

## Development of new additives of graphene oxide, lysozyme and lecithin with enhanced antimicrobial and mechanical properties for active food packaging applications.

Lucas Cavalcanti Escocard Freitas\*, Antonio Matias Navarrete de Toledo, Nathalia Cristina Cirone Silva, Ana Carla Kawazoe Sato, Carolina Siqueira Franco Picone.

### Abstract

The need to extend food shelf-life has driven the development of new conservation means. Recently, nanotechnology has been on the spotlight due to the enhancement of material's properties. In this sense, this present work aimed to study the association of graphene oxide, egg lysozyme and soy lecithin on nanoscale. The combination of their attributes achieved a boost of antimicrobial and mechanical properties and enabled the functionalization of low-ester pectin films.

### Key words:

Nanoparticle, biopolymer, electrostatic complex.

### Introduction

Antimicrobial activity of nanomaterials has motivated several researches to improve additives and packaging<sup>[1,2]</sup>. The emulsifying capacity of lecithin and the effect of lysozyme against bacteria are widely used as technology coadjuvants. However, graphene and its derivatives are yet to be explored. Recent studies reported cell membrane disruption due to tension made by their sheets<sup>[3]</sup>. Better understanding this mechanism would enable a complexation of the mentioned substances with a synergistic effect, as activity would be combined, and broaden the spectrum for food application. The main objective was the formation of graphene oxide, lysozyme and lecithin nanoparticles with antimicrobial and mechanical properties for food applications, functionalizing low-ester pectin films.

### Results and Discussion

Initially, soy lecithin (Lip), egg lysozyme (Lis) and graphene oxide (GO) were physico-chemically characterized. According to  $\zeta$ -potential analysis, pH 4 showed stronger complexation force due to better exposing phosphatidylcholine, the main negative group from lecithin, and positives aminoacids from lysozyme. Further experiments varying ratios and concentrations revealed optimal mass ratio to be 1:10 Lip:Lis at 100 $\mu$ g/mL, thus avoiding critical micellar concentration of lecithin and resulting on the charge density to remain positive for binding GO sheets. Then, complexes prepared were mixed with GO under the same conditions, at 1:1 ratio. The final particles had an overall zeta potential slightly more positive, in spite of the negative charge density of GO. This could be explained by a conformation rearrangement of the enzyme, better exposing its active site. Moreover, microbiological studies of the nanoparticles presented interesting results on *Staphylococcus aureus*. Minimal inhibitory concentration (MIC) carried out exhibited Lip:Lis to be the particle with the least amount to be effective, followed by LipLis:GO. However, minimal bactericidal concentration (MBC) analysis exhibited a unique result for LipLis:GO. It was the only nanoparticle complex produce a bactericidal effect, as the combined effects of lecithin, lysozyme and graphene oxide were successful. First, lysozyme, whose activity is the catalysis of peptidoglycan, degraded bacteria wall, making it more susceptible to graphene oxide. Then, the tension made by the GO sheets have disrupted bacteria membrane, finally destroying them. In

addition, lecithin has also contributed with its emulsifying factor, as it has improved particle adhesion by bacteria. Ultimately, low-ester pectin films with nanoparticles added were prepared and mechanical experiments were carried out. Due to LipLis:GO insertion into the structure, the properties of the films have been improved. In comparison to a control, the LipLis:GO one has presented enhanced strain capacity, useful for improving more durable, active packaging. This was possible because graphene and graphene oxide have remarkable flexibility attributes, well appreciated with its insertion among the pectin network.

Chart 1. Particles properties overview.

	Size (nm)	$\zeta$ -pot. (mv)	MIC (ppm)	MBC (ppm)
Lecithin	79.4 $\pm$ 26.0 <sup>a</sup>	-62.5 $\pm$ 1.7 <sup>a</sup>	-	-
Lysozyme	3.0 $\pm$ 0.1 <sup>b</sup>	31.4 $\pm$ 0.6 <sup>b</sup>	<156.3	-
Graphene Oxide	668.0 $\pm$ 111.0 <sup>c</sup>	-37.6 $\pm$ 2.0 <sup>c</sup>	62.5	-
Lip:Lis	119.0 $\pm$ 16.0 <sup>d</sup>	20.9 $\pm$ 0.8 <sup>d</sup>	46.9	-
LipLis:GO	1875.0 $\pm$ 238.0 <sup>e</sup>	28.3 $\pm$ 0.6 <sup>e</sup>	93.8	375

Different letters in the same column means significant difference at 95% (p value < 0.05).

### Conclusions

Size and  $\zeta$ -potential comparison showed successful lysozyme and lecithin complexation, as well as GO binding. The union implied in a better exposure of the active site from the enzyme, which corroborated the hypothesis of synergistic antimicrobial activity, decisive for its unique MBC results on *Staphylococcus aureus*. Furthermore, it was also possible to appreciate GO's mechanical properties attributes into the pectin film. Briefly, the developed low-cost technique had mild preparation conditions without organic solvents, which provided a reduction of preservatives amount and enhanced properties, opening path food packaging functionalization and other applications.

### Acknowledgement

To FAPESP (2015/26359-0), CNPq and FEA/UNICAMP for financial investment and scientific support.

[1] Aminlari, L.; Hashemi, M. M.; Aminlari, M. *J. Food Science*. **2014**, 79, 1077.

[2] Barbolina, I.; Woods, C. R.; Lozano, N.; Kostarelos, K.; Novoselov, K. S.; Roberts, I. S. *2D Materials*. **2016**, 3, 13.

[3] Souza, M. P.; Vaz, A. F. M.; Correia, M. T. S.; Cerqueira, M. A.; Vicente, A. A.; Carneiro-da-cunha, M. G. *Food and Bioprocess Tech*. **2014**, 7, 1159.