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(54) Title: AMYLASE VARIANTS

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1    HE[REDACTED]T[REDACTED]TMM QYFEWYLPND G[REDACTED]WNRLRSD ASNLKDKGIT AVWIPPAWKG ASQNDVGYGA
60   YDLYDLGEFN QKGTVRTKYG TRNQLQAAVT ALKS[REDACTED]IQVY GDVVM[REDACTED]KGG ADATEWVRAV
120  EVNPSNRNQE VSGDYTIEAW TKFDFPGRG[REDACTED]HSNFKWRWY HFDGVDWDQS RQLQNRIYKF
180  RGDGKGWDWE VDTE[REDACTED]NYDY IMYADIDMDH PEVVNELRNW GVWYT[REDACTED]LGL DGFRIDAVKH
240  IKYSFTRDWL THVR[REDACTED]TGKN MFVAEFWKN DIGAIENYLS KTNW[REDACTED]SVFD VPLHYNLYNA
300  SRSGGNYDMR QIF[REDACTED]TVVQR HPTHAVTFVD [REDACTED]DSQPEEAL ESFVEEWFKP LAYALTLTRD
360  QGYPSVFGD YYGIPTHGVP AMKSKIDPIL EARQKYAYGK QNDYLDHHNM IGWTREG[REDACTED]A
420  HP[REDACTED]GLATIM SDGPGGNKWM YVGRNKAGQV WRDITGNRSG TVTINADGWG NFSV[REDACTED]GSVS
480  I[REDACTED]WV[REDACTED]

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Fig. 10

(57) Abstract: The present invention pertains to the field of amylases, particularly alpha-amylases, and compositions comprising such amylases. Also described are means and methods for producing such amylases, and uses of amylases and amylase-comprising compositions, for example for cleaning starch-containing stains, textile desizing, starch liquefaction and saccharification, baking and brewing. The invention also discloses means for increasing amylase purity during fermentation, formulation and storage of amylase or amylase- containing formulations.



Amylase Variants

FIELD OF THE INVENTION

5 The present invention pertains to the field of amylases, particularly alpha-amylases, and compositions comprising such amylases. Also described are means and methods for producing such amylases, and uses of amylases and amylase-comprising compositions, for example for cleaning starch-containing stains, textile desizing, starch liquefaction and saccharification, baking and brewing. The invention also discloses means for increasing
10 amylase purity during fermentation, formulation and storage of amylase or amylase-containing formulations.

BACKGROUND

15 Starch is a mixture of amylose, which consists of alpha-1-4 linked glucose, and amylopectin, which comprises alpha-1-4 linked glucose and alpha-1-6 linked glucose. Amylases catalyse the cleavage of glycosidic bonds in amylose, amylopectin, glycogen and related polysaccharides. Specifically, alpha-amylases catalyse the cleavage of hydrolysis of random (1-4)-alpha-D-glucosidic linkages. They belong to family 13 of the CAZY
20 classification of glycosyl hydroxylases.

Amylases and particularly alpha-amylases have been used for different purposes, including cleaning starch-containing stains - particularly in laundry and dishwashing-, textile desizing, starch modification, liquefaction and saccharification - particularly in the pulp & paper
25 industry, for syrup production and in the feed industry-, baking and brewing.

For industrial applications of enzymes, homogeneity of product batches is important. In particular for such applications in which enzymes and enzyme compositions are brought into direct contact with humans or animals, e.g. in cleaning compositions and food or feed
30 production, strict quality control standards have to be employed. One object of such quality control is to ascertain that no adverse substances are accidentally comprised in the composition. Ideally, an enzyme composition should not comprise degraded forms of the respective enzyme which may for example influence the substrate specificity of the enzyme or lead to precipitation. Another object is to ascertain that the enzyme preparation analysed
35 in quality controls is representative for the total lot to be sold. Further, it must be established if and how the enzyme of the enzyme composition will change in the time between sampling for quality control and sales to a customer and/or in the time to final use.

It has now been found that homogeneity of commercially available amylases is to a large
40 scale insufficient. Particularly, it has been unexpectedly found that what is sold as commercial compositions comprising only one enzyme (e.g. Termamyl® or Stainzyme®), i.e. one amylase as ascertained by SDS-PAGE, generally comprises several polypeptide

species which can be separated by isoelectric focusing or ion exchange chromatography (herein also termed “parent amylase” and “ghost”/“ghosts”).

Given that protein sequencing is cumbersome and costly, the exact amino acid sequence of polypeptides including enzymes is generally predicted only on the basis of nucleic acid sequences coding for the polypeptides to be produced. However, the above finding that allegedly pure enzyme compositions, i.e. compositions comprising only one species of polypeptide, actually comprise several polypeptide species necessitates the conclusion that the compositions sold comprise significant amounts of polypeptides of unknown primary, secondary and tertiary structure. Thus, unless a complete protein sequencing of all individual amylase species of a composition is performed - which generally is not done -, it is presently not possible to provide enough information to perform a proper freedom-to-operate analysis by amylase manufacturers or customers thereof.

SUMMARY

The general object of the present invention is to alleviate, reduce or remove the above described problems. In particular it was the object of the present invention to provide means and methods for improving homogeneity of compositions comprising one or more amylases (herein also termed “amylase compositions”), particularly one or more alpha-amylases. Further, it was an object of the invention to provide compositions having improved polypeptide homogeneity.

According to the invention there are provided amylase variants facilitating the production of amylase compositions having increased homogeneity compared to compositions prepared using the corresponding wild type amylase, or, more generally, corresponding amylases not comprising the two or more substitutions as described herein.

Particularly, amylase variants are preferred according to the invention which have a sequence identity of at least 65% to SEQ ID NO. 1 and differ therefrom by two or more substitutions, independently chosen from the following groups:

i) positions in the numbering according to SEQ ID NO. 1:

3, 475, 314,

6, 95, 195,

423,

150, 226, 255, 418,

22, 106, 285, 484,

ii) 4, 476, 315,

7, 96, 196,

424,

151, 227, 256, 419,

23, 107, 286, 485,

wherein the substitutions of group i) are selected from any of A, D, E, F, G, H, I, K, L, P, R, S, T, V, W, Y, and preferably are selected from any of A, G, K, R, S, T, and wherein the substitutions of group ii) are selected from any of F, Y, L, I, W, R, E, M.

- 5 The invention also provides amylase compositions with reduced disposition to form of ghost polypeptides as defined herein compared to corresponding amylase compositions comprising the respective parent amylase as defined herein.

Also, the invention provides amylase compositions having a reduced disposition to form
10 isoaspartic acid ("isoAsp" or "J" hereinafter) and/or isoglutamic acid compared to corresponding amylase compositions comprising the respective parent amylase as defined herein.

The amylase compositions provided according to the invention preferably are chosen from
15 cleaning compositions, food compositions, feed compositions, textile desizing compositions and pulp treatment compositions.

Also, the invention provides methods for preparing amylase compositions having increased
20 homogeneity compared to corresponding amylase compositions comprising the respective parent amylase.

Further, the invention provides methods for analysing amylase composition quality,
preferably for amylase composition lot homogeneity determination.

- 25 Further aspects of the invention are described in the detailed description section, examples, figures, sequences and claims.

BRIEF DESCRIPTION OF THE SEQUENCES

- 30 SEQ ID NO. 1 mature section of the alpha-Amylase of Bacillus sp. A 7-7 (DSM 12368)
SEQ ID NO. 2 S707 (mature section) Uniprot ID P19571
SEQ ID NO. 3 LAMY (mature section) Uniprot ID O82839
SEQ ID NO. 4 Sequence SEQ ID NO. 3 of US8017351
SEQ ID NO. 5 Sequence SEQ ID NO. 8 of US7713723
35 SEQ ID NO. 6 Sequence SEQ ID NO. 1 of US7629158

BRIEF DESCRIPTION OF THE FIGURES

- FIG. 1 SDS-PAGE (A) and isoelectric focusing (B) of alpha-amylases. Total protein
40 load was approximately 40 µg of each alpha-amylase on SDS-PAGE and 65 µg of each alpha-amylase on isoelectric focusing gel.

FIG. 2 Chromatographic separation of purified alpha-amylases by cation exchange chromatography (Protein-Pak Hi Res CM, Waters). A: SEQ ID NO. 1; B, C: the aforementioned commercial amylases. The alpha-amylases stock solutions described in the "Methods" section of the detailed description were diluted 1:5 with buffer A. Injection volume was 50 μ l (equal to a total protein injection of approximately 25 – 100 μ g). Starting Buffer (A): MES 25mM pH 5.4 Elution Buffer (B): MES 25mM pH 5.4 + 0.5M NaCl. Elution was effected with a linear gradient 5-32% B in 22 min at a flow rate of 1 ml / min.

FIG. 3 Preparative separation of alpha-amylase SEQ ID NO. 1 by cation exchange chromatography (Protein Pak SP 8HR, waters). Five fractions, F1 – F5 were collected as indicated in orange. A total amount of 3 mg protein was loaded onto the column. Starting buffer (A): 25mM MES pH 5.4 Elution buffer: 25mM MES pH 5.4 + 0.5M NaCl. Elution was effected with a linear gradient 0 - 40% B in 40 min.

FIG. 4 Isoelectric focusing of SEQ ID NO. 1 fractions gained from preparative separation on ion exchange chromatography, shown in figure 3. All samples were adjusted to the same protein concentration (Bradford). 25 μ l containing 35 μ g total protein was loaded on lane A, 25 μ l containing 12.5 μ g total protein were loaded on all other lanes. F1-F5 = fraction 1-5 from figure 3, A = untreated SEQ ID NO. 1, M = marker. The theoretical pI of SEQ ID NO. 1 was calculated to be 6.61.

FIG. 5 Specific alpha-amylase activities of SEQ ID NO. 1 fractions gained from preparative separation on ion exchange chromatography, determined by EPS assay described in the "Methods" section of the detailed description. The measured activity was divided by the concentration of the respective samples (estimated by absorption at 280 nm) to gain the specific activity. Sample activities were normalized to the activity of Fraction 5. Five-times replicates were performed. The error bars show the standard deviations.

FIG. 6 Retention time shift of alpha-Amylase SEQ ID NO. 1 induced by forced degradation as observed on cation exchanger (Protein-Pak Hi Res CM, Waters). Fraction 5 from figure 2 was incubated in 25 mM phosphate buffer pH 8.0 at 37°C. Chromatography was performed at different time points. 20 μ l the incubated sample was diluted with 80 μ l buffer A. Injection volume was 50 μ l. Elution was effected with a linear gradient 5-32% B in 22 min at a flow rate of 1 ml / min.

FIG. 7 Charge shift in isoelectric focusing of SEQ ID NO. 1 induced by forced degradation. All samples were adjusted to the same protein concentration (Bradford). 15 μ l containing 50 μ g total protein was loaded on lane A, 15 μ l containing 25 μ g total protein were loaded on all other lanes. A = untreated SEQ ID NO. 1, M = marker.

FIG. 8 Charge shift in isoelectric focusing of Termamyl® induced by forced degradation. All samples were adjusted to the same protein concentration (Bradford). 15 μ l

containing 50 µg total protein were loaded on the sample lanes. M = marker.

FIG. 9 Total ion count chromatogram of alpha-Amylase SEQ ID NO. 1 after Trypsin digestion. Fraction 5 untreated (red) and fraction 5 after 145 h incubation in 20 mM phosphate buffer at pH 8 / 37°C (blue) are shown. The chromatograms of both samples appear identical, except the complete absence of the N-terminal peptide ($m = 3220.29$ Da) in the stressed sample and an increase of the $m + 1$ N-terminal peptide ($m = 3221.28$ Da) in the degraded sample.

FIG. 10 Depiction of positions within SEQ ID NO. 1 which may be substituted according to the invention.

FIG. 11 Alignment of SEQ ID NO. 1-4.

FIG. 11a Alignment of SEQ ID NO. 1-6.

FIG. 12-15 Alignments of preferred amylase sequences, wherein each X independently from each other is selected according to the corresponding above groups i) or ii), respectively. That is, where X replaces an N according to SEQ ID NO. 1, X is selected from any of A, D, E, F, G, H, I, K, L, P, R, S, T, V, W, Y, and preferably are selected from any of A, G, K, R, S, T, and when X replaces an amino acid at a position according to SEQ ID NO. 1 corresponding to group ii), X is selected from any of F, Y, L, I, W, R, E, M.

DETAILED DESCRIPTION

It has now been found that spontaneous deamidation of amylases is one of or the major contributor to amylase composition inhomogeneity and occurs already in fermentation and post-fermentation purification processing and formulation of amylases.

This finding was surprising, because deamidation so far has only been considered to be a factor influencing stability of amylases at temperatures above 60°C; deamidation has so far not been considered to contribute to amylase composition lot homogeneity, nor has it been envisaged that deamidation could occur significantly during common fermentation and post-fermentation processing conditions. For example, Tomazic et al. (J Biol. Chem 1988, 3093-3096) after comparing *Bacillus amyloliquefaciens* and *Bacillus licheniformis* amylase stability at 60°C and 95°C conclude that deamidation is highly likely to contribute to irreversible thermal inactivation of amylases. Likewise, Declerck et al. (J Mol. Biol. 2000, 1041-1057) discuss increasing the thermostability of *Bacillus licheniformis* amylase, i.e. its half-life at 80°C at pH 5.6 in 0.1mM CaCl₂, by single amino acid substitutions. The authors found three substitutions which increase thermostability of the amylase compared to the wild type form (RS greater than or equal to 3), the substitutions being, in the numbering of SEQ ID NO. 1, at positions 135, 195 and 214, wherein at position 195 asparagine is

replaced by phenylalanine to increase thermostability. Again, the authors do not discuss amylase lot homogeneity nor the occurrence of deamidation at temperatures lower than 60°C. Thermostability of *Bacillus* sp. KR-8104 alpha-amylase has been discussed by Rahimzadeh et al. (Int J Biol Macromol 2012, 1175-1182); the authors analyse the stability of said amylase and variants thereof at 65°C and 70°C. The authors do not investigate or discuss amylase lot homogeneity nor the occurrence of deamidation at temperatures lower than 60°C. The aforementioned documents thus provide no indication to the skilled person how to improve amylase lot homogeneity. They also provide no hint that deamination could contribute to lot inhomogeneity or that deamination would occur significantly also at temperatures below 60°C. Instead, the documents lead the skilled person to believe that deamidation is a problem only relevant at temperatures higher than 60°C. As typical fermentation and post-fermentation purification and formulation processes are performed at temperatures of 37°C, and as higher temperatures are generally avoided by the skilled person to minimise random denaturation and thus thermal inactivation, the skilled person had no reason to fear that during fermentation, purification and/or formulation of amylases deamidation could be a factor of post-translational amylase modification of greater impact than random denaturation processes. The skilled person is thus effectively distracted from the present invention by the above documents. It is important to note that deamidation is not necessarily correlated to a general loss of amylase activity.

Also, patent literature seems to be devoid of any indication for improving amylase lot homogeneity. Instead, patent literature describes individual substitutions, insertions or deletions for specific amylases to improve desired traits, mostly wash performance and suppression of aggregate formation, as exemplified by WO2006037483 and WO2013063460. These documents do not discuss deamidation processes, particularly at temperatures below 60°C, and do not allow to extrapolate how individual mutations disclosed therein should or even could be combined to achieve protection from deamidation during fermentation, purification and formulation of amylases. In particular the skilled person has to take into account that a mutation at one position in the primary structure of an amylase can unexpectedly reduce the degree of freedom for mutations at other positions in the primary structure. Thus, the skilled person is generally hesitant to haphazardly combine individual mutations, as he normally would have to check in vitro if the result of combined mutations does have the desired effect and does not result in undesired effects, particularly loss or decrease of enzymatic activity.

According to the invention there are thus provided amylase variants facilitating the production of amylase compositions having increased amylase homogeneity compared to compositions prepared using the corresponding parent amylase.

For the purposes of the present invention, an amylase is considered to be an enzyme which catalyses the hydrolysis of starch into sugars, and an alpha-amylase is considered to be an enzyme capable of hydrolysis of random (1-4)-alpha-D-glucosidic linkages. Unless

specifically stated the terms "amylase" and "alpha-amylase" are used interchangeably herein. Polypeptides incapable of such catalysis are according to the invention not considered to be amylases and/or alpha-amylases. Particularly, degradation products of amylases and alpha-amylases incapable of performing the aforementioned respective catalysis are not considered to be amylases or alpha-amylases, respectively.

Within the context of the present invention, a "ghost" or "ghost polypeptide" of a composition is a polypeptide resulting from deamidation of a corresponding "parent" amylase or alpha-amylase originally added to the composition. In particular, polypeptides incorporating isoAsp are considered to be ghosts within the context of the present invention. It is to be noted that the designation of a polypeptide as "ghost" is independent of its amylase or alpha-amylase catalytic activity.

According to the present invention, amylase composition homogeneity (also called amylase lot homogeneity) is measured as the molar ratio of ghosts to their respective parent amylase.

When referring to amino acid sequence identity, alignment or numbering of amino acid sequences for the purposes of the present invention, the alignment and scoring algorithm of Needleman-Wunsch is meant. The alignment and scoring is made using the BLOSUM50 matrix, a gap open penalty of 12 and a gap extension penalty of 2. Percentage of amino acid sequence identity is preferably calculated as the number of identical amino acids after pairwise alignment divided by the total of amino acid pairs obtained by the alignment; amino acid sequence similarity is preferably calculated as the number of amino acid pairs after alignment having a BLOSUM50 matrix score of greater than 0 divided by the total number of amino acid pairs obtained by the alignment. Preferably alignments and scores (identity and similarity) are calculated using the MatGAT v2.0 program described by Campanella et al., BMC Bioinformatics 2003, doi:10.1186/1471-2105-4-29. In figures 11, 11a, 12-15, alignments of varying mature amylase sequences are shown. The top sequence is always SEQ ID NO. 1, and the position numbering is according to the amino acid positions in SEQ ID NO. 1. In each alignment, only those amino acids of sequences other than SEQ ID NO. 1 are explicitly indicated which differ from the corresponding amino acid according to SEQ ID NO. 1; where amino acids are identical to the amino acid at the corresponding position in SEQ ID NO. 1, a dot "." Indicates such amino acid identity. Deletions relative to SEQ ID NO. 1 are indicated by "-" for each deleted amino acid; insertions relative to SEQ ID NO. 1 are indicated by "-" in the sequence of SEQ ID NO. 1. Thus, where for example SEQ ID NO. 1 according to a figure comprises the letters "YGI---PTH", the actual amino acid sequence of the amylase according to SEQ ID NO. 1 at this location is "YGIPTH", because the bars "---" indicate that at least one other sequence in this figure has an insertion of three amino acids at this location when compared to SEQ ID NO. 1. And where for sequence SEQ ID NO. 5 at this location the letters "..TKGDSQ" are given in a figure, the actual amylase sequence SEQ ID NO. 5 at this location is "YGTKGDSQ", because the dots ".." are replaced by the

corresponding amino acids of SEQ ID NO. 1 – “YG” – and all remaining amino acids in the example for SEQ ID NO. 5 are explicitly spelled out in the figure; they are thus different from the sequence given for SEQ ID NO. 1 in the figure. The amino acid sequences according to SEQ ID NO. 1-4 are also given in figure 11 of the priority document, for the purposes of describing the invention relating to these sequences said figure 11 and the corresponding description of the priority document is incorporated herein.

The amylases of the present invention are generally coded for by nucleic acids also coding for two or three protein domains: A signal-domain responsible for excretion of the amylase, a pro-domain for temporary inactivation of the amylase, and the amylase as such. After cleavage of the (signal and) pro-domain a resulting mature amylase (the "amylase as such") is obtained. For the purposes of the present invention, the term amylase or alpha-amylase refers to the mature form of the polypeptide. Whenever amino acid sequences comprising pro- and/or signal-pro-domains are aligned to a mature amylase as given by the sequences according to the present invention, the amino acids of the pro- and signal-pro-domains are numbered by negative integers descending as exemplified for example in the sequence protocol section of WO2006037484.

Within the frame of the present invention amino acids are nucleotides are generally referred to by their 3-letter or 1-letter codes.

Particularly, amylase variants are preferred according to the invention which have a sequence identity of at least 65% to SEQ ID NO. 1 and differ therefrom by two or more substitutions, independently chosen from the following groups:

i) positions in the numbering according to SEQ ID NO. 1:

3, 475, 314,
6, 95, 195,
423,
150, 226, 255, 418,

22, 106, 285, 484,

ii) 4, 476, 315,

7, 96, 196,
424,
151, 227, 256, 419,
23, 107, 286, 485,

wherein each substitution of group i) is independently selected from any of A, D, E, F, G, H, I, K, L, P, R, S, T, V, W, Y, and preferably are selected from any of A, G, K, R, S, T, and wherein each substitution of group ii) is independently selected from any of F, Y, L, I, W, R, E, M.

As indicated above it has now been found that deamidation of amylases occurs spontaneously and in a significant extent also during fermentation, purification and

formulation processes wherein heating of amylase-containing compositions to temperatures >45°C are avoided and generally do not occur for more than 30 min, preferably for less than 15 min, even more preferably for less than 8 min, even more preferably for less than 4 min, and most preferably do not occur at all. The invention thus provides means, particularly
5 amylases, which reduce the formation of "ghosts" (deamidation products) during heating for 4 min of amylase-containing compositions to a temperature of 40°C, preferably during heating for 8 min, more preferably during heating for up to 15 min, even more preferably during heating for up to 30 min. The pH of the composition during heat treatment is not less than 5 and preferably at most 9, more preferred at most 8.5, even more preferred at most 8,
10 even more preferred at most 7.5, even more preferred at most 7, even more preferred at most 6.5 and even more preferred at most 6.

It has also been found according to the present invention that amylases having a sequence identity of at least 65% to SEQ ID NO. 1 are particularly prone to deamidation at position 3
15 of SEQ ID NO. 1. A purified amylase of SEQ ID NO. 1 is effectively converted into a ghost thereof, i.e. having position 3 deamidated at >50% within 24h of storage in aqueous 20mM phosphate buffer at pH 8 and 37°C. Deamidated ghosts comprise, at the deamidated position, isoAsp or aspartic acid. The incorporation of isoAsp into an amylase is particularly unfavourable, as this beta amino acid strongly distorts the tertiary structure of the amylase.
20 In wild-type beta-sheet domains isoAsp prevents the direct hydrogen bonding required to support beta-sheet formation at the site of the deamination, thus altering the surface properties of the deamidated polypeptide, facilitating aggregation of ghosts and/or formation of ghost-amylase aggregates and promoting conformational changes also in the non-deaminated "parent" amylase. Further, presence of isoAsp is generally implicated in the
25 formation of protein aggregates in neurodegradative diseases and cataract formation. Thus, incorporation of isoAsp into polypeptides is undesirable, particularly for applications where humans or animals come into direct contact with polypeptides, in particular in cleaning compositions, food compositions, feed compositions, textile desizing compositions, pulp treatment compositions and corresponding uses thereof. The present invention
30 advantageously allows to reduce the rate of or avoid the formation of isoAsp and isoglutamic acid in amylases, thereby alleviating or avoiding the aforementioned concerns.

The substitutions of group i) are designed to remove a potential deamidation site according to SEQ ID NO. 1. Within group i), substitutions in the first line of potential substitution
35 positions are preferred over substitutions involving only positions nominated in the second or following lines, substitutions in the second line are preferred over substitutions involving only positions nominated in the third or following lines etc. Thus, substitutions at any of positions 3, 475, 314 are preferred over amylases wherein only positions listed in the second or following lines of group i) are substituted according to the invention.

40 Within the group of substitutions at positions 3, 475 and 314, substitution according to the invention at position 3 are most preferred. Thus, an amylase variant according to the

present invention preferably comprises, when compared to SEQ ID NO. 1, a substitution of position 3 as defined above and at least one further substitution at another position defined in group i) and/or ii) above.

5 The amylases of the present invention preferably comprise, at their N-terminus, a XXXNGTXMQ motif. This motif fits to the oligopeptide starting at position 3 of SEQ ID NO. 1. Thus, the 4th amino acid of said motif – “N” – corresponds to the 6th amino acid of SEQ ID NO. 1. Preferably, the first X is replaced by any of A, D, E, F, G, H, I, K, L, P, R, S, T, V, W, Y, and preferably by any of A, G, K, R, S, T. Preferred motifs are thus: AGTNGTMMQ,
10 DGTNGTMMQ, EGTNGTMMQ, FGTNGTMMQ, GGTNGTMMQ, HGTNGTMMQ, IGTNGTMMQ, KGTNGTMMQ, LGTNGTMMQ, PGTNGTMMQ, RGTNGTMMQ, SGTNGTMMQ, TGTNGTMMQ, VGTNGTMMQ, WGTNGTMMQ, YGTNGTMMQ, with AGTNGTMMQ, GGTNGTMMQ, KGTNGTMMQ, RGTNGTMMQ, SGTNGTMMQ, TGTNGTMMQ being particularly preferred. For reasons described later herein, the following
15 motifs are more preferred: AGTNGTLMQ, DGTNGTLMQ, EGTNGTLMQ, FGTNGTLMQ, GGTNGTLMQ, HGTNGTLMQ, IGTNGTLMQ, KGTNGTLMQ, LGTNGTLMQ, PGTNGTLMQ, RGTNGTLMQ, SGTNGTLMQ, TGTNGTLMQ, VGTNGTLMQ, WGTNGTLMQ, YGTNGTLMQ, with AGTNGTLMQ, GGTNGTLMQ, KGTNGTLMQ, RGTNGTLMQ, SGTNGTLMQ, TGTNGTLMQ being even more preferred. In all of these
20 motifs, the tripeptide “TNG” can be exchanged against any of “VNG” and “LNG”.

The substitutions of group ii) as defined above are specifically designed to reduce deamidation of any Asn immediately preceding the positions listed in group ii). The skilled person understands that according to the invention by exchanging a small amino acid as
25 encountered in SEQ ID NO. 1 at the positions indicated in group ii) for an amino acid described above with respect to group ii), deamidation of a preceding Asn is sterically hindered. Thus, in case it is desired to maintain a particular Asn which would fall within the definition of group i), substitution of the adjacent C-terminal amino acid by one of the amino acids cited above for group ii) should be made.

30 The skilled person understands that according to the invention deamidation can be avoided by substitution of an Asn according to group i). Thus, it is generally not recommended to substitute two amino acids immediately adjacent to each other, for example substituting at amino acid position 3 by a replacement according to the rules pertinent to group i) and
35 additionally substituting at amino acid position 4 by a replacement according to the rules pertinent to group ii).

In addition to position 3, another deamidation hotspot of SEQ ID NO. 1 is located at position 475 of SEQ ID NO. 1. It was particularly surprising that Asn at this position could be
40 substituted as this Asn is generally conserved among alpha-amylases, particularly among amylases having at least 80% sequence identity to SEQ ID NO. 1. For example, even in the commercially available amylase Termamyl® or the *Bacillus licheniformis* alpha amylase

according to Uniprot ID P06278 ("BLA"), the Asn at position 475 in the numbering of SEQ ID NO. 1 (position 473 of the mature Termamyl®/BLA amylase) is conserved. However, by preventing deamidation at position 3 and 475 the two most important causes of reduction of amylase lot homogeneity can be eliminated, e.g. by substitution at positions 3 and 475, at positions 4 and 475, at positions 3 and 476 or at positions 4 and 476 of SEQ ID NO. 1. Preferably, positions 3 and 475 are substituted according to the rules of group i) given above. Also a mature amylase according to the invention is preferred having at least 80% sequence identity to SEQ ID NO. 1, wherein (a) the amino acid at position 475 of SEQ ID NO. 1 is substituted according to the rules of group i), i.e. by an amino acid selected from any of A, D, E, F, G, H, I, K, L, P, R, S, T, V, W, Y, and preferably selected from any of A, G, K, R, S, T, wherein preferably the substitution according to (a) is the only substitution according to the rules of group i), or (b) wherein the amino acid at position 476 of SEQ ID NO. 1 is substituted according to the rules of group ii), i.e. by an amino acid selected from any of F, Y, L, I, W, R, E, M, wherein preferably the substitution according to (b) is the only substitution according to the rules of group ii). Thus, the invention allows to provide a composition comprising a mature amylase having at least 80% amino acid sequence identity to SEQ ID NO. 1, wherein the molar ratio of said mature amylase, i.e. without deamidation at position 475 according to SEQ ID NO. 1, to the total of amylases comprising a deamidation at position 475 according to SEQ ID NO. 1 is

- at most 1:1,
- preferably <1:2,
- more preferably <1:5,
- most preferably the composition does not comprise any ghost of a mature amylase having at least 80% sequence identity to SEQ ID NO. 1,

and wherein the ratio is determined after 24h of storage in aqueous 20mM phosphate buffer at pH 8 and 37°C.

To a lesser extent it is preferred to prevent deamidation at position 314 of SEQ ID NO. 1, by substitution at position 314 according to group i) and/or substitution at position 315 according to group ii). Again, the Asn at position 314 is generally conserved among alpha-amylases, particularly among amylases having at least 80% sequence identity to SEQ ID NO. 1, so it was surprising that this position could be prone to significant deamidation, or could be substituted without generally interfering with amylase activity. However, by preventing deamidation at positions 3 and 315 according to SEQ ID NO. 1, and preferably also at position 475 according to SEQ ID NO. 1, two major contributor sites for deamidation are defused. It is thus preferable to substitute according to the rules of respective groups i and/or ii), the amino acids at positions 3 and 314, 3 and 315, 4 and 314 or 4 and 315 in the numbering of SEQ ID NO. 1.

Particularly preferred are substitutions to remove all of the aforementioned deamination hotspots at positions 3, 475 and 314 according to SEQ ID NO. 1. It is thus preferred to substitute according to the rules of respective groups i and/or ii), the amino acids at

positions 3 and 314 and 475, 4 and 314 and 475, 3 and 315 and 475, 4 and 315 and 475, 3 and 314 and 476, 4 and 314 and 476, 3 and 315 and 476, or 4 and 315 and 476. In particular preferred is the triple substitutions at positions 3 and 314 and 475.

- 5 Further beneficial mutations are described in DE102012209289A1; this document is incorporated herein in its entirety. According to the invention it is particularly preferred, in addition to any of the substitutions indicated above to provide an amylase variant differing from the corresponding parent amylase by one or more of the following substitutions:
- the amino acid of position 5 according to SEQ ID NO. 1 by A and/or
- 10 the amino acid of position 167 according to SEQ ID NO. 1 by R and/or
- the amino acid of position 170 according to SEQ ID NO. 1 by P and/or
- the amino acid of position 177 according to SEQ ID NO. 1 by L and/or
- the amino acid of position 202 according to SEQ ID NO. 1 by L and/or
- the amino acid of position 204 according to SEQ ID NO. 1 by V and/or
- 15 the amino acid of position 271 according to SEQ ID NO. 1 by D and/or
- the amino acid of position 330 according to SEQ ID NO. 1 by D and/or
- the amino acid of position 377 according to SEQ ID NO. 1 by R and/or
- the amino acid of position 385 according to SEQ ID NO. 1 by S and/or
- the amino acid of position 445 according to SEQ ID NO. 1 by Q.

20 Preferably, the further substitutions are performed according to any of the patterns (1) to (39) described in paragraph [0028] of DE 10 2012 209 289 A1, even more preferred the further substitutions conform to patterns (10), (28), (31), (35), (38) or (39) of DE 10 2012 209 289 A1, and even more preferred the further substitutions conform to pattern (31) of DE

25 10 2012 209 289 A1.

Further beneficial mutations are:

- deletion at positions 183 and 184 according to SEQ ID NO. 1, and substitution of
- the amino acid of position 118 according to SEQ ID NO. 1 by K and/or
- 30 the amino acid of position 195 according to SEQ ID NO. 1 by F and/or
- the amino acid of position 320 according to SEQ ID NO. 1 by K and/or
- the amino acid of position 458 according to SEQ ID NO. 1 by K.

Preferably, the further mutations comprise the deletions at positions 183 and 184 according

35 to SEQ ID NO. 1, and substitution of the amino acid of position 118 according to SEQ ID NO. 1 by K, and substitution of

the amino acid of position 195 according to SEQ ID NO. 1 by F, and substitution of the amino acid of position 320 according to SEQ ID NO. 1 by K, and substitution of the amino acid of position 458 according to SEQ ID NO. 1 by K.

40 During deamidation, Asn is replaced by Asp and/or isoAsp, wherein the ratio of Asp to isoAsp is generally approximately at least 1:2 or even 1:3. Thus, unless deamidation

hotspots are defused as indicated herein according to the invention, the final amylase-containing composition will contain significant amounts of undesirable beta-amino acids, particularly of isoAsp. Following the above teachings according to the invention, it is now possible to provide compositions containing one or more amylases having improved
5 homogeneity over compositions comprising the corresponding wild type amylase, and particularly having a reduced content of beta-amino acids, particularly of isoAsp.

The amylase substituted according to the invention preferably is an amylase having at least 67% sequence identity to SEQ ID NO. 1, preferably at least 68% sequence identity to SEQ
10 ID NO. 1, more preferably at least 74% sequence identity to SEQ ID NO. 1, more preferably at least 80% sequence identity to SEQ ID NO. 1, more preferably at least 85% sequence identity to SEQ ID NO. 1, more preferably at least 87% sequence identity to SEQ ID NO. 1, more preferably at least 90% sequence identity to SEQ ID NO. 1, more preferably at least 92% sequence identity to SEQ ID NO. 1, more preferably at least 94% sequence identity to
15 SEQ ID NO. 1, more preferably at least 95% sequence identity to SEQ ID NO. 1, more preferably at least 97% sequence identity to SEQ ID NO. 1, more preferably at least 99% sequence identity to SEQ ID NO. 1.

Preferably, the substituted amylase according to the invention is an amylase having at least
20 80% sequence identity, preferably at least 85% sequence identity, more preferably at least 87% sequence identity, more preferably at least 90% sequence identity, more preferably at least 92% sequence identity, more preferably at least 94% sequence identity, more preferably at least 95% sequence identity, more preferably at least 97% sequence identity, more preferably at least 99% sequence identity, to any amino acid sequence preferred
25 according to the examples. It is understood that for such sequences, the beneficial substitution(s) according to the invention are conserved.

The invention also provides a nucleic acid comprising a sequence coding for an amylase according to any of the previous claims, and further provides an expression cassette
30 comprising a sequence coding for an amylase according to the invention, wherein the sequence is operably linked to a promotor.

The invention also provides a recombinant host cell comprising the nucleic acid and/or the expression cassette as described herein according to the invention.

35 The invention also provides an enzyme composition comprising one or more amylases, wherein the molar ratio of the total of amylases substituted according to the invention to the total of amylases not according to invention is

- at most 1:1,
- 40 - preferably <1:2,
- more preferably <1:5,

- most preferably the composition does not comprise any amylase not according to the invention,
and wherein the ratio is determined after 24h of storage in aqueous 20mM phosphate buffer at pH 8 and 37°C.

5

Thus, the invention also provides an enzyme composition comprising one or more amylases, wherein the molar ratio of the total deaminated amylases to the total of not deaminated amylases is

- at most 1:1,
- 10 - preferably <1:2,
- more preferably <1:5,
- most preferably the composition does not comprise any deaminated amylase,
and wherein the ratio is determined after 24h of storage in aqueous 20mM phosphate buffer at pH 8 and 37°C.

15

Correspondingly, the invention provides an enzyme composition comprising one or more amylases, wherein the molar ratio of amylases comprising any of L-isoAsp and D-isoAsp to amylases not comprising any of L-isoAsp and D-isoAsp is

- at most 1:1,
- 20 - preferably <1:2,
- more preferably <1:5,
- most preferably the composition does not comprise any amylase comprising any of L-isoAsp and D-isoAsp,
and wherein the ratio is determined after 24h of storage in aqueous 20mM phosphate buffer at pH 8 and 37°C.

25

Said enzyme composition as described above preferably is a cleaning composition, food composition, feed composition, textile desizing composition or pulp treatment composition.

30 The invention also provides a method for improving amylase lot homogeneity, comprising the steps of

- a) providing a parent amylase amino acid sequence having a sequence identity of at least 65% to SEQ ID NO. 1, and
- b) substituting at least 2 positions of the parent amylase amino acid sequence, wherein
35 the positions and substitutions are independently chosen from the following groups:
 - i) positions in the numbering according to SEQ ID NO. 1:
3, 475, 314,
6, 95, 195,
423,
40 150, 226, 255, 418,
22, 106, 285, 484,
 - ii) 4, 476, 315,

7, 96, 196,
424,
151, 227, 256, 419,
23, 107, 286, 485,

- 5 wherein each substitution of group i) is independently selected from any of A, D, E, F, G, H, I, K, L, P, R, S, T, V, W, Y, and preferably are selected from any of A, G, K, R, S, T, and wherein each substitution of group ii) is independently selected from any of F, Y, L, I, W, R, E, M,
- c) providing a host microorganism expressing the amylase having the amino acid
10 sequence obtained in step b),
- d) fermenting, using the host microorganism according to step c), to express the amylase, and
- e) purifying the amylase obtained in step d), and optionally
- f) formulating the amylase obtained in step e) into a cleaning composition, food
15 composition, feed composition, textile desizing composition or pulp treatment composition.

The parent amylase amino acid sequence according to step a) may comprise, as indicated above, an additional signal-, pro- or signal-pro-domain without affecting the numbering according to SEQ ID NO. 1. Also, presence of any of the additional domain(s) does not
20 influence the teaching according to the present invention, because the additional domain(s) are removed either during fermentation and/or purification and/or formulation of the final amylase composition, such that the additional domain(s) do not give rise to "ghosts" in the amylase composition obtained after purification and formulation.

- 25 The parent amylase amino acid sequence according to step a) may comprise further beneficial mutations as described in DE102012209289A1; this document is incorporated herein in its entirety. According to the invention it is particularly preferred, in addition to any of the substitutions indicated above to provide an amylase variant differing from the corresponding parent amylase by one or more of the following substitutions:
- 30 the amino acid of position 5 according to SEQ ID NO. 1 by A and/or
the amino acid of position 167 according to SEQ ID NO. 1 by R and/or
the amino acid of position 170 according to SEQ ID NO. 1 by P and/or
the amino acid of position 177 according to SEQ ID NO. 1 by L and/or
the amino acid of position 202 according to SEQ ID NO. 1 by L and/or
35 the amino acid of position 204 according to SEQ ID NO. 1 by V and/or
the amino acid of position 271 according to SEQ ID NO. 1 by D and/or
the amino acid of position 330 according to SEQ ID NO. 1 by D and/or
the amino acid of position 377 according to SEQ ID NO. 1 by R and/or
the amino acid of position 385 according to SEQ ID NO. 1 by S and/or
40 the amino acid of position 445 according to SEQ ID NO. 1 by Q.

Preferably, the further substitutions conform to any of the patterns (1) to (39) described in

paragraph [0028] of DE 10 2012 209 289 A1, even more preferred the further substitutions conform to patterns (10), (28), (31), (35), (38) or (39) of DE 10 2012 209 289 A1, and even more preferred the further substitutions conform to pattern (31) of DE 10 2012 209 289 A1.

- 5 The parent amylase amino acid sequence according to step a) may comprise further beneficial mutations:

deletion at positions 183 and 184 according to SEQ ID NO. 1, and substitution of

the amino acid of position 118 according to SEQ ID NO. 1 by K and/or

the amino acid of position 195 according to SEQ ID NO. 1 by F and/or

- 10 the amino acid of position 320 according to SEQ ID NO. 1 by K and/or

the amino acid of position 458 according to SEQ ID NO. 1 by K.

Preferably, the further mutations comprise the deletions at positions 183 and 184 according to SEQ ID NO. 1, and substitution of the amino acid of position 118 according to SEQ ID

- 15 NO. 1 by K, and substitution of

the amino acid of position 195 according to SEQ ID NO. 1 by F, and substitution of the

amino acid of position 320 according to SEQ ID NO. 1 by K, and substitution of the amino

acid of position 458 according to SEQ ID NO. 1 by K.

- 20 The invention also provides a method for reducing the content of beta-amino acids, particularly of isoAsp, in a cleaning composition, food composition, feed composition, textile desizing composition or pulp treatment composition compared to a corresponding composition comprising a corresponding parent amylase. The steps of the method are the steps a)-f) as described above, wherein, as described above, step f) is optional.

- 25 According to the invention there is also provided a cleaning composition, food composition, feed composition, textile desizing composition or pulp treatment composition having a content of polypeptides (preferably amylase polypeptides, optionally also other polypeptides) comprising one or more beta amino acids in said polypeptides, preferably
- 30 having a content of polypeptides comprising one or more isoAsp moieties, of at most 20 mol% of total polypeptides of the composition, preferably at most 10 mol% of total polypeptides of the composition, more preferably at most 5 mol% of total polypeptides of the composition, more preferably at most 2 mol% of total polypeptides of the composition, and preferably devoid of beta amino acids, preferably devoid of isoAsp moieties.

- 35 "Polypeptide" in the sense of the previous sentence is a polypeptide having at least 65% sequence identity to the amino acid sequence of SEQ ID NO. 1, and the content of beta amino acids and preferably of isoAsp is determined after 24h of storage in aqueous 20mM phosphate buffer at pH 8 and 37°C. The skilled person understands that the composition is easily prepared by formulating an amylase composition according to the invention into a
- 40 respective cleaning composition, food composition, feed composition, textile desizing composition or pulp treatment composition. Thus, the invention provides the aforementioned compositions and thereby realises the benefits of the invention described

above. The skilled person understands that in these compositions other sources of beta amino acids and particularly of isoAsp in addition to amylases of the invention should be avoided.

5 Particularly preferred cleaning compositions, food compositions, feed compositions, textile desizing compositions and pulp treatment compositions are described in DE 10 2012 209 289 A1 and WO2013063460, which are incorporated herein in their entirety. The skilled person understands that the cleaning compositions, food compositions, feed compositions, textile desizing compositions and pulp treatment compositions according to the invention
10 differs from the respective compositions described in the documents cited in the previous sentence in that the amylases according to the invention of the respective document is replaced by the amylase(s) of the present invention.

Further methods, embodiments and examples according to the invention are described in
15 the following sections:

METHODS

Amylase purification and analytic ion exchange chromatography

20 For purification as described in the following section the GE Akta system used. Purification and adjustment of buffers were done at room temperature. If not otherwise stated all chemicals were analytical grade.

25 For purification of an amylase out of fermentation broth or formulated products an initial anion exchange chromatography (Q-Sepharose) was performed by using a TRIS buffer containing 1 mM CaCl_2 as loading buffer. Samples were desalted if necessary by dialysis using loading buffer as reservoir buffer. Elution of the anion exchange chromatography was performed by a linear salt gradient to 500 mM NaCl. To enable binding using the loading
30 buffer pH for the purification was depending on the individual amylase. To avoid unwanted deamidations a pH higher than pH 8 was avoided. Fractions resulting during this step as well as from the other were monitored for alpha-amylase activity by using an EPS based amylase assay from Roche Diagnostics according to the manufacturer's protocol.

35 As second purification step a hydrophobic interaction chromatography was performed (Butyl-Sepharose). Herefore the alpha-amylase containing fractions were supplemented for loading with ammonium sulfate to a final concentration of 35 %. Protein elution was performed by an elution buffer containing 50 mM MOPS (pH 7.2) and 1 mM CaCl_2 . Alpha-amylase containing fractions contained after as judged by SDS-PAGE analysis an alpha-
40 amylase content of 90%+ mature alpha-amylase protein. Samples containing alpha-amylase were pooled and dialysed using 50 mM MES (pH 5.4) and 1 mM CaCl_2 as reservoir buffer.

For further analysis and separation of charge variants an analytical ion exchange chromatography was performed. Herefore a Mono S cation exchanger was used with 50 mM MES (pH 5,4) and 1 mM CaCl₂ as loading buffer. Elution was performed by an elution buffer containing 50 mM MES (pH 5,4), 500 mM NaCl and 1 mM CaCl₂. The gradient was adjusted to enable a clear separation of the individual charge variants. Hereby individual peaks appeared corresponding to charge variants of the purified alpha-amylase.

Forced deamidation conditions

For forced degradation studies, the alpha-amylases were incubated at specific conditions that favor deamidation. To induce forced deamidation, the alpha-amylases were incubated at elevated temperature (37° C) in slightly alkaline pH (8.0) and a phosphate based buffer system. Those conditions were experimentally verified to induce deamidation processes. The pH values of the buffers used for forced degradation studies were adjusted at room temperature (23.5°C), but also check at 37°C. Samples were incubated in aliquots of 1 - 5 ml volume in 15 ml tubes without shaking for a given period of time.

Digest of alpha-amylases for MS analysis

The alpha-amylase samples were precipitates by mixing them with 50 % TCA to a final concentration of 12.5 % TCA (v/v). The sample volumes were chosen depending on the actual alpha-amylase concentration to gain a protein pellet of at least 100 µg (protein concentration measured by absorption at 280 nm). The samples were centrifuged for 5 min at 14.000 rpm at 4°C. Supernatants were discarded and the protein pellets were washed twice with 1 ml of an ethanol / diethyl ether 1:1 (v/v) solution.

For digest all proteases were purchased from Roche in a sequencing grade quality.

For digestion with endoprotease trypsin, 25 µg of trypsin (sequencing grade) was dissolved in 2 ml phosphate buffer 20 mM, pH 8.0 and mixed gently. 150 µl of the trypsin solution was added to the alpha-amylase pellet (equals 1.875 µg trypsin per 150 µg protein). Digestion was performed for 4h at 37°C without shaking.

For digestion with endoprotease Lys-C, 5 µg of Lys-C (sequencing grade) was dissolved in 1.5 ml phosphate buffer 20 mM, pH 8.0 and mixed gently. 150 µl of the Lys-C solution was added to the alpha-amylase pellet (equals 0.5 µg Lys-C per 150 µg protein). Digestion was performed for 4h at 37°C without shaking.

For digestion with endoprotease Asp-N, 2 µg of Asp-N (sequencing grade) was dissolved in 1.5 ml phosphate buffer 20 mM, pH 8.0 and mixed gently. 150 µl of the Asp-N solution was added to the alpha-amylase pellet (equals 0.2 µg Asp-N per 150 µg protein). Digestion was performed for 4h at 37°C without shaking.

Samples were stored for a maximum of two days at 4°C before LC-MS analysis was performed.

5 SDS-PAGE

The α -amylase samples were precipitated with 50 % TCA to a final volume of 12.5 % TCA (v/v). The sample volumes were chosen depending on the actual α -amylase concentration but with at least 50 μ g total protein (protein concentration measured by absorption at 280 nm). The samples were centrifuged for 5 min at 14.000 rpm at 4°C. Supernatant was discarded and the protein pellet was washed twice with 1 mL of an ethanol / diethyl ether 1:1 (v/v) solution. After drying the protein pellets were dissolved in at least 50 μ L SDS sample buffer (4% SDS, 2% DTT, 80 mM Tris, 0.1 %, bromphenol blue, pH 6.8) and heated at 95°C for 15 minutes.

SDS-PAGE was performed on Novex NuPAGE® 4-12% Bis-Tris gels in a Novex XCell SureLock® mini cell electrophoresis chamber, coupled with a Pharmacia Biotech EPS 3501 XL power supply unit. MES SDS NuPAGE® running buffer was used. The settings for the SDS-PAGE are 150V, 90mA, 15W, 40min, temperature not regulated. 15 μ L protein sample were mixed with 45 μ L SDS sample buffer (4% SDS, 2% DTT, 80 mM Tris, 0.1 %, bromophenol blue, pH 6.8) and heated at 95°C for 15 minutes. The protein concentrations and amount of protein load per lane was in the range of 20-40 μ g protein. Protein molecular weight was estimated by using 5 μ L BioRad Precision Plus Protein™ marker as reference.

Isoelectric focusing was performed on a Pharmacia Biotech Multiphor II horizontal electrophoresis chamber coupled with a Pharmacia Biotech EPS 3500 XL electrophoresis power supply unit. Temperature of the electrophoresis chamber was regulated by a Huber Ministate 230 cooling circulator. Samples were applied to the anodic side of SERVA vertical focus gels pH 3-10 (Cat.No.: 43327.01) using 1 cm² Pharmacia Biotech sample application pieces. 15 μ L SERVA IEF-Marker liquid mix pH 3-10 (Cat.No.: 39212), 1:5 diluted with water was used as pI marker on all gels. The power supply settings for the focusing process are

Step	Voltage set [V]	Current Set [mA]	Power Set [W]	Time [min]
Temperature [C°]				
Pre focusing (without sample)	1000	50	10	20
Sample entrance	500	30	10	30
Focusing	1500	18	20	90
Band sharpening	2000	15	25	30

40 EXAMPLES

The following mature alpha-amylases are preferred according to the invention due to their

reduced or abolished tendency of deamidation during normal storage conditions as described above: Amylases comprising or consisting of any of the sequences SEQ-A.1 to SEQ-A.16, sequences SEQ-B.1 to SEQ-B.16, sequences SEQ-C.1 to SEQ-C.16 and sequences SEQ-D.1 to SEQ-D.33 according to Figures 12-16, wherein each X

5 independently from any other X in the same sequence is defined as explained in the above figure description. Also preferred are amylases of at least 80% amino acid sequence identity to SEQ ID NO. 6 and comprising a substitution according to group i) at positions 475 and 314 according to SEQ ID NO. 1.

10 As described above, amylases are also preferred according to the invention which comprise, in addition to any of the sequences of this "Examples" section, one or more further domains, e.g. a propeptide and/or a signal peptide.

15 Examples relating to the preparation and use of compositions and amylases according to the present invention:

Detergent composition [wt. %]

	FAEOS	5%
	C12/14 7 EO	12%
20	APG	2%
	Fatty acids C12-18	5%
	Glycerine	5%
	Tinopal ® CBS-X	0.1%
	Citrate	1%
25	Polyacrylate	2%
	Protease	+
	Amylase	+
	Water	made up to 100%

30 A built laundry detergent composition having the following formulation:

	Weight%
Linear alkylbenzene sulphonate	23.00
Cationic surfactant (C12-14 alkyl dimethyl	0.80
35 hydroxyethyl ammonium chloride)	
Sodium tripolyphosphate	14.50
Sodium carbonate	17.50
Sodium silicate	7.00
Sodium sulphate	28.52
40 Sodium carboxymethyl cellulose	0.37
Fluorescers	0.19
Enzymes (protease, lipase, amylase)	0.94

Blue colour, perfume 0.44
Moisture etc 6.92

Ingredient	Powder Formulation (concentration in final product in final in final [weight %])	Liquid Detergent Formulation (concentration in final product in final in final [weight %])	Concentrated Liquid Detergent (concentration in final product in final in final [weight %])
Linear alkyl benzene sulfonate	10	12	24
Alcohol ethoxylate	5	6	12
Zeolite builder	20		
Sodium carbonate	20		
Amylase according to the invention	1	1	2
Whitening agent	0.1	0.1	0.1
Bleach	15		
Bleach activator	1		
Copolymer(s)/Polymer(s)	1.5	1	2
Sodium citrate		5	5
Sodium chloride		2	2
Sodium hydroxide		1	1
Dispersent (e.g. polycarboxylate)		1	2
Water, perfume, foam control & other minors	balance	balance	balance

- 5 In one embodiment of the present invention, (A) an amylase according to the invention, preferably having a sequence as indicated above in this example section, is a component of a laundry care composition that additionally comprises at least one anionic surfactant (B) and at least one builder (C).
- 10 Examples of suitable anionic surfactants (B) are alkali metal and ammonium salts of C₈-C₁₂-alkyl sulfates, of C₁₂-C₁₈-fatty alcohol ether sulfates, of C₁₂-C₁₈-fatty alcohol polyether sulfates, of sulfuric acid half-esters of ethoxylated C₄-C₁₂-alkylphenols (ethoxylation: 3 to 50 mol of ethylene oxide/mol), of C₁₂-C₁₈-alkylsulfonic acids, of C₁₂-C₁₈ sulfo fatty acid alkyl esters, for example of C₁₂-C₁₈ sulfo fatty acid methyl esters, of C₁₀-C₁₈-alkylarylsulfonic acids, preferably of n-C₁₀-C₁₈-alkylbenzene sulfonic acids, of C₁₀-C₁₈ alkyl alkoxy
- 15

carboxylates and of soaps such as for example C₈-C₂₄-carboxylic acids. Preference is given to the alkali metal salts of the aforementioned compounds, particularly preferably the sodium salts.

5 In one embodiment of the present invention, anionic surfactants (B) are selected from n-C₁₀-C₁₈-alkylbenzene sulfonic acids and from fatty alcohol polyether sulfates, which, within the context of the present invention, are in particular sulfuric acid half-esters of ethoxylated C₁₂-C₁₈-alkanols (ethoxylation: 1 to 50 mol of ethylene oxide/mol), preferably of n-C₁₂-C₁₈-alkanols.

10 Examples of builders (C) are complexing agents, hereinafter also referred to as complexing agents (C), ion exchange compounds, and precipitating agents (C). Examples of builders (C) are citrate, phosphates, silicates, carbonates, phosphonates, amino carboxylates and polycarboxylates.

15 Examples of complexing agents (C) ("sequestrants") are selected from complexing agents such as, but not limited to citrate, phosphates, phosphonates, silicates, and ethylene amine derivatives selected from ethylene diamine tetraacetate, diethylene pentamine pentaacetate, methylglycine diacetate, and glutamine diacetate. Complexing agents (C) will
20 be described in more details below.

Examples of precipitating agents (C) are sodium carbonate and potassium carbonate.

25 In one embodiment of the present invention, the use according to the invention comprises the use of amylase (A) together with at least one further enzyme (D). Useful enzymes are, for example, one or more lipases, hydrolases, proteases, cellulases, hemicellulases, phospholipases, esterases, pectinases, lactases and peroxidases, and combinations of at least two of the foregoing types of the foregoing.

30 Compositions according to the invention may comprise one or more bleaching agent (E) (bleaches).

Preferred bleaches (E) are selected from sodium perborate, anhydrous or, for example, as the monohydrate or as the tetrahydrate or so-called dihydrate, sodium percarbonate,
35 anhydrous or, for example, as the monohydrate, and sodium persulfate, where the term "persulfate" in each case includes the salt of the peracid H₂SO₅ and also the peroxodisulfate.

40 Formulations according to the invention can comprise one or more bleach catalysts. Bleach catalysts can be selected from oxaziridinium-based bleach catalysts, bleach-boosting transition metal salts or transition metal complexes such as, for example, manganese-, iron-, cobalt-, ruthenium- or molybdenum-salen complexes or carbonyl complexes. Manganese,

iron, cobalt, ruthenium, molybdenum, titanium, vanadium and copper complexes with nitrogen-containing tripod ligands and also cobalt-, iron-, copper- and ruthenium-amine complexes can also be used as bleach catalysts.

- 5 Formulations according to the invention can comprise one or more bleach activators, for example tetraacetyl ethylene diamine, tetraacetylmethylenediamine, tetraacetylglycoluril, tetraacetylhexylenediamine, acylated phenolsulfonates such as for example n-nonanoyl- or isononanoyloxybenzene sulfonates, N-methylmorpholinium-acetonitrile salts ("MMA salts"), trimethylammonium acetonitrile salts, N-acylimides such as, for example, N-
- 10 nonanoylsuccinimide, 1,5-diacetyl-2,2-dioxohexahydro-1,3,5-triazine ("DADHT") or nitrile quats (trimethylammonium acetonitrile salts).

- Formulations according to the invention can comprise one or more corrosion inhibitors. In the present case, this is to be understood as including those compounds which inhibit the
- 15 corrosion of metal. Examples of suitable corrosion inhibitors are triazoles, in particular benzotriazoles, bisbenzotriazoles, aminotriazoles, alkylaminotriazoles, also phenol derivatives such as, for example, hydroquinone, pyrocatechol, hydroxyhydroquinone, gallic acid, phloroglucinol or pyrogallol.

- 20 Formulations according to the invention may comprise at least one additional surfactant, selected from non-ionic surfactants and amphoteric surfactants.

Non-ionic surfactants

- 25 Examples of surfactants are in particular nonionic surfactants. Preferred nonionic surfactants are alkoxyated alcohols and alkoxyated fatty alcohols, di- and multiblock copolymers of ethylene oxide and propylene oxide and reaction products of sorbitan with ethylene oxide or propylene oxide, furthermore alkylphenol ethoxylates, alkyl glycosides, polyhydroxy fatty acid amides (glucamides) and so-called amine oxides.

- 30 Further suitable nonionic surfactants are selected from di- and multiblock copolymers, composed of ethylene oxide and propylene oxide. Further suitable nonionic surfactants are selected from ethoxylated or propoxylated sorbitan esters. Amine oxides such as lauryl dimethyl amine oxide ("lauramine oxide") or alkylphenol ethoxylates or alkyl polyglycosides
- 35 or polyhydroxy fatty acid amides (glucamides) are likewise suitable. An overview of suitable further nonionic surfactants can be found in EP-A 0 851 023 and in DE-A 198 19 187.

- Mixtures of two or more different nonionic surfactants may also be present. Examples of
- 40 amphoteric surfactants are C₁₂-C₁₈-alkylbetaines and sulfobetaines. Further optional ingredients may be but are not limited to viscosity modifiers, cationic surfactants, foam boosting or foam reducing agents, perfumes, dyes, optical brighteners, dye transfer inhibiting agents and preservatives.

CLAIMS

1. Amylase having a sequence identity of at least 65% to SEQ ID NO. 1 and differing therefrom by two or more substitutions, independently chosen from the following groups:

i) positions in the numbering according to SEQ ID NO. 1:

3, 475, 314,

6, 95, 195,

423,

150, 226, 255, 418,

22, 106, 285, 484,

ii) 4, 476, 315,

7, 96, 196,

424,

151, 227, 256, 419,

23, 107, 286, 485,

wherein each substitution of group i) is independently selected from any of A, D, E, F, G, H, I, K, L, P, R, S, T, V, W, Y, and preferably are selected from any of A, G, K, R, S, T, and

wherein each substitution of group ii) is independently selected from any of F, Y, L, I, W, R, E, M.

2. Amylase according to claims 1 having at least 67% sequence identity to SEQ ID NO. 1, preferably at least 68% sequence identity to SEQ ID NO. 1, more preferably at least 80% sequence identity to SEQ ID NO. 1, more preferably at least 85% sequence identity to SEQ ID NO. 1, more preferably at least 87% sequence identity to SEQ ID NO. 1, more preferably at least 90% sequence identity to SEQ ID NO. 1, more preferably at least 92% sequence identity to SEQ ID NO. 1, more preferably at least 94% sequence identity to SEQ ID NO. 1, more preferably at least 95% sequence identity to SEQ ID NO. 1, more preferably at least 97% sequence identity to SEQ ID NO. 1, more preferably at least 99% sequence identity to SEQ ID NO. 1.

3. Amylase according to any of the previous claims, having at least 80% sequence identity, preferably at least 85% sequence identity, more preferably at least 87% sequence identity, more preferably at least 90% sequence identity, more preferably at least 92% sequence identity, more preferably at least 94% sequence identity, more preferably at least 95% sequence identity, more preferably at least 97% sequence identity, more preferably at least 99% sequence identity, to any amino acid sequence according to the examples.

4. Nucleic acid comprising a sequence coding for an amylase according to any of the previous claims.

5. Expression cassette comprising a sequence coding for an amylase according to any of the previous claims, wherein the sequence is operably linked to a promotor.
- 5 6. Recombinant host cell comprising the nucleic acid and/or the expression cassette of any of the previous claims.
7. Enzyme composition comprising one or more amylases, wherein the molar ratio of the total of amylases according to any of the previous claims to the total of amylases not according to the previous claims is
- 10 - at most 1:1,
- preferably <1:2,
- more preferably <1:5,
- most preferably the composition does not comprise any amylase not according
- 15 to any of the previous claims,
and wherein the ratio is determined after 24h of storage in aqueous 20mM phosphate buffer at pH 8 and 37°C.
8. Enzyme composition comprising one or more amylases, wherein the molar ratio of amylases comprising any of L-isoAsp and D-isoAsp to amylases not comprising any of L-isoAsp and D-isoAsp is
- 20 - at most 1:1,
- preferably <1:2,
- more preferably <1:5,
- 25 - most preferably the composition does not comprise any amylase comprising any of L-isoAsp and D-isoAsp,
and wherein the ratio is determined after 24h of storage in aqueous 20mM phosphate buffer at pH 8 and 37°C.
- 30 9. Enzyme composition according to any of the previous claims 7 or 8, wherein the composition is a cleaning composition, food composition, feed composition, textile desizing composition or pulp treatment composition.
10. Method for treating starch-containing substances, preferably for cleaning fabrics from starch-containing stains, comprising exposing the substance, preferably the stained
- 35 woven or non-woven fabric, to an enzyme composition according to any of the previous claims to allow the amylase comprised in the composition to cleave glycosidic bonds.

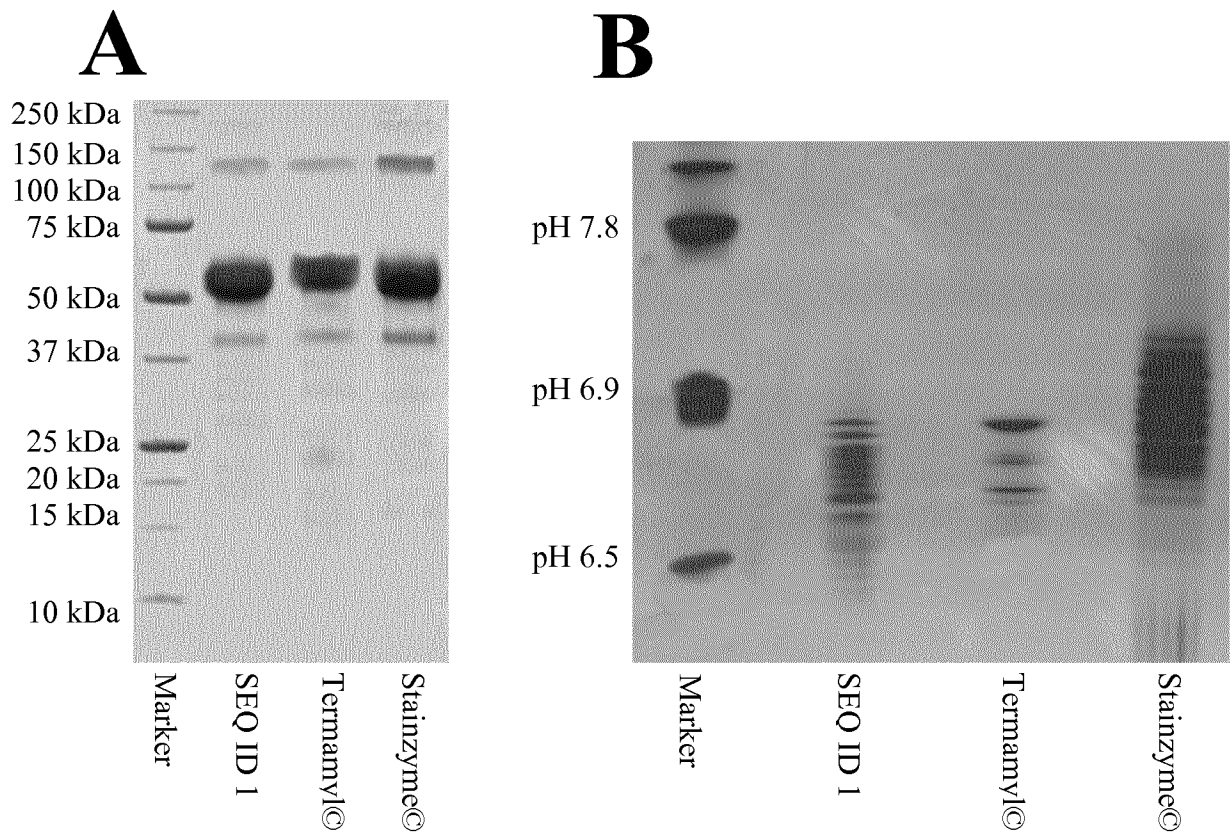


Figure1

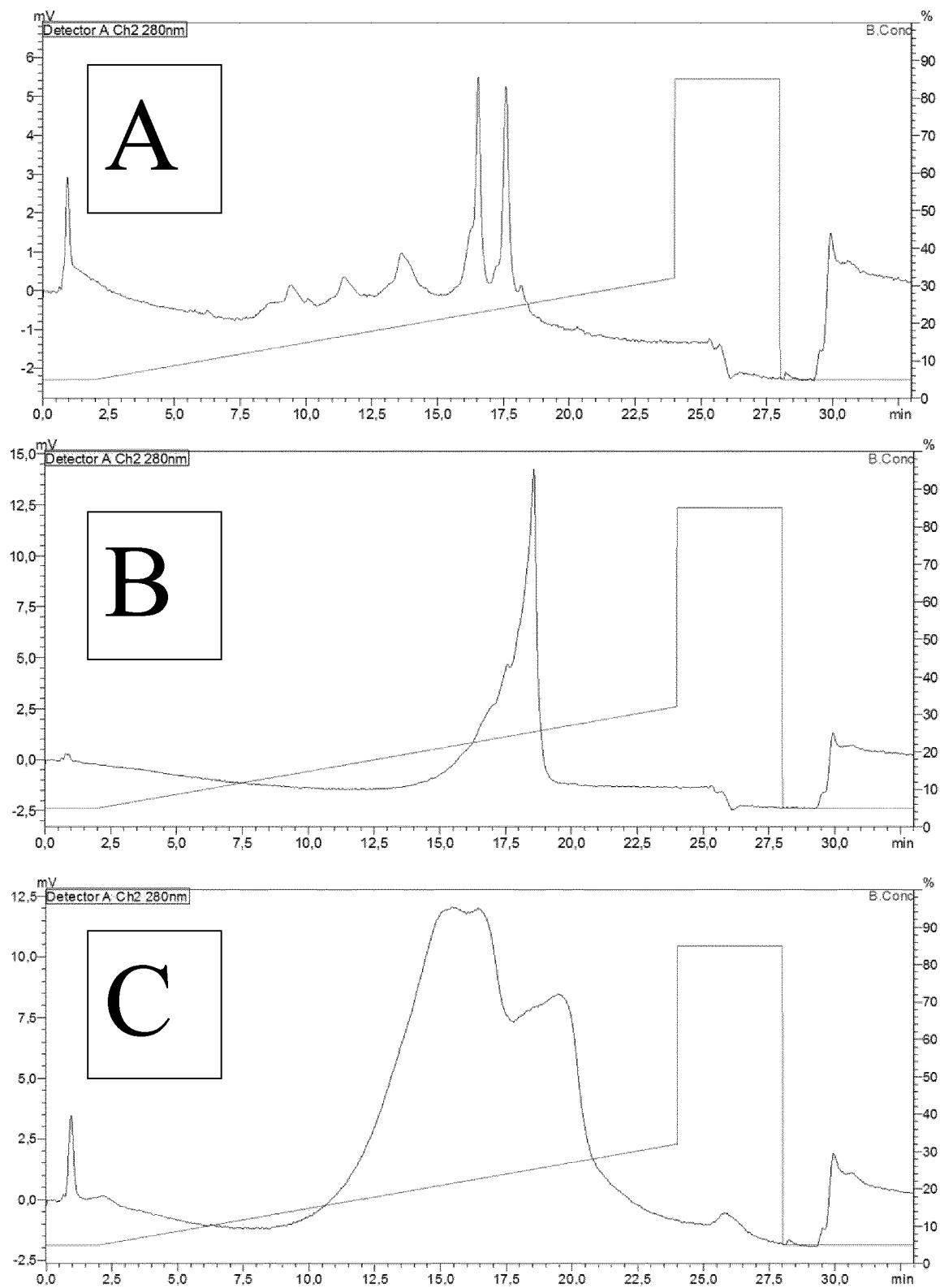


Fig. 2

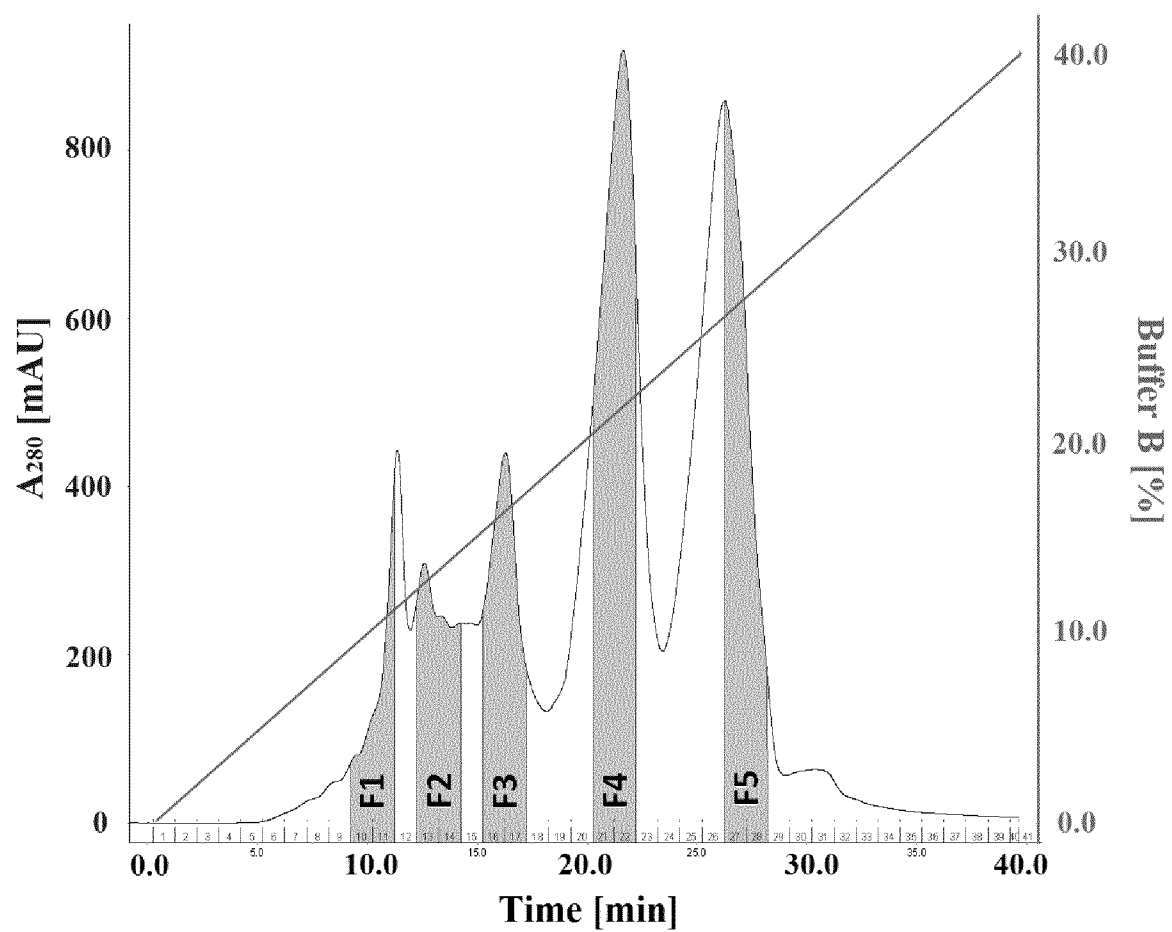
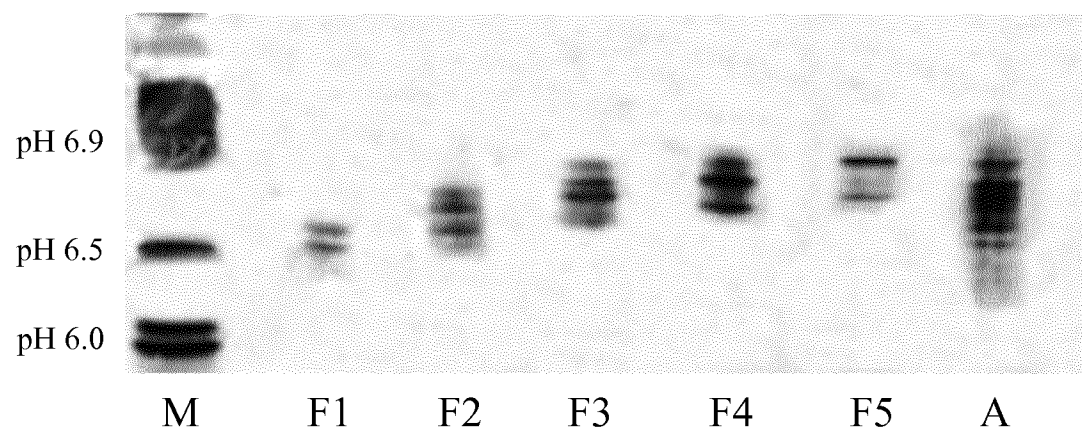


Fig. 3

**Fig. 4**

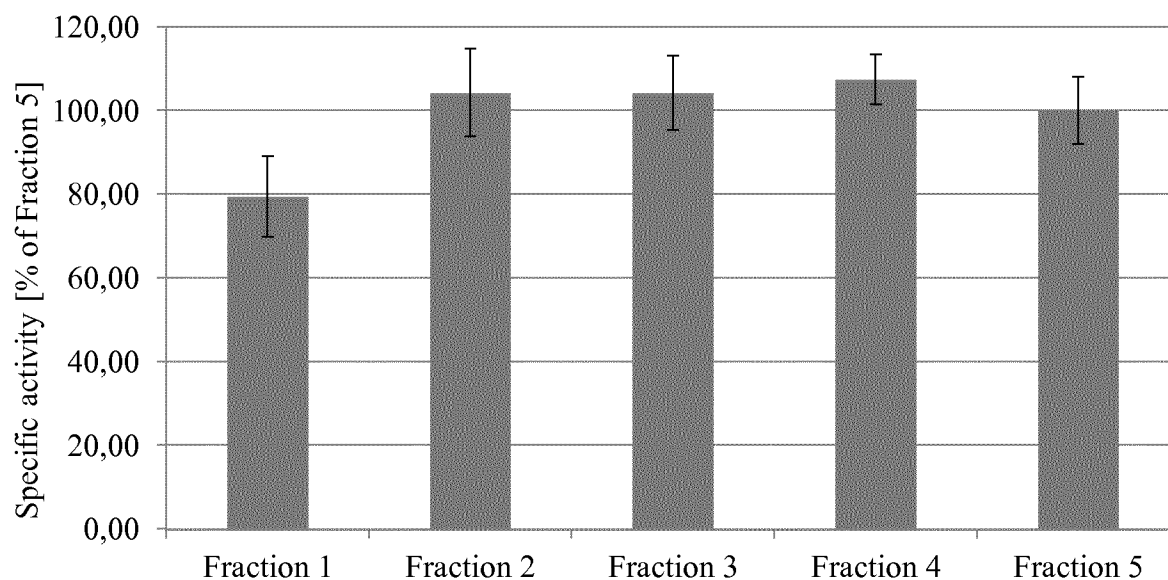


Fig. 5: no statistically significant reduction of specific activity relative to the pure amylase of SEQ ID NO.1 (= fraction 5) was detected even after deamidation at position 3 (fraction 4), 475 and 314, respectively (fractions 3 and 2).

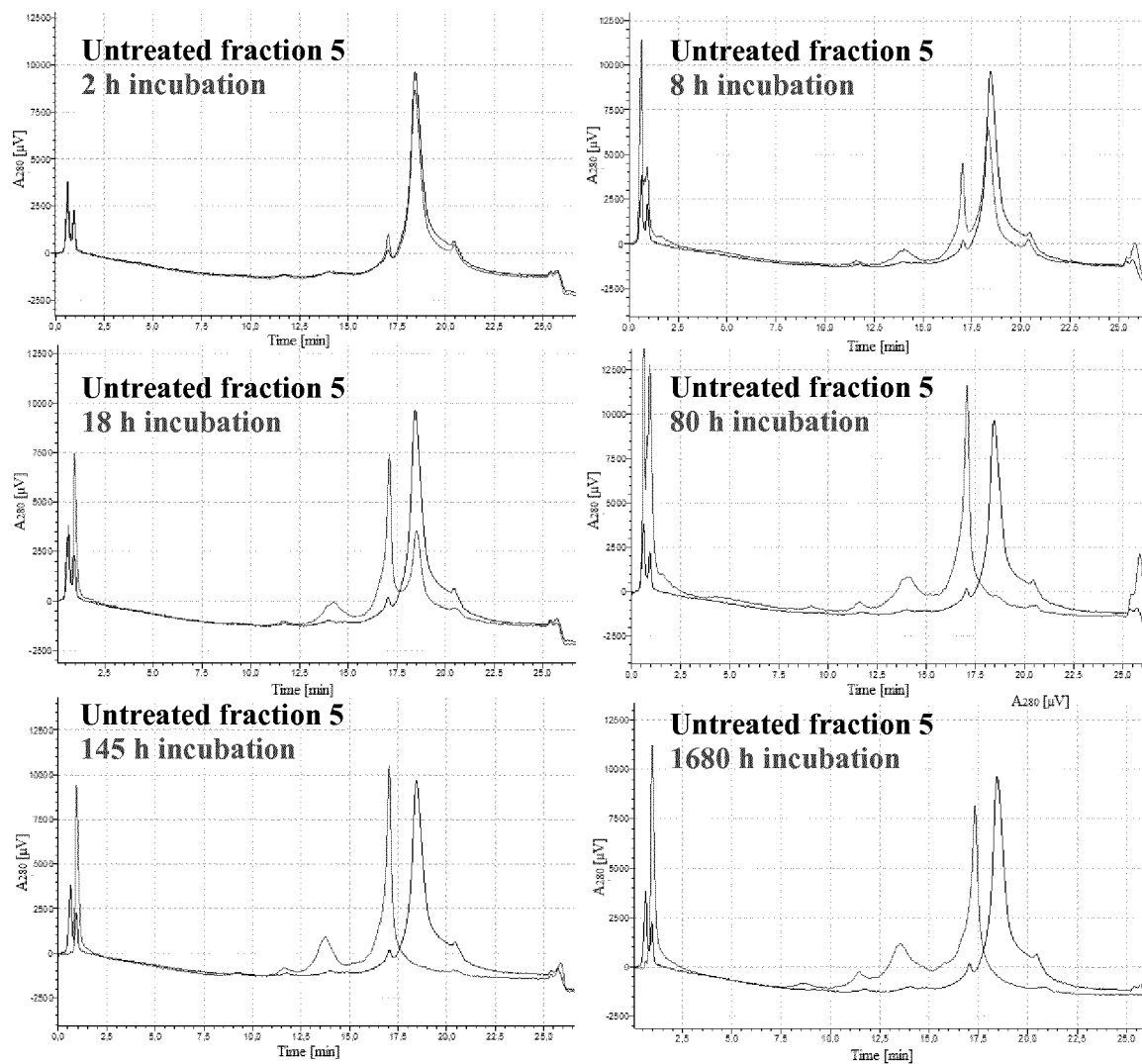


Fig. 6

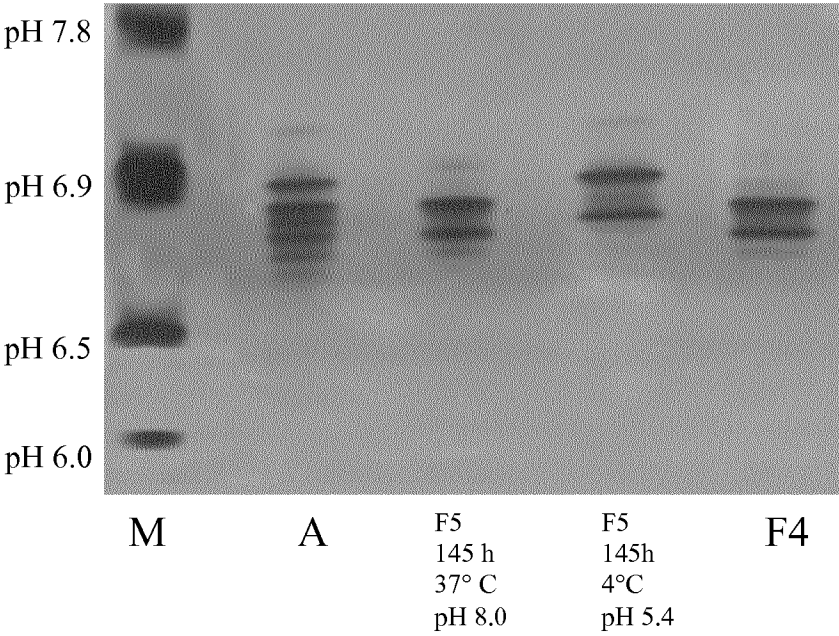


Fig. 7

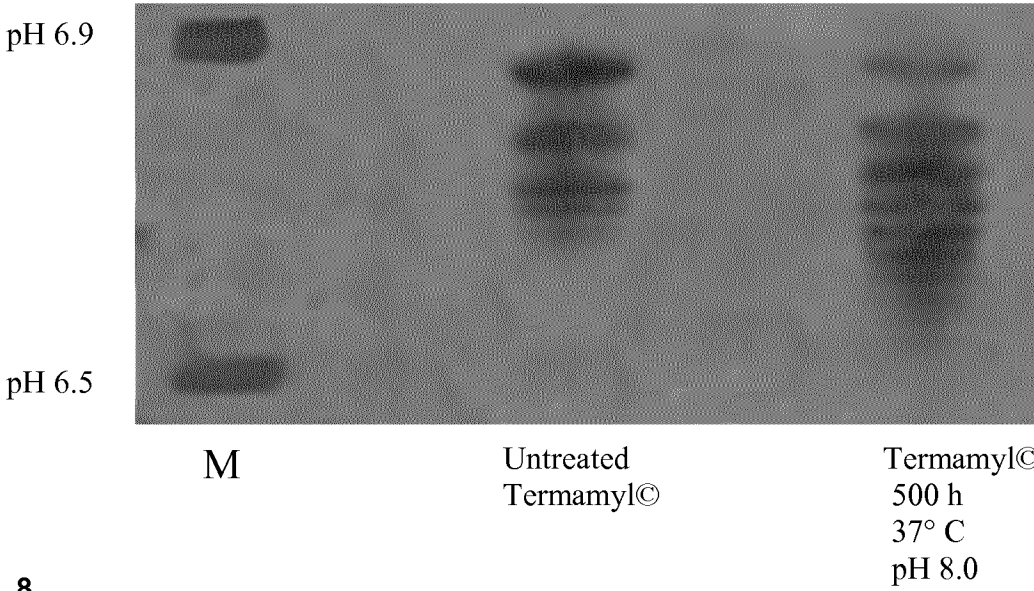
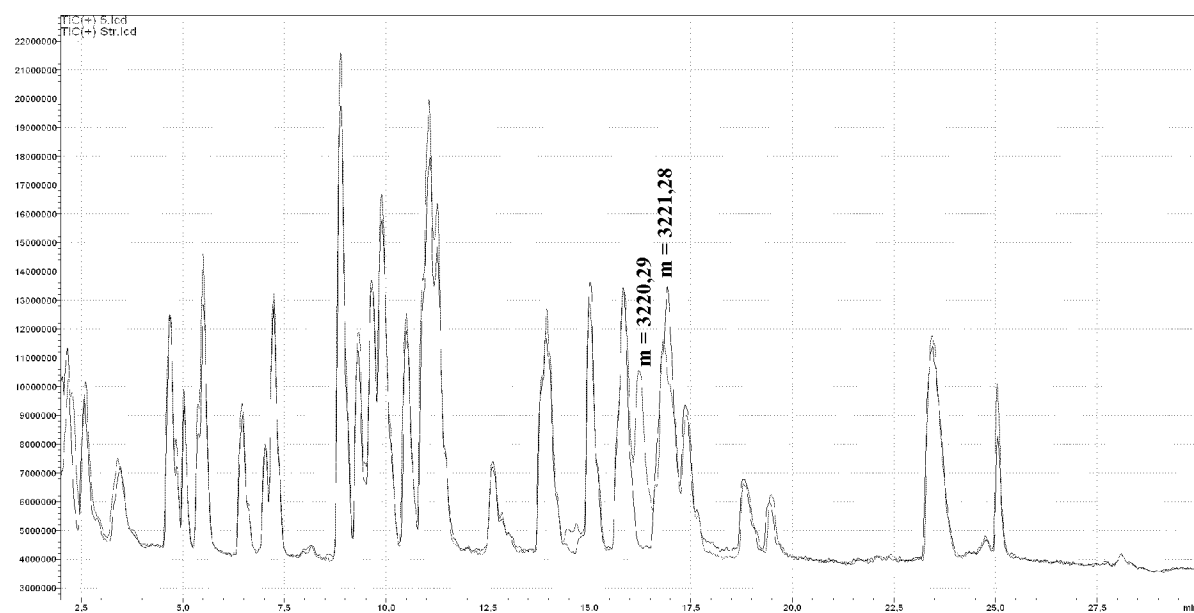


Fig. 8

**Fig. 9**

1 HHNGTNGTMM QYFEWYLPND GNIWNRLRSD ASNLKDKGIT AVWIPPAWKG ASQNDVGYGA
60 YDLYDLGEFN QKGTVRTKYG TRNQLQAAVT ALKSNGIQVY GDVVMNHKGG ADATEWVRVAV
120 EVNPSNRNQE VSGDYTIEAW TKFDFPGRGNIHSNFKWRWY HFDGVDWDQS RQLQNRIYKF
180 RGDGKGWDWE VDTENGNYDY LMYADIDMDH PEVVNELRNW GVWYTNILGL DGFRIDAVKH
240 IKYSFTRDWL THVRITTGKN MFAVAEFWKN DIGAIENYLS KTNWIRSVFD VPLHYNLNA
300 SRSGGNYDMR QIFNGTVVQR HPTHAVTFVD NIDSQPEEAL ESFVEEWFKP LAYALTLTRD
360 QGYPSVFIGD YYGIPTHGVP AMKSKIDPIL EARQKYAYGK QNDYLDHHNM IGWTREGNIA
420 HPNSGLATIM SDGPGGNKWM YVGRNKAGQV WRDITGNRSG TVTINADGWG NFSVNGGSVS
480 IWVNN

Fig. 10


```

      10      20      30      40      50      60      70
SEQ ID NO 1 HHNGTNGTMMQYFEWYLPNDGNNHNNRLRSDASNLKDKGITAVWIPPAWKGASQNDVGYGAYDLYDLGEFN
SEQ ID NO 3 .....H.....D..A..S.....T.....
SEQ ID NO 2 .....N.....S.....
SEQ ID NO 4 .....S.....
SEQ ID NO 5 --ANL...L...M...Q...R..QN.SAY.AEH.....Y..T..A.....H
SEQ ID NO 6 --ANL...L...T...Q...K..QN.SAY.AEH.....Y..T..A.....H

      80      90      100      110      120      130      140
SEQ ID NO 1 QKGTVRTKYGTRNQLQAAVTALKSNGIQVYGDVVMNHKGGADATEWVRAVEVNPSPNRNQEVSGDYITIEAW
SEQ ID NO 3 .....S...G...S...N.....G..M..N.....R.....I..E.....
SEQ ID NO 2 .....S.....S..N.....M.....N.....T.E.....
SEQ ID NO 4 .....I.....N.....M.K.....N.....E.....
SEQ ID NO 5 .....KGE..S..IKS.H.RD.N.....I.....D.T...D..AD..RVI..EHL.K..
SEQ ID NO 6 .....KGE..S..IKS.H.RD.N.....I.....D.T...D..AD..RVI..E.L.K..

      150      160      170      180      190      200      210
SEQ ID NO 1 TKFDFFPGRGNTHSNFKWRWYHFDGVDWDQSRQLQNRIYKFRGDGKGWDWEVDTENGNYDYLMYADIDMDH
SEQ ID NO 3 .....T.....K.....T..A.....I.....
SEQ ID NO 2 .R.....S.....R.N.....H..A.....
SEQ ID NO 4 .....K.N.....--.....F.....
SEQ ID NO 5 .H.H.....S.Y.D..H.....T..E..K.-.....Q--..A.....SN.....Y..
SEQ ID NO 6 .H.H.....S.Y.D..H.....T..E..K.-.....Q--..A.....SS.....Y..

      220      230      240      250      260      270      280
SEQ ID NO 1 PEVVNELRNWGVWYTNLTGLDGFRIIDAVKHIKYSFTRDWLTHVRNTTGKNMFAVAEFWKNDIGAIENYLS
SEQ ID NO 3 ...I.....N.....Y.....P.....LA.....N
SEQ ID NO 2 .....IN...SA.....L.....Q
SEQ ID NO 4 .....IN...SA.....L.....N
SEQ ID NO 5 .D.AA.IKR..T..A.E.Q...L.....F..L..VN..EK...E..T...Y.Q..L..L...N
SEQ ID NO 6 .D..A.IKR..T..A.E.Q...L.....F..L..VN..EK...E..T...Y.Q..L..L...N

      290      300      310      320      330      340      350
SEQ ID NO 1 KTNWNHVSFVDFPLHYNLNYSRSGGNVDMRQIFNGTVVQRHPHTHAVTFVDNHDSPQEEALESFVEEWFKP
SEQ ID NO 3 ...S.....N...YF...N.L.S...K..I.....G.....QS...
SEQ ID NO 2 .....K.....N.....S.....
SEQ ID NO 4 .....K.....K..M.....
SEQ ID NO 5 ...F.....QFHA..TD..G...KLL...SK..LKS.....T..GQS...T.QT...
SEQ ID NO 6 ...F.....QFHA..TQ..G...KLL...SK..LKS.....T..GQS...T.QT...

      360      370      380      390      400      410
SEQ ID NO 1 LAYALTLRDQGYPSVFYGDYYGI---PTHGVPAMKSKIDPILEARQKYAYGKQNDYLDHNNMIGWTREG
SEQ ID NO 3 ...I..E.....---S.....L.Q...T...T.H..F..DI...
SEQ ID NO 2 ...E.....---R.....I.....
SEQ ID NO 4 ...E.....---R.....I.....
SEQ ID NO 5 ...FI...ES...Q...M..TKGDSQREI..L.H..E...K..KQ...A.H..F..DIV...
SEQ ID NO 6 ...FI...ES...Q...M..TKGDSQREI..L.H..E...K..KQ...A.H..F..DIV...

      420      430      440      450      460      470      480
SEQ ID NO 1 NTAHPNSGLATIMSDGPGCNKWMYVGRNKAGQVWRDITGNRSGTWTINADGWGNFVNGGSVSIWVNN
SEQ ID NO 3 DSS.....KH.....T.....A..V..KQ
SEQ ID NO 2 .....A..S...F.....S.....T.....K
SEQ ID NO 4 .....A...F.....T.....KA.....K
SEQ ID NO 5 DSSVA.....ALIT...A.R...QN..ET.H.....EP.V..SE...E.H.....Y.QR
SEQ ID NO 6 DSSVA.....ALIT...A.R...QN..ET.H.....EP.V..SE...E.H.....Y.QR
```

Fig. 11a

	10	20	30	40	50	60	70
SEQ ID NO 1	HHNGTNGTMMQYFEWYLPNDGNHWNRLRSDASNLKDKGITAVWIPPAWKGASQNDVGYGAYDLYDLGEFN						
SEQ-A.1	.A						
SEQ-A.2	.D						
SEQ-A.3	.E						
SEQ-A.4	.F						
SEQ-A.5	.G						
SEQ-A.6	.H						
SEQ-A.7	.I						
SEQ-A.8	.K						
SEQ-A.9	.L						
SEQ-A.10	.P						
SEQ-A.11	.R						
SEQ-A.12	.S						
SEQ-A.13	.T						
SEQ-A.14	.V						
SEQ-A.15	.W						
SEQ-A.16	.Y						

	80	90	100	110	120	130	140
SEQ ID NO 1	QKGTVRTKYGTRNQLQAQAVTALKSNQIQVYGDVVMNHKGGADATEWVRAVEVNPSNRNQEYSGDYTTIEAW						
SEQ-A.1							
SEQ-A.2							
SEQ-A.3							
SEQ-A.4							
SEQ-A.5							
SEQ-A.6							
SEQ-A.7							
SEQ-A.8							
SEQ-A.9							
SEQ-A.10							
SEQ-A.11							
SEQ-A.12							
SEQ-A.13							
SEQ-A.14							
SEQ-A.15							
SEQ-A.16							

	150	160	170	180	190	200	210
SEQ ID NO 1	TKFDFFPCRGNTHSNFKWRWYHFDQVDWDQSRQLQNRIYKFRGDGKGWDWEVDTEGNYDYLMYADIDMDH						
SEQ-A.1							
SEQ-A.2							
SEQ-A.3							
SEQ-A.4							
SEQ-A.5							
SEQ-A.6							
SEQ-A.7							
SEQ-A.8							
SEQ-A.9							
SEQ-A.10							
SEQ-A.11							
SEQ-A.12							
SEQ-A.13							
SEQ-A.14							
SEQ-A.15							
SEQ-A.16							

	220	230	240	250	260	270	280
SEQ ID NO 1	PEVVNELRNWGVWYTNLTGLDGFRIIDAVKHIKYSFTRDWLTHVRNTTGKMMFAVAEFWKNDIGAIENYLS						
SEQ-A.1							
SEQ-A.2							
SEQ-A.3							
SEQ-A.4							
SEQ-A.5							
SEQ-A.6							
SEQ-A.7							
SEQ-A.8							
SEQ-A.9							
SEQ-A.10							
SEQ-A.11							
SEQ-A.12							
SEQ-A.13							
SEQ-A.14							
SEQ-A.15							
SEQ-A.16							

Fig. 12

SEQ ID NO 1	KTNWNH	SVFDV	PLHYN	LYN	ASR	SGG	NYDM	RQIF	NGT	VVQR	HPTH	AVT	FVD	NHDS	SQPEE	ALES	FFVEE	WFKP
SEQ-A.1																		
SEQ-A.2																		
SEQ-A.3																		
SEQ-A.4																		
SEQ-A.5																		
SEQ-A.6																		
SEQ-A.7																		
SEQ-A.8																		
SEQ-A.9																		
SEQ-A.10																		
SEQ-A.11																		
SEQ-A.12																		
SEQ-A.13																		
SEQ-A.14																		
SEQ-A.15																		
SEQ-A.16																		

SEQ ID NO 1	LAYAL	TLTRD	QCYP	SVFY	GDYY	GIP	THG	VPAM	ESKID	PILEA	RQKY	AYG	KQND	YLD	HNN	MIG	WTRE	GN	T
SEQ-A.1																			
SEQ-A.2																			
SEQ-A.3																			
SEQ-A.4																			
SEQ-A.5																			
SEQ-A.6																			
SEQ-A.7																			
SEQ-A.8																			
SEQ-A.9																			
SEQ-A.10																			
SEQ-A.11																			
SEQ-A.12																			
SEQ-A.13																			
SEQ-A.14																			
SEQ-A.15																			
SEQ-A.16																			

SEQ ID NO 1	HPNS	GLAT	IMSD	GP	CG	NK	W	MY	VGR	NK	AG	QV	WR	DIT	GN	RS	CT	V	TIN	AD	G	W	GN	F	S	V	N	G	S	V	S	I	W	V	N	N
SEQ-A.1																																				
SEQ-A.2																																				
SEQ-A.3																																				
SEQ-A.4																																				
SEQ-A.5																																				
SEQ-A.6																																				
SEQ-A.7																																				
SEQ-A.8																																				
SEQ-A.9																																				
SEQ-A.10																																				
SEQ-A.11																																				
SEQ-A.12																																				
SEQ-A.13																																				
SEQ-A.14																																				
SEQ-A.15																																				
SEQ-A.16																																				

Fig. 12 (continued)

	10	20	30	40	50	60	70
SEQ ID NO 1	HHNGTNGTMMQYFEWYLPNDGNNHWNRLRSDASNLKDKGITAVWIPPAWKGASQNDVGYGAYDLYDLGEFN						
SEQ ID NO 4	S			S			
SEQ-B.1	A			S			
SEQ-B.2	D			S			
SEQ-B.3	E			S			
SEQ-B.4	F			S			
SEQ-B.5	G			S			
SEQ-B.6	H			S			
SEQ-B.7	I			S			
SEQ-B.8	K			S			
SEQ-B.9	L			S			
SEQ-B.10	P			S			
SEQ-B.11	R			S			
SEQ-B.12	S			S			
SEQ-B.13	T			S			
SEQ-B.14	V			S			
SEQ-B.15	W			S			
SEQ-B.16	Y			S			

	80	90	100	110	120	130	140
SEQ ID NO 1	QKGTVRTKYGTRNQLQAAVTALKSNGIQVYGDVVMNHKKGADATEWVRRAVEVNPNSNRNQEVSGDYTTIEAW						
SEQ ID NO 4	I	N			M	K	N
SEQ-B.1	I	N			M	K	N
SEQ-B.2	I	N			M	K	N
SEQ-B.3	I	N			M	K	N
SEQ-B.4	I	N			M	K	N
SEQ-B.5	I	N			M	K	N
SEQ-B.6	I	N			M	K	N
SEQ-B.7	I	N			M	K	N
SEQ-B.8	I	N			M	K	N
SEQ-B.9	I	N			M	K	N
SEQ-B.10	I	N			M	K	N
SEQ-B.11	I	N			M	K	N
SEQ-B.12	I	N			M	K	N
SEQ-B.13	I	N			M	K	N
SEQ-B.14	I	N			M	K	N
SEQ-B.15	I	N			M	K	N
SEQ-B.16	I	N			M	K	N

	150	160	170	180	190	200	210
SEQ ID NO 1	TKFDFFPGRGNTHSNFKWRWYHFDGVDWDQSRQLQNRIYKFRGDKGKGDWEVDTEGNGYDYLMYADIDMDH						
SEQ ID NO 4			K	N	--		F
SEQ-B.1			K	N	--		F
SEQ-B.2			K	N	--		F
SEQ-B.3			K	N	--		F
SEQ-B.4			K	N	--		F
SEQ-B.5			K	N	--		F
SEQ-B.6			K	N	--		F
SEQ-B.7			K	N	--		F
SEQ-B.8			K	N	--		F
SEQ-B.9			K	N	--		F
SEQ-B.10			K	N	--		F
SEQ-B.11			K	N	--		F
SEQ-B.12			K	N	--		F
SEQ-B.13			K	N	--		F
SEQ-B.14			K	N	--		F
SEQ-B.15			K	N	--		F
SEQ-B.16			K	N	--		F

	220	230	240	250	260	270	280
SEQ ID NO 1	PEVVNELRNWGVWYNTLTGLDGFRIDAVKHKYSFTRDWLTHVRNTTGKNNMFAVAEFAWKNDIGAIENYLS						
SEQ ID NO 4				IN	SA		L
SEQ-B.1				IN	SA		L
SEQ-B.2				IN	SA		L
SEQ-B.3				IN	SA		L
SEQ-B.4				IN	SA		L
SEQ-B.5				IN	SA		L
SEQ-B.6				IN	SA		L
SEQ-B.7				IN	SA		L
SEQ-B.8				IN	SA		L
SEQ-B.9				IN	SA		L
SEQ-B.10				IN	SA		L
SEQ-B.11				IN	SA		L
SEQ-B.12				IN	SA		L
SEQ-B.13				IN	SA		L
SEQ-B.14				IN	SA		L
SEQ-B.15				IN	SA		L
SEQ-B.16				IN	SA		L

Fig. 13

	290	300	310	320	330	340	350
SEQ ID NO 1	KTNWNHNSVFDVPLHYNLYNASRSGGNYDMRQIFNGTIVVQRHPHTHAVTFVDNNHDSQPPEEALESFVEEWFKP						
SEQ ID NO 4	K.....K.M.....						
SEQ-B.1	K.....X.....K.M.....						
SEQ-B.2	K.....X.....K.M.....						
SEQ-B.3	K.....X.....K.M.....						
SEQ-B.4	K.....X.....K.M.....						
SEQ-B.5	K.....X.....K.M.....						
SEQ-B.6	K.....X.....K.M.....						
SEQ-B.7	K.....X.....K.M.....						
SEQ-B.8	K.....X.....K.M.....						
SEQ-B.9	K.....X.....K.M.....						
SEQ-B.10	K.....X.....K.M.....						
SEQ-B.11	K.....X.....K.M.....						
SEQ-B.12	K.....X.....K.M.....						
SEQ-B.13	K.....X.....K.M.....						
SEQ-B.14	K.....X.....K.M.....						
SEQ-B.15	K.....X.....K.M.....						
SEQ-B.16	K.....X.....K.M.....						

	360	370	380	390	400	410	420
SEQ ID NO 1	LAYALTLTRDQGYPSVIFYGDIYGIPTHCVPAMKSKIDPILEARQKYAYGKQNDYLDHHNMIGWTREGNTA						
SEQ ID NO 4	E.....R.....I.....						
SEQ-B.1	E.....R.....I.....						
SEQ-B.2	E.....R.....I.....						
SEQ-B.3	E.....R.....I.....						
SEQ-B.4	E.....R.....I.....						
SEQ-B.5	E.....R.....I.....						
SEQ-B.6	E.....R.....I.....						
SEQ-B.7	E.....R.....I.....						
SEQ-B.8	E.....R.....I.....						
SEQ-B.9	E.....R.....I.....						
SEQ-B.10	E.....R.....I.....						
SEQ-B.11	E.....R.....I.....						
SEQ-B.12	E.....R.....I.....						
SEQ-B.13	E.....R.....I.....						
SEQ-B.14	E.....R.....I.....						
SEQ-B.15	E.....R.....I.....						
SEQ-B.16	E.....R.....I.....						

	430	440	450	460	470	480
SEQ ID NO 1	HPNSGLATIMSDGPGGNKWMYVGRNKACQVWRDITGNRSGTGTINADGWGNFVSNGGCVSIWVNN					
SEQ ID NO 4	A.....F.....T.....KA.....K					
SEQ-B.1	A.....F.....T.....KA.....K					
SEQ-B.2	A.....F.....T.....KA.....K					
SEQ-B.3	A.....F.....T.....KA.....K					
SEQ-B.4	A.....F.....T.....KA.....K					
SEQ-B.5	A.....F.....T.....KA.....K					
SEQ-B.6	A.....F.....T.....KA.....K					
SEQ-B.7	A.....F.....T.....KA.....K					
SEQ-B.8	A.....F.....T.....KA.....K					
SEQ-B.9	A.....F.....T.....KA.....K					
SEQ-B.10	A.....F.....T.....KA.....K					
SEQ-B.11	A.....F.....T.....KA.....K					
SEQ-B.12	A.....F.....T.....KA.....K					
SEQ-B.13	A.....F.....T.....KA.....K					
SEQ-B.14	A.....F.....T.....KA.....K					
SEQ-B.15	A.....F.....T.....KA.....K					
SEQ-B.16	A.....F.....T.....KA.....K					

Fig. 13 (continued)

	10	20	30	40	50	60	70
SEQ ID NO 1	HHNGTNGCTMMQYFEWYLPNDGNHWNRLRSDASNLKDKGITAVWIPPAWKASQNDVGYGAYDLYDLGEFN						
SEQ ID NO 4				S			
SEQ-C.1	A			S			
SEQ-C.2	D			S			
SEQ-C.3	E			S			
SEQ-C.4	F			S			
SEQ-C.5	G			S			
SEQ-C.6	H			S			
SEQ-C.7	I			S			
SEQ-C.8	K			S			
SEQ-C.9	L			S			
SEQ-C.10	P			S			
SEQ-C.11	R			S			
SEQ-C.12	S			S			
SEQ-C.13	T			S			
SEQ-C.14	V			S			
SEQ-C.15	W			S			
SEQ-C.16	Y			S			

	80	90	100	110	120	130	140
SEQ ID NO 1	QKGTVRTKYGTRNQLQAAVTALKSNGIQVYGDVVMNHKGGADATEWVRAVEVNPNSNRNQEVSGDYTTIEAW						
SEQ ID NO 4	I	N		M	K	N	E
SEQ-C.1	I	N		M	K	N	E
SEQ-C.2	I	N		M	K	N	E
SEQ-C.3	I	N		M	K	N	E
SEQ-C.4	I	N		M	K	N	E
SEQ-C.5	I	N		M	K	N	E
SEQ-C.6	I	N		M	K	N	E
SEQ-C.7	I	N		M	K	N	E
SEQ-C.8	I	N		M	K	N	E
SEQ-C.9	I	N		M	K	N	E
SEQ-C.10	I	N		M	K	N	E
SEQ-C.11	I	N		M	K	N	E
SEQ-C.12	I	N		M	K	N	E
SEQ-C.13	I	N		M	K	N	E
SEQ-C.14	I	N		M	K	N	E
SEQ-C.15	I	N		M	K	N	E
SEQ-C.16	I	N		M	K	N	E

	150	160	170	180	190	200	210
SEQ ID NO 1	TKFDFFPGRGNTHSNFKWRWYHFDGVDWDQSRQLQNRIYKFRGDGKGWDWEVDTEGNYDYLMYADIDMDH						
SEQ ID NO 4			K	N	--	F	
SEQ-C.1			K	N	--	F	
SEQ-C.2			K	N	--	F	
SEQ-C.3			K	N	--	F	
SEQ-C.4			K	N	--	F	
SEQ-C.5			K	N	--	F	
SEQ-C.6			K	N	--	F	
SEQ-C.7			K	N	--	F	
SEQ-C.8			K	N	--	F	
SEQ-C.9			K	N	--	F	
SEQ-C.10			K	N	--	F	
SEQ-C.11			K	N	--	F	
SEQ-C.12			K	N	--	F	
SEQ-C.13			K	N	--	F	
SEQ-C.14			K	N	--	F	
SEQ-C.15			K	N	--	F	
SEQ-C.16			K	N	--	F	

	220	230	240	250	260	270	280
SEQ ID NO 1	PEVYNELRNWGVWYNTNTLGLDGFRIIDAVKHIIKYSFTRDWLTHVRNTTGKNMFAVAEFWKNDIGAIENYLS						
SEQ ID NO 4				I	N	S	A
SEQ-C.1				I	N	S	A
SEQ-C.2				I	N	S	A
SEQ-C.3				I	N	S	A
SEQ-C.4				I	N	S	A
SEQ-C.5				I	N	S	A
SEQ-C.6				I	N	S	A
SEQ-C.7				I	N	S	A
SEQ-C.8				I	N	S	A
SEQ-C.9				I	N	S	A
SEQ-C.10				I	N	S	A
SEQ-C.11				I	N	S	A
SEQ-C.12				I	N	S	A
SEQ-C.13				I	N	S	A
SEQ-C.14				I	N	S	A
SEQ-C.15				I	N	S	A
SEQ-C.16				I	N	S	A

Fig. 14

	290	300	310	320	330	340	350
SEQ ID NO 1	KTNWNH	SVFDVPLHYN	LYNASRSGGNYDMRQIFNGTVVQRHPTHAVTFVDN	HDSQP	EEALESF	VEEWF	KP
SEQ ID NO 4		K.....		K.M.....			
SEQ-C.1		K.....		K.M.....			
SEQ-C.2		K.....		K.M.....			
SEQ-C.3		K.....		K.M.....			
SEQ-C.4		K.....		K.M.....			
SEQ-C.5		K.....		K.M.....			
SEQ-C.6		K.....		K.M.....			
SEQ-C.7		K.....		K.M.....			
SEQ-C.8		K.....		K.M.....			
SEQ-C.9		K.....		K.M.....			
SEQ-C.10		K.....		K.M.....			
SEQ-C.11		K.....		K.M.....			
SEQ-C.12		K.....		K.M.....			
SEQ-C.13		K.....		K.M.....			
SEQ-C.14		K.....		K.M.....			
SEQ-C.15		K.....		K.M.....			
SEQ-C.16		K.....		K.M.....			

	360	370	380	390	400	410	420
SEQ ID NO 1	LAYALT	LTLDQGYPSV	FYGYGDIPTHGVPAMKSKIDPILEARQKYAYGKQNDYLDH	HN	MIGW	TREG	NTA
SEQ ID NO 4	E.....			R.....	I.....		
SEQ-C.1	E.....			R.....	I.....		
SEQ-C.2	E.....			R.....	I.....		
SEQ-C.3	E.....			R.....	I.....		
SEQ-C.4	E.....			R.....	I.....		
SEQ-C.5	E.....			R.....	I.....		
SEQ-C.6	E.....			R.....	I.....		
SEQ-C.7	E.....			R.....	I.....		
SEQ-C.8	E.....			R.....	I.....		
SEQ-C.9	E.....			R.....	I.....		
SEQ-C.10	E.....			R.....	I.....		
SEQ-C.11	E.....			R.....	I.....		
SEQ-C.12	E.....			R.....	I.....		
SEQ-C.13	E.....			R.....	I.....		
SEQ-C.14	E.....			R.....	I.....		
SEQ-C.15	E.....			R.....	I.....		
SEQ-C.16	E.....			R.....	I.....		

	430	440	450	460	470	480
SEQ ID NO 1	HPNSCLATIMSDGPGCNKWMYVGRNKAGQVWRDITGNRSGTVTINADGWGNF	SVN	GGSV	SIW	VNN	
SEQ ID NO 4	A.....F.....T.....KA.....	X.....	K.....			
SEQ-C.1	A.....F.....T.....KA.....	X.....	K.....			
SEQ-C.2	A.....F.....T.....KA.....	X.....	K.....			
SEQ-C.3	A.....F.....T.....KA.....	X.....	K.....			
SEQ-C.4	A.....F.....T.....KA.....	X.....	K.....			
SEQ-C.5	A.....F.....T.....KA.....	X.....	K.....			
SEQ-C.6	A.....F.....T.....KA.....	X.....	K.....			
SEQ-C.7	A.....F.....T.....KA.....	X.....	K.....			
SEQ-C.8	A.....F.....T.....KA.....	X.....	K.....			
SEQ-C.9	A.....F.....T.....KA.....	X.....	K.....			
SEQ-C.10	A.....F.....T.....KA.....	X.....	K.....			
SEQ-C.11	A.....F.....T.....KA.....	X.....	K.....			
SEQ-C.12	A.....F.....T.....KA.....	X.....	K.....			
SEQ-C.13	A.....F.....T.....KA.....	X.....	K.....			
SEQ-C.14	A.....F.....T.....KA.....	X.....	K.....			
SEQ-C.15	A.....F.....T.....KA.....	X.....	K.....			
SEQ-C.16	A.....F.....T.....KA.....	X.....	K.....			

Fig: 14 (continued)

	10	20	30	40	50	60	70
SEQ ID NO 1	HHNGTNGTMMQYFEWYLPNDGNHWNRLRSDASNLKDKGITAVWIPPAWKGASQNDVGYGAYDLYDLGEFN						
SEQ ID NO 5	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.1	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.2	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.3	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.4	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.5	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.6	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.7	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.8	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.9	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.10	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.11	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.12	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.13	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.14	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.15	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.16	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.17	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.18	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.19	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.20	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.21	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.22	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.23	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.24	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.25	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.26	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.27	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.28	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.29	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.30	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.31	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.32	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						
SEQ-D.33	--ANL...L...M...Q...R...QN.SAY.AEH...Y...T...A...H						

	90	100	110	120	130	140
SEQ ID NO 1	QKGTVRTKYGTRNQLQAAVTALKSNGIQVYGDVVMNHKGGADATEWVRRAVEVNPSPNRNQEVSGDYTTIEAW					
SEQ ID NO 5	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.1	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.2	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.3	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.4	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.5	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.6	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.7	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.8	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.9	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.10	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.11	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.12	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.13	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.14	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.15	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.16	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.17	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.18	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.19	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.20	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.21	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.22	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.23	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.24	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.25	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.26	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.27	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.28	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.29	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.30	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.31	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.32	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					
SEQ-D.33	KGE...S.IKS.H.RD.N...I...D.T...D.AD.RVI.EHL.K..					

Fig. 15

	220	230	240	250	260	270	280
SEQ ID NO 1	PEVVNELRNWGVWYNTLGLDGRIDAVKHIKYSFTROWLTHVRNTTGKMMFAVAEFWKNDIGAIENYLS						
SEQ ID NO 5	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.1	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.2	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.3	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.4	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.5	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.6	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.7	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.8	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.9	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.10	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.11	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.12	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.13	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.14	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.15	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.16	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.17	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.18	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.19	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.20	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.21	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.22	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.23	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.24	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.25	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.26	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.27	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.28	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.29	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.30	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.31	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.32	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						
SEQ-D.33	D.AA.IKR.T.A.E.Q.L.F.L.VN.EK.E.T.Y.Q.L.L.L.N						

Fig. 15 (continued)

	290	300	310	320	330	340	350
SEQ ID NO 1	KTNWNH	SVFDVPLHYNLYNAS	RS	CGNYDMRQIFNGT	VVQRHPT	HA	VTFFVDNHDSQP
SEQ ID NO 5	F	QFHA	TQ	G	KLL	SK	LKS
SEQ-D.1	F	QFHA	TQ	G	KLL	SK	LKS
SEQ-D.2	F	QFHA	TQ	G	KLL	SK	LKS
SEQ-D.3	F	QFHA	TQ	G	KLL	SK	LKS
SEQ-D.4	F	QFHA	TQ	G	KLL	SK	LKS
SEQ-D.5	F	QFHA	TQ	G	KLL	SK	LKS
SEQ-D.6	F	QFHA	TQ	G	KLL	SK	LKS
SEQ-D.7	F	QFHA	TQ	G	KLL	SK	LKS
SEQ-D.8	F	QFHA	TQ	G	KLL	SK	LKS
SEQ-D.9	F	QFHA	TQ	G	KLL	SK	LKS
SEQ-D.10	F	QFHA	TQ	G	KLL	SK	LKS
SEQ-D.11	F	QFHA	TQ	G	KLL	SK	LKS
SEQ-D.12	F	QFHA	TQ	G	KLL	SK	LKS
SEQ-D.13	F	QFHA	TQ	G	KLL	SK	LKS
SEQ-D.14	F	QFHA	TQ	G	KLL	SK	LKS
SEQ-D.15	F	QFHA	TQ	G	KLL	SK	LKS
SEQ-D.16	F	QFHA	TQ	G	KLL	SK	LKS
SEQ-D.17	F	QFHA	TQ	G	KLLA	SK	LKS
SEQ-D.18	F	QFHA	TQ	G	KLLD	SK	LKS
SEQ-D.19	F	QFHA	TQ	G	KLLE	SK	LKS
SEQ-D.20	F	QFHA	TQ	G	KLLF	SK	LKS
SEQ-D.21	F	QFHA	TQ	G	KLLG	SK	LKS
SEQ-D.22	F	QFHA	TQ	G	KLLH	SK	LKS
SEQ-D.23	F	QFHA	TQ	G	KLLI	SK	LKS
SEQ-D.24	F	QFHA	TQ	G	KLLK	SK	LKS
SEQ-D.25	F	QFHA	TQ	G	KLLL	SK	LKS
SEQ-D.26	F	QFHA	TQ	G	KLLP	SK	LKS
SEQ-D.27	F	QFHA	TQ	G	KLLR	SK	LKS
SEQ-D.28	F	QFHA	TQ	G	KLLS	SK	LKS
SEQ-D.29	F	QFHA	TQ	G	KLLT	SK	LKS
SEQ-D.30	F	QFHA	TQ	G	KLLV	SK	LKS
SEQ-D.31	F	QFHA	TQ	G	KLLW	SK	LKS
SEQ-D.32	F	QFHA	TQ	G	KLLY	SK	LKS
SEQ-D.33	F	QFHA	TQ	G	KLLX	SK	LKS

	360	370	380	390	400	410
SEQ ID NO 1	LAYALT	LRDQGYPSV	FYCDYYGI	---	PTHGVP	AMKSKIDP
SEQ ID NO 5	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.1	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.2	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.3	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.4	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.5	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.6	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.7	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.8	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.9	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.10	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.11	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.12	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.13	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.14	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.15	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.16	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.17	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.18	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.19	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.20	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.21	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.22	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.23	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.24	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.25	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.26	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.27	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.28	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.29	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.30	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.31	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.32	FI	ES	Q	M	TKGDSQREI	L.H.E
SEQ-D.33	FI	ES	Q	M	TKGDSQREI	L.H.E

Fig. 15 (continued)

	420	430	440	450	460	470	480
SEQ ID NO 1	NTAHPNSGLATIMSDGPGGNKWMYVGRNKAGQVWRDITGNRSCTVTINADGWGNFSVNGGSVSIWVNN						
SEQ ID NO 5	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.....Y.QR
SEQ-D.1	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.A.....Y.QR
SEQ-D.2	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.D.....Y.QR
SEQ-D.3	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.E.....Y.QR
SEQ-D.4	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.F.....Y.QR
SEQ-D.5	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.G.....Y.QR
SEQ-D.6	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.H.....Y.QR
SEQ-D.7	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.I.....Y.QR
SEQ-D.8	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.K.....Y.QR
SEQ-D.9	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.L.....Y.QR
SEQ-D.10	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.P.....Y.QR
SEQ-D.11	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.R.....Y.QR
SEQ-D.12	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.S.....Y.QR
SEQ-D.13	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.T.....Y.QR
SEQ-D.14	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.V.....Y.QR
SEQ-D.15	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.W.....Y.QR
SEQ-D.16	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.Y.....Y.QR
SEQ-D.17	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.....Y.QR
SEQ-D.18	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.....Y.QR
SEQ-D.19	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.....Y.QR
SEQ-D.20	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.....Y.QR
SEQ-D.21	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.....Y.QR
SEQ-D.22	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.....Y.QR
SEQ-D.23	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.....Y.QR
SEQ-D.24	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.....Y.QR
SEQ-D.25	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.....Y.QR
SEQ-D.26	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.....Y.QR
SEQ-D.27	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.....Y.QR
SEQ-D.28	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.....Y.QR
SEQ-D.29	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.....Y.QR
SEQ-D.30	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.....Y.QR
SEQ-D.31	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.....Y.QR
SEQ-D.32	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.....Y.QR
SEQ-D.33	DSSVA	ALIT	A.R	QN	ET.H	EP.V	SE..E.H.X.....Y.QR

Fig. 15 (continued)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/058126

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N9/26 C12N15/52
ADD. C11D3/386

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

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, Sequence Search, WPI Data, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Further documents are listed in the continuation of Box C.



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Date of the actual completion of the international search

1 July 2015

Date of mailing of the international search report

20/07/2015

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/058126

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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International application No

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