

[0006] It is an object of the present invention to provide improved or alternative recombinant β -lactoglobulin proteins, and/or to at least provide the public with a useful choice.

[0007] In this specification, where reference has been made to external sources of information, including patent specifications and other documents, this is generally for the purpose of providing a context for discussing the features of the present invention. Unless stated otherwise, reference to such sources of information is not to be construed, in any jurisdiction, as an admission that such sources of information are prior art or form part of the common general knowledge in the art.

SUMMARY

[0008] In one aspect, the present disclosure refers to a composition comprising two or more recombinant β -lactoglobulin proteins of differing amino acid sequence, the recombinant β -lactoglobulin proteins having at least 70% sequence identity to a wild type, mature β -lactoglobulin, and wherein at least one of the β -lactoglobulin proteins is an elongated β -lactoglobulin protein; wherein the composition comprises one or more recombinant, elongated β -lactoglobulin proteins having an amino acid sequence comprising or consisting of a) a core sequence having at least 70% sequence identity to the sequence of a wild type, mature β -lactoglobulin; and b) an N-terminal elongation of the core sequence, the N-terminal elongation having a sequence consisting of the sequence: i) KREAEAM, KREAEAEAM, or KREAEAEAEAM; ii) REAEAEAEAM, REAEAEAM, or REAEAM; EAEAEAEAM, EAEAEAM, or EAEAM; iv) EAEAEAEA, EAEAEA, or EAEA; v) EAM; vi) EA; or vii) R.

[0008a] In another aspect, the present disclosure refers to a recombinant, elongated β -lactoglobulin protein having an amino acid sequence comprising or consisting of a) a core sequence having at least 70% sequence identity to the sequence of a wild type, mature β -lactoglobulin; and b) an N-terminal elongation of the core sequence, the N-terminal elongation having a sequence consisting of i) KREAEAEAEAM, KREAEAEAM, or KREAEAM; ii) REAEAEAEAM, REAEAEAM, or REAEAM; iii) EAEAEAEAM, EAEAEAM, or EAEAM; iv) EAEAEAEA, EAEAEA, or EAEA; v) EAM; vi) EA; or vii) R.

[0008b] In another aspect, the present disclosure refers to a food product comprising the composition or recombinant, elongated β -lactoglobulin protein as disclosed herein. In yet another aspect, the present disclosure refers to a method for preparing a food product, the method comprising a) providing the composition or recombinant, elongated β -lactoglobulin protein as disclosed herein, and b) mixing the

composition or the recombinant, elongated β -lactoglobulin protein with one or more additional ingredients to produce the food product.

[0008c] In one example the disclosure relates to a composition comprising a plurality of recombinant β -lactoglobulin proteins heterogeneous in amino acid sequence, the recombinant proteins having at least 70% sequence identity to a wild type, mature β -lactoglobulin, wherein the plurality comprises at least one modified β -lactoglobulin protein comprising or consisting of an amino acid sequence that comprises

- a) an N-terminal truncation of from about 1 to about 20 amino acids relative to the sequence of a wild type, mature β -lactoglobulin;
- b) an N-terminal elongation of from about 1 to about 20 amino acids relative to the sequence of a wild type, mature β -lactoglobulin; or
- c) one or more substitutions of from about 1 to about 20 amino acids relative to the sequence of a wild type, mature β -lactoglobulin,

and wherein

- A) the amount of modified β -lactoglobulin protein is at least 40% of the total recombinant β -lactoglobulin proteins in the composition;
- B) the amount of modified β -lactoglobulin protein comprising or consisting of an amino acid sequence that comprises an N-terminal elongation of from about 1 to about 20 amino acids relative to the sequence of a wild type, mature β -lactoglobulin, is at least about 10% of the total recombinant β -lactoglobulin proteins in the composition;
- C) the amount of modified β -lactoglobulin protein comprising or consisting of an amino acid sequence that comprises an N-terminal truncation of from about 1 to about 20 amino acids relative to the sequence of a wild type,

mature β -lactoglobulin, is at least about 30% of the total recombinant β -lactoglobulin proteins in the composition; or

D) any combination of any two or more of (A) to (C).

[0009] In another example the disclosure relates to a composition comprising a plurality of recombinant β -lactoglobulin proteins heterogeneous in amino acid sequence, the recombinant proteins having at least 70% sequence identity to SEQ ID No: 1, wherein the plurality comprises at least one modified β -lactoglobulin protein comprising or consisting of an amino acid sequence that comprises:

- a) an N-terminal truncation of from about 1 to about 20 amino acids relative to the sequence of SEQ ID No: 1;
- b) an N-terminal elongation of from about 1 to about 20 amino acids relative to the sequence of SEQ ID No: 1; or
- c) one or more substitutions of from about 1 to about 20 amino acids relative to the sequence of SEQ ID No: 1,

and wherein

- A) the amount of modified β -lactoglobulin protein is at least 40% of the total recombinant β -lactoglobulin proteins in the composition;
- B) the amount of modified β -lactoglobulin protein comprising or consisting of an amino acid sequence that comprises an N-terminal elongation of from about 1 to about 20 amino acids relative to the sequence of SEQ ID No: 1, is at least about 10% of the total recombinant β -lactoglobulin proteins in the composition;
- C) the amount of modified β -lactoglobulin protein comprising or consisting of an amino acid sequence that comprises an N-terminal truncation of from about 1 to about 20 amino acids relative to the sequence of SEQ ID No: 1, is at least about 30% of the total recombinant β -lactoglobulin proteins in the composition; or
- D) any combination of any two or more of (A) to (C).

[0010] In another example the disclosure relates to a composition comprising a plurality of recombinant β -lactoglobulin proteins heterogeneous in amino acid sequence, the recombinant proteins having at least 70% sequence identity to SEQ ID No: 2,

wherein the plurality comprises at least one modified β -lactoglobulin protein comprising or consisting of an amino acid sequence that comprises:

- a) an N-terminal truncation of from about 1 to about 20 amino acids relative to the sequence of SEQ ID No: 2;
- b) an N-terminal elongation of from about 1 to about 20 amino acids relative to the sequence of SEQ ID No: 2; or
- c) one or more substitutions of from about 1 to about 20 amino acids relative to the sequence of SEQ ID No: 2,

and wherein

- A) the amount of modified β -lactoglobulin protein is at least 40% of the total recombinant β -lactoglobulin proteins in the composition;
- B) the amount of modified β -lactoglobulin protein comprising or consisting of an amino acid sequence that comprises an N-terminal elongation of from about 1 to about 20 amino acids relative to the sequence of SEQ ID No: 2, is at least about 10% of the total recombinant β -lactoglobulin proteins in the composition;
- C) the amount of modified β -lactoglobulin protein comprising or consisting of an amino acid sequence that comprises an N-terminal truncation of from about 1 to about 20 amino acids relative to the sequence of SEQ ID No: 2, is at least about 30% of the total recombinant β -lactoglobulin proteins in the composition; or
- D) any combination of any two or more of (A) to (C).

[0011] In one example the disclosure relates to a composition comprising two or more recombinant β -lactoglobulin proteins of differing amino acid sequence, the recombinant β -lactoglobulin proteins having at least 70% sequence identity to a wild type, mature β -lactoglobulin, and wherein at least one of the β -lactoglobulin proteins is an elongated β -lactoglobulin protein. Preferably, the elongated β -lactoglobulin protein comprises an N-terminal elongation.

[0012] In another example the disclosure relates to a food product comprising a recombinant, elongated β -lactoglobulin protein having at least 70% sequence identity to a wild type, mature β -lactoglobulin. Preferably, the elongated β -lactoglobulin protein comprises an N-terminal elongation.

[0013] In another example the disclosure relates to a food product comprising a composition disclosed herein.

[0014] In a further example the disclosure relates to a method for preparing a food product, the method comprising

- a) providing a composition disclosed herein, and
- b) mixing the composition with one or more additional ingredients to produce the food product.

[0015] In a further example the disclosure relates to a method for preparing a food product, the method comprising

- a) providing a recombinant, elongated β -lactoglobulin protein having at least 70% sequence identity to a wild type, mature β -lactoglobulin, and
- b) mixing the composition with one or more additional ingredients to produce the food product.

[0016] In various examples the recombinant, elongated β -lactoglobulin protein comprises a protein or composition of the disclosure.

[0017] In one example the disclosure relates to a polynucleotide expression vector for expressing a plurality of recombinant β -lactoglobulin proteins heterogeneous in amino acid sequence, the polynucleotide expression vector encoding

- a) a signal sequence,
- b) a leader sequence,
- c) a β -lactoglobulin protein, and
- d) a processing site selected from KR, KREA, KREAEA and KREAEAM located between the leader sequence and the mature dairy protein.

[0018] In a further example the disclosure provides a polynucleotide expression vector for expressing a composition or recombinant, elongated β -lactoglobulin described herein, the polynucleotide expression vector encoding

- a) a signal sequence;
- b) a leader sequence;

- c) a β -lactoglobulin protein; and
- d) a processing site selected from KR, KREA, KREAEA and KREAEAM located between the leader sequence and the mature dairy protein.

[0019] In another example the disclosure relates to a host cell comprising an expression vector described herein.

[0020] In a further example the disclosure relates to a host cell for expressing a plurality of recombinant β -lactoglobulin proteins heterogeneous in amino acid sequence, wherein the species of the host cell is selected from *Pichia pastoris*, *Kluyveromyces lactis* and *Aspergillus niger*, and wherein the host cell comprises a polynucleotide encoding

- a) a signal sequence,
- b) a leader sequence,
- c) a β -lactoglobulin protein, and
- d) a processing site selected from KR, KREA, KREAEA and KREAEAM located between the leader sequence and the β -lactoglobulin protein.

[0021] In a further example the disclosure relates to a host cell for expressing a plurality of recombinant β -lactoglobulin proteins heterogeneous in amino acid sequence, wherein the species of the host cell is selected from *Pichia pastoris*, *Kluyveromyces lactis*, *Saccharomyces cerevisiae* and *Aspergillus niger*, and wherein the host cell comprises a polynucleotide encoding

- a) a signal sequence,
- b) a leader sequence,
- c) a β -lactoglobulin protein, and
- d) a processing site selected from KR, KREA, KREAEA and KREAEAM located between the leader sequence and the β -lactoglobulin protein.

[0022] In another example the disclosure provides a host cell for expressing a composition or recombinant, elongated β -lactoglobulin described herein, wherein the species of the host cell is selected from *Pichia pastoris*, *Kluyveromyces lactis*, *Saccharomyces cerevisiae* and *Aspergillus niger*, and wherein the host cell comprises a polynucleotide encoding

- a) a signal sequence;
- b) a leader sequence;
- c) a β -lactoglobulin protein; and
- d) a processing site selected from KR, KREA, KREAEA and KREAEAM located between the leader sequence and the β -lactoglobulin protein.

[0023] In one example the disclosure relates to a method for producing a plurality of recombinant β -lactoglobulin proteins heterogeneous in amino acid sequence, comprising

- a) culturing a host cell described herein in a culture medium under conditions sufficient to allow for expression of the one or more recombinant β -lactoglobulin proteins, and
- b) isolating the one or more recombinant proteins from the culture medium.

[0024] In a further example the disclosure provides a method for producing a composition or recombinant, elongated β -lactoglobulin described herein, the method comprising

- a) culturing a host cell described herein in a culture medium under conditions sufficient to allow for expression of one or more recombinant β -lactoglobulin proteins; and
- b) isolating the one or more recombinant β -lactoglobulin proteins from the culture medium.

[0025] In various examples the recombinant β -lactoglobulin proteins have at least 70%, 75%, 80%, 85%, 90%, 95% or 99% sequence identity to any one of SEQ ID Nos: 1-3.

[0026] In various examples the β -lactoglobulin protein may be selected from a mature β -lactoglobulin protein or a full length β -lactoglobulin protein.

[0027] In various examples the polynucleotide expression vector or the polynucleotide may encode a wild type β -lactoglobulin protein. In various examples the polynucleotide expression vector or the polynucleotide may encode a wild type, mature β -lactoglobulin protein. In various examples the wild type β -lactoglobulin protein is a bovine β -lactoglobulin protein, such as a bovine wild type mature β -lactoglobulin protein.

[0028] In various examples the polynucleotide encodes a β -lactoglobulin protein comprising, or consisting of a sequence of any one of SEQ ID Nos:1 to 3, 34, 36, 38, 40, 42, 44, 45, or 47. In various examples the polynucleotide encodes a β -lactoglobulin protein comprising, or consisting of a sequence of any one of SEQ ID Nos:1 to 3.

[0029] In various examples the processing site may be selected from KR, KREA, and KREAEAM. In other examples the processing site may be selected from KREA, KREAEA and KREAEAM.

[0030] In various examples the host cell lacks an operative *pep4* gene, or has been modified to produce less PEP4 protein than a wild type control cell. In one example the host cell lacks an operative *pep4* gene.

[0031] In another example the disclosure relates to a recombinant, elongated β -lactoglobulin protein having an amino acid sequence comprising or consisting of

- a) a core sequence having at least 70% sequence identity to the sequence of a wild type, mature β -lactoglobulin; and
- b) an N-terminal elongation of the core sequence, the N-terminal elongation having a sequence comprising or consisting of
 - i) 2 or more contiguous amino acids from the sequence KREAEAM; or
 - ii) R.

[0032] In various examples the N-terminal elongation has a sequence comprising or consisting of 2 or more contiguous amino acids from the sequence

- a) REAEAM
- b) EAEAM; or
- c) EAEA.

[0033] In various examples the N-terminal elongation has a sequence comprising or consisting of any one of:

- a) EAEAM,
- b) EAEA,
- c) EAM,

d) EA; and

e) R.

[0034] In various examples the N-terminal elongation has a sequence comprising or consisting of EA, or two or more repeats of EA, for example three or more repeats of EA, four or more repeats of EA, or five or more repeats of EA. For example, the N-terminal elongation has a sequence comprising or consisting of EA, EAEA, EAEAEA, EAEAEAEA, or EAEAEAEAEA.

[0035] In various examples the recombinant, elongated β -lactoglobulin protein has an amino acid sequence comprising or consisting of a sequence of any one of SEQ ID Nos: 4, 5, 7, 19-20, 22 and 71-74.

[0036] In one example the disclosure relates to a composition comprising one or more recombinant, elongated β -lactoglobulin proteins of the disclosure.

[0037] The following examples may relate to any of the above examples.

[0038] In various examples the wild type, mature β -lactoglobulin has a sequence of one of SEQ ID Nos: 1 to 3, 34, 36, 38, 40, 42, 44, 45, or 47. In some examples the wild type, mature β -lactoglobulin has a sequence of any one of SEQ ID Nos: 1-3. In some examples the wild type, mature β -lactoglobulin has a sequence of any one of SEQ ID Nos: 1 or 2.

[0039] In various examples the amount of recombinant β -lactoglobulin protein having an amino acid sequence comprising an aspartic acid at a position equivalent to position 80 of SEQ ID No: 1 and a valine at a position equivalent to position 134 of SEQ ID No: 1, is at least 90%, 95% or at least 99% of the total recombinant β -lactoglobulin protein in the composition.

[0040] In various examples the amount of recombinant β -lactoglobulin protein having an amino acid sequence comprising a glycine at a position equivalent to position 80 of SEQ ID No: 2 and an alanine at a position equivalent to position 134 of SEQ ID No: 2, is at least 90%, 95% or at least 99% of the total recombinant β -lactoglobulin proteins in the composition.

[0041] In various examples the amount of modified β -lactoglobulin protein in the composition is at least 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99% of the total recombinant β -lactoglobulin proteins in the composition, and various ranges may be selected from between any two of these values.

For example, from about 1 to about 100, about 1 to about 99, about 1 to about 90, about 1 to about 80, about 1 to about 70, about 1 to about 60, about 1 to about 50, about 10 to about 100, about 10 to about 99, about 10 to about 90, about 10 to about 80, about 10 to about 70, about 10 to about 60, about 10 to about 50, about 20 to about 100, about 20 to about 99, about 20 to about 90, about 20 to about 80, about 20 to about 70, about 20 to about 60, about 20 to about 50, about 30 to about 100, about 30 to about 99, about 30 to about 90, about 30 to about 80, about 30 to about 70, about 30 to about 60, about 30 to about 50, about 40 to about 100, about 40 to about 99, about 40 to about 90, about 40 to about 80, about 40 to about 70, about 40 to about 60, about 40 to about 50, about 50 to about 100, about 50 to about 99, about 50 to about 90, about 50 to about 80, about 50 to about 70, about 50 to about 60, about 60 to about 100, about 60 to about 99, about 60 to about 90, about 60 to about 80, about 60 to about 70, about 70 to about 100, about 70 to about 99, about 70 to about 90, about 70 to about 80, about 80 to about 100, about 80 to about 99, or about 80 to about 90% of the total recombinant β -lactoglobulin proteins in the composition.

[0042] In various examples the recombinant β -lactoglobulin proteins, or the one or more recombinant, elongated β -lactoglobulin proteins, may have at least about 70, 75, 80, 85, 90, 95, 99 or 100% sequence identity to

- a) a wild type, mature β -lactoglobulin;
- b) any one of SEQ ID Nos:1 to 3, 34, 36, 38, 40, 42, 44, 45, or 47;
- c) any one of SEQ ID Nos:1-3;
- d) SEQ ID No:1 or 2;
- e) SEQ ID No:1; or
- f) SEQ ID No:2.

[0043] In various examples the N-terminal elongation consists of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or about 20 amino acids, and various ranges may be selected from between any two of these values, for example, from about 1 to about 20, about 1 to about 10, about or 1 to about 5 amino acids.

[0044] In various examples the N-terminal truncation consists of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or about 20 amino acids, and various ranges may be selected from between any two of these values, for example, from about 1 to about 20, about 1 to about 10, about or 1 to about 5 amino acids.

[0045] In various examples the plurality comprises at least one modified β -lactoglobulin protein comprising or consisting of an amino acid sequence that comprises one or more substitutions of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or about 20 amino acids relative to the sequence of one of SEQ ID Nos: 1 to 3, and various ranges may be selected from between any two of these values, for example, from about 1 to 10, 1 to 5, 1 to 4, or 1 to 3 amino acids relative to the sequence of one of SEQ ID Nos: 1 to 3.

[0046] In various examples the one or more substitutions comprises one or more or from 1 to about 20, about 1 to about 10, about 1 to about 5, about 1 to about 3 or 1 or 2 essential amino acids.

[0047] In various examples the one or more essential amino acids comprise or consist of one or more histidine residues.

[0048] In various examples the amount of recombinant β -lactoglobulin protein comprising or consisting of an amino acid sequence of any one of a) a wild type, mature β -lactoglobulin; b) SEQ ID Nos: 1 to 3, 34, 36, 38, 40, 42, 44, 45, or 47; c) SEQ ID Nos 1 to 3, or d) SEQ ID No 1 or 2, is less than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 65, 70, 75, 80, 85, 90, 95, 99 or about 100% of the total recombinant β -lactoglobulin proteins in the composition, and various ranges may be selected from between any of these values, for example, from about 1 to about 99, about 1 to about 90, about 1 to about 80, about 1 to about 75, about 1 to about 70, about 1 to about 65, about 1 to about 60, about 1 to about 55, about 1 to about 50, about 1 to about 45, about 1 to about 40, about 1 to about 35, about 1 to about 30, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, or about 1 to about 5% of the total recombinant β -lactoglobulin proteins in the composition.

[0049] In various examples the food product or composition may comprise a recombinant, elongated β -lactoglobulin protein.

[0050] In various examples the food product or composition may comprise one or more, two or more, three or more or four or more recombinant elongated β -lactoglobulin proteins of differing amino acid sequence. In various examples the food product or composition may comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 15, 16, 18 or 20 recombinant elongated β -lactoglobulin proteins of differing amino acid sequence, and suitable ranges may be selected from between any of these values, for example, from about 1 to about 20, about 1 to about 15, about 1 to about 10, about 1 to about 5, about 2 to about 20, about 2 to about 15, about 2 to about 10, about 2 to about 5, about 3 to about 20, about 3 to about 15, about 3 to about 10, or about 3 to about 5.

[0051] In various examples the one or more recombinant, elongated β -lactoglobulin proteins may have an amino acid sequence comprising or consisting of

- a) a core sequence having at least 70% sequence identity to the sequence of a wild type, mature β -lactoglobulin; and
- b) an N-terminal elongation of the core sequence, the N-terminal elongation having a sequence comprising or consisting of from about 1 to about 20 amino acids.

[0052] In various examples the one or more recombinant, elongated β -lactoglobulin proteins may have an amino acid sequence comprising or consisting of

- a) a core sequence having at least 70% sequence identity to the sequence of a wild type, mature β -lactoglobulin; and
- b) an N-terminal elongation of the core sequence, the N-terminal elongation having a sequence comprising or consisting of 1 amino acid or 2 or more contiguous amino acids from the sequence KREAEAM, KREAEAEAM, or KREAEAEAEAM.

[0053] In various examples the recombinant, elongated β -lactoglobulin protein may have an amino acid sequence comprising or consisting of a core sequence having at least about 70, 75, 80, 85, 90, 95, 99 or 100% sequence identity to

- a) a wild type, mature β -lactoglobulin;
- b) any one of SEQ ID Nos:1 to 3, 34, 36, 38, 40, 42, 44, 45, or 47;
- c) any one of SEQ ID Nos:1-3;
- d) SEQ ID No:1 or 2;
- e) SEQ ID No:1; or
- f) SEQ ID No:2.

[0054] In various examples the N-terminal elongation may have a sequence comprising or consisting of 1 amino acid or 2 or more contiguous amino acids from the sequence

- a) REAEAEAEAM, REAEAEAM, or REAEAM

- b) EAEAEAEAM, EAEAEAM, or EAEAM; or
- c) EAEAEAEA, EAEAEA, or EAEA.

[0055] In various examples the one or more recombinant, elongated β -lactoglobulin proteins may have an N-terminal elongation having a sequence comprising or consisting of any one of:

- a) EAEAM
- b) EAEA
- c) EAM
- d) EA
- e) three or more repeats of EA
- f) M; and
- g) R.

[0056] In various examples the composition further comprises

- a) one or more recombinant, truncated β -lactoglobulin proteins that comprise or consist of an amino acid sequence having an N-terminal truncation of from about 1 to about 20 amino acids relative to the sequence of a wild type, mature β -lactoglobulin; and/or
- b) one or more recombinant, substituted β -lactoglobulin proteins that comprise or consist of an amino acid sequence having one or more substitutions of from about 1 to about 20 amino acids relative to the sequence of a wild type, mature β -lactoglobulin.

[0057] In various examples the amount of modified β -lactoglobulin protein comprising or consisting of an amino acid sequence that comprises an N-terminal elongation of from about 1 to about 20 amino acids relative to the sequence of any one of a) a wild type, mature β -lactoglobulin; b) SEQ ID Nos: 1 to 3, 34, 36, 38, 40, 42, 44, 45, or 47; c) SEQ ID Nos 1 to 3, or d) SEQ ID No 1 or 2, is at least about 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 65, 70, 75, 80, 85, 90, 95, 99 or about 100% of the total recombinant β -lactoglobulin proteins in the composition, and various ranges may be selected from between any of these values, for example, from about 0 to about 100, about 0 to about 99, about 0 to about 90, about 0 to about 80, about 0 to about 70,

about 0 to about 60, about 0 to about 50, about 0 to about 40, about 0 to about 30, about 0 to about 20, about 0 to about 10, about 1 to about 100, about 1 to about 99, about 1 to about 90, about 1 to about 80, about 1 to about 70, about 1 to about 60, about 1 to about 50, about 10 to about 100, about 10 to about 99, about 10 to about 90, about 10 to about 80, about 10 to about 70, about 10 to about 60, about 10 to about 50, about 20 to about 100, about 20 to about 99, about 20 to about 90, about 20 to about 80, about 20 to about 70, about 20 to about 60, about 20 to about 50, about 30 to about 100, about 30 to about 99, about 30 to about 90, about 30 to about 80, about 30 to about 70, about 30 to about 60, about 30 to about 50, about 40 to about 100, about 40 to about 99, about 40 to about 90, about 40 to about 80, about 40 to about 70, about 40 to about 60, about 40 to about 50, about 50 to about 100, about 50 to about 99, about 50 to about 90, about 50 to about 80, about 50 to about 70, about 50 to about 60, about 60 to about 100, about 60 to about 99, about 60 to about 90, about 60 to about 80, about 60 to about 70, about 70 to about 100, about 70 to about 99, about 70 to about 90, about 70 to about 80, about 80 to about 100, about 80 to about 99, or about 80 to about 90% of the total recombinant β -lactoglobulin proteins in the composition.

[0058] In various examples the amount of the one or more recombinant, elongated β -lactoglobulin proteins in the composition may be at least about 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 65, 70, 75, 80, 85, 90, 95, 99 or about 100% of the total recombinant β -lactoglobulin proteins in the composition, and various ranges may be selected from between any of these values, for example, from about 0 to about 100, about 0 to about 99, about 0 to about 90, about 0 to about 80, about 0 to about 70, about 0 to about 60, about 0 to about 50, about 0 to about 40, about 0 to about 30, about 0 to about 20, about 0 to about 10, about 1 to about 100, about 1 to about 99, about 1 to about 90, about 1 to about 80, about 1 to about 70, about 1 to about 60, about 1 to about 50, about 10 to about 100, about 10 to about 99, about 10 to about 90, about 10 to about 80, about 10 to about 70, about 10 to about 60, about 10 to about 50, about 20 to about 100, about 20 to about 99, about 20 to about 90, about 20 to about 80, about 20 to about 70, about 20 to about 60, about 20 to about 50, about 30 to about 100, about 30 to about 99, about 30 to about 90, about 30 to about 80, about 30 to about 70, about 30 to about 60, about 30 to about 50, about 40 to about 100, about 40 to about 99, about 40 to about 90, about 40 to about 80, about 40 to about 70, about 40 to about 60, about 40 to about 50, about 50 to about 100, about 50 to about 99, about 50 to about 90, about 50 to about 80, about 50 to about 70, about 50 to about 60, about 60 to about 100, about 60 to about 99, about 60 to about 90, about 60 to about 80, about 60 to about 70, about 70 to about 100, about 70 to about 99, about 70 to about 90, about 70 to about 80, about 80 to about 100, about 80 to about 99, or about 80 to about 90% of the total recombinant β -lactoglobulin proteins in the composition.

[0059] In various examples the amount of modified β -lactoglobulin protein comprising or consisting of an amino acid sequence that comprises an N-terminal truncation of from about 1 to about 20 amino acids relative to the sequence of any one of a) a wild type, mature β -lactoglobulin; b) SEQ ID Nos: 1 to 3, 34, 36, 38, 40, 42, 44, 45, or 47; c) SEQ ID Nos 1 to 3, or d) SEQ ID No 1 or 2, is at least about 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 65, 70, 75, 80, 85, 90, 95, 99 or about 100% of the total recombinant β -lactoglobulin proteins in the composition, and various ranges may be selected from between any of these values, for example, from about 1 to about 100, about 1 to about 99, about 1 to about 90, about 1 to about 80, about 1 to about 70, about 1 to about 60, about 1 to about 50, about 10 to about 100, about 10 to about 99, about 10 to about 90, about 10 to about 80, about 10 to about 70, about 10 to about 60, about 10 to about 50, about 20 to about 100, about 20 to about 99, about 20 to about 90, about 20 to about 80, about 20 to about 70, about 20 to about 60, about 20 to about 50, about 30 to about 100, about 30 to about 99, about 30 to about 90, about 30 to about 80, about 30 to about 70, about 30 to about 60, about 30 to about 50, about 40 to about 100, about 40 to about 99, about 40 to about 90, about 40 to about 80, about 40 to about 70, about 40 to about 60, about 40 to about 50, about 50 to about 100, about 50 to about 99, about 50 to about 90, about 50 to about 80, about 50 to about 70, about 50 to about 60, about 60 to about 100, about 60 to about 99, about 60 to about 90, about 60 to about 80, about 60 to about 70, about 70 to about 100, about 70 to about 99, about 70 to about 90, about 70 to about 80, about 80 to about 100, about 80 to about 99, or about 80 to about 90 of the total recombinant β -lactoglobulin proteins in the composition.

[0060] In various examples:

- a) the amount of recombinant β -lactoglobulin protein comprising or consisting of an amino acid sequence of any one of SEQ ID Nos 1 to 3, is from about 1 to about 10% of the total recombinant β -lactoglobulin proteins in the composition;
- b) the amount of modified β -lactoglobulin protein comprising or consisting of an amino acid sequence that comprises an N-terminal elongation of from about 1 to about 20 amino acids relative to the sequence of any one of SEQ ID Nos 1 to 3, is about 50 to about 99% of the total recombinant β -lactoglobulin proteins in the composition; and
- c) the amount of modified β -lactoglobulin protein comprising or consisting of an amino acid sequence that comprises an N-terminal truncation of from about 1 to about 20 amino acids relative to the sequence of any one of

SEQ ID Nos 1 to 3, is at least about 30% of the total recombinant β -lactoglobulin proteins in the composition.

[0061] In various examples:

- a) the amount of recombinant β -lactoglobulin protein comprising or consisting of an amino acid sequence of any one of SEQ ID Nos 1 to 3, is from about 1 to about 50% of the total recombinant β -lactoglobulin proteins in the composition;
- b) the amount of modified β -lactoglobulin protein comprising or consisting of an amino acid sequence that comprises an N-terminal elongation of from about 1 to about 20 amino acids relative to the sequence of any one of SEQ ID Nos 1 to 3, is about 0 to about 10% of the total recombinant β -lactoglobulin proteins in the composition; and
- c) the amount of modified β -lactoglobulin protein comprising or consisting of an amino acid sequence that comprises an N-terminal truncation of from about 1 to about 20 amino acids relative to the sequence of any one of SEQ ID Nos 1 to 3, is from about 50 to about 99% of the total recombinant β -lactoglobulin proteins in the composition.

[0062] In various examples the modified β -lactoglobulin protein comprises one or more recombinant proteins each comprising or consisting of a sequence of any one of SEQ ID Nos: 4-7 and SEQ ID Nos: 19-22. In various examples the modified β -lactoglobulin protein comprises one or more recombinant proteins each comprising or consisting of a sequence of any one of SEQ ID Nos: 4-7, 19-22 and 71-74.

[0063] In various examples the composition or food product comprises one or more recombinant, elongated β -lactoglobulin proteins comprising or consisting of a sequence of any one of SEQ ID Nos: 4-7, 19-22 and 71-74.

[0064] In various examples the composition or food product comprises two or more, three or more, five or more, six or more or seven or more recombinant, elongated β -lactoglobulin proteins of differing amino acid sequence. In various examples the composition comprises at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 15, 16, 18 or 20 recombinant, elongated β -lactoglobulin proteins of differing amino acid sequence, and various ranges may be selected from between these values, for example from about 1 to about 20, about 1 to about 15, about 1 to about 10, about 1 to about 8, about 1 to about 6, about 1 to about 5, or about 2 to about 20, about 2 to about 15, about 2 to about 10, about 2 to about 8, about 2 to about 6, about 2 to about 5, or about 3 to about 20, or

about 3 to about 10 recombinant, elongated β -lactoglobulin proteins of differing amino acid sequence. In various examples the composition or food product may comprise one or more, two or more, three or more or four or more recombinant β -lactoglobulin proteins having an amino acid sequence selected from SEQ ID Nos: 4-7, 19-22 and 71-74.

[0065] In various examples the composition or food product may comprise one or more, two or more, three or more or four or more recombinant β -lactoglobulin proteins having an amino acid sequence selected from

- a) SEQ ID Nos:4-7, 71 and 72; or
- b) SEQ ID Nos:19-22, 73 and 74.

[0066] In various examples the modified β -lactoglobulin protein comprises one or more recombinant proteins each comprising or consisting of a sequence of any one of SEQ ID Nos: 8-18, SEQ ID Nos: 23-30 and SEQ ID NO: 61.

[0067] In various examples the plurality of recombinant β -lactoglobulin proteins has a secondary protein structure comprising or consisting of:

- a) from about 0 to about 6% α -helix;
- b) from about 40 to about 55% anti-parallel β -sheet;
- c) from about 0 to about 3% parallel β -sheet;
- d) from about 8 to about 20% turns; and
- e) about 35 to about 45% unspecified or unordered structure.

[0068] In various examples, wherein the wild type, mature β -lactoglobulin has a sequence of SEQ ID No:1, the plurality of recombinant β -lactoglobulin proteins has a secondary protein structure comprising or consisting of

- a) from about 0.5 to about 6%, about 1 to about 6% or about 2 to about 6% α -helix;
- b) from about 40 to about 55%, about 40 to about 50%, or about 40 to about 45% anti-parallel β -sheet;
- c) from about 0 to about 3%, or about 0 to about 2.5%, parallel β -sheet;

- d) from about 8 to about 20% about 10 to about 20%, or about 10 to about 15% turns; and
- e) about 35 to about 45% unspecified or unordered structure.

[0069] In various examples, wherein the wild type, mature β -lactoglobulin has a sequence of SEQ ID No:2, the plurality of recombinant β -lactoglobulin proteins has a secondary protein structure comprising or consisting of

- a) from about 0 to about 5%, about 0 to about 4%, about 0 to about 3% or about 0 to about 2% or about 0 to about 1% α -helix;
- b) from about 40 to about 55%, about 45 to about 55%, or about 45 to about 50% anti-parallel β -sheet;
- c) from about 0 to about 3%, about 0 to about 2%, or about 0 to about 1%, parallel β -sheet;
- d) from about 8 to about 20% about 10 to about 20% or about 10 to about 15% turns; and
- e) about 35 to about 45% unspecified or unordered structure.

[0070] In various examples the plurality of recombinant β -lactoglobulin proteins has a mean DIAAS score of at least 0.8.

[0071] In various examples the plurality of recombinant β -lactoglobulin proteins denatures at a higher temperature than a protein consisting of the sequence of SEQ ID No:1 or 2 as measured by differential scanning calorimetry.

[0072] In various examples, the plurality of recombinant β -lactoglobulin proteins denatures at a lower temperature than a protein consisting of the sequence of SEQ ID No:1 or 2 as measured by differential scanning calorimetry.

[0073] In various examples the storage modulus of a heat set gel comprising the composition at a pH of about 7 is at least equivalent to that of NZMP SureProtein™ WPI895 when measured at a frequency of 1Hz and at 20°C, wherein the heat set gel is formed by heating a solution comprising 10% by weight of the plurality of recombinant β -lactoglobulin proteins or WPI895 by heating from 20 to 80°C at a rate of 1°C/min, holding the solution at 80°C for 30 min, and cooling the solution from 80 to 20°C at a rate of 1°C/min and holding the temperature at 20°C for 20 min on a rheometer to form the gel.

[0074] In various examples the food product is a yoghurt and the yoghurt exhibits increased, similar or reduced firmness and/or viscosity compared with a control food product having the same ingredient composition and total protein content, except that the recombinant β -lactoglobulin protein is replaced with milk-derived β -lactoglobulin.

[0075] In various examples the stiffness (G') at 20°C of a heat set gel comprising the composition at a pH of about 7 is at least about 7,000 when measured at a frequency of 0.1 Hz and a strain of 0.25%, wherein the heat set gel is formed by heating a solution comprising 10% by weight of the protein(s) from 20°C to 90°C at a rate of 5°C/min, holding at 90°C for 30 min, and cooling to 20°C at a rate of 5°C/min. In various examples, the stiffness (G') is at most about 30,000, or from 8,000 to 30,000.

[0076] In various examples the stiffness (G') at 20°C of a heat set gel comprising the composition at a pH of about 7 is at least about 80% of that of a heat set gel formed with wild type β -lactoglobulin when measured at a frequency of 0.1 Hz and a strain of 0.25%, wherein the heat set gel is formed by heating a solution comprising 10% by weight of the protein(s) from 20°C to 90°C at a rate of 5°C/min, holding at 90°C for 30 min, and cooling to 20°C at a rate of 5°C/min. In various examples, the stiffness (G') is at most about 200%, or from 85% to 180% of that of a heat set gel formed with wildtype β -lactoglobulin.

[0077] In various examples the final stiffness of an acid-set gel formed by combining milk and 1.0% by weight of the protein(s), heating to 80°C for 30 minutes, cooling to 30°C, and acidifying with 2% w/w glucono- δ -lactone (GDL) is at least 400 Pa, or at least 100% of that of an acid-set gel formed using wild type bovine β -lactoglobulin.

[0078] In various examples a shelf-stable protein beverage comprising 3.5% by weight of the plurality of recombinant β -lactoglobulin proteins exhibits no visible sedimentation after 4 weeks at ambient temperature.

[0079] In various examples a solution having a pH of about 7 and comprising 10% by weight of the plurality of recombinant β -lactoglobulin proteins has an overrun of at least 500% after 10 minutes whipping, and/or wherein a foam produced by whipping a solution of neutral pH comprising 10% by weight of the plurality of recombinant proteins exhibits no serum leakage for at least about 5 minutes after whipping.

[0080] In various examples a solution of neutral pH comprising 2.5% by weight of the plurality of recombinant proteins has a foam volume after 70 seconds whipping of

at least 75mL, and/or produces a foam after 70 seconds of whipping that retains at least 50% of its volume after 45 minutes.

[0081] In various examples an emulsion comprising the composition exhibits increased, the same or reduced stability compared with an emulsion comprising a protein having a sequence of any one of SEQ ID Nos:1 or 2, wherein the emulsion is formed by combining the composition with oil to in an amount of from 2.5 to 17.5% and water, pre-homogenizing and homogenizing by sonication at 100% amplitude for 10 min, and measuring particle size using laser light scattering.

[0082] In various examples the emulsion activity index (EAI) of an emulsion comprising the composition is increased, the same or decreased compared with an emulsion comprising a protein having a sequence of SEQ ID Nos:1 or 2, wherein the emulsion is formed by preparing a solution comprising 0.5% of the plurality of recombinant β -lactoglobulin proteins, 20% soya oil and water and homogenizing the solution at 200 bar, and wherein the EAI is determined by measuring the specific surface area of the emulsion droplets using laser light scattering.

[0083] In various examples, an emulsion comprising the composition exhibits increased, the same or decreased emulsifying capacity (EC) compared with an emulsion comprising a protein of SEQ ID No:1 or 2. In some examples the emulsion is formed by combining the composition, using different levels of oil to give a range of oil to protein ratios and homogenizing and immediately measuring the electrical conductivity to determine EC.

[0084] In various examples, an emulsion comprising a composition with 0.5% w/w protein has an EC of at least 900 mL oil/g protein and/or has an EC of at least 90% of that of wild type β -lactoglobulin.

[0085] In various examples the composition may be substantially free of aspartyl protease-like activity.

[0086] In various examples, when the composition is added to a skim milk composition comprising 10% by weight total solids to a concentration of the one or more recombinant proteins of about 1% by weight and the skim milk composition is heated at 80°C for 30 minutes then held at 5°C for 6 hours,

- a) no degradation of κ -casein is observed, and/or
- b) no production of para- κ -casein is observed.

[0087] In various examples, when the composition is added to a skim milk composition comprising 10% by weight total solids to a concentration of the one or more recombinant proteins of about 1% by weight and incubated at ambient temperature for 24 hours, less than about 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5% or 1%, or 0% of κ -casein in the skim milk composition is degraded to form para- κ -casein.

[0088] In various examples, when the composition is added to a skim milk composition comprising 10% by weight total solids to a concentration of the one or more recombinant proteins of about 1% by weight and the skim milk composition is held at ambient temperature for 15 minutes then heated at 140 °C, the composition has not coagulated after about 14 minutes.

[0089] In some examples, when the composition is added to a skim milk composition comprising 10% by weight total solids to a concentration of the one or more recombinant proteins of about 1% by weight, and the skim milk composition is heated at 40 °C for 12 hours, the skim milk composition does not reach gelation point.

[0090] In various examples the composition is substantially free of aspartyl protease

- a) consisting of an amino acid sequence of SEQ ID no. 62 or 63, or
- b) having at least 75%, 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID Nos: 62 or 63.

[0091] In various examples the food product is in bar or solid moulded form. "Solid moulded form" means that the food product has been moulded into a shape that holds its form.

[0092] In various examples the food product may be selected from the group consisting of a fermented food, a yoghurt, a soup, a sauce, a bar, a gel, a foam, a nutritional formulation, a beverage, a beverage whitener, a cheese, a dairy tofu, a food emulsion and a dessert.

[0093] In various examples the food product may be a yoghurt, drinking yoghurt, a bar, a gel, a foam, a nutritional formulation, medical food, dairy beverage, a product that requires the protein to form a heat-set gel, an acid protein fortified beverage, a jelly drink, a protein water, a heat-set foam extruded food product, or a food emulsion.

[0094] In various examples the nutritional formulation or food may be an infant formula, toddler milk, growing up formula, maternal formula, a sports beverage, food for active lifestyles, a medical food or supplement.

[0095] In various examples the nutritional formulation may be selected from the group comprising an infant formula, a follow-on formula, a toddler milk, a growing up formula, a maternal formula, a food for active lifestyles, a medical food and a supplement.

[0096] In various examples the cheese may be fresh cheese. In various examples the cheese may be processed cheese, Petit-Suisse, cottage cheese, or quark.

[0097] In various examples the beverage is selected from the group comprising a dairy beverage, a sports beverage, a smoothie, a protein fortified fruit or vegetable juice, a drinking yoghurt, an acid protein fortified beverage, a jelly drink, protein water, liquid coffee, liquid tea, and a liquid beverage whitener.

[0098] In various examples the food product may comprise an additional source of protein. In various examples the additional source of protein is a non-dairy source. In various examples the non-dairy source may comprise a plant source, algal protein, mycoprotein, or a combination thereof.

[0099] In various examples the plant source may comprise one or more legumes, grains, seeds, nuts, tubers, or any combination of any two or more thereof.

[00100] In various examples

- a) the legumes may comprise soy, pea, lentil, chickpea, peanut, bean or any combination of any two or more thereof;
- b) the grains may comprise wheat, rice, oat, corn or any combination of any two or more thereof;
- c) the seeds may comprise canola, flaxseed (linseed), hemp, sunflower, quinoa, chia, or any combination of any two or more thereof;
- d) the nuts may comprise almond, cashew, walnut or any combination of any two or more thereof; and/or
- e) the tuber may comprise potato.

[00101] In various examples the food product is suitable for those on a vegan diet.

[00102] In various examples the food product may not comprise any milk proteins other than the recombinant, β -lactoglobulin proteins.

[00103] In various examples the food product comprises casein. In various examples the food product comprises κ -casein.

[00104] In various examples the method for preparing the food product comprises mixing the composition with one or more additional ingredients to produce the food product, preferably wherein at least one ingredient comprises κ -casein.

[00105] In various examples the food product comprises milk-derived casein, one or more recombinant casein proteins, or a combination thereof.

[00106] In various examples the food product comprises milk-derived κ -casein, recombinant κ -casein, or a combination thereof.

[00107] In various examples the food product or the one or more additional ingredients may comprise whole milk, skim milk, a milk protein concentrate (MPC), a milk protein isolate (MPI), micellar casein, or a combination of any two or more thereof.

[00108] The term "heterogeneous" as used herein with reference to a plurality of recombinant proteins means that the plurality of recombinant proteins comprises at least two or two or more, three or more, four or more, five or more, six or more, or seven or more proteins of differing amino acid sequence.

[00109] The term "mature" as used herein with reference to β -lactoglobulin proteins refers to the β -lactoglobulin protein, or amino acid sequence of the protein, after cleavage of the signal sequence. The term "full length" as used herein with reference to β -lactoglobulin proteins refers to the β -lactoglobulin protein, or amino acid sequence of the protein, comprising the signal sequence. Examples of mature and full length β -lactoglobulin sequences are provided in Table 1 herein.

[00110] The term "sequence identity" as used herein in the context of amino acid sequences is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in a selected sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can

be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared.

[00111] The terms "variant" or "modified β -lactoglobulin protein" as used herein may include proteins comprising an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or at least about 99% sequence identity to the sequence of a wild type (native) β -lactoglobulin (either full length or mature β -lactoglobulin lacking a signal sequence, but preferably the mature sequence), but particularly any wild type bovine, ovine, caprine, buffalo, equine, donkey or reindeer β -lactoglobulin sequence, including any sequence of SEQ ID Nos:1 to 3 or 31-48. In some examples the amino acid sequence of such variants may comprise truncations or elongations at the N-terminus and/or the C-terminus relative to the wild type sequence, for example, elongations or truncations of from about 1 to about 20 amino acids. In some examples, variants or modified β -lactoglobulin proteins may contain from 1 to 20 amino acid insertions, deletions, and/or substitutions (collectively) with respect to the wild type sequence. Such proteins may be referred to herein as "elongated β -lactoglobulin proteins", "truncated β -lactoglobulin proteins" or "substituted β -lactoglobulin proteins". In some examples the variants or modified β -lactoglobulin proteins may comprise one or more post-translational modifications that differ to a wild type β -lactoglobulin protein, including glycosylation and or phosphorylation at one or more residues.

[00112] The term "wild type" as used herein with reference to proteins or polynucleotides refers to a protein or polynucleotide having an amino acid or nucleotide sequences that is the same as that expressed naturally in any species. This term includes all naturally occurring variants of a particular protein, for example, all naturally occurring variants of β -lactoglobulin. Furthermore, this term includes both full length β -lactoglobulin proteins and mature β -lactoglobulin proteins and polynucleotides that encode wild type full length and mature β -lactoglobulin. The term is generally synonymous with the term "native".

[00113] The term "comprising" as used in this specification and claims means "consisting at least in part of". When interpreting statements in this specification and claims which include the term "comprising", other features besides the features prefaced by this term in each statement can also be present. Related terms such as "comprise" and "comprised" are to be interpreted in similar manner.

[00114] As used herein the term "and/or" means "and" or "or", or both.

[00115] It is intended that reference to a range of numbers disclosed herein (for example, 1 to 10) also incorporates reference to all rational numbers within that range (for example, 1, 1.1, 2, 3, 3.9, 4, 5, 6, 6.5, 7, 8, 9 and 10) and also any range of rational numbers within that range (for example, 2 to 8, 1.5 to 5.5 and 3.1 to 4.7) and, therefore, all sub-ranges of all ranges expressly disclosed herein are hereby expressly disclosed. These are only examples of what is specifically intended and all possible combinations of numerical values between the lowest value and the highest value enumerated are to be considered to be expressly stated in this application in a similar manner.

[00116] The disclosure may also be said broadly to consist in the parts, elements and features referred to or indicated in the specification of the application, individually or collectively, in any or all combinations of two or more of said parts, elements or features, and where specific integers are mentioned herein which have known equivalents in the art to which the disclosure relates, such known equivalents are deemed to be incorporated herein as if individually set forth.

[00117] Although the present disclosure is broadly as defined above, those persons skilled in the art will appreciate that the disclosure is not limited thereto and that the disclosure also includes examples of which the following description gives examples.

BRIEF DESCRIPTION OF THE DRAWINGS

[00118] The invention will now be described by way of example only and with reference to the drawings in which:

[00119] Figure 1 provides plasmid maps of the following expression constructs: (A) pLGAAOX-005 for expression of β -lactoglobulin variant A; and (B) pLGBAOX-005 for expression of the β -lactoglobulin variant B.

[00120] Figure 2 provides a plasmid map of the expression construct pLGATOP-002 for expression of the β -lactoglobulin variant A.

[00121] Figure 3 provides a plasmid map of the expression construct pLGBTOP-002 for expression of the β -lactoglobulin variant B.

[00122] Figure 4 provides a map of the marker-gene containing vector pGBAAS-3.

[00123] Figure 5 provides a plasmid map of the expression construct pCASPP-05.

CLAIMS

1. A composition comprising two or more recombinant β -lactoglobulin proteins of differing amino acid sequence, the recombinant β -lactoglobulin proteins having at least 70% sequence identity to a wild type, mature β -lactoglobulin, and wherein at least one of the β -lactoglobulin proteins is an elongated β -lactoglobulin protein;

wherein the composition comprises one or more recombinant, elongated β -lactoglobulin proteins having an amino acid sequence comprising or consisting of

- a) a core sequence having at least 70% sequence identity to the sequence of a wild type, mature β -lactoglobulin; and
- b) an N-terminal elongation of the core sequence, the N-terminal elongation having a sequence consisting of the sequence:
 - i) KREAEAM, KREAEAEAM, or KREAEAEAEAM;
 - ii) REAEAEAEAM, REAEAEAM, or REAEAM;
 - iii) EAEAEAEAM, EAEAEAM, or EAEAM;
 - iv) EAEAEAEA, EAEAEA, or EAEA;
 - v) EAM;
 - vi) EA; or
 - vii) R.

2. The composition of claim 1, wherein the composition comprises:

- a) one or more recombinant, elongated β -lactoglobulin proteins comprising or consisting of a sequence of any one of SEQ ID Nos: 4-7, 19-22 and 71-74; and/or
- b) two or more or three or more recombinant, elongated β -lactoglobulin proteins of differing amino acid sequence.

3. The composition of claim 1 or 2, wherein the amount of recombinant, elongated β -lactoglobulin proteins in the composition is:

- a) at least about 4% of total recombinant β -lactoglobulins in the composition; and/or
 - b) at least about 40%, 50% or 60% of total recombinant β -lactoglobulins in the composition.
4. The composition of any one of claims 1 to 3, wherein the composition further comprises
- a) one or more recombinant, truncated β -lactoglobulin proteins that comprise or consist of an amino acid sequence having an N-terminal truncation of from 1 to 20 amino acids relative to the sequence of a wild type, mature β -lactoglobulin; and/or
 - b) one or more recombinant, substituted β -lactoglobulin proteins that comprise or consist of an amino acid sequence having one or more substitutions of from 1 to 20 amino acids relative to the sequence of a wild type, mature β -lactoglobulin.
5. The composition of claim 4, wherein the one or more substituted β -lactoglobulin proteins each comprise or consist of an amino acid sequence that comprises one or more substitutions of from 1 to 10, 1 to 5, 1 to 4, 1 to 3 amino acids, or one amino acid, relative to the sequence of
- a) a wild type, mature β -lactoglobulin;
 - b) any one of SEQ ID Nos: 1-3, 34, 36, 38, 40, 42, 44, 45, or 47;
 - c) any one of SEQ ID Nos: 1-3;
 - d) SEQ ID No: 1 or 2;
 - e) SEQ ID No: 1; or
 - f) SEQ ID No: 2.
6. The composition of claim 4 or 5, wherein the one or more truncated β -lactoglobulin proteins each comprise or consist of a sequence of any one of SEQ ID Nos: 8-18 and SEQ ID Nos: 23-30.
7. The composition of any one of claims 1 to 6, wherein the composition is free of aspartyl protease-like activity.

8. A recombinant, elongated β -lactoglobulin protein having an amino acid sequence comprising or consisting of

- a) a core sequence having at least 70% sequence identity to the sequence of a wild type, mature β -lactoglobulin; and
- b) an N-terminal elongation of the core sequence, the N-terminal elongation having a sequence consisting of
 - i) KREAEAEAEAM, KREAEAEAM, or KREAEAM;
 - ii) REAEAEAEAM, REAEAEAM, or REAEAM;
 - iii) EAEAEAEAM, EAEAEAM, or EAEAM;
 - iv) EAEAEAEA , EAEAEA, or EAEA;
 - v) EAM;
 - vi) EA; or
 - vii) R.

9. The recombinant, elongated β -lactoglobulin protein of claim 8, wherein the recombinant, elongated β -lactoglobulin protein has an amino acid sequence comprising or consisting of a sequence of any one of SEQ ID Nos: 4, 5, 7, 19-20, 22 and 71-74.

10. The composition of any one of claims 1 to 7, or the recombinant, elongated β -lactoglobulin protein of claim 8 or 9, wherein the wild type, mature β -lactoglobulin has a sequence of any one of SEQ ID Nos: 1-3, 34, 36, 38, 40, 42, 44, 45, or 47.

11. The composition or recombinant, elongated β -lactoglobulin protein of claim 10, wherein the one or more recombinant β -lactoglobulin proteins have at least 75%, 80%, 85%, 90% or 95% sequence identity to

- a) a wild type, mature β -lactoglobulin;
- b) any one of SEQ ID Nos: 1-3, 34, 36, 38, 40, 42, 44, 45, or 47;
- c) any one of SEQ ID Nos: 1-3;
- d) SEQ ID No: 1 or 2;
- e) SEQ ID No: 1; or

f) SEQ ID No:2.

12. A food product comprising the composition or recombinant, elongated β -lactoglobulin protein of any one of claims 1 to 11.
13. A method for preparing a food product, the method comprising
 - a) providing the composition or recombinant, elongated β -lactoglobulin protein of any one of claims 1 to 11, and
 - b) mixing the composition or the recombinant, elongated β -lactoglobulin protein with one or more additional ingredients to produce the food product.
14. The food product or method of claim 12 or 13, wherein the food product is:
 - a) in bar or solid moulded form;
 - b) selected from the group consisting of a fermented food, a yoghurt, a soup, a sauce, a bar, a gel, a foam, a nutritional formulation, a beverage, a beverage whitener, a cheese, a dairy tofu, a food emulsion and a dessert;
 - c) an infant formula, toddler milk, growing up formula, maternal formula, a sports beverage, food for active lifestyles, or a medical food or supplement; or
 - d) selected from the group consisting of a dairy beverage, a sports beverage, a smoothie, a protein fortified fruit or vegetable juice, a drinking yoghurt, an acid protein fortified beverage, a jelly drink, protein water, liquid coffee, liquid tea, and a liquid beverage whitener.
15. The food product or method of any one of claims 12 to 14, wherein the food product:
 - a) comprises an additional source of protein;
 - b) comprises an additional source of non-dairy protein;
 - c) comprises an additional source of non-dairy protein comprising a plant source, algal protein, mycoprotein, or a combination thereof;

- d) comprises an additional source of non-dairy protein comprising a plant source comprising one or more legumes, grains, seeds, nuts, tubers, or a combination thereof;

is suitable for those on a vegan diet; and/or

does not comprise any milk proteins other than the recombinant, β -lactoglobulin proteins.