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Separation of bromelain from crude pineapple waste mixture by a two-stage ceramic ultrafiltration process

M.Z.M. Nor[a,b], L. Ramchandran[a], M. Duke[c], T. Vasiljevic[a,*]

[a] Advanced Food Systems Research Unit, College of Health and Biomedicine, Victoria University, PO Box 14428, Melbourne 8001, Australia
[b] Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, UPM, 43400 Serdang, Selangor, Malaysia
[c] Institute for Sustainability and Innovation, College of Engineering and Science, Victoria University, PO Box 14428, Melbourne 8001, Australia

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ABSTRACT

Membrane technology is an attractive technique for purification of bromelain enzyme from pineapple. In this study, the feasibility of bromelain purification from pineapple's crude waste mixture (CWM) extract was investigated using a two-stage ceramic ultrafiltration (UF) scheme involving 75 kDa and followed by 10 kDa tubular ceramic membranes. Both UF stages were performed at specific processing conditions and determination on the permeate flux values and the extract properties of each UF stage were conducted. Observations were also employed at different feed pH and processing temperatures in both UF stages for processing optimization. Results showed the proposed technique managed to retain 96.8% enzyme recovery in the UF stage 1 and increase the bromelain purity up to 2.5-fold, which is within the targeted purity requirement, in the UF stage 2. The permeate flux values for UF stages 1 and 2 were 11.6 and 6.2 kg/m^2 h, respectively. Higher VRF of the process in UF stage 2 leads to higher enzyme purity, thus the process can be adjusted depending on the enzyme final applications. For a better flux behaviour of the process and preservation of the bromelain enzyme during the process, this study suggests to adjust the feed pH to 7.0 and performing the process at room temperature (~20 °C).

1. Introduction

Bromelain, known as stem bromelain (EC 3.4.22.32) and fruit bromelain (EC 3.4.22.33), is a crude enzyme that can be obtained from pineapple plant including its inedible parts which are normally considered as waste in the industry. This proteolytic enzyme has unique functions useful to the food, pharmaceutical and cosmetic industries (Ketnawa et al., 2012). Researchers have conducted many investigations on ways to isolate this enzyme from the crude pineapple extract. The common techniques for the purification of bromelain from the crude pineapple extract include reverse micellar system, aqueous two phase extraction, cation exchange chromatography and ammonium sulphate precipitation (Nadzirah et al., 2013).

Bromelain can also be purified from the crude pineapple extract using a membrane process. The membrane process is considered a more attractive purification technology compared to the other processing methods since it offers major advantages in high throughput of product, easy to scale up,

* Corresponding author. Tel.: +61 3 9919 8062; fax: +61 3 9919 8284.
E-mail address: todor.vasiljevic@vu.edu.au (T. Vasiljevic).
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Abbreviations and nomenclatures

ANOVA	analysis of variance
CDU	casein digestion unit
CWM	crude waste mixture of pineapple
Da	Dalton (1.67×10^{-24} g)
EA	enzyme activity (CDU/ml)
EA_f	enzyme activity of the feed (CDU/ml)
EA_p	enzyme activity of the permeate (CDU/ml)
EDTA	ethylene diaminetetraacetic acid
ER	enzyme recovery (%)
J	permeate flux (kg/m^2 h)
MF	microfiltration
MWCO	molecule weight cut-off (Da)
PC	protein content (mg/ml)
PES	polyether sulfone
PE	purification fold (dimensionless)
pI	isoelectric point
PR	permeate recovery (%)
SA	specific activity of enzyme (CDU/mg protein)
SA_f	specific activity of enzyme in the feed (CDU/mg protein)
SA_r	specific activity of enzyme in the retentate (CDU/mg protein)
SAS	statistical analysis system
T	processing temperature (°C)
t	operating time (min)
TCA	trichloroacetic acid
TMP	*trans*-membrane pressure (bar)
TN	total nitrogen
TOC	total organic carbon
TS	total solid (%)
TSS	total soluble solid (°Brix)
UF	ultrafiltration
v	cross-flow velocity (m/s)
V_f	volume of feed (ml)
V_p	volume of permeate (ml)
V_r	volume of retentate (ml)
VRF	volume reduction factor (dimensionless)

environmental friendly and cost effective. Studies on the usage of membrane technology particularly by microfiltration (MF) and ultrafiltration (UF) for bromelain purification either as a single process or being coupled with other techniques have been successfully reported (Doko et al., 1991; Hebbar et al., 2012; Lopes et al., 2009). Nevertheless, most of the related studies were only focused on achieving high bromelain enzyme purity without much consideration about the feasibility of the process e.g. flux behaviour. The gaps on understanding the separation mechanisms during the membrane process need to be filled to support wider industrial uptake of novel membrane processes for bromelain recovery from pineapple waste.

The present work focused on separation of bromelain from pineapple's crude waste mixture (CWM) employing a two-stage UF scheme using ceramic membrane. A two-stage membrane strategy has been successfully applied for protein isolation (Cheang and Zydney, 2004; Datta et al., 2009; Takaç et al., 2000). In most cases, it involves the separation of the protein of interest as permeate from bigger size molecules during the first stage of the UF. The permeate from the first UF stage is then fed to a membrane with pore size below the molecular size of the protein for capturing and concentration of the protein in the retentate. An increment of 10-fold purity of α-lactalbumin from whey protein isolate with 90% yield has been reported by a two-stage UF using 100 and 30 kDa composite regenerated cellulose membranes in series (Cheang and Zydney, 2004). Ovalbumin with 98.7% purity has been successfully separated from chicken egg white using two-stage UF employed with 50 and 30 kDa polyether sulfone (PES) membranes (Datta et al., 2009). However, most of the studies used polymeric membranes while ceramic membranes can also be used for the protein separation.

The use of ceramic membrane has several benefits in purifying high value components such as bromelain. Ceramic membranes can offer a more robust and long-term alternative to polymeric membranes in membrane process due to its superior chemical, thermal and mechanical properties, and can be cleaned at extreme conditions (Lee et al., 2015). One important feature for recovery of low concentration of high value compounds such as bromelain, is the narrow pore size distribution of ceramic membranes enabling a more selective separation with reduced losses. Ceramic membranes also offer the possibility for lower organic fouling potential compared to their polymeric counterparts (Hofs et al., 2011). Unlike polymeric membrane which is widely used commercially in various industries, further research is needed for the successful applications of this membrane in many areas especially in the food industry.

The objective of this study was thus to establish the performance of a two-stage UF system using commercial ceramic membranes for the purification of bromelain enzyme from pineapple's crude waste mixture (CWM) extract, a mixture that consisted of waste parts of pineapple including the crown, peel and core. Experimental data were first obtained with fixed operating parameters including *trans*-membrane pressure (TMP), temperature (T), pH and cross-flow velocity (v) to determine the permeate flux and physico-chemical properties of the extracts. The flux behaviour, percentage enzyme recovery and enzyme purity were then compared at different pH and temperature during the two-stage UF system for the determination of the optimum related operating conditions.

2. Materials and methods

2.1. Chemicals

Casein, ethylene diaminetetraacetic acid (EDTA), L-tyrosine, L-cysteine HCl and trichloroacetic acid (TCA) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). All other chemicals used in the experiment were analytical grade.

2.2. Preparation of pineapple crude waste mixture (CWM) extracts

The pineapple crude waste mixture (CWM) extract was made from commercial grade pineapples (*Ananus comosus* L.) of Smooth Cayenne cultivars, provided by a local supplier (Werribee, Victoria, Australia). The CWM was prepared to represent a real form of raw materials obtained in the pineapple industry, and the preparation procedures was following a method described by Nor et al. (2015). The pineapples were washed, air dried and then manually peeled by knife. The different waste parts (crown, peel and core) were chopped into small pieces and mix at a specific weight ratio of 28% crown, 57% peel and 15%. The mixture was blended (8011ES, Waring, Torrington, Connecticut, USA) with an equal weight of cold Mili-Q water