Pepsin activity as a function of pH and digestion time under static in vitro conditions

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Background

The activity of pepsin, the gastric protease, is generally considered to be negligible for pH ≥ 4, based on the results obtained with a few purified globular proteins. The present study aimed at studying the activity of porcine pepsin on egg white proteins and casein micelle micro-aggregates over a broad range of pH (from 1 to 7) for short (3 min) and long (2 h) digestion times.

Materials & Methods

Two series of static *in vitro* gastric digestions (INFOGEST protocol) were conducted with porcine pepsin at different pH with both caseins and egg white proteins. The first series was used to assess the initial reaction rate of hydrolysis (after 3 min) using the OPA method. The second series was used to monitor the kinetics of protein hydrolysis for 2 h of digestion with a high temporal resolution using the pH-STAT method and converting the results in degree of hydrolysis (DH) thanks to OPA analyses of the end samples.

Key Results

After 3 min: The pH activity profile of pepsin strongly depends on the substrate and can be substantial for pH ≥ 4.

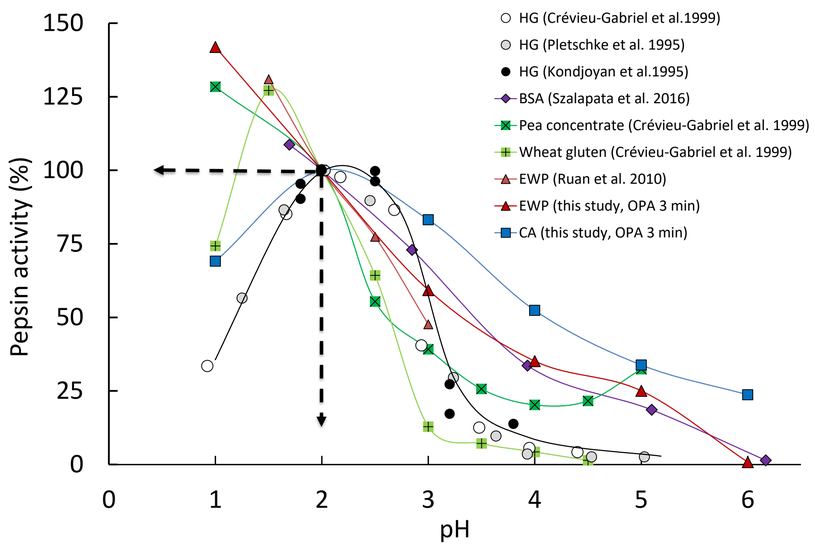


***Fig. 1**. pH dependence of porcine pepsin activity at short time on different protein substrates (our results and literature data), arbitrary setting 100% at pH = 2. Lines are guides for the eyes.*

After 2h: The extent of casein hydrolysis was constant in a remarkably broad range of pH: from 1 to 5 !

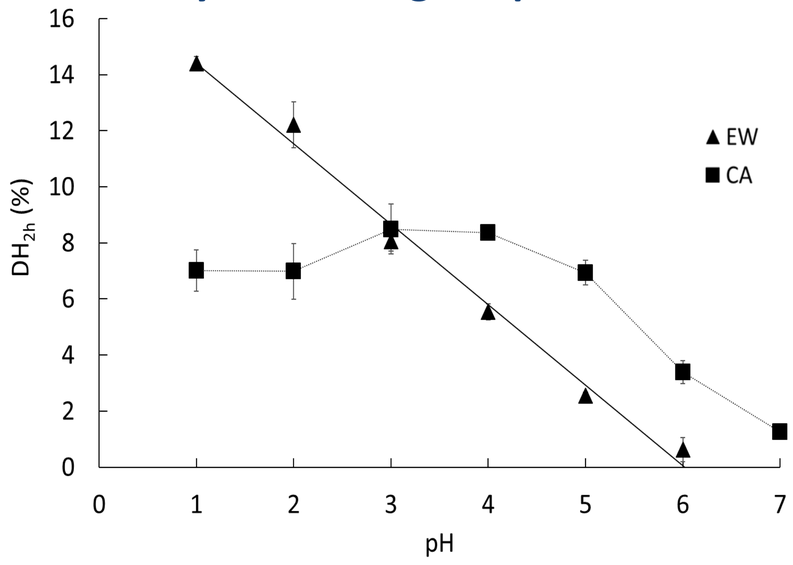
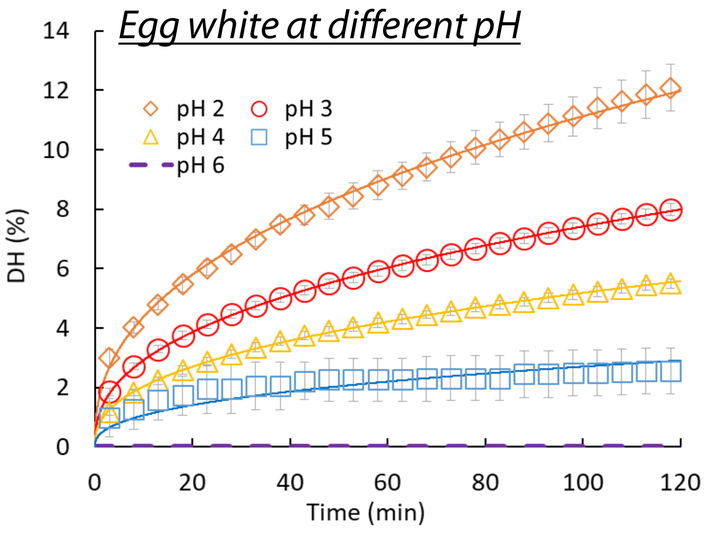
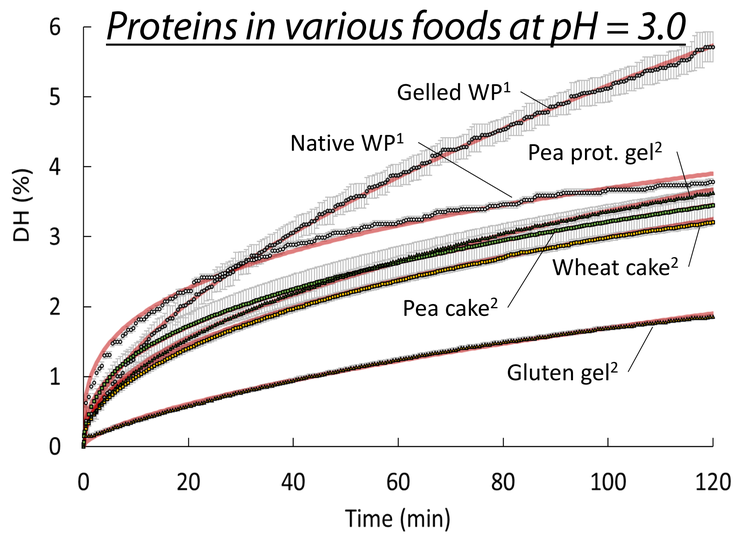


***Fig. 2.** DH_{2h} at different pH for egg white (EW) and caseins (CA) (mean ± sd over 3 replicates). The dotted line is a guide for eyes. The solid line is a linear regression ($DH_{2h} = -2.87 \times pH + 17.27$, $R^2 = 0.99$)*

During the course of digestion: Pepsin hydrolysis profiles can be accurately modelled by a simple power law

(with a scaling factor, α, and a shape parameter, β, that seems largely pH independent and characteristic of the substrate)





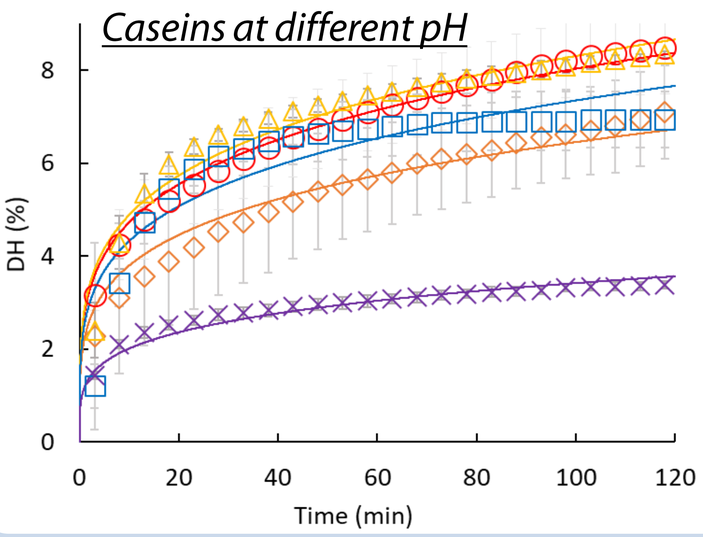


Fig. 3.** DH monitored by pH-STAT during 2 h of static gastric in vitro digestions (mean ± sd over at least 3 replicates). **All lines represent fittings of the power law model:

$$DH(\%) = \alpha \times time^{\beta}$$

***Table 1.** R^2 and estimated parameters (α, β) of the power law model on various foods.*

Food	pH	α	β	R^2
Egg white proteins	2	1.72	0.41	> 0.99
	3	1.15		> 0.99
	4	0.80		> 0.99
	5	0.42		0.96
Caseins	2	2.23	0.23	> 0.99
	3	2.78		> 0.99
	4	2.88		0.92
	5	2.55		0.74
	6	1.18		0.98
Native whey proteins[1]	3	0.91	0.30	> 0.99
Gelled whey proteins[1]	3	0.37	0.57	> 0.99
Wheat based cake[2]	3	0.38	0.45	> 0.99
Pea based cake[2]	3	0.53	0.39	> 0.99
Gluten gel[2]	3	0.09	0.64	> 0.99
Pea protein gel[2]	3	0.37	0.48	> 0.99

[1] Mat et al., *Food Chemistry*, 2020, 311
[2] Unpublished data of ours

Main Conclusions

- Pepsin activity under weakly acidic conditions (pH ≥ 4) should not always be neglected, in particular, for milk caseins.
- Pepsin instantaneous activity seems to evolve proportionally to the power of time during static *in vitro* gastric digestion.
- For complementary information: **Salelles L., Floury J. & Le Feunteun S. (2021) *Food Funct.*, 12, 12468**

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