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**PRODUCTION OF AN ENZYMIC CASEIN HYDROLYSATE
USING A
CONTINUOUS MEMBRANE BIOREACTOR**

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JULIE MAREE O'SULLIVAN

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To Derek, Ryan and Lauren

ABSTRACT

Milk protein hydrolysates suitable for inclusion in hypoallergenic infant formulae should, in general, contain no material greater than 5000 daltons molecular weight and have less than 10% free amino acids. The aim of this study was to investigate the suitability of a continuous stirred tank membrane bioreactor for the production of such a hydrolysate from casein.

Thirteen commercial protease preparations were evaluated for their effect on casein. The most suitable enzymes for production of the target hydrolysate were selected on the basis of molecular weight profiles obtained by the use of high performance size exclusion chromatography. Novo Alcalase 2.4L and Amano Protease A were selected for the bioreactor experiments.

Hollow fibre polysulphone and spiral wound cellulose acetate membranes, each with nominal molecular weight cut off values of 10,000 and 30,000 daltons, were evaluated for their potential effectiveness in the bioreactor system. The spiral wound membrane with a nominal molecular weight cut off of 30,000 daltons was selected for the bioreactor experiments on the basis of molecular weight profiles of permeates obtained from the ultrafiltration of a casein hydrolysate. This membrane had a high flux, gave the highest recovery of peptides, was not susceptible to particulate fouling and gave the most desirable permeate molecular weight profile relative to the target product.

The effect of hydraulic residence time on the molecular weight profile of products produced using the bioreactor and on the productivity of the bioreactor was investigated. Using Amano Protease A and a short hydraulic residence time it was possible to produce a casein hydrolysate with a low proportion of free amino acids and a large proportion of material in the molecular weight range 3000-5000 daltons. Although the hydrolysate produced contained a small amount of material (approximately 2%) greater than 5000 daltons molecular weight, in all other respects the molecular weight profile of this hydrolysate met the criteria defined above.

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LIST OF ABBREVIATIONS OF UNITS

°C	degrees Celsius
g	gram
h	hour
kPa	kiloPascal
L	litre
m	metre
μm	micrometre
mg	milligram
min	minute
ml	millilitre
mm	millimetre
mol%	percent of peptides by number
nm	nanometre
psi	pounds per square inch
v/w	volume/weight

w/w weight/weight

w/v weight/volume

LIST OF OTHER ABBREVIATIONS

CPP	casein phosphopeptide
CSTMR	continuous stirred tank membrane reactor
CSTR	continuous stirred tank reactor
DH	degree of hydrolysis
E:S	enzyme to substrate ratio
HPSEC	high performance size exclusion chromatography
HPLC	high performance liquid chromatography
HRT	hydraulic residence time
MW	molecular weight
MWCO	molecular weight cut off
MWP	molecular weight profile
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis