~~Claims~~

CLAIMS

1. A variant of porcine trypsin comprising or consisting of

an amino acid sequence that has at least ~~90~~91% sequence identity to SEQ ID NO: 1,

wherein said amino acid sequence differs from SEQ ID NO: 1 at least by one or more amino acid substitutions at one or more positions corresponding to F24, S44, D56, G78, Y131, S172 and W193 of native porcine trypsin according to SEQ ID NO: 1, with the proviso that said amino acid sequence is not the sequence of SEQ ID NOs: 1 and 2.

~~with the proviso that said amino acid sequence is not native porcine trypsin according to SEQ ID NO: 1; and~~

~~with the proviso that said amino acid sequence is not porcine variant trypsin S172A according to SEQ ID NO: 2.~~

2. The variant of porcine trypsin according to claim 1,

wherein the amino acid at the position corresponding to F24 is substituted by an amino acid selected from the group consisting of Ala, Asn, Arg, Gln, Ile, Leu, Lys, Met, Ser, Thr, and Val~~;~~.

~~wherein the amino acid at the position corresponding to S44 is substituted by an amino acid selected from the group consisting of Leu and Pro;~~

~~wherein the amino acid at the position corresponding to D56 is substituted by an amino acid selected from the group consisting of Ala, Asn, His, and Trp;~~

~~wherein the amino acid at the position corresponding to G78 is substituted by an amino acid selected from the group consisting of Ala, Glu; Pro, Ser, and Tyr;~~

~~wherein the amino acid at the position corresponding to Y131 is substituted by an amino acid selected from the group consisting of Ala, Asn, Asp, Cys, Gln; Glu, Gly, His, Ile, Leu, Met, Ser, Thr, Trp, Val;~~

~~wherein the amino acid at the position corresponding to S172 is substituted by an amino acid selected from the group consisting of Ala, Cys, and Thr; and/or~~

~~wherein the amino acid at the position corresponding to W193 is substituted by an amino acid selected from the group consisting of Phe, Ser, Thr, and Tyr.~~

3. The variant of porcine trypsin according to ~~any one of claims~~claim 1 ~~to 2~~, wherein said amino acid sequence additionally differs from SEQ ID NO: 1 at least by one or more amino acid

substitutions at one or more positions corresponding to R99, R107, K125, and K170 of native porcine trypsin according to SEQ ID NO: 1.

4. The variant of porcine trypsin according to claim 3,
wherein the amino acid at the position corresponding to R99 is substituted by an amino acid selected from the group consisting of Ala, Asn, Asp, Glu, Gly, His, Leu, Phe, Thr, Trp, and Tyr;.
wherein the amino acid at the position corresponding to R107 is substituted by an amino acid selected from the group consisting of Asp, Gly, Pro, Ser, and Thr;
wherein the amino acid at the position corresponding to K125 is substituted by an amino acid selected from the group consisting of Ala, Cys, Gln, Glu, Gly, His, Leu, Ser, and Tyr; and/or
wherein the amino acid at the position corresponding to K170 is substituted by an amino acid selected from the group consisting of Ala, Asn, Gly, and Tyr.

5. The variant of porcine trypsin according to any one of claimsclaim 1 to 4, wherein said amino acid sequence is SEQ ID NO: 3,
wherein
– Xaa24 is an amino acid selected from the group consisting of Ala, Asn, Arg, Gln, Ile, Leu, Lys, Met, Phe, Ser, Thr, and Val;–, with the proviso that SEQ ID NO: 3 is not the sequence of SEQ ID NOs:1 and 2.
- Xaa44 is an amino acid selected from the group consisting of Leu, Pro, and Ser;
- Xaa56 is an amino acid selected from the group consisting of Ala, Asn, Asp, His, and Trp;
- Xaa78 is an amino acid selected from the group consisting of Ala, Glu, Gly, Pro, Ser, and Tyr;
- Xaa99 is an amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Glu, Gly, His, Leu, Phe, Thr, Trp, and Tyr;
- Xaa107 is an amino acid selected from the group consisting of Arg, Asp, Gly, Pro, Ser, and Thr;
- Xaa125 is an amino acid selected from the group consisting of Ala, Cys, Gln, Glu, Gly, His, Leu, Lys, Ser, and Tyr;
- Xaa131 is an amino acid selected from the group consisting of Ala, Asn, Asp, Cys, Gln; Glu, Gly, His, Ile, Leu, Met, Ser, Thr, Trp, Tyr, and Val;
- Xaa170 is an amino acid selected from the group consisting of Ala, Asn, Gly, Lys, and Tyr;
- Xaa172 is an amino acid selected from the group consisting of Ala, Cys, Ser, and Thr; and/or
- Xaa193 is an amino acid selected from the group consisting of Phe, Ser, Thr, Trp, and Tyr;
with the proviso that SEQ ID NO: 3 is not porcine wild-type trypsin according to SEQ ID NO: 1; and

~~with the proviso that SEQ ID NO: 3 is not porcine variant trypsin S172A according to SEQ ID NO: 2.~~

6. The variant of porcine trypsin according to ~~any one of claims 1 to 5~~claim 1, wherein said amino acid sequence is selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO:, 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, and SEQ ID NO: 119.

7. The variant of porcine trypsin according to ~~any one of claims 1 to 6~~claim 1, wherein

~~(i)~~ said variant is capable of cleaving a peptide with the general formula A-Lys-Thr-Arg-Arg-B according to SEQ ID NO: 131 to yield a cleavage product of the general formula A-Lys-Thr-Arg-Arg according to SEQ ID NO: 132 in a yield of at least 80%,

wherein A is an amino acid sequence consisting of one or more amino acids; and

wherein B is an amino acid sequence consisting of one or more amino acids;.

and/or

(ii)

8. The variant of porcine trypsin according to claim 1, wherein the variant exhibits an increased selectivity for the cleavage of a pre-pro-human insulin, pre-pro-insulin glargine, pre-pro-insulin lispro, pre-pro-insulin aspart, or pre-pro-insulin glulisine at a position C-terminally to position B32-Arg as compared to porcine variant trypsin S172A according to SEQ ID NO: 2.

8. A nucleic acid molecule encoding

a9. The variant of porcine trypsin according to any one of claims claim 1, wherein

- the amino acid at the position corresponding to 7, orF24 is substituted by Arg.

a trypsinogen precursor molecule of a

10.The variant of porcine trypsin as defined in any one of claims 1 to 7,

wherein said nucleic acid molecule is optionally contained in a vector or is optionally integrated into a genome.

9. A host cell containing a nucleic acid molecule according to claim 81, wherein the amino acid at the position corresponding to S44 is substituted by an amino acid selected from the group consisting of Leu and Pro.

10. A method of producing a11.The variant of porcine trypsin according to any one of claims claim 1, wherein the amino acid at the position corresponding to 7 comprising the steps of:D56 is substituted by an amino acid selected from the group consisting of Ala, Asn, His, and Trp.

cultivating a host cell according to claim 9; and

isolating the trypsinogen precursor molecule of the

12. The variant of porcine trypsin from the culture medium or from the host cell; andaccording to claim 1,

optionally activating the trypsinogen precursor molecule, thereby obtaining said variant of porcine trypsin.

11. Awherein the amino acid at the position corresponding to G78 is substituted by an amino acid selected from the group consisting of Ala, Glu; Pro, Ser, and Tyr;

wherein the amino acid at the position corresponding to Y131 is substituted by an amino acid selected from the group consisting of Ala, Asn, Asp, Cys, Gln; Glu, Gly, His, Ile, Leu, Met, Ser, Thr, Trp, Val;

wherein the amino acid at the position corresponding to S172 is substituted by an amino acid selected from the group consisting of Ala, Cys, and Thr; or

wherein the amino acid at the position corresponding to W193 is substituted by an amino acid selected from the group consisting of Phe, Ser, Thr, and Tyr.

13. The variant of porcine trypsin according to ~~any one~~claim 3, wherein the amino acid at the position corresponding to R107 is substituted by an amino acid selected from the group consisting of Asp, Gly, Pro, Ser, and Thr.

14. The variant of porcine trypsin according to claim 3, wherein the amino acid at the position corresponding to K125 is substituted by an amino acid selected from the group consisting of ~~claims 1 to 7 or a nucleic acid molecule according to claim 8 or a host cell according to claim 9 for use within a process of manufacturing a food ingredient or a feed ingredient~~Ala, Cys, Gln, Glu, Gly, His, Leu, Ser, and Tyr.

~~12. A use~~15. The variant of porcine trypsin according to claim 3, wherein the amino acid at the position corresponding to K170 is substituted by an amino acid selected from the group consisting of ~~the~~Ala, Asn, Gly, and Tyr.

16. The variant of porcine trypsin according to ~~any one~~claim 1, wherein said amino acid sequence is SEQ ID NO: 3, wherein Xaa44 is an amino acid selected from the group consisting of ~~claims 1 to 7 in a method for the production~~ Leu, Pro, and Ser, with the proviso that SEQ ID NO: 3 is not the sequence of ~~human insulin, an insulin analogue or a derivative of insulin~~SEQ ID NOs:1 and 2.

~~13~~17. The ~~use of~~ variant of porcine trypsin according to claim ~~12~~1, wherein ~~the insulin analogue~~ said amino acid sequence is SEQ ID NO: 3, wherein Xaa56 is an amino acid selected from the

group consisting of insulin aspart, insulin lispro, insulin glulisineAla, Asn, Asp, His, and insulin glargine.

14. Use Trp, with the proviso that SEQ ID NO: 3 is not the sequence of aSEQ ID NOs:1 and 2.

18. The variant of porcine trypsin according to any one of claims 1 to 7 to cleave a protein or peptide with the general formula A-Lys-Thr-Arg-Arg-B,
wherein A is an amino acid sequence consisting of one or more amino acids; and
claim 1, wherein BSaid amino acid sequence is SEQ ID NO: 3,
wherein
- Xaa78 is an amino acid sequence selected from the group consisting of Ala, Glu, Gly, Pro, Ser, and Tyr;
- Xaa99 is an amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Glu, Gly, His, Leu, Phe, Thr, Trp, and Tyr;
- Xaa107 is an amino acid selected from the group consisting of one or more amino acids.Arg, Asp, Gly, Pro, Ser, and Thr;

15. The use of claim 14, wherein the cleavage yields a cleavage product A-Lys-Thr-Arg-Arg.

- 16Xaa125 is an amino acid selected from the group consisting of Ala, Cys, Gln, Glu, Gly, His, Leu, Lys, Ser, and Tyr;
- Xaa131 is an amino acid selected from the group consisting of Ala, Asn, Asp, Cys, Gln; Glu, Gly, His, Ile, Leu, Met, Ser, Thr, Trp, Tyr, and Val;
- Xaa170 is an amino acid selected from the group consisting of Ala, Asn, Gly, Lys, and Tyr;
- Xaa172 is an amino acid selected from the group consisting of Ala, Cys, Ser, and Thr; or
- Xaa193 is an amino acid selected from the group consisting of Phe, Ser, Thr, Trp, and Tyr;
with the proviso that SEQ ID NO: 3 is not the sequence of SEQ ID NOs:1 and 2.

19. The use of variant of porcine trypsin according to claim 151, wherein the cleavage yields the cleavage product A-Lys-Thr-Arg-Arg in a yield of amino acid at least 80.the position corresponding to S44 is substituted by Pro.

20. The variant of porcine trypsin according to claim 1, wherein
the amino acid at the position corresponding to D56 is substituted by His;
the amino acid at the position corresponding to G78 is substituted by Pro;
the amino acid at the position corresponding to Y131 is substituted by Met;
the amino acid at the position corresponding to S172 is substituted by Ala; or
the amino acid at the position corresponding to W193 is substituted by Ser.