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1 Preparation and characterization of calcium alginate-chitosan complexes 2 loaded with lysozyme

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10 **Abstract:** The objective of this study was to study the effect of calcium ions on the
11 binding of lysozyme to chitosan (CS)/sodium alginate (SA) complexes (CS-SA-Ps),
12 in order to develop a complex system for lysozyme delivery which might be of
13 practical interest in the field of food preservatives. Result showed that with the
14 increase of calcium ions (Ca^{2+}) concentration, the value of Z-Ave decreased while the
15 value of ζ -potential and *LE* increased, where the addition of 5.0 mM Ca^{2+} contributed
16 to uniform distribution of the complexes. Sulphate-polyacrylamide gel electrophoresis
17 (SDS-PAGE) revealed that there was no significant difference of molecular integrity
18 when lysozyme was released from CS-SA-Ps. The exothermic interaction between
19 lysozyme and SA were proved by thermodynamic measurements, in which the
20 binding stoichiometry showed a slight decrease in the presence of calcium chloride.
21 Moreover, the release rate of lysozyme from CS-SA-Ps could be slowed by the
22 increase of Ca^{2+} addition concentration.

23 **Key words:** chitosan; alginate; lysozyme; calcium cation; binding; release

24 1. Introduction

25 Lysozymes are known as effective antimicrobial agents against food pathogens, and
26 have been recognized as safe for use as food additives. Due to its abundant resources
27 and functional characteristics, lysozyme is extensively noticeable at present (Bayarri,
28 Oulahal, Degraeve, & Gharsallaoui, 2014; Souza, Da, Souza, Tosin, & Garciarajas,
29 2017). However, the widespread utilization of lysozyme by the food industry may be
30 limited because of its potential interactions with food matrices (i.e. proteins, lipids,
31 and metabolic enzymes), which can lead to diminished bioactivities. Furthermore,
32 when used alone, lysozyme exhibits weak inhibitory effect against Gram-negative
33 bacteria like *Enterobacteriaceae* and *Pseudomonadaceae* that are relatively common
34 contaminants of meat-based products (Wu et al., 2017; Barbiroli et al., 2012).

35 To improve the quality of lysozyme, many methods have been extensively
36 investigated. The number of publications concerning the incorporation of this protein
37 into different biopolymeric materials based on polysaccharides is on the rise (Ma,
38 Tang, Yin, Yang, & Qi, 2013; Zhang, Tao, Niu, Li, & Chen, 2017). Wherein, chitosan

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