A methodology to simulate the protein conversion rate and the degree of hydrolysis kinetics for enzymatic hydrolysis process

Sophie Beaubier1,2,Claire Defaix1,2,Xavier Framboisier1, Olivier Galet2, Romain Kapel1\*

1 Laboratoire Réactions et Génie des Procédés, Université de Lorraine, CNRS, LRGP, F-54000 Nancy, France

2 Avril SCA, 11 rue de Monceau, F-75008 Paris, France

Corresponding author: \* romain.kapel@univ-lorraine.fr

**Highlights**

* Identification of models for proteolysis kinetics was realized
* Kinetic terms were correlated with operating conditions (T, pH and E/S)
* Pareto front were generated toward cost and time of reaction

**1. Introduction**

Enzymatic proteolysis is an effective process to improve protein properties (digestibility and functionalities). However, the enzyme cost often remains a killer for industrial proteolysis implementation. Proteolysis kinetics and performances depend on several operating conditions (pH, temperature (T), ratio Enzyme/Substrate (E/S)). To date, there is a lack of rationality in proteolysis process implementation. Several approaches were proposed to model proteolysis kinetics. At the best, these approaches were able to predict degree of hydrolysis (DH) kinetics as a function of T and protein initial concentration or E/S. But to our knowledge, no methodology for multi-objective optimization (enzymatic cost and reaction duration) has ever been proposed.

The aim of this communication is to present a new methodology for establishing Pareto front (reaction duration vs enzymatic cost) of proteolysis with a given protease / protein couple at chosen protein conversion rate (Xp) or DH values. The proposed methodology was applied to the enzymatic hydrolysis of rapeseed albumins using Alcalase 2.4L. Because of its balanced amino acid profile and good functional properties [1], this isolate can be of interest as an alternative to animal proteins for food applications. However, its digestibility is poor, requiring a comprehensive study of its proteolysis.

**2. Methods**

The first part of the method consisted in a simulation of DH and Xp kinetics as a function of pH, E/S and T. In order to describe this complex system 3 assumptions were made: (i) **Xp and DH kinetics follow 2nd order reaction models [2], (ii) maximum hydrolysis terms (Xpmax and DHmax) only depend on pH, (iii) hydrolysis kinetic terms (kXp and kDH) depend on** T, pH and E/S**. Xpmax and DHmax were determined experimentally. kXp and kDH** were modelled as a function of the operating conditions by an original methodology. A full factorial design of experiments (DoE) with 3 factors (T, pH and E/S) was implemented. Proteolysis kinetics (Xp and DH) were monitored during 3 h in the conditions of the DoE matrix. Kinetics were monitored by a recently published method [3] and regressed using second order model to get the corresponding kinetic terms. Then, correlation models between operating conditions (T, pH and E/S) and kinetic terms were obtained by non-linear regressions. The second part of the method consisted in implementing the obtained models with genetic-evolutionary algorithms to generate the Pareto front. These front presented the best compromises between enzymatic cost and time of reaction for desired DH or Xp value.

**3. Results and discussion**

First, mean peptide size *vs* DH was studied in the operating pH and T range of Alcalase at E/S between 1/15 and 1/150 for rapeseed albumin hydrolysis by Alcalase. The same trend was observed, suggesting that the same proteolysis mechanism (one-by-one type) occurred whatever the operating conditions. Then the methodology described above was applied. **Xpmax and DHmax varied between 89% and 100% and 15% and 23% respectively for pH ranging from 7 to 10.** DoE methodology enabled to establish correlations between pH, T and E/S and the kinetic terms (k(Xp) and k(DH)). The ANOVA showed that the 2 models were reliable. R² for k(Xp) and k(DH) correlations were 0.9 and 0.85 respectively. Furthermore, p-value was inferior to 0.05 and no significant lack of fit was observed.

The simulation methodology was validated with experimental kinetics in two set of operating conditions totally different from DoE matrix and with different initial protein concentration. Relative errors less than 15 % were founded between the predicted and observed values of Xp and DH for the validation conditions (Figure 1A). Then, Pareto fronts were successfully generated for different DH and Xp values.

Finally, the best DH trade-off in terms of functional properties (emulsifying and foaming properties) and *in vitro* digestibility for rapeseed albumin hydrolysis with Alcalase was searched and found at 18% DH. The Pareto front was established as explained above (Figure 1B). The figure shows that 16 mg protease /g substrate could be saved for a prolongation of 1 hour of reaction (6 instead of 5 hours of hydrolysis) in order to get the targeted DH value.

A

B

**Figure 1.** Experimental kinetics (cross type and round points) and predicted kinetics (dotted and solid lines) of Xp (round points; solid lines) and DH (cross type points; dotted lines) for validation points (S0= 10 g/L in blue; S0= 50 g/L in green) (A) and Pareto front generation (B) for DH value of the identified compromise digestibility/functionnalities.

**4. Conclusions**

The modelling study is a powerful tool to predict the Xp and DH values with defined operating conditions and for a given time in order to produce hydrolysates. That means significant time savings in order to characterize chosen hydrolysates toward functional properties or bioactivities (antimicrobial or antioxidant)**.**

**References**

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