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(81) **Designated States** (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(88) **Date of publication of the international search report:**
31 December 2014

(54) **Title:** PLANT ASPARTIC PROTEASES WITH DELETED PSI MOTIFS

(57) **Abstract:** The invention relates to aspartic proteases, and particularly to aspartic proteases for plants. Disclosed are modified plant aspartic proteases, and methods for their manufacture, and uses thereof. Particularly contemplated are the uses of aspartic proteases in clotting milk.



INTERNATIONAL SEARCH REPORT

International application No

PCT/PT2014/000017

A. CLASSIFICATION OF SUBJECT MATTER
 INV. C07K14/415
 ADD.

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)
 C07K A23C

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 practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/75283 A1 (INST DE CIENCIA APLIC E TECNOL [PT]; SOARES PAIS MARIA SALOME [PT]; CA) 14 December 2000 (2000-12-14) the whole document	1-36
X	----- SAMPAIO P N ET AL: "Production and characterization of recombinant cyprosin B in Saccharomyces cerevisiae (W303-1A) strain", JOURNAL OF BIOSCIENCE AND BIOENGINEERING, ELSEVIER, AMSTERDAM, NL, vol. 105, no. 4, 1 April 2008 (2008-04-01), pages 305-312, XP022672643, ISSN: 1389-1723, DOI: 10.1263/JBB.105.305 [retrieved on 2008-04-01] the whole document ----- -/--	1-36



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international 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)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"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 invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to 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

"&" document member of the same patent family

Date of the actual completion of the international search

7 July 2014

Date of mailing of the international search report

06/11/2014

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No

PCT/PT2014/000017

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	K. TORMAKANGAS: "A Vacuolar Sorting Domain May Also Influence the Way in Which Proteins Leave the Endoplasmic Reticulum", THE PLANT CELL ONLINE, vol. 13, no. 9, 1 September 2001 (2001-09-01), pages 2021-2032, XP055125636, ISSN: 1040-4651, DOI: 10.1105/tpc.13.9.2021 the whole document	1-36
X	WHITE PAUL C ET AL: "Processing, activity, and inhibition of recombinant cyprosin, an aspartic proteinase from Cardoon (Cynara cardunculus)", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, US, vol. 274, no. 24, 11 June 1999 (1999-06-11), pages 16685-16693, XP002149833, ISSN: 0021-9258, DOI: 10.1074/JBC.274.24.16685 the whole document	1-22, 24-30
X	PRASAD B D ET AL: "Heterologous expression and characterization of recombinant OsCDR1, a rice aspartic proteinase involved in disease resistance", PROTEIN EXPRESSION AND PURIFICATION, ACADEMIC PRESS, SAN DIEGO, CA, vol. 72, no. 2, 1 August 2010 (2010-08-01), pages 169-174, XP027052627, ISSN: 1046-5928, DOI: 10.1016/J.PEP.2010.03.018 [retrieved on 2010-05-15] the whole document	1-12, 15-19, 21,24-26
X	MAZORRA-MANZANO M A ET AL: "Expression and characterization of the recombinant aspartic proteinase A1 from Arabidopsis thaliana", PHYTOCHEMISTRY, PERGAMON PRESS, GB, vol. 69, no. 13, 1 October 2008 (2008-10-01), pages 2439-2448, XP025535878, ISSN: 0031-9422, DOI: 10.1016/J.PHYTOCHEM.2008.07.012 [retrieved on 2008-09-14] the whole document	1-12, 15-22, 24-26, 28-30
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INTERNATIONAL SEARCH REPORT

International application No

PCT/PT2014/000017

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	CURTO PEDRO ET AL: "Establishing the yeast <i>Kluyveromyces lactis</i> as an expression host for production of the saposin-like domain of the aspartic protease cirsin", APPLIED AND ENVIRONMENTAL MICROBIOLOGY, AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 80, no. 1, 1 January 2014 (2014-01-01), pages 86-96, XP009178777, ISSN: 1098-5336 [retrieved on 2013-10-11] the whole document -----	17,31
X	MILISAVLJEVIC M DJ ET AL: "Two types of aspartic proteinases from buckwheat seed - Gene structure and expression analysis", JOURNAL OF PLANT PHYSIOLOGY, URBAN UND FISCHER VERLAG, DE, vol. 165, no. 9, 16 June 2008 (2008-06-16), pages 983-990, XP022691027, ISSN: 0176-1617, DOI: 10.1016/J.JPLPH.2007.03.016 [retrieved on 2007-10-17] the whole document -----	1-12, 15-19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/PT2014/000017

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international 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:
2. ☐ 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:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-36

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-36

Plant aspartic protease modified to lack functional PSI domain. Use thereof in milk clotting, preparation of cheese.

2. claims: 37-40

Promotion of accumulation of peptide of interest in cell vacuole by linkage to PSI domain.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/PT2014/000017

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0075283	A1	14-12-2000	
		AU 783323 B2	13-10-2005
		AU 5258200 A	28-12-2000
		BR 0011364 A	16-07-2002
		CA 2376189 A1	14-12-2000
		CN 1355839 A	26-06-2002
		EP 1196542 A1	17-04-2002
		PT 102318 A	29-12-2000
		US 2006003435 A1	05-01-2006
		WO 0075283 A1	14-12-2000
