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MACROALGA EXTRACTS AND MEAT PRODUCTS. MATCH OR MISMATCH?

S. Ray[1], M. Kuijpers[1], R. Salvador[1], M. Porta[1], C. Peteiro[2] and T. Aymerich[1]

[1] IRTA, Food Safety and Functionality, Monells, Spain [2] CSIC-IEO, Santander, Spain

*Corresponding author email: teresa.aymerich@irta.cat

I. INTRODUCTION

Research on bioactive compounds from macroalgae has gained relevance due to their potential use as new or alternative ingredients or natural additives to extend shelf life or improve food safety. Although synthetic additives are very important in the food industry and are well established under EU legislation, consumer demand for natural preservatives has risen in recent decades. Marine macroalgae (or seaweeds), particularly brown macroalgae are known to produce a wide variety of chemical compounds with potential bioactivities. Rubiño et al [1] [2] [3] [4] reported significant *in vitro* activity of mid-polarity extracts from *Ericaria selaginoides* against *Listeria monocytogenes* and *Bacillus cereus*, along with promising potential for application as antimicrobial agents in fresh cheese. *In situ* testing is critical to observe the real effect on a specific food matrix and to understand the positive or negative interactions with the food matrix and preservation conditions.

L. monocytogenes remains one of the most significant concerns in the EU. Although the number of reported cases is relatively low, it is the food-borne pathogens with the highest-mortality [5]. Moreover, its ability to survive food processing environments [6] and to grow even at refrigeration temperatures poses a serious public health concern.

The objective of this study was to evaluate the antimicrobial efficacy of mid-polarities extracts from *E. selaginoides*, previously shown to be highly active *in vitro*, in poultry burger meat preparations with spinach. Samples were packed either in MAP (80% O_2 and 20% CO_2), air, or vacuum, with or without traditional additive such as sulfite. Although *L. monocytogenes* is especially critical in RTE-food products [7], this study investigates the potential use of the extracts in burger meat preparations that may pose a risk if undercooked or intended for further processing.

II. MATERIALS AND METHODS

E. selaginoides was collected from the northern Spanish Cantabrian Sea. This perennial, arborescent brown macroalga forms extensive stands and is widely distributed with abundant populations along the European Atlantic coast. Collected samples were freed of epiphytes and cleaned with seawater. Afterwards, they were sent refrigerated to the laboratory for further processing.

To obtain mid-polarity extracts, the samples were ground, and 90-minute homogenization extraction with a mix of food-grade solvents containing isopropanol and hexane was performed. Macroalga residues were removed by centrifugation at 5,000 rpm for 10 minutes. The resulting extracts were dried by using a rotary evaporator and nitrogen efflux. For food application, extracts were redissolved in a food-grade compatible TGA buffer (10:10:80 of glycerol: tween 80: water). A microemulsion was prepared through 10 minutes of ultrasound treatment at 37 MHz, combined with 60 seconds of vortexing to enhance solubility and further homogenization. Poultry burger meat with spinach, without additives or with sulfites, was produced in the pilot plant. Challenge tests were conducted by artificially inoculating the samples with *L. monocytogenes* CTC 1011 at approximately 2 log CFU/g, in order to evaluate the potential for *L. monocytogenes* growth with or without the extracts during the established refrigerated shelf life at 8ºC (slightly refrigerated temperature abuse) for 10 days (considering 1/3 extended shelf life as recommend in the LM-guides) [8]. The different lots spiked with *L. monocytogenes* were analyzed along the shelf life (until 10 days for MAP and air and 21 days for vacuum pack). Samples were analyzed in duplicates to assess *L. monocytogenes* growth behavior in the presence or absence of different concentrations of the mid-polarity macroalga extracts. Concentrations applied were related to previously *in vitro* determined MIC (minimal inhibitory concentration) of the extracts, 2.5X, 5X and 10X MIC. Packing conditions included modified atmosphere (MAP, 80%O_2 and 20% CO_2), air, and vacuum.

Statistical analyses were performed to determine the μmax, growth potential, time to 2-Log increase and percentage of remaining expected safe shelf life compared to control batch. Significant differences were evaluated using one-way ANOVA and Tukey-Kramer post hoc test ($p<0.05$) in JMP17 software (SAS Institute).

III. RESULTS AND DISCUSSION

Macroalga extracts were not able to completely inhibit the growth of the *L. monocytogenes* at any of the concentrations or conditions tested. Nevertheless, under MAP (simulated commercial preservation), 5 X MIC significantly ($p<0.1$) reduced μmax, increased the time to achieve 2-Log increase, and consequently extended the remaining safe shelf life. Despite these effects, the extracts did not significantly reduce the total growth potential after 10 days storage at 8ºC (no significant differences in final counts compared to the control batch were observed at the end of storage). Under vacuum conditions, after 21 days of storage at 8ºC, the extract applied at a concentration equivalent to 10 X the *in vitro* MIC also slightly decreased the growth rate and extended the safe shelf life. Under air conditions (simulating secondary shelf life) and compared to control, no significant differences were observed when the extract was applied at 10 X MIC. Macroalga extracts were less effective in meat burgers packed in air compared to their previous application in Catalan fresh cheese, probably due to the interaction of with meat fat and proteins or due to atmosphere interactions with active compounds, which may interfere with the activity of the extracts against *L. monocytogenes*. Nevertheless, the overall growth potential of *L. monocytogenes* was significantly lower in burger samples compared to fresh cheese matrix. The batch containing sulfite extended lag phase but did not inhibited growth, probably due to the neutral pH of the product, 6.4. Macroalga extracts under these conditions also did not provide any additional antimicrobial effect.
Macroalga extracts containing natural chlorophyll pigment, added to enhance the color of the burger with lyophilized vegetables, visually preserved the green color under tested conditions throughout shelf life.

IV. CONCLUSION

The microemulsions of macroalga extracts containing the natural green chlorophyll pigment maintained stable color throughout the entire shelf life and could therefore be proposed to improve color of burger meat with added lyophilized vegetables. However, future studies should focus on optimizing emulsion design to enable the controlled release of active compounds of the extract, while minimizing negative interactions with meat fats and proteins that may inhibit their antimicrobial efficacy.

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