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BIO-BASED PACKAGING FOR CHICKEN MEAT PRESERVATION: ALGINATE COATINGS ENRICHED WITH PLANT EXTRACTS AND NISIN

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I. INTRODUCTION

The growing demand for sustainable and eco-friendly alternatives has led to the research of innovative preservation techniques for perishable products. The application of edible films and coatings has emerged as one of the most promising approaches to achieve this challenging and important relationship between preservation efficacy and environmental responsibility. This study investigated the effectiveness of sodium alginate-based coatings enriched with laurel leaf extract, olive leaf extract, and nisin in preserving the microbial quality of chicken breast meat packed with polylactic acid, providing a bio-based and eco-friendly alternative to traditional packaging.

II. MATERIALS AND METHODS

Laurel and olive leaves extracts were obtained by ultrasonic-assisted extraction technique in an ultrasound bath using 70:30 (v/v) ethanol:water. The coating solutions were prepared in distilled water by mixing 1% (w/v) of sodium alginate (SA) and 0.5% (w/v) of glycerol under agitation overnight at room temperature. Lyophilized extracts of laurel and olive leaves were dissolved in distilled water, stirred for 1 h, filtered under vacuum and added to the film-forming solutions in the following concentrations: LLE: Laurel at 2% (w/v); MIX: LLE at 1% (w/v) + OLE at 1% (w/v); and NIS: Nisin at 1% (w/v) (dissolved in 0.01 N HCl). All solutions were stirred for 1 h.

Chicken breast pieces (24 h *post mortem*) were portioned with ca. 50-70 g and divided in 5 groups. Three groups were coated by spraying at room temperature (LLE, MIX, and NIS samples) until the entire surface was covered. Then, a solution of $CaCl_2$ at 1.5% (w/v) was sprayed to achieve enhanced crosslinking. Coated and uncoated samples (PLA samples) were packed in polylactic acid (PLA) biopolymer trays + PLA films. Uncoated samples (CNT samples) were also packed in conventional polypropylene trays and polythene films. The samples were stored at 4 ºC and analyzed over 13 days and 6 sampling times. The study was conducted in triplicate and on three different occasions. In total, 270 samples were analyzed. Microbial analyses were performed in duplicate.

Serial decimal dilutions were prepared using Tryptone salt. Total aerobic mesophilic bacteria and psychrotrophic bacteria were determined using Plate Count agar with incubation at 30 °C for 72 h and 7 °C for 10 days, respectively. The enumeration of *Pseudomonas* spp. was performed using Pseudomonas agar + Cetrimide Fucidin, and Cephaloridine Pseudomonas supplement at 25°C for 48 hours. Lactic acid bacteria were enumerated on de Man Rogosa Sharpe Agar at 30 °C for 72 hours. Baird-Parker agar with egg yolk–tellurite emulsion 20% and rabbit plasma fibrinogen supplement II at 37 °C for 24–48 h was used for *S. aureus*. The results were expressed as log CFU g^{-1}.

III. RESULTS AND DISCUSSION

Table 1 shows the changes in microbial counts (log CFU g^{-1}) of chicken breast fillets during 13 days at 4 °C. LLE, MIX, and NIS treatments consistently showed lower microbial counts for mesophilic microorganisms during storage time with significant differences observed over time compared to CNT and PLA, mainly at 1 ($P < 0.001$), 9 ($P < 0.05$) and 13 days ($P < 0.001$). The CNT and PLA samples reached approximately 9 log at day 9, while LLE, MIX, and NIS only reached that level at day 13. For psychrotrophic microorganisms, NIS treatment led to a significant reduction ($P < 0.01$) by ~1 log after 24 h of storage, while LLE and MIX showed a slight decrease. At day 13, CNT and PLA packaging showed the highest values with NIS, LLE, and MIX reductions of ~0.6-0.8 log. For LAB, the NIS treatment showed the lowest counts during the storage period up to day 9. At days 1 and 3, NIS effectively reduced LAB counts to 0 log, which corresponds to a reduction of ~1.5 log when compared to CNT and PLA. MIX and NIS treatments significantly inhibited *Pseudomonas* spp. growth compared to uncoated samples after 24 h of storage ($P < 0.01$). The mean count for CNT and PLA represented a 1.58 and 1.67 log increase, while MIX and NIS showed an increase of 0.27 and 0.41 log, respectively. This represents more than 50% less growth than CNT and PLA.

Table 1 – Counts (mean ± standard deviation, log CFU g^{-1}) for each treatment during storage time.

	Time (days)	Treatment					*P*
		CNT	PLA	LLE	MIX	NIS	
Mesophilic	0#	3.47±0.21^{D}	3.50±0.45^{D}	3.39±0.23^{D}	3.38±0.31^{D}	3.47±0.24^{E}	ns.
	1	3.48±0.28$^{ac\ D}$	3.89±0.38$^{a\ C}$	3.28±0.31$^{c\ D}$	3.25±0.29$^{bc\ D}$	2.73±0.50$^{b\ D}$	***
	3	5.00±0.48^{C}	4.19±0.48^{C}	4.95±0.55^{C}	4.72±0.60^{C}	4.71±0.81^{C}	ns.
	6	7.50±0.66^{B}	7.42±0.44^{B}	7.12±0.53^{B}	7.10±0.60^{B}	6.95±0.40^{B}	ns.
	9	9.12±0.36$^{a\ A}$	8.90±0.65$^{ab\ A}$	8.43±0.56$^{b\ A}$	8.49±0.19$^{ab\ A}$	8.45±0.46$^{ab\ A}$	*
	13	9.57±0.17$^{a\ A}$	9.45±0.13$^{a\ A}$	8.92±0.35$^{b\ A}$	8.96±0.23$^{b\ A}$	9.01±0.23$^{b\ A}$	***
	P	***	***	***	***	***	
Psychrotrophic	0#	3.75±0.38^{D}	3.55±0.44^{D}	3.51±0.41^{D}	3.71±0.43^{CD}	3.73±0.36^{D}	ns.
	1	3.53±0.47$^{a\ D}$	3.87±0.18$^{a\ CD}$	3.35±0.42$^{ab\ D}$	3.43±0.47$^{ab\ D}$	2.83±0.46$^{b\ E}$	**
	3	5.34±0.31^{C}	4.79±0.68^{C}	5.02±0.42^{C}	4.79±0.51^{C}	4.61±0.46^{C}	ns.
	6	8.26±1.49^{B}	8.54±1.01^{B}	7.77±1.30^{B}	7.77±1.55^{B}	7.74±1.10^{B}	ns.
	9	10.74±0.38$^{a\ A}$	10.60±0.39$^{ab\ A}$	9.92±0.51$^{c\ A}$	10.04±0.22$^{bc\ A}$	10.19±0.40$^{abc\ A}$	**
	13	11.37±0.14$^{a\ A}$	11.10±0.17$^{ab\ A}$	10.65±0.38$^{c\ A}$	10.62±0.22$^{c\ A}$	10.76±0.26$^{bc\ A}$	***
	P	***	***	***	***	***	
LAB	0#	1.42±1.12^{BC}	1.41±1.15^{B}	1.51±1.20^{B}	1.23±1.21^{C}	1.71±0.84^{B}	ns.
	1	1.64±0.90$^{a\ BC}$	1.46±0.95$^{a\ B}$	1.19±1.03$^{ab\ B}$	1.05±1.18$^{ab\ C}$	0.00±0.00$^{b\ C}$	**
	3	1.32±1.01$^{a\ C}$	1.43±0.89$^{a\ B}$	1.35±1.03$^{a\ B}$	1.09±1.06$^{ab\ C}$	0.00±0.00$^{b\ C}$	**
	6	2.86±0.87$^{a\ B}$	3.09±0.88$^{a\ A}$	3.17±0.28$^{a\ A}$	2.18±1.23$^{ab\ BC}$	1.01±1.53$^{b\ BC}$	**
	9	4.62±0.89$^{a\ A}$	4.40±0.66$^{ab\ A}$	3.87±0.56$^{ab\ A}$	3.52±0.46$^{b\ AB}$	3.64±0.29$^{b\ A}$	**
	13	4.52±0.85^{A}	4.49±1.00^{A}	3.85±0.80^{A}	4.44±0.49^{A}	4.16±0.33^{A}	ns.
	P	***	***	***	***	***	
Pseudomonas spp.	0#	0.83±0.82^{F}	0.87±0.84^{E}	0.60±0.91^{E}	0.86±0.87^{D}	0.66±0.80^{D}	ns.
	1	2.41±0.41$^{ab\ E}$	2.54±0.49$^{a\ D}$	1.70±0.80$^{ab\ D}$	1.13±1.22$^{b\ D}$	1.07±1.20$^{b\ D}$	**
	3	4.11±0.36$^{a\ D}$	3.29±0.50$^{a\ D}$	3.74±0.47$^{a\ C}$	3.48±0.69$^{a\ C}$	3.36±0.41$^{a\ C}$	*
	6	5.67±1.02^{C}	5.64±0.92^{C}	5.11±0.74^{B}	5.26±0.81^{B}	5.23±0.45^{B}	ns.
	9	7.47±0.56^{B}	7.24±0.58^{B}	6.87±0.54^{A}	6.90±0.39^{A}	6.96±0.63^{A}	ns.
	13	8.71±0.61$^{a\ A}$	8.30±0.69$^{ab\ A}$	7.53±0.37^{CA}	7.80±0.31$^{b\ CA}$	7.78±0.32$^{b\ CA}$	***
	P	***	***	***	***	***	
S. aureus	0#	1.71±1.63^{B}	1.96±1.48^{AB}	1.57±1.51	2.14±1.23	1.56±1.50^{AB}	ns.
	1	1.84±1.43^{B}	1.95±1.52^{B}	1.77±1.38	2.45±1.21	0.51±1.25^{AB}	ns.
	3	2.91±0.24$^{a\ AB}$	3.03±0.52$^{a\ AB}$	2.40±1.18^{a}	2.89±0.25^{a}	0.00±0.00$^{b\ B}$	***
	6	2.33±1.65^{AB}	2.62±0.23^{AB}	1.45±1.70	2.21±1.09	1.34±1.47^{AB}	ns.
	9	3.12±1.64^{AB}	3.64±1.01^{AB}	2.19±1.74	1.59±1.46	1.94±1.84^{AB}	ns.
	13	4.41±0.53^{A}	4.46±0.38^{A}	3.53±1.69	2.91±1.73	2.76±1.96^{A}	ns.
	P	**	***	ns.	ns.	*	

LAB – lactic acid bacteria; # Before treatment; ns., non-significant; For storage time (rows), means with different letters (lowercase) differ significantly, *P < 0.05, **P < 0.01, ***P < 0.001. For treatments (columns), means with different letters (uppercase) differ significantly, *P < 0.05, **P < 0.01, ***P < 0.001.

The NIS treatment effectively inhibited *S. aureus*, reaching 0 log at day 3 ($P < 0.001$), whereas all other treatments showed counts above 2 log. On day 3 and 6, *S. aureus* was able to grow. Despite this, it was possible to maintain lower bacterial counts during storage compared to CNT and PLA, achieving a reduction of ~1.7 log on day 13.

IV. CONCLUSION

The findings revealed the ability of the coatings to significantly retard microbial growth. The incorporation of nisin showed greater efficacy in controlling microbial growth during the initial stages of storage. In contrast, the antimicrobial effects of the formulations with laurel and olive leaves were more gradual, likely due to a slower and controlled release of the active compounds over the storage period. The simultaneous incorporation of LLE and OLE demonstrated useful interaction, promoting the use of an industrial by-product (olive leaves). Future research could focus on exploring new combinations of antimicrobial compounds and optimizing coating formulations.

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