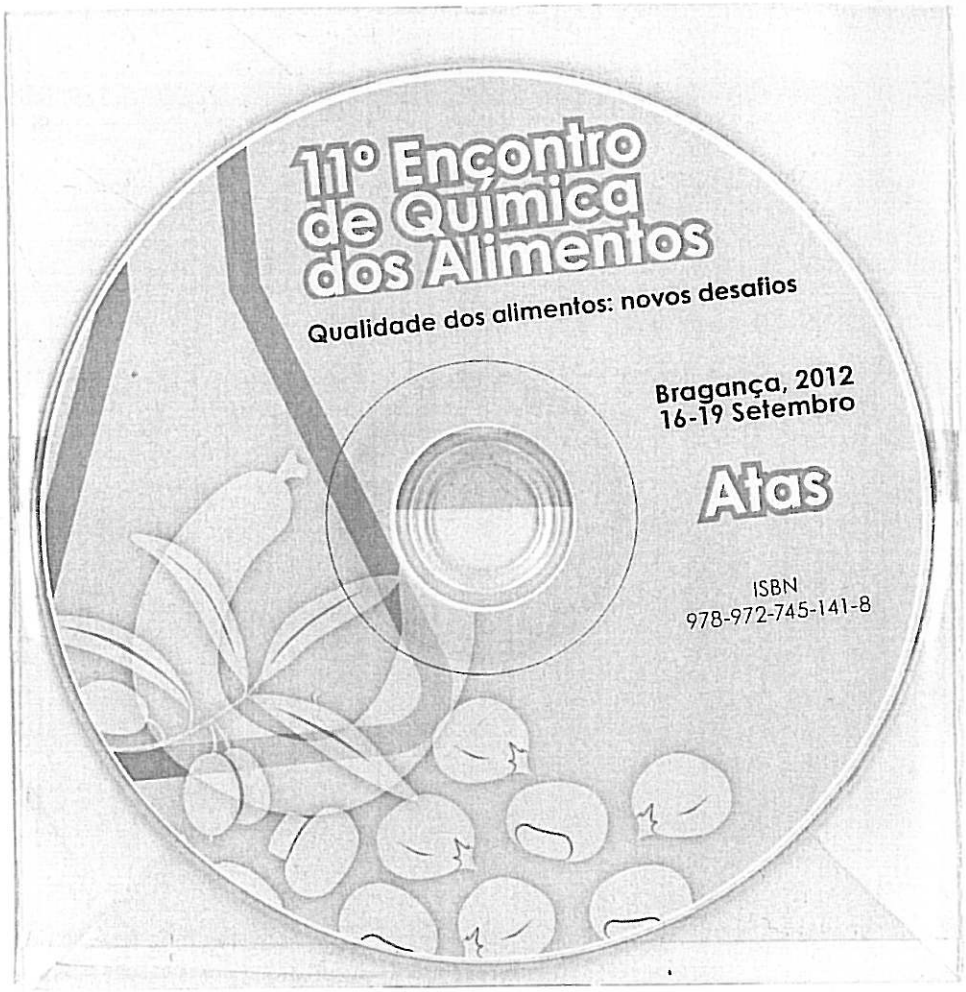

Production of chitosan based films enriched with oregano essential oil for increased antibacterial activity

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Keywords: chitosan; films; oregano essential oil; antibacterial activity

ABSTRACT

During the last years, there has been an increasing interest in developing bio-based active films to improve food safety, extend food shelf life and reduce the use of chemical preservatives. Chitosan, a deacetylated derivative of chitin, is a linear polysaccharide consisting of β-(1→4) glucosamine and *N*-acetylglucosamine residues with potential to be used as a food packaging/coating material. This biopolymer can be used in a wide range of applications in the food industry due to several interesting properties such as its biodegradability, biocompatibility, non-toxicity, antimicrobial activity and versatile physical properties such as its film-forming capacity. Recently, different strategies have been explored to improve its natural properties for the development of food packaging/coating materials with enhanced antimicrobial activity. In particular, the incorporation in chitosan films of essential oils (EO) with acknowledged antibacterial properties, as an alternative of synthetic preservatives, is a matter of great interest since they are generally perceived by consumers as being "natural" food additives.

Thus, the objective of this work was the production of chitosan films enriched with oregano EO to further improve the natural antimicrobial properties of chitosan. The obtained films which were then evaluated for its antibacterial activity.

1. INTRODUCTION

Chitosan, a deacetylated derivative of chitin, is a natural linear polysaccharide composed of β-(1→4) glucosamine and *N*-acetylglucosamine residues. Due to its safety, non-toxicity, biocompatibility and biodegradability, its use has been suggested in several areas, including the food industry [1]. In addition to its film-forming capacity, this biopolymer has been described as having antimicrobial activity against several bacteria and fungi, thus making it a very interesting material for application in food coatings, improving food shelf life and safety [2]. In the last years, different strategies have been suggested and explored to improve

chitosan natural properties, towards the development of food packaging/coating materials with enhanced antimicrobial activity. In particular, the incorporation in chitosan films of essential oils (EO) with acknowledged antibacterial properties, as an alternative of synthetic preservatives, is a matter of great interest since they are generally perceived by consumers as being "natural" food additives [3]. Among these oils, oregano is known as a source of molecules, such as tymol and carvacrol, with known biological activity, namely antibacterial and antifungal properties. Thus, oregano EO can be a feasible alternative to be incorporated in food coating/packaging films contributing to the reducing the use of synthetic additives, namely preservatives.

In this work different chitosan based films enriched with oregano essential oil were produced at room temperature by solution casting method, with different parameters being tested. Subsequently, the obtained films were assayed for its antibacterial activity, against a common foodborne microorganism.

2. MATERIALS AND METHODS

2.1 Materials

Chitosan 90/200/A1 (flakes with size <200 µm, deacetylation degree of 93.1%, dynamic viscosity of 135 mPA s (1% at 20ºC in 1% acetic acid solution)) was acquired to Biolog Biotechnologie GmbH (Germany). Oregano essential oil (EO) was acquired to Aromol (Lisbon, Portugal).

2.2 Chitosan films preparation

Chitosan solution (2%, w/v) was prepared by dissolving chitosan in 2% (v/v) acetic acid and stirring overnight (200 rpm at 45ºC). For citric acid, the same procedure was done, using 6% (w/v). The solutions were cast into plastic containers, dried at ambient temperature and the obtained films were used as blank controls (without essential oil (EO) addition). Chitosan films added with oregano EO were prepared using two different emulsifiers (1%, v/v), with Hydrophilic-Lipophylic Balance (HLB) of 16.3 and HLB=13.0, and different concentrations of oregano EO (1%, 3% and 5%, v/v). The oil-in-water emulsion was prepared by homogenizing the chitosan solution (2%, w/v), essential oil and emulsifier at 21000 rpm during 1 min using a CAT Unidrive. The films were produced at room temperature by solution casting method.

2.3 Antibacterial activity evaluation

After preparing the films, antimicrobial activity against *Escherichia coli* ATCC 10536 was assayed by the disc diffusion method. The bacterial culture was djusted to 0.5 McFarland turbidity standard and spread over the plates containing Mueller-Hinton agar using a sterile cotton swab. Discs of 0.9cm diameter were cut from the prepared films and placed on the surface of the inoculated plates, which were then incubated at 37ºC for 24h under aerobic conditions.

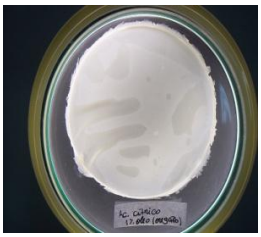
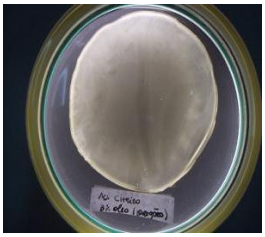
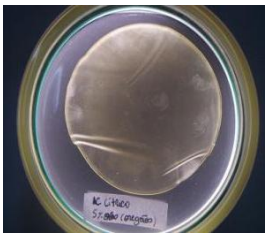
3. RESULTS AND DISCUSSION

3.1. Chitosan films preparation

In this work, two emulsifiers with different HLB values were assayed using identical preparation conditions, namely 2% chitosan in 3% acetic acid, added with 3% EO and 0.5% (v/v) of each emulsifier. The best results were obtained with the emulsifier with higher HLB, giving rise to more homogeneous films, with less precipitated chitosan. Considering that chitosan precipitation was still noticed in some films prepared with acetic acid and the emulsifier with HLB=16.3, especially the ones with higher EO concentration, an increase of emulsifier concentration (1%, v/v) was tested. For all concentrations of EO assayed, better results were obtained, since a considerable decrease of the precipitation phenomena was achieved, although some was still observed in the higher concentration of EO (5%).

Subsequently, using the same referred conditions, citric acid, instead of acetic acid, was tested for chitosan solution preparation and film casting. Since it was noticed that using 3% citric acid solution resulted in a poor dissolution of chitosan, a higher concentration was chosen (6% citric acid solution) to proceed with the assays. Comparing the films produced using different acids (acetic and citric) it was noticed that softer, more flexible and thicker films were obtained using citric acid, probably due to its capacity to form multiple linkages. Moreover, for all tested EO concentrations, the films produced with citric acid were more homogeneous compared to acetic acid. It was also visible that, in general, the films obtained with citric acid were more opaque than the ones with acetic acid. Figure 1 shows the films obtained with the tested % of oregano EO.

Figure 1. Chitosan films prepared with chitosan in citric acid solution (6%, v/v) added with 1% emulsifier HLB=16.3.

1% oregano EO	3% oregano EO	5% oregano EO
		

3.1. Antibacterial activity

Figure 2 shows the results obtained in the disk diffusion assays, for the chitosan films added with different percentages of EO. During the assay, it was observed that the film disks when placed in contact with the agar, rapidly swelled (in less than a minute). So, immediately after placing the disks, the application area was marked on the glass Petri dish (as can be seen in Fig. 2). For this reason, some images show disks with somewhat undefined edges, since the initial rounded shape was lost due swelling.

The obtained results evidenced the presence of inhibition halos for all the tested films, with higher inhibition of bacteria growth achieved with the films containing higher EO concentrations.

Figure 2. Disk diffusion assays results, using the chitosan films prepared with added oregano EO in different concentrations.

Chitosan film without EO (control)	Chitosan film 1% EO	Chitosan film 3% EO	Chitosan film 5% EO
			
TOTALDIAMETER=2.1 CM	TOTALDIAMETER =2.2 CM	TOTALDIAMETER =2.7 CM	TOTALDIAMETER=3.3 CM

4. CONCLUSIONS

The incorporation of oregano essential oil in chitosan films resulted in films with better attractive properties (transparency, flexibility and softness) and a significant increase in antimicrobial capacity, thereby increasing its area of application.

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