

# PATENT COOPERATION TREATY

From the  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

## PCT

### NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(PCT Rule 71.1)

To:

EP&C  
P.O. Box 3241  
2280 GE Rijswijk  
PAYS-BAS (ROYAUME DES)

Date of mailing  
(day/month/year)

20.06.2024

Applicant's or agent's file reference  
P35427PC00

#### IMPORTANT NOTIFICATION

International application No.  
PCT/EP2023/064848

International filing date (day/month/year)  
02.06.2023

Priority date (day/month/year)  
02.06.2022

Applicant  
Rousselot BV

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

#### 4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international  
preliminary examining authority:



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# PATENT COOPERATION TREATY

## PCT

### INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                          |                                                                               |                       |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------|-------------------------------------------------------------------------------|-----------------------|
| Applicant's or agent's file reference<br>P35427PC00                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | <b>FOR FURTHER ACTION</b>                                |                                                                               | See Form PCT/IPEA/416 |
| International application No.<br>PCT/EP2023/064848                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | International filing date (day/month/year)<br>02.06.2023 | Priority date (day/month/year)<br>02.06.2022                                  |                       |
| International Patent Classification (IPC) or national classification and IPC<br>INV. A23L33/18                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                          |                                                                               |                       |
| Applicant<br>Rousselot BV                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                          |                                                                               |                       |
| <p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of <u>7</u> sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p>a. <input checked="" type="checkbox"/> a total of <u>11</u> sheets, as follows:</p> <p><input checked="" type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and/or sheets containing rectifications authorized by this Authority, unless those sheets were superseded or cancelled, and any accompanying letters (see Rules 46.5, 66.8, 70.16, 91.2, and Section 607 of the Administrative Instructions).</p> <p><input type="checkbox"/> sheets containing rectifications, where the decision was made by this Authority not to take them into account because they were not authorized by or notified to this Authority at the time when this Authority began to draw up this report, and any accompanying letters (Rules 66.4bis, 70.2(e), 70.16 and 91.2).</p> <p><input type="checkbox"/> superseded sheets and any accompanying letters, where this Authority either considers that the superseding sheets contain an amendment that goes beyond the disclosure in the international application as filed, or the superseding sheets were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in item 4 of Box No. I and the Supplemental Box (see Rule 70.16(b)).</p> <p>b. <input type="checkbox"/> a separate electronic file containing a sequence listing (<i>sent to the International Bureau only</i>)</p> |                                                          |                                                                               |                       |
| <p>4. This report contains indications relating to the following items:</p> <p><input checked="" type="checkbox"/> Box No. I Basis of the report</p> <p><input type="checkbox"/> Box No. II Priority</p> <p><input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p><input type="checkbox"/> Box No. IV Lack of unity of invention</p> <p><input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p><input type="checkbox"/> Box No. VI Certain documents cited</p> <p><input checked="" type="checkbox"/> Box No. VII Certain defects in the international application</p> <p><input type="checkbox"/> Box No. VIII Certain observations on the international application</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                          |                                                                               |                       |
| Date of submission of the demand<br><br>02.04.2024                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                          | Date of completion of this report<br><br>20.06.2024                           |                       |
| Name and mailing address of the international preliminary examining authority:<br> European Patent Office P.B. 5818 Patentlaan 2<br>NL-2280 HV Rijswijk - Pays Bas<br>Tel. +31 70 340 - 2040<br>Fax: +31 70 340 - 3016                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                          | Authorized officer<br><br>Heirbaut, Marc<br><br>Telephone No. +31 70 340-2570 |                       |



# INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.  
PCT/EP2023/064848

## Box No. I Basis of the report

1. With regard to the **language**, this report is based on
  - ☒ the international application in the language in which it was filed
  - ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
    - ☐ international search (under Rules 12.3(a) and 23.1(b))
    - ☐ publication of the international application (under Rule 12.4(a))
    - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a) and (b))
2. With regard to the **elements\*** of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):

### Description, Pages

1-47 as originally filed

### Claims, Numbers

1-40 filed with the letter of

02-04-2024

### Drawings, Sheets

1/5-5/5 as originally filed

- ☐ a sequence listing - see Supplemental Box Relating to Sequence Listing.
3. ☐ The amendments have resulted in the cancellation of:
    - ☐ the description, pages
    - ☐ the claims, Nos.
    - ☐ the drawings, sheets/figs
    - ☐ the sequence listing (*specify*):
  4. ☒ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since either they are considered to go beyond the disclosure as filed, or they were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in the Supplemental Box (Rules 70.2(c) and (c-bis)):
    - ☐ the description, pages
    - ☒ the claims, Nos. 33-40
    - ☐ the drawings, sheets/figs
    - ☐ the sequence listing (*specify*):
  5. ☐ This report has been established:
    - ☐ taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rules 66.1(d-bis) and 70.2(e)).
    - ☐ without taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91(Rules 66.4bis and 70.2(e)).

**INTERNATIONAL PRELIMINARY REPORT  
ON PATENTABILITY**

International application No.  
PCT/EP2023/064848

6. ☒ With regard to top-up searches (Rules 66.1 *ter* and 70.2(f)):
- ☒ A top-up search was carried out by this Authority on 17.04.2024 (all discovered documents are listed in the Supplemental Box Relating to Top-up Search).
  - ☐ Additional relevant documents have been discovered during the top-up search.
  - ☐ No top-up search was carried out by this Authority because it would serve no useful purpose.
7. ☐ Supplementary international search report(s) from Authority(ies) has/have been received and taken into account in establishing this report (Rule 45bis.8(b) and (c)).

\* If item 4 applies, some or all of those sheets may be marked "superseded".

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**Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

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1. Statement

|                               |             |             |
|-------------------------------|-------------|-------------|
| Novelty (N)                   | Yes: Claims | <u>1-40</u> |
|                               | No: Claims  |             |
| Inventive step (IS)           | Yes: Claims | <u>1-40</u> |
|                               | No: Claims  |             |
| Industrial applicability (IA) | Yes: Claims | <u>1-40</u> |
|                               | No: Claims  |             |

2. Citations and explanations (Rule 70.7):

**see separate sheet**

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**Box No. VII Certain defects in the international application**

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The following defects in the form or contents of the international application have been noted:

**see separate sheet**

## **ITEM I**

The amended claims filed with the letter dated 02.04.2024 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT.

The amendment concerned is the following:

The deletion of the feature "*obtainable by the method according to any of claims 15-32*" in present claim 33. In the letter dated 02.04.2024, the Applicant referred to page 21, lines 27 and 30 in the application as originally filed as providing adequate support for this amendment. However, said passage does not disclose the combination of the remaining features of this claim. It is considered that the step of subjecting the collagen-containing material to a combination of enzymes as set out in claims 15-32 confers distinctive properties to the resulting hydrolysed collagen, which go beyond the remaining features of present claim 33.

Consequently, the omission of the reference to the method of preparation is considered to infringe Article 34(2)(b) PCT. Claims 34-40 refer back to claim 33, and therefore infringe Article 34(2)(b) PCT as well.

The International Preliminary Report on Patentability is established as if said amendment had not been made.

## **ITEM V**

### **1 PCT-Direct procedure**

The Applicant has requested to have the present application processed under the PCT Direct procedure (PCT Guidelines B-IV, 1.2.1). In drafting the International Preliminary Report on Patentability, the Applicant's comments submitted with the PCT Direct letter of 02.06.2023 were taken into account.

## **2 Cited prior art**

Reference is made to the following prior art (**D1** and **D2**):

D1            JP 2009 235064 A (HOKKAIDO; UNIV HOKKAIDO) (2009-10-15)

D2            JP 2012 116773 A (NIPPI INC) (2012-06-21)

As these documents were published before the effective date of the present application, they form part of the prior art pursuant to Rule 64.1 PCT.

## **3 Novelty (Articles 33(1)(2) PCT)**

The subject-matter of present independent claims 1, 12, 15, 33, 40 is considered to meet the requirements of novelty, for the following reasons.

None of the prior art documents cited in the search report teaches the claimed combination of features, in particular:

Enzymatic hydrolysis of a collagen-containing material with a combination of enzymes, comprising a neutral protease and one or both of a carboxypeptidase and an aminopeptidase.

This treatment confers a particular molecular weight and molecular weight distribution to the hydrolysed collagen, as demonstrated in Table 6.

The use of a novel hydrolysed collagen composition is also novel.

Accordingly, the subject-matter of dependent claims 2-11, 13-14, 16-32 and 34-39 also meets the requirements of novelty.

## **4 Inventive step (Articles 33(1)(3) PCT)**

The subject-matter of present independent claims 1, 12, 15, 33, 40 is considered to meet the requirements of inventive step.

### **4.1 Closest prior art**

#### ***4.1.1 Selection of closest prior art***

Documents **D1** and **D2** pertain to collagen hydrolysates for use in reducing blood glycaemic level (D1) or increasing IGF-1 level (D2). As this is the general technical problem underlying the present application and these documents share a number of

features with the present claims, they can be considered to represent the closest prior art and the skilled person seeking to solve the technical problem underlying the present application at the effective date would have consulted their teachings.

#### *4.1.2 Disclosure of document D1*

Document **D1** discloses a blood-glucose-increase inhibitor which uses as an active ingredient the starfish collagen peptide contained in the hydrolysate obtained by protease-treating star fishes (D1, claim 1). Preferably, a neutral protease is used (D1, claim 3). In a particular embodiment, Sumizyme P is used (D1, paragraph [0020], example 1). The blood-glucose-increase inhibitor is effective also in type-2 diabetes patients who have insulin resistance (D1, paragraph [0013]). Preferably, hydrolysis is carried out at 60°C for 5 hours (D1, paragraph [0022]).

#### *4.1.3 Disclosure of document D2*

Document **D2** discloses an IGF-1 value increasing agent including a collagen peptide extracted by processing fish scales and/or fish skin origin collagen by a protease (D2, claim 1; paragraphs [0018] to [0030]; example 1). Preferably, said protease is derived from *Aspergillus oryzae* (D2, claim 2); and the weight average molecular weight of the said collagen peptide is 1 000-10 000 (D2, claim 3). Preferably, extraction is carried out at 60-100°C for 0.5 to 10 hours (D2, paragraph [0024]).

### 4.2 Distinguishing features

The subject-matter of present independent claims 1, 12, 15, 33, 40 is distinguished from the teachings of documents **D1** and **D2** in that it is specified that enzymatic hydrolysis of the collagen-containing material is carried out with a combination of enzymes comprising a neutral protease and one or both of a carboxypeptidase and an aminopeptidase.

### 4.3 Unexpected technical effects

This distinguishing feature unexpectedly provides the following technical effects: a further improved blood sugar level reducing effect and glucagon-like peptide 1 (GLP-1) level increasing effect.

This has been demonstrated plausibly for the claimed embodiments in the Examples, in particular in Tables 3-4 and 6, as well as in the supplementary experimental evidence filed with the letter dated 02.04.2024. In view of the experimental evidence provided, it is considered sufficiently plausible that the technical effects can be achieved over a broad range of gelatine precursor concentrations, pH and temperature levels.

#### 4.4 Objective technical problem

In view of the achievement of these unexpected technical effects, the objective technical problem facing the skilled person working in the technical field of nutritional product technology at the effective date of the present application can be formulated as providing a collagen hydrolysate with a further improved blood sugar reducing effect and GLP-1 increasing effect.

#### 4.5 Proposed solution of the technical problem

This technical problem was solved by the implementation of enzymatic hydrolysis of the collagen-containing material with a combination of enzymes comprising a neutral protease and one or both of a carboxypeptidase and an aminopeptidase.

#### 4.6 Why the proposed solution was not obvious for certain claimed embodiments

There was no indication or suggestion in documents **D1** and **D2** read alone or in combination with the other prior art documents cited in the search report that would have rendered obvious this solution to the skilled person at the effective date.

Documents **D1** and **D2** advocate the use of specific single proteolytic enzymes, rather than a combination of enzymes as claimed.

### **5 Industrial applicability (Articles 33(1)(4) PCT)**

The present application meets the requirements of industrial applicability, as it can be applied in the nutritional product industry.

### **ITEM VII**

The following objection related to formal issues is raised:

The present application does not meet the requirements of Rule 5.1(a)(ii) PCT, as the relevant background art disclosed in prior art documents **D1** and **D2** has not been mentioned in the description, nor have these documents been identified therein.

P35427PC00

March, 2024

Appl. No. PCT/EP2023/064848

**Amended C L A I M S**

1. Hydrolyzed collagen for use in ameliorating hyperglycemia or a risk factor for hyperglycemia, wherein the hydrolyzed collagen is obtained by enzymatic hydrolysis of a collagen-containing material with a combination of enzymes comprising a neutral protease and one or both of a carboxypeptidase and an aminopeptidase.
2. Hydrolyzed collagen for use according to claim 1, wherein the combination of enzymes comprises a neutral protease, a carboxypeptidase, and an aminopeptidase.
3. Hydrolyzed collagen for use according to any one of the previous claims, wherein the risk factor for hyperglycemia is one or more selected from the group consisting of insulin resistance, type 2 diabetes, gestational diabetes, high body mass index, obesity, and hyperglucagonemia.
4. Hydrolyzed collagen for use according to any one of the previous claims, for use in one or more selected from the group consisting of:
  - increasing blood glucagon-like peptide-1;
  - decreasing blood glucagon;
  - increasing blood insulin;
  - increasing blood insulin/glucose ratio.
5. Hydrolyzed collagen for use according to any one of the previous claims, wherein the hydrolyzed collagen is administered at a daily dose in an amount of 1 – 100 gram, preferably 2 - 50 g, more preferably 5 – 25 g, wherein the amount is the dry weight amount.
6. Hydrolyzed collagen for use according to any one of the previous claims, wherein the hydrolyzed collagen is administered orally, preferably as a food supplement.
7. Hydrolyzed collagen for use according to any one of the previous claims, wherein the hydrolyzed collagen is administered no more than 60 min separated from a meal.

- 5
8. Hydrolyzed collagen for use according to any one of the previous claims, wherein the collagen-containing material is derived from one or more tissues selected from the group consisting of skin, scale, cartilage, bone, tendon, ligament, and connective tissue.
- 10
9. Hydrolyzed collagen for use according to any one of the previous claims, wherein the collagen is one or more types selected from the group consisting of porcine collagen, bovine collagen, and fish collagen.
- 15
10. Hydrolyzed collagen for use according to any one of the previous claims, wherein the collagen-containing material is gelatin.
11. Hydrolyzed collagen for use according to any one of the previous claims, wherein the hydrolyzed collagen has an average molecular weight of 1000-7000 Da and/or a polydispersity of 1.2-1.8.
- 20
12. Use of hydrolyzed collagen for non-therapeutic lowering of blood glucose, wherein the hydrolyzed collagen is as defined by any one of claims 1-11.
- 25
13. Use according to claim 12, wherein the use is in ameliorating postprandial glucose.
- 30
14. Use according to claim 12 or 13, wherein the hydrolyzed collagen is administered as defined in any one of claims 5-7.
- 35
15. Method for obtaining hydrolyzed collagen, comprising
- a) providing a collagen-containing material in a liquid formulation; and
  - b) subjecting the collagen-containing material to a combination of enzymes comprising a neutral protease and one or both of a carboxypeptidase and an aminopeptidase.
16. Method according to claim 15, wherein the combination of enzymes comprises a neutral protease, a carboxypeptidase, and an aminopeptidase.
17. Method according to claim 15 or 16, wherein the neutral protease is one or more selected from the group consisting of serine protease, aspartic protease, cysteine protease, and metalloprotease.
18. Method according to any one of claims 15-17, wherein the aminopeptidase is leucine aminopeptidase.

19. Method according to any one of claims 15-18, wherein one or more of the neutral protease, carboxypeptidase, and aminopeptidase are derived from a microorganism of the *Aspergillus* genus, preferably from *Aspergillus oryzae*.

5

20. Method according to any one of claims 15-19, wherein the collagen-containing material is subjected to an amount of neutral protease defined by an enzyme activity of 0.2 - 25000 U/g, preferably 2 - 2500 U/g, more preferably 20 - 250 U/g, wherein the weight in g is the total weight of the collagen-containing material and the liquid formulation.

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21. Method according to any one of claims 15-20, wherein the collagen-containing material is subjected to an amount of carboxypeptidase defined by an enzyme activity of 0.001 - 500 U/g, preferably 0.01 – 50 U/g, more preferably 0.1 - 5 U/g, wherein the weight in g is the total weight of the collagen-containing material and the liquid formulation.

15

22. Method according to any one of claims 15-21, wherein the collagen-containing material is subjected to an amount of aminopeptidase defined by an enzyme activity of 0.002 - 500 U/g, preferably 0.02 – 50 U/g, more preferably 0.2 - 5 U/g, wherein the weight in g is the total weight of the collagen-containing material and the liquid formulation.

20

23. Method according to any one of claims 15-22, wherein the collagen-containing material is subjected to one or more enzymes in the combination of enzymes for 60 – 180 min.

25

24. Method according to any one of claims 15-22, wherein the collagen-containing material is subjected to one or more enzymes in the combination of enzymes at 30 – 60 °C.

30

25. Method according to any one of claims 15-24, wherein the collagen-containing material is subjected to one or more enzymes in the combination of enzymes at pH 5 – 8.

35

26. Method according to any one of claims 15-25, wherein the collagen-containing material is subjected successively to two or more enzymes in the combination of enzymes.

27. Method according to any one of claims 15-26, wherein the collagen-containing material is subjected simultaneously to two or more enzymes in the combination of enzymes.
- 5 28. Method according to claim 27, wherein the collagen-containing material is subjected to a mixture comprising the combination of enzymes.
- 10 29. Method according to any one of claims 15-28, wherein the collagen-containing material is provided in a liquid formulation in an amount of 20-50 wt.%, preferably 30-40 wt.%.
- 15 30. Method according to any one of claims 15-29, wherein the collagen-containing material is derived from one or more tissues selected from the group consisting of skin, scale, cartilage, bone, tendon, ligament, and connective tissue.
- 20 31. Method according to any one of claims 15-30, wherein the collagen is one or more types selected from the group consisting of porcine collagen, bovine collagen, and fish collagen.
32. Method according to any one of claims 15-31, wherein the collagen-containing material is gelatin.
- 25 33. Hydrolyzed collagen, wherein the hydrolyzed collagen:
- has a weight average molecular weight of 2000-4500 Da, preferably 2500-400 Da; and
  - comprises 35-60% by weight of collagen peptides with a molecular weight in the range of 2000-5000 Da, calculated on total weight of collagen peptides in the hydrolyzed collagen.
- 30 34. Hydrolyzed collagen according to claim 33, wherein the hydrolyzed collagen comprises more than 40% by weight of collagen peptides with a molecular weight in the range of 2000-5000 Da, calculated on total weight of collagen peptides in the hydrolyzed collagen.
- 35 35. Hydrolyzed collagen according to claim 33 or 34, wherein the hydrolyzed collagen comprises:
- 1-20% by weight of collagen peptides with a molecular weight below 1000 Da; and/or

- 20-40% by weight of collagen peptides with a molecular weight of 1000-2000 Da;  
and/or

- 5-25% by weight of collagen peptides with a molecular weight of 5000-10000 Da;  
and/or

5        - 0-10% by weight of collagen peptides with a molecular weight more than 10000  
Da, calculated on the total weight of collagen peptides in the hydrolyzed collagen.

10       36. Hydrolyzed collagen according to any one of claims 33-35, wherein the hydrolyzed  
collagen has a polydispersity of 1.2-1.8.

37. Hydrolyzed collagen according to any one of claims 33-36, wherein the hydrolyzed  
collagen is capable of stimulating glucagon-like peptide-1 secretion.

15       38. Hydrolyzed collagen according to any one of claims 33-37, wherein the hydrolyzed  
collagen is obtainable by the method according to any one of claims 15-32.

39. Hydrolyzed collagen according to any one of claims 33-38, for use in ameliorating  
hyperglycemia or a risk factor of hyperglycemia as defined in any one of claims 1-  
11.

20       40. Use of hydrolyzed collagen according to any one of claims 33-38 in non-therapeutic  
lowering of blood glucose as defined in any one claims 12-14.

EUROPEAN PATENT OFFICE  
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Rijswijk, April 2, 2024

Our ref. : P35427PC00/MCR  
Contact : Tel. 31-70-4145471/ email: Boyle@epc.nl

Application no. : PCT/EP2023/064848  
Country : PCT Application  
In the name of : Rousselot B.V.

Dear Sir, Madam,

This letter accompanies the filing of a demand for international preliminary examination.

We herewith report our arguments addressing the objections made by the Examiner in charge of the WO-ISA (herein "the Examiner"). When referring to the amended claims, we herein mean the replacement claims submitted under Article 34 PCT (enclosed in marked up and clean versions).

#### **Amendments**

Claims 1 and 15 are amended to specify that the hydrolyzed collagen (for use) is obtained by enzymatic hydrolysis of a collagen-containing material with a combination of enzymes comprising a *neutral protease and one or both of a carboxypeptidase and an aminopeptidase*.

Basis for this amendment can be found on p.17 line 12 in the application as filed.

Claim 33 has been amended. The phrase "*obtainable by the method according to ...*" has been deleted hence the product is not described anymore in terms of a process of manufacture. This amendment finds basis in the application as filed on p.21 line 27 and p.21 line 30.

Furthermore, in claim 33 the range of the weight average molecular weight is amended from "*2000-4000 Da, preferably 2500-3500 Da*" to "*2000-4500, preferably 2500-4000 Da*". This amendment finds basis in the application as filed on p.13 line 30.

Present claim 38 is new and describes a hydrolyzed collagen obtainable by the method according to claims 15-32. Claim 38 is the same as claim 33 as filed.

All other claims are unchanged.

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### **Novelty (Article 33(2) PCT)**

The WO-ISA already acknowledged novelty of the claims as originally filed.

Claims 1-32 as originally filed were directed to use of a hydrolyzed collagen obtained by hydrolysis with a combination of enzymes comprising *two or more of a neutral protease, a carboxypeptidase and an aminopeptidase*. Present claims 1-32 are directed to use of a hydrolyzed collagen obtained by hydrolysis with a combination of enzymes comprising *a neutral protease and one or both of a carboxypeptidase and an aminopeptidase*.

Present claims 1-32 have a narrower scope than the claims originally as filed and are also novel.

In present claims 33-40, the weight average molecular weight of the hydrolyzed collagen is specified as being “2000-4500, preferably 2500-4000 Da”, while this was originally “2000-4000 Da, preferably 2500-3500 Da”. The amendment in the definition of the hydrolyzed collagen does not affect the novelty of claims 33-40 over D1 and D2.

Present claims are novel.

### **Inventive step (Article 33(3) PCT)**

Present claims have been amended to a hydrolyzed collagen obtained by hydrolysis with a combination of enzymes comprising *a neutral protease and one or both of a carboxypeptidase and an aminopeptidase*. The enzyme combinations covered by present claims can be written out as the following three combinations:

1. neutral protease + carboxypeptidase
2. neutral protease + aminopeptidase
3. neutral protease + carboxypeptidase + aminopeptidase

The Examiner considers that D1 or D2 can represent the closed prior art.

As closest embodiment with the present claims, D1 or D2 disclose collagen peptides obtained by enzymatic hydrolysis of collagen with a neutral protease and a use of said collagen peptides in reducing blood sugar levels.

The difference of present claims over D1 is the presence of at least one further enzyme in enzymatic hydrolysis of the collagen, namely a carboxypeptidase, an aminopeptidase or both a carboxypeptidase and aminopeptidase.

The technical effect of providing, in addition to a neutral protease, a further carboxypeptidase, an aminopeptidase or both a carboxypeptidase and aminopeptidase is:  
***a hydrolyzed collagen with improved blood sugar level reducing effect.***

The objective technical problem to be solved can be formulated as:  
***how to achieve an improved blood sugar level reducing effect?***

The problem is not solved starting with D1 or D2 as closest prior art.

The Examiner has previously contested that the essential features were not recited in the independent claims. However, it is clear from the application as filed that a hydrolyzed collagen suitable for lowering glucose is achieved by hydrolysis with a combination of enzymes comprising a *neutral protease and one or both of a carboxypeptidase and an aminopeptidase*, see Table 2 and page 42 lines 1-4 recited herein:

*"As illustrated in Table 2, the use of a neutral protease in enzymatic hydrolysis of gelatin leads to a gelatin hydrolysate that has a glucose-lowering activity albeit limited. A combination of a neutral protease with a carboxypeptidase alone or an aminopeptidase alone provides a stronger glucose-lowering activity relative to the blank or the use of a neutral protease alone."*

In case of any doubt, the applicant herewith puts forward even further comparative data to show that the objective technical problem is solved if the combination of enzymes as specified by the claims.

Table A shows the suitability of hydrolyzed collagen products obtained with different enzymes. Table A is a further elaboration of Table 2 already present in the original application. Group VII is a new comparative group that has been included.

Present inventors consider that the biological activity of the hydrolyzed collagen is associated with the molecular weight distribution thereof. The molecular weight distribution is considered to be desirable (+ or ++) if the average weight molecular weight is in the range of 2000-45000 Da and 35-60% by weight of collagen peptides have a molecular weight in the range of 2000-5000 Da (i.e. falling in the scope of present claim 34).

The molecular weight distribution is considered to be most desirable (++) if the distribution is further defined as:

- 1-20% by weight of collagen peptides with a molecular weight below 1000 Da; and
  - 20-40% by weight of collagen peptides with a molecular weight of 1000-2000 Da; and
  - 5-25% by weight of collagen peptides with a molecular weight of 5000-10000 Da; and
  - 0-10% by weight of collagen peptides with a molecular weight more than 10000 Da, calculated on the total weight of collagen peptides in the hydrolyzed collagen.
- (i.e. having all preferred features of present claim 35).

Table A:

| <b>Group</b>                 | <b>Enzyme(s)</b>                                                                                          | <b>Biological activity</b> | <b>Desirable MW distribution</b> |
|------------------------------|-----------------------------------------------------------------------------------------------------------|----------------------------|----------------------------------|
| <b>I<br/>(comparative)</b>   | Blank                                                                                                     | Very low/absent            | n/a                              |
| <b>II<br/>(comparative)</b>  | 1. Neutral protease (microbial)                                                                           | Low                        | +/-                              |
| <b>IIIa</b>                  | 1. Neutral protease (microbial) +<br>2. Carboxypeptidase (microbial)                                      | High                       | +                                |
| <b>IIIb</b>                  | 1. Neutral protease (microbial) +<br>2. Carboxypeptidase (recombinant/                                    | High                       | +                                |
| <b>IV</b>                    | 1. Neutral protease (microbial) +<br>2. Aminopeptidase (microbial)                                        | High                       | ++                               |
| <b>Va</b>                    | 1. Neutral protease (microbial) +<br>2. Carboxypeptidase (microbial) +<br>3. Aminopeptidase (microbial)   | Very High                  | ++                               |
| <b>Vb</b>                    | 1. Neutral protease (microbial) +<br>2. Carboxypeptidase (recombinant) +<br>3. Aminopeptidase (microbial) | Very High                  | ++                               |
| <b>VI</b>                    | 1. Neutral protease (microbial) +<br>2. Carboxypeptidase (microbial) +<br>3. Aminopeptidase (microbial)   | Very High                  | ++                               |
| <b>VII<br/>(comparative)</b> | 1. Aminopeptidase (microbial) +<br>2. Carboxypeptidase (recombinant)                                      | Very low/absent            | No                               |

It can be seen that the hydrolyzed collagens according to groups III-VI (according to the invention) have desirable molecular weight distribution and are considered suitable for reducing blood glucose. However, the hydrolyzed collagens according to groups I, II or VII (comparative groups) do not have a desirable molecular weight distribution and/or are not considered suitable for reducing blood glucose. More specifically, the hydrolyzed collagen of group II has an average molecular weight distribution >4500 Da and the hydrolyzed collagen of group VII even has an average molecular weight >10000 Da and are therefore deemed inappropriate.

Thus, the presence of at least a neutral protease and one or more further enzymes contributes to the technical effect. More specially, the objective technical problem is solved for the further selection of enzymes from:

1. neutral protease + carboxypeptidase
2. neutral protease + aminopeptidase
3. neutral protease + carboxypeptidase + aminopeptidase

As already put forward in the PCT direct letter, the broad working conditions tested by the Applicant (Table 4 in the original application) encompass conditions typically used in enzymatic hydrolysis, and which shows that a satisfactory hydrolyzed collagen can be achieved over a broad range of conditions tested (precursor concentration, pH, temperature). The skilled person could therefore easily achieve satisfactory results, without any undue burden or without any technical difficulties, based on the features of the present claims.

Thus, it is clear that the objective technical problem as formulated by the Examiner is solved over the entire scope of the claims.

The present claims involve an inventive step.

### **Other objections**

The Examiner considered the claims not sufficiently disclosed as allegedly the effect is not achieved over the whole claimed scope of this claim.

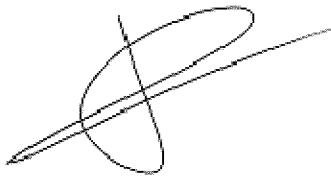
However, as elaborated already under Inventive step, the objective technical problem is solved over the entire scope of the claims after amending the claims towards a further selection from:

1. neutral protease + carboxypeptidase
2. neutral protease + aminopeptidase
3. neutral protease + carboxypeptidase + aminopeptidase

In view of the above, a positive examination is kindly requested.

We look further to your further communication. If the examiner persists and no positive IPRP can be issued, we request that the undersigned is contacted directly to discuss the matter. This can be done by telephone (+31 (0)70 414 54 71) or mail (robbertjan.delang@epc.nl).

EP&C  
Association no. 497

A handwritten signature in black ink, consisting of a series of loops and a long horizontal stroke extending to the right.

Robbert-Jan de Lang

Annex:

- Replacement claims filed under art. 34 PCT (marked up and clean).

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