PHYSICAL AND ANTIMICROBIAL PROPERTIES OF *Chlorella sorokiniana* BASED FILMS

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ABSTRACT

Films are an alternative to replacing part of synthetic plastic packaging in a sustainable way. Thus, the aim of this work was to develop and characterize biopolymeric films based on the biomass from the microalgae *Chlorella sorokiniana*. The films were obtained by the casting method, in triplicate, with microalgae, gelatin and glycerol, using an experimental design. The films with the best appearance were evaluated for their antimicrobial potential, having the following composition: 0.5 g of microalgae, 2.5 g of gelatin and 1.5 g of glycerol in 100 mL of water (F3) and 1.5 g of microalgae, 2.5 g of gelatin and 1.5 g of glycerol in 100 mL of water (F4). The control film contained 1.0 g of microalgae, 2.0 g of gelatin and 2.0 g of glycerol in 100 mL of water (F9). The films have been shown to be a strong barrier against the development of microorganisms *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus epidermidis*, and *Salmonella typhimurium*.

Keywords: Microalgae. Packaging. Biodegradable. Bactericide.

1 INTRODUCTION

The availability, biodegradability and functionality of edible films are advantages that encourage the exploration of new raw materials[1]. Microalgae, for example, have numerous advantages, including their high growth rate, cell productivity, and CO_2 absorption, and especially their ability to produce several biomolecules, such as proteins and carbohydrates of high digestibility, which are highly demanded by the food industry[2].

Thus, the aim of this work was to develop films based on the combination of the biomass of the microalgae *Chlorella sorokiniana*, gelatin and glycerol and to characterize them in terms of physical and antimicrobial properties.

2 MATERIAL & METHODS

Obtaining of microalgal biomass. The microalgae *Chlorella sorokiniana* (Trebouxiophyceae) was acquired from the André Tosello Foundation (Ref. 211-32; CTT 7727; IBVF 211-32, University of Seville- Spain). It was pre-cultivated in 500 mL Erlenmeyer flasks containing 250 mL of defined medium in an orbital shaker incubator with constant agitation (200 rpm) at controlled temperature of 25 ± 2°C and photoperiod (12 h light / 12 h dark) provided by fluorescent lamps with 2,500 lux for 7 days. The main cultivations were carried out by transferring the volume of 2 flasks of the pre-culture for a 4 L polypropylene gallon containing 2,5 L of the same defined Sueoka medium, totaling 3 L. Cultivations were performed under constant aeration provided by an air pump, at the same temperature and photoperiod conditions described above. The microalgae were harvested at the stationary phase after 28 days of cultivation. The biomass was separated by electroflocculation and then lyophilized. These processes were repeated until obtaining enough biomass for the development of the films.

Obtaining of the films. The films were obtained in triplicate from microalga, gelatin and glycerol at different concentrations (Table 1), using the casting technique, as described elsewhere[3].

Subjective evaluation of the films. The following parameters were determined for the films using visual and tactile analyzes: (a) continuity (absence of rupture after drying), homogeneity (absence of insoluble particles, bubbles of air or opacity zone) and flexibility (handling without risk of rupture)[4].

Antimicrobial Activity. The films obtained with T4, T7, and T9 were evaluated in triplicate for the antimicrobial activity. A film without microalgal biomass was utilized as a negative control. The utilized bacteria were the Gram (−) *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhimurium* ATCC 14028, and the Gram (+) *Staphylococcus epidermidis* ATCC 12228. An inoculum of each microorganism was prepared by direct suspension in a sterile saline solution of a loopful of colonies isolated from a nonselective PCA medium Petry dish with the corresponding bacteria previously incubated at 35 °C for 24 h. The optical density of the suspensions was adjusted using a spectrophotometer. The absorbance of the McFarland solution at 0.5 was evaluated from 0.08 to 0.10 at 625 nm. Mueller Hinton agar plates were inoculated with microorganisms by rubbing a swab of sterile cotton moistened with the inoculum solution. On the surface of the inoculated agar were placed discs (2.0 cm diameter) of the films, previously sterilized in a chamber with UV light for 3 min. Plates were incubated at 35 °C, and after 24 h the inhibition halos were measured, including the disc diameter[5].

3 RESULTS & DISCUSSION

The visual characteristics determined for the microalgae biomass-based films are shown in Table 1. All the films were symmetrical and presented a brownish green color (Figure 1). The films that presented optimal characteristics for continuity, homogeneity and handling were those corresponding to T4, T8, T9, T10, T11, without fractures or ruptures after drying, while T5 and T6 were difficult to remove them from the Petri dishes, requiring care to do not cause any rupture.

Table 1. Factorial planning matrix, with the coded and real values for the development of the *Chlorella sorokiniana* based films and the subjective evaluation of the corresponding films.

Treatment	Coded variables			Real variables (g 100 mL^{-1} solution)			Characteristics of the films		
	M	GE	GL	M	GE	GL	C	H	M
T1	-1	-1	-1	0.5	1.5	1.5	xx	x	xx
T2	+1	-1	-1	1.5	1.5	1.5	xxx	xx	xxx
T3	-1	+1	-1	0.5	2.5	1.5	xxx	xx	xxx
T4	+1	+1	-1	1.5	2.5	1.5	xxx	xxx	xxx
T5	-1	-1	+1	0.5	1.5	2.5	x	x	x
T6	+1	-1	+1	1.5	1.5	2.5	x	x	x
T7	-1	+1	+1	0.5	2.5	2.5	xx	xx	xx
T8	+1	+1	+1	1.5	2.5	2.5	xxx	xxx	xxx
T9	0	0	0	1.0	2.0	2.0	xxx	xxx	xxx
T10	0	0	0	1.0	2.0	2.0	xxx	xxx	xxx
T11	0	0	0	1.0	2.0	2.0	xxx	xxx	xxx

M: microalga; GE: gelatin; GL: glycerol; C: continuity; H: homogeneity; M: handling; xxx: excellent; xx: good; x: disabled.

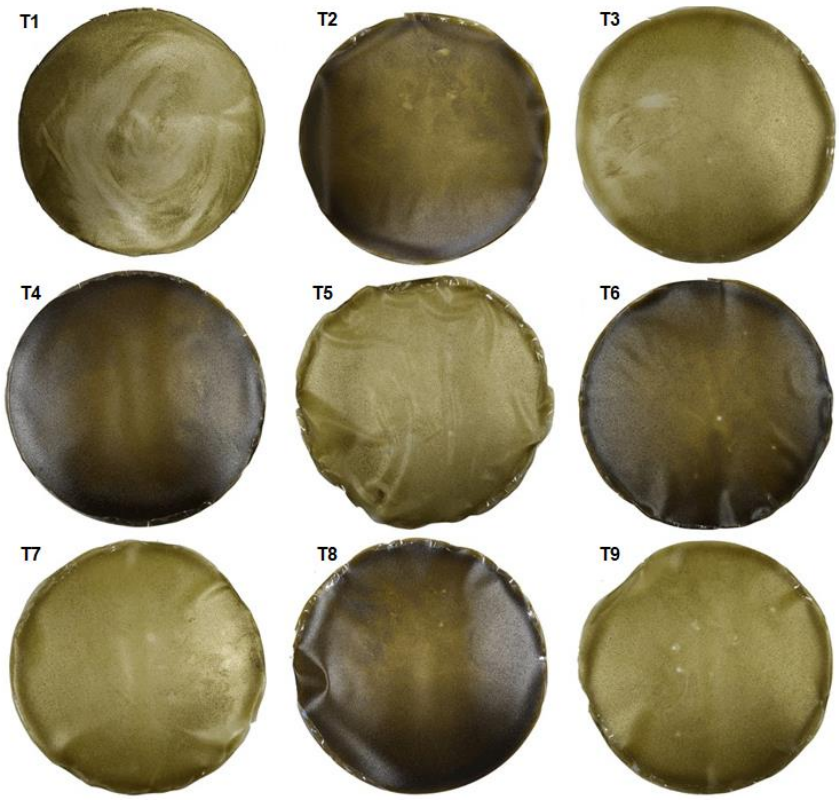

Figure 1 Microalgae-based films.

Three films with different characteristics were selected for the antimicrobial analysis: T4: 1.5 g of microalgae, 2.5 g of gelatin, and 1.5 g of glycerol 100mL^{-1} (more resistant), T7: 0.5 g of microalgae, 2.5 g of gelatin, and 2.5 g of glycerol 100mL^{-1} (more elastic), and T9: 1.0 g of microalgae, 2.0 g of gelatin, and 2.0 g of glycerol 100 mL^{-1} (control). The results of the antimicrobial test (agar diffusion test) of the films against the activity of *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 14028, *Bacillus cereus* ATCC 11778, and *Klebsiella pneumoniae* ATCC 13883 are shown in Figure 2.

The films did not present a halo of inhibition. However, it was observed that there was no microbial growth in the area in contact with the film (Figure 2), which may indicate a limited activity against this microorganism or even that the film acted as a barrier to these microorganisms at the three treatments evaluated (T4, T7, and T9). The antimicrobial activity of microalgae extracts has been reported elsewhere[6,7]. In a study with 10 macro- and microalgae, the methanolic, ethanolic, and chloroform extracts were evaluated for their antimicrobial activity revealing the higher activity for the *Chlorella* sp. extract in all solvent forms when compared to the other organisms[8].

In another work, the aqueous extract of *C sorokiniana* showed not only an antimicrobial activity, but also a great antioxidant capacity, which was 50 times higher when compared to ascorbic acid[7]. In the present work, the antimicrobial activity of *C. sorokiniana* was verified even without the extract obtaining. It is presumed that these compounds have been released to the filmogenic solution during the formation of the cross-linking interactions between the biomolecules of all film compounds.

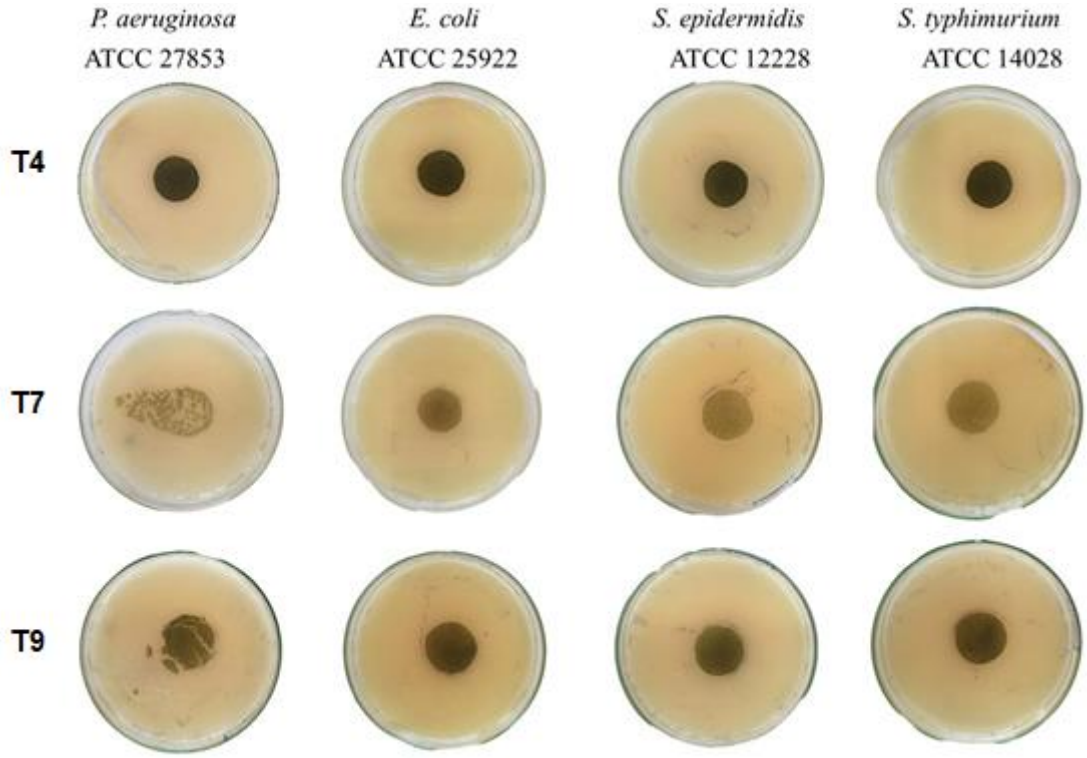

Figure 2. Antimicrobial activity analysis for selected microalgae-based films. T4, T7 and T9 according to Table 1

4 CONCLUSION

According to the parameters evaluated, the films with the better characteristics for food packaging were produced with 1.5g of microalgae, 2.5g of gelatin and 1.5g of glycerol in 100 mL^{-1} (T4), while for covering food the best treatments were obtained with 0.5 g of microalgae, 2.5 g of gelatin, and 2.5g of glycerol 100mL^{-1} (T7). These films were among the more resistant and more elastic respectively. Both these films acted as a barrier against the development of the microorganisms *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus epidermidis*, *Salmonella typhimurium*.

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