

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:

Høiberg P/S
Adelgade 12
1304 Copenhagen K
DANEMARK

Date of mailing
(day/month/year)

06.06.2025

Applicant's or agent's file reference
P6581PC00

IMPORTANT NOTIFICATION

International application No.
PCT/EP2024/055170

International filing date (day/month/year)
29.02.2024

Priority date (day/month/year)
01.03.2023

Applicant
Carlsberg A/S

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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NL-2280 HV Rijswijk - Pays Bas
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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 P6581PC00	FOR FURTHER ACTION		See Form PCT/IPEA/416
International application No. PCT/EP2024/055170	International filing date (<i>day/month/year</i>) 29.02.2024	Priority date (<i>day/month/year</i>) 01.03.2023	
International Patent Classification (IPC) or national classification and IPC INV. C12C11/00			
Applicant Carlsberg A/S			
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. 2. This REPORT consists of a total of <u>8</u> sheets, including this cover sheet. 3. This report is also accompanied by ANNEXES, comprising: a. ☒ a total of <u>9</u> sheets, as follows: ☒ 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). ☐ 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). ☐ 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)). b. ☐ a separate electronic file containing a sequence listing (<i>sent to the International Bureau only</i>)			
4. This report contains indications relating to the following items: ☒ Box No. I Basis of the report ☒ Box No. II Priority ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability ☐ Box No. IV Lack of unity of invention ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement ☐ Box No. VI Certain documents cited ☐ Box No. VII Certain defects in the international application ☒ Box No. VIII Certain observations on the international application			
Date of submission of the demand 30.10.2024		Date of completion of this report 06.06.2025	
Name and mailing address of the international preliminary examining authority:  European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040		Authorized officer Du, Mingliu Telephone No. +31 70 340-3307	



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

1-67 as originally filed

Sequence listings, SEQ ID NO

1-4 as originally filed

Claims, Numbers

1-33 filed in electronic form on 08-01-2025

Drawings, Sheets

1/4-4/4 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.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):

**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

International application No.
PCT/EP2024/055170

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)).
6. ☒ With regard to top-up searches (Rules 66.1ter and 70.2(f)):
- ☒ A top-up search was carried out by this Authority on 04.11.2024 (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".

Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
- ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
 - ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).
2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:
- see separate sheet**

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

1. Statement

Novelty (N)	Yes: Claims	<u>1-28, 30-33</u>
	No: Claims	<u>29</u>
Inventive step (IS)	Yes: Claims	<u>1-28, 30-33</u>
	No: Claims	<u>29</u>
Industrial applicability (IA)	Yes: Claims	<u>1-33</u>
	No: Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search and/or examination,
 - ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
 - c. ☐ furnished to this Authority as an amendment* under PCT Article 34 on .
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established to the extent that a meaningful opinion referred to in Article 33(1) could be formed without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

* If item 4 in Box No. I applies, the sequence listing, which forms part of the basis of the report, may be marked "superseded."

Re Item II

Priority

- 1 Current application claims priority from EP23159456.5 ("priority application"). The priority claim is not valid for the following reasons:
- Claim 1 requires that the first parental yeast strain and the progeny strain both carry a mutation in a gene encoding ILV2 resulting in a mutation at position 498 of SEQ ID NO:1 or its functional homologue.
- The priority application only discloses one parental yeast strain M49 (which carries the mutation required in current claim 1) but does not disclose any sequencing result of any progeny strain. The priority application does not mention ILV2 anywhere in the whole application. Therefore, the priority claim is not valid for the subject-matter of claim 1.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

- 2 Reference is made to the following documents:
- | | |
|----|---|
| D1 | ZHAO-YUE WANG ET AL: "Construction of self-cloning industrial brewing yeast with high-glutathione and low-diacetyl production", INTERNATIONAL JOURNAL OF FOOD SCIENCE AND TECHNOLOGY, BLACKWELL SCIENTIFIC PUBLICATIONS, OXFORD, GB, vol. 43, no. 6, 25 March 2008 (2008-03-25), pages 989-994, XP071855334, ISSN: 0950-5423, DOI: 10.1111/J.1365-2621.2007.01546.X |
| D2 | WO 2022/002960 A1 (CARLSBERG AS [DK]) 6 January 2022 (2022-01-06)cited in the application |
| D3 | WO 2016/101960 A2 (CARLSBERG BREWERIES AS [DK]) 30 June 2016 (2016-06-30)cited in the application |
| D4 | KRISTOFFER KROGERUS: "An Improved Model for Prediction of Wort Fermentation Progress and Total Diacetyl Profile", JOURNAL OF THE AMERICAN SOCIETY OF BREWING CHEMISTS., |

vol. 73, no. 1, 1 January 2015 (2015-01-01), pages 90-99,
XP093162897,
US
ISSN: 0361-0470, DOI: 10.1094/ASBCJ-2015-0106-01
Retrieved from the Internet:
URL: [https://www.tandfonline.com/doi/abs/10.1094/
ASBCJ-2015-0106-01](https://www.tandfonline.com/doi/abs/10.1094/ASBCJ-2015-0106-01)

D5 WO 2021/034191 A1 (HEINEKEN SUPPLY CHAIN BV [NL]) 25
February 2021 (2021-02-25)

D6 UTE E B DONALIES ET AL: "Improvement of Saccharomyces
Yeast Strains Used in Brewing, Wine Making and Baking", 1
January 2008 (2008-01-01), FOOD BIOTECHNOLOGY,
SPRINGER BERLIN / HEIDELBERG, BERLIN / HEIDELBERG,
PAGE(S) 67 - 98, XP008106069,
ISBN: 978-3-540-70535-2
[retrieved on 2008-05-08]

3 Claim 29 relates to a product defined by its method of manufacturing, namely the method of claim 26. As the method of claim 26 can comprise additional steps to steps i, ii, and iii, such as filtration, the obtained product may not comprise the yeast used in the method. In addition, the method of claim 26 does not limit the end point of fermentation and does not exclude treatments that can alter the composition of the fermented product after fermentation, such that substrates can be added or removed from the fermented product. The fermented aqueous extract of claim 29 cannot be distinguished from commonly known fermented wort or beer therefore is not novel (Article 33(2) PCT).

4 Claim 1 meets the requirements of the PCT with respect to novelty and inventive step (Article 33(2) and (3) PCT).

4.1 D2 is regarded as being the prior art closest to the subject-matter of claim 1, and shows in Example 1:

A progeny yeast strain ("ZDA1", see p.59, l.5 "hybrid"), wherein said progeny yeast strain is the progeny of a first parental yeast strain and a second parental yeast strain (definition of "hybrid"), wherein one parental yeast strain carries a

gene encoding ILV2 as set forth in SEQ ID NO: 1 (SEQ ID NO:32 of D2 is 100% identical to SEQ ID NO:1 of the present application, see Fig.4 for sequence of ILV2 gene in ZDA1 strain).

- 4.2 The subject-matter of claim 1 therefore differs from this known strain in that the first parental yeast strain is *Saccharomyces cerevisiae*, the first parental yeast strain and the progeny yeast strain both carry a mutation in the ILV2 gene which results in an amino acid substitution at position 498 of SEQ ID NO: 1, and in that said first parental yeast strain and said progeny yeast strain are each capable of producing a fermented test solution upon incubation in a test solution as defined in claim 1 of the present application, wherein said fermented test solution does not reach a level of total diacetyl above 50 ppb at any time during fermentation.

The subject-matter of claim 1 is therefore novel (Article 33(2) PCT).

- 4.3 The technical effect relating to the distinguishing features is that the total diacetyl amount can be kept low throughout the fermentation while good brewing properties can be kept, so that the fermentation can be stopped at any time, e.g. for the production of low alcohol beer, without the need of additional maturation to reduce the unfavourable flavour from diacetyl. As ILV2 encodes the acetolactate synthase directly responsible for the formation of α -acetolactate (D6, Fig.1), it is plausible that the mutation in ILV2 is related to the feature of low α -acetolactate (precursor of diacetyl) release. It is also shown by the examples that the ability of reducing total diacetyl throughout the fermentation can be transmitted to progeny strains by routine crossing and screening, and it is feasible to create progeny strains with reduced total diacetyl generation while having good brewing properties. The technical effect can be considered achievable over the whole scope of the claim.

- 4.4 The problem to be solved by the present invention may therefore be regarded as providing a strain for improved flexibility and efficiency in brewing.

- 4.5 The solution to this problem proposed in claim 1 of the present application is considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

Neither strain M49, nor a parental yeast strain having the required mutation is revealed in the cited documents. None of the cited documents reveal another strain capable of keeping a total diacetyl level of at most 50 ppb throughout the fermentation, or pointing to the position of mutation in ILV2 gene. The solution of claim 1 is neither known from, nor rendered obvious by the available prior art.

- 5 The method of independent claim 24 comprises all the features of the first parental strain of claim 1, which is not disclosed in any cited documents. The first parental strain is shown to be related to the technical effect of reduced total diacetyl generation while possible to give rise to progeny strains maintaining this feature and additionally have good brewing properties by means of routine screening, which solves the problem of providing yeast strains for brewing with reduced total diacetyl content. As neither the strain M49 nor the mutation at position 498 of ILV2 gene is disclosed or hinted in the cited documents, method of claim 24 can also be considered involving an inventive step.
- 6 The method of independent claim 26 comprises all the features of claim 1, therefore can also be considered novel and inventive.
- 7 Claims 2-23, 25, 27, 28 and 30-33 are dependent claims of claims 1, 24, 26 and as such also meets the requirements of the PCT with respect to novelty and inventive step.

Re Item VIII

Certain observations on the international application

- 8 Claim 29 relates to a product defined by its method of manufacturing, namely the method of claim 26. As the method of claim 26 does not exclude other steps, it is unclear what technical characteristics is in the product of claim 29 (Article 6 PCT).
- 9 The relative terms "significantly slower" in claims 15 and 17 do not have any well recognised meaning, especially as the comparison is made to unspecified "other yeasts". Claims 15 and 17 are therefore unclear.

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Claims

1. A progeny yeast strain, wherein said progeny yeast strain is the progeny of a first parental yeast strain and a second parental yeast strain, wherein
- 5 • the first parental yeast strain is of the species *Saccharomyces cerevisiae*; and
- said first parental yeast strain and said progeny yeast strain carry a mutation in a gene encoding ILV2, wherein ILV2 is as set forth in SEQ ID NO: 1 or a functional homologue of SEQ ID NO: 1 with at least 90%,
- 10 such as at least 95%, such as at least 98% sequence identity to SEQ ID NO: 1, wherein said mutation results in an amino acid substitution at position 498 of SEQ ID NO: 1 or an amino acid substitution at the corresponding position of said functional homologue thereof,
- wherein said first parental yeast strain and said progeny yeast strain are each
- 15 capable of producing a fermented test solution upon incubation in a test solution, wherein the test solution is an extract of malt and/or cereal, and wherein said fermented test solution does not reach a level of total diacetyl above 50 ppb, such as above 40 ppb, or such as above 30 ppb at any time during fermentation, wherein
- 20 • said test solution is a wort with an apparent extract of at least 9° Plato and a pH in the range of 4.0 to 5.0, and
- said incubation in said test solution is performed for between 2 to 6 days at a temperature in the range of 12°C to 18 °C after inoculation of in the range of 7.000.000 to 20.000.000 viable yeast cells per mL test
- 25 solution.
2. The progeny yeast strain according to claim 1, wherein said test solution comprises at the most 3500 mg/L, such as at the most 3000 mg/L, such as at the most 2500 mg/L amino acids.
- 30
3. The progeny yeast strain according to any one of the preceding claims, wherein said test solution comprises at least 40 g/kg maltose.
4. The progeny yeast strain according to any one of the preceding claims, wherein
- 35 said test solution comprises from 1,0 to 3,5 g fructose per L test solution, such

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as from 1,5 to 3,0 g fructose per L test solution, or such as from 2,0 to 2,5 g fructose per L test solution, such as approximately 2,2 g fructose per L test solution.

5 5. The progeny yeast strain according to any one of the preceding claims, wherein said test solution comprises from 5,0 to 8,0 g glucose per L test solution, such as from 5,5 to 7,5 g glucose per L test solution, or such as from 6,0 to 7,0 g glucose per L test solution, such as approximately 6,5 g glucose per L test solution.

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6. The progeny yeast strain according to any one claims 1 to 4, wherein the test solution comprises at least 40 g/L glucose, such as at least 50 g/L glucose, such as at least 60 g/L glucose, such as at least 70 g/L glucose, such as at least 80 g/L glucose, such as at least 90 g/L glucose, such as at least 100 g/L glucose, or such as at least 110 g/L glucose.

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7. The progeny yeast strain according to any one of the preceding claims, wherein said test solution comprises from 1,0 to 5,0 g sucrose per L test solution, such as from 1,5 to 4,0 g sucrose per L test solution, or such as from 2,0 to 3,0 g sucrose per L test solution, such as approximately 2,5 g sucrose per L test solution.

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8. The progeny yeast strain according to any one of the preceding claims, wherein said mutation results in the substitution of a non-polar amino acid to a charged amino acid, such as a negatively charged amino acid.

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9. The progeny yeast strain according to any one of the preceding claims, wherein said mutation results in the substitution of a non-polar amino acid to aspartate (D) or glutamate (E).

30

10. The progeny yeast strain according to any one of the preceding claims, wherein said mutation results in the substitution of a non-polar amino acid to glutamate (E).

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11. The progeny yeast strain according to any one of the preceding claims, wherein said mutation results in the substitution of a glycine (G) to glutamate (E), such as wherein said mutation results in a G498E substitution.
- 5 12. The progeny yeast strain according to any one of the preceding claims, wherein said second parental yeast strain is of the genus *Saccharomyces*.
13. The progeny yeast strain according to any one of the preceding claims, wherein said second parental yeast strain is of the species *Saccharomyces cerevisiae*.
- 10 14. The progeny yeast strain according to any one of the preceding claims, wherein said second parental yeast strain is maltose intolerant, such as wherein said second parental yeast strain is not capable of completely converting maltose into ethanol.
- 15 15. The progeny yeast strain according to any one of the preceding claims, wherein said second parental yeast strain is maltose intolerant, such as wherein said second parental yeast strain is not capable of growing on maltose as sole carbon source or grows significantly slower on maltose as sole carbon source
- 20 compared to other yeasts.
16. The progeny yeast strain according to any one of the preceding claims, wherein said progeny yeast strain is maltose intolerant, such as wherein said progeny yeast strain is not capable of completely converting maltose into ethanol.
- 25 17. The progeny yeast strain according to any one of the preceding claims, wherein said progeny yeast strain is maltose intolerant, such as wherein said progeny yeast strain is not capable of growing on maltose as sole carbon source or grows significantly slower on maltose as sole carbon source compared to other
- 30 yeasts.
18. The progeny yeast strain according to any one of the preceding claims, wherein said progeny yeast strain is maltotriose intolerant, such as wherein said progeny yeast strain is not capable of completely converting maltotriose into
- 35 ethanol.

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19. The progeny yeast strain according to any one of the preceding claims, wherein said fermented test solution has an alcohol content of at the most 1,0% ABV, such as at the most 0,8% ABV, or such as at the most 0,6% ABV.
20. The progeny yeast strain according to any one of the preceding claims, wherein said progeny yeast strain produces at the most 1,0% ABV, such as at the most 0,8% ABV, or such as at the most 0,6% ABV after incubation in the test solution.
21. The progeny yeast strain according to any one of the preceding claims, wherein said second parental yeast strain is PPU121 as deposited with DSMZ under the accession number DSM 34497.
22. The progeny yeast strain according to any one of the preceding claims, wherein said progeny yeast strain is not capable of converting more than 25% of *p*-coumaric acid into 4-ethylphenol when incubated in an aqueous solution comprising *p*-coumaric acid.
23. The progeny yeast strain according to any one of the preceding claims, wherein said second parental strain and/or said progeny yeast strain is not capable of converting more than 25% of *p*-coumaric acid into 4-ethylphenol when incubated in an aqueous solution comprising *p*-coumaric acid.
24. A method of producing a progeny yeast strain, said method comprising the steps of:
- Providing spores of a first parental yeast strain, wherein said first parental yeast strain carries a mutation in a gene encoding ILV2, wherein ILV2 is as set forth in SEQ ID NO: 1 or a functional homologue of SEQ ID NO: 1 with at least 90%, such as at least 95%, such as at least 98% sequence identity to SEQ ID NO: 1, wherein said mutation results in an amino acid substitution at position 498 of SEQ ID NO: 1 or an amino acid substitution at the corresponding position of said functional homologue thereof, optionally wherein said first parental yeast strain is as defined in any one of the preceding claims,

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- Mating said spore with spores of a second parental yeast strain, wherein at least one spore of the first parental yeast strain has a mating type different to at least one spore of the second parental yeast strain.

5 25. The method according to claim 24, wherein said second parental yeast strain is as defined in any one of claims 12 to 15.

26. A method of producing a fermented aqueous extract, said method comprising the steps of:

- 10 i) providing an aqueous extract of malt and/or cereal;
 ii) providing a progeny yeast strain according to any one of claims 1 to 23;
 and
 iii) fermenting the aqueous extract provided in step i) with said yeast strain of step ii), thereby obtaining a fermented aqueous extract.

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27. The method according to claim 26, wherein the aqueous extract is wort.

28. The method according to any one of claims 26 to 27, wherein the aqueous extract has an apparent extract of at the most 16° Plato, such as in the range of
 20 6 to 16° Plato, for example in the range of 8 to 16° Plato, preferably in the range 6 to 12° Plato, for example in the range of 8 to 10° Plato, such as approx. 9° Plato.

29. A fermented aqueous extract prepared by the method according to any one of
 25 claims 26 to 28.

30. A method for producing a beverage, said method comprising the steps of:

- a) preparing a fermented aqueous extract according to claim 26,
 and
 30 b) processing said fermented aqueous extract into a beverage.

31. The method according to claim 30, wherein the steps of processing comprise one or more of the following:

- a) Filtration,
 35 b) Carbonation,

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- c) Maturation, or
- d) Bottling

5 32. The method according to claims 30 to 31, wherein the method further comprises a step of reducing the alcohol content of the fermented aqueous extract.

33. The method according to claim 32, wherein said step of reducing the alcohol content is rectification or debrewing.

The International Bureau of WIPO
34, Chemin des Colombettes
CH-1211 Geneva 20
Switzerland

8 January 2025

Filed electronically

Response to second Written Opinion

International Patent Application No. PCT/EP2024/055170
Title: LOW DIACETYL YEAST PROGENY STRAINS
Applicant: Carlsberg A/S
Our ref.: P6581PC00

This is in response to the Written Opinion of the International Preliminary Examining Authority dated 12 November 2024.

Request

The applicant respectfully requests that the Examiner acknowledges the patentability of the claims amended as described below. In case the Examiner intends to issue a preliminary report on patentability not acknowledging patentability of the claimed invention, the applicant requests a telephone interview (Rule 66.6 PCT).

Claims

We enclose a set of claims which should replace all pending claims. The claims have been amended as follows:

Claim 1 has been amended by defining that the progeny yeast strain and the first parental yeast strain both carry the mutation at position 498 of ILV2. Basis for this amendment can be found in the application as filed p. 12, l. 15-24.

Claims 8-11 which further define the nature of this mutation have been amended to also refer to the mutation of the progeny yeast strain. Basis for these amendments can be found in the application as filed p. 12, l. 26-34.

Claim 24 has been amended to insert the genotype of the first parental yeast strain according to amended claim 1. Basis for this amendment can be found in the claim as originally filed and in claim 1 as originally filed.

Novelty

Claim 29 was found to lack novelty. This claim is directed to the fermented aqueous extract, e.g. wort, produced by the claimed yeast strains.

The examiner has argued that due to possibility of adding extra steps after fermentation, such as filtration, which removes the yeasts, the claim cannot be considered novel. However, the presence of the yeast used in the final aqueous extract is not necessary to render this composition novel. Due to the low production of diacetyl during fermentation with the presently claimed yeast strains, the fermented wort will necessarily have a different chemical composition compared to worts prepared by fermentation with conventional yeasts that produce normal amounts of total diacetyl, as the total diacetyl content will constantly be very low, while the other produced compounds will be at normal levels. As is known to the skilled person, it is not possible to after fermentation selectively reduce the diacetyl content in a conventionally prepared wort without also affecting the other desirable taste compounds that have been produced by the fermenting yeast.

Since the fermented aqueous extracts produced by the yeasts of the invention have a different chemical composition compared to fermented aqueous extracts produced by conventional yeasts, and since it is not possible to selectively reduce only the total diacetyl content in a conventional wort without affecting other compounds, the aqueous extracts according to claim 26 are therefore novel, even in the absence of the yeasts themselves in the aqueous extract.

It is thereby submitted that claim 26 is novel.

Inventive step and sufficiency

All claims, except claims 24-25 directed to methods of producing the progeny yeast strains, were found to lack inventive step. This lack of inventive step was found due to the invention allegedly not being achievable over the whole scope of claim 1 without undue burden.

Without necessarily agreeing with the examiner, all claims have now been limited to the progeny yeast strain also harbouring a mutation at position 498 of ILV2.

The technical effect of claim 1, i.e. the very low total diacetyl production during fermentation, is already stated in the claim. As the technical effect is stated in the claims, it is submitted that all claims therefore involve an inventive step as none of the prior art discloses a yeast strain that produces at the most 50 ppb total diacetyl at all times during fermentation, as also extensively explained in the demand for international preliminary examination filed on 30 October 2024.

It is submitted that all claims are also sufficiently disclosed. Claim 1 now defines that the progeny yeast strain must also have a mutation in ILV2 in the same position as M49. As shown in Examples 8, 10, 13 and 14 of the application as filed, the progeny yeast strains of M49 have the above-mentioned low total diacetyl phenotype. Example 2 demonstrates that M49 has the claimed point mutation in ILV2.

The skilled person is well aware of how to introduce the claimed point mutation in ILV2 into a given strain, e.g. as described in Example 6 or by standard genetic engineering techniques.

It is thereby submitted that all claims are sufficiently disclosed.

Other

Independent claim 24 has been amended as suggested by the examiner to include the genotypic limitations of the first parental yeast strain according to claim 1.

By the above explanations, the claims of the patent application are believed to comply with the criteria for patentability and we look forward to receiving an International Preliminary Report on Patentability reflecting this opinion.

Yours sincerely

HØIBERG

André Brannvoll

Enclosed

Clean copy of amended claims 1-33

Marked-up copy of amended claims 1-33

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