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Understanding proteases' mechanisms for production of pea protein hydrolysates

Laure Degrand^{1,2}, Kevin Crouvisier-Urion³, Wafa Guiga^{1,2}, Rebeca Garcia* ^{1,2}

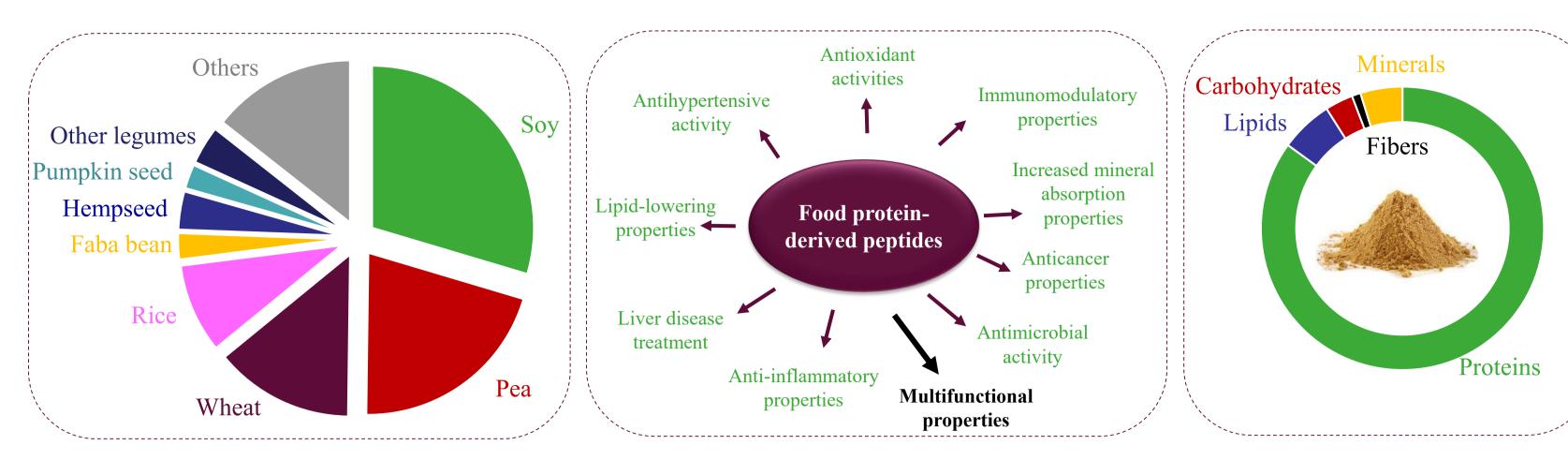
- ¹ INRAE, AgroParisTech, UMR SayFood (Paris-Saclay Food and Bioproduct Engineering), Paris-Saclay university, France
- ² Le Cnam, UMR SayFood, Paris, France
- ³ Oniris, Nantes Université, CNRS, GEPEA, UMR 6144, F-44000 Nantes, France

Introduction

GEPEA

UMR CNRS 6144

Plant-based proteins are arising as an interesting alternative to their animal counterparts, due to their contribution to food system sustainability. Among plant sources, pea proteins represent more than 20% [1]. Such a success can be explained by their health benefits, as they contain interesting amounts of essential amino acids. Unfortunately, pea proteins have poor functional properties which can limit their use in food products but solubility can be highly improved by controlled enzymatic hydrolysis [2, 3]. Moreover, the peptides released by enzymatic action can also have interesting bioactive properties [4]. Many enzyme preparations are on the market for them to be used in food products. They consist of a mixture of activities (endoprotease, exoprotease) in order to achieve efficient protein hydrolysis[5]. Therefore, it can be difficult to control the use of these preparations and achieve a selective peptide production. The understanding of enzymes' mechanism will help to control selective production of peptides with interesting activities or protein functional properties modifications. The aim of this work is to investigate the activities of two commonly used food-grade proteases: Alcalase and Flavourzyme.



Plant-based protein ingredients [1] (626 ingredients found from 20 countries) Potential health benefits of bioactive peptides [4]

pH = 8

Pea protein isolate composition (Bulk Powder)

Material and methods

Flavourzyme (FL) Alcalase (AL) Alcalase® Commercial name Flavourzyme® 1000L Type of protease Mainly exoprotease Endoprotease (aminopeptidase, EC 3.4.11) (Subtilisin) Small proportion of endopeptidase (3.4.24) (EC 3.4.21.62) Metalloprotease (neutral protease) Serine protease (alkaline protease) Family Mainly after Leu and Pro residues Carboxyl side of hydrophobic Cleavage specificity amino acids Optimum pH 7.0

*PPI:Super pea protein isolate from yellow pea (Bulk Powders)

pH-drop experiments: pH was only recorded

• Peptide's molecular weight distribution (size exclusion chromatography) Yara SEC2000 column, isocratic elution (phosphate buffer pH 6.9), detection at 214 nm (peptide bond).

+ enzyme (O) 144 (O) 0 O) Non-controlled pH conditions

Pea protein isolate (PPI*) hydrolysis using Flavourzyme® or Alcalase®

Controlled pH conditions (pH-stat) (pH-drop) Two-hours reaction, 50 °C, Enzyme/Substrate = 238 μ l.g⁻¹

Comparison between hydrolysis conditions pH-stat vs pH-drop

 α : Mean dissociation coefficient of α aminated groups m_p: amount of protein (g)

B: Base volume (mL)

N : Base normality (N)

h_{total}: peptidic bounds in the substrate (meqv/g protein)

Calculation of the degree of hydrolysis (DH)

 $DH(\%) = \frac{B*N}{\alpha * m_p * h_{total}} * 100$

DH: degree of hydrolysis (%)

Peptides characterisation

• Antioxidant properties: Ferric Reducing Ability of Plasma assay (FRAP) Reducing power was determined using potassium ferrocyanide and an absorbance measurement at 700 nm [3].

Results

6,2 0

Optimum temperature

Enzyme inactivation

Hydrolyses were performed with Flavourzyme or Alcalase (E/S = 238 μ L.g⁻¹ protein) in pH-stat (lines) and pH-drop (dashes) conditions.

Plateau

Plateau

50-55

100 °C − 15 min

pH-stat experiments: pH was maintained at 8.0 and DH was calculated

♦ In **pH-drop** conditions:

50-60

100 °C − 15 min

• Both AL and FL can hydrolyse PPI efficiently

• AL is 1.3 times more efficient than FL for PPI hydrolysis

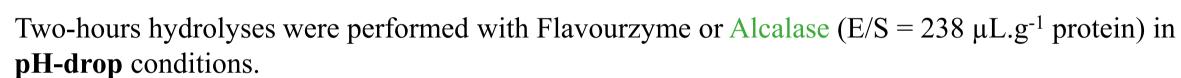
⋄ In pH-stat conditions:

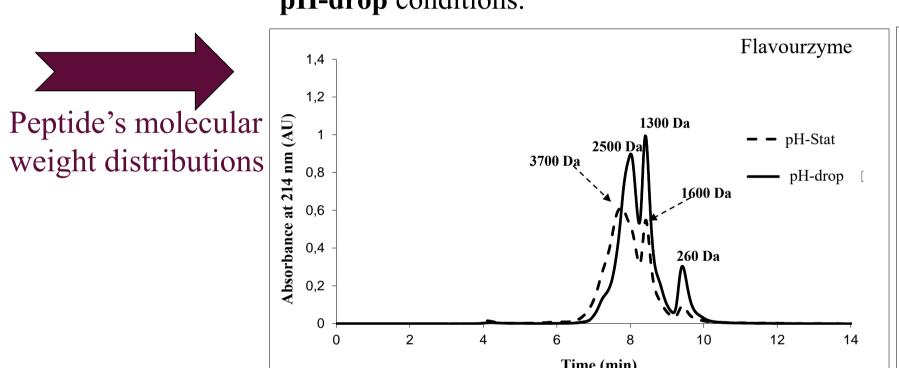
• AL is 1.7 times more efficient than FL for PPI hydrolysis

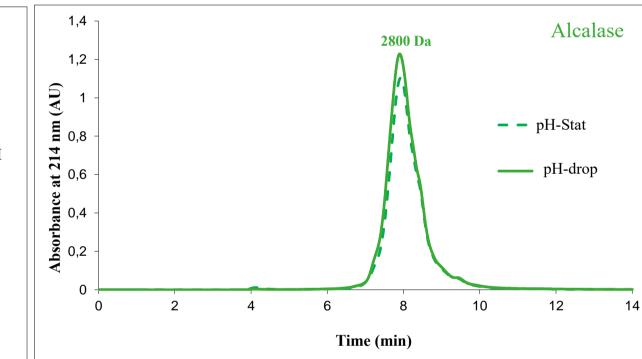
a plateau is reached at the end of the hydrolysis.

Lack of substrate? → NO, the enzyme is saturated Autolysis of the enzyme? → NO, control experiments Lack of enzyme during hydrolysis? → Probably YES

Hydrolysis mechanisms investigation





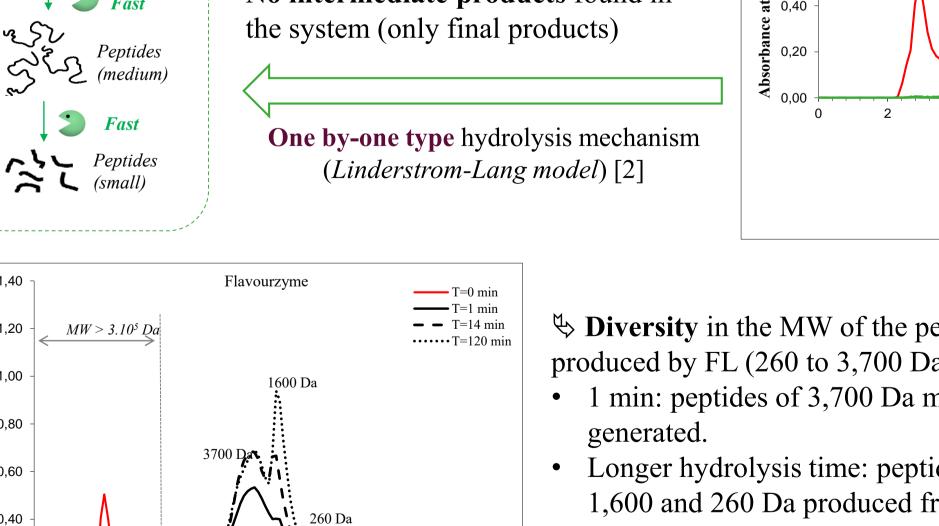


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- For FL: can be related to its optimal pH (in pH-stat conditions, only 60% of FL activity is expressed)
- For AL: less difference in rates (between pH 6 and 8, 90% of AL activity is expressed)
- More diversity in the peptide's molecular weight when hydrolysis is conducted with FL than AL

All peptides in the hydrolysate have nearly the same molecular weight (around 2600 Da) regardless the reaction time. No intermediate products found in the system (only final products) **S** Fast

Alcalase $MW > 3.10^5 Da$ (AU) 0,80 ••••• T=120 min 0,20

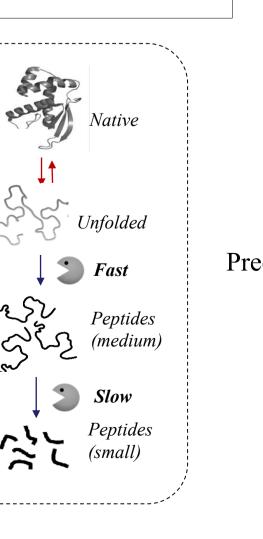


> **Diversity** in the MW of the peptides produced by FL (260 to 3,700 Da).

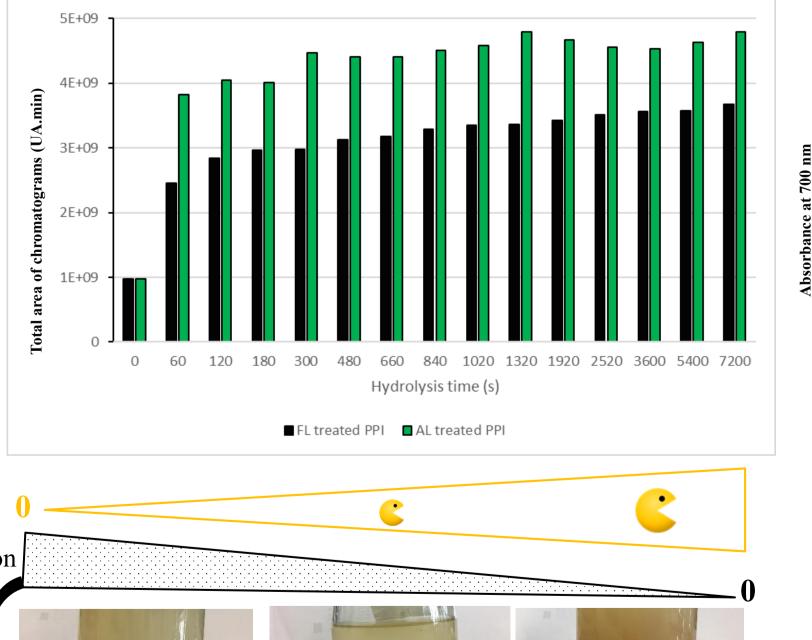
1 min: peptides of 3,700 Da mainly

Longer hydrolysis time: peptides of 1,600 and 260 Da produced from the intermediate peptides.

> Zipper type hydrolysis mechanism (Linderstrom-Lang model) [2]



Effect of hydrolysis on functional and bioactive properties of pea protein



Precipitation

PPI hydrolysates obtained with increasing volumes of Flavourzyme

Both AL and FL action increase protein solubility

+196 % FRAP assay +130 % +100 % +36 % p-value < 0.05

Pea protein isolate has a ferric reducing power.

AL action only slightly improves PPI reducing power

> FL action highly improves the reducing power

Conclusions and perspectives

PPI hydrolysis by Alcalase generates peptides of homogeneous size whereas hydrolysis with Flavourzyme generates peptides with heterogenous sizes (260 to to 3,700 Da). This allow us to conclude that Alcalase and Flavourzyme hydrolysis mechanisms are different in the conditions used in this study (E/S = 238 μ L.g⁻¹ protein, pH 8.0, 50 ° C): one by one for Alacalse and zipper for Flavourzyme. Both enzymes are more efficient in pH-drop conditions so the hydrolysis reaction can be implemented in more eco-friendly conditions. Both enzymes increase protein solubility (by size-reduction) but Alcalase is more efficient than Flavourzyme. Flavourzyme generates peptides with higher reducing power values. These results show that the choice of protease is important depending on the targeted properties / applications.

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Le Cnam, UMR SAYFOOD; GéPro Team 292, rue Saint Martin F-75003 Paris, France







* Contact: Rebeca GARCIA 5-7 July 2023 rebeca.garcia@lecnam.net **EDUCATION AND INDUSTRY**