(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 July 2005 (21.07.2005)

PCT

(10) International Publication Number
WO 2005/066351 A2

(51) International Patent Classification[7]: C12P 7/64

(21) International Application Number:
PCT/IB2004/004374

(22) International Filing Date:
23 December 2004 (23.12.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

0330016.7	24 December 2003 (24.12.2003)	GB
PCT/IB2004/000655	15 January 2004 (15.01.2004)	IB
0416023.0	16 July 2004 (16.07.2004)	GB
10/898,775	26 July 2004 (26.07.2004)	US

(71) Applicant *(for all designated States except US)*: **DANISCO A/S** [DK/DK]; Langebrogade 1, P.O. Box 17, DK-1001 Copenhagen K (DK).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **KRISTENSEN, Anna, Cecilie, Jentoft** [DK/DK]; Vesterbrogade 10A, 4. Sal, 8000, DK-Århus C (DK). **WASSELL, Paul** [GB/DK]; Michael Anchers Vej 3, DK-8270 Højbjerg, Århus (DK). **MIKKELSEN, Jørn, Dalgaard** [DK/DK]; Engkær 38, DK-2650 Hividovre (DK). **SØE, Jørn, Borch** [DK/DK]; Orøvænget 11, DK-8381 Tilst (DK).

(74) Agents: **WILLIAMS, Aylsa** et al.; D Young & Co, 120 Holborn, London EC1N 2DY (GB).

(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.

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

Published:
— *without international search report and to be republished upon receipt of that report*

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

WO 2005/066351 A2

(54) Title: METHOD

(57) Abstract: The present invention relates to a method of reducing and/or removing diglyceride from an edible oil, comprising a) admixing an edible oil with an acyl acceptor substrate and a fatty-acid CoA independent diglyceride: glycerol acyltransferase, wherein the fatty-acid CoA independent diglyceride: glycerol acyltransferase is characterized as an enzyme which in an edible oil is capable of transferring an acyl group from a diglyceride to glycerol. Preferably, the diglyceride: glycerol acyltransferase comprises the amino acid sequence motif GDSX, wherein X is one or more of the following amino acid residues L, A, V, I, F, Y, H, Q, T, N, M or S. Furthermore the present invention relates to the use of a fatty-acid CoA independent diglyceride: glycerol acyltransferase characterized as an enzyme which in an edible oil is capable of transferring an acyl group from a diglyceride to glycerol, in the manufacture of an edible oil, for reducing and/or removing (preferably selectively reducing and/or removing) diglyceride from said edible oil, and to the use of said enzyme in the manufacture of a foodstuff comprising an edible oil for improving the crystallization properties of said foodstuff.