Packaging from renewable sources with antimicrobial properties: development and applications

L. Ramos da Silva[1], A. C. A. B. Casari[1], B. R. C. Santos[1], K. R. S. Avelino[1], M. Ferraz[1], P. N. Justi[1], R. P. Lima[1], J. I. Velasco[2] and F. M. Fakhouri[1,2]

[1] Faculty of Engineering (FAEN), Federal University of Grande Dourados (UFGD), Dourados, Brazil
[2] Centre Català del Plàstic, Universitat Politècnica de Catalunya, Terrassa, Spain

A large amount of packaging has been used and discarded daily, and most of them are discarded in the nature and require many years to decompose. The use of synthetic polymers, due to its advantages and processability, is widely diffused industrially on the development of packaging, however raw materials from renewable sources are an attractive alternative for this purpose. Polymers from renewable sources in the production of packaging results in a product capable of degrading in a shorter period of time when compared to traditional ones, therefore influencing for a greater sustainability in the world. Several chemical compounds exhibit antimicrobial behavior and they can be found in plant extracts or in essential oils. Therefore, in order to confer antimicrobial properties on edible films and coatings, it is possible to add in its composition plant extracts and / or essential oils from various plant species. Thus, this chapter is aimed to describe packaging from renewable sources with antimicrobial properties, antimicrobial agents capable to be added in theses packaging, the incorporation form of those agents and the application of an antimicrobial packaging in food science field.

Keywords: Antimicrobial agents; Edible films and coating; Packaging technology.

1. Introduction

A large amount of packaging has been used and discarded daily, and most of them are discarded in the nature and require many years to decompose. The use of synthetic polymers, due to its advantages and processability, is widely diffused industrially on the development of packaging, however raw materials from renewable sources are an attractive alternative for this purpose. Polymers from renewable sources in the production of packaging results in a product capable of degrading in a shorter period of time when compared to traditional ones, therefore influencing for a greater sustainability in the world.

Edible films and coatings are examples of packaging capable to be made by raw materials from renewable sources, such as hydrocolloids. Starches are natural polymers from plants, mainly in cereals and tubers, which have presented a thermoplastic behavior and have many applications in food science field. This type of system is widely applicable in various types of food products, such as fruits and vegetables, cheeses, shrimps, among others. The starch capacity to behave as a true thermoplastic, when submitted to pressure and high temperature, is the most attractive characteristic of this polysaccharide in many studies.

Primary function of a food packaging is its behavior as a barrier that separates the product from the environment, thus reducing its exposure to deterioration factors such as microorganisms, oxygen, luminosity, humidity, among others. These packagings may still exhibit ability to inhibit microbial growth, by the addition of antimicrobial agents in their composition.

Several chemical compounds exhibit antimicrobial behavior and they can be found in vegetable extracts or in essential oils. Therefore, in order to confer antimicrobial properties on edible films and coatings, it is possible to add in its composition vegetable extracts and / or essential oils from various plant species.

The addition of these agents in the formulation of a package have been largely studied by researchers, to prove its effectiveness during storage of the packaged product. Those agents can be carried out in a variety of ways, by the direct dispersion during the preparation of the formulations, or by more sophisticated techniques, like its use as microcapsules. Both forms have advantages and disadvantages, which are being widely studied by several researchers and reported in the literature.

These packagings with antimicrobial agents can be applied in many types of food and promote a greater conservation of the products, increasing their shelf life, and guarantee their microbiological safety for consumers.

Thus, this chapter is aimed to describe packaging from renewable sources with antimicrobial properties, antimicrobial agents capable to be added in theses packaging, the incorporation form of those agents and the application of an antimicrobial packaging in food science field.

2. Renewable packaging

The last decades were marked by changes in habits and an increase in consumerism, which boosted technological innovations and consequently to higher production of industrial things, also generating a growth in the production of packaging. Packagings are present in several sectors, with emphasis on the food industry, which packaging has as one of the main function to contribute for food preservation. Different materials are used in the production of food packaging, such as plastics, metals, glass, cellulose and biopolymers. Each material has its single characteristics to conserve the product, among them the main ones are property of barrier to gases, aroma, light, water, microorganisms and mechanical resistance. However, despite its advantages, its use and disorderly discard generates a large amount of solid waste, which is associated with environmental impact. Based on that, alternative ways have been sought to reduce these impacts, such as the reuse and recycling of packagings, as well as the development of them with green polymers and biodegradable materials [1].

In order to reduce the environmental impact caused by conventional packaging, there is a growing interest of the scientific community and the industrial sector in the development of biodegradable packaging obtained from renewable sources [2].

Among the inputs that can be used as raw materials for the production of biodegradable packaging are the materials of agricultural origin, which are the most used, since they are cheaper, available all year and renewable sources. Between the products of this segment, starch receives special attention due to it is a natural polymer that has the property of forming films and foams when gelatinized and dried. Regarding to Brazilian agricultural production, cassava stands out as a good source of starch, presenting high availability associated with a low cost [3]. Although globally there are corn starch and others with a large popularity for industrial use.

Biopolymers use in the production of biodegradable packaging can be done in three ways:

i) by the mix of biopolymers with synthetic polymers, which leads to an increase of the biofragmentation of the packaging, in other words, it increases its decomposition in nature;

ii) by using agricultural products or by-products as substrates in the production of biopolymers by fermentation (PHB production);

iii) by the use of biopolymers directly in the production of films, which make them edible and/or biodegradable.

Edible films must be thin and flexible, and made with biological macromolecules that form a continuous matrix containing food additives [4].

2.1 Edible films

Edible and / or biodegradable films are those of various thicknesses, consisted by different natural and / or synthetic substances that polymerize itself and isolate the food, without presenting risks to consumer health, since the body does not metabolize them and its passage through the gastrointestinal tract is innocuously done. The function that films will play depends on the food product and the type of deterioration that the food may suffer [5].

Among the main functions of edible films are the inhibition of the migration of moisture, oxygen, aromas, lipids, carbon dioxide, and others. In addition, as they can carry food additives and antimicrobial agents, they contribute for the integrity of food products and the characteristics of food handling [5, 6].

In 1970, researches were focused on the introduction of starch in synthetic polymer matrices (proportion of 5 to 20%), resulting in the production of plastics considered biofragmentable, but not fully biodegradable. From 1990 to nowadays, there was an increase in the interest in developing thermoplastic materials composed mainly of starch, with the addition of plasticizers to improve the mechanical properties. Those plasticizers need to be compatible with the biopolymer and their proportion in dry matter will depend on the degree of stiffness of the material [7, 8, 9].

Formulations of the edible films must contain at least one component (biopolymer) capable of forming a suitable, cohesive and continuous matrix. This basic component for the constitution of the formulations can be classified into three categories: polysaccharides (vegetable or microbial gums, starches, celluloses, etc.), lipids or proteins. Polysaccharides have good properties for the formation of films, as they have hydrophilic characteristics, providing efficient barriers against oils and lipids [10, 11, 12].

Film formation is based on the dispersion or solubilization of a macromolecule in a solvent (water, alcohol or organic acids) and the addition of additives (binding agents, plasticizers, etc.), resulting in a filmogenic solution. pH adjuster or other additives such as antioxidants or antimicrobial agents can be added in the filmogenic solution, if necessary. After prepared, this solution must be dried for the proper formation of the film. In this step, there is an increase of the concentration of the biopolymer in the solution, due to the evaporation of the solvent, leading to the aggregation of the molecules and, consequently, in the formation of a three-dimensional network [13].

A packaging in the form of a film, coating or protective layer is characterized as edible when it integrates the food and is consumed. As a consequence of this dual function of packing and food constituent, films and coatings offer several advantages such as being consumed directly with food, materials with low cost, possibility of individual packaging, improvements of the mechanical, sensorial and even nutritional properties [14, 4, 15].

2.2 Edible coatings

Edible coating is a thin layer of filmogenic solution applied and formed directly on the surface of the product. Likewise films, they can be classified as edible and / or biodegradable, depending on the constituents used for their production and the amount of substances used [16, 17].

Films and coatings have the function of inhibiting or reducing the migration of moisture, oxygen, carbon dioxide, lipids, aromas, among others, as they act as a semipermeable barriers. Furthermore, they may carry food ingredients such as antioxidants, antimicrobials and flavorings, and / or improve mechanical integrity as well as food handling characteristics [18].

The most used biopolymers in the elaboration of edible films and coatings are:
i) proteins (gelatin, casein, albumin, wheat gluten, zein and myofibrillar proteins);
ii) polysaccharides (starch and its derivatives, pectin, cellulose and its derivatives, alginate and carrageenan);
iii) lipids (acetylated monoglycerides, stearic acid, waxes and fatty acid esters) or the combination of them [19].

3. Antimicrobial agents

In recent years, many plants have demonstrated efficacy in safety, food preservation and as health promoters by its extracts or essential oils. Essential oils have the majority of their properties, though the extracts present some secondary metabolites that may have action in health or other applications [20, 21, 22].

3.1 Essential oils

Essential oils from diverse plants may have different antimicrobial activity, due to their distinct chemical compositions, therefore the essential oils isolated from the flowering and fruit-bearing stages have larger amounts of monoterpene hydrocarbons with allylic groups and ether, alcohol, aldehydes, ketones, esters and phenols than the essential oil isolated from the vegetative stage [23]. In addition, Feitosa-Alcantara et al.[24] have studied the chemical composition of *Hyptis pectinata* (L.) Poit essential oils from many places and they have seen the variety on the composition among the oils tested.

Essential oils antimicrobial properties can be partially attributed to their lipophilic character, which promote the accumulation in membranes and energy exhaustion in microorganisms. The compounds contained sensitize the phospholipid bilayer of the cell membrane, resulting in increased permeability and leakage of the vital intracellular constituents or impairment of the microbial enzymatic system [25, 26, 27]. Several mechanisms were proposed to explain the actions of volatile oils. Due to antimicrobial activity is not completely understood by researchers. However, it is evidenced by studies that prove the efficacy of *Eucalyptus staigeriana* essential oil against *Staphylococcus aureus* and *Candida albicans* [26], *Origanum vulgare* against *Staphylococcus aureus, Listeria monocytogenes* and *Salmonella* ATCC [21], thyme and oregano against *Escherichia coli* [28, 29] and *Lippia sidoides* against *Salmonella enteritidis* and *Lactobacillus plantarum* [30]. In addition to the antimicrobial activity, the essential oils present an antioxidant, anxiolytic, preventive of chronic diseases and food preservation due to their compounds [20, 31, 32, 33].

Heredia-Vieira et al. [34] have tested *Piper diospyrifolium* Kunth (Piperaceae) essential oil against many kinds of *Candida* sp., and demonstrated thats it has shown significant potential antifungal activity.

According to Santos et al. [35] *Campomanesia guazumifolia* (Cambess.) O. Berg essential oil strongly inhibited *Staphylococcus aureus* (MIC 15 ± 0.1 µg/mL), *Escherichia coli* (MIC 25 ± 0.2 µg/mL) and *Candida albicans* (MIC 5 ± 0.1 µg/mL).

The essential oil of *Myrcia oblongata* DC has the antimicrobial activity higher against Gram-positive bacteria when compared to Gram-negative bacteria. The highest activity was against *Enterococcus faecalis*, followed by *Staphylococcus aureus*, *Bacillus subtillis* and *Staphylococcus epidermidis.* This essential oil have showed no activity against *Proteus mirabilis*, *Klebsiella. pneumoniae* and *Staphylococcus gallinarum* [36].

3.2 Vegetable extracts

Vegetable extracts are those made by one or more plants (the whole plant or parts of it) with the addition of solvents. All conditions are important to extract the maximum compounds from the material. Also, a previously knowledge of the plant composition and/or polarity of solvents are necessary to use the most effective method. Furthermore, the conditions of preparing extracts demonstrate difference in antimicrobial activity of them, and it can be seen in many vegetable species [37].

Lima et al. [38] have done antimicrobial analyses of ethanolic extract of *Piper tuberculatum* Jacq. and they have concluded that all botanical structures tested in the microbiological assay presented bactericidal activity on *Staphylococcus aureus*, with an emphasis on stalks, since it presented growth inhibition when compared positive and negative controls, but new methods and concentrations need to be tested for a better understanding of the relationship between bacteria and plant.

Costa et al. [39] have studied the antimicrobial activity of extract vegetable garlic in fungi phytopathogenic control and they have concluded that ethanolic garlic extract presented intense antimicrobial activity especially for the fungus *Curvularia lunata* and appearance of important secondary metabolites directed to the antifungal action.

4. Packaging incorporation

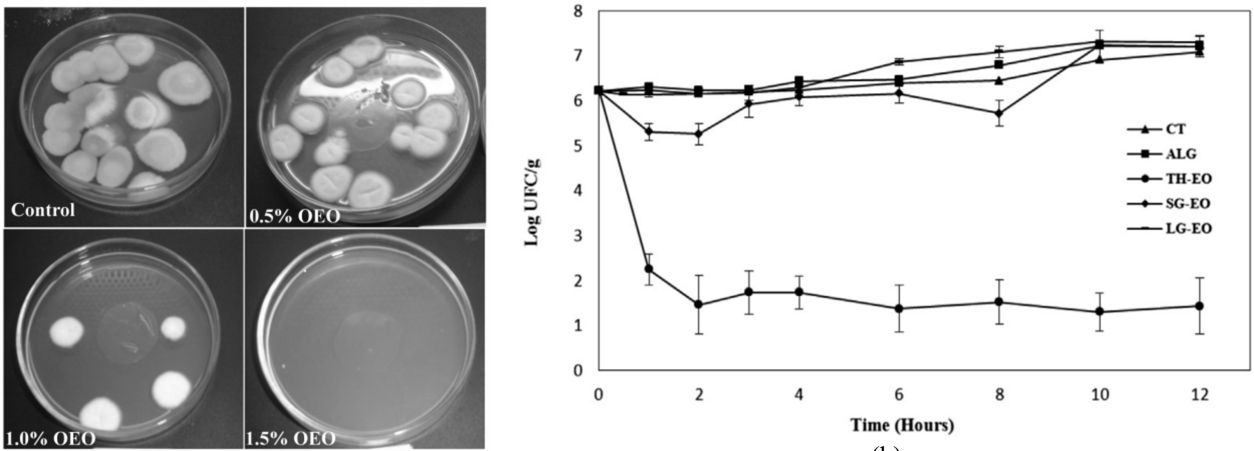
4.1 Direct dispersion

There are several methods for the incorporation of antimicrobial agents into polymeric materials, such as direct dispersion. Which means the deposition through coating or the application of very thin layers, by spraying on polymeric surfaces, immobilization by chemical grafting or the use of polymers having properties intrinsic antimicrobials. One of the major difficulties on the application of natural antimicrobial compounds is their interaction with the polymer matrix and the occurrence of organoleptic changes [40].

Essential oils and vegetal extracts act as antioxidant and antimicrobial agents, due to their natural characteristics, and generate several researches aimed at its industrial applicability. However, volatility, susceptibility to oxygen and light, and the possible alteration of sensorial characteristics, are factors that may limit its use in the food packaging industry [41, 42].

Many researches have shown the possibility of using essential oils in food systems as a way of avoiding the growth of deteriorating microorganisms and increasing the shelf life of foods [43]. A variety of studies have highlighted the effectiveness of direct dispersion of essential oils and vegetable extracts into a polymer matrix resulting in an antibacterial action.

Oliveira et al. [44] have studied the incorporation of oregano essential oil in whey protein based films, and they have presented a great inhibition halo on tests with *Penicillium commune* (Figure 1a). Acevedo-Fani et al. [45] have incorporated some essential oils in alginate films and have obtained a great antimicrobial activity for films with thyme oil (Figure 1b).



(a) (b)

Fig. 1 (a) Diffusion test for whey protein-based films incorporated with different concentrations of oregano essential oil against *Penicillium commune* [44]. (b) Antimicrobial activity of alginate films containing essential oils against *Escherichia coli* inoculated on TSA-NaCl plates. CT = control without films; ALG = alginate films; TH-EO = thyme oil film; SG-EO = sage oil film; LG-EO = lemongrass oil film [45].

There are still many discrepancies between laboratory and industrial scale results in terms of material performance and antimicrobial effectiveness, due to conventional methods for the production of packaging, such as extrusion and blow molding or compression, use high temperatures (160-190 ° C), which end up degrading the antimicrobial agents incorporated into the polymer matrix. Thus, new approaches to the development of antimicrobial packaging are required, using moderate production conditions and fast processing [46, 47].

Using the conventional high-temperature processing method, Ha et al. [48] used a temperature profile of 160°C and 190°C for the extrusion of films of Linear Low Density Polyethylene incorporated with grape seed extract, under these conditions there was a drastic reduction of the extract, not presenting antimicrobial activity. Ramos et al. [49] have developed antimicrobial packages based on propylene incorporated with carvacrol and thymol, and then submitted to 190ºC for 18 minutes by the hot pressing process. The percentages established were 4, 6 and 8% for carvacrol and thymol, and after processing only 1, 2 and 3.5% of the antimicrobial agents were retained in the propylene film. The antimicrobial activity against *Staphylococcus aureus* was effective only for the formulations that containing the highest compound concentration at the end of the process.

Thus, it is necessary to introduce and implement techniques that make these active compounds compatible with the processing requirements for both laboratory and industrial scale, allowing better yield and performance of essential oils and vegetable extracts incorporated into the polymer matrix.

4.2 Microcapsules

Another way to add antimicrobial agents into packages is by the use of microcapsules. The formation of them occurs by the microencapsulation method, which can be defined as the technology of packing solid, liquid or gaseous materials with polymer coatings, forming small particles. Polymer will act as a protective agent forming a membrane so that the compound of interest is isolated in a core, avoiding the undesired effects of its inadequate exposure [50], this membrane is called wall material.

Microencapsulation has several applications in different areas, such as pharmaceutical, medical, agricultural and food industries. Microcapsule technologies include homogenization, spray drying and coacervation, and they have been applied for the purpose of controlled release of active compounds and increase their stability against environmental damage. The encapsulation has been widely used for essential oils, dyes, microorganisms, among others [42, 51].

Efficiency of microencapsulation is directly linked with the retention of the active agent within the membrane. It is possible to reduce evaporation or degradation of the active compounds, improving their handling and controlled release during storage [52, 53]. The conditions that may affect this accumulation are:

i) the chemical nature of the core including its molecular weight, polarity, chemical functionality and volatility;
ii) the properties of the encapsulating material;
iii) the technique for forming microcapsules.

One of the most important factors is the choice of a wall material, due to its directly influence on the effectiveness and stability of the microcapsule. There is a diversity of wall materials among the carbohydrate, protein and lipid groups. Desirable characteristics for the wall materials are:

i) do not be reactive with the nucleus;
ii) be able to seal and maintain the core into the microcapsule;
iii) provide maximum protection to the core;
iv) do not be distasteful, in the case of food applications;
v) be economically viable

Bustos et al. [54] developed antimicrobial films incorporated with lemon grass essential oil microcapsules using as encapsulating agent sodium caseinate, and they obtained a total yield of 98.3% microcapsule formation with microencapsulation efficiency of 97.3% and the lipid content of 51%. In the oil release kinetics of the films, the maximum concentrations reached were 11.4 $mg.L^{-1}$, 15.3 $mg.L^{-1}$ and 19.9 $mg.L^{-1}$ for the films containing 1250, 2500 and 5000 ppm, respectively. The fractions of essential oil released were 22.7%, 30.5% and 39.9% of the entire content. Kim et al. [42] incorporated cinnamon oil microcapsules into the printing ink, which was applied to the low density polyethylene film and its surface was laminated with a layer of polypropylene. In this study, microencapsulation increased thermal stability and effectively prevented the volatilization of cinnamon oil.

On the application of bioactive compounds (from essential oils or vegetable extracts) in packaging and to have the efficiency of their action, it is important to adopt practices that:

i) increase their thermal stability, preventing their volatilization and degradation;
ii) improving the rate of release;
iii) reducing the necessary dose of additives;
iv) improving the efficacy and expanding the applications of interested compounds;
v) be a viable industrial application alternative.

5. Packaging applications

The primary function of packaging is to isolate the food from the external environment and protect it against light, heat, presence or absence of moisture, contamination and deterioration by microorganisms. Among several packages, active packaging has been a promising way to use in food products. The term "active" corresponds to the addition of additives in polymeric films or inside other kind of packaging, with the purpose of maintaining and prolonging the shelf life of the products, following a principle of interaction between packaging and food [47, 55].

The effectiveness of the addition of antimicrobial agents in packaging can be observed in many researches and some of them are related bellow.

Films developed by Bustos et al. [54], incorpored with lemon grass microcapsules, have shown a great antimicrobial activity against *Escherichia coli* and *Listeria monocytogenes*. Therefore, it could be used as packaging of fatty food as fish, meat and cheese.

Kim et al. [42] have incorporated cinnamon oil microcapsules in the printing ink, with this application it was possible to repel and prevent the invasion of moth larvae, according to the profile of minimum concentration of repulsion, the films became active during the period 21 days for cookies, 21 days for chocolate and 10 days for caramel.

Llana-Ruiz-Cabello et al. [56] have developed poly lactic acid (PLA) films incorporated with extract of *Allium* ssp., in order to be applied as packaging for salads ready for consumption. The antimicrobial efficacy was confirmed for the film and the salad, being effective during all storage (7 days) for all analyzed microorganisms, like molds, yeasts and enterobacteria. It has demonstrated strong development potential as active packaging.

Cruz-Gálvez et al. [57] have used acetanic and methanolic extracts of *Hibiscus sabdariffa* as an antimicrobial agent, being incorporated into the potato starch films. In both formulations, that containing the extracts, have presented antimicrobial activity against *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli* and *Shigella flexneri*. The film containing the acetone extract had a higher antimicrobial effect, producing inhibition halos of more than 14 mm against *Escherichia coli* and *Shigella flexneri*.

Alves et al. [58] have studied the shelf life of refrigerated Salmon (*Salmo salar*) packaged with chitosan films with grape seed extract-carvacrol microcapsules, and those films delayed the deterioration of the samples due to the active compounds with antimicrobial and antioxidant properties that affect the cellular membranes and retard microbial growth.

Rosemary and thyme extracts have been incorporated into whey protein concentrate active films and even it was added in a low quantity, they were able to inhibit *Staphylococcus aureus* and *Listeria monocytogenes* [59].

According to Aquino et al. [60], guavas coated with chitosan–cassava starch and chitosan–cassava starch containing *Lippia gracilis* Schauer genotype mixtures have shown better microbiological qualities in terms of yeast and mold counts during storage at room temperature (25 ºC) for 10 days, when compared with uncoated guavas. Also, the authors have concluded that those edible coatings have delayed the ripening process, reduced browning and inhibited color development in guavas, increasing its shelf life.

Pequi (*Caryocar brasiliense* Camb.) peel extract has antifungal activity against some fungal pathogens and its activity was maintaned on the edible coating applied on tomatoes. Breda et al. [61] have observed its effectiveness in tomatoes coated with chitosan and pequi peel extract edible coating, which stabilizing mold and yeast counts during 16 days of evaluation.

Dannenberg et al. [62] have incorporated essential oil of pink pepper in films and have observed the increase in growth reduction of *Listeria monocytogenes* and *Staphylococcus aureus*, indicating the potential antimicrobial activity (Figure 2a). Likewise, the antimicrobial activity was observed when those films were applied in sliced chesse (Figure 2b). That activity is because about seventeen compounds representing 98.55% of the essential oil of Pink Pepper (*Schinus terebinthifolius*) and the major or them is the α-pinene [63], which has a great antimicrobial potential [64].

Several natural antimicrobial agents have been the focus of research, being an alternative to substitute chemical additives for packaging, considered safer and less risky for consumer's health [43]. Essential oils and vegetable extracts have present great potential for application for the development of active packaging.

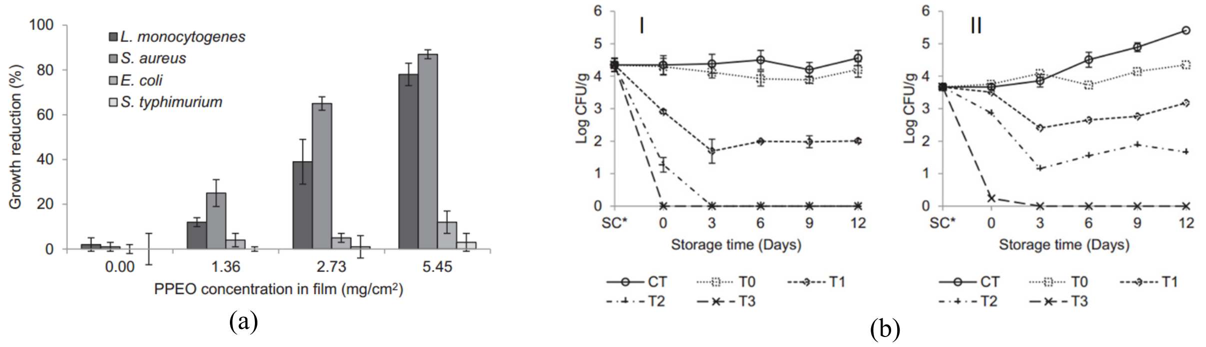


Fig. 2 (a) Antimicrobial activity of films with different concentrations of Pink Pepper Essential Oil (PPEO), by volatilization in micro-atmosphere, on the development of pathogenic bacteria. Results are expressed as mean (n = 3) ± standard deviation of percentage reduction for each treatment compared to the control (CT). (b) Antimicrobial activity of films with different concentrations of PPEO on *Staphylococcus aureus* (I) and *Listeria monocytogenes* (II) in sliced cheese. Results are expressed as mean (n = 3) ± standard deviation. CT = control treatment (without film); T0 = film without EO; T1 = 1.36 mg/cm^2; T2 = 2.73 mg/cm^2; T3 = 5.45 mg/cm^2 (EO mass per film area); SC* = Starting concentration (Count of bacteria in the cheese before the application of the films) [62].

6. Conclusions

As the natural sources are becoming scarce, industries need to look for new technologies and methods to use. Based on that packagings of renewable sources are a way to avoid it and make industries more suitanables, making low waste of materials and reducing environmental impact.

Bacterias classified as Gram negative have in their composition a double cell membrane constituted by lipopolysaccharide which makes them more resistant to antimicrobial agents since it is difficult to be penetrated by essential oils components.Therefore, the antimicrobial activity of new essential oils have to be tested, due to it may not work properly. However, there are vegetable extracts that may act as antimicrobial agent.

In addition, food industries need products with longer shelf life, to be exported and transported for longer periods without lose quality. Therefore, antimicrobial packaging is a very promising system for those food products, mainly if it has essential oils or vegetable extracts as antimicrobial agents.

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