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(54) Title: MODIFIED CASEIN PROTEINS

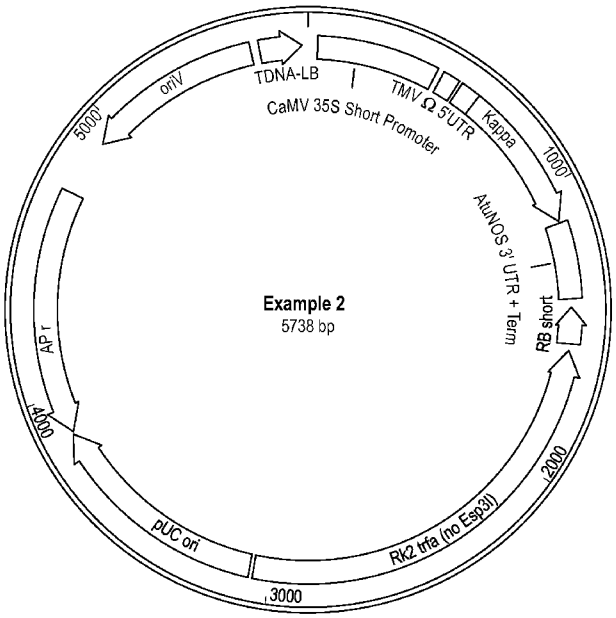



FIG. 1

(57) Abstract: Compositions, methods and systems for producing functional non-glycosylated, under- glycosylated, or differentially-glycosylated recombinant casein proteins in plants which allow for the safe, sustainable, and humane production of milk proteins for commercial use, such as in food compositions. The disclosure provides modified non-glycosylated, under-glycosylated, or differentially-glycosylated recombinant casein protein, or fragments thereof. The disclosure also provides methods for producing modified non-glycosylated, under-glycosylated, or differentially- glycosylated recombinant proteins, and food compositions comprising the same.

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[Continued on next page]

DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

**Declarations under Rule 4.17:**

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

**Published:**

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

**(88) Date of publication of the international search report:**
09 November 2023 (09.11.2023)

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/65558

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C07K 14/00, A23J 3/10, C07K 14/47 (2023.01)
ADD. C12P 21/02 (2023.01)

CPC - INV. C07K 14/4732
ADD. A23C 19/053

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)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>----<br>Y | US 2010/0223682 A1 (KATZ et al.) 2 September 2010 (02.09.2010) Abstract; para [0014]; [0103] | 1-2, 5, 10/5<br>----------<br>3-4 |
| Y | PEDERSEN et al., Caseinomacropeptide specifically stimulates exocrine pancreatic secretion in the anesthetized rat. Peptides, 2000, Vol. 21, No. 10, p 1527- 1535. Abstract; p1527, col 1, para 1 | 3-4 |

☐ Further documents are listed in the continuation of Box C. ☐ 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
"D" document cited by the applicant in the international application
"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 |
|---|---|
| 8 July 2023 | SEP 28 2023 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-8300 | Authorized officer<br>Kari Rodriquez<br>Telephone No. PCT Helpdesk: 571-272-4300 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/65558

**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as fi led.

   b. ☐ furnished subsequent to the international fi ling date for the purposes of international search (Rule 13ter.1(a)),

      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/65558

**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.: 19-35, 47, 68-90
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:
----Please see continuation in first extra 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 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.:
1-5, 10 (in part), limited to a single Ala to Arg substitution

**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.

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US 23/65558

Continuation of Box No. III. Observations where unity of invention is lacking.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+, Claims 1-18, 36-46, 48-67, directed to a mutant or modified casein protein. The mutant or modified casein protein will be searched to the extent that the mutant or modified casein protein encompasses a mutant casein protein comprising a single mutation compared with a wild-type casein protein counterpart, wherein the mutation comprises changing a hydrophobic amino acid A to a hydrophilic amino acid R (note, this is the first claimed mutation for the inventive mutant casein protein). The first named invention was determined based on the first claimed mutation (claim 5) and amino acid substitution (claim 10) for the inventive mutant casein protein embodiment. This first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. It is believed that claims 1-5, 10 (in part), encompass this first named invention, and thus these claims will be searched without fee to the extent that the mutant casein protein encompasses a mutant casein protein comprising a single mutation compared with a wild-type casein protein counterpart, wherein the mutation comprises changing a hydrophobic amino acid A to a hydrophilic amino acid R. Additional mutant casein protein(s) comprising additional amino acid substitution(s) and/or combination thereof, or modified casein protein(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected mutant casein protein(s) comprising additional amino acid substitution(s) and/or combination thereof. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a mutant casein protein comprising a single mutation compared with a wild-type casein protein counterpart, wherein the mutation comprises changing a hydrophobic amino acid A to a hydrophilic amino acid H (claims 1-5, 10 (in part)).

The inventions listed as Group I+ do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features

The inventions of Groups I+ each include the special technical feature of a unique amino acid sequence. Each amino acid sequence comprises a unique peptide, and is considered a distinct technical feature.

Common technical features

No technical features are shared between the mutant or modified casein protein amino acid sequences in each of Group I+ inventions and, accordingly, these inventions lack unity a priori.

Additionally, even if Group I+ inventions were considered to share the technical features of including:
a mutant casein protein comprising at least one mutation compared with a wild-type casein protein counterpart, wherein the at least one mutation provides mammalian glycan-related protein functionality;

a modified casein protein comprising a modification near the C-terminus of the casein protein to increase hydrophilicity of the C-terminus region;
these shared technical features are made obvious by US 2017/0273328 A1 to Perfect Day , Inc, (hereinafter 'PDI') in view of US 2021/0155946 A1 to Mozza (hereinafter 'Mozza').

PDI teaches a mutant casein protein comprising at least one mutation compared with a wild-type casein protein counterpart (Abstract - 'Disclosed herein are methods and compositions including casein, and methods for making these compositions'; Claim 101 - 'A composition comprising: (i) one or more recombinant proteins selected from the group consisting of a ß-lactoglobulin protein, a kappa-casein protein, an a-lactalbumin protein, a ß-casein protein, an a-S2-casein protein, an a-S1-casein protein, and a serum albumin protein, wherein at least one of the one or more recombinant proteins comprises a sequence that is at least 70% identical to the bovine protein amino acid sequence, and is produced in a fungal cell;'; para [0174] - 'A wide range of caseins including casein with substantial homology to the wild-type casein, variants, mutants of casein are expressed and incorporated as a component of milk protein.'; para [0077] - 'For instance, an endogenous coding sequence is considered "recombinant" if it contains an insertion, deletion, or a point mutation introduced artificially, e.g., by human intervention'; para [0083] - 'The term "mutated" when applied to nucleic acid sequences means that nucleotides in a nucleic acid sequence may be inserted, deleted, or changed compared to a reference nucleic acid sequence. A single alteration may be made at a locus (a point mutation) or multiple nucleotides may be inserted, deleted, or changed at a single locus. In addition, one or more alterations may be made at any number of loci within a nucleic acid sequence'). PDI does not teach wherein the at least one mutation provides mammalian glycan-related protein functionality.

----Please see continuation in next extra sheet ----------

Continuation of Box No. III. Observations where unity of invention is lacking.

Mozza teaches expression of recombinant kappa casein in a plant cell system that provides mammalian glycan-related protein functionality (Abstract - 'A method of in vivo assembly of a recombinant micelle including: introducing a plasmid into a plant cell, wherein: the plasmid includes a segment of deoxyribonucleic acid (DNA) for encoding a ribonucleic acid (RNA) for a protein in a casein micelle, the segment of DNA is transcribed and translated; forming recombinant casein proteins in the plant cell, wherein: the recombinant casein proteins include a k-casein and at least one of an aS1-casein, an aS2-casein, a ß-casein; and assembling in vivo a recombinant micelle within the plant cell, wherein: an outer layer of the recombinant micelle is enriched with the k-casein, an inner matrix of the recombinant micelle include at least one of the aS1-casein, the aS2-casein, the ß-casein.'; para [0036] - 'In other embodiments and examples, one or more post-translational modifications of the recombinant casein proteins can occur, including phosphorylation, glycosylation, ubiquitination, nitrosylation, methylation, acetylation, lipidation and proteolysis. In other embodiments no post-translational modifications occur on the recombinant casein proteins, or in other words, the post-translation modification is optional.'). It would have been obvious to one of ordinary skill in the art that by using the casein variants of PDI with the method of Mozza, casein variants with mammalian glycan-related protein functionality could be obtained.

PDI also fails to expressly teach a modified casein protein comprising a modification near the C-terminus of the casein protein to increase hydrophilicity of the C-terminus region. However, since PDI teaches Kappa-casein is inherently hydrophilic (para [0272] - 'The following casein proteins a-s1casein, a-s2casein, and ß-casein are inherently hydrophobic, which precipitate out when secreted from the yeast and come into contact with water. Purification from the reactor media involves collection of the protein from the surface of the media, followed by drying to isolate pure protein. Kappa-casein is inherently hydrophilic and purification of the k-caseins involves the change in pH of the solution to 4.6, followed by centrifugation at 10,000 rcf. Combined casein cassette works the same way as k-casein.'), it would have been obvious that the variants of k-casein could include increased hydrophilic amino acids by amino acid substitutions or insertion of extraneous hydrophilic amino acids e.g., a short hydrophilic peptide, to further increase hydrophilicity of the recombinant casein, to facilitate its purification. One of ordinary skill could have determined the optimal sites for said introduction of modifications/mutations, e.g., at the C-terminus akin to adding a C-terminal tag, using routine experimentation.

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I+ inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

Continuation of item 4 above: claims 19-35, 47, 68-90 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).