

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
5 August 2004 (05.08.2004)

PCT

(10) International Publication Number
WO 2004/064537 A3

(51) International Patent Classification⁷: C12N 15/54,
15/55, 9/10, 9/18, 11/00, C12P 7/62, 7/64, A23L 1/035,
C12Q 1/44, 1/48

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(21) International Application Number:
PCT/IB2004/000655

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(22) International Filing Date: 15 January 2004 (15.01.2004)

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,
ZW.

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0301117.8 17 January 2003 (17.01.2003) GB
0301118.6 17 January 2003 (17.01.2003) GB
0301119.4 17 January 2003 (17.01.2003) GB
0301120.2 17 January 2003 (17.01.2003) GB
0301121.0 17 January 2003 (17.01.2003) GB
0301122.8 17 January 2003 (17.01.2003) GB
60/489,441 23 July 2003 (23.07.2003) US
0330016.7 24 December 2003 (24.12.2003) GB

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), Euro-
pean (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR,
GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
ML, MR, NE, SN, TD, TG).

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Published:
— with international search report

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(88) Date of publication of the international search report:
23 March 2006

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: METHOD FOR THE IN SITU PRODUCTION OF AN EMULSIFIER IN FOODSTUFF

(57) Abstract: A method for the *in situ* production of an emulsifier in a foodstuff, wherein a lipid acyltransferase is added to the foodstuff. Preferably the emulsifier is produced without an increase or without a substantial increase in the free fatty acid content of the foodstuff. Preferably, the lipid acyltransferase is one which is capable of transferring an acyl group from a lipid to one or more of the following acyl acceptors: a sterol, a stanol, a carbohydrate, a protein or a sub-unit thereof, glycerol. Preferably, in addition to an emulsifier one or more of a stanol ester or a stanol ester or a protein ester or a carbohydrate ester or a diglyceride or a monoglyceride may be produced. One or more of these may function as an additional emulsifier.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/54 C12N15/55 C12N9/10 C12N9/18 C12N11/00
 C12P7/62 C12P7/64 A23L1/03 A23L1/035 C12Q1/44
 C12Q1/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12P A23L C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, PAJ, FSTA, EMBL, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/05396 A1 (DANISCO A/S; SOEE, JOERN, BORCH) 3 February 2000 (2000-02-03)	1-9, 14-21, 26, 32, 33
Y	page 1, line 3 - page 16, line 20	10-13, 22-25
A	----- EP 1 275 711 A (COGNIS DEUTSCHLAND GMBH & CO. KG) 15 January 2003 (2003-01-15) page 2, line 24 - page 3, line 50; claims 1-40; sequences 1,2 page 5, line 27 - line 33 page 10, line 54 - page 11, line 2	1-33
A	----- US 6 066 482 A (STEFFENS ET AL) 23 May 2000 (2000-05-23) column 1 - column 3; examples 4-6	1-26, 32, 33
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 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

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T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search

26 July 2005

Date of mailing of the international search report

09. 08. 2005

Name and mailing address of the ISA

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 Fax: (+31-70) 340-3016

Authorized officer

Devijver, K

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IB2004/000655

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	UPTON C ET AL: "A new family of lipolytic enzymes?" TIBS TRENDS IN BIOCHEMICAL SCIENCES, ELSEVIER PUBLICATION, CAMBRIDGE, EN, vol. 20, no. 5, May 1995 (1995-05), pages 178-179, XP004222260 ISSN: 0968-0004 cited in the application	27-31
Y	the whole document	10-13, 22-25
X	----- BRUMLIK MICHAEL J ET AL: "Identification of the catalytic triad of the lipase/acyltransferase from Aeromonas hydrophila" JOURNAL OF BACTERIOLOGY, vol. 178, no. 7, 1996, pages 2060-2064, XP002315734 ISSN: 0021-9193 cited in the application the whole document	27-31
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A	----- NEUGNOT VIRGINIE ET AL: "The lipase/acyltransferase from Candida parapsilosis. Molecular cloning and characterization of purified recombinant enzymes" EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 269, no. 6, March 2002 (2002-03), pages 1734-1745, XP002320424 ISSN: 0014-2956 page 1734	1-33
X	----- HILTON S ET AL: "PURIFICATION AND SPECTRAL STUDY OF A MICROBIAL FATTY ACYLTRANSFERASE ACTIVATION BY LIMITED PROTEOLYSIS" BIOCHEMISTRY, vol. 29, no. 38, 1990, pages 9072-9078, XP002337706 ISSN: 0006-2960	27-31
Y	the whole document	10-13, 22-25
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INTERNATIONAL SEARCH REPORT

IB2004/000655

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	-& DATABASE UniProt 'Online! 1 July 1989 (1989-07-01), "Phosphatidylcholine-sterol acyltransferase precursor (EC 2.3.1.43) (Glycerophospholipid-cholesterol acyltransferase) (GCAT)." XP002337710 retrieved from EBI accession no. UNIPROT:GCAT_AERHY Database accession no. P10480 the whole document	
X	----- NERLAND A H: "The nucleotide sequence of the gene encoding GCAT from Aeromonas salmonicida ssp. salmonicida" JOURNAL OF FISH DISEASES, vol. 19, no. 2, 1996, pages 145-150, XP008049669 ISSN: 0140-7775	27-31
Y	the whole document	10-13, 22-25
	-& DATABASE UniProt 'Online! 1 November 1996 (1996-11-01), "Glycerophospholipid-cholesterol acyltransferase precursor." XP002337711 retrieved from EBI accession no. UNIPROT:Q44268 Database accession no. Q44268 the whole document	
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Y	the whole document	10-13, 22-25
A	----- "USE OF LIPOLYTIC ENZYME FROM AEROMONAS IN DETERGENTS" RESEARCH DISCLOSURE, KENNETH MASON PUBLICATIONS, WESTBOURNE, GB, no. 390, October 1996 (1996-10), pages 661-662, XP000639927 ISSN: 0374-4353 the whole document	1-33

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
1-26,32,33 (in part, as far as applicable) (invention 1); 27-32 (in part as far as applicable) (inventions 2 and 3)

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-26,32,33 (in part, as far as applicable)

Method for the in situ production of an emulsifier in a foodstuff, wherein the method comprises the step of adding a lipid acyltransferase to the foodstuff. Such a method wherein at least 2 emulsifiers are produced. Such a method wherein the emulsifier is produced without increasing the free fatty acids in the foodstuff. Such a method wherein the lipid acyltransferase is capable of transferring an acyl group from a lipid to a sterol. Such a method wherein a sterol ester is produced. Such a method wherein the lipid acyltransferase is an enzyme which possesses acyltransferase activity and comprises the amino acid sequence motif GDSX. Use of a lipid acyltransferase according to said method. Foodstuff obtainable by said method.

1.1. claims: 1-26,32,33 (in part, as far as applicable)

As invention 1, but relating to a stanol ester.

1.2. claims: 1-26,32,33 (in part, as far as applicable)

As invention 1, but relating to a carbohydrate ester.

1.3. claims: 1-26,32,33 (in part, as far as applicable)

As invention 1, but relating to a protein ester or a protein subunit ester.

1.4. claims: 1-26,32,33 (in part, as far as applicable)

As invention 1, but relating to a glycerol ester.

2. claims: 27-32 (in part, as far as applicable)

Food or feed enzyme composition which contains a lipid acyltransferase enzyme obtainable from Aeromonas, in particular Aeromonas hydrophila. Such an enzyme relating to SEQ ID NO: 2 and 32.

3. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Aeromonas, in particular Aeromonas salmonicida. Such an enzyme relating to SEQ ID NO: 3 and 34.

4. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Streptomyces, in particular Streptomyces coelicolor. Such an enzyme relating to SEQ ID NO: 4, 5, 20, 22, 24, 26 and 28.

5. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Streptomyces, in particular Streptomyces rimosus. Such an enzyme relating to SEQ ID NO: 30.

6. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Saccharomyces, in particular Saccharomyces cerevisiae. Such an enzyme relating to SEQ ID NO: 6.

7. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Lactococcus.

8. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Mycobacterium.

9. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Streptococcus.

10. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Lactobacillus.

11. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Desulfitobacterium.

12. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Bacillus.

13. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Campylobacter.

14. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Vibrionaceae.

15. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Xylella.

16. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Sulfolobus.

17. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Aspergillus.

18. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Schizosaccharomyces.

19. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Listeria.

20. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Neisseria.

21. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Mesorhizobium.

22. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Ralstonia. Such an enzyme relating to SEQ ID NO: 12.

23. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Xanthomonas.

24. claims: 27-32 (in part, as far as applicable)

As invention 2, but relating to a lipid acyltransferase enzyme obtainable from Candida.

25. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Aeromonas, in particular Aeromonas hydrophila. Such an enzyme relating to SEQ ID NO: 2 and 32.

26. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Aeromonas, in particular Aeromonas salmonicida. Such an enzyme relating to SEQ ID NO: 3 and 34.

27. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Streptomyces, in particular Streptomyces coelicolor. Such an enzyme relating to SEQ ID NO: 4, 5, 20, 22, 24, 26 and 28.

28. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Streptomyces, in particular Streptomyces rimosus. Such an enzyme relating to SEQ ID NO: 30.

29. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Saccharomyces, in particular Saccharomyces cerevisiae. Such an enzyme relating to SEQ ID NO: 6.

30. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Lactococcus.

31. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Mycobacterium.

32. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Streptococcus.

33. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Lactobacillus.

34. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Desulfitobacterium.

35. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Bacillus.

36. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Campylobacter.

37. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Vibrionaceae.

38. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Xylella.

39. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Sulfolobus.

40. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Aspergillus.

41. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Schizosaccharomyces.

42. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Listeria.

43. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Neisseria.

44. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Mesorhizobium.

45. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Ralstonia. Such an enzyme relating to SEQ ID NO: 12.

46. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from Xanthomonas.

47. claims: 34-38 (in part, as far as applicable)

Immobilized lipid acyltransferase enzyme obtainable from
Candida.

48. claims: 39-43

Method of identifying a lipid acyltransferase comprising the
steps of testing an enzyme using one or more of the
"transferase assay in a low water environment", the
"transferase assay in high water egg yolk" or the
"transferase assay in buffered substrate". Lipid
acyltransferase identified using said method.

INTERNATIONAL SEARCH REPORT

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