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(54) Title: HIGH PH METHOD FOR PURIFICATION OF HYDROPHOBIC PROTEINS

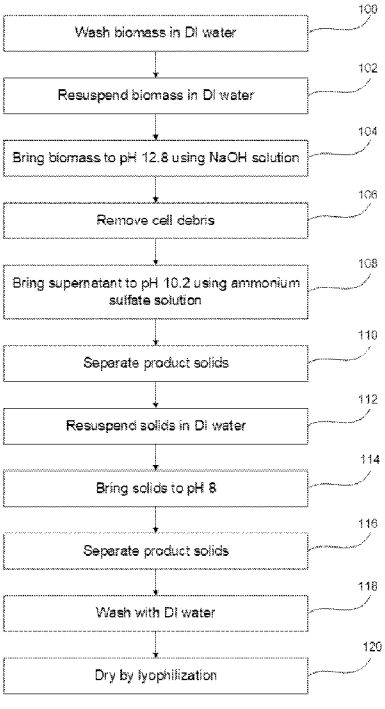


FIG. 1

(57) Abstract: In one aspect, the disclosure relates to a process for purification of hydrophobic proteins and/or proteins that have a tendency to aggregate when produced at high levels in cell culture. In a further aspect, the process involves subjecting intact cells to a strong base in a solution with a pH of greater than 12.5, with an optional initial pretreatment at a pH of greater than 13. The process operates efficiently with a high biomass loading of up to or greater than about 20% (w/v) and does not require the use of external flocculants. The target protein can purified by the disclosed process inexpensively and efficiently, producing a high-quality end product having few to no contaminants. This abstract is intended as a scanning tool for purposes of searching in the particular art and is not intended to be limiting of the present disclosure.

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*[Continued on next page]*

**Declarations under Rule 4.17:**

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

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

**Published:**

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

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

**(88) Date of publication of the international search report:**
15 May 2025 (15.05.2025)

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 24/32894

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C07K 14/435, A23J 1/14 (2024.01)
ADD. A23L 2/66, A23J 3/14, A23L 29/00 (2024.01)

CPC - INV. C07K 14/43504, A23J 1/148
ADD. A23L 33/185, A23V 2002/00

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 2019/0216126 A1 (INNOVATIVE PROTEINS HOLDING LLC) 18 July 2019 (18.07.2019) Abstract; Claim 1; Claim 2; Claim 4; Claim 22; para [0006]; [0039]; [0046]; [0054]; [0055]; [0063]; [0075] | 1, (9-13, 15-16, 20, 22-28)/1<br>---<br>2-4, 8, (9-13, 15-16)/(2-4), 17, 20/(2-4), 21, (22-28)/(2-4) |
| Y | US 2020/0352195 A1 (EAT JUST INC) 12 November 2020 (12.11.2020) Abstract; para [0051]; [0164]; [0174]; [0337] | 2-4, (8-13, 15-16)/(2-4), 17, 20/(2-4), 21, (22-28)/(2-4) |
| Y | FRIEDMAN et al., Factors Governing Lysinoalanine Formation in Soy Proteins. J. Food Sci. September 1984, Volume 49, Issue 5, Pages 1282-1288. pg 1282, col 2, para 10 | 8/(1-4) |

☐ 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 |
|---|---|
| 25 October 2024 (25.10.2024) | NOV 13 2024 |
| 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 24/32894

**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. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing 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 24/32894

**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:
----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.:
1-4, (8-13,15-17, 20-28) (in part), limited to the first noted invention

**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 24/32894

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 1-28, directed to a method for separating a target protein from an intact cell. The method will be searched to the extent that the method encompasses wherein the one or more additives comprise sodium chloride, wherein steps (c) and (e) are carried out using a precipitation agent. The first named invention was determined based on the first claimed additive (claim 3) and precipitation agent (claim 12) for the inventive method 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 1-4, (8-13,15-17, 20-28) (in part), encompass this first named invention, and thus these claims will be searched without fee to the extent that the method encompasses wherein the one or more additives comprise sodium chloride, wherein steps (c) and (e) are carried out using a precipitation agent.
Additional method(s) comprising additional additive(s), acid(s) for step (c) and (e), and/or combination(s) thereof will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected method(s) comprising additional additive(s), acid(s) for step (c) and (e), and/or combination(s) 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 method comprising wherein the one or more additives comprise calcium chloride, wherein steps (c) and (e) are carried out using a precipitation agent (claims 1-3, 5, (8-13,15-17, 20-28) (in part)).

Group II+, claims 29-44, directed to a target protein. Group II+ will be searched upon payment of additional fees. The target protein may be searched, for example, to encompass wherein the target protein comprises VEGF165 for an additional fee and election as such. It is believed that claims 28-34 read on this exemplary invention. Additional target protein(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected target protein(s). 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. Another exemplary election would be a target protein wherein the target protein comprises IGF-1 (claims 28-34).

The inventions listed as Groups I+ and II+ 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 II+ 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.
Additionally,
Group I+ has the special technical feature of a method for separating a target protein from an intact cell, that is not required by Group II+.
Group II+ has the special technical feature of a target protein, that is not required by Group I+.

Common technical features

No technical features are shared between the amino acid sequences in each of Group II+ inventions and, accordingly, these inventions lack unity a priori.

Additionally, even if Groups I+ and II+ were considered to share the technical features of including:
a target protein produced by the method of separating a target protein from an intact cell, the method comprising:
(a) optionally pretreating a solution comprising the intact cell at a pH greater than or equal to 13 for a period of time;
(b) lysing the cell at a pH value greater than 12.5 to create a first solution that includes lysed cell components;
(c) optionally, precipitating non-target molecules from the first solution;
(d) removing cell debris and precipitates from the first solution;
(e) decreasing solubility of the target protein in the first solution;
(f) separating target protein solids from the first solution;
(g) resuspending the target protein solids to create a second solution;
(h) optionally, adjusting a pH of the second solution; and
(i) separating target protein solids from the second solution.

However, these shared technical features are made obvious by US 2019/0216126 A1 to Innovative Proteins Holding LLC, (hereinafter 'IPH').

------continued on next extra sheet ----------

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

IPH teaches a target protein produced by the method of separating a target protein from a cell, the method comprising:
(a) optionally pretreating a solution comprising the intact cell at a pH greater than or equal to 13 for a period of time;
(b) lysing the cell at a pH value greater than 7.5 to create a first solution that includes lysed cell components (Claim 1 - 'A method for making a plant protein composition from pulse particles, the method comprising: (a) conducting an alkaline extraction on an aqueous slurry of the pulse particles; (b) removing water-insoluble proteins and other water-insoluble components from the aqueous slurry to provide a water-soluble, protein-rich liquid fraction'; Claim 2 - 'The method of claim 1, wherein the alkaline extraction is conducted at a pH in the range from 7.5 to 9.5 for a duration sufficient to provide the plant proteins with a degree of hydrolysis of greater than zero and less than 15.');
(d) removing cell debris and precipitates from the first solution (Claim 1 - '(c) separating water-soluble proteins in the protein-rich liquid fraction from water-soluble lower molecular weight peptides and water-soluble non-proteins in the protein-rich liquid fraction via ultrafiltration using an ultrafiltration membrane that concentrates the albumin proteins to provide a protein-rich retentate');
(e) decreasing solubility of the target protein in the first solution (para [0075]);
(f) separating target protein solids from the first solution (para [0075] - 'The pH of the clarified extract was adjusted to the pH of 4.3 with hydrochloric acid (10% concentration) and the precipitated clarified extract was fed to a Sharples P-660 decanter to separate precipitated protein (curds) from the acid whey. The precipitated clarified curd had a solids level of 6.61% and a protein dry basis of 48.1%, and the whey had a solids level of 2.56% and a protein dry basis of 18.4%. The protein recovery in this separation was 84.2%, and the overall protein recovery was 74.5% of the protein contained in the raw material');
(g) resuspending the target protein solids to create a second solution;
(h) optionally, adjusting a pH of the second solution; and
(i) separating target protein solids from the second solution (para [0006] - 'In Step 3, the protein-enriched extract obtained in Step 2 is further processed to separate the water-soluble proteins from other water-soluble non-protein components in the protein-enriched extract. This step is most commonly carried out using an isoelectric precipitation (also referred to as acid precipitation).'; Claim 22 - 'conducting an acid precipitation on the soluble fraction to separate the water-soluble pulse proteins into curds that comprise precipitated pulse proteins and an acid whey that comprises unprecipitated pulse proteins, including albumin proteins'). IPH fails to expressly teach an intact cell, or lysing a cell. However, it would have been obvious to one of ordinary skill in the art that the method of IPH could also be applied to an intact cell and treatment with alkali would result in lysis of the cell. IPH also fails to expressly teach a pH greater than 12.5. However, it would have been obvious to one of ordinary skill in the art that the method of IPH could be further optimized during the course of routine experimentation by increasing the pH from 9.5 to about 13, for any improvement in protein extraction.

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+ and II+ inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.