reported controlled-release or diffusion of various antimicrobial agents including carvacrol [38], thymol [39] and rosemary Eos[33] from polymer composite films. In the present study, during the first days of the storage period, the concentration of the EOs was a significant factor in the inhibition of pathogens growth. It must be highlighted that, as storage time increased, the film effectiveness decreased. As can be seen from the growth curve, in all pathogens, growth was reduced in the 3 initial days of the storage period and then the population of bacteria increased until the end of the storage period. This phenomenon can be explained by the evaporation of volatile compounds responsible for the antimicrobial activity and/or by the migration of EOs components into the agar medium [35]. On other hand,

after the films were placed on the inoculated surface of TSA-NaCl, the composite film hydrophilic matrices absorbed water, which induced changes in the film structure. This condition facilitates the evaporation and liberalization of EOs components from the polymer matrix. Consequently, the effectiveness of the films tends to decrease during the storage period.

CONCLUSION

The antimicrobial effects of pure EOs of clove, coriander, marjoram, cinnamon and cumin against *E. coli*, *S. aureus* and *L. monocytogenes* were determined by an agar diffusion test. Marjoram essential oils showed the highest antimicrobial effect, followed by clove and cinnamon, respectively. Next, the antimicrobial films were prepared by incorporating different concentrations of marjoram, clove and cinnamon into methyl cellulose films. During the storage period in this study, the concentration of the EOs, rather than the type of EOs, was the most relevant parameter in the inhibition of all 3 pathogens' growth. The results also revealed that the films with EOs incorporated were more effective against gram-positive bacteria (*S. aureus* and *L. monocytogenes*) than gram-negative bacteria (*E. coli*). Furthermore, complete growth inhibition was observed for the films containing the highest concentration of marjoram essential oil in each of the three pathogens utilized in this study.

REFERENCES

1. Perez-Perez, C., C. Regolado-Gonzales, C.A. Rodriquez-Rodriquez, J.R. Barbosa-Roderiquez and F. Villasenor-Ortega, 2006. Incorporation of antimicrobial agents in food packaging films and coatings. In: Advances in Agricultural and Food Biotechnology. Eds., Guevara-Gonzales, R.G. and and I. Torres-Pacheco., Chapter, 9: 193-216.
2. Ayana, B., 2007. Production and characterization of antimicrobial edible films, M. S. thesis, Mersin University, Institute of Science, Department of Food Engineering.
3. Debeaufort, F. and A. Voilley, 1997. Methylcellulose-based edible films and coatings: 2. Mechanical and thermal properties as a function of plasticizer content. J. Agric. Food Chem., 45: 685-689.
4. Turhan, K.N. and F. Şahbaz, 2004. Water vapor permeability, tensile properties and solubility of methylcellulose-based edible films, Journal of Food Engineering, 61: 459-466.
5. Greener, I.K. and O. Fennema, 1989. Barrier Properties and Surface Characteristics of Edible, Bilayer Films. J. Food Science., 54: 1393-1399.
6. Kamper, S.L. and O. Fennema, 1984. Water Vapor Permeability of Edible Bilayer Films. J. Food Science, 49: 1478-1481.
7. Kester, J. and O. Fennema, 1989. An Edible Film of Lipids and Cellulose Ethers: Barrier Properties to Moisture Vapor Transmission and Structural Evaluation. J. Food Science, 54: 1383-1389.
8. Koelsch, C.M. and T.P. Labuza, 1992. Functional, physical and morphological properties of methyl cellulose and fatty acid-based edible barriers. Lebensmittel-Wissenschaft und-Technologie, 25: 404-411.
9. Park, H.J. and M.S. Chinnan, 1995. Effect of plasticizer level and temperature on water vapor transmission of cellulose-based edible films. J. Food Process Engineering, 18: 497-507.
10. Debeaufort, F. and A. Voilley, 1997. Methylcellulose-Based Edible Films and Coatings:? 2. Mechanical and Thermal Properties as a Function of Plasticizer Content. J. Agricultural and Food Chemistry, 45: 685-689.
11. Turhan, K.N. and F. Sahbaz, 2004. Water vapour permeability, tensile properties and solubility of methylcellulosebased edible films. J. Food Eng., 61: 459-466.
12. Sanchez-Gonzalez, L., M. Vargas, C. Gonzalez-Matinez, A. Chiralt and M. Chafer, 2011. Use of Essential Oils in Bioactive Edible Coatings. Food Eng. Rev., 3: 1-16.
13. Lee, S.J., K. Umano, T. Shibamoto and K.G. Lee, 2005. Identification of volatile components in basil (Ocimumbasilicum L.) and thymes leaves (Thymus vulgaris L.) and their antioxidant properties. Food Chem., 91: 131-137.
14. Lambert, R.J.W., P.N. Skandamis, P. Coote and G.J.E. Nychas, 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol, J. Appl. Microbiol., 91: 453-462.
15. Smith-Palmer, A., J. Stewart and L. Fyfe, 2001. The potential application of plant essential oils as natural food preservatives in soft cheese. Food Microbiol., 18: 463-470.
16. Khairruddin, K., 2005. Study on antimicrobial starch-based active packaging system, University of Technology of Chemical Engineering and Natural Resources Engineering, Department of Bioprocess Engineering, A Research, pp: 7.

17. Gómez-Estaca, J., A. López de Lacey, M. López-Caballero, M. Gómez-Guillén and P. Montero, 2010. Biodegradable gelatinechitosan films incorporated with essential oils as antimicrobial agents for fish preservation. Food Microbiology, 27: 889e896.
18. Lv, F., H. Liang, Q.P. Yuan and C.F. Li, 2011. In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. Food Research International, 44: 3057-3064.
19. Ait-Ouazzou, A., S. Loran, A. Arakrak, A. Laglaoui, C. Rota, A. Herrera, R. Pagan and P. Conchello, 2012. Evaluation of the chemical composition and antimicrobial activity of Mentha pulegium, Juniperus phoenicea and Cyperus longus essential oils from Morocco. Food Research International, 45: 313-319.
20. Ojagh, S.M., M. Rezaei, S.H. Razavi and M.H. Hosseini, 2010. Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water Food Chemistry, 122: 161-166.
21. Sánchez-González, L., M. Cháfer, M. Hernández, A. Chiralt and C. González-Martínez, 2011. Antimicrobial activity of polysaccharide films containing essential oils. Food Control, 22: 1302-1310.
22. Kristo, E., K.P. Koutsoumanis and C.G. Biliaderis, 2008. Thermal, mechanical and water vapor barrier properties of sodium caseinate films containing antimicrobials and their inhibitory action on Listeria monocytogenes. Food Hydrocolloids, 22: 373-386.
23. Benavides, S., R. Villalobos-Carvajal and J. Reyes, 2012. Physical, mechanical and antibacterial properties of alginate film: effect of the crosslinking degree and oregano essential oil concentration. Journal of Food Engineering, 110: 232-239.
24. Pranoto, Y., S. Rakshit and V. Salokhe, 2005. Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. LWT- Food Science and Technology, 38: 859-865.
25. Lis-Balchin, M. and S. Deans, 2003. Bioactivity of selected plant essential oils against Listeria monocytogenes. Journal of Applied Microbiology, 82: 759-762.
26. Sanchez-Garcia, M.D., A. Lopez-Rubio and J.M. Lagaron, 2010. Natural micro and nanobiocomposites with enhanced barrier properties and novel functionalities for food biopackaging applications. Trends in Food Science & Technology, 21: 528-536.
27. Gómez-Estaca, J., A. López de Lacey, M. López-Caballero, M. Gómez-Guillén and P. Montero, 2010. Biodegradable gelatinechitosan films incorporated with essential oils as antimicrobial agents for fish preservation. Food Microbiology, 27: 889-896.
28. Winward, G.P., L.M. Avery, T. Stephenson and B. Jefferson, 2008. Essential oils for the disinfection of grey water. Water Research, 42: 2260-2268.
29. Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods-review. International Journal of Food Microbiology, 94: 223-253.
30. Castellano, P., C. Belfiore, S. Fadda and G. Vignolo, 2008. A review of bacteriocinogenic lactic acid bacteria used as bioprotective cultures in fresh meat produced in Argentina. Meat Science, 79: 483-499.
31. Tajkarimi, M., S. Ibrahim and D. Cliver, 2010. Antimicrobial herb and spice compounds in food. Food Control, 21: 1199-1218.
32. Hosseini, M., S. Razavi and M. Mousavi, 2009. Antimicrobial, physical and mechanical properties of chitosan based films incorporated with thyme, clove and cinnamon essential oils. Journal of Food Processing and Preservation, 33: 727-743.
33. Abdollahi, M., M. Rezaei and G. Farzi, 2012. A novel active bionanocomposite film incorporating rosemary essential oil and nanoclay into chitosan. Journal of Food Engineering, 111: 343-350.
34. Fisher, K. and C. Phillips, 2008. Potential antimicrobial uses of essential oils in food: is citrus the answer? Trends in Food Science & Technology, 19: 156-164.
35. Sánchez-González, L., M. Cháfer, M. Hernández, A. Chiralt and C. González-Martínez, 2011. Antimicrobial activity of polysaccharide films containing essential oils. Food Control, 22: 1302-1310.
36. Papadokostaki, K., S. Amarantos and J. Petropoulos, 1998. Kinetics of release of particulate solutes incorporated in cellulosic polymer matrices as a function of solute solubility and polymer swellability. II. Highly soluble solute. Journal of Applied Polymer Science, 69: 1275-1290.
37. Oussalah, M., S. Caillet, S. Salmieri, L. Saucier and M. Lacroix, 2006. Antimicrobial effects of alginate-based film containing essential oils for the preservation of whole beef muscle. Journal of Food Protection, 69: 2364-2369.

38. Tunç, S. and O. Duman, 2011. Preparation of active antimicrobial methylcellulose/carvacrol /montmorillonite nanocomposite films and investigation of carvacrol release. LWT-Food Science and Technology, 44: 465-472.

39. Sánchez-García, M.D., E. Gimenez, M.J. Ocio and J.M. Lagaron, 2008. Novel polycaprolactone nanocomposites containing thymol of interest in antimicrobial film and coating applications. Journal of Plastic Film and Sheeting, 24: 239-251.