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# How inclusion of semi-dynamic digestion conditions impact the proteolysis and amylolysis kinetics of cooked lentils?

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Semi-dynamic *in vitro* digestion models allow to strategically choose which dynamic factors to include, filling the gap between static and dynamic approaches. However, few systems are available allowing independent sampling throughout digestion to determine kinetics. Hence, we introduced an automated system (BioXplorer100, H.E.L Group) to simulate semi-dynamic digestion in multiple reactors simultaneously. The digestion conditions used in the different reactors in the first part of this work were (i) gradual pH change and enzyme secretion in the stomach (based on INFOGEST protocols) and (ii) gradual enzyme and bile salt secretion in the small intestine.

Different digestion patterns were observed when comparing static and semi-dynamic kinetics for cooked lentils. For the static approach, proteolysis increased rapidly until a plateau was reached. For the semi-dynamic approach, the start of proteolysis was retarded, which was related to the decreasing pH and large pH-dependency of pepsin activity in the stomach. Additionally, during intestinal digestion, a shorter lag phase was noticed, yet clearly present. This was explained by the gradual enzyme secretion, which resulted in a period of enzyme competition before proteolysis occurred at maximum rate.

In the second part, the choice of which additional parameters to include in such semi-dynamic simulations and their impact on digestion kinetics was investigated. For example, salivary  $\alpha$ -amylase remains active during the initial period of the gastric phase due to the gradual pH decrease. However, static models mostly do not introduce this enzyme as it is immediately inactivated at the static pH of 3. The action of salivary  $\alpha$ -amylase can impact the subsequent amylolysis kinetics of, e.g., pulses.

Introducing salivary  $\alpha$ -amylase followed by a semi-dynamic gastric phase showed that starch was significantly hydrolyzed in the orogastric phase. Consequently, amylolysis extent was higher at the start of the small intestine compared to static conditions. This difference was not compensated by the action of pancreatic  $\alpha$ -amylase, resulting in a higher final extent of amylolysis. This was most probably related to the specific actions of both enzymes, hydrolyzing different starch bonds.

Overall, we showed that the BioXplorer100 could be used to mimic semi-dynamic digestion conditions and determine more physiological relevant digestion kinetics.