Supplementary Material

Pepsin activity as a function of pH and digestion time on caseins and egg white proteins in static in vitro conditions

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As illustrated in Figure 3 and Table 2 of our article, a power law enabled a fair modelling of the hydrolysis kinetics of both egg white proteins and caseins during the course of static *in vitro* gastric digestions (pH-STAT results). More precisely, the degree of hydrolysis of proteins were modelled using the following relation:

$$DH = a \times t^b$$

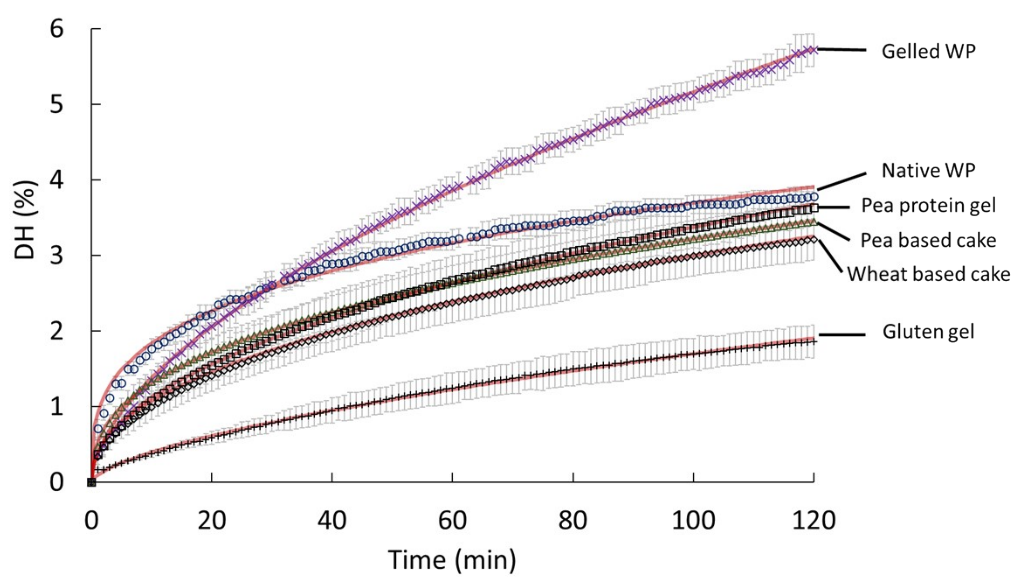
where a is a pre-factor, t is the time (min) and b is a power exponent.

To check whether this power law model could be satisfactory applied to other substrates during static *in vitro* gastric digestions, we tested it on other pH-STAT data of ours. These data were all obtained using the INFOGEST protocol of Minekus et al. (2014)[1] with the later recommendation of replacing $NaHCO_3$ by NaCl at the same molar ratio in the electrolyte solutions to avoid unwanted pH drifts[2,3]. The digested foods include:

- a solution of native dairy whey proteins (Native WP)
- a heat-induced gel of dairy whey proteins (Gelled WP)
- a wheat based cake made of wheat flour, eggs, oil and sugars (Wheat based cake)
- a pea based cake made of pea flour, eggs, oil and sugars (Pea based cake)
- a gluten gel (cooked in a plastic film for 1 h at 85°C in a water bath) made of 50 wt% of gluten (Gluten Vital, Roquette, France) and 50 wt% of salty water (2 wt% NaCl).

- a pea protein gel (cooked in a plastic film for 1 h at 85°C in a water bath) made of 40 wt% of pea proteins (Nutralys S85F, Roquette, France) and 50 wt% of salty water (2 wt% NaCl).

The results relative to the solution of native whey proteins and to the whey protein gel have been previously published in the Fig. 1A of Mat et al. (2020)[4]. The other results are unpublished data that have been obtained by Alicia Dérand during her internship in the UMR SayFood (Thiverval-Grignon, France) under the supervision of Steven Le Feunteun and Isabelle Souchon. Experimental data and their fittings by the power law model are presented in S-Fig. 1. The coefficients of determination and the estimated values of the model parameters are given in S-Table 1. Results show that the power law enabled a very accurate modelling of the protein hydrolysis kinetics by pepsin, with coefficients of determination all > 0.99. These results, together with the ones presented as part of our article, therefore suggest that a power law might be suitable to model the gastric proteolysis of various edible proteins, and even some complex foods. The extensive use of the INFOGEST static *in vitro* protocol now calls for standardized ways of analysing and presenting *in vitro* digestion data to enable comparisons across studies. In this regard, the use of a power law might prove useful for its capability to summarize the gastric proteolysis kinetics in only two well-defined parameters.



<u>S-Fig. 1:</u> *Degree of hydrolysis (DH) of proteins for various foods during static in vitro gastric digestions at pH 3 by pepsin during 2 h (INFOGEST protocol). Data represent mean ± std over at least 3 replicates, and red lines represent model fittings.*

S-Table 1: Coefficients of determination (R^2) and estimated parameters of the power law model ($DH = a \times t^b$) on various foods.

Food	a	b	R^2
Native WP	0.91	0.30	0.991
Gelled WP	0.37	0.57	> 0.999
Wheat based cake	0.38	0.45	> 0.999
Pea based cake	0.53	0.39	> 0.999
Gluten gel	0.09	0.64	0.998
Pea protein gel	0.37	0.48	> 0.999

References

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