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EVALUATION OF THREE SUSTAINABLE SOLUTIONS FOR EXTENDING THE SHELF LIFE OF BEEF BURGERS UNDER MODIFIED ATMOSPHERE AT 4°C

Matteo Carletta*, Chiara Purgatorio, Luigi Esposito, Matteo Quaranta, Francesca Maggio, Annalisa Serio, Maria Martuscelli, Antonello Paparella

[1] Department of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Via R. Balzarini, 1, 64100 Teramo, Italy

*Corresponding author email: mcarletta@unite.it

I. INTRODUCTION

Biopreservation strategies are gaining attention as alternatives to conventional methods (use of additives) to improve the stability of refrigerated meat products. These methods typically involve the use of plant-derived extracts and essential oils, bacteriocins, and/or starter cultures, which mitigate the effects of microbial growth and extend products' shelf-life [1]. In addition to their antimicrobial potential, biopreservatives align with circular economy principles. The valorization of natural compounds from food industry by-products supports resource efficiency, waste reduction, and sustainability, minimizing their environmental impact and promoting the development of functional ingredients that enhance food safety and quality [2]. Moreover, the use of natural compounds in food formulations may have the advantage of keeping food labels clean, by removing the additives from the list of ingredients, provided that they can be considered as processing aids according to art. 3 Regulation (EC) no. 1333/2008 of food additives. The aim of this study was to evaluate the efficacy of three biopreservatives: (1) *Citrus* × *paradisi* essential oil (EO), (2) a commercial phenolic extract derived from olive processing by-products, and (3) a commercial aromatic extract from fruits and spices, to extend the shelf life of beef burgers.

II. MATERIALS AND METHODS

Four beef burger formulations were prepared: (i) control samples without biopreservatives (Ctrl), (ii) samples supplemented with *Citrus* × *paradisi* EO (Flora srl, Lorenzana, Italy) at 0.03% (Bp1), (iii) samples containing *Citrus* × *paradisi* EO at 0.01% and olive phenolic extract at 0.05% (Bp2), and (iv) samples incorporating a fruit and spice aromatic extract at 0.4% (Bp3). Following production, samples were packaged in Modified Atmosphere Packaging (MAP; oxygen 65%, nitrogen 10%, carbon dioxide 25%) and stored in a laboratory refrigerated cabinet at 4°C. The samples were analyzed in triplicate at three time points: after production (T0), at the end of the shelf-life (T5), and 3 days after the shelf life (T8).

For microbiological analyses, twenty-five grams of each sample were aseptically collected and homogenized with 225 mL of saline solution. Ten-fold serial dilutions were prepared and inoculated into Plate Count Agar media for enumeration of aerobic mesophilic count (AMC), after incubation at 30°C for 48 h. Two replications for each plated dilution were performed. The pH of the meat-water mixtures was assessed over time using a pH meter (Mettler Toledo, FE20, Columbus, OH, USA). Colour assessments were carried out using a CR-5 colorimeter (Spectrally based, Konica Minolta, Tokyo, Japan) with a D65 light source in the CIE Lab colorimetric space. The colour differences (ΔE) between the enriched burgers (L_2^*, a_2^*, b_2^*) and the control (L_1^*, a_1^*, b_1^*) was determined according to equation 1 [3]:

$$\Delta E = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2} \quad (1)$$

Samples were evaluated through Quantitative Descriptive Analysis (QDA), targeting four categories of sensory descriptors: appearance, odour, flavour, and texture. The selected attributes were rated by the panelists using a 9-point Likert scale, where 1 corresponded to the lowest perceived intensity and 9 to the highest. The statistical analysis was performed with Minitab® 19.1 software (USA, 2019). The significance level for the one-way ANOVA was set to $p < 0.05$, and the Tukey test was performed to assess pairwise significant differences.

III. RESULTS AND DISCUSSION

Microbiological results showed a significant reduction of aerobic mesophilic count for all three treatments compared to the control, as observed in Table 1. The reduction was similar for the three experimental batches, even if the aromatic fruit and spice extract (Bp3) showed a slightly higher load reduction. A maximum antimicrobial effect was observed at T0 (about 1 Log CFU/g reduction), still remaining significant over time.

Colorimetric evaluation showed a ΔE value ≈3.0 throughout the shelf-life (T0 and T5) for all the enriched samples, suggesting that the biopreservatives did not cause any perceivable change in appearance. ΔE values were significantly higher at the end of the observation period (T8); visual inspection (Figure 1-B) confirmed these findings,

with enriched samples exhibiting more vivid red coloration and less discoloration than the control. Sensory profiles revealed that key attributes were preserved across all formulations, although the samples with Bp1 were rated higher for the typical *Citrus* × *paradisi* EO flavour.

	Log CFU/g Reduction			pH			Color change ΔE		
	T0	T5	T8	T0	T5	T8	T0	T5	T8
Ctrl	/	/	/	5.87 ± 0.03 aA	5.84 ± 0.05 aA	5.51 ± 0.03 aB	/	/	/
Bp1	1.05 ±0.11 aA	0.91 ±0.12 aA	0.45 ± 0.11 abB	5.84 ± 0.03 aA	5.84 ± 0.03 aA	5.52 ± 0.04 aB	2.74 ± 1.08 aA	3.01 ± 1.30 aA	5.3 ± 0.99 aB
Bp2	0.84 ±0.22 aA	0.97 ±0.07 aA	0.39 ± 0.06 aB	5.83 ± 0.03 aA	5.79 ± 0.03 aA	5.52 ± 0.04 aB	3.09 ± 1.16 aA	2.62 ± 0.97 aA	5.00 ± 0.81 aB
Bp3	1.07 ±0.09 aA	0.98 ±0.11 aA	0.51 ± 0.04bB	5.72 ± 0.027 bA	5.71 ± 0.038 bA	5.44 ± 0.031 bB	3.08 ± 1.75 aA	3.34 ± 1.37 aA	7.36 ± 0.58 bB

Table 1 *- Reduction of aerobic mesophilic count (Log CFU/g), pH values and colour change (ΔE) of control (Ctrl) and treated beef burgers (Bp1, Bp2, Bp3) at T0, T5, and T8. Different lowercase letters indicate statistically significant differences between formulations at the same time point; different uppercase letters indicate significant differences between time points within the same formulation.*

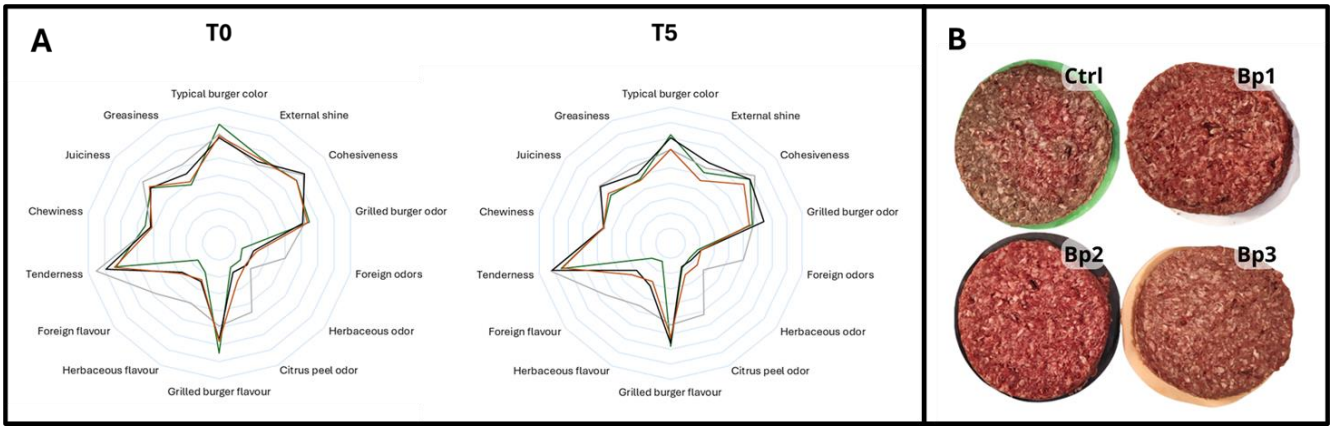


Figure 1- *QDA profiles of control (Ctrl) and beef burgers treated with biopreservatives (Bp1, Bp2, Bp3) immediately after production (T0) and at the end of their commercial shelf-life (T5) (**A**); visual appearance of control and treated burgers at T8 (**B**).*

IV. CONCLUSION

This study confirms that the selected biopreservatives could potentially enhance the stability of beef burgers. Among the tested formulations, Bp2 and Bp3 emerged as the most promising options, as they combine effective microbial inhibition with favorable sensory retention. These results support the feasibility of incorporating clean-label preservation strategies in meat preparations, responding to both industry needs and consumer demand for natural and sustainable alternatives to food additives.

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