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*C13K 1/06* (2006.01)      *C12P 19/02* (2006.01)  
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(54) Title: ALPHA-AMYLASES WITH MUTATIONS THAT IMPROVE STABILITY IN THE PRESENCE OF CHELANTS

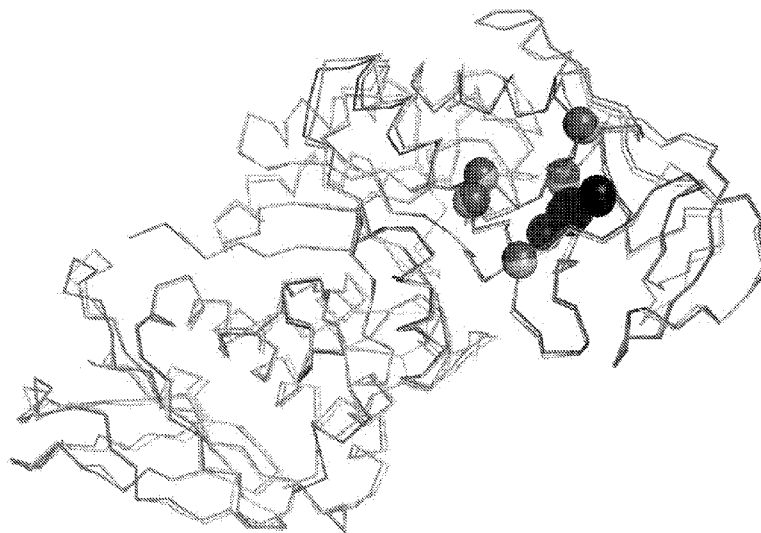


Fig. 1

(57) Abstract: Disclosed are variant  $\alpha$ -amylases having mutations that improve enzyme stability in the presence of chelants, methods of designing such variants, and methods of use, of the resulting variants. The variant  $\alpha$ -amylases are particularly useful, for use in cleaning and desizing composition that include significant amounts of chelants.



TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,  
KM, ML, MR, NE, SN, TD, TG).

**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:**  
30 July 2020 (30.07.2020)

**INTERNATIONAL SEARCH REPORT**

International application No  
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**A. CLASSIFICATION OF SUBJECT MATTER**  
 INV. C12N9/28 C11D3/386 C12P19/14 C13K1/06 D06L1/14  
 C12C5/00 A21D8/04 C12P19/04 C12P19/02  
 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)  
 C12N C11D C12P C13K D06Q D06L C12C A21D  
 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, Sequence Search, FSTA, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/080352 A1 (NOVOZYMES AS [DK]; ANDERSEN CARSTEN [DK]; POULSEN THOMAS AGERSTEN [DK]) 7 July 2011 (2011-07-07) the whole document examples 1-7	1,2,4, 6-11,13, 15,16
X	WO 2011/098531 A1 (NOVOZYMES AS [DK]) 18 August 2011 (2011-08-18) the whole document examples 1-9	1,6-10, 15,16
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Further documents are listed in the continuation of Box C.  See patent family annex.

\* Special categories of cited documents :

<p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"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)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"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</p> <p>"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</p> <p>"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</p> <p>"&amp;" document member of the same patent family</p>
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Date of the actual completion of the international search <b>11 February 2020</b>	Date of mailing of the international search report <b>29/06/2020</b>
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer  <b>van de Kamp, Mart</b>
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## INTERNATIONAL SEARCH REPORT

International application No  
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LI Z. ET AL: "Improving the thermostability and enhancing the Ca <sup>2+</sup> binding of the maltohexaose-forming [alpha]-amylase from Bacillus stearothermophilus", JOURNAL OF BIOTECHNOLOGY, vol. 222, 8 February 2016 (2016-02-08), pages 65-72, XP029453248, ISSN: 0168-1656, DOI: 10.1016/J.JBIOTEC.2016.02.013 the whole document abstract	1,6-10
X	----- WO 2015/044448 A1 (NOVOZYMES AS [DK]) 2 April 2015 (2015-04-02)  the whole document examples 1-4 claims 1,4	1-4, 6-13,15, 16
X	----- WO 2014/164777 A1 (DANISCO US INC [US]) 9 October 2014 (2014-10-09) cited in the application the whole document examples 1-17,19(2) claim 12	1-4, 6-13,15, 16
X	----- WO 2018/184004 A1 (DANISCO US INC [US]) 4 October 2018 (2018-10-04)  the whole document example 1 claims 3,4	1-4, 6-13,15, 16
X	----- WO 2017/100720 A1 (DANISCO US INC [US]; CUEVAS WILLIAM [US] ET AL.) 15 June 2017 (2017-06-15) the whole document examples 1,2,5,6 claims 1,2 -----	1-4, 6-13,15, 16

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2019/056067

## 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.: **5, 14(completely); 2-4, 11-13(partially)**  
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:  
**see FURTHER INFORMATION sheet PCT/ISA/210**
  
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.:  
**1-16(partially)**

### 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: 1-16(partially)

A recombinant variant of a parental Family 13 alpha-amylase, wherein the variant has a mutation (i) in the side chain of an amino acid residue that is not a ligand to a calcium or sodium ion, (ii) wherein the mutation is capable of altering the conformational freedom, the hydrogen bonding interactions, the pi stacking interactions, or the van der Waals interactions of the backbone loop that surrounds the Ca<sup>2+</sup>-Na<sup>+</sup>-Ca<sup>2+</sup> site, and (iii) wherein the variant has increased stability in the presence of a predetermined amount of chelant compared to a parental Family 13 alpha-amylase lacking the mutation, wherein the mutation is at amino acid position E190 or E187. Compositions containing said variant amylase. A method for increasing the stability of a Family 13 alpha-amylase in the presence of a chelant, comprising introducing to a parent Family 13 a-amylase a mutation (i) in the side chain of an amino acid residue that is not a ligand to a calcium or sodium ion, (ii) wherein the mutation is capable of altering the conformational freedom, the hydrogen bonding interactions, the pi stacking interactions, or the van der Waals interactions of the backbone loop that surrounds the Ca<sup>2+</sup>- Na<sup>+</sup>- Ca<sup>2+</sup> site, and (iii) wherein the variant has increased stability in the presence of a predetermined amount of chelant compared to a parental Family 13 a-amylase lacking the mutation, wherein the mutation is at amino acid position E190 or E187. Methods involving use of said variant amylase.

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2. claims: 1-16(partially)

As invention 1, but wherein the mutation is at amino acid position V206 or I203.

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3. claims: 1-16(partially)

As invention 1, but wherein the mutation is at amino acid position H201 or H207.

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4. claims: 1-16(partially)

As invention 1, but wherein the mutation is at amino acid position S244 or S241.

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5. claims: 1-16(partially)

As invention 1, but wherein the mutation is at amino acid position F245 or F242.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

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**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

Continuation of Box II.2

Claims Nos.: 5, 14(completely); 2-4, 11-13(partially)

The specific sequences of claims 2-5, 11-14 have, according to PCT Rule 13ter.1.d, not been searched since the Sequence Listing as present in the description does not comply with WIPO Standard ST 25 prescribed in the administrative instructions under Rule 5.2. The Sequence Listing has been furnished neither in paper form nor in machine readable form as provided for in the same instructions and the applicant has not remedied the disclosed deficiencies within the time limit fixed in the invitation pursuant to PCT Rule 13ter.1.a.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) declaration be overcome.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2019/056067

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