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Datasheet for the decision of 10 June 2022

Case Number: T 3092/19 - 3.3.08

Application Number: 15175891.9

Publication Number: 2977453

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Language of the proceedings: EN

Title of invention:
Enzyme preparations yielding a clean taste

Applicant:
DSM IP Assets B.V.

Headword:
Enzyme preparation/DSM

Relevant legal provisions:
EPC Art. 84, 111(1), 123(2)

Keyword:
Main request - added subject-matter (no)
Main request - clarity - (yes)
Appeal decision - remittal to the department of first instance (yes)

Decisions cited:

Catchword:

Europäisches Patentamt
European Patent Office
Office européen des brevets

Beschwerdekammern

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Case Number: T 3092/19 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 10 June 2022

Appellant: (Applicant)	Stuart Raynor, Lizanne van Grieken-Plooster DSM IP Assets B.V. Het Overloon 1 6411 TE Heerlen (NL)
Representative:	DSM Intellectual Property P.O. Box 4 6100 AA Echt (NL)
Decision under appeal:	**Decision of the Examining Division of the European Patent Office posted on 15 July 2019 refusing European patent application No. 15175891.9 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman	B. Stolz
Members:	M. Montrone
	L. Bühler

Summary of Facts and Submissions

I. The appeal lies against the decision of an examining division to reject the European patent application No. 2 977 453 ("patent application"). This application is based on the patent application No. 15175891.9, which is a divisional application of European patent application No. 06819802.7, published as International patent application WO 2007/060247 ("earlier patent application").

II. The examining division held in the decision under appeal that claim 1 of the main request comprised added subject-matter, while the auxiliary request was not admitted under Rule 116(2) EPC, because claims 1 and 2 comprised added subject-matter, and lacked clarity.

III. With their statement of grounds of appeal, the applicant ("appellant") submitted a main request that was identical to the main request dealt with in the decision under appeal.

IV. A third party observation was filed.

V. In a communication pursuant to Article 15(1) RPBA, the appellant was informed of the board's provisional, non-binding opinion. A new objection under lack of clarity was raised therein.

VI. In their reply, the appellant submitted a new main request, a new auxiliary request 1, and three declarations (D105A to D105C).

VII. Oral proceedings before the board were held on

10 June 2022 in the form of a videoconference. During the oral proceedings the appellant filed four new auxiliary requests. Auxiliary request 2 filed at 15:15 became the new main request.

VIII. Claim 1 of the main request (filed as auxiliary request 2 at 15:15 hrs during the oral proceedings) reads as follows:

"1. Process to produce a dairy product which comprises adding an enzyme preparation comprising a neutral lactase to a dairy product which comprises lactose, wherein said lactase is lactase expressed in an industrial production strain, and wherein said industrial production strain is *Bacillus licheniformis*, said lactase has a pH optimum between pH=6 and pH=8, and wherein said enzyme composition has no arylsulfatase activity when determined using p-nitrophenylsulfate as a substrate, for activity measurements 0.5 ml of substrate solution, this being 20 mM p-nitrophenylsulfate in 100 mM NaP_i buffer pH 6.5, is mixed with 0.5 ml sample solution containing the arylsulfatase activity, the solution is incubated at 37°C for 3 hours, then the reaction is stopped by addition of 1.5 ml 0.5M NaOH, the OD at 410 nm is determined using a 1 cm pathlength against a blank experiment in which water is added instead of sample solution, as reference a solution is prepared in which the enzyme is added after the reaction was stopped with NaOH, the OD_{410} of this reference solution is subtracted from the OD_{410} determined for the solution in which the enzyme was active for three hours, an aryl sulfatase unit (ASU) is expressed as the change in OD_{410} *10E6/hr, for liquid products the arylsulfatase activity can be expressed as the change in OD_{410} *10E6/ hr per ml of product."

IX. The following documents are cited in this decision:

D105A: Declaration of Peter J.T. Dekker, dated 7 October 2015;

D105B: Declaration of Prof. Henk Noorman, dated 9 October 2015;

D105C: Declaration of Prof. Eric Cator, dated 8 October 2015;

A1: Declaration of Christina Bongiomi, dated 27 October 2017;

A2: Declaration of Eugenio Ferrari, dated 26 October 2017;

A3: Declaration of Olivier Berteau, dated 15 October 2017.

X. The appellant's submissions, insofar as relevant to the present decision, may be summarised as follows:

Main request (filed as auxiliary request 2 during the oral proceedings at 15:15 hrs)

Added subject-matter

The process of claim 1 found a basis in claim 9 in combination with claims 4, 5 and 7 as filed. A further basis was found on page 4, line 11, page 22, lines 27, and 30, page 23, lines 19 and 23, and page 29, line 27 to page 30, line 4 of the earlier patent application.

Clarity and support

The expression "*no arylsulfatase activity*" in claim 1 was clear, because this activity was determined by the assay likewise cited in the claim.

Furthermore, the term "*10E6*" in claim 1 was clear (see documents D105A to D105C), because within the relevant technical field this term was used as a reference to one million.

XI. The appellant requested that the decision under appeal be set aside, and that a patent be granted on the basis of the main request filed during the oral proceedings as auxiliary request 2 at 15:15 hrs.

Reasons for the Decision

Main request (filed as auxiliary request 2 during the oral proceedings at 15:15 hrs)

Added subject-matter

1. The patent application is a divisional application of an earlier patent application. Accordingly, the claimed subject-matter has to comply with the requirements of Articles 76(1) and 123(2) EPC. Since the description of the patent application and the earlier patent application are identical, except for the claims, the following references are made solely to the earlier patent application.

2. In the decision under appeal, the examining division took the view that the main request added subject-matter. This was so because in claim 1 any reference to an arylsulfatase was deleted and, hence, the claim

comprised any activity thereof, "*be it increased, not modified, reduced or absent*" (see page 4, second paragraph to third paragraph). Furthermore, the earlier patent application did not disclose *Bacillus licheniformis* (*B. licheniformis*) as preferred embodiment (see page 22, line 24 to page 23, line 28). Page 8, line 26 to page 9, last line of the earlier patent application mentioned methods of reducing arylsulfatase levels in lactase preparations. However, the earlier patent application did not disclose that *B. licheniformis* had a reduced, or no arylsulfatase activity. Moreover, the earlier patent application was silent on using any of the methods disclosed on page 8, line 26 to page 9, last line to reduce or eliminate arylsulfatase activity from lactase preparations obtained from *B. licheniformis*.

As regards the auxiliary request, the examining division took the view that there was no direct and unambiguous link between *B. licheniformis* and the absence of any arylsulfatase or arylsulfatase activity for the reasons set out for the main request. Rather the skilled person had to cherry pick from different passages of the earlier patent application (see claims 1 and 9 as filed, page 10, line 32 to page 11, line 9 and page 23, line 23), and had to assume that *B. licheniformis* was arylsulfatase free.

3. The process of claim 1 of the main request differs from claim 1 of the main request before the examining division, in that the features "*said lactase has a pH optimum between pH=6 and pH=8, and wherein said enzyme composition has no arylsulfatase activity when determined using p-nitrophenylsulfate as a substrate, for activity measurements 0.5 ml of substrate solution, this being 20 mM p-nitrophenylsulfate in 100 mM* NaP_i

*buffer pH 6.5, is mixed with 0.5 ml sample solution containing the arylsulfatase activity, the solution is incubated at 37°C for 3 hours, then the reaction is stopped by addition of 1.5 ml 0.5M NaOH, the OD at 410 nm is determined using a 1 cm pathlength against a blank experiment in which water is added instead of sample solution, as reference a solution is prepared in which the enzyme is added after the reaction was stopped with NaOH, the OD_{410} of this reference solution is subtracted from the OD_{410} determined for the solution in which the enzyme was active for three hours, an aryl sulfatase unit (ASU) is expressed as the change in OD_{410} *10E6/hr, for liquid products the arylsulfatase activity can be expressed as the change in OD_{410} *10E6/hr per ml of product*" have been added.

4. These features likewise distinguish claim 1 of the main request from claim 1 of the auxiliary request dealt with in the decision under appeal. Furthermore, the term "*free from aryl sulfatase*" in claim 1 of that auxiliary request has been replaced by "*no arylsulfatase activity*" in claim 1 of the main request.

5. The appellant indicated various passages in the earlier patent application as basis for the process of claim 1.

5.1 Claim 9 as filed reads: "*Process to produce a dairy product which comprises adding a lactase of any one of the claims 1 to 6, or a composition of claim 7 to a dairy product which comprises lactose*" (emphasis added).

5.2 Furthermore, claims 1, 4, 5, and 7 as filed read:

- "*Lactase which comprises less than 40 units arylsulfatase activity per NLU of lactase activity*";

- "*Lactase according to any preceding claim, which is a neutral lactase, preferably a K. lactis lactase*";

- "*Lactase according to any preceding claim, which has a pH optimum between pH=6 and pH = 8*"; and

- "*Composition comprising lactase of any one of the claims 1 to 6*", respectively (emphasis added).

5.3 Thus, the process of claim 9 as filed in conjunction with claims 1, 4, 5, and 7 as filed refers to a process for the production of a dairy product which comprises adding a composition that comprises a neutral lactase having a pH optimum between pH=6 and pH=8, and comprises less than 40 units arylsulfatase activity per NLU of lactase activity to a dairy product which comprises lactose.

5.4 In the board's view, the range "*less than 40 units arylsulfatase activity per NLU of lactase activity*" in claim 9 as filed in conjunction with claim 1 as filed comprises no arylsulfatase activity. This range in claim 1 defines as its upper limit "*less than 40 units*", but leaves the lower limit open, thereby encompassing 0 units. This view is likewise supported by the disclosure of the earlier patent application which states in the context of the summary of the invention on page 4, lines 8 to 12 that: "*Surprisingly it is now found that the presence of arylsulfatase as contaminating side activity in enzyme preparations, even at very low levels, can lead to a strong development of off-flavor in a product when a substrate is treated with the preparation, and that the use of an enzyme preparation having no or a reduced aryl sulfatase activity results in a strong reduction of off-flavor development*" (emphasis added).

5.5 In other words, the earlier patent application discloses in the claims and the passage cited above a process for the production of a dairy product by adding as a process step an enzyme preparation that comprises a neutral lactase (with defined properties) but no arylsulfatase activity to a dairy product that contains lactose. This disclosure encompasses any such enzyme preparation that contains lactase and lacks any arylsulfatase activity, irrespective of how this preparation has been obtained, or prepared before it is added to the dairy product.

5.6 The term "*arylsulfatase activity*" is defined on page 11, lines 10 to 13 of the earlier patent application which reads: "*By arylsulfatase activity is meant the sulphuric ester hydrolase activity able to cleave a phenol sulfate into the phenol and sulfate moiety as described for EC 3.1.6.1. Definition for the arylsulfatase unit is provided in the Materials & Methods section (and example 2) of the present application*".

5.7 The respective passage in the "*Materials & Methods*" section on page 29, line 27 to page 30, line 4 of the earlier patent application reads as follows: "*Activity assay arylsulfatase: Arylsulfatase activity was determined using p-nitrophenylsulfate (obtained from Sigma) as a substrate. For activity measurements, 0.5 ml of substrate solution (20 mM p-nitrophenylsulfate in 100 mM NaP_i buffer pH6.5) was mixed with 0.5 ml sample solution containing the arylsulfatase activity. The solution was incubate at 37°C for 3 hours. Than the reaction was stopped by addition of 1.5 ml 0.5M NaOH. The OD at 410 nm was determined (1 cm pathlength) against a blank experiment in which water was added*

*instead of sample solution. As reference, a solution was prepared in which the enzyme was added after the reaction was stopped with NaOH. The OD_{410} of this reference solution was subtracted from the OD_{410} determined for the solution in which the enzyme was active for three hours. An aryl sulfatase unit (ASU) is expressed as the change in OD_{410} *10E6/hr. For liquid products, the aryl sulfatase activity can expressed as the change in OD_{410} *10E6/hr per ml of product*".

5.8 As regards the preparation of enzyme preparations suitable for the claimed process, the earlier patent application discloses on page 15 that "*[I]ndustrially available, food grade enzyme preparations are typically obtained*" from various sources, including "*Bacillus species*" (see page 15, lines 25 to 28). Bacillus species as industrial host strains are further mentioned as being "*very suitable as hosts because of their capability to secrete proteins into the culture medium*" (see page 22, lines 28 to 31). Furthermore, examples "*of preferred industrial production strains within the scope of the present invention*" are mentioned on page 23, lines 19 to 23, including a list of "*especially*" preferred strains such as, for example, "*Bacillus licheniformis*". Thus, the earlier patent application discloses that each of these strains is equally suitable, and preferably used for the preparation of an enzyme preparation with a desired activity (here lactase), irrespective of how an enzyme preparation lacking any arylsulfatase activity is ultimately prepared.

5.9 The board agrees with the examining division that the earlier patent application is silent about the fact that *B. licheniformis* lacks a gene encoding an arylsulfatase, and hence, inherently produces a lactase

preparation without arylsulfatase activity. It seems that this advantageous property of *B. licheniformis* was not known at the filing date of the patent application (see declarations A1 to A3).

5.10 However, while claim 1 requires that the lactase of the enzyme preparation is expressed in *B. licheniformis*, claim 1 does not require that the whole enzyme preparation is obtained from *B. licheniformis* which comprises this lactase as one component, and lacks inherently any arylsulfatase activity too. The claim requires, however, that the enzyme preparation lacks any arylsulfatase activity, irrespective of how this is achieved (see page 4, lines 10 to 12).

5.11 The earlier patent application discloses therefore in the claims and passages indicated above, the process of claim 1 wherein:
the lactase of the enzyme preparation is expressed in *B. licheniformis*, and
an enzyme preparation is used that comprises at least this lactase but lacks any arylsulfatase activity (irrespective of how this is achieved) as determined by a specific assay, and
the addition of this preparation to a dairy product containing lactose.

6. Claim 2 finds literal support in claim 17 as filed.

7. Claim 3 is literally disclosed on page 17, lines 16 to 19 of the earlier patent application.

8. The term "*milk*" as dairy product cited in claim 4 is disclosed in claim 18 as filed.

9. The term "*cow milk*" of claim 5 is mentioned on page 12, line 28 of the earlier patent application.

10. The term "*UHT milk*" of claim 6 is, for example, mentioned on page 9, line 88 of the earlier patent application.

11. In view of the considerations above, the main request complies with the requirements of Articles 76(1) and 123(2) EPC.

Clarity and support

12. In the decision under appeal, the examining division was of the view that claim 1 of the auxiliary request contained an unclear term ("*free from arylsulfatase*") (see page 6, fourth paragraph). This term has been deleted from claim 1 of the main request.

13. The board raised in its communication likewise an objection under lack of clarity against the subject-matter of claim 1. Amended claim 1 of the main request remedies this deficiency.

14. Furthermore, the term "*10E6*" of claim 1 is clear in the relevant technical field, since it refers to one million (see declarations D105A, D105B, and D105C).

15. Hence, the main request complies with Article 84 EPC.

Remittal

16. Since a substantive examination of the main request has not been carried out, the case is remitted to the examining division for further prosecution (Article 111(1) EPC).

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the examining division for further prosecution on the basis of the main request filed as auxiliary request 2 on 10 June 2022 at 15:15 hrs.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated