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D E C I S I O N
of 26 October 1999

Case Number: T 0867/97 - 3.3.4

Application Number: 90118761.7

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Language of the proceedings: EN

Title of invention:
Protein hydrolysates

Patentee:
Novartis Nutrition AG

Opponent:
N.V. Verenigde Bedrijven Nutricia

Headword:
Protein hydrolysates/NOVARTIS

Relevant legal provisions:
EPC Art. 83, 54, 56

Keyword:
"Sufficient disclosure - yes"
"Novelty - yes"
"Inventive step - yes"

Decisions cited:

-

Catchword:

-



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Boards of Appeal

Chambres de recours

Case Number: T 0867/97 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 26 October 1999

Appellant: Novartis Nutrition AG
(Proprietor of the patent) Monbijoustrasse 118
3007 Bern (CH)

Representative: Smolders, Walter
Novartis AG
Geistiges Eigentum Konzern
Patent- und Markenabteilung CH
Postfach
4002 Basel (CH)

Respondent: N.V. Verenigde Bedrijven Nutricia
(Opponent) P.O. Box 1
2700 MA Zoetermeer (NL)

Representative: Jorritsma, Ruurd
Nederlandsch Octrooibureau
Scheveningseweg 82
2517 KZ Den Haag (NL)

Decision under appeal: Decision of the Opposition Division of the
European Patent Office posted 27 June 1997
revoking European patent No. 0 421 309 pursuant
to Article 102(1) EPC.

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: D. D. Harkness
S. C. Perryman

Summary of Facts and Submissions

- I. The appeal lies from the decision of the Opposition Division to revoke European patent No. 0 421 309 relating to a whey protein hydrolysate.
- II. The opposition filed against the grant of the patent was based on objections of lack of novelty, inventive step and sufficiency of disclosure under Articles 100(a) and (b), 54, 56 83 and for other grounds under Article 84 EPC.
- III. In reply the patentee maintained the claims as granted as main request and filed an auxiliary request having four process claims. Claim 1 of this request read:
- "A process for preparing a whey protein hydrolysate characterized by subjecting a whey protein fraction which is substantially free of proteins having a molecular weight of more than 60,000 to the steps of
- a) heating a solution of said whey protein in water to $43 \pm 4^{\circ}\text{C}$ and subjecting said solution to a pepsin prehydrolysis at pH between 2.0 and 3.0;
- b) adjusting the pH of the mixture of step a) at a temperature in the range of from 35° to 50°C to a pH between 7.0 and 9.0 and submitting said mixture to an enzymatic trypsin-chymotrypsin hydrolysis in the presence of a cationic serine endoprotease type elastase 2;
- c) pasteurizing the mixture of step b), subjecting it to an ultrafiltration and concentrating and drying the permeate."

IV. Both the main and auxiliary requests were refused by the opposition division for lack of inventive step.

The documents relevant to the opposition division's decision and cited in this decision are as follows:

- (1): EP-0 250 501
- (2): Mullally M. M. et al., J. Agric. Food Chem. 1994, 42, pages 2973 to 2981
- (4): US-4 293 571
- (5): Jakobsson I, et al., J. Pediatr. Gastroenter. Nutr., 2(4), 1983, pages 613 to 616
- (6): Asselin J., et al., J. Food Sci., 54, (4), 1989, pages 1037 to 1039
- (7): EP-A-0 226 221
- (9): EP-A-0 065 663
- (10): EP-A-0 022 019
- (11): Technical Information on @Corolase PP by Röhm GmbH
- (13): Weström B. J. et al., Pancreas, Vol. 2, 1987, pages 589 to 596
- (14): Ohlsson B. et al., Int. J. Biochem, Vol. 19, 1987, pages 633 to 639

(18): M. Gestin et al., Digestive Diseases and Sciences, Vol. 42, No. 6, June 1997, pages 1302 to 1311.

(19): E. G. Del Mar et al., Biochemistry. 1980, Vol. 19, pages 468 to 472.

(20): M. Gestin et al., Lait, 1997, Vol. 77, pages 399 to 409.

V. The appellant (patentee) filed an appeal and submitted a statement of grounds.

VI. Further submissions were filed on 24 September 1999 together with a new main and two auxiliary requests.

The respondent (opponent) submitted a reply to the grounds of appeal.

VII. Oral proceedings were appointed for the 26 October 1999. In a fax received on 25 October 1999 the respondent stated that he would not be represented.

VIII. During the oral proceedings the appellant withdrew the main and first auxiliary request and maintained the second auxiliary request as sole request, which is identical to the auxiliary request before the Opposition Division (see section III above).

IX. The appellant's arguments are summarised as follows;

Novelty

The preparation of a whey protein hydrolysate starting

from a whey protein fraction which is substantially free of proteins having a MW of more than 60 000 was a decisive distinction between the invention and the prior art. None of the citations disclosed a process in which such a starting material was subjected to the two stage enzyme hydrolysis (a) with pepsin at pH 2 to 3 and at 43°C + or - 3°C, and (b) with trypsin, chymotrypsin plus cationic serine endoprotease type 2 elastase (elastase 2) at pH 7 to 9 and at a temperature of 35° to 50°C. Because the starting material was different from what had previously been employed to produce a hydrolysate the enzyme hydrolysis resulted in a different peptide pattern being produced. The processes described in documents (7) an (10) were also distinguished because after filtration they continued to treat the retentate and not the filtrate as was the case in the patent in suit. The process was therefore novel.

Inventive step

Document (6) represented the nearest prior art since this document was concerned with the preparation of non-allergenic whey hydrolysate compositions for use in milk formulae. However, there was not any disclosure of the removal of proteins of MW above 60,000 as a first step in the process, nor was there any conclusive evidence that elastase 2 was present in the pancreatin enzyme referred to in document (6). It was stated that the prior art disclosures of documents (13) and (14) had employed the wrong substrates for elastase 2 and that the correct substrate for it was however attacked by chymotrypsin and elastase 1, thus the disclosures of documents (18) and (19) were in conflict with that of

documents (13) and (14). Also the teaching of document (6) was that enzyme hydrolysis should be carried out with pepsin and alpha-chymotrypsin (which would not contain elastase 2) rather than with pepsin, trypsin, chymotrypsin and elastase 2, and further a separate elastase digestion was not disclosed. Again there was considerable doubt whether or not the porcine enzymes of the prior art did contain elastase 2, this being because only enzyme preparations derived from very young piglets, ie less than 56 days old, had been shown to contain significant quantities.

- X. The respondent's written arguments concerning the process of preparation of a whey hydrolysate are summarised as follows;

Sufficiency

The appellant had indicated that the specificity of the enzymes determined which peptides and (free) amino acids were present in the final product and therefore had omitted any reference to the fact that the composition of the final product did not solely depend on the use of specific enzymes but was also inextricably bound up with enzyme concentration, ratio of enzymes, enzyme activity, hydrolysis time, other hydrolysis conditions and starting material.

Inventive step

Document (1) explicitly provided a method for producing products that solve the problem postulated in the patent in suit.

The method described by document (1) differed from that disclosed in the patent in suit for preparing the claimed products merely in the omission of a gastric hydrolysis step and of a pasteurisation step after hydrolysis. The cited document however also disclosed the use of **at least one protease** thereby clearly suggesting that combinations of proteases can be applied. No inventive contribution can be derived from carrying out a pasteurisation step as such a step is standard procedure when preparing nutrients in order to prevent bacterial growth. In addition no explanation was given for the pasteurisation step so it merely served the purpose for which it is usually applied in other processes of the state of the art in preparing nutrients in particular whey hydrolysates.

A person skilled in the art interested in whey hydrolysates with reduced allergenicity would be familiar with document (6). An evaluation of the allergenicity of the products was disclosed. It stated explicitly that "Selective proteolysis by pepsin and chymotrypsin was the most efficient combination of enzymes to reduce the allergenicity of both α -lactalbumin and β -lactoglobulin". Most specifically on page 1038 in Table 3 it was quite clear that the combined use of pepsin and pancreatin provided the lowest and thus most favourable value for α -lactalbumin. It was pointed out that pancreatin comprises a mixture of trypsin, chymotrypsin and elastase and that document (6) disclosed "The above hydrolysate could be used to develop an ingredient for infant milk formula with lower allergenicity."

Therefore the person skilled in the art would readily incorporate use of the gastric and pancreatic proteinases into the teaching of document (1) which in fact already incited a person skilled in the art to use more than one proteinase.

The subject-matter of claim 1 thus lacks inventive step over the teaching of the cited documents (1) and (6) as such and in combination.

The problem of document (7) was defined as follows "It has been attempted to produce mothers milk substitutes from e.g. whey by a combination of enzymatic hydrolysis, heat treatment and ultrafiltration". The peptides obtained thereby had a size of 5,000 to 10,000 Dalton and are often allergenic.

A method for producing the products was disclosed, said method comprising ultrafiltration and hydrolysis of whey characterised by

- (a) diafiltrating essentially casein free whey with water on an about 20 000 Dalton membrane, if desired after a preceding concentration of the whey,
- (b) enzymatically hydrolysing the whey protein retentate from (a) in one or more steps each hydrolysis step being terminated with ultrafiltration through an about 6,000 Dalton membrane to harvest the resulting peptides in the permeate.

Claim 6 claimed performing the hydrolysis in one or two steps with two different enzymes and claim 7 claimed the use of one or more proteases. Corolase PP was provided as an example of a suitable protease.

Document (6) would readily be consulted by a person skilled in the art. In the line of further development of the method disclosed in document (7) it would be obvious to apply the gastric and pancreatic hydrolyses in an embodiment of the process of claim 6 or 7 of the cited patent application. The only steps which differentiated the method according to the combined teaching of documents (6) and (7) from the method described in claim 1 were those of pasteurisation, concentration and drying. These steps were not explicitly described in the state of the art teaching. However such steps were routine and cannot be regarded as contributing any inventive step to the process for preparing the products. Moreover these steps do not seem to be relevant for arriving at a non-allergenic product with a particular peptide composition which peptide composition is dependent on the starting material and the means of hydrolysis and any steps that would remove peptide components or amino acids.

Element (a) concerning the molecular weight less than 60,000 was specifically mentioned in documents (1), (7) and (10). Thus a combination of any of these three documents with document (6) would most definitely provide all elements of the claim.

If element (a) provided the only difference from the teaching of document (6) this could not provide an

inventive step over the teaching of document (6). The inventive step was presented in the description as the combination of a gastric and peptic hydrolysis step. It was not presented as nor remotely discernible as the removal of large molecules prior to hydrolysis.

XI. The appellant requested that the decision under appeal be set aside and that the patent be maintained on the basis of the claims 1 to 4 submitted as auxiliary request 2 by telefax on 24 September 1999.

XII. The respondent requested in writing that the appeal be dismissed.

Reasons for the Decision

1. The appeal is admissible.
2. *Disclosure of the invention, Article 83 EPC.*

The features which determine the outcome of the process, namely, starting material, enzymes, pH and temperature conditions, are all recited in the description particularly in the numerous examples where they occur in combination with the details, see inter alia Examples 1 to 4 in the patent in suit. Thus the skilled person would not have any difficulty in carrying out the process of the invention because all those essential features necessary to do that are disclosed in an enabling manner. The insufficiency objection therefore fails.

3. *Novelty, Article 54 EPC*

Document (1) describes a single stage hydrolysis at pH 7,5 or above, therefore alkaline, whereas the patent in suit first hydrolyses at pH 2 to 3 with the pepsin prehydrolysis step and afterwards at pH 7 to 9. It was acknowledged by the respondent in his written submissions that this citation did not disclose a process having a gastric hydrolysis step. The MWs of the products are also different, much lower MW values being obtained for the product of the patent in suit than for that of document (1). Therefore said document (1) does not anticipate the process of the patent in suit.

4. The process described in document (6) does not anticipate that of the patent in suit because it fails to mention that the starting material has been freed from proteins having a MW of above 60,000.

5. In the process according to document (7) there is only one alkaline enzyme hydrolysis step, see column 5 paragraph 2, and there is no reference to the necessary acidic gastric hydrolysis. On page 1, column 2 last paragraph, a MW of less than 2,000 is to be avoided, thus the product has a different MW spread of up to 6,000, preferably 2,000 to 6,000, whereas the patent in suit prepares products having MW below 2,000, e.g., below 1,400, see pages 8, 15 and 17.

6. The disclosures of claim 2 of document (10) and page 5, lines 18 to 20 of the patent in suit are similar, however the former relates to a total enzyme hydrolysis (pH 7 to 9) (foot of page 11 et seq) without any acid hydrolysis, thus this document is not detrimental to the novelty of the process of the patent in suit.

Thus, novelty of the subject-matter of the claims of this request is accepted.

Inventive step, Article 56 EPC

The closest prior art

7. Document (6) relates to a study of the effects of *in vitro* proteolysis on the allergenicity of whey proteins alpha-lactalbumin and beta-lactoglobulin and describes hydrolysis of whey proteins by a pepsin hydrolysis at pH 2 for 30 mins, followed by a pancreatic enzyme hydrolysis at pH 7.5 for 60 mins, both being carried out at 37°C, with a subsequent heat treatment at 80°C to inactivate the enzymes. The conclusion of document (6) was that hydrolysed whey proteins obtained by successively using pepsin and chymotrypsin might be a promising ingredient in adapted cow's milk formulas of low allergenicity.

Thus this document relates to the problem solved by the patent in suit which is to provide a process for the production of whey protein hydrolysates of low allergenicity which may be incorporated into milk formulas. None of the remaining prior art documents relates to a process to reduce allergenicity in hydrolysed whey proteins or discloses a two stage

hydrolysis using enzyme combinations which are more akin to those of the process of the patent in suit than those of document (6). In the light of this disclosure document (6) is regarded as the nearest prior art.

The problem to be solved

8. Thus the problem to be solved by the patent in suit is that of providing an alternative process for the hydrolysis of whey proteins to produce hydrolysates of low allergenicity.

The solution to the problem

9. The solution is provided by the process of claim 1 of the sole request.

Assessment of inventive step

10. What was recommended by document (6) in order to reduce "allergenicity" in the hydrolysate products, which aim is not necessarily linked with the highest degree of "hydrolysis" of the proteins, (see document (6), page 1038, column 2, paragraphs 2 and 3), was that the degree of hydrolysis of alpha-lactalbumin and beta-lactoglobulin is not the only factor influencing allergenicity. Hence the recommendation for maximum reduction in allergenicity (see document (6), page 1038, Table 3) was the hydrolysis of whey proteins with pepsin and chymotrypsin successively, (see document (6), page 1038, column 2), because these enzymes were specific in their action to reduce allergenicity in beta-lactoglobulin and alpha-lactalbumin respectively. The highest degree of

- "hydrolysis" was obtained by employing pepsin plus pancreatin, (see document (6), page 1038, Table 2), but this was not proposed as the best combination to reduce "allergenicity".
11. Document (6) at page 1038, column 1 states that the degree of hydrolysis obtained with pancreatin was high because it contains trypsin and chymotrypsin and that pretreatment with pepsin before hydrolysis with pancreatic enzymes did not change the degree of "hydrolysis" by very much. This teaching therefore does not imply that the pretreatment with pepsin would improve the reduction in "allergenicity" in the final product.
 12. The primary teaching of document (6) with respect to a reduction in allergenicity in hydrolysed whey proteins is therefore that hydrolysis be carried out with pepsin followed by chymotrypsin and there was no mention of the use of pepsin followed by the trypsin-chymotrypsin-elastase 2 combination.
 13. From Table 3 of document (6) the skilled person would have taken the pepsin-chymotrypsin combination as the most promising line to follow when seeking to reduce whey protein hydrolysate allergenicity. Although Tables 2 and 3 of document (6) specify hydrolysis with and allergy values for whey protein hydrolysates produced with pepsin followed by pancreatin there is no hint to choose this option and even if elastase 2 were to be found in pancreatin there is no pointer to the use of elastase 2 in hydrolysis with the other enzymes for the purpose of reducing allergenicity. The opposition division combined documents (6) and (14),

the former stated that pancreatin contained chymotrypsin, trypsin and carboxypeptidase B, whilst the latter showed the presence of elastase 2 in pancreatic enzymes. The appellant denied this and cited document (18) which refers to difficulties in measuring the quantitative elastase 2 activity. Document (13) at Table 1 shows presence of elastase 2 in pigs and document (20) showed that elastase 2 was present in porcine pancreatic powder. The appellant disputed this in as much as elastase 2 was said not to be present in porcine enzymes after the piglet was more than 56 days old.

14. There is much evidence for and against the presence of elastase 2 in porcine enzymes and this must remain in doubt. The Board is not convinced (a) that it was obvious to choose pepsin and pancreatin for the purpose of further reducing allergenicity and (b) that an obvious connection (documents (6) and (14)) exists between elastase 2 and the problem of reducing allergenicity.

15. Further, the choice of enzymes to be used in hydrolysis of the whey proteins is not the only important factor in the determination of the constitution of the final protein hydrolysate. A very significant step in the process of the patent in suit is the removal of proteins of MW above 60,000 from the starting material and this has a considerable effect upon the hydrolysis process and the composition of the subsequent hydrolysate.

16. The disclosure of document (1) does not relate to the problem of the production of whey hydrolysates having

low allergenicity. It is directed to a method for producing products which are heat resistant, non-bitter, and easily water-soluble, and does not refer to elastase 2 or to an initial filtration step with cut-off at MW 60,000. It does not therefore give any hints with respect to the attainment of low allergenic properties nor to a combination of features with document (6) which would obviously lead to the process of the patent in suit.

17. The disclosure of document (2) shows that elastase was present in Corolase PP and pancreatin, however, forms 1 and 2 of elastase were not identified in this document published in 1994 after the filing date of the patent in suit.

18. The process according to document (4) relates to the hydrolysis of various protein sources including whey by enzyme hydrolysis (pancreatin) to remove allergens, followed by heating to denature unhydrolysed proteins and subsequent filtration. This teaching does not add anything to that of document (6) which would render the solution to the problem proposed by the patent in suit obvious.

19. Even though it was said in document (5) that cathodal elastase (elastase 2) might be a crucial enzyme for the hydrolysis of alpha-lactalbumin and beta-lactoglobulin the relevance of elastase 2 to the reduction in allergenicity of whey proteins hydrolysed therewith is not convincingly stated because at page 615, column 2 two researchers found that there was no correlation between low content of elastase 2 in duodenal juice and the diagnosis of cow's milk protein intolerance.

20. Document (7) relates to a process in which casein-free whey is diafiltrated on a 20,000 Dalton membrane followed by enzyme hydrolysis of the "retentate" in one or more steps. This process therefore does not have any significance in respect of the process of the patent in suit which treats the filtrate and therefore uses a different protein fraction.

21. The process described in document (9) relates to the hydrolysis of whey protein principally lactalbumin by removing lactose followed by hydrolysis of an aqueous slurry of the whey protein using fungal protease from *Aspergillus oryzae* to give a mixture of amino acids and di- and tri-peptides which is then heated and filtered to give a filtrate of desired protein hydrolysate. Again this process does not add any teaching which would in combination with that of document (6) give rise to an obviousness objection.

22. The process of document (10) also involves an enzymatic hydrolysis of the retentate after filtration which hydrolysis is continued until there are no residual proteins present. This document is also not relevant to the process of the patent in suit.

23. For the above given reasons there is no single document or combination of documents which obviously leads to the solution to the problem solved by the appellant. The subject-matter of claim 1 of the patent in suit therefore fulfills the requirements of Article 56 EPC.

24. The remaining three claims of the request are all appendant to claim 1 and also involve an inventive step for the same reasons.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The matter is remitted to the first instance with the order to maintain the patent on the basis of claims 1 to 4 submitted as auxiliary request 2 by telefax on 24 September 1999 and a description to be adapted.

The Registrar:

The Chairwoman:

A. Townend

U. Kinkeldey