To Replace Toxins Linked with Breast Cancer: The Development of Antimicrobial Sustainable Food Packaging Films Utilizing Bilayer Emulsion Compositions

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Introduction

Food packaging presents a distinct challenge for breast cancer prevention due to concerns regarding the toxicity of synthetic preservatives and other chemicals that may leach into food. Previous studies have found common food preservatives, such as sulfites, sorbates, nitrates, benzoates, and formaldehyde to elicit negative health effects including allergic reactions.[1] Plastics used to contain food have also been a source of concern. Bisphenol A (BPA) and perfluorooctanoic acid (PFOA), common chemicals used in plastic food packaging, have been linked to breast cancer.[2,3] These chemicals are attractive targets for replacement through green chemistry.

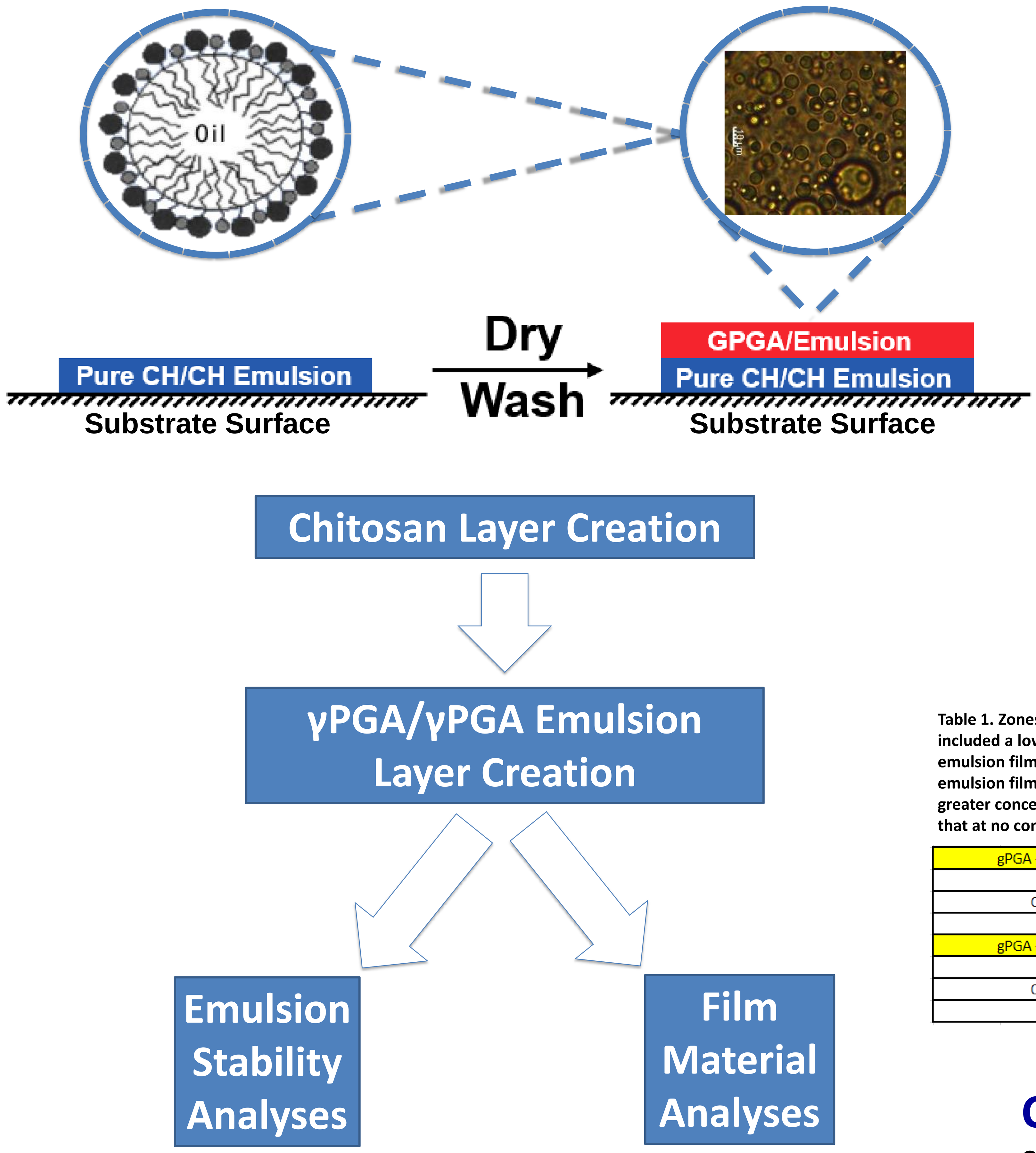
To develop a nontoxic, biodegradable food packaging film, emulsion systems present a novel opportunity. Previous films have employed monolayer systems, such as whey protein isolate films, which have been tested to great effect as targeted antimicrobials[4]. However, these materials lack the broad-range antimicrobial capabilities necessary for an applicable film. Instead, bilayer systems utilizing emulsions present a more feasible option. Through the use of water, chitosan (CH)[5], essential oils[6], poly-γ-glutamic acid (gPGA)[7], and sophorolipid-butyl ester (SLBE)[8], a food packaging film with these desired properties could potentially be developed. Knowledge of optimal formulation compositions for emulsion stability, mechanical strength, and antimicrobial capability are essential to assessing the applicability of these materials. Understanding these factors may, in the near future, allow for the development of a safer, more sustainable option in food packaging.

Methods

Materials for preparing films were provided by Dr. Gross and Rensselaer Polytechnic Institute. Oregano, thyme, and lemon oils were used as the essential oil phases in the emulsion systems used within the bilayer films. Essential oil concentrations of 1 wt%, 5 wt% and 10 wt% were tested. Bilayer film compositions were also varied.

Imaging of emulsion vials, microscopy of samples, pH readings, viscosity measurements, and dynamic light scattering (DLS) tests for droplet size and zeta potential of systems were analyzed. Greater emulsion stability results from these measurements were indicative of optimal film materials.

Formed bilayer films were tested for functionality, mechanical stability, and antimicrobial capability. Visible film integrity, thickness, moisture content, and water solubility were recorded. For mechanical strength, Young's Moduli, elongation at break, and ultimate tensile strength were also measured. To test antimicrobial activity, films were further tested for their inhibition of *Bacillus cereus*, *Candida tropicalis*, or *Escherichia coli.*



Results

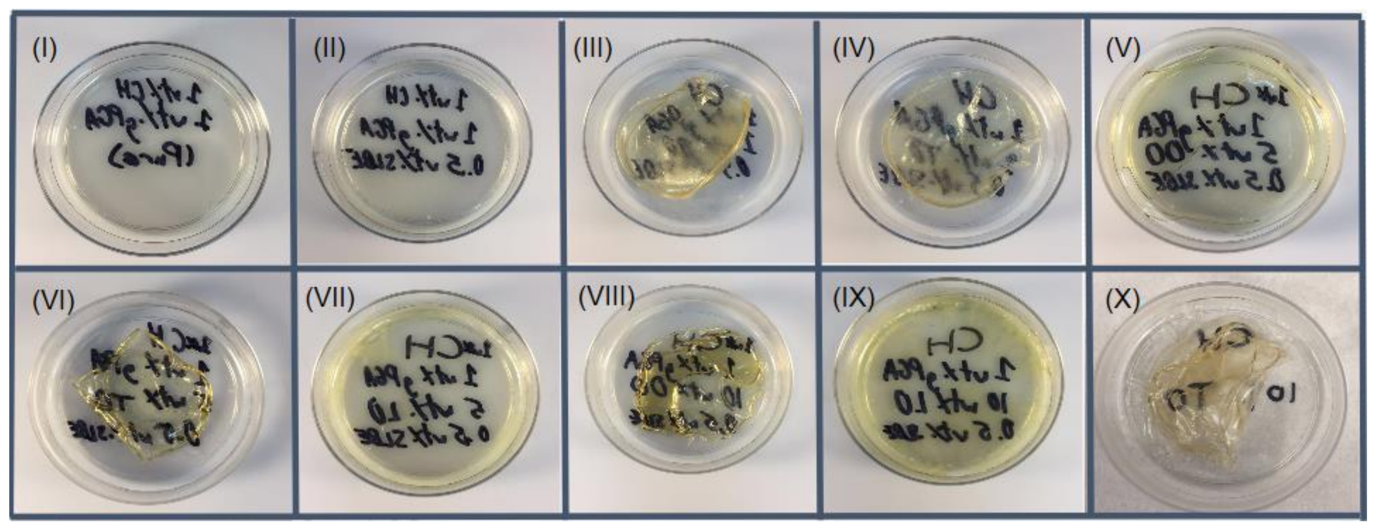


Figure 1. Images of casted bilayer films of pure low molecular weight chitosan bottom layers and gPGA solution or emulsion top layers. The above images show film systems which all contained a bottom layer of a casted 1 wt% low molecular weight chitosan solution. The systems varied by containing top layers (I) 1 wt% gPGA solution, (II) 1 wt% gPGA and 0.5 wt% SLBE solution, (III) 1 wt% gPGA, 0.5 wt% SLBE, and 1 wt% oregano oil emulsion, (IV) 1 wt% gPGA, 0.5 wt% SLBE, and 1 wt% thyme oil emulsion, (V) 1 wt% gPGA, 0.5 wt% SLBE, and 5 wt% oregano oil emulsion, (VI) 1 wt% gPGA, 0.5 wt% SLBE, and 5 wt% thyme oil emulsion, (VII) 1 wt% gPGA, 0.5 wt% SLBE, and 5 wt% lemon oil emulsion, (VIII) 1 wt% gPGA, 0.5 wt% SLBE, and 10 wt% oregano oil emulsion, (IX) 1 wt% gPGA, 0.5 wt% SLBE, and 10 wt% lemon oil emulsion, or (X) 1 wt% gPGA, 0.5 wt% SLBE, and 10 wt% thyme oil emulsion. Graphic by author.

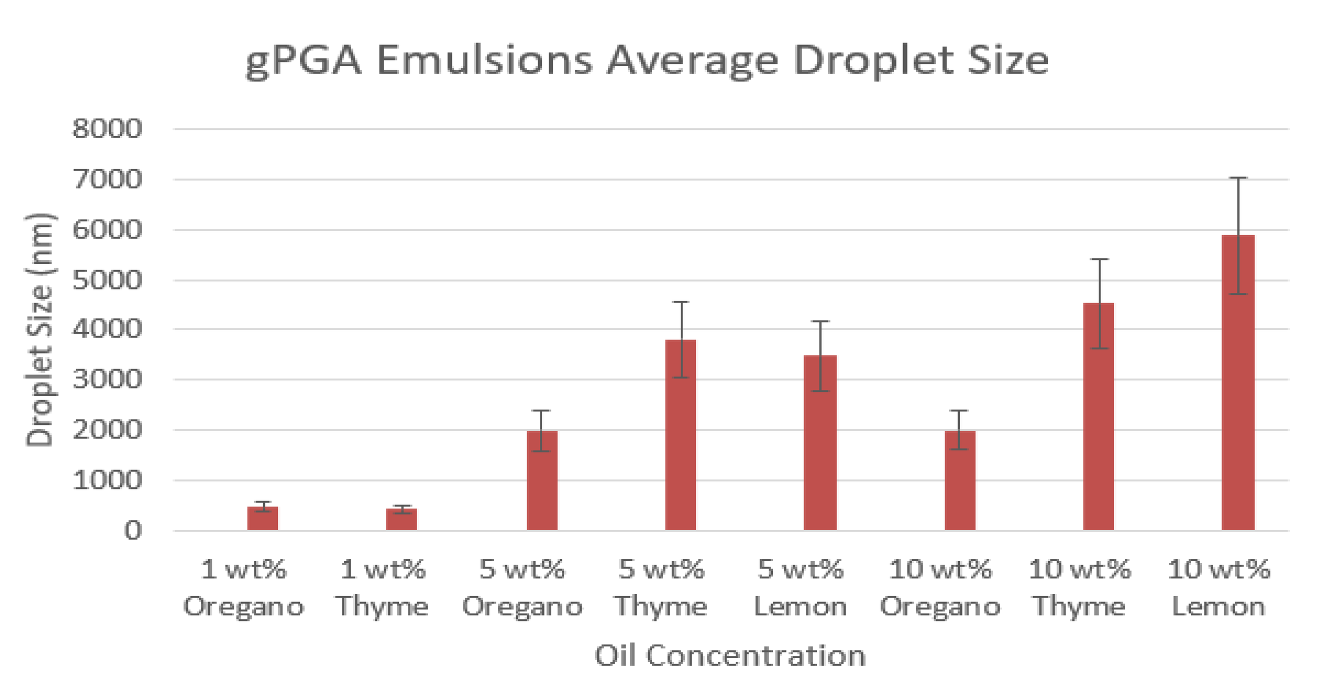


Figure 2. gPGA Emulsions Average Droplet Size. Error bars represent +/- 1 S.E. Note that increasing oil concentrations resulted in increasing droplet sizes in most cases. Oregano oil systems generally exhibited lower average droplet sizes when compared to lemon oil and thyme oil systems of similar oil concentration, as seen in the 5 wt% and 10 wt% compositions. Also note that while thyme oil and lemon oil systems experienced a notable increase in droplet size between 5 wt% and 10 wt% oil concentrations, oregano oil emulsions did not experience a similar increase, suggesting different molecular interactions with SLBE. Graphic by author.

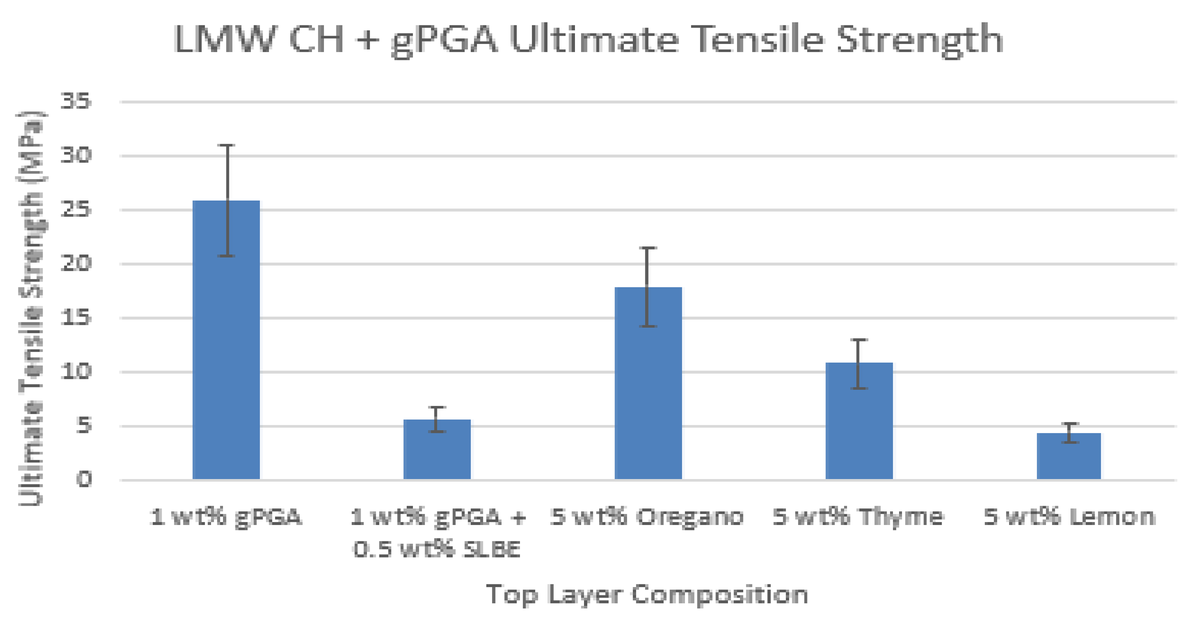


Figure 3. Ultimate Tensile Strength of bilayer films of pure low molecular weight chitosan bottom layers and gPGA solution or emulsion top layers. All bottom layers were composed of 1 wt% low molecular weight chitosan. The top layers varied between tested films. In general, films with greater ultimate tensile strengths, and thus greater elasticity values, were preferable. Note that films with top layers of 1 wt% gPGA solutions had the most elasticity, materials with top layers of 5 wt% oregano oil, 1 wt% gPGA, and 0.5 wt% SLBE emulsions remained comparably elastic. Graphic by author.

Table 1. Zones of inhibition generated from the antimicrobial tests. All measurements were taken in cm^2. These tested films included a low molecular weight chitosan bottom layer and a gPGA solution or emulsion top layer. Note that oregano oil emulsion film systems exhibited activity against the gram-positive bacteria *B. cereus* and fungi *C. tropicalis*, and thyme oil emulsion film systems exhibited activity against the gram-negative bacteria *E. coli*. This activity became more pronounced with greater concentrations of oil, until at 10 wt% oil, the top layer emulsions began to fail prior to the antimicrobial tests. Also note that at no concentration did the lemon oil emulsion film systems gain notable antifungal capabilities. Table by author.

gPGA + LMW Chitosan	gPGA (Pure)	gPGA + SLBE	1 wt% OO	1 wt% TO	5 wt% OO
B. cereus	None	3.9741	0.785	0.785	8.2916
C. tropicalis	None	None	3.14	2.4041	33.1663
E. coli	None	None	1.7663	3.14	2.4041
gPGA + LMW Chitosan	5 wt% TO	5 wt% LO	10 wt% OO	10 wt% TO	10 wt% LO
B. cereus	None	None	2.355	11.0391	5.9366
C. tropicalis	17.7116	None	4.9063	4.9063	0.785
E. coli	9.6163	0.785	1.2266	None	None

Conclusion

SLBE was most effective in stabilizing oregano oil emulsion systems. Pure 1 wt% gPGA top layers yielded the greatest elasticity, with 5 wt% oregano oil emulsion top layers with the second greatest ultimate tensile strength. Oregano oil exhibited the greatest antimicrobial activity against the gram-positive bacteria and fungi, while thyme oil had the greatest activity against the gram-negative bacteria. Employing 5 wt% oregano oil, 1 wt% gPGA, 0.5 wt% SLBE, and 1 wt% CH, antimicrobial hydrocolloid bilayer films with favorable mechanical properties could be applied to circumvent the use of suspected carcinogens in food packaging.

Acknowledgments

This work was made possible by the many contributions of the Great Neck Breast Cancer Coalition, Dr. Richard Gross, and Xue Wang from the Center for Biotechnology and Interdisciplinary Studies of Rensselaer Polytechnic Institute.

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