***In vitro digestion and release of bioactive peptides from chitosan-alginate polyelectrolyte complexes***

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**Abstract**

In previous work we have characterized the physicochemical characteristics of polyelectrolyte complexes (PECs) formed from mixtures of chitosan (CS) and alginate (ALG), plus the effects of incorporating a mixture of low molecular weight bioactive peptides (BAPs) into these complexes.1 In this work we investigate the effects of *in vitro* gastric and intestinal digestion conditions on the physical properties of the PECs and the concomitant release of the peptides from them. The molecular weights of the chitosan and alginate were 111 and 21 kDa, respectively. The *in vitro* gastric and intestinal digestion fluids were prepared according to the International Network on Food Digestion (INFOGEST) protocol with key enzymes pepsin, trypsin, and chymotrypsin. Dynamic light scattering (DLS) and mixed-mode phase analysis light scattering (M3-PALS) were used to assess the changes in the PEC size and structure. Ultra-high-performance liquid chromatography (UHPLC) was used to identify and quantify the peptides released under the digestion conditions and the bioactivity of the digestates was analyzed with respect to angiotensin-converting enzyme (ACE).

There are differences in the initial sizes of the PECs depending on whether they are formed in the presence of excess CS (molar charge ratio of ALG : CS = 0.5) or excess ALG (molar charge ratio ALG: CS = 10), as well as their initial zeta potentials but, in general, under gastric conditions (pH 3 + pepsin) the changes in the sizes of the PECs are minimal over 2 h digestion time. At the same time, there is a steady release of peptides over 2 h of up to 60 ± 5 %. Under intestinal conditions (pH 7 + trypsin and chymotrypsin) the PECs formed with excess ALG are more stable in terms of size change than those formed with excess CS, but both PECs show almost complete (100%) release of the peptides after 2 h, though the release is slightly delayed with the latter. Thus, the PECs show reasonably good resistance to gastric digestion, but less stability to intestinal conditions. It should be noted that when digestive conditions are applied but in the absence of the enzymes, the complexes are more stable and peptide release tends to be reduce. Since the free non-encapsulated peptide itself shows susceptibility to intestinal enzyme breakdown (but of course not the CS or ALG) the overall release kinetics are the result of a complex interplay between the enzymes, the encapsulated peptide and the pH and salt conditions moderating the electrostatic interaction between the biopolymers and the peptides. Overall, these types of PECs show good promise for significant protection of the ACE bioactivity of the peptide on consumption.

1Atma, Y., Sadeghpour, A., Murray, B.S., Goycoolea, F.M. (2024). Chitosan-alginate polyelectrolyte complexes for encapsulation of low molecular weight fish bioactive peptides. *Food Hydrocolloids,* **160,** 110789.

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