ANTIMICROBIAL ACTIVITY OF SODIUM ALGINATE FILMS INCORPORATED WITH LAUREL LEAVES EXTRACT FOR MEAT PRESERVATION

Márcio Moura-Alves[1,2*], Alexandra Esteves[1,2], Maria Ciríaco[1,2], José A. Silva[1,2], Kamila Soares[1,2], Lorenzo M. Pastrana[3], Miguel A. Cerqueira[3] and Cristina Saraiva[1,2]

[1]Veterinary and Animal Research Centre (CECAV), University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal

[2]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Portugal

[3]International Iberian Nanotechnology Laboratory (INL), Av. Mestre José Veiga s/n, 4715-330 Braga, Portugal

*Corresponding author email: mmalves@utad.pt

I. INTRODUCTION

The proliferation of microorganisms can cause undesirable biochemical and sensory changes in meat, and can also compromise its food safety [1]. To minimize these problems, the use of sustainable polymers with antimicrobial agents may be an alternative approach. This study aimed to gather data about the antimicrobial activity of sodium alginate-based active films incorporated with laurel (*Laurus nobilis*) leaves extract.

II. MATERIALS AND METHODS

To obtain the extracts, leaves of laurel were harvested, washed, and dried at 25ºC under air circulation until constant weight. Then, dried leaves were 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 in 100 mL of 70:30 (v/v) ethanol:water solution was sealed in an Erlenmeyer flask and placed into the bath with 3 L of 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) was dissolved in distilled water, stirred for 1 h, filtered under vacuum, and added to the film-forming solutions at 1 and 2%. All solutions were stirred for 1 h, homogenised 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 at ~ $5x10^5$ CFU/mL. The antimicrobial activity of films was determined by the viable cell count assay method according to Nouri *et al*. [2] 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

For LLE, minimal inhibitory concentration (MIC) was achieved at 1% extract for *S. aureus* and *L. monocytogenes* and 1.8% for *Enterococcus faecalis*. For other microorganisms, MIC was not achieved, which indicates that it is higher than 2%. Table 1 shows the counts for the tested microorganisms after 24h of incubation with the addition of the different films and control (BHI only).

Table 1. Counts (mean, log UFC/mL) at 0 h and after 24 h of incubation for tested microorganisms.

Sample	*S. aureus*		*L. monocytogenes*		*E. faecalis*		*S.* Typhimurium	
	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
CNT	5.97	9.33^{a}	5.95	9.16^{a}	6.01	8.85^{a}	5.84	9.36^{ab}
SA		9.38^{a}		8.61^{a}		8.70^{a}		9.44^{a}
SA + LLE 1%		6.18^{b}		5.07^{b}		7.96^{a}		8.44^{bc}
SA + LLE 2%		4.99^{c}		4.26^{b}		6.08^{b}		7.90^{c}
RSD%		27.77		34.13		15.09		8.15
P		<0.001		<0.001		<0.01		<0.001

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

In general, no antimicrobial activity was observed for the alginate-based films without LLE compared to the control. With the addition of LLE, lower counts were always observed at 24 h compared to the control, which indicates an increased difficulty for microbial development in the presence of these compounds. Significant differences (P <0.001 for *S. aureus*, *L. monocytogenes,* and *S*. Typhimurium; P <0.01 for *E. faecalis*) were obtained between the different films for all the tested microorganisms. Compared to the control, the greatest difference in counts after 24 h was observed against *L. monocytogenes* with the application of SA + LLE 2% which presented an average of 4.9 log CFU/mL levels less of this microorganism. For *S*. Typhimurium, although counts increased after 24 hours in all treatments, lower counts were observed compared to the control, mainly with the addition of LLE 2%. Antimicrobial activity was observed against *S. aureus* and *L. monocytogenes* with the addition of SA + LLE 1% and SA + LLE 2% and against *E. faecalis* with the addition of SA + LLE 2%. Compared to 0 h, the highest reduction obtained was against *L. monocytogenes*, with a reduction of 1.69 log CFU/mL, which demonstrates a good antimicrobial activity, although not enough to consider the compound bactericidal (≥ 3 $\log_{10}$ reduction) at 2%.

IV. CONCLUSION

LLE extracts and films incorporated demonstrated good antimicrobial activity. Better results were achieved with the incorporation of LLE 2% on Gram-positive bacteria, mainly for *L. monocytogenes* and *S. aureus*. Nevertheless, it was also possible to observe a bacteriostatic effect at 1% for *L. monocytogenes* and *S. aureus*. The study of the antimicrobial activity of the extracts and films shows their potential to be used in the control of microorganisms in meat, and consequently the possibility of extending its shelf-life and also reducing food waste using biodegradable compounds.

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