# **PCT**

WORLD INTELLECTU

## INTERNATIONAL APPLICATION PUBLISHED

(51) International Patent Classification 6:

A3



WO 9529999A3

(21) International Application Number:

\_\_\_\_

tion Number: PCT/DK95/00171

(22) International Filing Date:

25 April 1995 (25.04.95)

(30) Priority Data:

0505/94

C12N 9/64

3 May 1994 (03.05.94)

DK

(71) Applicant (for all designated States except US): CHR. HANSEN A/S [DK/DK]; Bøge Allé 10-12, P.O. Box 407, DK-2970 Hørsholm (DK).

(72) Inventors; and

(75) Inventors/Applicants (for US only): LARSEN, Robert [DK/DK]; Hasselvej 32, DK-2830 Virum (DK). HARBOE, Marianne, Kirsten [DK/DK]; Kulsviervej 149, DK-2800 Lyngby (DK). LADSTEN, Pål, Martin [NO/DK]; 4th floor, Kastelsvej 22, DK-2100 Copenhagen Ø (DK).

(74) Agent: PLOUGMANN & VINGTOFT A/S; Sankt Annæ Plads 11, P.O. Box 3007, DK-1021 Copenhagen K (DK).

(81) Designated States: AM, AT, AT (Utility model), AU, BB, BG, BR, BY, CA, CH, CN, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, ES, FI, FI (Utility model), GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), TJ, TM, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

### Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report: 8 February 1996 (08.02.96)

# (54) Title: A PROCESS FOR SEPARATING MILK CLOTTING ENZYMES, AND STABLE RENNET COMPOSITIONS

### (57) Abstract

A process of separating milk clotting enzymes in extracts of animal stomach tissue which comprises contacting a partially purified extract with an ion exchange resin under conditions where chymosin is bound to the resin and recovering the chymosin, the process optionally comprising the further step of recovering pepsin, from a pepsin-rich extract fraction using an ion exchange resin, and a liquid or powdered rennet composition containing at least 90 % of its milk clotting activity as chymosin activity, the composition comprising a chymosin stabilizing agent which is typically selected from a protein, a peptide, an amino acid or ascorbic acid.

## FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

Austria	GB	United Kingdom	MR	Mauritania
Australia	GE	Georgia	MW	Malawi
Barbados	GN	Guinea	NE	Niger
Belgium	GR	Greece	NL	Netherlands
-	HU	Hungary	NO	Norway
Bulgaria	IE	Ireland	NZ	New Zealand
_	IT	Italy	PL	Poland
=	JP	Japan	PT	Portugal
	KE	Kenya	RO	Romania
+	KG	Kyrgystan	RU	Russian Federation
	KP	Democratic People's Republic	SD	Sudan
•		of Korea	SE	Sweden
•	KR	Republic of Korea	SI	Slovenia
<b>-</b>	KZ	Kazakhstan	SK	Slovakia
	LI	Liechtenstein	SN	Senegal
•		Sri Lanka	TD	Chad
<del></del>			TG	Togo
		Latvia	TJ	Tajikistan
•	MC	Monaco	TT	Trinidad and Tobago
•	MD	Republic of Moldova	UA	Ukraine
	MG	-	US	United States of America
-	ML	•	UZ	Uzbekistan
			VN	Viet Nam
	Australia	Australia GE Barbados GN Belgium GR Burkina Faso HU Bulgaria IE Benin IT Brazil JP Belarus KE Canada KG Central African Republic KP Congo Switzerland KR Côte d'Ivoire KZ Cameroon LI China LK Czechoslovakia LU Czech Republic LV Germany MC Denmark MD Spain MG Finland ML France MN	Australia GE Georgia Barbados GN Guinea Belgium GR Greece Burkina Faso HU Hungary Bulgaria IE Ireland Benin IT Italy Brazil JP Japan Belarus KE Kenya Canada KG Kyrgystan Central African Republic KP Democratic People's Republic Congo of Korea Switzerland KR Republic of Korea Côte d'Ivoire KZ Kazakhstan Cameroon LI Liechtenstein China LK Sri Lanka Czechoslovakia LU Luxembourg Czech Republic G MOnaco Denmark MD Republic of Moldova Spain MG Madagascar Finland ML Mali France MN Mongolia	Australia GE Georgia MW Barbados GN Guinea NE Belgium GR Greece NL Burkina Faso HU Hungary NO Bulgaria IE Ireland NZ Benin IT Italy PL Brazil JP Japan PT Belarus KE Kenya RO Canada KG Kyrgystan RU Central African Republic KP Democratic People's Republic SD Congo of Korea SE Switzerland KR Republic of Korea SI Côte d'Ivoire KZ Kazakhstan SK Cameroon LI Liechtenstein SN China LK Sri Lanka TD Czechoslovakia LU Luxembourg TG Czech Republic LV Latvia TJ Germany MC Monaco TT Denmark MD Republic of Moldova UA Spain MG Madagascar US Finland ML Mali UZ France MN Mongolia

Internati Application No PCT/DK 95/00171

CLASSIFICATION OF SUBJECT MATTER IPC 6 C12N9/64 According to International Patent Classification (IPC) or to both national classification and IPC **B. FIELDS SEARCHED** Minimum documentation searched (classification system followed by classification symbols) IPC 6 C12N Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practical, search terms used) C. DOCUMENTS CONSIDERED TO BE RELEVANT Category ' Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No. X GB, A, 2 106 521 (NOVO INDUSTRI A/S) 13 30 - 48April 1983 cited in the application Y see the whole document 14, 15 Y WO,A,90 15866 (GENENCOR INTERNATIONAL) 27 1-8, December 1990 10-29 cited in the application see page 16 - page 17, paragraph 3 see page 19, paragraph 2 - page 20 Y EP,A,O 137 710 (GENEX CORPORATION) 17 1-8, April 1985 10-29 see the whole document -/--Further documents are listed in the continuation of box C. X X Patent family members are listed in annex. Special categories of cited documents: "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the "A" document defining the general state of the art which is not considered to be of particular relevance invention "E" earlier document but published on or after the international "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "O" document referring to an oral disclosure, use, exhibition or "P" document published prior to the international filing date but later than the priority date claimed "&" document member of the same patent family Date of the actual completion of the international search Date of mailing of the international search report 94, 12 95 4 December 1995 Name and mailing address of the ISA Authorized officer European Patent Office, P.B. 5818 Patentiaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Van der Schaal, C Fax: (+31-70) 340-3016

Form PCT/ISA/219 (second sheet) (July 1992)

internat: 'Application No PCT/DK 95/00171

C.(Continue	DOCUMENTS CONSIDERED TO BE RELEVANT	
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Υ	WO,A,88 02220 (SANOFI BIO INGREDIENTS) 7 April 1988 cited in the application see the whole document	28
<b>A</b>	PREPARATIVE BIOCHEMISTRY, vol. 17, no. 3, pages 297-312, S. SUBRAMANIAN 'Separation of chymosin and pepsin in calf rennet by dye-ligand affinity chromatography'	

Inte ional application No.

PCT/DK95/00171

Box i	Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)
This inter	ernational search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1.	Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
,	Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
b	Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
	Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
	national Searching Authority found multiple inventions in this international application, as follows:
- c	laims 1-29: A process of separating milk clotting aspartic endopeptidases present in an extract of animal stomach tissue
- c1	laims 30-48: Stabilized chymosin preparations
1. X A	as all required additional search fees were timely paid by the applicant, this international search report covers all earchable claims.
2. A	as all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment f any additional fee.
3. As	s only some of the required additional search fees were timely paid by the applicant, this international search report overs only those claims for which fees were paid, specifically claims Nos.:
. No	o required additional search fees were timely paid by the applicant. Consequently, this international search report is stricted to the invention first mentioned in the claims; it is covered by claims Nos.:
emark on i	Protest  The additional search fees were accompanied by the applicant's protest.  X  No protest accompanied the payment of additional search fees.

l. .. mation on patent family members

Internate 'Application No PCT/UK 95/00171

Patent document cited in search report	Publication date	Patent family member(s)		Publication date	
GB-A-2106521	13-04-83	BE-A-	894447	21-03-83 05-05-83	
		DE-A-	3234761	09-09-83	
		FR-A,B	2522681	18-04-83	
		NL-A-	8203631	19-06-84	
		US- <b>A-</b>	4455375 	13-00-04	
WO-A-9015866	27-12-90	AT-T-	126531	15-09-95	
MO X 3013000	<b></b>	AU-B-	624968	25-06-92	
		AU-B-	5842190	08-01-91	
		AU-B-	624969	25-06-92	
		AU-B-	5842790	08-01-91	
		CA-A-	2058934	14-12-90	
		CA-A,C	2058948	14-12-90	
		DE-D-	69021729	21-09-95	
		EP-A-	0477284	01-04-92	
		EP-A-	0477285	01-04-92	
		JP-T-	5500602	12-02-93	
		JP-T-	5500603	12-02-93	
		WO-A-	9015864	27-12-90	
		US-A-	5151358	29-09-92	
		US-A-	5139943	18-08-92	
	17_04_0E	US-A-	4721673	26-01-88	
EP-A-0137710	17-04-85	JP-A-	60070074	20-04-85	
		UP-A-			
WO-A-8802220	07-04-88	US-A-	4745063	17-05-88	
	0, 0, 00	AU-B-	605221	10-01-91	
		AU-B-	8278387	21-04-88	
		CA-A-	1286620	23-07-91	
		DE-A-	3780809	03-09-92	
		EP-A,B	0288554	02-11-88	
		IE-B-	60464	13-07-94	