Antimicrobial Properties of Sodium Alginate Films Loaded with Laurel and Olive Leaves Extracts for Extending the Shelf-life of Fresh Meat

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I. INTRODUCTION

The application of edible films and coatings has emerged as one of the most promising approaches to achieve the challenging and important relationship between preservation efficacy and environmental responsibility. This study aimed to perform *in vitro* studies to investigate the effectiveness of alginate films impregnated with laurel and olive leaf extracts for extending the freshness and quality of meat.

II. MATERIALS AND METHODS

Leaves of laurel and olive were harvested, washed, dried at 25ºC under air circulation until constant weight and milled in a 1 mm sieve. Extractions were carried out by ultrasonic-assisted extraction technique in an ultrasound bath. Twenty grams of dried milled leaves were mixed with 100 mL of 70:30 (v/v) ethanol:water solution, sealed in an Erlenmeyer flask and placed into the bath with 3 L of destilled water at 25ºC ±5ºC for 1h. The mixture was centrifuged at 5000 × g for 10 min. After centrifugation, the solvent was removed in a rotary evaporator at 38 ºC, under vacuum, and freeze-dried.

The films were obtained by mixing 1% (w/v) of sodium alginate (SA) and 0.5 % (w/v) of glycerol in distilled water under agitation overnight. Then, laurel leaves extract (LLE) and olive leaves extract (OLE) were dissolved in distilled water, stirred for 1 h, filtered under vacuum, and added to the film-forming solutions at 1:1 and a final concentration of 1 and 2%. All solutions were stirred for 1 h, homogenized with an Ultra-Turrax at 10000 rpm for 2 minutes, and degassed under vacuum. The film-forming solutions were cast in polystyrene petri plates, dried at 35 ºC (air circulation) for 24 h, and conditioned in desiccators containing a saturated solution of $Mg(NO_3)_2.6H_2O$ at 53% of relative humidity and 20 ºC before analysis. Antimicrobial activity for a maximum of 2% extract was conducted following the broth microdilution method using an ELISA plate reader at 600 nm against *Listeria monocytogenes* ATCC 7973, *Staphylococcus aureus* ATCC 25923, *Salmonella* Typhimurium ATCC 14028, *Enterococcus faecalis* ATCC 19433 and *Escherichia coli* ATCC 11775 at ~$5x10^5$ CFU/mL. The antimicrobial activity of films was determined by the viable cell count assay method according to Nouri et al. (1) with slight modifications. Samples with 0.1 g were immersed in 2 mL of brain heart infusion broth (BHI) inoculated with ~10^6 CFU/mL of the microorganisms previously mentioned. Samples were incubated at 37 ºC and counts were obtained at 0 and 24h. Microorganisms concentrations were standardized by OD600. All analyses were performed in duplicate.

III. RESULTS AND DISCUSSION

When OLE was combined with LLE (1:1), the minimal inhibitory concentration MIC value was achieved at 1% for *S. aureus* and 2% for *L. monocytogenes*. For other microorganisms, MIC was not achieved, which indicated that it is higher than 2%. Despite this, it was possible to observe that the absorbance decreased for higher extract concentrations. The minimum bactericidal concentration (MBC) was achieved at 2% for *S. aureus*.

Table 1 – Antimicrobial activity of alginate films impregnated with LLE+OLE extracts: counts (mean, log UFC/g) at 0 h and after 24 h of incubation.

Sample	*S. aureus*	*L. monocytogenes*	*E. faecalis*	*S.* Typhimurium	*E. coli*
CNT 0h	7.26±0.06^{b}	7.32±0.03^{d}	7.19±0.05^{c}	7.09±0.04^{c}	7.25±0.08^{c}
CNT 24h	10.66±0.24^{a}	10.48±0.19^{a}	10.17±0.09^{a}	10.68±0.05^{a}	12.05±0.02^{a}
SA	10.70±0.06^{a}	9.93±0.13^{ab}	10.02±0.14^{a}	10.77±0.03^{a}	12.05±0.03^{a}
SA+LLE0.5%+OLE0.5%	6.00±0.26^{c}	9.55±0.27^{bc}	9.65±0.02^{ab}	9.51±0.06^{b}	11.45±0.11^{b}
SA+LLE1%+OLE1%	5.31±0.05^{d}	8.84±0.19^{c}	9.03±0.33^{b}	9.35±0.04^{b}	11.20±0.05^{b}
P	<0.001	<0.001	<0.001	<0.001	<0.001

CNT – control; SA – sodium alginate; LLE – laurel leaves extract; OLE – olive leaves extract; Means with different letters (columns) differ significantly, P <0.05.

No antimicrobial activity was observed for the alginate-based film (SA) without extracts compared to the control (CNT 24h). With the addition of extracts, lower counts were observed at 24 h compared to CNT 24h, mainly for Gram-positive microorganisms.

After 24 h, and compared to CNT at 0 h, the best results were observed for SA + LLE 1% + OLE 1% against *S. aureus* with a reduction of 1.95 log CFU/g which demonstrates a good antimicrobial activity, although not enough to consider the compound bactericidal (≥ 3 log10 reduction). SA+LLE0.5%+OLE 0.5% also demonstrated good results against *S. aureus* with a reduction of 1.32 CFU/g. The antimicrobial effect observed for the other microorganisms was less desirable. However, a reduction in counts was observed after 24 hours compared to CNT24h. Notably, lower counts were obtained for *L. monocytogenes*, with a difference of 1.64 log CFU/g compared to CNT24h (P<0.001).

IV. CONCLUSION

The incorporation of plant-derived extracts demonstrated encouraging outcomes in reducing microbial counts within 24 hours, with a particular focus on Gram-positive microorganisms. The observed antimicrobial efficacy was notably pronounced against *S. aureus*. Future investigations could explore the potential of fine-tuning the concentrations or synergistic combinations of these extracts to enhance their antimicrobial potency, thereby advancing their application in meat preservation methods.

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