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A potent peptide emulsifier from potato storage proteins and its natural isoforms: Insight into the structure/function relationship of amphipathic, α-helical peptide emulsifiers, their targeted release, and applicability.

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Peptide emulsifiers derived from plant proteins are gathering growing interest as green and sustainable replacements for chemical additives in food. Potato (*Solanum tuberosum*) is one of the most important crops for both human consumption and industrial processing. Globally, the annual production of potato starch exceeds 3,000,000 MT with more than 200,000 MT of potato protein isolated as a side-stream, providing an enormous source of raw protein. The direct isolation of food-grade protein is in many cases regarded as cost-ineffective. Nevertheless, potato proteins may be a valuable source of functional peptides.

Previously, we demonstrated amphiphilicity-based bioinformatic prediction of peptide emulsifiers embedded in potato proteins[1,2]. Amongst the predicted peptides, especially one (γ1), derived from the storage protein patatin, showed exceptional emulsifying activity *in vitro*. Although patatin is the most abundant protein in potatoes, it is not a single protein, but a family of highly homologous isoforms.

Using bottom-up proteomics in combination with multiple sequence alignment and *in silico* digestion, we identified several γ1 variants. The variants consist of both full-length isoforms with single amino acid substitutions and tryptic variants/truncations from different patatin isoforms. The emulsifying activity of the γ1 variants, physical stability of the emulsions during storage, and interfacial properties were investigated[3]. Furthermore, the interfacial conformation of the peptides was investigated by SRCD and supplemented by NMR and benchtop CD for selected peptides in micellar model systems.

Based on these results, we are able to i) evaluate the full potential of using γ1 variants as peptide emulsifiers in food; ii) provide novel insight on the structure/function relationship of amphipathic, α-helical peptide emulsifiers; iii) combine *in vitro* functional validation with *in silico* proteolysis to design a scalable and targeted enzymatic hydrolysis, resulting in a hydrolysate with improved emulsifying properties; and iv) apply the hydrolysates as stabilizers for encapsulation of fish oil for foods.

[1]García-Moreno, Pedro J., et al. "Emulsifying peptides from potato protein predicted by bioinformatics: Stabilization of fish oil-in-water emulsions." Food Hydrocolloids 101 (2020): 105529. https://doi.org/10.1016/j.foodhyd.2019.105529
[2]García-Moreno, Pedro J., et al. "Identification of emulsifier potato peptides by bioinformatics: application to omega-3 delivery emulsions and release from potato industry side streams." Scientific reports 10.1 (2020): 1-22. https://doi.org/10.1038/s41598-019-57229-6
[3]García-Moreno, Pedro J., et al. "The structure, viscoelasticity and charge of potato peptides adsorbed at the oil-water interface determine the physicochemical stability of fish oil-in-water emulsions." Food Hydrocolloids 115 (2021): 106605. https://doi.org/10.1016/j.foodhyd.2021.106605