Electronic Supplementary Material (ESI)

Simulated gastrointestinal digestion of protein alginate complexes: Effects of whey protein cross-linking and composition and degradation of alginate

Mikkel Madsen,[a] Mette E. Rønne,[a] Ruifen Li,[b] Ines Greco,[b] Richard Ipsen[b] and Birte Svensson[a*]

[a]Enzyme and Protein Chemistry, Department of Biotechnology and Biomedicine, Technical University of Denmark, Søltofts Plads, Building 224, DK-2800 Kgs. Lyngby, Denmark.

[b]Department of Food Science, University of Copenhagen, DK-1958 Frederiksberg, Denmark.

[*]Corresponding author

Methods

S1 Cloning, production, purification and activity assay of alginate lyase (AL) (gene number: BSCG_01542)from *Bacteriodes* sp. *2_2_4*

Alginate lyase (AL) gene (number: BSCG_01542) from *Bacteroides* sp. *2_2_4* (locus: EEO54617.1) encoding an N-terminal His-tag and a thrombin cleavage site (MGSSHHHHHHSSGLPRGSH) was codon optimized for *E. coli* as purchased from Genscript in a pET-28a(+) vector, containing kanamycin resistance gene, and transformed into *E. coli* BL21. One colony from an LB kanamycin agar plate was cultured in 10 mL LB medium (37°C, overnight, 160 rpm), inoculated in 750 mL LB kanamycin medium added 10 mM glucose, and grown (37°C, 160 rpm) to OD_{600} 0.6–0.8. Expression was induced by 0.2 mM isopropyl β-D-thiogalactopyranoside followed by incubation for 18 h (19°C, 160 rpm). Cells were harvested by centrifugation (5000 *g*, 4°C, 20 min) and stored at −20°C. Pellet corresponding to 325 mL culture was resuspended in 20 mL cold 50 mM HEPES, 150 mM NaCl, pH 7.0, lysed (pressure cell homogenizer; Stansted Fluid Power, UK) and centrifuged (20,000 *g*, 20 min). The supernatant was added 2 mL pre-equilibrated HisPur™ nickel-nitrilotriacetic acid resin (ThermoFisher, USA) (4°C, 30 min, gentle mixing), then packed in a gravity flow column, washed by 20 mL cold buffer A (50 mM HEPES, 300 mM NaCl pH 7.0), 15 mL cold buffer A containing 20 mM imidazole and eluted by 5 mL 300 mM imidazole in buffer A. The eluate was immediately gel-filtered (HiLoad® 16/600 Superdex® 75 pg, GE Healthcare) in 50 mM HEPES, 150 mM NaCl, pH 7.0 at a flowrate 0.5–1 ml/min. Protein containing fractions were collected yielding 3.6 mg purified enzyme per gram of cells as determined

spectrophotometrically at 280 nm using ε = 85,385 $M^{-1}cm^{-1}$ (ExPASy's ProtParam tool).[1] The purity of recombinant AL (section S1) was assessed by SDS-PAGE (Fig. S1). Activity of AL towards 0.125% alginate AlgM, AlgMG and AlgG was determined spectrophotometrically in 50 mM HEPES, 150 mM, pH 7.0 in UV-star 96-well microplate (Greiner Bio-One, Austria). Substrates (250 µL) were equilibrated (5 min, 37°C) and added enzyme (50 µL) to a final concentration of 75 nM. Formation of unsaturated products was monitored spectrophotometrically at 235 nm[2] in a plate reader (Bio-Tek Powerwave XS, Holm & Halby, Denmark) every 10 s for 2 h, corresponding to the intestinal phase digestion.

Figures

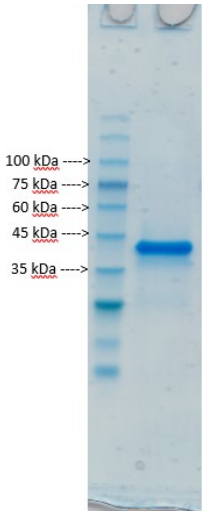

Fig. S1 SDS-PAGE of purified AL (1 µg). Theoretical molecular weight is 45,907 Da calculated by ProtParam.[3] Molecular weight marker (Prestained Protein Marker, Proteintech Group, UK) to the left.

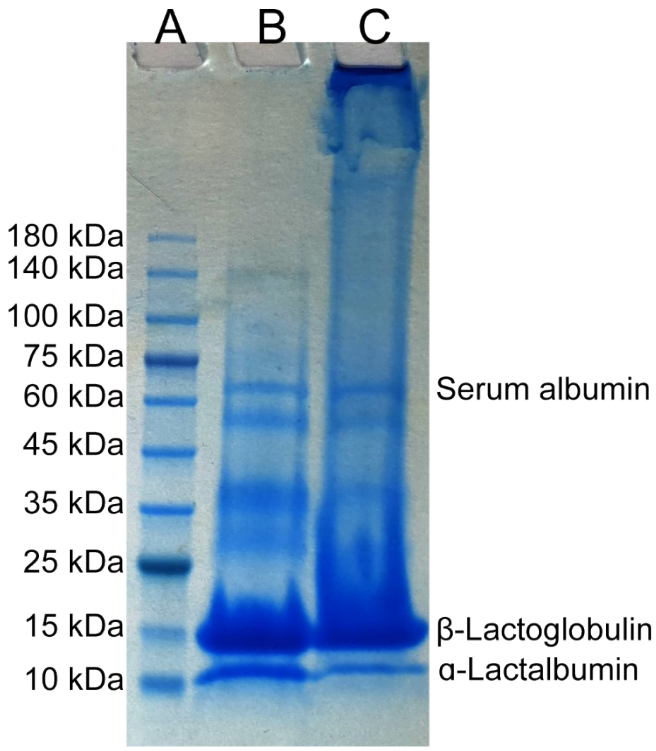

Fig. S2 SDS-PAGE of native and XL SPC. Lane A: molecular weight marker (Prestained Protein Marker, Proteintech Group, UK). Lane B: 50 µg native SPC. Lane C: 50 µg XL SPC. Well known proteins in SPC are indicated.

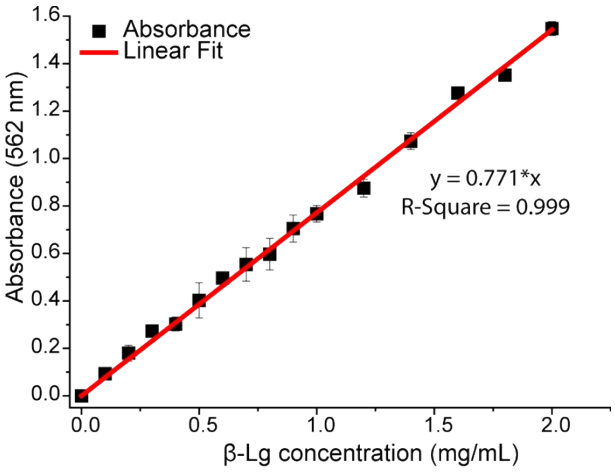

Fig. S3 BCA β-Lg standard curve.

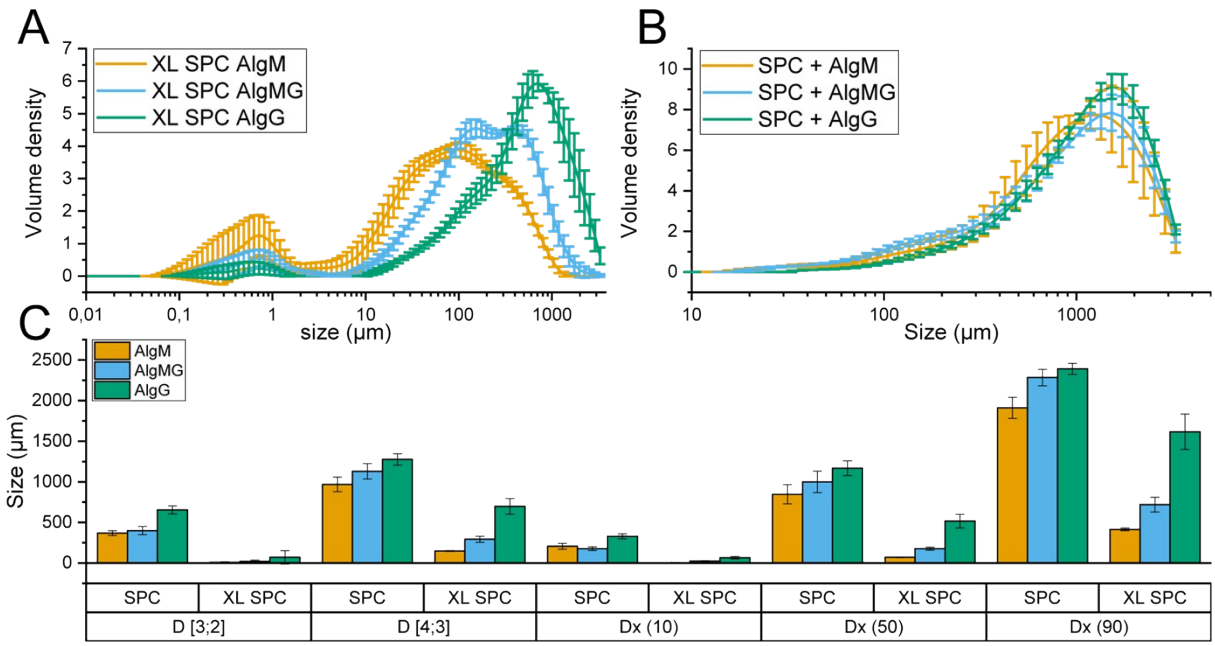

Fig. S4 Particle sizes of 2% cross-linked (XL) and native SPC in complex with 0.5% of three different alginates in SGF buffer (pH 3.0). A) Volume particle distribution of XL SPC complexes with AlgM (yellow), AlgMG (blue) or AlgG (green). B) Volume particle distribution of SPC alginate complexes (color codes as in A). C) Surface mean diameter (D[3;2]), volume mean diameter (D[4;3]), maximum size of the smallest 10% (D_{10}), the smallest 50% (D_{50}) and the smallest 90% (D_{90}) for XL SPC and native SPC alginate complexes (color code as in A). Error bars are the standard deviation of the mean from three individual measurements.

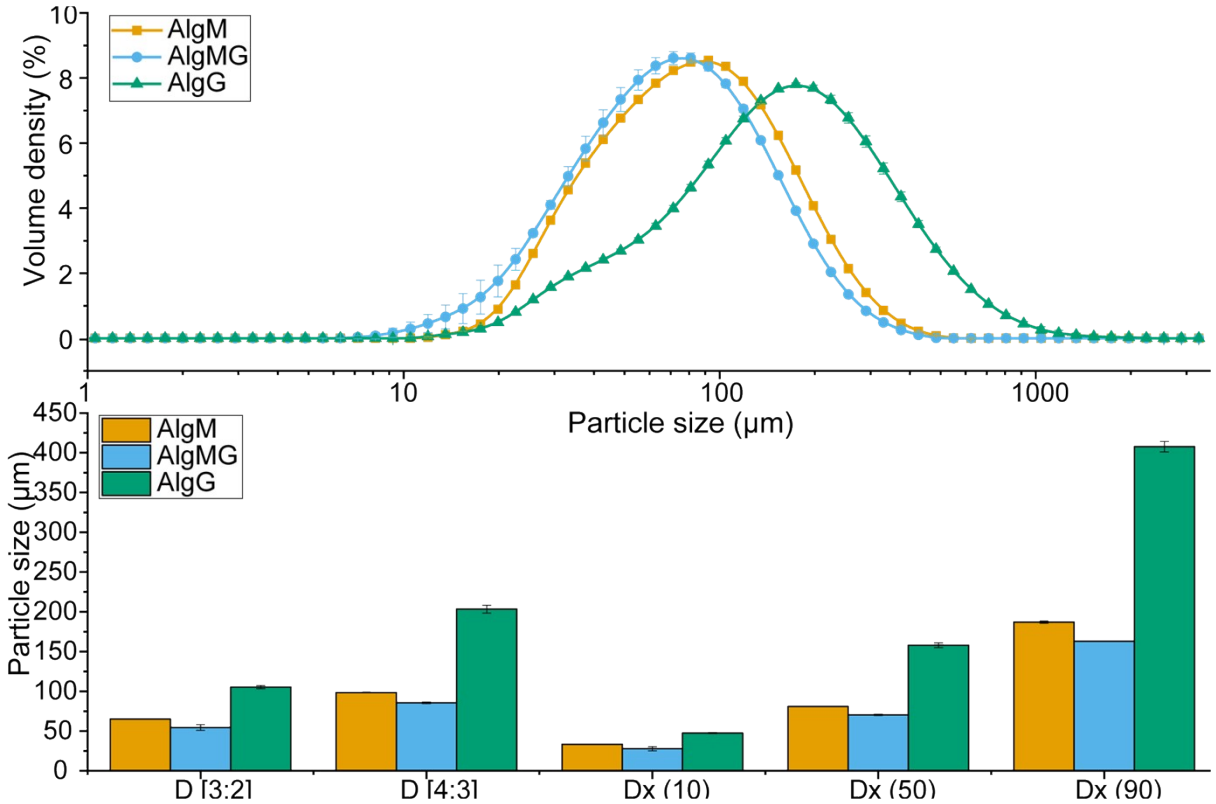

Fig. S5 Volume density distribution and particle sizes of 0.5% alginate in SGF buffer (pH 3.0). AlgM (orange), AlgMG (blue) or AlgG (green). Error bars result from three individual measurements.

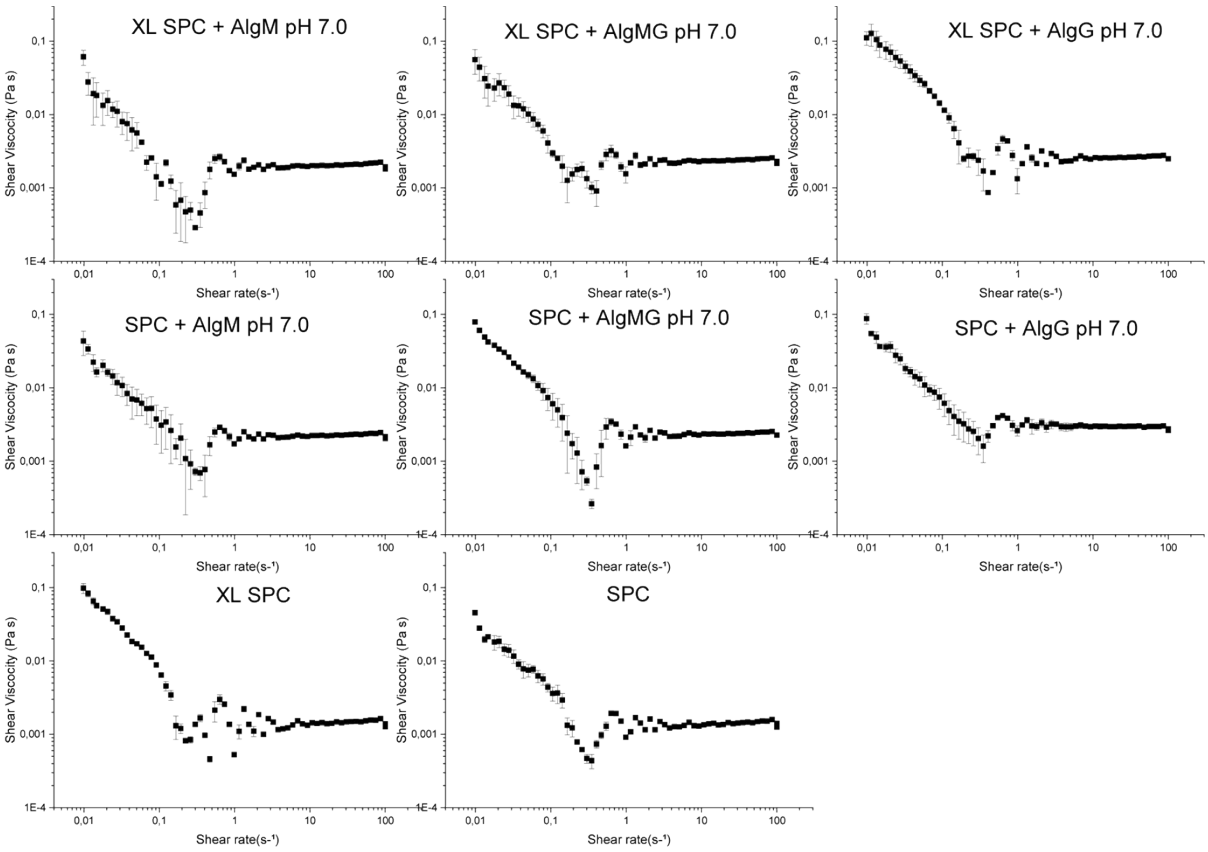

Fig. S6 Viscosity measurements of complexes (2% (w/v) protein + 0.25% alginate), free native SPC, free XL SPC and free alginate in SIF (pH 7.0) buffer (See Materials and methods, section 2.4[4]).

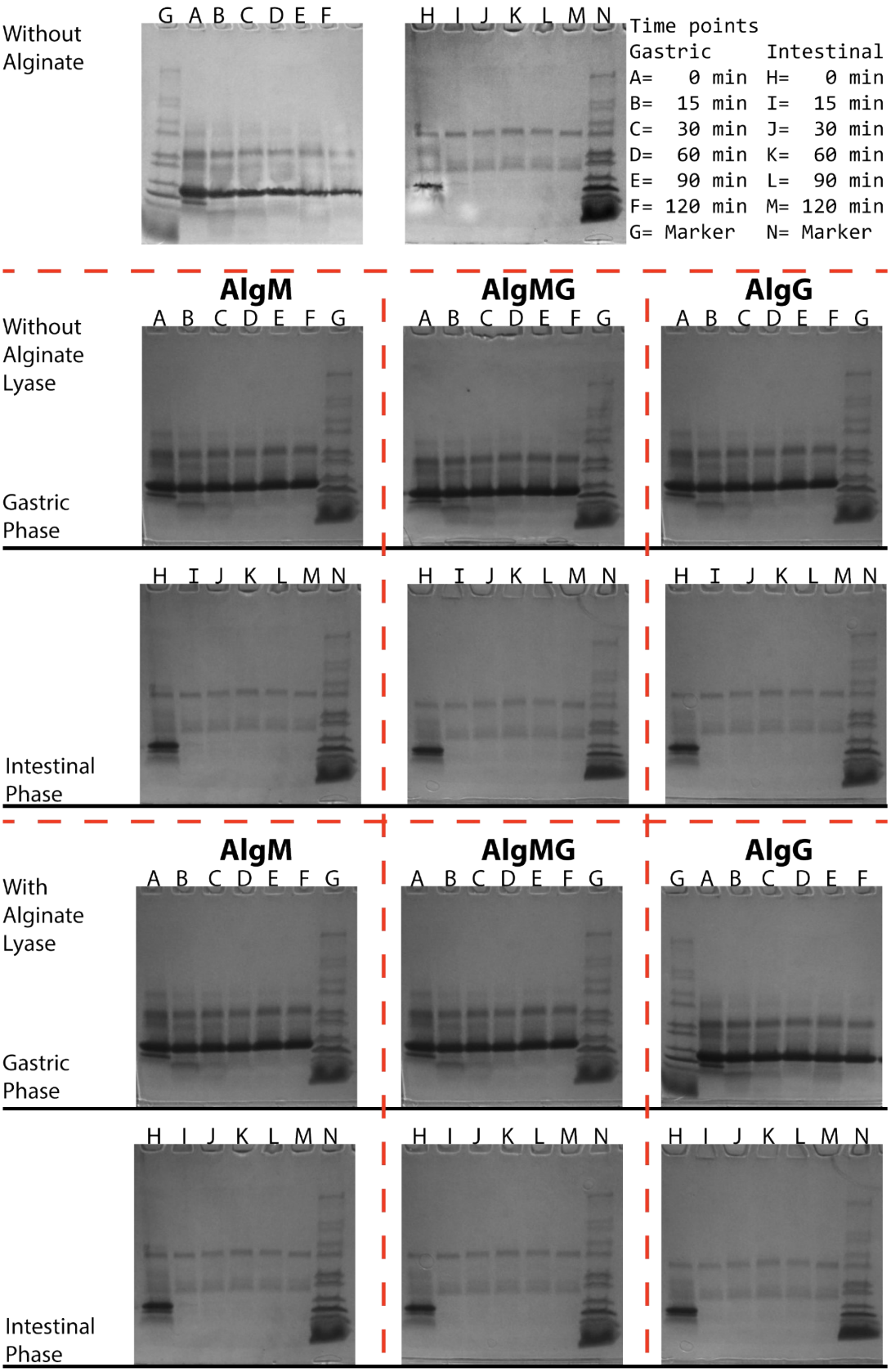

Fig. S7 SDS-PAGE of proteins in time point samples from *in vitro* simulated gastrointestinal digestion experiments. Native SPC (2% (w/v)) ± alginate (0.25% (w/v)) and ± AL. The molecular marker ranges 200–2.5 kDa (200, 116, 97, 66, 55, 37, 31, 22, 14, 6, 4, 3 kDa, top to bottom).

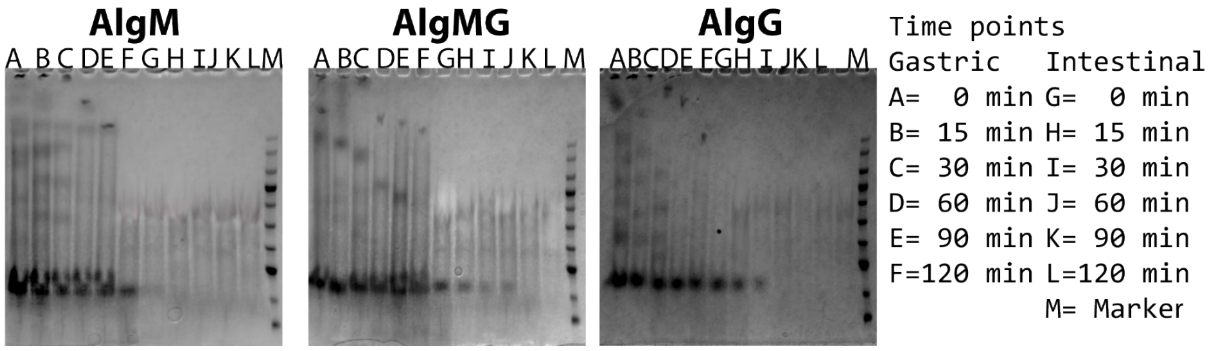

Fig. S8 SDS-PAGE of proteins in time point samples from *in vitro* simulated gastrointestinal digestion of XL SPC (2% (w/v)) + alginate (0.25% (w/v)). Molecular marker ranges 180 kDa – 10 kDa (180, 140, 100, 75, 60, 45, 35, 25, 15, 10 kDa, top to bottom).

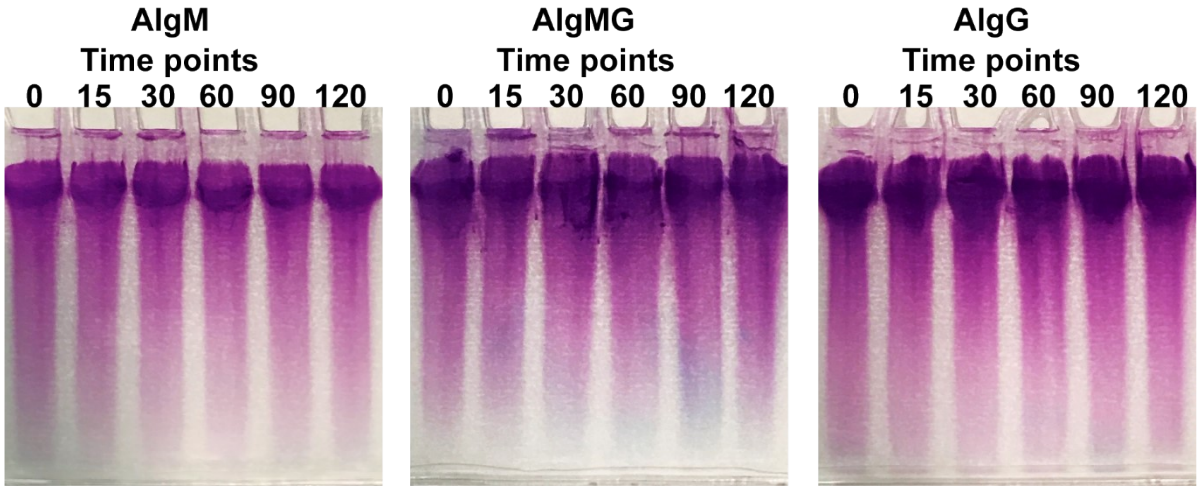

Fig. S9 C-PAGE from gastric phase of *in vitro* simulated gastrointestinal digestion for native SPC (2% (w/v)) in complex with 0.25% (w/v) AlgM, AlgMG or AlgG.

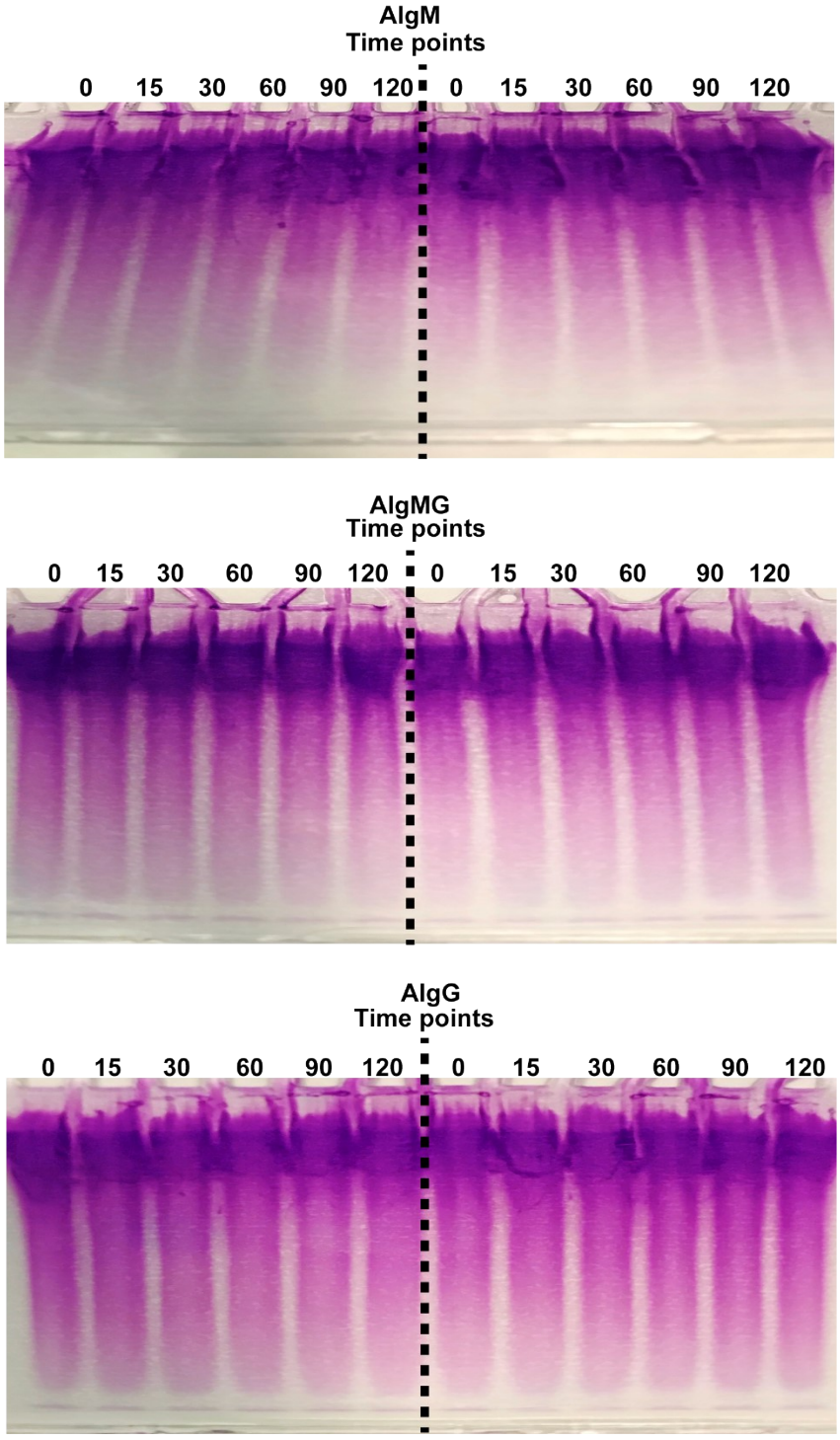

Fig. S10 C-PAGE from gastric (left) and intestinal (right) phases of 0.25% (w/v) AlgM, AlgMG and AlgG, in complex with SPC (2% w/v), but without AL added in the intestinal phase.

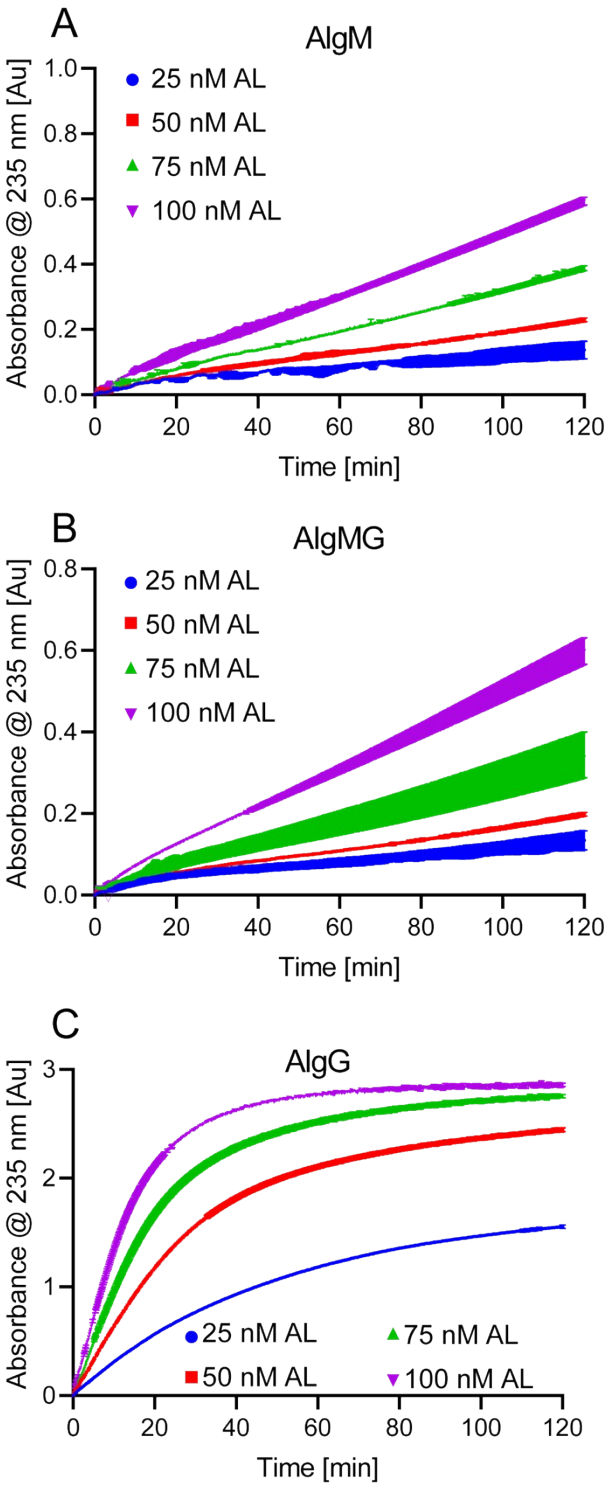

Fig. S11 Alginate (0.125 % (w/v)) degraded by AL monitored by absorbance at 235 nm. A) AlgM degraded by 25 nM AL (purple), 50 nM AL (green), 75 nM AL (red) and 100 nM AL (blue). B) AlgG degraded by AL (codes as in A). C) AlgMG degraded by AL (codes as in A).

References

1. Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel, R. D., and Bairoch, A. Protein identification and analysis tools on the ExPASy server. in *The Proteomics Protocols Handbook*, pp. 571–608, 10.1385/1592598900

2. Preiss, J., and Ashwell, G. Alginic acid metabolism in bacteria. *J. Biol. Chem.* 1962. **237**, 309–316

3. Walker, J. M. *The Proteomics Protocols Handbook*, 10.1385/1-59259-890-0:067

4. Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R., Carrière, F., Clemente, A., Corredig, M., Dupont, D., Dufour, C., Edwards, C., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A. R., Martins, C., Marze, S., McClements, D. J., Ménard, O., Minekus, M., Portmann, R., Santos, C. N., Souchon, I., Singh, R. P., Vegarud, G. E., Wickham, M. S. J., Weitschies, W., and Recio, I. INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nat. Protoc.* 2019. **14**, 991–1014