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Production of whey protein hydrolysates with reduced allergenicity in a stable membrane reactor

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Abstract

In this paper, a process for the stable production of low allergenicity hydrolysates is presented. Whey protein was hydrolysed at 50 °C and pH 8.5 using a bacterial protease in a continuous stirred tank membrane reactor including a polyethersulfone plate and frame ultrafiltration module with a molecular weight cut-off of 3 kDa. The reactor was maintained in operation for 16 h. Conversion reached a steady value around 80% after 10 h of operation, while a steady permeate flow was achieved after 13 h. A slight, first order enzyme thermal inactivation was detected. A hydrolysate with an average peptide chain length around 4 amino acids was obtained. The antigenic whey protein in the product was reduced 99.97%, which suggests that it can be incorporated as nitrogen source in infant formula and enteral nutrition.

Keywords: Membrane reactor; Protein hydrolysis; Whey protein; Antigenicity

1. Introduction

Enzymatic hydrolysis of whey proteins is a well-known method to modify their solubility, viscosity and emulsifying and foaming properties and, more importantly, to improve their nutritional properties (Nielsen, 1997). Whey proteins hydrolysates are considered to be ideal ingredients in the formulation of human milk substitutes due to their high nutritional value, low bitterness and low antigenicity. Allergic reactions are caused by the presence of specific sequences of amino acids in the native protein, as those identified in β-lactoglobulin (Otani, 1987), the major whey protein. By employing appropriate proteases, such sequences can be hydrolysed and, as a consequence, antigenicity is reduced. As an example, Nakamura, Sado, Syukunobe, and Hirota (1993) used two proteases to reduce the antigenicity of whey proteins by 1000 times. Unfortunately, antigenic peptide structures may remain intact even after an extensive hydrolysis. In this case, membrane processing can be a solution as short peptides can be obtained in the permeate of a ultrafiltration unit while long, antigenic sequences are retained. On the other hand, it has to be considered that an excessive hydrolysis should be avoided since it produces a high content in free amino acids involving negative effects such as bad sensory properties and high osmolality. Therefore, the hydrolytic reaction has to be strictly controlled at an industrial level.

The most common method for the production of protein hydrolysates is the batch reactor. However, there are several problems involved such as high costs because of the large quantity of enzyme, energy and labour required (Mannheim & Cheryan, 1990). A number of attempts (see, for example, Lasch, Koelsch,& Kretschmer, 1987) have been made employing immobilised enzyme reactors. Unfortunately, their use has not

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Nomenclature

A	absorbance (–)
DH	degree of hydrolysis (–)
h_T	number of peptide bonds in the substrate (mol/g)
k_d	enzyme decay constant (min^{-1})
I	inhibition (%)
K_W	ionic product of water (mol^2/L^2)
M_B	base consumption (mol)
N_F	nitrogen concentration in the feed (g/L)
N_P	nitrogen concentration in the permeate (g/L)
NPN_F	nonprotein nitrogen in the feed (g/L)
P_T	transmembrane pressure (kPa)
PCL	average peptide chain length (–)
Q_F	permeate flow (mL/min)
Q_{F0}	initial permeate flow (mL/min)
Q_R	recycling rate (L/h)
R_F	fouling resistance (min kPa/mL)
R_G	polarisation layer resistance (min kPa/mL)
R_M	clean membrane resistance (min kPa/mL)
S_F	protein concentration in the feed (g/L)
$t_{1/2}$	enzyme half life (h)
t_R	retention time (min)
V_P	cumulated permeate volume (mL)
V_R	reaction volume (L)
X	conversion (–)

Greek symbols

α	average degree of dissociation of the α-amino groups (–)
ΔP	pressure drop (kPa)

been widespread because of high losses in activity (mainly due to diffusional restrictions) and expensive immobilisation procedures.

An alternative is the continuous stirred tank membrane reactor (CSTMR). This approach is based on the difference in molecular weight between the enzyme and the hydrolysis products. The CSTMR has the ability to separate products from the reaction media in order to increase the yield. The soluble enzyme is confined in the retentate side of the membrane, where it is in contact with the substrate (which is retained as well), while the product is small enough to permeate through the membrane. The major advantages of the CSTMR are that it allows the reuse of the enzyme and facilitates the control of the molecular weight of the product by selecting the appropriate membrane pore size.

Deeslie and Cheryan (1982) studied the continuous hydrolysis of soy protein with Promine-D in a hollow fiber CSTMR. An initial conversion of 90% was obtained but it dropped to 60% in 10 h due to leakage of enzyme through the membrane and thermal deactivation. Bressollier, Petit, and Julian (1988) studied the effect of the operational variables on the performance of a hollow fiber CSTMR for the hydrolysis of plasma proteins with Alcalase. The major drawback observed during operation for 35 h was the drop of permeate flux due to membrane fouling. Mannheim and Cheryan (1990) hydrolysed casein with Alcalase in a hollow fiber CSTMR. Long-term experiments were carried out to determine the stability of the reactor at 50 and 37 °C. The conversion after 15 h changed from 96% to 62% at 50 °C and from 75% to 51% at 37 °C. The decline in the reactor performance was due to enzyme leakage, enzyme-membrane interactions and thermal deactivation. Chiang, Cordle, and Thomas (1995) produced casein hydrolysate with protease from Aspergillus oryzae in a formed-in-place membrane reactor, which operated steadily longer than 17 h. Perea and Ugalde (1996) hydrolysed whey proteins with Alcalase in a hollow fiber CSTMR. The process was only operational for 7 h due to membrane fouling and enzyme inactivation. Chiang, Shih, and Chu (1999) produced soy protein hydrolysates with Alcalase and Flavourzyme in a hollow fiber CSTMR which maintained a steady state over 16 h.

Significant recent works have focused on the production of specific bioactive peptides by means of the tryptic hydrolysis of caseinomacropeptide in a CSTMR (Martin-Orue, Henry, & Saïd Bouhallab, 1999; Prata-Vidal, Bouhallab, Henry, & Aimar, 2001). Goat whey hydrolysates have also been studied in terms of the generation of bioactive compounds (Bordenave, Sannier, Ricart, & Piot, 1999), purification of β-lactoglobulin (Sannier, Bordenave, & Piot, 2000) and detailed characterisation of the peptides generated (Bordenave, Sannier, Ricart, & Piot, 2000).

The objective of this research work was to design a stable process for the production of low antigenicity whey protein hydrolysates, which should work for a long-term period. In this sense, the decline in the enzyme activity should be due to thermal deactivation only and membrane fouling should be mitigated in order to reach a steady permeate flow. First, the membrane module is characterised and the enzyme stability in the system is studied. Then, the hydrolytic process is carried out and its performance is monitored. Finally, the product obtained is analysed in terms of molecular weight distribution and reduction in antigenicity compared with the original substrate.