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(54) Title: AUTOMATIC DISHWASHING DETERGENT COMPOSITION

(57) Abstract: An automatic dishwashing cleaning composition having a new protease.

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AUTOMATIC DISHWASHING DETERGENT COMPOSITION

FIELD OF THE INVENTION

The present invention is in the field of detergents. In particular, it relates to an automatic dishwashing detergent comprising a specific protease. The composition provides improved removal of proteinaceous soils versus compositions comprising conventional proteases.

BACKGROUND OF INVENTION

There is a permanent desire to improve the performance of automatic dishwashing compositions and their environmental profile.

Due to environmental concerns phosphate is increasingly being replaced by biodegradable complexing agents. These complexing agents can have a strong binding capacity for metals and/or are used in high levels and can negatively affect the stability of enzymes, in particular complexing agents can negatively affect proteases by extracting the structural calcium metal ions of the protease. The proteases can be affected in product and/or in-use. While compositions having a high level of bleach can provide good cleaning the bleach can also impair on the performance of enzymes, specifically proteases. This effect can be exacerbated by high level of complexing agents, high temperature and long cycles.

Automatic dishwashing compositions can be designed to have optimum performance under certain in-use conditions, for example a composition can be designed to have optimum performance in a soft water cycle, however a composition that has optimum performance in soft water might not have optimum performance in a hard water cycle and vice versa.

The object of the present invention is to provide a dishwashing composition that provides better removal of proteinaceous soils. Preferably, the removal should be good when the composition is used in soft water and preferably under different water hardness conditions. It is also desirable that the composition has improved stability and provides improved performance even under stressed conditions such as heavily soiled load washed in hot, long cycles.

SUMMARY OF THE INVENTION

According to the first aspect of the present invention, there is provided an automatic dishwashing detergent composition comprising a specific protease. The composition is preferably a phosphate-free automatic dishwashing cleaning composition. More preferably, the composition comprises a complexing agent system, and more preferably the composition comprises a complexing agent system and high level of a bleaching system. The composition presents

improved stability and/or performance on egg and/or crème brulee removal. The composition of the invention can be suitable for soft water and/or high temperatures and/or long cycles are used in automatic dishwashing.

According to the second aspect of the invention there is provided a method of automatic dishwashing using the composition of the invention. There is also provided the use of the composition of the invention to provide crème brulee removal in automatic dishwashing.

The elements of the composition of the invention described in connexion with the first aspect of the invention apply *mutatis mutandis* to the other aspects of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention encompasses an automatic dishwashing cleaning composition comprising a specific protease. The composition is preferably phosphate-free and preferably comprises a complexing agent system. The composition has improved stability and delivers improved cleaning versus cleaning compositions comprising conventional proteases under a plurality of conditions. The composition provides good proteinaceous cleaning, in particular on egg and/or crème brulee soils. The invention also encompasses methods of automatic dishwashing. The composition of the invention can provide good cleaning in hot, long cycles and when using soft water.

By "soft" water is herein meant water having a hardness of less than about 2 gpg (34.3 ppm). Grain per gallon (gpg) is a unit of water hardness defined as 1 grain (64.8 milligrams) of calcium carbonate dissolved in 1 US gallon of water (3.785412 L). It translates into 17.1 parts per million (ppm).

By "hot" cycle is herein understood a dishwashing program in which the main cycle is performed at a temperature above 50°C, preferably above 55°C.

By "long" cycle is herein understood a dishwashing program in which the main cycle has a duration of at least 25, preferably at least 30 and more preferably at least 35 minutes.

The composition of the invention comprises a variant protease, the variant proteases have a defined percentage of identity with respect to a reference protease (protease of SEQ ID NO: 1).

The protease of the composition of the invention is herein sometimes referred to as "the protease of the invention". The protease having sequence ID NO:1 is herein sometimes referred to as "the reference protease" or "the parent protease".

The relatedness between two amino acid sequences or between two nucleotide sequences is described by the parameter "sequence identity".

The term "variant" means a protease comprising a mutation, i.e., a substitution, insertion, and/or deletion, at one or more (e.g., several) positions relative to the reference protease. A substitution means replacement of the amino acid occupying a position with a different amino acid; a deletion means removal of the amino acid occupying a position; and an insertion means adding an amino acid adjacent to and immediately following the amino acid occupying a position. The variants of the present invention have at least 90%, preferably at least 92% more preferably a least 95% and especially 99% identity with the reference protease.

SEQ ID NO: 1 corresponds to B. *gibsonii* subtilisin Bgi02446 with S039E substitution

The term "wild-type" protease means a protease expressed by a naturally occurring microorganism, such as a bacterium, yeast, or filamentous fungus found in nature.

The present invention provides compositions comprising a variant comprising an amino acid sequence having a glutamate at a position corresponding to position 39 of SEQ ID NO:1 and further comprising one or more additional amino acid substitutions. The variants provided herein demonstrate one or more improved properties, such as an improved cleaning performance, or improved stability, or both an improved cleaning performance and an improved stability when compared to a composition comprising a protease having the amino acid sequence of SEQ ID NO: 1.

Enzyme related terminology

Nomenclature for amino acid modifications

In describing enzyme variants herein, the following nomenclature is used for ease of reference: Original amino acid(s):position (s):substituted amino acid(s).

According to this nomenclature, for instance the substitution of glutamic acid for glycine in position 195 is shown as G195E. A deletion of glycine in the same position is shown as G195*, and insertion of an additional amino acid residue such as lysine is shown as G195GK. Where a specific enzyme contains a "deletion" in comparison with other enzyme and an insertion is made in such a position this is indicated as *36D for insertion of an aspartic acid in position 36. Multiple mutations are separated by pluses, i.e.: S99G+V102N, representing mutations in positions 99 and 102 substituting serine and valine for glycine and asparagine, respectively. Where the amino acid in a position (*e.g.* 102) may be substituted by another amino acid selected from a group of amino acids, e.g. the group consisting of N and I, this will be indicated by V102N, I.

In all cases, the accepted IUPAC single letter or triple letter amino acid abbreviation is employed.

Protease Amino Acid Numbering

The numbering used in this patent is versus SEQ ID NO:1.

Amino acid identity

The relatedness between two amino acid sequences is described by the parameter "identity". For purposes of the present invention, the alignment of two amino acid sequences is determined by using the Needle program from the EMBOSS package (http://emboss.org) version 2.8.0. The Needle program implements the global alignment algorithm described in Needleman, S. B. and Wunsch, C. D. (1970) J. Mol. Biol. 48, 443-453. The substitution matrix used is BLOSUM62, gap opening penalty is 10, and gap extension penalty is 0.5.

The degree of identity between an amino acid sequence of an enzyme used herein ("invention sequence") and a different amino acid sequence ("foreign sequence") is calculated as the number of exact matches in an alignment of the two sequences, divided by the length of the "invention sequence" or the length of the "foreign sequence", whichever is the shortest. The result is expressed in percent identity. An exact match occurs when the "invention sequence" and the "foreign sequence" have identical amino acid residues in the same positions of the overlap. The length of a sequence is the number of amino acid residues in the sequence.

The term "succinate based compound" and "succinic acid based compound" are used interchangeably herein.

As used herein, articles such as "a" and "an" when used in a claim, are understood to mean one or more of what is claimed or described.

Unless otherwise noted, all component or composition levels are in reference to the active portion of that component or composition, and are exclusive of impurities, for example, residual solvents or by-products, which may be present in commercially available sources of such components or compositions.

All percentages and ratios are calculated by weight unless otherwise indicated. All percentages and ratios are calculated based on the total composition unless otherwise indicated.

The protease of the invention

The variant of the present invention has at least 90%, more preferably at least 92% more preferably at least 95% and specially at least 99% identity with the protease of SEQ ID NO: 1.

The variant has a glutamate (E) residue at position 39 and further comprising one or more amino acid substitutions at one or more positions selected from:

(i) 3V, 4T, 8V, 9A/C/E/G/H/K/M/N/Q/W/Y, 10A/K/M/N/Q/W, 11A/I/S/T, 12A/C/D/G/M/N/R/S/T/V/W, 14D, 15D/E/F/H/I/K/M/P/Q/V/W/Y, 16L/M/S, 17C/E/F/G/I/L/N/V/W/Y, 18A/C/D/E/F/G/L/M/Q/T, 19A/C/D/E/F/H/I/K/L/N/Q/S/T/W/Y, 20A/C/D/M/N/T, 24A/E/, 25A/C/D/E/M/N, 26A/I, 33T, 36C/E/I/L/M/Q/T/V, 42C/D/E/M/Q, 43L, 44C/E/F/G/H/I/K/L/N/Q/T/V/W/Y, 47I/Y, 50I, 52A/C/D/H/L/M/N/S/T/Y, 54A/C/G/L/M/N/T/V, 55A/C/D/E/H/N/S/Y, 57D/E/H/M/N/Q/T, 59A/C/D/E/M/N/Q/T, 60S, 69S, 76A/D/E/F/H/K/L/M/N/R/T/Y, 82A, 84D/F/H/Y, 95A/N, 96M/Q, 97E/H/K, 101T, 102L/M, 104A/D/H/M/N/T/V/W/Y, 105V, 107K/M, 110L, 113T/V, 114V, 115E/H/Q, 116E/H, 118D/E/N, 120V, 128G, 129A/H/N/Y, 131A/D/E/I/M/N/P/Q/V, 133M, 135A/E/F/H/I/K/L/M/Q/S/T/V/W/Y, 136M, 137L, 139E/S, 141E/H/N, 142A/D/E/H/M/N/Q, 143E/H/M/N/V, 144E/N, 145C, 147C, 148L/V, 150M, 156C/D/N/T, 157A/C/D/E/N/Q, 158A/C/F/L/M/N/Q/V/W/Y, 159L, 160A/C/D/M/T, 161W, 164A/K/M/Q/Y, 166D/E/I/P/Q/V, 167E, 170G, 174V, 176A/C/D/L/M/N/S, 177A/C/D/E/G/H/K/L/M/Q/S/W/Y, 178D, 179A/C/E/F/G/H/I/K/M/Q/S/V/W/Y, 180K, 182A/C/D/E/G/H/I/K/L/P/Q/T/V/W/Y, 186F, 188C/D/E/I/L/M/N/Q/S/V/W/Y, 189C/D/E, 190M, 191E, 192C/M, 193A/M, 198D/E, 200H/I/K/M/V/Y, 207K/L/N/Q, 209P, 210C/D/E/F/G/L/N/P/Q/Y, 211E/L/Q/R, 212A/C/Q, 218C/S, 227M/Q, 228L, 230A/D/L/M/N, 231C/E/H/I/L/N/Q/S/T, 232F/H/Q/R/W, 234A/D/E/M/T/W/Y, 236G/S/T, 238A/D/E/M/V, 239D/E/L/M/N/T, 242A, 245E, 246A/L, 247E/Q, 249C/D/E/F/I/L/S/Y, 250S/T, 253E, 254P/Y, 255A/C/D/E/F/I/M/V/W, 256C/F/H/M/W/Y, 257C/M, 259D/E/M/N, 262L, 263D/Q, 264T, 265A/M/N/Q, 266L/M/N/Q/R, 268A/C/D/E, and 269H/P/W;

wherein the amino acid positions of the variant are numbered by correspondence with the amino acid sequence of SEQ ID NO: 1.

The variant has at least 90% identity with the amino acid sequence of SEQ ID NO:1. The variant comprises at least two, more preferably at least three, more preferably at least four amino acid substitutions (using the SEQ ID NO:1 numbering) selected from the group consisting of:

(ii) 3V, 4T, 8V, 9A/C/E/G/H/K/M/N/Q/W/Y, 10A/K/M/N/Q/W, 11A/I/S/T, 12A/C/D/G/M/N/R/S/T/V/W, 14D, 15D/E/F/H/I/K/M/P/Q/V/W/Y, 16L/M/S, 17C/E/F/G/I/L/N/V/W/Y, 18A/C/D/E/F/G/L/M/Q/T, 19A/C/D/E/F/H/I/K/L/N/Q/S/T/W/Y, 20A/C/D/M/N/T, 24A/E/, 25A/C/D/E/M/N, 26A/I, 33T, 36C/E/I/L/M/Q/T/V, 42C/D/E/M/Q, 43L, 44C/E/F/G/H/I/K/L/N/Q/T/V/W/Y, 47I/Y, 50I, 52A/C/D/H/L/M/N/S/T/Y, 54A/C/G/L/M/N/T/V, 55A/C/D/E/H/N/S/Y, 57D/E/H/M/N/Q/T, 59A/C/D/E/M/N/Q/T, 60S, 69S, 76A/D/E/F/H/K/L/M/N/R/T/Y, 82A, 84D/F/H/Y, 95A/N, 96M/Q, 97E/H/K, 101T, 102L/M, 104A/D/H/M/N/T/V/W/Y, 105V, 107K/M, 110L, 113T/V, 114V, 115E/H/Q, 116E/H, 118D/E/N, 120V, 128G, 129A/H/N/Y, 131A/D/E/I/M/N/P/Q/V, 133M, 135A/E/F/H/I/K/L/M/Q/S/T/V/W/Y, 136M, 137L, 139E/S, 141E/H/N, 142A/D/E/H/M/N/Q, 143E/H/M/N/V, 144E/N, 145C, 147C, 148L/V, 150M, 156C/D/N/T, 157A/C/D/E/N/Q, 158A/C/F/L/M/N/Q/V/W/Y, 159L, 160A/C/D/M/T, 161W, 164A/K/M/Q/Y, 166D/E/I/P/Q/V, 167E, 170G, 174V, 176A/C/D/L/M/N/S, 177A/C/D/E/G/H/K/L/M/Q/S/W/Y, 178D, 179A/C/E/F/G/H/I/K/M/Q/S/V/W/Y, 180K, 182A/C/D/E/G/H/I/K/L/P/Q/T/V/W/Y, 186F, 188C/D/E/I/L/M/N/Q/S/V/W/Y, 189C/D/E, 190M, 191E, 192C/M, 193A/M, 198D/E, 200H/I/K/M/V/Y, 207K/L/N/Q, 209P, 210C/D/E/F/G/L/N/P/Q/Y, 211E/L/Q/R, 212A/C/Q, 218C/S, 227M/Q, 228L, 230A/D/L/M/N, 231C/E/H/I/L/N/Q/S/T, 232F/H/Q/R/W, 234A/D/E/M/T/W/Y, 236G/S/T, 238A/D/E/M/V, 239D/E/L/M/N/T, 242A, 245E, 246A/L, 247E/Q, 249C/D/E/F/I/L/S/Y, 250S/T, 253E, 254P/Y, 255A/C/D/E/F/I/M/V/W, 256C/F/H/M/W/Y, 257C/M, 259D/E/M/N, 262L, 263D/Q, 264T, 265A/M/N/Q, 266L/M/N/Q/R, 268A/C/D/E, and 269H/P/W;

wherein the amino acid positions of the variant are numbered by correspondence with the amino acid sequence of SEQ ID NO: 1.

The compositions are very good for the removal of egg, crème brulee and/or present good stability.

Especially preferred compositions, in terms of crème brulee removal, comprise a protease wherein the protease is a variant having at least 90% identity with the amino acid sequence of SEQ ID NO:1 and the variant comprises one or more amino acid substitutions at one or more positions selected from: 3V; 9A/C/E/K; 10A/M/N/Q; 11A/I; 12C/D; 14D; 15D/E/H/I/M/V/Y; 16M; 17C/F/I/L/W; 18D/E; 19A/C/D/E/H/I/L/Q/S/T/W; 24A/E;

36C/E; 42C/D/E; 44C/E/W/Y; 52A/C/D/H; 54L/M;55A/D/H/S; 57D/E/; 59A/C/D/E/N; 60S; 76E/H/K/L/M/N/T; 84H/Y;; 95N; 96Q; 97E; 104A/D; 107K; 110L; 116E; 129H/N/Y; 131D/E; 135A/E/H/I/L/M/S/T/V/W/Y; 136M; 141E; 142E; 144E; 156C/D; 157A/C/D/E; 158A/C; 160A/M; 164A/M/Q/Y; 166D/E; 176C/D; 177C/D/M/S/Y; 178D; 179A/C/E/F/G/H/I/K/M/Q/S/V/W/Y; 182D/E; 188C/D/E/M; 189C/D/E; 193A/M; 198D/E; 200I/Y; 207K/L/Q; 209P; 210D/E/N; 238A/D/E/M; 239D/E; 241C/G/L/Q/T/Y; 245E; 247E/ 249C/D/E/Y; 253E; 255C/D/E; 256C/Y; 259D/E; 262L; 268D/E; and 269H/W.

The variant has at least 90% identity with the amino acid sequence of SEQ ID NO:1 and preferably at least three substitutions.

Especially preferred variants for use in the composition of the invention are selected from the group consisting of variants having at least 90%, more preferably at least 92%, more preferably at least 95% and specially at least 99% identity with the amino acid sequence SEQ ID NO:1.

In one embodiment, the composition provide herein comprises a protease wherein the protease is a variant having at least 90% identity with the amino acid sequence of SEQ ID NO:1 and having a glutamate residue (E) at a position corresponding to position 39 of SEQ ID NO:1 and further comprises one or more amino acid substitutions at one or more positions selected from

(iii) 3V, 4T, 8V, 9A/C/E/G/H/K/M/N/Q/W/Y, 10A/K/M/N/Q/W, 11A/I/S/T, 12A/C/D/G/M/N/R/S/T/V/W, 14D, 15D/E/F/H/I/K/M/P/Q/V/W/Y, 16L/M/S, 17C/E/F/G/I/L/N/V/W/Y, 18A/C/D/E/F/G/L/M/Q/T, 19A/C/D/E/F/H/I/K/L/N/Q/S/T/W/Y, 20A/C/D/M/N/T, 24A/E/, 25A/C/D/E/M/N, 26A/I, 33T, 36C/E/I/L/M/Q/T/V, 42C/D/E/M/Q, 43L, 44C/E/F/G/H/I/K/L/N/Q/T/V/W/Y, 47I/Y, 50I, 52A/C/D/H/L/M/N/S/T/Y, 54A/C/G/L/M/N/T/V, 55A/C/D/E/H/N/S/Y, 57D/E/H/M/N/Q/T, 59A/C/D/E/M/N/Q/T, 60S, 69S, 76A/D/E/F/H/K/L/M/N/R/T/Y, 82A, 84D/F/H/Y, 95A/N, 96M/Q, 97E/H/K, 101T, 102L/M, 104A/D/H/M/N/T/V/W/Y, 105V, 107K/M, 110L, 113T/V, 114V, 115E/H/Q, 116E/H, 118D/E/N, 120V, 128G, 129A/H/N/Y, 131A/D/E/I/M/N/P/Q/V, 133M, 135A/E/F/H/I/K/L/M/Q/S/T/V/W/Y, 136M, 137L, 139E/S, 141E/H/N, 142A/D/E/H/M/N/Q, 143E/H/M/N/V, 144E/N, 145C, 147C, 148L/V, 150M, 156C/D/N/T, 157A/C/D/E/N/Q, 158A/C/F/L/M/N/Q/V/W/Y, 159L, 160A/C/D/M/T, 161W, 164A/K/M/Q/Y, 166D/E/I/P/Q/V, 167E, 170G, 174V, 176A/C/D/L/M/N/S, 177A/C/D/E/G/H/K/L/M/Q/S/W/Y, 178D,

179A/C/E/F/G/H/I/K/M/Q/S/V/W/Y, 180K,
182A/C/D/E/G/H/I/K/L/P/Q/T/V/W/Y, 186F, 188C/D/E/I/L/M/N/Q/S/V/W/Y,
189C/D/E, 190M, 191E, 192C/M, 193A/M, 198D/E, 200H/I/K/M/V/Y,
207K/L/N/Q, 209P, 210C/D/E/F/G/L/N/P/Q/Y, 211E/L/Q/R, 212A/C/Q, 218C/S,
227M/Q, 228L, 230A/D/L/M/N, 231C/E/H/I/L/N/Q/S/T, 232F/H/Q/R/W,
234A/D/E/M/T/W/Y, 236G/S/T, 238A/D/E/M/V, 239D/E/L/M/N/T, 242A, 245E,
246A/L, 247E/Q, 249C/D/E/F/I/L/S/Y, 250S/T, 253E, 254P/Y,
255A/C/D/E/F/I/M/V/W, 256C/F/H/M/W/Y, 257C/M, 259D/E/M/N, 262L,
263D/Q, 264T, 265A/M/N/Q, 266L/M/N/Q/R, 268A/C/D/E, and 269H/P/W;

wherein the amino acid positions of the variant are numbered by correspondence with the amino acid sequence of SEQ ID NO:1. In some embodiments the composition demonstrates an improved stability and improved performance, especially in egg and/or crème brulee removal versus a composition comprising a protease of SEQ ID NO:1.

Another embodiment is directed to a composition comprising a protease wherein the protease is a variant having at least 90% identity with the amino acid sequence of SEQ ID NO:1 and having a glutamate residue (E) at a position corresponding to position 39 of SEQ ID NO:1 and further comprising one or more amino acid substitutions at one or more positions selected from

(iv) 3V, 4T, 8V, 9A/C/E/G/H/K/M/N/Q/W/Y, 10A/K/M/N/Q/W, 11A/I/S/T,
12A/C/D/G/M/N/R/S/T/V/W, 14D, 15D/E/F/H/I/K/M/P/Q/V/W/Y, 16L/M/S,
17C/E/F/G/I/L/N/V/W/Y, 18A/C/D/E/F/G/L/M/Q/T,
19A/C/D/E/F/H/I/K/L/N/Q/S/T/W/Y, 20A/C/D/M/N/T, 24A/E/,
25A/C/D/E/M/N, 26A/I, 33T, 36C/E/I/L/M/Q/T/V, 42C/D/E/M/Q, 43L,
44C/E/F/G/H/I/K/L/N/Q/T/V/W/Y, 47I/Y, 50I, 52A/C/D/H/L/M/N/S/T/Y,
54A/C/G/L/M/N/T/V, 55A/C/D/E/H/N/S/Y, 57D/E/H/M/N/Q/T,
59A/C/D/E/M/N/Q/T, 60S, 69S, 76A/D/E/F/H/K/L/M/N/R/T/Y, 82A,
84D/F/H/Y, 95A/N, 96M/Q, 97E/H/K, 101T, 102L/M,
104A/D/H/M/N/T/V/W/Y, 105V, 107K/M, 110L, 113T/V, 114V, 115E/H/Q,
116E/H, 118D/E/N, 120V, 128G, 129A/H/N/Y, 131A/D/E/I/M/N/P/Q/V, 133M,
135A/E/F/H/I/K/L/M/Q/S/T/V/W/Y, 136M, 137L, 139E/S, 141E/H/N,
142A/D/E/H/M/N/Q, 143E/H/M/N/V, 144E/N, 145C, 147C, 148L/V, 150M,
156C/D/N/T, 157A/C/D/E/N/Q, 158A/C/F/L/M/N/Q/V/W/Y, 159L,
160A/C/D/M/T, 161W, 164A/K/M/Q/Y, 166D/E/I/P/Q/V, 167E, 170G, 174V,
176A/C/D/L/M/N/S, 177A/C/D/E/G/H/K/L/M/Q/S/W/Y, 178D,

179A/C/E/F/G/H/I/K/M/Q/S/V/W/Y, 180K,
182A/C/D/E/G/H/I/K/L/P/Q/T/V/W/Y, 186F, 188C/D/E/I/L/M/N/Q/S/V/W/Y,
189C/D/E, 190M, 191E, 192C/M, 193A/M, 198D/E, 200H/I/K/M/V/Y,
207K/L/N/Q, 209P, 210C/D/E/F/G/L/N/P/Q/Y, 211E/L/Q/R, 212A/C/Q, 218C/S,
227M/Q, 228L, 230A/D/L/M/N, 231C/E/H/I/L/N/Q/S/T, 232F/H/Q/R/W,
234A/D/E/M/T/W/Y, 236G/S/T, 238A/D/E/M/V, 239D/E/L/M/N/T, 242A, 245E,
246A/L, 247E/Q, 249C/D/E/F/I/L/S/Y, 250S/T, 253E, 254P/Y,
255A/C/D/E/F/I/M/V/W, 256C/F/H/M/W/Y, 257C/M, 259D/E/M/N, 262L,
263D/Q, 264T, 265A/M/N/Q, 266L/M/N/Q/R, 268A/C/D/E, and 269H/P/W;

wherein the amino acid positions of the variant are numbered by correspondence with the amino acid sequence of SEQ ID NO:1. In some embodiments, the composition demonstrates an improved stability and performance, especially on egg and/or crème brûlée removal compared to a composition comprising a protease having a glutamate at a position corresponding to position 39 in SEQ ID NO:1.

Another embodiment is directed to a composition comprising a protease wherein the protease is a variant having at least 90% identity with the amino acid sequence of SEQ ID NO:1 and the variant has a glutamate residue (E) at a position corresponding to position 39 of SEQ ID NO:1 and further comprising one or more amino acid substitutions at one or more positions selected from 3V; 9A/C/E/K; 10A/M/N/Q; 11A/I; 12C/D; 14D; 15D/E/H/I/M/V/Y; 16M; 17C/F/I/L/W; 18D/E; 19A/C/D/E/H/I/L/Q/S/T/W; 24A/E; 36C/E; 42C/D/E; 44C/E/W/Y; 52A/C/D/H; 54L/M;55A/D/H/S; 57D/E/; 59A/C/D/E/N; 60S; 76E/H/K/L/M/N/T; 84H/Y;; 95N; 96Q; 97E; 104A/D; 107K; 110L; 116E; 129H/N/Y; 131D/E; 135A/E/H/I/L/M/S/T/V/W/Y; 136M; 141E; 142E; 144E; 156C/D; 157A/C/D/E; 158A/C; 160A/M; 164A/M/Q/Y; 166D/E; 176C/D; 177C/D/M/S/Y; 178D; 179A/C/E/F/G/H/I/K/M/Q/S/V/W/Y; 182D/E; 188C/D/E/M; 189C/D/E; 193A/M; 198D/E; 200I/Y; 207K/L/Q; 209P; 210D/E/N; 238A/D/E/M; 239D/E; 241C/G/L/Q/T/Y; 245E; 247E/ 249C/D/E/Y; 253E; 255C/D/E; 256C/Y; 259D/E; 262L; 268D/E; and 269H/W;

wherein the amino acid positions of the variant are numbered by correspondence with the amino acid sequence of SEQ ID NO:1. In some embodiments, the composition demonstrates an improved cleaning performance, especially on egg removal compared to a composition comprising a protease having a glutamate at a position corresponding to position 39 in SEQ ID NO:1.

Another embodiment is directed to a composition comprising a protease wherein the protease is a variant having at least 90% identity with the amino acid sequence of SEQ ID NO:1

and the variant has a glutamate residue (E) at a position corresponding to position 39 of SEQ ID NO:1 and further comprises one or more amino acid substitutions at one or more positions selected from 9A/C/E/M/N/Y, 10A/K/M/N/Q/W, 11A/T, 12A/C/D/M, 14D, 15D/E/H/I/M/V/W/Y, 16L/M, 17C/E, 18C/D/E/M, 19A/C/D/E/F/H/I/K/L/N/Q/S/T/W/Y, 20C/D, 24A/E, 25A/C/D/E/M/N, 26A, 36C/E/Q/V, 42C/D/E, 43L, 44C/E/G/H/I/L/N/Q/T, 52A/C/D/L/M/N, 54A/C/L/M/V, 55A/C/D/E, 57D/E, 59A/C/D/E/M/N/Q/T, 60S, 76D/E/N, 82A, 84D, 96Q, 97E/H, 104A/D/H/N/V/Y, 115H, 116E, 129H, 131D/E, 135A/E/F/H/I/K/L/M/S/T/V/W/Y, 139E, 141E, 142D/E, 143E, 144E, 147C, 148L, 156C/D/N/T, 157C/D/E, 158C/L/Q/Y, 159L, 164A/K/M/Q/Y, 166D/E, 167E, 174V, 176A/C/D/N, 177C/D, 178D, 179A/C/E/F/G/H/I/K/M/Q/S/V/W/Y, 182C/D/E, 188C/D/E, 189C/D/E, 193A/M, 198D/E, 207K/L/Q, 209P, 210C/D/E/L/N/Y, 212C, 228L, 231C/E/L/N/Q, 232F, 234D/E/T/W/Y, 236T, 238A/D/E/M/V, 239D/E/M/N, 241C/G/L/Q/T/Y, 245E, 246A/L, 247E/Q, 249C/D/E/L/Y, 253E, 254Y, 255A/C/D/E/, 256C/Y, 257C, 259D/E/M/N, 262L, 263D, 268C/D/E, and 269H/P/W; wherein the amino acid positions of the variant are numbered by correspondence with the amino acid sequence of SEQ ID NO:1. In some embodiments, the composition demonstrates an improved cleaning performance, in particular crème brûlée removal compared to a composition comprising a protease having a glutamate at a position corresponding to position 39 in SEQ ID NO:1.

In another embodiment, compositions comprising variants are provided that have an improved crème brûlée cleaning performance compared to a parent protease (e.g. SEQ ID NO:1), where the variant comprises an amino acid substitution at one or more positions selected from 9, 10, 12, 14, 15, 17, 18, 19, 20, 24, 25, 36, 42, 44, 52, 54, 55, 57, 59, 76, 84, 97, 104, 116, 131, 135, 139, 141, 142, 143, 147, 156, 157, 158, 164, 166, 167, 176, 177, 178, 179, 180, 182, 188, 189, 198, 207, 210, 212, 231, 234, 238, 239, 245, 247, 249, 253, 254, 255, 156, 257, 259, 263, 268, and 269, where the positions are numbered corresponding to SEQ ID NO: 1, and where the substitution introduces an overall negative net charge relative to the parent subtilisin in the application. In some embodiments, the variant comprises one or more negatively charged amino acid substitutions at one or more positions or replaces a positively charged amino acid at one or more positions, selected from 9C/E/Y, 10A/K/M/N/Q/W, 12C/D, 14D, 15D/E/Y, 17C/E, 18C/D/E, 19A/C/D/E/F/H/I/K/L/N/Q/S/T/W/Y, 20C/D, 24E, 25C/D/E, 36C/E, 42C/D/E, 44C/E/G/H/I/L/N/Q/T, 52C/D, 54C, 55C/D/E, 57D/E, 59C/D/E, 76D/E, 84D, 97E, 104D/Y, 116E, 131D/E, 135A/E/F/H/I/K/L/M/S/T/V/W/Y, 139E, 141E, 142D/E, 143E, 147C, 156C/D, 157C/D/E, 158C/Y, 164A/K/M/Q/Y, 166D/E, 167E, 176C/D, 177C/D, 178D, 179A/C/E/F/G/H/I/K/M/Q/S/V/W/Y, 180K, 182C/D/E, 188C/D/E, 189C/D/E, 198D/E, 207K/L/Q, 210C/D/E/Y, 212C, 230D, 231C/E/L/N/Q, 234D/E/Y, 238D/E, 239D/E, 245E, 247E,

249C/D/E/Y, 253E, 254Y, 255C/D/E, 256C/Y, 257C, 259D/E, 263D, 268C/D/E, and 269H/P/W, where the positions are numbered corresponding to SEQ ID NO: 1.

Another embodiment is directed to a composition comprising a protease wherein the protease is a variant having at least 90% identity with the amino acid sequence of SEQ ID NO:1 and the variant comprises an amino acid sequence having a glutamate residue (E) at a position corresponding to position 39 of SEQ ID NO:1 and further comprising one or more amino acid substitutions at one or more positions selected from 9N, 11A, 12A/M, 15I/V, 16L/M, 19C/K/L/Q, 20D, 24A, 25A/D/N, 52D, 54A/L/M/V, 55A/D, 59A/M/N, 60S, 96Q, 129H, 157D, 158Q, 159L, 177D, 179A/K, 182D, 207L, 210E, 232F, , and 256Y; wherein the amino acid positions of the variant are numbered by correspondence with the amino acid sequence of SEQ ID NO:1. In some embodiments, the variant demonstrates an improved cleaning performance on egg and a crème brûlée removal compared to a composition comprising a protease having a glutamate at a position corresponding to position 39 in SEQ ID NO:1.

Another embodiment is directed to a composition comprising a protease wherein the protease is a variant having at least 90% identity with the amino acid sequence of SEQ ID NO:1 and the variant comprises an amino acid sequence having a glutamate residue (E) at a position corresponding to position 39 of SEQ ID NO:1 and further comprising one or more amino acid substitutions at one or more positions selected from 3V, 4T, 8V, 9A/E/G/H/K/N/Q/W/Y, 10Q, 11A, 12A/C/G/M/N/T, 15F/H/M/P/Q/W, 16S, 17C/E/F/I/L/N/V/W/Y, 18A/D/E/L/M/Q, 19C/D/Y, 20C/D/M/N, 24A/E, 25C/D/N, 26I, 33T, 36C/I/L/M/Q/V, 42C/D/E/M/Q, 44C/E/F/G/H/I/K/L/N/Q/T/V/W/Y, , 47I/Y, 50I, 52A/M/N/S/T/Y, 54N/V, 55C/D/E/N, 57E/H/M/N/Q/T, 59N, 76A/D/E/F/H/K/L/M/N/R/T/Y, 82A, 84D/F/H/Y, 95A/N, 96M, 97K, 101T, 102L/M, 104M/N/T/V/W, 105V, 107M, 113V, 114V, 115Q, 116E/H, 118D/E, 131A/D/E/I/M/N/P/Q/V, 133M, 135A/H/I/K/L/M/S/T/V/W/Y, 136M, 142A/D/E/H/M/N/Q, 143E/H/M/N, 147C, 148V, 150M, 156N/T, 157A/C/N, 158C/F/L/M/N/Q/V/W/Y, 159L, 160A/C/M/T, 166D/E/P/Q, 170G, 176C/M, 177A/C/D/H/L/M/Q/W/Y, 179M/Q, 180K, 182A/C/E/G/H/I/K/L/P/Q/T/V/W/Y, 188C/D/E/I/L/M/N/Q/V/W/Y, 189D, 192C/M, 193M, 200H/I/K/M/V/Y, 209P, 210E/F/P, 218C/S, 228L, 231C/E/H/N/T, 232F/H, 234D/M, 236G/S/T, 238A/D/E/M/V, 239E/L/M/T, 242A, 246A/L, 249E/F/I/L/S/Y, 250S, 253E, 254P, 255C/D/E/F/I/M/V/W, 256C/F/H/W/Y, 264T, 266L/M/N, and 268C; wherein the amino acid positions of the variant are numbered by correspondence with the amino acid sequence of SEQ ID NO:1. In some embodiments, the composition demonstrates an improved stability as compared to a composition comprising a protease having a glutamate at a position corresponding to position 39 in SEQ ID NO:1.

Another embodiment is directed to a composition comprising a protease wherein the protease is a variant having at least 90% identity with the amino acid sequence of SEQ ID NO:1 and the variant comprises an amino acid sequence having a glutamate residue (E) at a position corresponding to position 39 of SEQ ID NO:1 and further comprising one or more amino acid substitutions at one or more positions selected from 9A/E/H/K/N/W/Y, 10Q, 11A, 12A/C/M/N, 15F/H/M/W, 17C/E/F/I/L/N/V/W, 18D/E/M, 19C/D/Y, 20C/D/M/N, 24A/E, 25C/D/N, 36C/L/Q/V, 42C/D/E, 44C/E/G/H/I/L/N/Q/T, 52A/M/N, 54V, 55C/D/E, 57E/S, 59N, 76D/E/K/L/N, 82A, 84D, 95N, 97K, 102L/M, 104N/V, 116E, 118D, 131D/E/M/N/P, 135A/H/I/K/L/M/S/T/V/W/Y, 136M, 142D/E, 143E/N, 147C, 156N/T, 157A/C, 158C/L/Q/Y, 159L, 160M, 166D/E, 170G, 176C, 177A/C/D/L/M/Y, 179M/Q, 180K, 182A/C/E/Y, 188C/D/E/M, 189D, 193M, 209P, 210E, 218S, 228L, 231C/E/N, 232F, 234D, 236T, 238A/D/E/M/V, 239E/M, 246A/L, 249E/L/Y, 253E, 255C/D/E, 256C/Y, and 268C; wherein the amino acid positions of the variant are numbered by correspondence with the amino acid sequence of SEQ ID NO:1. In some embodiments, the composition demonstrates an improved cleaning performance, in particular on egg or crème brûlée removal. The composition also presents improved stability compared to a composition comprising a protease having a glutamate at a position corresponding to position 39 in SEQ ID NO:1.

In some embodiments, the parent protease or variant also comprise at least one, two, three, or more additional substitutions selected from Q012E, Q037E, N060D, N097D, Q107E, N115D, N154D, N167D, Q176E, Q185E, Q200E, N205D, Q230E, N236D, N242D, N250D, N253D, Q256E, N253D-Q256E, G025R-M117I-H118N, A149S, R044P-D175N-Y208N-Q230H, L041F-G078D-P084A, S101G-T174A, I021V-N177I, I021V-S142G-T188A, I021V-M122L-A222S, Q012L-I021V-M122L-A222S, I021V-M122L-N253D, I021V-N177V-V228I, I021V-S039T-M122L-N177E, I021V-V079L-D087E-A209N-A222S, I021V-M122L-A222S-T247N, I021V-M122L, S039E-N074D-D087E, N253P, S039E-N074D-D087E-N253D, I021V-S039E-N074D-D087E-N253D, S039E-N074D-D087E-M122L-N253D, I021V-S039E-N074D-D087E-M122L-N253D, I021V, M122L, M211S, P212N, Q012L, N177V, A222S, V228I, T274N, R099E, N097D-R099E, S097D, S099E, I043V, M122L-N145S-T156A, M211N-P212D, M211L-P212D, G160S, D127P-M211L-P212D, P212H, Q012L-M122L-A222S, D127P, N145S, T156A, M211N, and P212D.

The disclosure includes variants having one or more modifications at a surface exposed amino acid. Surface modifications in the enzyme variants can be useful in a detergent composition by having a minimum performance index for wash performance, stability of the enzyme in detergent compositions and thermostability of the enzyme, while having at least one of these

characteristics improved from a parent subtilisin enzyme. In some embodiments, the surface modification changes the hydrophobicity and/or charge of the amino acid at that position. Hydrophobicity can be determined using techniques known in the art, such as those described in White and Wimley (White,S.H. and Wimley, W.C,. (1999) Annu. Rev. Biophys. Biomol. Struct. 28:319-65). Net charge of an amino acid at a pH of interest can be calculated using the pK_a values of titratable chemical groups in amino acids, such as those described in Hass and Mulder (Hass, M.A.S and Mulder, F.A.A (2015) Annu. Rev. Biophys. 44:53–75)

As used herein, "surface property" can be used in reference to electrostatic charge, as well as properties such as the hydrophobicity and hydrophilicity exhibited by the surface of a protein. The variants provided herein that have at least one of the surface modifications as suitable modifications include positions 76, 84, 97, 104, 116, 131, 135, 139, 141, 142, 143, 157, where the amino acid positions of the variant are numbered by correspondence with the amino acid sequence in SEQ ID NO:1.

The term “enhanced stability” or “improved stability” in the context of an oxidation, chelator, denaturant, surfactant, thermal and/or pH stable protease refers to a higher retained proteolytic activity over time as compared to a reference protease, for example, a wild-type protease or parent protease.

A further embodiment is directed to a method of cleaning a crème brûlée stain comprising contacting a surface or an item in need of cleaning with a composition comprising one or more protease wherein the protease is a variant having at least 90% identity with the amino acid sequence of SEQ ID NO:1 and the variant comprises an amino acid sequence having a glutamate at a position corresponding to position 39 of SEQ ID NO: 1 and further comprising one or more substitutions at one or more positions corresponding to SEQ ID NO:1 positions selected from: 9A/C/E/M/N/Y, 10A/K/M/N/Q/W, 11A/T, 12A/C/D/E/M, 14D, 15D/E/H/I/M/V/W/Y, 16L/M, 17C/E, 18C/D/E/M, 19A/C/D/E/F/H/I/K/L/N/Q/S/T/W/Y, 20C/D, 24A/E, 25A/C/D/E/M/N, 26A, 27K, 36C/E/Q/V, 42C/D/E, 43L, 44C/E/G/H/I/L/N/Q/S/T, 52A/C/D/L/M/N, 54A/C/L/M/V, 55A/C/D/E/M, 57D/E, 59A/C/D/E/M/N/Q/T, 60S, 76D/E/N, 82A, 84D, 96Q, 97E/H, 104A/D/H/N/V/Y, 115H, 116E, 129H, 131D/E, 135A/E/F/H/I/K/L/M/S/T/V/W/Y, 139E, 141E, 142D/E, 143E, 144E, 147C, 148L, 154D, 156A/C/D/N/T, 157C/D/E, 158C/L/Q/T/Y, 159L, 164A/K/M/Q/Y, 166D/E, 167E, 169L, 174V, 176A/C/D/E/N, 177C/D/E, 178D, 179A/C/E/F/G/H/I/K/M/Q/S/V/W/Y, 180K, 182C/D/E, 188C/D/E, 189C/D/E, 193A/M, 198D/E, 206D, 207K/L/Q, 209P, 210C/D/E/L/N/Y, 211K/L, 212C, 228L, 230A/D/E/H/M/N, 231C/E/L/N/Q, 232F, 234D/E/T/W/Y, 236D/T, 238A/D/E/M/V, 239D/E/M/N, 245E, 246A/L, 247E/Q, 249C/D/E/L/Y, 250D, 252A/Q, 253D/E/P, 254Y, 255A/C/D/E, 256C/E/Y, 257C,

259D/E/M/N, 262L, 263D, 268C/D/E, and 269H/P/W and combinations; wherein the amino acid positions of the variant are numbered by correspondence with the amino acid sequence of SEQ ID NO:1. A still further embodiment is directed to a method of cleaning a crème brûlée stain comprising contacting a surface or an item in need of cleaning with a composition comprising one or more variant, wherein said variant comprises an amino acid sequence having a glutamate at a position corresponding to position 39 of SEQ ID NO:1 and further comprising one or more substitutions at one or more positions corresponding to SEQ ID NO:1 positions selected from: 9A/C/E/M/N/Y, 10A/K/M/N/Q/W, 11A/T, 12A/C/D/E/M, 14D, 15D/E/H/I/M/V/W/Y, 16L/M, 17C/E, 18C/D/E/M, 19A/C/D/E/F/H/I/K/L/N/Q/S/T/W/Y, 20C/D, 24A/E, 25A/C/D/E/M/N, 26A, 27K, 36C/E/Q/V, 42C/D/E, 43L, 44C/E/G/H/I/L/N/Q/S/T, 52A/C/D/L/M/N, 54A/C/L/M/V, 55A/C/D/E/M, 57D/E, 59A/C/D/E/M/N/Q/T, 60S, 76D/E/N, 82A, 84D, 96Q, 97E/H, 104A/D/H/N/V/Y, 115H, 116E, 129H, 131D/E, 135A/E/F/H/I/K/L/M/S/T/V/W/Y, 139E, 141E, 142D/E, 143E, 144E, 147C, 148L, 154D, 156A/C/D/N/T, 157C/D/E, 158C/L/Q/T/Y, 159L, 164A/K/M/Q/Y, 166D/E, 167E, 169L, 174V, 176A/C/D/E/N, 177C/D/E, 178D, 179A/C/E/F/G/H/I/K/M/Q/S/V/W/Y, 180K, 182C/D/E, 188C/D/E, 189C/D/E, 193A/M, 198D/E, 207K/L/Q, 209P, 210C/D/E/L/N/Y, 211K/L, 212C, 228L, 231C/E/L/N/Q, 232F, 234D/E/T/W/Y, 236D/T, 238A/D/E/M/V, 239D/E/M/N, 245E, 246A/L, 247E/Q, 249C/D/E/L/Y, 250D, 253D/E/P, 254Y, 255A/C/D/E, 256C/E/Y, 257C, 259D/E/M/N, 262L, 263D, 268C/D/E, and 269H/P/W and combinations; wherein the amino acid positions of the variant are numbered by correspondence with the amino acid sequence of SEQ ID NO:1.

A further embodiment is directed to a method of cleaning egg stain comprising contacting a surface or an item in need of cleaning with a composition containing one or more subtilisin variant, where the variant has one or more substitutions at one or more positions corresponding to SEQ ID NO:1 positions selected from: 9H/K/N/W, 11A/I, 12A/M/N/R/S/V, 15F/I/K/V, 16L/M, 17F/G/I/L/N/V/W, 18F, 19C/K/L/Q, 20A/D/M/N/T, 24A, 25A/D/N, 36L, 52D/H, 54A/G/L/M/V, 55A/D/H/S/Y, 57S, 59A/M/N, 60S, 69S, 76K/L, 95N, 96Q, 97K, 102L/M, 107K, 110L, 113T, 118D, 120V, 129A/H/N/Y, 131M/N/P, 136M, 143N, 144N, 145C, 157A/D, 158Q, 159L, 160M, 166I, 170G, 176L, 177A/D/G/K/L/M/S/Y, 179A/K, 182A/D/Y, 188M, 191E, 207L, 210E/G/Q, 211R, 218S, 227M, 232F/W, 256Y, 263Q, 265A/M/Q, and 268A and combinations; wherein the amino acid positions of the variant are numbered by correspondence with the amino acid sequence of SEQ ID NO:1. A still further embodiment is directed to a method of cleaning an egg stain comprising contacting a surface or an item in need of cleaning with a composition containing one or more variant, wherein said variant comprises an amino acid sequence having a glutamate at a position corresponding to position 39 of SEQ ID NO:1 and further comprising one or more

substitutions at one or more positions corresponding to SEQ ID NO:1 positions selected from: 9H/K/N/W, 11A/I, 12A/M/N/R/S/V, 15F/I/K/V, 16L/M, 17F/G/I/L/N/V/W, 18F, 19C/K/L/Q, 20A/D/M/N/T, 24A, 25A/D/N, 36L, 52D/H, 54A/G/L/M/V, 55A/D/H/S/Y, 59A/M/N, 60S, 69S, 76K/L, 95N, 96Q, 97K, 102L/M, 107K, 110L, 113T, 118D, 120V, 129A/H/N/Y, 131M/N/P, 136M, 143N, 144N, 145C, 157A/D, 158Q, 159L, 160M, 166I, 170G, 176L, 177A/D/G/K/L/M/S/Y, 179A/K, 182A/D/Y, 188M, 191E, 207L, 210E/G/Q, 211R, 218S, 227M, 232F/W, 256Y, 263Q, 265A/M/Q, and 268A and combinations; wherein the amino acid positions of the variant are numbered by correspondence with the amino acid sequence of SEQ ID NO:1.

The protease of the invention performs very well in phosphate-free compositions even when the compositions are used in soft water.

Preferred levels of protease in the composition of the invention include from about 0.04 to about 5 mg, more preferably from about 0.05 to about 2 mg of active protease per gram of the composition.

Automatic dishwashing cleaning composition

The automatic dishwashing cleaning composition can be in any physical form. It can be a loose powder, a gel or presented in unit dose form. Preferably it is in unit dose form, unit dose forms include pressed tablets and water-soluble packs. The automatic dishwashing cleaning composition of the invention is preferably presented in unit-dose form and it can be in any physical form including solid, liquid and gel form. The composition of the invention is very well suited to be presented in the form of a multi-compartment pack, more in particular a multi-compartment pack comprising compartments with compositions in different physical forms, for example a compartment comprising a composition in solid form and another compartment comprising a composition in liquid form. The composition is preferably enveloped by a water-soluble film such as polyvinyl alcohol. Especially preferred are compositions in unit dose form wrapped in a polyvinyl alcohol film having a thickness of less than 100 μm, preferably from 20 to 90 μm. The detergent composition of the invention weighs from about 8 to about 25 grams, preferably from about 10 to about 20 grams. This weight range fits comfortably in a dishwasher dispenser. Even though this range amounts to a low amount of detergent, the detergent has been formulated in a way that provides all the benefits mentioned herein above.

The composition is preferably phosphate free. By “phosphate-free” is herein understood that the composition comprises less than 1%, preferably less than 0.1% by weight of the composition of phosphate.

The composition of the invention is preferably phosphate-free and comprises a complexing agent system.

Complexing agent system

For the purpose of this invention a "complexing agent" is a compound capable of binding polyvalent ions such as calcium, magnesium, lead, copper, zinc, cadmium, mercury, manganese, iron, aluminium and other cationic polyvalent ions to form a water-soluble complex. The complexing agent has a logarithmic stability constant ([log K]) for Ca2+ of at least 3. The stability constant, log K, is measured in a solution of ionic strength of 0.1, at a temperature of 25° C.

The composition of the invention comprises from 10% to 50% by weight of the composition of a complexing agent system. Preferably, the composition comprises a complexing agent selected from the group consisting of citric acid, methyl glycine diacetic acid (MGDA), glutamic-N,N-diacetic acid (GLDA), iminodisuccinic acid (IDS), carboxy methyl inulin, L-Aspartic acid N, N-diacetic acid tetrasodium salt (ASDA) and mixtures thereof. For the purpose of this invention, the term "acid", when referring to complexing agents, includes the acid and salts thereof.

In a preferred embodiment, the composition comprises from 15% to 40% by weight of the invention of MGDA, more preferably the tri-sodium salt of MGDA. Compositions comprising this high level of MGDA perform well in the presence of hard water and also in long and/or hot cycles.

In a preferred embodiment, the composition comprises from 15% to 28% by weight of the invention of citric acid, more preferably sodium citrate. Compositions comprising citric acid perform well in the presence of soft water.

In a preferred embodiment, the complexing agent system comprises citric acid and MGDA, preferably in a weight ratio of from about 0.5:1 to about 2:1, more preferably from about 0.5:1 to about 2.5:1.

Dispersant polymer

A dispersant polymer can be used in any suitable amount from about 0.1 to about 20%, preferably from 0.2 to about 15%, more preferably from 0.3 to % by weight of the composition.

The dispersant polymer is capable to suspend calcium or calcium carbonate in an automatic dishwashing process.

The dispersant polymer has a calcium binding capacity within the range between 30 to 250 mg of Ca/g of dispersant polymer, preferably between 35 to 200 mg of Ca/g of dispersant polymer, more preferably 40 to 150 mg of Ca/g of dispersant polymer at 25°C. In order to determine if a

polymer is a dispersant polymer within the meaning of the invention, the following calcium binding-capacity determination is conducted in accordance with the following instructions:

Calcium binding capacity test method

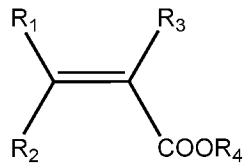
The calcium binding capacity referred to herein is determined via titration using a pH/ion meter, such as the Meettler Toledo SevenMulti™ bench top meter and a PerfectION™ comb Ca combination electrode. To measure the binding capacity a heating and stirring device suitable for beakers or tergotometer pots is set to 25 °C, and the ion electrode with meter are calibrated according to the manufacturer's instructions. The standard concentrations for the electrode calibration should bracket the test concentration and should be measured at 25 °C. A stock solution of 1000 mg/g of Ca is prepared by adding 3.67 g of $CaCl_2$-$2H_2O$ into 1 L of deionised water, then dilutions are carried out to prepare three working solutions of 100 mL each, respectively comprising 100 mg/g, 10 mg/g, and 1 mg/g concentrations of Calcium. The 100 mg Ca/g working solution is used as the initial concentration during the titration, which is conducted at 25 °C. The ionic strength of each working solution is adjusted by adding 2.5 g/L of NaCl to each. The 100 mL of 100 mg Ca/g working solution is heated and stirred until it reaches 25 °C. The initial reading of Calcium ion concentration is conducted at when the solution reaches 25 °C using the ion electrode. Then the test polymer is added incrementally to the calcium working solution (at 0.01 g/L intervals) and measured after 5 minutes of agitation following each incremental addition. The titration is stopped when the solution reaches 1 mg/g of Calcium. The titration procedure is repeated using the remaining two calcium concentration working solutions. The binding capacity of the test polymer is calculated as the linear slope of the calcium concentrations measured against the grams/L of test polymer that was added.

The dispersant polymer preferably bears a negative net charge when dissolved in an aqueous solution with a pH greater than 6.

The dispersant polymer can bear also sulfonated carboxylic esters or amides, in order to increase the negative charge at lower pH and improve their dispersing properties in hard water. The preferred dispersant polymers are sulfonated / carboxylated polymers, i.e., polymer comprising both sulfonated and carboxylated monomers.

Preferably, the dispersant polymers are sulfonated derivatives of polycarboxylic acids and may comprise two, three, four or more different monomer units. The preferred copolymers contain:

At least one structural unit derived from a carboxylic acid monomer having the general formula (III):

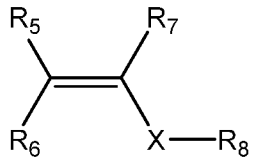


(III)

wherein R_1 to R_3 are independently selected from hydrogen, methyl, linear or branched saturated alkyl groups having from 2 to 12 carbon atoms, linear or branched mono or polyunsaturated alkenyl groups having from 2 to 12 carbon atoms, alkyl or alkenyl groups as aforementioned substituted with –NH2 or -OH, or –COOH, or $COOR_4$, where R_4 is selected from hydrogen, alkali metal, or a linear or branched, saturated or unsaturated alkyl or alkenyl group with 2 to 12 carbons;

Preferred carboxylic acid monomers include one or more of the following: acrylic acid, maleic acid, maleic anhydride, itaconic acid, citraconic acid, 2-phenylacrylic acid, cinnamic acid, crotonic acid, fumaric acid, methacrylic acid, 2-ethylacrylic acid, methylenemalonic acid, or sorbic acid. Acrylic and methacrylic acids being more preferred.

Optionally, one or more structural units derived from at least one nonionic monomer having the general formula (IV):



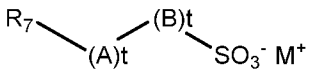
(IV)

Wherein R_5 to R_7 are independently selected from hydrogen, methyl, phenyl or hydroxyalkyl groups containing 1 to 6 carbon atoms, and can be part of a cyclic structure, X is an optionally present spacer group which is selected from $-CH_2-$, -COO-, -CONH- or $-CONR_8-$, and R_8 is selected from linear or branched, saturated alkyl radicals having 1 to 22 carbon atoms or unsaturated, preferably aromatic, radicals having from 6 to 22 carbon atoms.

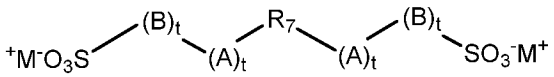
Preferred non-ionic monomers include one or more of the following: butene, isobutene, pentene, 2-methylpent-1-ene, 3-methylpent-1-ene, 2,4,4-trimethylpent-1-ene, 2,4,4-trimethylpent-2-ene, cyclopentene, methylcyclopentene, 2-methyl-3-methyl-cyclopentene, hexene, 2,3-dimethylhex-1-ene, 2,4-dimethylhex-1-ene, 2,5-dimethylhex-1-ene, 3,5-dimethylhex-1-ene, 4,4-dimethylhex-1-ene, cyclohexene, methylcyclohexene, cycloheptene, alpha olefins having 10 or more carbon atoms such as, dec-1-ene, dodec-1-ene, hexadec-1-ene, octadec-1-ene and docos-1-ene, preferred aromatic monomers are styrene, alpha methylstyrene, 3-methylstyrene, 4-dodecylstyrene, 2-ethyl-4-bezylstyrene, 4-cyclohexylstyrene, 4-propylstyrol, 1-vinylnaphtalene, 2-vinylnaphtalene; preferred carboxylic ester monomers are methyl (meth)acrylate, ethyl

(meth)acrylate, propyl (meth)acrylate, t-butyl (meth)acrylate, pentyl (meth)acrylate, hexyl (meth)acrylate, 2-ethylhexyl (meth)acrylate, octyl (meth)acrylate, lauryl (meth)acrylate, stearyl (meth)acrylate and behenyl (meth)acrylate; preferred amides are N-methyl acrylamide, N-ethyl acrylamide, N-t-butyl acrylamide, N-2-ethylhexyl acrylamide, N-octyl acrylamide, N-lauryl acrylamide, N-stearyl acrylamide, N-behenyl acrylamide.

and at least one structural unit derived from at least one sulfonic acid monomer having the general formula (V) and (VI):



(V)



(VI)

wherein R_7 is a group comprising at least one sp2 bond, A is O, N, P, S, an amido or ester linkage, B is a mono- or polycyclic aromatic group or an aliphatic group, each t is independently 0 or 1, and M+ is a cation. In one aspect, R_7 is a C2 to C6 alkene. In another aspect, R7 is ethene, butene or propene.

Preferred sulfonated monomers include one or more of the following: 1-acrylamido-1-propanesulfonic acid, 2-acrylamido-2-propanesulfonic acid, 2-acrylamido-2-methyl-1-propanesulfonic acid, 2-methacrylamido-2-methyl-1-propanesulfonic acid, 3- methacrylamido-2-hydroxy-propanesulfonic acid, allylsulfonic acid, methallylsulfonic acid, allyloxybenzenesulfonic acid, methallyloxybenzenesulfonic acid, 2-hydroxy-3- (2-propenyloxy) propanesulfonic acid, 2-methyl-2-propen-1-sulfonic acid, styrenesulfonic acid, vinylsulfonic acid, 3-sulfopropyl, 3-sulfo-propylmethacrylate, sulfomethacrylamide, sulfomethylmethacrylamide and mixtures of said acids or their water-soluble salts.

Preferably, the polymer comprises the following levels of monomers: from about 40 to about 90%, preferably from about 60 to about 90% by weight of the polymer of one or more carboxylic acid monomer; from about 5 to about 50%, preferably from about 10 to about 40% by weight of the polymer of one or more sulfonic acid monomer; and optionally from about 1% to about 30%, preferably from about 2 to about 20% by weight of the polymer of one or more non-ionic monomer. An especially preferred polymer comprises about 70% to about 80% by weight of the polymer of at least one carboxylic acid monomer and from about 20% to about 30% by weight of the polymer of at least one sulfonic acid monomer.

In the polymers, all or some of the carboxylic or sulfonic acid groups can be present in neutralized form, i.e. the acidic hydrogen atom of the carboxylic and/or sulfonic acid group in some or all acid groups can be replaced with metal ions, preferably alkali metal ions and in particular with sodium ions.

The carboxylic acid is preferably (meth)acrylic acid. The sulfonic acid monomer is preferably 2-acrylamido-2-propanesulfonic acid (AMPS).

Preferred commercial available polymers include: Alcosperse 240, Aquatreat AR 540 and Aquatreat MPS supplied by Alco Chemical; Acumer 3100, Acumer 2000, Acusol 587G and Acusol 588G supplied by Rohm & Haas; Goodrich K-798, K-775 and K-797 supplied by BF Goodrich; and ACP 1042 supplied by ISP technologies Inc. Particularly preferred polymers are Acusol 587G and Acusol 588G supplied by Rohm & Haas.

Suitable dispersant polymers include anionic carboxylic polymer of low molecular weight. They can be homopolymers or copolymers with a weight average molecular weight of less than or equal to about 200,000 g/mol, or less than or equal to about 75,000 g/mol, or less than or equal to about 50,000 g/mol, or from about 3,000 to about 50,000 g/mol, preferably from about 5,000 to about 45,000 g/mol. The dispersant polymer may be a low molecular weight homopolymer of polyacrylate, with an average molecular weight of from 1,000 to 20,000, particularly from 2,000 to 10,000, and particularly preferably from 3,000 to 5,000.

The dispersant polymer may be a copolymer of acrylic with methacrylic acid, acrylic and/or methacrylic with maleic acid, and acrylic and/or methacrylic with fumaric acid, with a molecular weight of less than 70,000. Their molecular weight ranges from 2,000 to 80,000 and more preferably from 20,000 to 50,000 and in particular 30,000 to 40,000 g/mol. and a ratio of (meth)acrylate to maleate or fumarate segments of from 30:1 to 1:2.

The dispersant polymer may be a copolymer of acrylamide and acrylate having a molecular weight of from 3,000 to 100,000, alternatively from 4,000 to 20,000, and an acrylamide content of less than 50%, alternatively less than 20%, by weight of the dispersant polymer can also be used. Alternatively, such dispersant polymer may have a molecular weight of from 4,000 to 20,000 and an acrylamide content of from 0% to 15%, by weight of the polymer.

Dispersant polymers suitable herein also include itaconic acid homopolymers and copolymers.

Alternatively, the dispersant polymer can be selected from the group consisting of alkoxylated polyalkyleneimines, alkoxylated polycarboxylates, polyethylene glycols, styrene co-polymers, cellulose sulfate esters, carboxylated polysaccharides, amphiphilic graft copolymers and mixtures thereof.

Bleaching system

The composition of the invention comprises a bleaching system comprising a high level of bleach, preferably percarbonate in combination with a bleach activator or a bleach catalyst or both. Preferably the bleach activator is TAED and the bleach catalyst is a manganese bleach catalyst.

Bleach

The composition of the invention preferably comprises from about 10 to about 20%, more preferably from about 12 to about 18% of bleach, preferably percarbonate, by weight of the composition.

Inorganic and organic bleaches are suitable for use herein. Inorganic bleaches include perhydrate salts such as perborate, percarbonate, perphosphate, persulfate and persilicate salts. The inorganic perhydrate salts are normally the alkali metal salts. The inorganic perhydrate salt may be included as the crystalline solid without additional protection. Alternatively, the salt can be coated. Suitable coatings include sodium sulphate, sodium carbonate, sodium silicate and mixtures thereof. Said coatings can be applied as a mixture applied to the surface or sequentially in layers.

Alkali metal percarbonates, particularly sodium percarbonate is the preferred bleach for use herein. The percarbonate is most preferably incorporated into the products in a coated form which provides in-product stability.

Potassium peroxymonopersulfate is another inorganic perhydrate salt of utility herein.

Typical organic bleaches are organic peroxyacids, especially dodecanediperoxoic acid, tetradecanediperoxoic acid, and hexadecanediperoxoic acid. Mono- and diperazelaic acid, mono- and diperbrassylic acid are also suitable herein. Diacyl and Tetraacylperoxides, for instance dibenzoyl peroxide and dilauroyl peroxide, are other organic peroxides that can be used in the context of this invention.

Further typical organic bleaches include the peroxyacids, particular examples being the alkylperoxy acids and the arylperoxy acids. Preferred representatives are (a) peroxybenzoic acid and its ring-substituted derivatives, such as alkylperoxybenzoic acids, but also peroxy-α-naphthoic acid and magnesium monoperphthalate, (b) the aliphatic or substituted aliphatic peroxy acids, such as peroxylauric acid, peroxystearic acid, ε-phthalimidoperoxycaproic acid[phthaloiminoperoxyhexanoic acid (PAP)], o-carboxybenzamidoperoxycaproic acid, N-nonenylamidoperadipic acid and N-nonenylamidopersuccinates, and (c) aliphatic and araliphatic peroxydicarboxylic acids, such as 1,12-diperoxycarboxylic acid, 1,9-diperoxyazelaic acid,

diperoxysebacic acid, diperoxybrassylic acid, the diperoxyphthalic acids, 2-decyldiperoxybutane-1,4-dioic acid, N,N-terephthaloyldi(6-aminopercaproic acid).

Bleach Activators

Bleach activators are typically organic peracid precursors that enhance the bleaching action in the course of cleaning at temperatures of 60° C and below. Bleach activators suitable for use herein include compounds which, under perhydrolysis conditions, give aliphatic peroxoycarboxylic acids having preferably from 1 to 12 carbon atoms, in particular from 2 to 10 carbon atoms, and/or optionally substituted perbenzoic acid. Suitable substances bear O-acyl and/or N-acyl groups of the number of carbon atoms specified and/or optionally substituted benzoyl groups. Preference is given to polyacylated alkylenediamines, in particular tetraacetylethylenediamine (TAED), acylated triazine derivatives, in particular 1,5-diacetyl-2,4-dioxohexahydro-1,3,5-triazine (DADHT), acylated glycolurils, in particular tetraacetylglycoluril (TAGU), N-acylimides, in particular N-nonanoylsuccinimide (NOSI), acylated phenolsulfonates, in particular n-nonanoyl- or isononanoyloxybenzenesulfonate (n- or iso-NOBS), decanoyloxybenzoic acid (DOBA), carboxylic anhydrides, in particular phthalic anhydride, acylated polyhydric alcohols, in particular triacetin, ethylene glycol diacetate and 2,5-diacetoxy-2,5-dihydrofuran and also triethylacetyl citrate (TEAC). If present the composition of the invention comprises from 0.01 to 5, preferably from 0.2 to 2% by weight of the composition of bleach activator, preferably TAED.

Bleach Catalyst

The composition herein preferably contains a bleach catalyst, preferably a metal containing bleach catalyst. More preferably the metal containing bleach catalyst is a transition metal containing bleach catalyst, especially a manganese or cobalt-containing bleach catalyst.

Bleach catalysts preferred for use herein include manganese triazacyclononane and related complexes; Co, Cu, Mn and Fe bispyridylamine and related complexes; and pentamine acetate cobalt(III) and related complexes. Especially preferred bleach catalyst for use herein are 1,4,7-trimethyl-1,4,7-triazacyclononane (Me-TACN) and 1,2, 4,7- tetramethyl-1,4,7-triazacyclononane (Me/Me-TACN).

Preferably the composition of the invention comprises from 0.001 to 0.5, more preferably from 0.002 to 0.05%, more preferably from 0.005 to 0.075% of bleach catalyst by weight of the composition. Preferably the bleach catalyst is a manganese bleach catalyst.

Inorganic builder

The composition of the invention preferably comprises an inorganic builder. Suitable inorganic builders are selected from the group consisting of carbonate, silicate and mixtures thereof. Especially preferred for use herein is sodium carbonate. Preferably the composition of the invention comprises from 5 to 60%, more preferably from 10 to 50% and especially from 15 to 45% of sodium carbonate by weight of the composition.

Surfactant

Surfactants suitable for use herein include non-ionic surfactants, preferably the compositions are free of any other surfactants. Traditionally, non-ionic surfactants have been used in automatic dishwashing for surface modification purposes in particular for sheeting to avoid filming and spotting and to improve shine. It has been found that non-ionic surfactants can also contribute to prevent redeposition of soils.

Preferably the composition of the invention comprises a non-ionic surfactant or a non-ionic surfactant system, more preferably the non-ionic surfactant or a non-ionic surfactant system has a phase inversion temperature, as measured at a concentration of 1% in distilled water, between 40 and 70°C, preferably between 45 and 65°C. By a "non-ionic surfactant system" is meant herein a mixture of two or more non-ionic surfactants. Preferred for use herein are non-ionic surfactant systems. They seem to have improved cleaning and finishing properties and better stability in product than single non-ionic surfactants.

Phase inversion temperature is the temperature below which a surfactant, or a mixture thereof, partitions preferentially into the water phase as oil-swollen micelles and above which it partitions preferentially into the oil phase as water swollen inverted micelles. Phase inversion temperature can be determined visually by identifying at which temperature cloudiness occurs.

The phase inversion temperature of a non-ionic surfactant or system can be determined as follows: a solution containing 1% of the corresponding surfactant or mixture by weight of the solution in distilled water is prepared. The solution is stirred gently before phase inversion temperature analysis to ensure that the process occurs in chemical equilibrium. The phase inversion temperature is taken in a thermostable bath by immersing the solutions in 75 mm sealed glass test tube. To ensure the absence of leakage, the test tube is weighed before and after phase inversion temperature measurement. The temperature is gradually increased at a rate of less than 1°C per minute, until the temperature reaches a few degrees below the pre-estimated phase inversion temperature. Phase inversion temperature is determined visually at the first sign of turbidity.

Suitable nonionic surfactants include: i) ethoxylated non-ionic surfactants prepared by the reaction of a monohydroxy alkanol or alkyphenol with 6 to 20 carbon atoms with preferably at least 12 moles particularly preferred at least 16 moles, and still more preferred at least 20 moles of ethylene oxide per mole of alcohol or alkylphenol; ii) alcohol alkoxylated surfactants having a from 6 to 20 carbon atoms and at least one ethoxy and propoxy group. Preferred for use herein are mixtures of surfactants i) and ii).

Another suitable non-ionic surfactants are epoxy-capped poly(oxyalkylated) alcohols represented by the formula:

$$R1O[CH2CH(CH3)O]x[CH2CH2O]y[CH2CH(OH)R2] \quad (I)$$

wherein R1 is a linear or branched, aliphatic hydrocarbon radical having from 4 to 18 carbon atoms; R2 is a linear or branched aliphatic hydrocarbon radical having from 2 to 26 carbon atoms; x is an integer having an average value of from 0.5 to 1.5, more preferably about 1; and y is an integer having a value of at least 15, more preferably at least 20.

Preferably, the surfactant of formula I, at least about 10 carbon atoms in the terminal epoxide unit [CH2CH(OH)R2]. Suitable surfactants of formula I, according to the present invention, are Olin Corporation's POLY-TERGENT® SLF-18B nonionic surfactants, as described, for example, in WO 94/22800, published October 13, 1994 by Olin Corporation.

Enzymes

Other proteases

The composition of the invention can comprise a protease in addition to the protease of the invention. A mixture of two or more proteases can contribute to an enhanced cleaning across a broader temperature, cycle duration, and/or substrate range, and provide superior shine benefits, especially when used in conjunction with an anti-redeposition agent and/or a sulfonated polymer.

Suitable proteases for use in combination with the variant proteases of the invention include metalloproteases and serine proteases, including neutral or alkaline microbial serine proteases, such as subtilisins (EC 3.4.21.62). Suitable proteases include those of animal, vegetable or microbial origin. In one aspect, such suitable protease may be of microbial origin. The suitable proteases include chemically or genetically modified mutants of the aforementioned suitable proteases. In one aspect, the suitable protease may be a serine protease, such as an alkaline microbial protease or/and a trypsin-type protease. Examples of suitable neutral or alkaline proteases include:

(a) subtilisins (EC 3.4.21.62), especially those derived from Bacillus, such as Bacillus sp., B. lentus, B. alkalophilus, B. subtilis, B. amyloliquefaciens, B. pumilus , B. gibsonii, and B. akibaii described in WO2004067737, WO2015091989, WO2015091990, WO2015024739, WO2015143360, US 6,312,936 B1, US 5,679,630, US 4,760,025, DE102006022216A1, DE102006022224A1, WO2015089447, WO2015089441, WO2016066756, WO2016066757, WO2016069557, WO2016069563, WO2016069569.

(b) trypsin-type or chymotrypsin-type proteases, such as trypsin (e.g., of porcine or bovine origin), including the Fusarium protease described in WO 89/06270 and the chymotrypsin proteases derived from Cellumonas described in WO 05/052161 and WO 05/052146.

(c) metalloproteases, especially those derived from Bacillus amyloliquefaciens decribed in WO07/044993A2; from Bacillus, Brevibacillus, Thermoactinomyces, Geobacillus, Paenibacillus, Lysinibacillus or Streptomyces spp. Described in WO2014194032, WO2014194054 and WO2014194117; from Kribella alluminosa described in WO2015193488; and from Streptomyces and Lysobacter described in WO2016075078.

(d) protease having at least 90% identity to the subtilase from Bacillus sp. TY145, NCIMB 40339, described in WO92/17577 (Novozymes A/S), including the variants of this Bacillus sp TY145 subtilase described in WO2015024739, and WO2016066757.

Especially preferred additional proteases for the detergent of the invention are polypeptides demonstrating at least 90%, preferably at least 95%, more preferably at least 98%, even more preferably at least 99% and especially 100% identity with the wild-type enzyme from Bacillus lentus, comprising mutations in one or more, preferably two or more and more preferably three or more of the following positions, using the BPN' numbering system and amino acid abbreviations as illustrated in WO00/37627, which is incorporated herein by reference:V68A, N76D, N87S, S99D, S99SD, S99A, S101G, S101M, S103A, V104N/I, G118V, G118R, S128L, P129Q, S130A, Y167A, R170S, A194P, V205I, Q206L/D/E, Y209W and/or M222S.

Most preferably the additional protease is selected from the group of proteases comprising the below mutations (BPN' numbering system) versus either the PB92 wild-type (SEQ ID NO:2 in WO 08/010925) or the subtilisin 309 wild-type (sequence as per PB92 backbone, except comprising a natural variation of N87S).

(i) G118V + S128L + P129Q + S130A

(ii) S101M + G118V + S128L + P129Q + S130A

(iii) N76D + N87R + G118R + S128L + P129Q + S130A + S188D + N248R

(iv) N76D + N87R + G118R + S128L + P129Q + S130A + S188D + V244R

(v) N76D + N87R + G118R + S128L + P129Q + S130A

(vi) V68A + N87S + S101G + V104N

(vii) S99AD

Suitable commercially available additional protease enzymes include those sold under the trade names Alcalase®, Savinase®, Primase®, Durazym®, Polarzyme®, Kannase®, Liquanase®, Liquanase Ultra®, Savinase Ultra®, Ovozyme®, Neutrase®, Everlase®, Coronase®, Blaze®, Blaze Ultra® and Esperase® by Novozymes A/S (Denmark); those sold under the tradename Maxatase®, Maxacal®, Maxapem®, Properase®, Purafect®, Purafect Prime®, Purafect Ox®, FN3®, FN4®, Excellase®, Ultimase® and Purafect OXP® by Dupont; those sold under the tradename Opticlean® and Optimase® by Solvay Enzymes; and those available from Henkel/Kemira, namely BLAP (sequence shown in Figure29 of US 5,352,604 with the following mutations S99D + S101 R + S103A + V104I + G159S, hereinafter referred to as BLAP), BLAP R (BLAP with S3T + V4I + V199M + V205I + L217D), BLAP X (BLAP with S3T + V4I + V205I) and BLAP F49 (BLAP with S3T + V4I + A194P + V199M + V205I + L217D); and KAP (Bacillus alkalophilus subtilisin with mutations A230V + S256G + S259N) from Kao.

Especially preferred for use herein in combination with the variant protease of the invention are commercial proteases selected from the group consisting of Properase®, Blaze®, Ultimase®, Everlase®, Savinase®, Excellase®, Blaze Ultra®, BLAP and BLAP variants.

Preferred levels of protease in the product of the invention include from about 0.05 to about 10, more preferably from about 0.5 to about 7 and especially from about 1 to about 6 mg of active protease/g of composition.

Amylases

Preferably the composition of the invention may comprise an amylase. Suitable alpha-amylases include those of bacterial or fungal origin. Chemically or genetically modified mutants (variants) are included. A preferred alkaline alpha-amylase is derived from a strain of Bacillus, such as Bacillus licheniformis, Bacillus amyloliquefaciens, Bacillus stearothermophilus, Bacillus subtilis, or other Bacillus sp., such as Bacillus sp. NCBI 12289, NCBI 12512, NCBI 12513, DSM 9375 (USP 7,153,818) DSM 12368, DSMZ no. 12649, KSM AP1378 (WO 97/00324), KSM K36 or KSM K38 (EP 1,022,334). Preferred amylases include:

(a) variants described in WO 96/23873, WO00/60060, WO06/002643 and WO2017/192657, especially the variants with one or more substitutions in the following positions versus SEQ ID NO. 11:

26, 30, 33, 82, 37, 106, 118, 128, 133, 149, 150, 160, 178, 182, 186, 193, 202, 214, 231, 246, 256, 257, 258, 269, 270, 272, 283, 295, 296, 298, 299, 303, 304, 305, 311, 314, 315, 318, 319, 339, 345, 361, 378, 383, 419, 421, 437, 441, 444, 445, 446, 447, 450, 461, 471, 482, 484, preferably that also contain the deletions of D183* and G184*.

(b) variants exhibiting at least 90% identity with SEQ ID No. 4 in WO06/002643, the wild-type enzyme from Bacillus SP722, especially variants with deletions in the 183 and 184 positions and variants described in WO 00/60060, WO2011/100410 and WO2013/003659which are incorporated herein by reference.

(c) variants exhibiting at least 95% identity with the wild-type enzyme from Bacillus sp.707 (SEQ ID NO:7 in US 6,093, 562), especially those comprising one or more of the following mutations M202, M208, S255, R172, and/or M261. Preferably said amylase comprises one or more of M202L, M202V, M202S, M202T, M202I, M202Q, M202W, S255N and/or R172Q. Particularly preferred are those comprising the M202L or M202T mutations.

(d) variants described in WO 09/149130, preferably those exhibiting at least 90% identity with SEQ ID NO: 1 or SEQ ID NO:2 in WO 09/149130, the wild-type enzyme from Geobacillus Stearophermophilus or a truncated version thereof.

(e) variants exhibiting at least 89% identity with SEQ ID NO:1 in WO2016091688, especially those comprising deletions at positions H183+G184 and additionally one or more mutations at positions 405, 421, 422 and/or 428.

(f) variants exhibiting at least 60% amino acid sequence identity with the "PcuAmyl α-amylase" from Paenibacillus curdlanolyticus YK9 (SEQ ID NO:3 in WO2014099523).

(g) variants exhibiting at least 60% amino acid sequence identity with the "CspAmy2 amylase" from Cytophaga sp. (SEQ ID NO:1 in WO2014164777).

(h) variants exhibiting at least 85% identity with AmyE from Bacillus subtilis (SEQ ID NO:1 in WO2009149271).

(i) variants exhibiting at least 90% identity with the wild-type amylase from Bacillus sp. KSM-K38 with accession number AB051102.

(j) variants exhibiting at least 80% identity with the mature amino acid sequence of AAI10 from Bacillus sp (SEQ ID NO:7 in WO2016180748)

(k) variants exhibiting at least 80% identity with the mature amino acid sequence of Alicyclobacillus sp. amylase (SEQ ID NO:8 in WO2016180748)

Preferably the amylase is an engineered enzyme, wherein one or more of the amino acids prone to bleach oxidation have been substituted by an amino acid less prone to oxidation. In particular it is preferred that methionine residues are substituted with any other amino acid. In particular it is preferred that the methionine most prone to oxidation is substituted. Preferably the methionine in a position equivalent to 202 in SEQ ID NO:2 is substituted. Preferably, the methionine at this position is substituted with threonine or leucine, preferably leucine.

Suitable commercially available alpha-amylases include DURAMYL®, LIQUEZYME®, TERMAMYL®, TERMAMYL ULTRA®, NATALASE®, SUPRAMYL®, STAINZYME®, STAINZYME PLUS®, FUNGAMYL®, ATLANTIC®, INTENSA® and BAN® (Novozymes A/S, Bagsvaerd, Denmark), KEMZYM® AT 9000 Biozym Biotech Trading GmbH Wehlistrasse 27b A-1200 Wien Austria, RAPIDASE® , PURASTAR®, ENZYSIZE®, OPTISIZE HT PLUS®, POWERASE®, PREFERENZ S® series (including PREFERENZ S1000® and PREFERENZ

S2000® and PURASTAR OXAM® (DuPont., Palo Alto, California) and KAM® (Kao, 14-10 Nihonbashi Kayabacho, 1-chome, Chuo-ku Tokyo 103-8210, Japan). In one aspect, suitable amylases include ATLANTIC®, STAINZYME®, POWERASE®, INTENSA® and STAINZYME PLUS® and mixtures thereof.

Preferably, the product of the invention comprises at least 0.01 mg, preferably from about 0.05 to about 10, more preferably from about 0.1 to about 6, especially from about 0.2 to about 5 mg of active amylase/ g of composition.

Preferably, the protease and/or amylase of the composition of the invention are in the form of granulates, the granulates comprise more than 29% of sodium sulfate by weight of the granulate and/or the sodium sulfate and the active enzyme (protease and/or amylase) are in a weight ratio of between 3:1 and 100:1 or preferably between 4:1 and 30:1 or more preferably between 5:1 and 20:1.

Crystal growth inhibitor

Crystal growth inhibitors are materials that can bind to calcium carbonate crystals and prevent further growth of species such as aragonite and calcite.

Examples of effective crystal growth inhibitors include phosphonates, polyphosphonates, inulin derivatives, polyitaconic acid homopolymers and cyclic polycarboxylates.

Suitable crystal growth inhibitors may be selected from the group comprising HEDP (1-hydroxyethylidene 1,1-diphosphonic acid), carboxymethylinulin (CMI), tricarballylic acid and cyclic carboxylates. For the purposes of this invention the term carboxylate covers both the anionic form and the protonated carboxylic acid form.

Cyclic carboxylates contain at least two, preferably three or preferably at least four carboxylate groups and the cyclic structure is based on either a mono- or bi-cyclic alkane or a heterocycle. Suitable cyclic structures include cyclopropane, cyclobutane, cyclohexane or cyclopentane or cycloheptane, bicyclo-heptane or bicyclo-octane and/or tetrhaydrofuran. One preferred crystal growth inhibitor is cyclopentane tetracarboxylate.

Cyclic carboxylates having at least 75%, preferably 100% of the carboxylate groups on the same side, or in the “cis” position of the 3D-structure of the cycle are preferred for use herein. It is preferred that the two carboxylate groups, which are on the same side of the cycle are in directly neighbouring or “ortho” positions.

Preferred crystal growth inhibitors include HEDP, tricarballylic acid, tetrahydrofurantetracarboxylic acid (THFTCA) and cyclopentanetetracarboxylic acid (CPTCA).

The THFTCA is preferably in the 2c,3t,4t,5c-configuration, and the CPTCA in the cis,cis,cis,cis-configuration. Especially preferred crystal growth inhibitor for use herein is HEDP.

Also preferred for use herein are partially decarboxylated polyitaconic acid homopolymers, preferably having a level of decarboxylation is in the range of 50 mole % to 90 mole %. Especially preferred polymer for use herein is Itaconix TSI® provided by Itaconix.

The crystal growth inhibitors are present preferably in a quantity from about 0.01 to about 10 %, particularly from about 0.02 to about 5 % and in particular, from 0.05 to 3 % by weight of the composition.

Metal Care Agents

Metal care agents may prevent or reduce the tarnishing, corrosion or oxidation of metals, including aluminium, stainless steel and non-ferrous metals, such as silver and copper. Preferably the composition of the invention comprises from 0.1 to 5%, more preferably from 0.2 to 4% and especially from 0.3 to 3% by weight of the product of a metal care agent, preferably the metal care agent is benzo triazole (BTA).

Glass Care Agents

Glass care agents protect the appearance of glass items during the dishwashing process. Preferably the composition of the invention comprises from 0.1 to 5%, more preferably from 0.2 to 4% and specially from 0.3 to 3% by weight of the composition of a metal care agent, preferably the glass care agent is a zinc containing material, specially hydrozincite. Other suitable glass care agents are polyethyleneimine (PEI). A particularly preferred PEI is Lupasol® FG, supplied by BASF.

The automatic dishwashing composition of the invention preferably has a pH as measured in 1% weight/volume aqueous solution in distilled water at 20°C of from about 9 to about 12, more preferably from about 10 to less than about 11.5 and especially from about 10.5 to about 11.5. The automatic dishwashing composition of the invention preferably has a reserve alkalinity of from about 10 to about 20, more preferably from about 12 to about 18 at a pH of 9.5 as measured in NaOH with 100 grams of product at 20°C.

A preferred automatic dishwashing composition of the invention comprises:

i) from 10 to 20% by weight of the composition of sodium percarbonate;

ii) from 10% to 50% by weight of the composition of an organic complexing agent system, preferably the complexing agent system comprises MGDA;

iii) TAED;

iv) amylases;

v) optionally but preferably from 5 to 50% by weight of the composition of an inorganic builder, preferably sodium carbonate;

vi) optionally but preferably from 2 to 10% by weight of the composition of a non-ionic surfactant;

vii) other optional ingredients include: a crystal growth inhibitor, preferably HEDP, and glass care agents.

A preferred automatic dishwashing composition of the invention comprises:

i) from 10 to 20% by weight of the composition of bleach, preferably sodium percarbonate;

ii) from 10% to 50% by weight of the composition of an organic complexing agent system;

iii) a manganese bleach catalyst and optionally TAED;

iv) amylases;

v) optionally but preferably from 5 to 50% by weight of the composition of an inorganic builder, preferably sodium carbonate;

vi) optionally but preferably from 2 to 10% by weight of the composition of a non-ionic surfactant;

vii) optionally but preferably a glass care agent.

The dimensions and values disclosed herein are not to be understood as being strictly limited to the exact numerical values recited. Instead, unless otherwise specified, each such dimension is intended to mean both the recited value and a functionally equivalent range surrounding that value. For example, a dimension disclosed as "40 mm" is intended to mean "about 40 mm."

EXAMPLES

The compositions displayed in Table 1 were used. 3 g of each composition were dissolved in a litre of deionized water to produce a cleaning solution having a pH of 11. The protease of the invention is added to the cleaning solution at a level between 0.25-0.75ppm. Good crème brulee removal is obtained.

Table 1: Automatic Dishwashing Compositions

Ingredients (active weight %)	ADW Formula A	ADW Formula B	ADW Formula C
Solid ingredients			
Sodium carbonate	41.7	41.7	41.7
Sodium sulphate	0.00	1.68	2.03
MGDA	21.0	0.00	10.1
Sodium citrate	0.00	19.2	10.1
TAED	1.68	1.68	1.68
Sodium percarbonate	12.6	12.6	12.6
Sulfonated polymer	2.5	2.5	2.5
Bleach catalyst	1.2	1.2	1.2
Amylase	0.11	0.11	0.11
Liquid ingredients			
Lutensol TO7	19.3	19.3	19.3

Table 1: Automatic Dish Washing (ADW) Compositions

Amylase	Stainzyme® Plus supplied by Novozymes
TAED	Tetraacetylethylenediamine
MGDA	Three-sodium methyl glycine diacetate supplied by BASF
Bleach catalyst	MnTACN (Manganese 1,4,7-Triazacyclononane)
Sulfonated polymer	Acusol 588 supplied by Dow Chemicals
Lutensol TO7	Nonionic surfactant supplied by BASF

Cleaning Performance in Detergent

Egg Yolk stain

The automatic dishwashing (ADW) cleaning performance of the protease variants described herein was tested relative to the reference protease having the amino acid sequence of SEQ ID NO:1 using egg yolk (PAS-38, Center for Testmaterials BV, Vlaardingen, Netherlands) microswatches and the GSM-B detergent (see below Table 1), pH 10.5 and in microtiter plates (MTPs). Pre-punched PAS-38 (to fit on MTP) rinsed and unrinsed swatches were used in this assay. Rinsed swatches were prepared by adding 180µL 10mM CAPS buffer pH 11 to MTPs containing the PAS-38 microswatches and shaking for 30 min at 60°C and 1100 rpm. After

incubation, the buffer was removed, the swatches rinsed with deionized water to remove any residual buffer, and the MTPs air dried prior to use in the assay. All microswatch plates were filled prior to enzyme addition with 3 g/l GSM-B detergent adjusted to 374ppm water hardness. After incubating the PAS-38 swatches with detergent and enzymes for 30 min at 40°C, absorbance was read at 405nm with a SpectraMax plate reader. Absorbance results were obtained by subtracting the value for a blank control (no enzyme) from each sample value (hereinafter "blank subtracted absorbance"). For each condition and variant, a performance index (PI) was calculated by dividing the blank subtracted absorbance by that of the reference protease at the same concentration. The value for the reference protease was determined from a standard curve of the reference protease which was included in the test and which was fitted to a Langmuir fit or Hill Sigmoidal fit, as appropriate.

Crème Brûlée stain

The cleaning performance of the variants on crème brûlée stain was tested by using custom ordered melamine dishwasher monitors (tiles) prepared by CFT in Vlaardingen, the Netherlands as set forth herein, and labeled DM10c. The DM10c tiles used in this study are prepared using 2.7 g of the same material used to prepare the commercially available DM10 monitors (crème brûlée Debic.com product) but baked at 140°C for 2 hours, instead of 150°C. The melamine tiles were used as a lid and tightly pressed onto a microtiter plate (MTP). 3 g/L of GSMB or MGDA detergent (Tables 1 and 2, respectively) adjusted to 374ppm water hardness and each enzyme sample were added to the MTP prior to attaching the melamine tile lid to the MTP. The volume capacity of the MTP, and therefore the volume of solution added thereto, may vary, wherein a minimal volume of solution should be added to the MTP that enables contact between solution and stain surface under the incubation conditions. In this example, a volume of 300µL of detergent containing enzyme was added to each well of an aluminum 96-well MTP. The MTPs were incubated in an Infors thermal shaker for 45 min at 40°C at 250 rpm. After incubation, the tiles were removed from the MTP and air-dried.

Stain removal was quantified by photographing the plates and measuring the RGB values from each stain area using custom software. Percent Soil removal (%SRI) values of the washed tiles were calculated by using the RGB values in the following formula:

$$\% \text{ SRI} = (\Delta E/\Delta E_{\text{initial}}) * 100$$

Where $\Delta E = \text{SQR}((R_{\text{after}} - R_{\text{before}})^2 + (G_{\text{after}} - G_{\text{before}})^2 + (B_{\text{after}} - B_{\text{before}})^2)$

Where $\Delta E_{\text{initial}} = \text{SQR}((R_{\text{white}} - R_{\text{before}})^2 + (G_{\text{white}} - G_{\text{before}})^2 + (B_{\text{white}} - B_{\text{before}})^2)$

Cleaning performance was obtained by subtracting the value of a blank control (no

enzyme) from each sample value (hereinafter "blank subtracted cleaning"). For each condition and variant, a performance index (PI) was calculated by dividing the blank subtracted cleaning by that of the reference protease (having amino acid SEQ ID NO: 1) at the same concentration. The value for the parent protease PI was determined from a standard curve of the parent protease which was included in the test and which was fitted to a Langmuir fit or Hill Sigmoidal fit, as appropriate.

Table 1: GSM-B pH 10.5 Phosphate-Free ADW Detergent Ingredients	
Component	Weight %
Sodium citrate dehydrate	30.0
Maleic acid/acrylic acid copolymer sodium salt (SOKALAN® CP5; BASF)	12.0
Sodium perborate monohydrate	5.0
TAED	2.0
Sodium disilicate: Protil A (Cognis)	25.0
Linear fatty alcohol ethoxylate	2.0
Sodium carbonate anhydrous	add to 100

Table 2: MGDA pH 10.5 ADW Detergent Ingredients	
Component	Weight %
MGDA	64.6
Plurafac SLF 18-45D	4.4
Bismuthcitrate	0.4
Phosphonates (Bayhibit S)	0.4
Acusol 420 / Acosul 587	1.6
PEG6000	2.4
PEG1500	5.9
Sodium percarbonate	16.1
TAED	4.1

AAPF Activity Assay

The protease activity of the reference protease (having amino acid SEQ ID NO:1) and the variants thereof was tested by measuring hydrolysis of N-suc-AAPF-pNA. The reagent solutions used for the AAPF hydrolysis assay were: 100 mM Tris/HCl pH 8.6, containing

0.005% TWEEN®-80 (Tris dilution buffer); 100 mM Tris buffer pH 8.6, containing 10 mM $CaCl_2$ and 0.005% TWEEN®-80 (Tris/Ca buffer); and 160 mM suc-AAPF-pNA in DMSO (suc-AAPF-pNA stock solution) (Sigma: S-7388). A substrate working solution was prepared by adding 1 mL suc-AAPF-pNA stock solution to 100 mL Tris/Ca buffer and mixed well. An enzyme sample was added to a MTP (Greiner 781101) containing 1 mg/suc-AAPF-pNA working solution and assayed for activity at 405 nm over 3 min with a SpectraMax plate reader in kinetic mode at room temperature (RT). The absorbance of a blank containing no protease was subtracted from each sample reading. The protease activity was expressed as $mOD \cdot min^{-1}$.

Stability Assay

The stability of the variants described herein was measured by diluting the variants in stress buffer and measuring the proteolytic activity of the variants before and after a heat incubation step using the AAPF assay described above. The temperature and duration of the heat incubation step were chosen such that the reference protease showed approximately 30 - 45% residual activity. Samples were incubated at 56 °C for 5 min in a 384-well thermocycler. Stability was measured in Tris-EDTA (50mM Tris pH 9; 5 mM EDTA; 0.005% Tween 80) buffered condition. Stability PIs were obtained by dividing the residual activity of variant by that of the reference protease.

Performance of the variants in Dish Applications

The cleaning performance of the reference protease (having amino acid sequence SEQ ID: NO 1) and variants thereof was evaluated in the following cleaning assays: the PAS-38 technical stain using the GSM-B detergent, and the Crème Brûlée stain using either GSM-B or MGDA detergents, and in the stability assay described herein above. The results for these evaluations of the reference protease and variants thereof are reported as Performance Index (PI) values calculated versus the reference protease are shown on Table 4.

SEQ ID NO:1 with the following amino acid substitutions	Stability in EDTA with respect to the reference protease (SEQ ID NO:1)	ADW EGG performance with respect to SEQ ID NO:1		Crème Brûlée performance with respect to SEQ ID NO:1	
		ADW Rinsed EGG Stain	ADW Unrinsed EGG Stain	GSM-B Detergent	MGDA Detergent
T003V	1.4	0.9	1.0	1.0	1.0
V004T	1.2	1.0	1.0	0.9	0.9

SEQ ID NO:1 with the following amino acid substitutions	Stability in EDTA with respect to the reference protease (SEQ ID NO:1)	ADW EGG performance with respect to SEQ ID NO:1		Crème Brûlée performance with respect to SEQ ID NO:1	
		ADW Rinsed EGG Stain	ADW Unrinsed EGG Stain	GSM-B Detergent	MGDA Detergent
I008V	1.2	1.0	1.0	1.0	1.1
T009A	1.3	1.0	1.0	1.2	1.1
T009C	1.0	1.0	0.9	1.5	1.7
T009E	1.2	< 0.9	0.9	1.3	1.5
T009G	1.1	< 0.9	0.9	0.9	1.0
T009H	1.2	1.1	1.1	1.0	1.1
T009K	1.1	1.1	1.2	< 0.9	< 0.9
T009M	1.0	1.0	1.0	1.3	1.2
T009N	1.1	0.9	1.1	1.2	1.3
T009Q	1.2	0.9	1.0	1.1	1.1
T009S	1.2	1.0	1.0	1.0	1.1
T009W	1.2	1.1	1.1	1.0	1.1
T009Y	1.2	1.0	1.0	1.2	1.1
R010A	1.0	1.0	0.9	1.8	1.7
R010K	0.9	0.9	1.0	1.2	1.0
R010M	1.0	0.9	1.0	2.1	1.8
R010N	1.0	0.9	< 0.9	1.7	1.8
R010Q	1.1	< 0.9	< 0.9	1.5	1.6
R010W	1.0	< 0.9	< 0.9	1.1	1.2
V011A	1.1	1.0	1.1	1.3	1.4
V011I	1.0	1.0	1.2	1.1	1.1
V011S	1.0	1.0	1.1	1.0	1.0
V011T	1.0	1.0	1.0	1.1	1.0
Q012A	1.1	1.0	1.1	1.1	1.2
Q012C	1.1	0.9	0.9	1.4	1.6
Q012D	1.0	0.9	0.9	1.5	1.6
Q012E	1.0	0.9	0.9	1.3	1.4
Q012G	1.1	1.0	1.1	0.9	1.0
Q012M	1.1	0.9	1.1	1.1	1.1
Q012N	1.2	0.9	1.1	1.1	1.1
Q012R	1.0	0.9	1.1	< 0.9	< 0.9
Q012S	1.0	0.9	1.1	0.9	< 0.9
Q012T	1.1	1.0	0.9	< 0.9	0.9
Q012V	1.0	0.9	1.1	< 0.9	< 0.9
Q012W	0.9	1.0	1.1	< 0.9	< 0.9
P014D	1.0	1.0	0.9	1.2	1.4

SEQ ID NO:1 with the following amino acid substitutions	Stability in EDTA with respect to the reference protease (SEQ ID NO:1)	ADW EGG performance with respect to SEQ ID NO:1		Crème Brûlée performance with respect to SEQ ID NO:1	
		ADW Rinsed EGG Stain	ADW Unrinsed EGG Stain	GSM-B Detergent	MGDA Detergent
A015D	1.0	1.0	1.0	1.2	1.4
A015E	1.0	1.0	0.9	0.9	1.4
A015F	1.1	1.1	1.1	< 0.9	1.0
A015H	1.1	1.0	1.0	1.4	1.0
A015I	1.0	1.1	1.1	1.4	1.0
A015K	1.0	1.0	1.1	1.0	< 0.9
A015M	1.1	0.9	1.1	1.8	1.2
A015P	1.1	1.0	1.0	< 0.9	1.0
A015Q	1.1	1.0	1.0	0.9	1.0
A015V	1.0	1.1	1.0	1.6	1.1
A015W	1.1	1.0	0.9	1.3	1.0
A015Y	1.0	1.0	0.9	1.7	1.1
V016L	1.0	1.0	1.1	1.1	0.9
V016M	1.0	1.0	1.1	1.6	1.0
V016S	1.1	0.9	0.9	0.9	0.9
H017C	1.3	1.0	0.9	1.2	1.3
H017E	1.2	< 0.9	0.9	1.2	1.0
H017F	1.3	1.1	1.1	0.9	< 0.9
H017G	1.0	1.1	1.0	< 0.9	0.9
H017I	1.4	1.1	1.1	< 0.9	< 0.9
H017L	1.4	1.1	1.1	< 0.9	< 0.9
H017N	1.1	0.9	1.1	1.0	0.9
H017V	1.2	1.1	1.0	< 0.9	< 0.9
H017W	1.3	1.1	1.0	< 0.9	< 0.9
H017Y	1.1	0.9	1.1	< 0.9	< 0.9
N018A	1.1	1.0	1.0	1.0	1.2
N018C	1.0	0.9	0.9	1.3	1.2
N018D	1.1	1.0	0.9	1.3	1.5
N018E	1.1	ND	< 0.9	1.2	1.4
N018F	1.0	1.1	1.0	1.0	1.1
N018G	1.0	1.0	1.0	1.0	1.2
N018L	1.1	0.9	1.1	1.0	1.1
N018M	1.1	1.0	1.1	1.0	1.3
N018Q	1.1	1.0	1.0	1.0	1.2
N018T	1.0	1.0	1.0	1.0	1.1
R019A	1.0	1.0	0.9	1.3	1.5

SEQ ID NO:1 with the following amino acid substitutions	Stability in EDTA with respect to the reference protease (SEQ ID NO:1)	ADW EGG performance with respect to SEQ ID NO:1		Crème Brûlée performance with respect to SEQ ID NO:1	
		ADW Rinsed EGG Stain	ADW Unrinsed EGG Stain	GSM-B Detergent	MGDA Detergent
R019C	1.1	1.1	< 0.9	1.3	1.4
R019D	1.1	ND	0.9	1.6	1.7
R019E	1.0	0.9	< 0.9	1.6	1.7
R019F	1.0	0.9	0.9	1.5	1.6
R019H	1.0	0.9	0.9	1.2	1.4
R019I	1.0	1.0	0.9	1.3	1.3
R019K	1.0	1.1	1.0	1.1	1.0
R019L	1.0	1.0	1.1	1.4	1.3
R019N	1.0	ND	1.0	1.3	1.3
R019Q	1.0	1.1	0.9	1.2	1.4
R019S	1.0	1.0	0.9	1.3	1.5
R019T	1.0	ND	1.0	1.3	1.4
R019W	1.0	1.0	1.0	1.2	1.4
R019Y	1.1	0.9	1.0	1.2	1.3
G020A	1.0	1.1	1.1	1.0	1.0
G020C	1.1	1.0	1.0	1.2	1.1
G020D	1.1	1.1	1.0	1.1	1.0
G020M	1.1	1.0	1.1	0.9	< 0.9
G020N	1.1	1.0	1.1	0.9	0.9
G020T	1.0	1.1	1.0	< 0.9	< 0.9
S024A	1.1	0.9	1.2	1.3	1.0
S024E	1.3	1.0	1.0	1.2	1.2
G025A	1.0	1.1	1.1	1.3	1.0
G025C	1.1	0.9	0.9	1.2	1.1
G025D	1.1	1.1	0.9	1.3	1.1
G025E	1.0	1.0	0.9	1.2	1.1
G025M	1.0	1.0	1.0	1.2	0.9
G025N	1.1	1.0	1.1	1.2	1.0
V026A	1.0	0.9	< 0.9	1.1	1.0
V026I	1.1	1.0	1.1	1.0	1.1
R027K	1.1	1.0	1.0	1.1	1.3
S033T	1.1	0.9	< 0.9	< 0.9	< 0.9
S036A	1.1	1.1	1.1	0.9	0.9
S036C	1.1	1.0	1.0	1.4	1.4
S036E	1.0	1.0	0.9	1.5	1.7
S036I	1.1	1.0	0.9	< 0.9	1.1

SEQ ID NO:1 with the following amino acid substitutions	Stability in EDTA with respect to the reference protease (SEQ ID NO:1)	ADW EGG performance with respect to SEQ ID NO:1		Crème Brûlée performance with respect to SEQ ID NO:1	
		ADW Rinsed EGG Stain	ADW Unrinsed EGG Stain	GSM-B Detergent	MGDA Detergent
S036L	1.1	1.1	0.9	0.9	1.0
S036M	1.2	1.0	0.9	1.0	1.1
S036Q	1.1	1.0	1.0	1.1	1.1
S036T	1.0	1.0	1.1	1.1	1.1
S036V	1.1	0.9	< 0.9	1.0	1.2
N042C	1.4	1.0	0.9	1.2	1.4
N042D	1.2	1.0	1.0	1.1	1.5
N042E	1.5	1.0	1.0	1.0	1.4
N042M	1.2	1.0	0.9	1.0	1.0
N042Q	1.1	1.0	0.9	0.9	1.1
I043L	1.0	1.0	1.0	1.2	1.3
R044C	1.3	1.0	1.1	1.6	1.7
R044E	1.3	1.0	< 0.9	1.3	1.5
R044F	1.2	0.9	< 0.9	< 0.9	1.0
R044G	1.1	1.0	0.9	1.1	1.3
R044H	1.2	1.0	1.1	1.1	1.3
R044I	1.2	0.9	< 0.9	0.9	1.2
R044K	1.1	1.0	1.1	1.0	1.1
R044L	1.2	0.9	< 0.9	1.0	1.2
R044N	1.2	1.0	ND	1.3	1.3
R044Q	1.2	0.9	1.0	1.2	1.3
R044S	1.2	0.9	0.9	1.0	1.3
R044T	1.2	0.9	< 0.9	1.1	1.2
R044V	1.2	0.9	0.9	0.9	1.2
R044W	1.3	0.9	< 0.9	0.9	0.9
R044Y	1.3	0.9	< 0.9	0.9	0.9
A047I	1.1	1.0	1.0	0.9	1.0
A047Y	1.1	0.9	1.0	< 0.9	1.0
V050I	1.1	1.0	1.0	< 0.9	1.0
G052A	1.1	0.9	1.1	1.3	1.5
G052C	1.0	< 0.9	< 0.9	1.2	1.5
G052D	1.0	1.1	1.0	1.5	1.7
G052H	1.0	1.0	1.2	< 0.9	1.2
G052L	1.0	< 0.9	< 0.9	0.9	1.3
G052M	1.1	0.9	1.1	1.2	1.6
G052N	1.2	0.9	1.0	1.1	1.2

SEQ ID NO:1 with the following amino acid substitutions	Stability in EDTA with respect to the reference protease (SEQ ID NO:1)	ADW EGG performance with respect to SEQ ID NO:1		Crème Brûlée performance with respect to SEQ ID NO:1	
		ADW Rinsed EGG Stain	ADW Unrinsed EGG Stain	GSM-B Detergent	MGDA Detergent
G052S	1.1	0.9	1.0	0.9	1.0
G052T	1.1	0.9	1.0	< 0.9	1.1
G052Y	1.1	< 0.9	0.9	< 0.9	1.0
P054A	1.0	1.0	1.1	1.5	1.4
P054C	1.0	1.0	0.9	1.3	1.4
P054G	1.0	1.1	< 0.9	0.9	< 0.9
P054L	1.0	1.0	1.2	0.9	1.5
P054M	1.0	0.9	1.3	1.8	2.1
P054N	1.1	0.9	1.1	1.0	1.0
P054T	1.0	1.0	1.0	< 0.9	< 0.9
P054V	1.1	1.0	1.1	1.1	1.6
T055A	1.0	0.9	1.2	1.3	1.2
T055C	1.1	0.9	0.9	1.1	1.1
T055D	1.1	1.0	1.2	1.5	1.4
T055E	1.1	0.9	1.0	1.3	1.4
T055H	1.0	0.9	1.2	< 0.9	0.9
T055M	1.0	0.9	1.1	1.2	0.9
T055N	1.1	0.9	1.1	0.9	0.9
T055S	1.0	0.9	1.2	< 0.9	0.9
T055Y	1.0	< 0.9	1.1	< 0.9	< 0.9
A057D	1.0	1.0	1.0	1.4	1.6
A057E	1.1	1.0	1.0	1.3	1.4
A057H	1.2	1.0	1.0	0.9	1.0
A057M	1.2	1.0	1.1	1.1	1.1
A057N	1.1	0.9	0.9	1.0	0.9
A057Q	1.1	0.9	1.0	0.9	1.0
A057T	1.1	1.0	1.0	< 0.9	< 0.9
L059A	1.0	1.1	0.9	1.5	1.2
L059C	1.0	0.9	< 0.9	1.7	1.3
L059D	0.9	1.0	1.0	1.9	1.6
L059E	1.0	1.0	1.0	1.6	1.4
L059M	1.0	1.1	ND	1.3	1.1
L059N	1.1	1.1	1.0	1.4	1.1
L059Q	1.0	ND	1.0	1.2	1.0
L059T	1.0	1.0	0.9	1.2	1.2
N060S	1.0	1.0	1.2	1.4	1.4

SEQ ID NO:1 with the following amino acid substitutions	Stability in EDTA with respect to the reference protease (SEQ ID NO:1)	ADW EGG performance with respect to SEQ ID NO:1		Crème Brûlée performance with respect to SEQ ID NO:1	
		ADW Rinsed EGG Stain	ADW Unrinsed EGG Stain	GSM-B Detergent	MGDA Detergent
T069S	1.0	1.1	1.1	< 0.9	0.9
S076A	1.2	0.9	1.0	0.9	1.0
S076D	1.2	1.0	1.1	1.2	1.3
S076E	1.4	1.0	1.0	1.2	1.2
S076F	1.2	0.9	1.0	1.0	1.1
S076H	1.5	1.0	1.0	0.9	0.9
S076K	1.3	1.0	1.1	< 0.9	< 0.9
S076L	1.3	< 0.9	1.1	1.0	1.0
S076M	1.5	1.0	1.0	1.0	1.1
S076N	1.4	1.0	1.1	1.1	1.0
S076R	1.2	1.0	1.0	< 0.9	< 0.9
S076T	1.4	0.9	1.0	1.0	1.0
S076Y	1.2	0.9	0.9	0.9	< 0.9
V082A	1.1	1.0	1.1	1.2	1.0
P084D	1.2	1.0	< 0.9	1.2	1.2
P084F	1.1	1.0	1.0	1.0	1.0
P084H	1.4	1.0	0.9	< 0.9	< 0.9
P084Y	1.3	0.9	1.0	< 0.9	< 0.9
N085S	1.1	0.9	1.0	0.9	1.0
G095A	1.1	1.0	1.0	< 0.9	1.1
G095N	1.1	1.0	1.2	< 0.9	< 0.9
A096M	1.1	ND	< 0.9	1.1	1.1
A096Q	1.0	0.9	1.2	1.1	1.0
N097E	0.9	1.0	1.1	1.4	1.6
N097H	1.0	0.9	< 0.9	1.0	1.3
N097K	1.1	1.0	1.1	< 0.9	0.9
S101T	1.1	1.0	0.9	< 0.9	< 0.9
V102L	1.1	1.1	1.0	< 0.9	0.9
V102M	1.2	1.1	0.9	< 0.9	1.1
G104A	1.0	0.9	< 0.9	1.0	1.7
G104D	1.0	< 0.9	< 0.9	1.4	2.4
G104H	1.0	1.0	< 0.9	1.2	1.2
G104M	1.1	1.0	1.0	< 0.9	1.0
G104N	1.1	1.0	1.0	1.2	1.4
G104T	1.1	0.9	0.9	0.9	0.9
G104V	1.1	1.0	< 0.9	1.1	1.5

SEQ ID NO:1 with the following amino acid substitutions	Stability in EDTA with respect to the reference protease (SEQ ID NO:1)	ADW EGG performance with respect to SEQ ID NO:1		Crème Brûlée performance with respect to SEQ ID NO:1	
		ADW Rinsed EGG Stain	ADW Unrinsed EGG Stain	GSM-B Detergent	MGDA Detergent
G104W	1.1	1.0	< 0.9	< 0.9	0.9
G104Y	1.0	< 0.9	< 0.9	1.0	1.2
I105V	1.1	0.9	< 0.9	0.9	ND
Q107K	1.0	1.0	1.2	< 0.9	0.9
Q107M	1.1	1.0	1.0	1.0	1.1
E110L	1.0	< 0.9	1.4	< 0.9	< 0.9
A113T	1.0	1.1	0.9	1.0	ND
A113V	1.1	1.0	0.9	1.0	ND
T114V	1.1	1.0	1.0	< 0.9	1.0
N115E	1.0	ND	< 0.9	1.0	ND
N115H	1.0	0.9	1.0	1.2	ND
N115Q	1.1	1.0	< 0.9	0.9	ND
N116E	1.1	1.0	< 0.9	1.1	1.4
N116H	1.1	0.9	0.9	1.0	1.1
H118D	1.1	1.1	1.0	1.0	1.1
H118E	1.2	ND	< 0.9	0.9	1.0
H118N	1.1	1.0	1.0	0.9	0.9
A120V	1.0	1.1	1.1	< 0.9	1.1
M122L	1.0	1.0	1.3	< 0.9	1.0
F128G	0.8	1.1	1.1	2.0	1.9
P129A	1.0	1.1	1.1	0.9	ND
P129H	1.0	1.1	1.3	1.1	ND
P129N	1.0	0.9	1.2	< 0.9	ND
P129Y	1.0	< 0.9	1.2	< 0.9	ND
S131A	1.2	0.9	1.1	0.9	0.9
S131D	1.1	0.9	0.9	1.6	1.7
S131E	1.1	< 0.9	1.0	1.6	1.7
S131I	1.1	1.0	1.0	< 0.9	< 0.9
S131M	1.1	0.9	1.2	< 0.9	1.0
S131N	1.1	0.9	1.1	0.9	0.9
S131P	1.1	< 0.9	1.1	0.9	< 0.9
S131Q	1.1	0.9	1.1	0.9	< 0.9
S131T	1.1	0.9	1.1	0.9	0.9
S131V	1.1	0.9	1.0	< 0.9	< 0.9
L133M	1.1	1.0	0.9	1.0	< 0.9
R135A	1.2	0.9	< 0.9	1.6	1.3

SEQ ID NO:1 with the following amino acid substitutions	Stability in EDTA with respect to the reference protease (SEQ ID NO:1)	ADW EGG performance with respect to SEQ ID NO:1		Crème Brûlée performance with respect to SEQ ID NO:1	
		ADW Rinsed EGG Stain	ADW Unrinsed EGG Stain	GSM-B Detergent	MGDA Detergent
R135E	1.0	< 0.9	< 0.9	1.3	1.3
R135F	1.0	< 0.9	< 0.9	1.3	1.0
R135H	1.1	1.0	< 0.9	1.6	1.4
R135I	1.1	0.9	< 0.9	1.6	1.2
R135K	1.1	1.0	0.9	1.3	0.9
R135L	1.1	0.9	1.0	2.2	1.2
R135M	1.2	0.9	1.0	1.6	1.2
R135S	1.1	0.9	0.9	1.7	1.4
R135T	1.1	1.0	< 0.9	1.5	1.3
R135V	1.1	< 0.9	< 0.9	1.6	1.0
R135W	1.1	0.9	0.9	1.7	1.0
R135Y	1.1	< 0.9	< 0.9	1.7	0.9
A136M	1.1	1.0	1.2	< 0.9	< 0.9
V137L	1.0	0.9	1.0	< 0.9	ND
Y139E	1.0	0.9	1.0	1.3	ND
Y139S	1.0	1.0	0.9	1.0	ND
T141E	1.0	< 0.9	0.9	1.3	1.6
T141H	1.0	0.9	0.9	0.9	1.0
T141N	1.0	< 0.9	1.0	0.9	1.1
S142A	1.1	0.9	1.0	1.0	1.0
S142D	1.1	0.9	< 0.9	1.2	1.3
S142E	1.1	1.0	0.9	1.3	1.3
S142H	1.2	1.0	< 0.9	< 0.9	< 0.9
S142M	1.2	0.9	1.0	1.0	< 0.9
S142N	1.1	1.0	1.1	1.1	0.9
S142Q	1.1	1.0	1.1	0.9	0.9
R143E	1.1	0.9	< 0.9	0.9	1.2
R143H	1.1	1.0	0.9	1.1	0.9
R143M	1.1	1.0	1.1	< 0.9	< 0.9
R143N	1.1	0.9	1.1	< 0.9	< 0.9
R143Q	1.1	1.0	0.9	1.0	1.1
R143V	1.0	1.0	< 0.9	0.9	0.9
D144E	1.0	ND	0.9	0.9	1.4
D144N	1.0	1.1	1.1	< 0.9	0.9
V145C	1.0	1.1	1.0	1.0	1.1
V147C	1.1	< 0.9	< 0.9	1.0	1.2

SEQ ID NO:1 with the following amino acid substitutions	Stability in EDTA with respect to the reference protease (SEQ ID NO:1)	ADW EGG performance with respect to SEQ ID NO:1		Crème Brûlée performance with respect to SEQ ID NO:1	
		ADW Rinsed EGG Stain	ADW Unrinsed EGG Stain	GSM-B Detergent	MGDA Detergent
I148L	1.0	1.0	0.9	1.0	1.2
I148V	1.1	1.0	< 0.9	1.0	1.1
A150M	1.2	< 0.9	< 0.9	< 0.9	< 0.9
N154D	1.0	1.0	0.9	1.4	2.1
S156A	1.0	0.9	1.0	1.1	1.3
S156C	1.0	< 0.9	0.9	1.3	1.8
S156D	0.9	0.9	< 0.9	1.5	2.1
S156N	1.1	0.9	0.9	1.1	1.1
S156T	1.1	1.0	0.9	1.1	1.0
G157A	1.1	1.0	1.2	0.9	0.9
G157C	1.1	ND	1.0	1.2	2.0
G157D	1.0	0.9	1.1	1.4	1.9
G157E	1.0	ND	0.9	1.6	2.1
G157N	1.1	1.0	1.0	1.0	1.1
G157Q	1.0	0.9	0.9	1.1	1.1
S158A	1.0	1.0	1.0	1.0	1.0
S158C	1.1	0.9	0.9	1.4	1.8
S158F	1.1	0.9	0.9	< 0.9	< 0.9
S158L	1.1	1.0	0.9	< 0.9	1.3
S158M	1.1	1.0	1.0	1.0	0.9
S158N	1.1	1.0	< 0.9	1.0	1.0
S158Q	1.1	1.0	1.1	1.2	1.2
S158T	1.1	1.1	1.3	1.3	1.2
S158V	1.1	0.9	0.9	0.9	1.1
S158W	1.1	0.9	< 0.9	< 0.9	< 0.9
S158Y	1.2	1.0	1.0	< 0.9	1.4
V159L	1.1	1.1	0.9	1.2	1.4
G160A	1.3	1.0	1.0	< 0.9	1.0
G160C	1.1	ND	1.0	< 0.9	1.1
G160D	0.7	1.2	1.5	<0.9	<0.9
G160M	1.1	0.9	1.2	< 0.9	< 0.9
G160S	1.2	ND	1.2	0.9	< 0.9
G160T	1.1	ND	< 0.9	0.9	0.9
Y161W	1.0	1.0	1.0	< 0.9	1.1
R164A	1.0	< 0.9	0.9	1.6	2.6
R164K	1.0	1.0	0.9	1.2	1.3

SEQ ID NO:1 with the following amino acid substitutions	Stability in EDTA with respect to the reference protease (SEQ ID NO:1)	ADW EGG performance with respect to SEQ ID NO:1		Crème Brûlée performance with respect to SEQ ID NO:1	
		ADW Rinsed EGG Stain	ADW Unrinsed EGG Stain	GSM-B Detergent	MGDA Detergent
R164M	1.0	< 0.9	< 0.9	1.4	2.3
R164Q	1.0	0.9	< 0.9	1.4	2.0
R164Y	1.0	0.9	< 0.9	1.7	2.2
A166D	1.2	0.9	1.1	1.4	ND
A166E	1.1	1.0	1.0	1.3	ND
A166I	1.0	1.0	1.1	0.9	ND
A166P	1.1	1.0	1.1	< 0.9	ND
A166Q	1.1	0.9	1.0	1.0	ND
A166V	1.0	0.9	0.9	1.0	ND
N167E	1.0	0.9	< 0.9	1.1	ND
M169L	1.0	1.0	1.1	1.2	0.9
A170G	1.1	1.1	1.0	1.1	0.9
T174V	1.0	1.0	1.1	1.1	< 0.9
Q176A	1.0	1.0	1.0	1.1	1.1
Q176C	1.1	0.9	< 0.9	1.2	1.6
Q176D	1.0	0.9	1.0	2.1	1.4
Q176E	1.0	0.9	1.0	2.0	1.8
Q176L	1.0	1.1	1.0	0.9	1.1
Q176M	1.1	1.0	1.0	1.0	1.1
Q176N	1.0	0.9	1.0	1.3	1.1
Q176S	1.0	0.9	1.0	1.0	1.0
N177A	1.1	0.9	1.1	0.9	1.0
N177C	1.1	< 0.9	1.0	1.1	1.5
N177D	1.1	1.1	0.9	1.4	1.6
N177E	1.0	< 0.9	1.0	1.5	1.7
N177G	1.0	1.1	1.0	0.9	0.9
N177H	1.1	1.0	1.0	0.9	< 0.9
N177K	1.0	1.0	1.1	< 0.9	< 0.9
N177L	1.1	1.0	1.1	0.9	< 0.9
N177M	1.2	< 0.9	1.2	0.9	0.9
N177Q	1.1	0.9	1.1	1.0	0.9
N177S	1.0	1.1	1.2	0.9	< 0.9
N177W	1.1	0.9	1.0	< 0.9	< 0.9
N177Y	1.1	< 0.9	1.2	1.0	< 0.9
N178D	1.0	1.0	0.9	1.5	1.8
R179A	1.0	< 0.9	1.1	1.9	1.5

SEQ ID NO:1 with the following amino acid substitutions	Stability in EDTA with respect to the reference protease (SEQ ID NO:1)	ADW EGG performance with respect to SEQ ID NO:1		Crème Brûlée performance with respect to SEQ ID NO:1	
		ADW Rinsed EGG Stain	ADW Unrinsed EGG Stain	GSM-B Detergent	MGDA Detergent
R179C	1.0	< 0.9	0.9	1.9	1.6
R179E	1.0	< 0.9	1.0	2.0	2.0
R179F	1.0	ND	0.9	1.5	1.7
R179G	1.0	0.9	1.1	1.7	1.7
R179H	1.0	< 0.9	1.0	1.6	1.6
R179I	1.0	< 0.9	1.1	1.9	1.8
R179K	1.0	< 0.9	1.2	1.4	1.3
R179M	1.1	< 0.9	0.9	1.5	1.4
R179Q	1.1	1.0	1.0	1.8	1.7
R179S	1.0	ND	1.0	1.8	1.6
R179V	1.0	1.0	1.0	1.9	1.9
R179W	1.0	0.9	ND	1.5	1.5
R179Y	1.0	< 0.9	1.0	1.9	1.8
R180K	1.1	0.9	1.0	1.2	0.9
N182A	1.1	0.9	1.1	0.9	< 0.9
N182C	1.1	< 0.9	1.0	< 0.9	1.3
N182D	1.0	1.0	1.2	1.7	1.5
N182E	1.1	1.0	1.0	1.5	1.6
N182G	1.1	1.0	1.0	1.0	1.1
N182H	1.1	0.9	< 0.9	< 0.9	0.9
N182I	1.1	1.0	1.0	< 0.9	0.9
N182K	1.1	1.0	1.0	< 0.9	< 0.9
N182L	1.1	< 0.9	1.1	< 0.9	1.0
N182P	1.2	1.0	0.9	< 0.9	1.0
N182Q	1.1	1.0	0.9	1.0	1.1
N182S	1.2	1.1	0.9	1.0	1.1
N182T	1.1	0.9	< 0.9	1.0	1.1
N182V	1.1	1.0	0.9	< 0.9	1.0
N182W	1.2	0.9	< 0.9	< 0.9	< 0.9
N182Y	1.2	1.1	1.0	< 0.9	1.1
Y186F	1.0	1.0	0.9	1.0	1.1
T188A	1.1	0.9	1.0	< 0.9	0.9
T188C	1.1	0.9	0.9	1.2	1.5
T188D	1.1	1.0	0.9	1.4	1.3
T188E	1.2	1.0	0.9	1.4	1.4
T188I	1.1	0.9	1.0	0.9	< 0.9

SEQ ID NO:1 with the following amino acid substitutions	Stability in EDTA with respect to the reference protease (SEQ ID NO:1)	ADW EGG performance with respect to SEQ ID NO:1		Crème Brûlée performance with respect to SEQ ID NO:1	
		ADW Rinsed EGG Stain	ADW Unrinsed EGG Stain	GSM-B Detergent	MGDA Detergent
T188L	1.1	0.9	1.0	0.9	< 0.9
T188M	1.1	0.9	1.2	0.9	0.9
T188N	1.1	1.0	1.1	0.9	0.9
T188Q	1.1	1.0	1.0	0.9	0.9
T188S	1.0	0.9	1.1	0.9	< 0.9
T188V	1.1	1.0	1.0	0.9	< 0.9
T188W	1.1	0.9	1.0	< 0.9	< 0.9
T188Y	1.1	< 0.9	ND	0.9	< 0.9
G189C	1.0	< 0.9	< 0.9	1.4	1.4
G189D	1.1	0.9	< 0.9	1.3	1.5
G189E	1.0	0.9	0.9	1.3	1.8
I190M	1.0	0.9	1.0	0.9	0.9
D191E	1.0	1.1	0.9	0.9	1.1
I192C	1.1	1.0	1.0	0.9	1.1
I192M	1.1	1.0	1.0	0.9	1.1
V193A	1.0	1.0	1.0	1.5	1.3
V193M	1.1	0.9	1.1	1.2	1.4
N198D	1.0	1.0	1.1	1.9	1.7
N198E	1.0	0.9	< 0.9	1.8	1.6
Q200H	1.1	0.9	0.9	0.9	1.0
Q200I	1.3	0.9	< 0.9	0.9	0.9
Q200K	1.2	0.9	1.0	< 0.9	< 0.9
Q200M	1.2	0.9	1.0	1.0	1.1
Q200V	1.1	1.0	0.9	0.9	0.9
Q200Y	1.3	1.0	0.9	1.0	1.1
R207K	1.0	ND	0.9	1.1	1.3
R207L	1.0	1.1	1.0	1.5	1.9
R207N	0.9	1.0	0.9	1.8	1.6
R207Q	0.9	1.0	0.9	1.7	1.6
R207T	0.6	1.0	0.9	1.6	1.7
V209P	1.1	1.0	0.9	1.4	1.2
S210C	1.0	0.9	< 0.9	1.0	1.3
S210D	0.9	ND	0.9	1.8	1.6
S210E	1.1	1.2	0.9	1.7	1.7
S210F	1.2	1.0	0.9	< 0.9	0.9
S210G	1.0	1.1	1.0	< 0.9	1.0

SEQ ID NO:1 with the following amino acid substitutions	Stability in EDTA with respect to the reference protease (SEQ ID NO:1)	ADW EGG performance with respect to SEQ ID NO:1		Crème Brûlée performance with respect to SEQ ID NO:1	
		ADW Rinsed EGG Stain	ADW Unrinsed EGG Stain	GSM-B Detergent	MGDA Detergent
S210L	1.0	1.0	1.0	0.9	1.2
S210N	0.9	1.0	1.0	1.2	1.4
S210P	1.2	1.0	1.0	0.9	0.9
S210Q	1.0	1.1	0.9	0.9	1.1
S210Y	1.0	0.9	1.0	0.9	1.2
M211E	0.6	1.1	1.4	1.7	1.6
M211K	1.1	1.0	< 0.9	0.9	1.3
M211L	1.0	1.0	0.9	1.1	1.9
M211Q	0.9	1.2	1.1	1.3	1.3
M211R	1.0	1.1	1.1	< 0.9	< 0.9
N212A	1.0	< 0.9	0.9	< 0.9	1.0
N212C	1.0	< 0.9	< 0.9	< 0.9	1.3
N212Q	0.9	1.0	<0.8	1.2	1.2
N212S	1.2	0.9	0.9	< 0.9	1.0
T218C	1.1	1.0	1.0	0.9	0.9
T218S	1.2	1.0	1.1	< 0.9	< 0.9
A224V	1.0	1.0	1.0	0.9	0.9
L227M	1.0	1.0	1.1	1.0	0.9
L227Q	1.0	0.9	1.0	0.9	0.9
V228L	1.1	0.9	ND	1.0	1.2
Q230E	1.2	0.9	1.1	1.2	1.2
R231C	1.1	0.9	< 0.9	1.2	1.2
R231E	1.1	0.9	< 0.9	1.1	1.3
R231H	1.1	0.9	< 0.9	1.1	1.1
R231I	1.0	1.0	< 0.9	1.0	1.0
R231L	1.0	0.9	1.0	1.1	1.2
R231N	1.1	< 0.9	1.0	1.1	1.2
R231Q	1.0	0.9	1.0	1.0	1.2
R231S	1.0	< 0.9	1.0	1.0	1.1
R231T	1.1	0.9	0.9	0.9	1.0
Y232F	1.1	1.1	0.9	1.0	1.2
Y232H	1.1	1.0	1.0	0.9	1.0
Y232Q	1.0	1.0	< 0.9	0.9	1.0
Y232R	1.0	1.0	< 0.9	< 0.9	< 0.9
Y232W	1.0	1.1	< 0.9	0.9	1.0
S234A	1.0	1.0	0.9	< 0.9	1.1

SEQ ID NO:1 with the following amino acid substitutions	Stability in EDTA with respect to the reference protease (SEQ ID NO:1)	ADW EGG performance with respect to SEQ ID NO:1		Crème Brûlée performance with respect to SEQ ID NO:1	
		ADW Rinsed EGG Stain	ADW Unrinsed EGG Stain	GSM-B Detergent	MGDA Detergent
S234D	1.1	0.9	0.9	0.9	1.2
S234E	1.0	0.9	1.0	1.0	1.2
S234M	1.1	1.0	1.0	< 0.9	1.1
S234T	1.0	1.0	0.9	< 0.9	1.2
S234W	1.0	1.0	0.9	< 0.9	1.2
S234Y	1.0	0.9	0.9	< 0.9	1.3
N236D	1.1	0.9	ND	1.2	1.2
N236G	1.0	0.9	ND	1.0	1.1
N236S	1.0	1.0	ND	1.0	1.1
N236T	1.0	0.9	ND	1.1	1.1
T238A	1.1	1.0	0.9	0.9	1.5
T238D	1.1	1.0	0.9	1.2	1.7
T238E	1.1	0.9	0.9	1.4	1.4
T238M	1.1	1.0	1.0	1.0	1.4
T238V	1.1	1.0	1.0	1.1	1.2
Q239D	1.0	< 0.9	< 0.9	1.2	1.6
Q239E	1.1	< 0.9	< 0.9	1.1	1.5
Q239L	1.1	1.0	< 0.9	0.9	1.1
Q239M	1.1	0.9	1.0	1.2	1.3
Q239N	1.0	1.0	< 0.9	1.0	1.2
Q239T	1.1	0.9	< 0.9	1.0	1.0
N242A	1.1	1.0	1.0	< 0.9	1.0
K245E	1.0	0.9	< 0.9	1.4	1.7
N246A	1.1	1.0	0.9	1.0	1.3
N246L	1.1	ND	0.9	1.3	1.1
N246S	1.1	0.9	0.9	0.9	1.0
T247E	1.0	< 0.9	ND	1.1	1.4
T247Q	1.0	1.0	< 0.9	0.9	1.2
T249C	1.0	1.0	< 0.9	1.0	1.6
T249D	1.0	0.9	< 0.9	1.2	1.5
T249E	1.1	0.9	< 0.9	1.3	1.4
T249F	1.1	1.0	0.9	1.0	< 0.9
T249I	1.1	1.0	1.0	< 0.9	1.0
T249L	1.1	1.0	0.9	1.0	1.2
T249S	1.1	1.0	1.0	0.9	0.9
T249Y	1.1	ND	0.9	1.0	1.4

SEQ ID NO:1 with the following amino acid substitutions	Stability in EDTA with respect to the reference protease (SEQ ID NO:1)	ADW EGG performance with respect to SEQ ID NO:1		Crème Brûlée performance with respect to SEQ ID NO:1	
		ADW Rinsed EGG Stain	ADW Unrinsed EGG Stain	GSM-B Detergent	MGDA Detergent
N250D	1.1	0.9	0.9	1.3	1.6
N250S	1.1	1.0	0.9	1.1	1.2
N250T	1.0	1.0	0.9	0.9	1.1
N253D	1.2	0.9	1.1	1.5	1.7
N253E	1.1	1.0	0.9	1.5	1.7
N253P	1.0	1.0	1.0	1.1	1.1
S254P	1.1	1.0	1.1	1.0	0.9
S254Y	1.0	ND	0.9	1.2	< 0.9
S255A	1.0	0.9	1.0	1.1	1.2
S255C	1.2	1.0	1.0	1.2	2.0
S255D	1.1	0.9	0.9	1.3	1.9
S255E	1.2	0.9	1.0	1.3	1.8
S255F	1.1	0.9	0.9	< 0.9	0.9
S255I	1.1	0.9	0.9	0.9	1.0
S255M	1.1	0.9	1.1	1.0	1.1
S255N	1.0	1.0	0.9	0.9	1.0
S255V	1.1	1.0	0.9	1.1	1.0
S255W	1.1	< 0.9	0.9	< 0.9	< 0.9
Q256C	1.1	ND	0.9	1.4	1.8
Q256E	1.1	1.0	0.9	1.4	1.8
Q256F	1.1	0.9	1.0	< 0.9	1.0
Q256H	1.1	1.0	1.0	1.0	1.1
Q256L	1.0	0.9	1.2	1.1	0.9
Q256M	1.0	1.0	1.1	< 0.9	1.0
Q256W	1.1	0.9	1.0	1.0	0.9
Q256Y	1.1	0.9	1.2	1.4	< 0.9
F257C	1.0	< 0.9	< 0.9	1.2	1.5
F257M	1.0	1.0	1.0	1.0	1.0
S259D	1.0	1.0	< 0.9	1.3	1.5
S259E	0.9	0.9	< 0.9	1.3	1.4
S259M	1.0	1.0	1.0	1.1	1.1
S259N	1.0	1.0	1.0	1.1	1.1
V262L	1.0	1.0	1.0	1.4	1.1
N263D	1.0	1.0	0.9	1.2	1.1
N263Q	1.0	0.9	1.1	< 0.9	0.9
A264T	1.1	0.9	1.0	1.1	0.9

SEQ ID NO:1 with the following amino acid substitutions	Stability in EDTA with respect to the reference protease (SEQ ID NO:1)	ADW EGG performance with respect to SEQ ID NO:1		Crème Brûlée performance with respect to SEQ ID NO:1	
		ADW Rinsed EGG Stain	ADW Unrinsed EGG Stain	GSM-B Detergent	MGDA Detergent
E265A	1.0	1.0	1.1	< 0.9	< 0.9
E265M	1.0	0.9	1.1	< 0.9	< 0.9
E265N	1.0	< 0.9	1.0	< 0.9	< 0.9
E265Q	1.0	0.9	1.1	< 0.9	< 0.9
A266L	1.1	1.0	1.0	0.9	< 0.9
A266M	1.1	1.0	1.0	< 0.9	1.1
A266N	1.1	< 0.9	0.9	0.9	< 0.9
A266Q	1.0	0.9	0.9	< 0.9	< 0.9
A266R	1.0	0.9	0.9	< 0.9	< 0.9
T268A	1.0	0.9	1.1	1.0	1.2
T268C	1.1	1.0	1.0	1.0	1.2
T268D	1.0	0.9	< 0.9	1.0	1.4
T268E	0.9	0.9	< 0.9	1.1	1.4
R269H	1.0	0.9	< 0.9	1.2	1.5
R269P	1.0	0.9	< 0.9	1.1	1.3
R269W	1.0	< 0.9	< 0.9	0.9	1.4

The following variants showed improved performance index (PI value of ≥ 1.1) compared to the reference protease on one of the following assays: BMI HDL cleaning, BMI HDD cleaning, PAS-38 ADW cleaning, Crème brûlée ADW cleaning, or stability in Tris-EDTA buffer: T003V, V004T, I008V, T009A/C/E/G/H/K/M/N/Q/S/W/Y, R010A/K/M/N/Q/W, V011A/I/S/T, Q012A/C/D/E/G/M/N/R/S/T/V/W, P014D, A015D/E/F/H/I/K/M/P/Q/V/W/Y, V016L/M/S, H017C/E/F/G/I/L/N/V/W/Y, N018A/C/D/E/F/G/L/M/Q/T, R019A/C/D/E/F/H/I/K/L/N/Q/S/T/W/Y, G020A/C/D/M/N/T, S024A/E, G025A/C/D/E/M/N, V026A/I, R027K, S033T, S036A/C/E/I/L/M/Q/T/V, N042C/D/E/M/Q, I043L, R044C/E/F/G/H/I/K/L/N/Q/S/T/V/W/Y, A047I/Y, V050I, G052A/C/D/H/L/M/N/S/T/Y, P054A/C/G/L/M/N/T/V, T055A/C/D/E/H/M/N/S/Y, A057D/E/H/M/N/Q/T, L059A/C/D/E/M/N/Q/T, N060S, T069S, S076A/D/E/F/H/K/L/M/N/R/T/Y, V082A, P084D/F/H/Y, N085S, G095A/N, A096M/Q, N097E/H/K, S101T, V102L/M, G104A/D/H/M/N/T/V/W/Y, I105V, Q107K/M, E110L, A113T/V, T114V, N115E/H/Q, N116E/H, H118D/E/N, A120V, M122L, F128G, P129A/H/N/Y, S131A/D/E/I/M/N/P/Q/T/V, L133M, R135A/E/F/H/I/K/L/M/S/T/V/W/Y, A136M, V137L, Y139E/S, T141E/H/N,

S142A/D/E/H/M/N/Q, R143E/H/M/N/Q/V, D144E/N, V145C, V147C, I148L/V, A150M, N154D, S156A/C/D/N/T, G157A/C/D/E/N/Q, S158A/C/F/L/M/N/Q/T/V/W/Y, V159L, G160A/C/D/M/S/T, Y161W, R164A/K/M/Q/Y, A166D/E/I/P/Q/V, N167E, M169L, A170G, T174V, Q176A/C/D/E/L/M/N/S, N177A/C/D/E/G/H/K/L/M/Q/S/W/Y, N178D, R179A/C/E/F/G/H/I/K/M/Q/S/V/W/Y, R180K, N182A/C/D/E/G/H/I/K/L/P/Q/S/T/V/W/Y, Y186F, T188A/C/D/E/I/L/M/N/Q/S/V/W/Y, G189C/D/E, I190M, D191E, I192C/M, V193A/M, N198D/E, Q200H/I/K/M/V/Y, R207K/L/N/Q/T, V209P, S210C/D/E/F/G/L/N/P/Q/Y, M211E/K/L/Q/R, N212A/C/Q/S, T218C/S, A224V, L227M/Q, V228L, Q230E, R231C/E/H/I/L/N/Q/S/T, Y232F/H/Q/R/W, S234A/D/E/M/T/W/Y, N236D/G/S/T, T238A/D/E/M/V, Q239D/E/L/M/N/T, N242A, K245E, N246A/L/S, T247E/Q, T249C/D/E/F/I/L/S/Y, N250D/S/T, N253D/E/P, S254P/Y, S255A/C/D/E/F/I/M/N/V/W, Q256C/E/F/H/L/M/W/Y, F257C/M, S259D/E/M/N, V262L, N263D/Q, A264T, E265A/M/N/Q, A266L/M/N/Q/R, T268A/C/D/E, and R269H/P/W.

The following variants showed improved ADW cleaning performance index (PI value of $\geq$ 1.1) compared to reference protease on one of the PAS-38 assays: T009H/K/N/W, V011A/I, Q012A/M/N/R/S/V, A015F/I/K/V, V016L/M, H017F/G/I/L/N/V/W, N018F, R019C/K/L/Q, G020A/D/M/N/T, S024A, G025A/D/N, S036A/L, G052D/H, P054A/G/L/M/V, T055A/D/H/S/Y, L059A/M/N, N060S, T069S, S076K/L, G095N, A096Q, N097K, V102L/M, Q107K, E110L, A113T, H118D, A120V, M122L, F128G, P129A/H/N/Y, S131M/N/P, A136M, R143N, D144N, V145C, G157A/D, S158Q/T, V159L, G160D/M/S, A166I, A170G, Q176L, N177A/D/G/K/L/M/S/Y, R179A/K, N182A/D/S/Y, T188M, D191E, R207L, S210E/G/Q, M211E/Q/R, T218S, L227M, Y232F/W, Q256L/Y, N263Q, E265A/M/Q, and T268A.

The following variants showed improved ADW cleaning performance index (PI value of $\geq$ 1.1) compared to reference protease on one of the Crème Brûlée assays: T009A/C/E/M/N/Y, R010A/K/M/N/Q/W, V011A/T, Q012A/C/D/E/M, P014D, A015D/E/H/I/M/V/W/Y, V016L/M, H017C/E, N018C/D/E/M, R019A/C/D/E/F/H/I/K/L/N/Q/S/T/W/Y, G020C/D, S024A/E, G025A/C/D/E/M/N, V026A, R027K, S036C/E/Q/V, N042C/D/E, I043L, R044C/E/G/H/I/L/N/Q/S/T, G052A/C/D/L/M/N, P054A/C/L/M/V, T055A/C/D/E/M, A057D/E, L059A/C/D/E/M/N/Q/T, N060S, S076D/E/N, V082A, P084D, A096Q, N097E/H, G104A/D/H/N/V/Y, N115H, N116E, F128G, P129H, S131D/E, R135A/E/F/H/I/K/L/M/S/T/V/W/Y, Y139E, T141E, S142D/E, R143E, D144E, V147C, I148L, N154D, S156A/C/D/N/T, G157C/D/E, S158C/L/Q/T/Y, V159L, R164A/K/M/Q/Y, A166D/E, N167E, M169L, T174V, Q176A/C/D/E/N, N177C/D/E, N178D, R179A/C/E/F/G/H/I/K/M/Q/S/V/W/Y, R180K, N182C/D/E, T188C/D/E, G189C/D/E,

V193A/M, N198D/E, R207K/L/N/Q/T, V209P, S210C/D/E/L/N/Y, M211E/K/L/Q, N212C/Q, V228L, Q230E, R231C/E/L/N/Q, Y232F, S234D/E/T/W/Y, N236D/T, T238A/D/E/M/V, Q239D/E/M/N, K245E, N246A/L, T247E/Q, T249C/D/E/L/Y, N250D, N253D/E/P, S254Y, S255A/C/D/E, Q256C/E/Y, F257C, S259D/E/M/N, V262L, N263D, T268C/D/E, and R269H/P/W.

The following variants showed improved cleaning performance index (PI value of ≥ 1.1) compared to reference protease on at least one PAS-38 assay and at least one crème brûlée assay : T009N, V011A, Q012A/M, A015I/V, V016L/M, R019C/K/L/Q, G020D, S024A, G025A/D/N, G052D, P054A/L/M/V, T055A/D, L059A/M/N, N060S, A096Q, F128G, P129H, G157D, S158Q/T, V159L, N177D, R179A/K, N182D, R207L, S210E, M211E/Q, Y232F, and Q256Y.

The following variants showed improved stability (PI value of ≥ 1.1) compared to reference protease in Tris-EDTA buffer: T003V, V004T, I008V, T009A/E/G/H/K/N/Q/S/W/Y, R010Q, V011A, Q012A/C/G/M/N/T, A015F/H/M/P/Q/W, V016S, H017C/E/F/I/L/N/V/W/Y, N018A/D/E/L/M/Q, R019C/D/Y, G020C/D/M/N, S024A/E, G025C/D/N, V026I, R027K, S033T, S036A/C/I/L/M/Q/V, N042C/D/E/M/Q, R044C/E/F/G/H/I/K/L/N/Q/S/T/V/W/Y, A047I/Y, V050I, G052A/M/N/S/T/Y, P054N/V, T055C/D/E/N, A057E/H/M/N/Q/T, L059N, S076A/D/E/F/H/K/L/M/N/R/T/Y, V082A, P084D/F/H/Y, N085S, G095A/N, A096M, N097K, S101T, V102L/M, G104M/N/T/V/W, I105V, Q107M, A113V, T114V, N115Q, N116E/H, H118D/E/N, S131A/D/E/I/M/N/P/Q/T/V, L133M, R135A/H/I/K/L/M/S/T/V/W/Y, A136M, S142A/D/E/H/M/N/Q, R143E/H/M/N/Q, V147C, I148V, A150M, S156N/T, G157A/C/N, S158C/F/L/M/N/Q/T/V/W/Y, V159L, G160A/C/M/S/T, A166D/E/P/Q, A170G, Q176C/M, N177A/C/D/H/L/M/Q/W/Y, R179M/Q, R180K, N182A/C/E/G/H/I/K/L/P/Q/S/T/V/W/Y, T188A/C/D/E/I/L/M/N/Q/V/W/Y, G189D, I192C/M, V193M, Q200H/I/K/M/V/Y, V209P, S210E/F/P, M211K, N212S, T218C/S, V228L, Q230E, R231C/E/H/N/T, Y232F/H, S234D/M, N236D/G/S/T, T238A/D/E/M/V, Q239E/L/M/T, N242A, N246A/L/S, T249E/F/I/L/S/Y, N250D/S, N253D/E, S254P, S255C/D/E/F/I/M/V/W, Q256C/E/F/H/W/Y, A264T, A266L/M/N, and T268C.

The following variants showed improved performance index (PI value of ≥ 1.1) compared to reference protease in ADW cleaning on at least one PAS-38 assay or at least one crème brûlée assay and improved stability (PI value of ≥ 1.1) in Tris-EDTA buffer: T009A/E/H/K/N/W/Y, R010Q, V011A, Q012A/C/M/N, A015F/H/M/W, H017C/E/F/I/L/N/V/W, N018D/E/M, R019C/D/Y, G020C/D/M/N, S024A/E, G025C/D/N, R027K, S036A/C/L/Q/V, N042C/D/E, R044C/E/G/H/I/L/N/Q/S/T, G052A/M/N, P054V, T055C/D/E, A057E, L059N,

S076D/E/K/L/N, V082A, P084D, G095N, N097K, V102L/M, G104N/V, N116E, H118D, S131D/E/M/N/P, R135A/H/I/K/L/M/S/T/V/W/Y, A136M, S142D/E, R143E/N, V147C, S156N/T, G157A/C, S158C/L/Q/T/Y, V159L, G160M/S, A166D/E, A170G, Q176C, N177A/C/D/L/M/Y, R179M/Q, R180K, N182A/C/E/S/Y, T188C/D/E/M, G189D, V193M, V209P, S210E, M211K, T218S, V228L, Q230E, R231C/E/N, Y232F, S234D, N236D/T, T238A/D/E/M/V, Q239E/M, N246A/L, T249E/L/Y, N250D, N253D/E, S255C/D/E, Q256C/E/Y, and T268C.

The following variants with a negative charge change showed improved performance index (PI value of $\geq$ 1.1) compared to the reference protease in at least one of the Crème Brûlée assays: T009C/E/Y, R010A/K/M/N/Q/W, Q012C/D/E, P014D, A015D/E/Y, H017C/E, N018C/D/E, R019A/C/D/E/F/H/I/K/L/N/Q/S/T/W/Y, G020C/D, S024E, G025C/D/E, R027K, S036C/E, N042C/D/E, R044C/E/G/H/I/L/N/Q/S/T, G052C/D, P054C, T055C/D/E, A057D/E, L059C/D/E, S076D/E, P084D, N097E, G104D/Y, N116E, S131D/E, R135A/E/F/H/I/K/L/M/S/T/V/W/Y, Y139E, T141E, S142D/E, R143E, V147C, N154D, S156C/D, G157C/D/E, S158C/Y, R164A/K/M/Q/Y, A166D/E, N167E, Q176C/D/E, N177C/D/E, N178D, R179A/C/E/F/G/H/I/K/M/Q/S/V/W/Y, R180K, N182C/D/E, T188C/D/E, G189C/D/E, N198D/E, R207K/L/N/Q/T, S210C/D/E/Y, M211E, N212C, Q230E, R231C/E/L/N/Q, S234D/E/Y, N236D, T238D/E, Q239D/E, K245E, T247E, T249C/D/E/Y, N250D, N253D/E, S254Y, S255C/D/E, Q256C/E/Y, F257C, S259D/E, N263D, T268C/D/E, and R269H/P/W.

The dimensions and values disclosed herein are not to be understood as being strictly limited to the exact numerical values recited. Instead, unless otherwise specified, each such dimension is intended to mean both the recited value and a functionally equivalent range surrounding that value. For example, a dimension disclosed as “40 mm” is intended to mean “about 40 mm”.

CLAIMS

What is claimed is:

1. An automatic dishwashing cleaning composition comprising a protease wherein the protease is a variant having at least 90% identity with the amino acid sequence of SEQ ID NO:1 and the variant has a glutamate (E) residue at position 39 and further comprises one or more amino acid substitutions at one or more positions selected from:
(i) 3V, 4T, 8V, 9A/C/E/G/H/K/M/N/Q/W/Y, 10A/K/M/N/Q/W, 11A/I/S/T, 12A/C/D/G/M/N/R/S/T/V/W, 14D, 15D/E/F/H/I/K/M/P/Q/V/W/Y, 16L/M/S, 17C/E/F/G/I/L/N/V/W/Y, 18A/C/D/E/F/G/L/M/Q/T, 19A/C/D/E/F/H/I/K/L/N/Q/S/T/W/Y, 20A/C/D/M/N/T, 24A/E/, 25A/C/D/E/M/N, 26A/I, 33T, 36C/E/I/L/M/Q/T/V, 42C/D/E/M/Q, 43L, 44C/E/F/G/H/I/K/L/N/Q/T/V/W/Y, 47I/Y, 50I, 52A/C/D/H/L/M/N/S/T/Y, 54A/C/G/L/M/N/T/V, 55A/C/D/E/H/N/S/Y, 57D/E/H/M/N/Q/T, 59A/C/D/E/M/N/Q/T, 60S, 69S, 76A/D/E/F/H/K/L/M/N/R/T/Y, 82A, 84D/F/H/Y, 95A/N, 96M/Q, 97E/H/K, 101T, 102L/M, 104A/D/H/M/N/T/V/W/Y, 105V, 107K/M, 110L, 113T/V, 114V, 115E/H/Q, 116E/H, 118D/E/N, 120V, 128G, 129A/H/N/Y, 131A/D/E/I/M/N/P/Q/V, 133M, 135A/E/F/H/I/K/L/M/S/T/V/W/Y, 136M, 137L, 139E/S, 141E/H/N, 142A/D/E/H/M/N/Q, 143E/H/M/N/V, 144E/N, 145C, 147C, 148L/V, 150M, 156C/D/N/T, 157A/C/D/E/N/Q, 158A/C/F/L/M/N/Q/V/W/Y, 159L, 160A/C/D/M/T, 161W, 164A/K/M/Q/Y, 166D/E/I/P/Q/V, 167E, 170G, 174V, 176A/C/D/L/M/N/S, 177A/C/D/E/G/H/K/L/M/Q/S/W/Y, 178D, 179A/C/E/F/G/H/I/K/M/Q/S/V/W/Y, 180K, 182A/C/D/E/G/H/I/K/L/P/Q/T/V/W/Y, 186F, 188C/D/E/I/L/M/N/Q/S/V/W/Y, 189C/D/E, 190M, 191E, 192C/M, 193A/M, 198D/E, 200H/I/K/M/V/Y, 207K/L/N/Q/T, 209P, 210C/D/E/F/G/L/N/P/Q/Y, 211E/L/Q/R, 212A/C/Q, 218C/S, 227M/Q, 228L, 230A/D/L/M/N, 231C/E/H/I/L/N/Q/S/T, 232F/H/Q/R/W, 234A/D/E/M/T/W/Y, 236G/S/T, 238A/D/E/M/V, 239D/E/L/M/N/T, 242A, 245E, 246A/L, 247E/Q, 249C/D/E/F/I/L/S/Y, 250S/T, 253E, 254P/Y, 255A/C/D/E/F/I/M/V/W, 256C/F/H/M/W/Y, 257C/M, 259D/E/M/N, 262L, 263D/Q, 264T, 265A/M/N/Q, 266L/M/N/Q/R, 268A/C/D/E, and 269H/P/W;

wherein the amino acid positions of the variant are numbered by correspondence with the amino acid sequence of SEQ ID NO: 1.

2. A composition according to claim 1 wherein the variant comprises one or more amino acid substitutions at one or more positions selected from: 3V; 9A/C/E/K; 10A/M/N/Q; 11A/I; 12C/D; 14D; 15D/E/H/I/M/V/Y; 16M; 17C/F/I/L/W; 18D/E; 19A/C/D/E/H/I/L/Q/S/T/W; 24A/E; 36C/E; 42C/D/E; 44C/E/W/Y; 52A/C/D/H; 54L/M;55A/D/H/S; 57D/E/; 59A/C/D/E/N; 60S; 76E/H/K/L/M/N/T; 84H/Y;; 95N; 96Q; 97E; 104A/D; 107K; 110L; 116E; 129H/N/Y; 131D/E; 135A/E/H/I/L/M/S/T/V/W/Y; 136M; 141E; 142E; 144E; 156C/D; 157A/C/D/E; 158A/C; 160A/M; 164A/M/Q/Y; 166D/E; 176C/D; 177C/D/M/S/Y; 178D; 179A/C/E/F/G/H/I/K/M/Q/S/V/W/Y; 182D/E; 188C/D/E/M; 189C/D/E; 193A/M; 198D/E; 200I/Y; 207K/L/Q; 209P; 210D/E/N; 238A/D/E/M; 239D/E; 241C/G/L/Q/T/Y; 245E; 247E/ 249C/D/E/Y; 253E; 255C/D/E; 256C/Y; 259D/E; 262L; 268D/E; and 269H/W.

3. A composition according to any of claims 1 or 2 wherein the protease is a variant having at least 95% identity with the amino acid sequence of SEQ ID NO:1.

4. A composition according to any of the preceding claims wherein the composition is phosphate free.

5. A composition according to any of the preceding claims wherein the composition comprises from 10% to 50% by weight of the composition of an organic complexing agent system.

6. A composition according to any of the preceding claims comprising more than 10% by weight of the composition of bleach.

7. A composition according to the preceding claim comprising a bleach activator and/or a bleach catalyst, preferably a manganese catalyst

8. A composition according to any of claims 5 to 7 wherein the complexing agent system comprises a complexing agent selected from the group consisting of citric acid, methyl glycine diacetic acid, glutamic-N,N-diacetic acid, iminodisuccinic acid, carboxy methyl inulin, their salts, and mixtures thereof, preferably a salt of methyl glycine diacetic acid.

9. A composition according to any of claims 5 to 8 wherein the complexing agent system comprises citric acid and methyl glycine diacetic acid preferably in a weight ratio of from about 0.5:1 to about 2:1.

10. A composition according to any of claims 5 to 9 wherein the bleach is percarbonate.

11. A composition according to any preceding claim wherein the composition further comprises an alpha amylase preferably having a mutation in position equivalent to 202 in SEQ ID No. 11.

12. A composition according to any preceding claim wherein the composition comprises a dispersant polymer, preferably a carboxylated/sulfonated polymer.

13. A composition according to any of the preceding claims comprising:

i) from 10% to 50% by weight of the composition of an organic complexing agent system.

ii) a bleaching system comprising at least 10% by weight of the composition of percarbonate and optionally a bleach activator and/or a bleach catalyst;

iii) a non-ionic surfactant;

iv) a dispersant polymer;

v) an amylase; and

vi) optionally but preferably a glass care agent.

14. A method of washing soiled dishware in a dishwasher in soft water comprising the steps of:

i) providing the soiled dishware;

ii) treating the dishware with a cleaning composition according to any preceding claim; and

iii) optionally rinsing the dishware.

15. Use of a composition according to any of claims 1 to 12 for the removal of crème brulee in automatic dishwashing.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/036888

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/54 C11D3/386
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C11D 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, WPI Data, BIOSIS, CHEM ABS Data, Sequence Search, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2016/205755 A1 (DANISCO US INC [US]) 22 December 2016 (2016-12-22) tables 4-5	1-15
Y	WO 2017/192692 A1 (DANISCO US INC [US]; BABE LILIA MARIA [US] ET AL.) 9 November 2017 (2017-11-09) table 4; sequences 333, 345, 353	1-15
X,P	WO 2019/108599 A1 (DANISCO US INC [US]) 6 June 2019 (2019-06-06) table 7	1-15
Y,P	WO 2018/118950 A1 (DANISCO US INC [US]) 28 June 2018 (2018-06-28) claim 8	1-15

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 August 2019	25/10/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Petri, Bernhard

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/036888

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

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

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

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

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

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-15(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-15(partially)

Automatic dishwashing cleaning composition comprising a protease, wherein the protease exhibits at least 90% sequence identity to SEQ ID NO 1 and has glutamate residue at position 39 and further comprises an amino acid substitution, related methods and uses, wherein the further substitution leads to V at position 3.

2-125. claims: 1-15(partially)

as invention 1, however wherein the further substitution leads to T at position 4, to A/C/E/G/H/K/M/N/Q/W/Y at position 9, ..., to H/P/W at position 269, respectively.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/036888

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	WO 2018/118917 A1 (DANISCO US INC [US]) 28 June 2018 (2018-06-28) table 8 -----	1-15

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2019/036888

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2016205755 A1	22-12-2016	CN 107849549 A	27-03-2018
		EP 3310911 A1	25-04-2018
		JP 2018521646 A	09-08-2018
		US 2018237761 A1	23-08-2018
		WO 2016205755 A1	22-12-2016
WO 2017192692 A1	09-11-2017	BR 112018072530 A2	26-03-2019
		CA 3022875 A1	09-11-2017
		CN 109715791 A	03-05-2019
		EP 3452585 A1	13-03-2019
		JP 2019518440 A	04-07-2019
		US 2019194636 A1	27-06-2019
		WO 2017192692 A1	09-11-2017
WO 2019108599 A1	06-06-2019	NONE	
WO 2018118950 A1	28-06-2018	CN 110312794 A	08-10-2019
		EP 3559226 A1	30-10-2019
		WO 2018118950 A1	28-06-2018
WO 2018118917 A1	28-06-2018	CN 110312795 A	08-10-2019
		EP 3559227 A1	30-10-2019
		WO 2018118917 A1	28-06-2018