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Analysis of the operational strategies for the enzymatic hydrolysis of food proteins in batch reactor

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ABSTRACT

The aim of this work was to validate a mathematical model and evaluate hydrolysis of salmon muscle proteins by Alcalase in batch reactor. The inhibition constants were correlated with conversion and temperature thus improving dramatically the agreement between model predictions and experimental hydrolysis time courses. The standard operation in batch reactor consisting in pre-heating, isothermal stage and inactivation of enzyme was evaluated through simulation of different operational strategies based on increasing temperature gradients. Operational conditions were 7.5%(w/w) protein, 1 g/l Alcalase and different temperatures from 50 °C to 73 °C. The optimal temperature for the standard process was 68 °C with a processing time of 41 min. Different operational strategies, included temperature profiles, resulted in longer operation times. The standard process was the best reactor performance due to the maximization of the enzyme efficiency.

1. Introduction

Enzymatic hydrolysis of proteins is an attractive technology to valorize food industry by-products and to produce protein hydrolysates with enhanced functional properties (Kristinsson and Rasco, 2000a). The fish protein hydrolysates have been widely characterized through their technological and bioactive properties (Kristinsson and Rasco, 2000a). Nevertheless, the enzymatic hydrolysis of proteins has several disadvantages. The most important are the high cost of enzymes and the need to inactivate the enzyme by pH or heat treatment after the required degree of hydrolysis is reached (Kristinsson and Rasco, 2000a). The standard process for producing fish protein hydrolysates is detailed in Fig. 1. In general, the process involves a reaction stage followed by an inactivation stage in a batch reactor. Both the reaction and inactivation stages require heat transfer. The reaction stage is generally operated on isothermal condition at an ideally optimal temperature; meanwhile the inactivation stage is operated on temperatures ranging from 75 °C to 100 °C for 5–30 min (Kristinsson and Rasco, 2000b). A kinetic model is needed to evaluate the batch reactor performance during both reaction and inactivation stages. The model has to be sensitive to temperature in order to evaluate the effects on enzyme efficiency and inactivation rate. The kinetic of the enzymatic hydrolysis of proteins has been modeled using the Michaelis–Menten equation for different enzymes and protein sources (Apar and Özbek, 2009; Barros and Xavier Malcata, 2004; O'Meara and Munro, 1985; Trusek-Holownia, 2008). The inhibition has been included in more complex models where the hydrolyzed proteins and peptides inhibit the enzyme (Demirhan et al., 2011; Sousa et al., 2004; Tardioli et al., 2005; Valencia et al., 2014). The reaction system in the hydrolysis of proteins is very complex in terms of substrate and products composition. The lack of a proper definition of these variables has been a constant problem in the cited articles. Nevertheless, during the study of the hydrolysis of salmon muscle proteins by Alcalase, Valencia et al. (2014) solved this problem by defining the substrate concentration as the peptide bond concentration and the product concentration as the concentration of α-amino groups released during hydrolysis. Both, the thermal inactivation of enzyme during the hydrolysis and the effects of the modulation exerted by the substrate and products on Alcalase, were also modeled by Valencia et al. (2014). It was found that substrate and products increased the thermal stability of Alcalase (positive modulation) approximately 10 times even at substrate concentrations as low

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Nomenclature

S	Substrate concentration (mM)
E	Enzyme concentration (g/l)
T	Temperature (K)
S_0	Initial substrate concentration (mM)
E_0	Initial enzyme concentration (g/l)
P	Product concentration (mM)
DH	Degree of hydrolysis
X	Degree of conversion
t	Reaction time (min)
k_{cat}	Catalytic constant ($mM \cdot l \cdot g^{-1} \cdot min^{-1}$)
k_d	Thermal inactivation constant (min^{-1})
K	Michaelis–Menten constant for substrate (mM)
K_S	Substrate uncompetitive inhibition constant (mM)
K_1	Product competitive inhibition (mM)
K_2	Product uncompetitive inhibition (mM)
$K_{i,0}$	Product inhibition constant independent of conversion (mM)
b_1	Slope of linear correlation between conversion and K_1 (mM)
b_2	Slope of linear correlation between conversion and K_2 (mM)
M, N	Slope and intercept of linear correlation between b_n and T
T_0	Initial temperature of operation (K)
m	Temperature rate of T-profiles ($^\circ C \cdot min^{-1}$)
a	Exponential value of T-profiles

as 15 mM of free amino groups from proteins. The kinetic model developed by Valencia et al. (2014) considers the effect of substrate inhibition, product inhibition, modulated enzyme inactivation and the temperature effect on the catalytic constant (k_{cat}), inactivation constant (k_d), Michaelis–Menten constant (K) and the substrate uncompetitive inhibition constant (K_S). However, the product inhibition constants, K_1 and K_2, were not correlated with temperature. Additionally, the evidence indicated that K_1 and K_2 values change during hydrolysis reaction, which agrees with the changing composition of hydrolysate. The challenge of the present work is to determine the evolution of inhibition constants during hydrolysis reaction and the dependence with temperature to complete the model. Finally, the model will be sensitive to changes in substrate and product concentration, and changes in temperature. Those characteristics are needed to evaluate the different operating strategies that involve temperature gradients in a batch reactor. The problem of the present work is to determine if the standard operation in batch reactor results in the optimal reactor performance. The aim of this work is to evaluate different operational strategies such as non-isothermal operation with temperature profile. The comparison of the different operational strategies will allow elucidating the optimal reactor performance.

2. Batch reactor model

The kinetic model for the enzymatic hydrolysis of salmon muscle proteins by Alcalase involves the variables substrate, product and enzyme concentration. The substrate concentration (S) was defined in terms of peptide bonds cleavable by Alcalase, which corresponds to the 26.2% of the total available peptide bonds (Valencia et al., 2014). The degree of hydrolysis (DH) of proteins is the number of peptide bonds cleaved divided by the total number of peptide bonds available in the reaction mixture. The degree of conversion (X), is defined as the ratio between hydrolyzed peptide bonds and those cleavable by Alcalase. A DH of 26.2% is equivalent to the total conversion of available peptide bonds, when the degree of conversion (X) is 1 (Valencia et al., 2014). The product concentration (P) was defined in terms of the free α-amino groups generated when a peptide bond is hydrolyzed. The enzyme concentration (E) corresponds to the mass of Alcalase per reaction volume. The model considers uncompetitive substrate inhibition and mixed product inhibition. The enzyme thermal inactivation kinetics was modeled by a first-order equation. The mass balance for the batch reactor considering the kinetic model for the rate of substrate conversion is presented in Eq. (1).

$$\frac{dS}{dt} = -\frac{k_{cat}(T) \cdot E_0 \cdot e^{-k_d(T) \cdot t} \cdot S}{K(T)\left[1 + \frac{P}{K_1}\right] + S\left[1 + \frac{P}{K_2} + \frac{S}{K_S(T)}\right]} \quad (1)$$

The kinetic, affinity and inhibition constants k_{cat}, k_d, K and K_S are function of temperature according to Arrhenius and van't Hoff

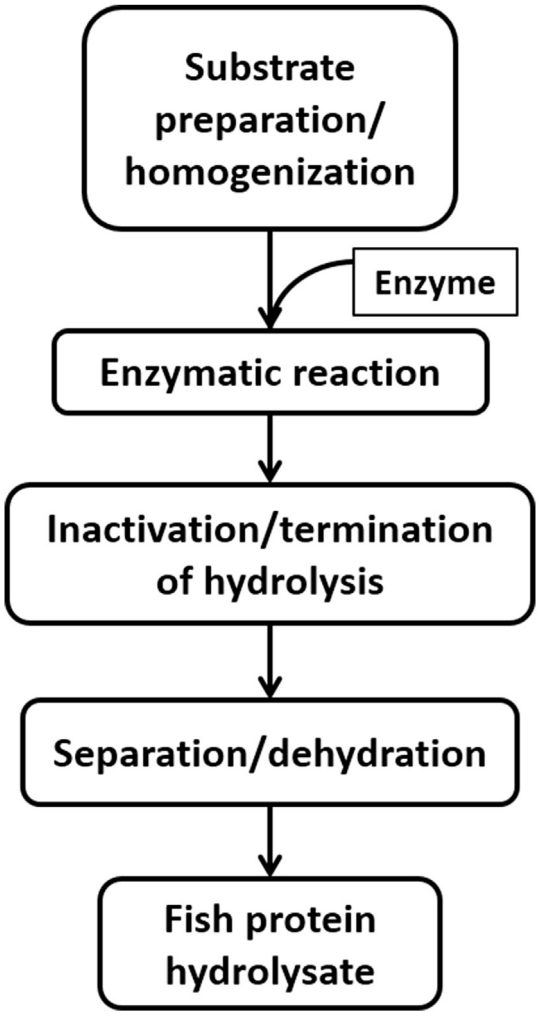

Fig. 1. Standard process to produce fish protein hydrolysate.