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The antibacterial properties of seaweed biopolymer based films incorporated with essential oils

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Abstract

The aim of this study was to obtain seaweed biopolymers based edible films (carrageenan and agar) and incorporated with peppermint, oregano, and thymus essential oils. The most intense antimicrobial activity was observed at films incorporated with peppermint essential oil, and the lowest was noticed at those with addition of oregano essential oil. It was evaluated the initial microbial contamination and after one week, during which time there were handeled and stored in open spaces. There were no yeasts and molds colonies, coliforms or *Escherichia Coli*, it has been noted total viable count, and the *Staphylococcus aureus* presence, but their total number decreased after one week. The addition of essential oils did not alter the physical properties, but the films got a pleasant taste and smell. Edible films developed from seaweed, incorporated with essential oils, can be used as food packaging material, being a viable alternative to synthetic conventional packaging.

Keywords: carrageenan, agar, essential oils, peppermint, oregano, thyme.

1. Introduction

Nowadays, edible films and coatings have become a real alternative to conventional packaging materials, mainly due to their specific characteristics, such as biodegradability, biocompatibility, improved aestethics, and oxygen barrier. [1] Although they are derived from various natural sources, those based on polysaccharides are the most widely used especially due to their regenerability, widespread, price and ease of handling. [2,3] The importance of films and coatings derived form the advantages of their use: extending shelf life, improving quality of fresh products, preventing loss of nutrients or volatile compounds, while maintaining or even improving the appearance of the products they protect. [4,5]; they are a selective barrier to moisture transfer, prevents access of oxygen, and thus lipid oxidation, but also harmful microorganisms. Furthermore, the replacement of conventional packages with edible or completely biodegradable materials reduce environmental pollution.

Into the matrix of edible films can be easily incorporated different substances, such as antioxidants, antimicrobial agents, flavors, spices or coloring agents, which can improve the existing appearance or can provide new qualities. [6] The initial food quality can be preserved if the product is propely packaged, into suitable materials. To prevent the contamination and the loss of nutritional and sensory qualities, studies were directed towards obtaining edible materials incorporated with essential oils. This innovative material for food packaging is able to keep the product unchanged, can be eat with it, resulting zero-waste, and solving the environmental pollution problems. [7]

Because of their special film-forming ability, the polysaccharides are the most widely used to obtain edible films and coatings. Carrageenans and agar are two major galactans groups present in red seaweed. The carrageenans are hidrophilic polymers with high potential to form biofilms.

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Agar has a good ability to encapsulate the antimicrobial agents: green tea extract [8], grapefruit seed extract [9], and essential oils, such as oregano, rosemary, thyme, lavander, peppermint, lemongrass, garlic, cinnamon. [10] Poliols, such as glycerol or sorbitol, are particularly used for plasticization because modifies the mechanical properties of hidrophilic films, by improving the extensibility, but reducing their elasticity; also, prevent destruction of the biofilms during handling or storage. [2]

In order to obtain food packaging with antimicrobial properties, manufacturers have added organic or inorganic compounds, metals, alcohols, or amino compounds. [11] However, the consumer concern and demand for healthy products have stimulated the use of bio preservatives. The incorporation of essential oils into the film matrix replaced the addition of synthetic antimicrobial agents used to obtain oxidative or microbial stability, and safety of food, but can replace the addition of lipids because reduces the affinity to water, as well. The incorporation of the essential oil into the matrix, in contrast with their direct application on the surface of the product, reduce the doses used, and the antimicrobial activity is unchanged. [12] The antimicrobial active packaging must fulfil certain requirements: to be effective against a large spectrum of microorganisms at low concentrations, to not cause undesirable changes in the sensory characteristics and must be in accordance with the current legislation. [13, 14]

The antimicrobial activity of peppermint is given by its active compounds, such as menthol, which prevents bacterial and exhibits anti-viral activity, menthone, with antifungal properties, and eugenol and carvone with effect against pathogens. [15] The active compounds from oregano essential oil – carvacrol, γ-terpinene, thymol – confer the antimicrobial activity.[16] Antibacterial compounds form the thyme essential oil – cineol, camphor, thymol, linalool – had increased the activity against yeasts. [17]

The aim of this work was to obtain edible films from carragennan and agar, incorporated with peppermint, oregano, and thyme essential oils. It was evaluated the antimicrobial activity against pathogenic bacteria or coliforms, and yeasts and molds, as well. The results provides important information about using these edible biofilms as a food active and intelligent packaging material.

2. Materials and Methods

2.1. Materials

Sodium alginate, agar and glycerol were purchased from Sigma-Aldrich; the essential oils were acquired from Solaris Plant, an autochthon company that sells natural porducts. Microorganisms culture media Dry Compact are produced by Nissui Pharma; were used specific cultures for *Escherichia Coli*, *Staphylococcus aureus*, coliforms, total count, and for yeasts and molds.

2.2. Preparation of antimicrobial films

Films were obtained through casting method from 1 g carrageenan, 0.5 g agar, 0.5 g glycerol and 100 ml distilled water. 10, 20, 30 µL of peppermint, oregano, and thyme essential oils were added into the film forming solution. The solution was stirred with high heat (90ºC); after 30 min maintenance, the solution was cast onto the silicone surface used for drying. Films were kept 48 hours at ambiental temperature (22ºC), until complete dryness.

2.3. The appearance and microstructure of films

It was observed the films adherence on the silicone support; the appearance was evaluated with the naked eye and the microstructure with digital Motic Microscopes. It were evaluated the homogeneity of structure, the presence of pores or cracks, the matrix appearance and the distribuition of essential oil particules into film structure.

2.4. Film thickness

The thickness was measured with an electronic digital micrometer (Mitutoyo, Japan). Measurements were teaken in at least five different areas of the film, the final value represent the average of them.

2.5. The determination of retraction ratio

The retraction ratio was calculated using the formula (1), where the initial film thickness is the thickness of the solution poured onto the drying surface, respectively 772 µm.

Retraction ratio, (%) = [(initial film thickness –dry film thickness)/ initial film thickness] x100 (1), [18]

2.6. Color evaluation

The luminosity (L*), and colour sistem a*, b*, were determinated through CIELAB method, and the film colour was evaluated with Chroma Meter colorimeter (Minolta, CR-200, Tokyo, Japonia). It were observed the color differences between films with and without essential oils incorporated into the matrix.

2.7. Swelling ratio capacity

For determinate the swelling ratio capacity, film samples were immersed into 50 ml distilles water at room temperature (22°C) and maintained various time intervals (1, 3, 5, 7, 10, 15, 20 minutes). The samples were weight before immersing and after completion timeframe, they were taken out and gently tapped with filter paper in order to remove the excess water, and then reweighted. Swelling ratio capacity was calculated using the formula (2), where W_0/W_t represents the sample film weight before water immersion and after *t* time of immersion; (g).

$$SR\ (\%) = [(W_t - W_0)/W_0] \times 100 \qquad (2),\ [19]$$

2.8. The moisture content determination

Film moisture or water content represents the amount of water from the material; it helps to highlight its hydrophilic nature. This determination is important as it provides information on the increasing of the shelf life of food packaged into biofilm and can establish the destination of film used as packaging material, for products with high or low water compositions, respectively. Thus, 3x3 cm film samples were weighted and dried in a hot air oven for 24 h at 110°C. After this time, were reweighed and the results were obtained using the formula (3), where W_0/W_1 is the weight of the sample before/ after dring; (g): [20]

$$MC,\ (\%) = [(W_0 - W_1)/W_0] \times 100 \qquad (3)$$

2.9. Evaluation of essential oils antimicrobial activity

For this determination have been used plates inoculated with specific culture media and were tested the total viable count, coliforms, *Escherichia Coli*, *Staphylococcus aureus*, and yeasts and molds presence. It was observed the initial microbiota (t_0) and after 7 days (t_1). During this time, films were unpacked, stored in open containers and took handled by different people. For antibacterial activity evaluation, the inoculated culture media were maintained for 24 h at 37^{0}C, excepted plates for yeasts and molds, which were maintained 72 h at 37°C.

All determinations were performed in triplicate and the average values were noted.

3. Results and Discussions

3.1. Film characterization

For drying process, the film forming solution was kept at ambient temperature for about 48 hours. The films presented low adherence to silicone support used for drying, and were easily removed from it.

If the control sample had no taste or smell, films with essential oils incorporated into matrix had taste and flavor of mint, oregano and thyme.

Table 1. Physical-chemical properties of biofilms

ASSAY*	THICK-NESS,(µm)	COLOR			RETRAC-TION RATIO, (%)	MC, (%)
		L*	a*	b*		
A1	34.2 ± 3.03	90.83± 0.24	-5.18±0.05	14.8±0.39	95.56 ± 0.39	20.04±0.02
A2	29.4 ± 2.96	90.74±0.33	-5.27±0.04	14.85±0.64	96.2 ± 0.38	19.62±0.01
A3	29 ± 1.51	90.39±0.19	-5.25±0.05	14.64±0.63	96.11 ± 0.24	21.37±0.03
A4	30.6 ± 1.51	91.34±0.33	-5.34±0.06	14.16±0.65	96.05 ± 0.18	18.66±0.33
A5	30.2 ± 2.16	90.65±0.41	-5.13±0.1	15.22±0.81	96.08 ±0.28	20.32±0.01
A6	41 ± 2.82	90.97±0.04	-5.25±0.06	14.52±0.68	94.68 ± 0.36	17.81±0.005
A7	35 ± 1.58	90.89±0.27	-5.23±0.03	14.72±0.38	95.46 ± 0.2	23.54±0.006
A8	34.6 ± 3.09	91.24±0.07	-5.22±0.01	14.44±0.12	95.51 ± 0.19	19.73±0.02
A9	37.2 ±1.64	90.41±0.11	-5.1±0.02	15.28±0.21	95.17 ± 0.21	16.96±0.01
A10-C	41.6 ± 1.67	90.91±0.7	-5.24±0.01	14.62±1.25	94.61 ± 0.21	14.83±0.005

Note* A1, A2, A3 – sample with 10, 20, 30 µL oregano essential oil, A4, A5, A6 – sample with 10, 20, 30 µL peppermint essential oil, A7, A8, A9 – sample with 10, 20, 30 µL thyme essential oil, and A10 – control sample, without essential oil added.

3.2. The thickness and films microstructre

All the values obtained by the averaging the measurements are noted in table 1. The addition of essential oils reduced the film thickness, which become thinner and more transparent than the control sample, without essential oil incorporated (control sample – 41 µm in contrast to the sample with 30µl oregano essential oil – 29 µm).

According to the research made by Han & Krochta [21], the film thickness is influenced by the solid content of film forming solution.

This aspect is reinforced by the results obtained in the determination of retraction ratio; thus, samples with essential oils added showed high values of retraction ratio. Observation of sample microstructure using digital microscope reveals the presence of pores into the film matrix, but they are not necessarily formed due to essential oils addition, as they are found in the control sample, as well (A10), fig. 1.

It is possible that a greater amount of plasticizer, glycerol in this case, would form a more compact matrix. The images obtained show a uniform distribution of the granules of hidrocolloids and essential oils particules into the film matrix; in this case we can eliminate the need for the addition of emulsifiers.

Table 2. Results of films sewlling ratio

TIME (min)	CH (%)						
	1'	3'	5'	7'	10'	15'	20'
A1	345	879.71	1051.19	1278.72	1477.9	1826.76	2210.34
A2	313.88	402.77	644.13	790	992.15	1337.81	1772.82
A3	427.53	589.4	812.58	1034.82	1039.5	1174.5	1338.1
A4	597.4	1129.16	1214.28	1298.68	1655.8	1677.36	1947.5
A5	480.85	882.85	964.22	1034.04	1066.4	1476.2	1508.33
A6	256	578.64	837.2	1017.83	1314.7	1199.03	1355.66
A7	654.54	703.54	877.77	1256.25	1785.1	2014.58	2849.4
A8	824.24	1055.4	1069.56	1100.73	1646.4	1662.23	2170.5
A9	494.06	897.81	932.06	1067.71	1325	1337.03	1436.36
A10-C	398	458.42	463.72	601	631	full solubility	full solubility

3.3. Determination of color properties

10 and 20 µL oregano essential oil added into the film matrix did not affect the control sample color, in contrast to film with 30µL oregano essential oil that has lower brightness and was darker. L*, a*,b* values are noted in table 1. A9 showed the most intense color due to the hue of thyme essential oil, whose tint is more developed than the mint or oregano essential oil. This aspect is important when the film is used as packaging material for food products; it will not change the products color and the consumer can directly appreciate their quality.

3.4. Swelling ratio determination

Determination is important for obtaining films used as food packaging material; films may hydrate in contact with high moisture products and may lose their properties.

In our case, the values noted in table 2 indicate the posibility of using this films for products with low moisture content or, in case of edible packaging, can be successfully disolved and eaten with food product (for example, for dehydrated vegetables, instant powder beverage, ramen noodles etc).

All measurements were carried out at 20°C; a higher temperature accelerates solubilization of films. Therefore, in hot water (90-100°C) they are completely dissolved and can be eaten with the product. The addition of essential oil reduced the swelling of the hydrocolloid granules. The values hepls to conclude that increasing the volume of essential oil added leads to a better film hydrophobicity, preventing complete solubilization after 15 and 20 minutes of immersion, as in the case of control sample. The essential oils added into the matrix modified the film solubilization, regardless of its type.

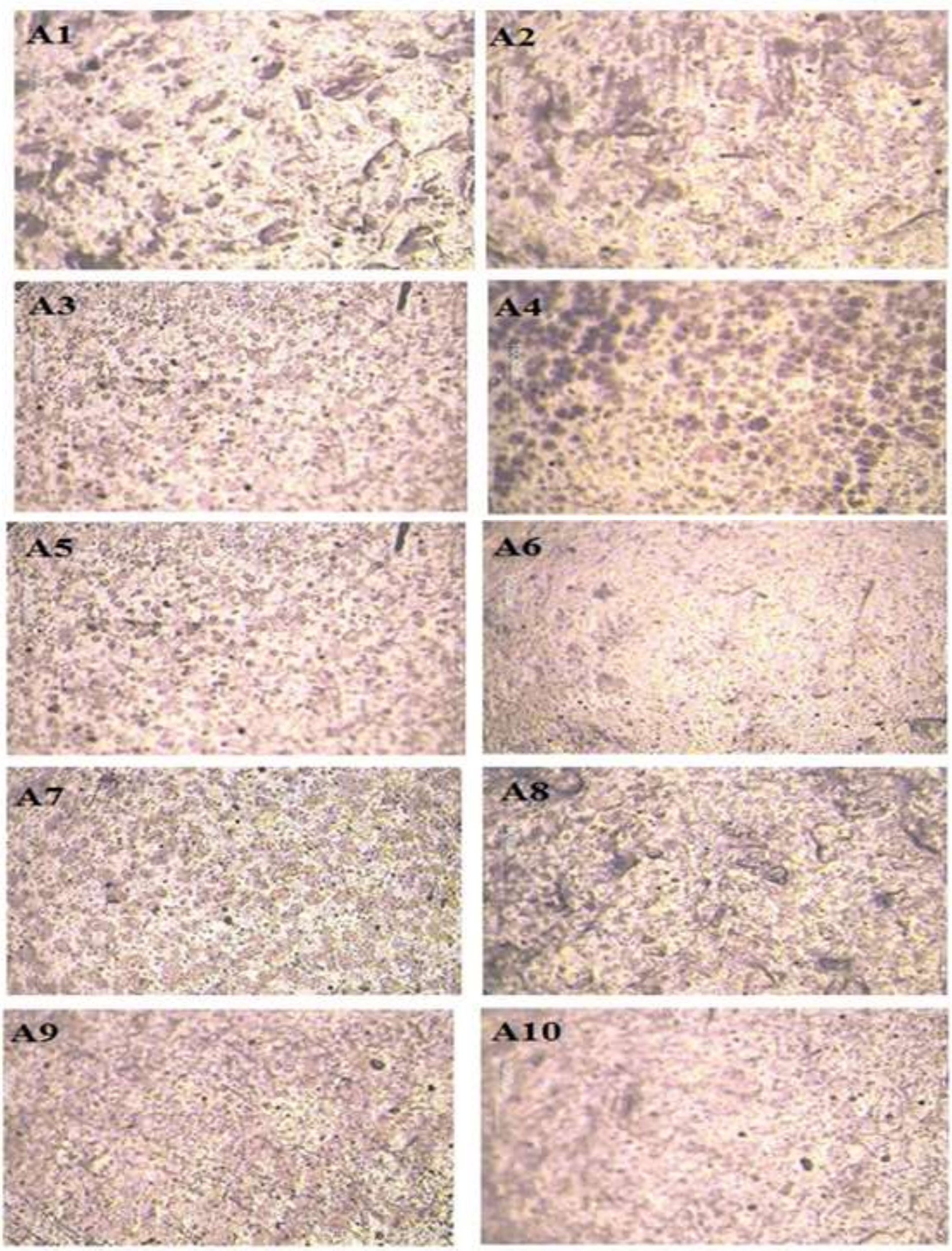

Figure 1. Images of microstructures of films

3.5. The antimicrobial activity

For this determination, the films were tested imediately after drying and after one week. If at t_0 time the microbial load was higher, it was reduced at t_1 time, for all microorganisms tested, indicating the posibility of using these biofilms as active packaging materials.

There were no yeasts and molds in all tested samples, but total count from the t_0 time indicates an initial microbial load, especially if we take into account that the control sample presented lower values than those with essential oils added. We can conclude that is very important a rigurous control of oils and manufacturers before their use in food industry because several times it was highlighted the susceptible quality of these.

Regarding the incidence of coliforms, *Escherichia Coli*, yeasts and molds, they have not developed into the culture media, at baseline or after one week.

Figures 1 and 2 showed that the most pronounced antibacterial activity was observed on the films incorporated with peppermint essential oil; even the initial load decreases with increasing concentration of essential oil added. The antimicrobial activity was carried out in the following order: peppermint essential oil> thyme essential oil> oregano essential oil. For oregano essential oil films, according to the data obtained, it can be appreciated that it presented a initial microbial load, whereas the increase of the added essential oil volume increased total germ count.

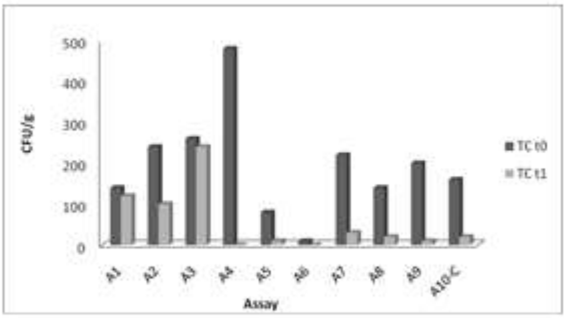

Figure 2. Antibacterial activity**.**
Total count determination

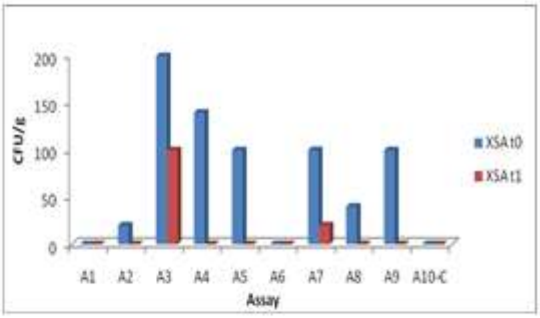

Figure 3. Antibacterial activity.
Staphylococcus aureus incidence

The antibacterial activity of chitosan based films incorporated with peppermint essential oil has been reported by Tsai et al., [22], as well.

Referring to the total viable count, all tested films are within the maximum permissible limits, according to ISO 4833-1:2003 [24] and 5000 CFU/g.

Only the control sample and film with 10 µL oregano essential oil added were within the maximum permissible limits for Staphylococcus aureus, according to SR EN ISO 6888-1.2/A1:2005 [25] and 10 CFU/g.

4. Conclusions

Antibacterial packaging is an innovative concept for food packaging that represent a noticeable interest of researchers and industry, but more of consumers who have refined their tastes and choices as they provide a variety of products.

This study aimed to evidence the antibacterial activity of carrageenan and agar based edible films incorporated with peppermint, oregano and thyme essential oils. We can appreciate that antimicrobial activity can be attributed to the film composition, which prevents the proliferation of microorganisms, not only due to essential oils addition. This films can represent a viable antibacterial packaging material and can be used for the whole range of food, replacing the synthetic preservatives used so far. Edible films incorporated with peppermint essential oil presented the most significant antibacterial activity. However, the lowest activity was observed at films obtained with oregano essential oil, whose main component, carvacrol, seems to have a less intense antimicrobial capacity, at least in this case.

Furthermore, it is necessary to study a variety of essential oils in order to highlight their antimicrobial capacity, which is closely related to the product, so that, there may be specific oils for certain types of food products. Films obtained can be used as material for food packaging, or coating for fresh products, increasing their shelf life, preserving freshness and sensory characteristics, and ensuring food safety, as well.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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