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(54) Title: METHOD OF CHEMICAL MONITORING THE FAT REMOVAL FROM SURFACES

(57) Abstract: The present invention concerns a method of chemically identifying the di- and tri-glyceride composition of a fat stain on a surface, said method comprising: a) stain surface with fat stain; b) treat fat stain by washing with an aqueous solution of a laundry detergent composition dosed at a 1 to 20g/L, wherein the laundry detergent composition comprises surfactant, preferably at levels of from 1 to 50 wt.%; then remove aqueous solution and optionally rinse the surface; c) optionally dry the residual stain in air; b) solubilise the residual fat from the surface using an organic solvent; e) measure the di- and tri-glyceride content of the residual fat using MALDI (matrix-assisted laser desorption ionization) mass spectrometry.

METHOD OF CHEMICAL MONITORING THE FAT REMOVAL FROM SURFACES**Field of Invention**

- 5 The present invention concerns a method for measuring the chemical changes in a fat stain due to washing with detergent ingredients.

Background of the Invention

- 10 Household detergent products are designed for the removal of soils from surfaces, such as textiles, work tops, windows and toilet bowls. Typically, the performance is measured by changes in the colour intensity of the stain on the surface. This method does not give detailed information on the change in the chemical composition of the soil caused by the cleaning processes.
- 15 Fats are ubiquitous in the home and are predominately composed of triglycerides which consist of a glycerol moiety bound via ester bonds to 3 fatty acids. Fats and oils are typically colourless. Removal of fats and oils from surfaces are a key function of household detergent products.
- 20 There is a desire to better identify the chemical composition of fat soil on treated and untreated surfaces so as to better design detergent products for household purposes.

Summary of the Invention

- 25 The invention relates to a method of chemically identifying the di- and tri-glyceride composition of a fat stain on a surface, said method comprising:-
- a) stain surface with fat stain;
 - b) treat fat stain by washing with an aqueous solution of a laundry detergent composition dosed at a 1 to 20g/L, wherein the laundry detergent composition comprises surfactant, preferably at levels of from 1 to 50 wt.%; then remove
 - 30 aqueous solution and optionally rinse the surface;
 - c) optionally dry the residual stain in air;
 - d) solubilise the residual fat from the surface using an organic solvent;
 - e) measure the di- and tri-glyceride content of the residual fat using MALDI (matrix-assisted laser desorption ionization) mass spectrometry.

Preferably the surface in step a) is selected from textiles and clothing.

After method step c), the stained surface is preferably stored for from 0 to 400 hours, preferably at a temperature below 313K, more preferably 253 to 303K, most preferably 283 to 298K.

Preferably the solubilisation step of method step d) has a solvent to fat weight ratio of 10:1 to 5000:1, more preferably 20:1 to 100:1. Preferably all the fat is solubilised.

Preferably the fat is selected from lard, butter, ghee, goose fat, duck fat, chicken fat, olive oil, suet, palm oil, sunflower oil, rapeseed oil, nut oils, soya oil, fish oil, cocoa butter, shea butter, sebum, borneo tallow, Illipe butter, sesame oil, safflower oil, linseed oil, poppy oil, pumpkin seed oil, wheat germ oil, corn germ oil.

Preferably the fat is a solid at 30°C, most preferably at 37 °C.
Most preferably the fat is selected from lard.

Palm kernels oils are preferably selected from: oil palm (*Elaeis guineensis*), coconut palm (*Cocos nucifera*), and babassu palm (*Orbignya speciosa*).

It is preferable to dilute extract by known amounts and remeasure to ensure spectrometer response is linear to the concentration of analyte. In MALDI the response often increases linearly as the concentration of dissolved fat decreases over a wide range.

The di- and tri-glycerides are preferably analysed in positive mode in the MALDI spectrometer.

The fat stains may be washed with a saline (NaCl) solution to increase signal in the MALDI spectrometer.

Preferably the treatment method in (b) is washing in aqueous solution with a laundry detergent composition dosed at a 1 to 20g/L, wherein the laundry detergent composition comprises surfactant, preferably at levels of from 1 to 50 wt.%. Most preferably the laundry detergent composition comprises an anionic surfactant.

Detailed Description of the Invention

Surface

Most preferably the surface in step a) is selected from textiles and clothing.

5 Fats

The fats are preferably of animal or plant origin and contain greater than 5 wt.%, more preferably greater than 30 wt.%, most preferably greater than 80wt.% of di- and tri-glyceride.

10 Preferably the solubilisation step of method step d) has a solvent to fat weight ratio of 10:1 to 5000:1, more preferably 20:1 to 100:1. Preferably all the fat is solubilised.

Preferably the fat is selected from lard, butter, ghee, goose fat, duck fat, chicken fat, olive oil, suet, palm oil, sunflower oil, rapeseed oil, nut oils, soya oil, fish oil, cocoa butter, shea butter, sebum, borneo tallow, Illipe butter, sesame oil, safflower oil, linseed oil, poppy oil, pumpkin
15 seed oil, wheat germ oil, corn germ oil.

Preferably the fat is a solid at 30°C, most preferably at 37 °C.

Most preferably the fat is selected from lard.

20 Palm kernels oils are preferably selected from: oil palm (*Elaeis guineensis*), coconut palm (*Cocos nucifera*), and babassu palm (*Orbignya speciosa*).

It is preferable to dilute extract by known amounts and remeasure to ensure spectrometer response is linear to the concentration of analyte. In MALDI the response often increases
25 linearly as the concentration of dissolved fat decreases over a wide range.

The di- and tri-glycerides are preferably analysed in positive mode in the MALDI spectrometer.

30 The fat stains may be washed with a saline (NaCl) solution to increase signal in the MALDI spectrometer.

The fat may be in the form of a food product, for example a pesto, gravy, ice cream, salad dressing, sauce for example cheese sauce, cheese, cream. chocolate and mayonnaise.

Triglycerides and fats are described in Food Chemistry, Belitz, H.-D., Grosch W., Schieberle P. (2009 Springer).

5 A triglyceride is described by the acyl carbon number, ACN, and the number of double bonds, DB, in the form ACN:DB. The ACN is the total number of carbons in the three fatty acid chain and DB is the total number of double bonds in the fatty acid chains. For example, triolein consisted of 3 oleic acid bound to glycerol and has an ACN:DB of 54:3; a triglyceride consisting of palmitic acid, lauric acid and myristic acid has an ACN:DB of 42:0; a triglyceride consisting of 3 linoleic acids has an ACN:DB of 54:6. The ACN:DB of a
10 triglyceride is expressed as for example C54:6. A diglyceride may be described in a similar manner, and is expressed for example by DG34:0 to distinguish it from the triglyceride C34:0.

15 Preferably fresh fat is used that is not rancid and has no rancid odour.

Lard is pig fat and may be obtained from part of the pig where there is a high concentration of fatty tissue. The lard may be rendered or unrendered, preferably unrendered. Preferably the lard is obtained from leaf and backfat, most preferably leaf fat. Leaf fat is obtained from the "flare" visceral fat deposit surrounding the kidneys and inside the loin.

20 Butter is preferably butter made from cows milk.

Washing method

The surface, preferably a textile garment or clothing, may be washed by any domestic wash habit, preferably by hand washing, washing in a top loading automatic washing machine or
25 washing in a front loading automatic washing machine. For rigid surfaces they are preferably attached to the side or window of a washing machine to prevent breakage.

Preferably water hardness of 6 to 48 degrees French hard are used for washes and rinses, with liquor to cloth ratios in the range of 2:1 to 40:1, preferably 5:1 to 30:1.

30 The wash water temperature is preferably 273 to 313K, more preferably 285 to 303K.

Rinse water temperatures are preferably ambient.

The laundry detergent composition used in the method may be in any suitable form such as liquids, powders, bars, unit dose powders, unit dose liquids. Laundry detergent compositions may preferably contain surfactants such as linear alkyl benzene sulfonates, alkyl ether sulfates, fatty alcohol ethoxylates, alkyl sulfates, quaternary ammonium salts (rinse conditioner active). Preferably the laundry detergent composition comprises anionic surfactant, preferably at a level of from 1 to 50 wt.%. 5

The laundry detergent compositions for testing may preferably be a powder detergent or a liquid detergent. The detergent may be in the form of a unit dose, for example a liquid unit dose. The laundry detergent compositions are preferably diluted in water before use. 10

Other common ingredient include: protease and amylase enzymes, sequesterants, builders, anti-reposition polymers.

The household detergent product, preferably when a laundry detergent product or machine dishwash product preferably comprises a lipid esterase (lipase). 15

Lipid Esterase

Cleaning lipid esterases are discussed in Enzymes in Detergency edited by Jan H. Van Ee,, Onno Misset and Erik J. Baas (1997 Marcel Dekker, New York). 20

Cleaning lipid esterases are preferable active at alkaline pH in the range 7 to 11, most preferably they have maximum activity in the pH range 8 to 10.5.

The lipid esterase may be selected from lipase enzymes in E.C. class 3.1 or 3.2 or a combination thereof. 25

Preferably the cleaning lipid esterases is selected from:

- (1) Triacylglycerol lipases (E.C. 3.1.1.3)
 - (2) Carboxylic ester hydrolase (E.C. 3.1.1.1)
 - (3) Cutinase (E.C. 3.1.1.74)
 - (4) Sterol esterase (E.C. 3.1.1.13)
 - (5) Wax-ester hydrolase (E.C. 3.1.1.50)
- 30

Suitable triacylglycerol lipases can be selected from variants of the Humicola lanuginosa (Thermomyces lanuginosus) lipase. Other suitable triacylglycerol lipases can be selected 35

from variants of *Pseudomonas* lipases, e.g., from *P. alcaligenes* or *P. pseudoalcaligenes* (EP 218 272), *P. cepacia* (EP 331 376), *P. stutzeri* (GB 1,372,034), *P. fluorescens*, *Pseudomonas* sp. strain SD 705 (WO 95/06720 and WO 96/27002), *P. wisconsinensis* (WO 96/12012), *Bacillus* lipases, e.g., from *B. subtilis* (Dartois et al. (1993), *Biochimica et Biophysica Acta*, 1131, 253-360), *B. stearothermophilus* (JP 64/744992) or *B. pumilus* (WO 91/16422).

Suitable carboxylic ester hydrolases can be selected from wild-types or variants of carboxylic ester hydrolases endogenous to *B. gladioli*, *P. fluorescens*, *P. putida*, *B. acidocaldarius*, *B. subtilis*, *B. stearothermophilus*, *Streptomyces chrysomallus*, *S. diastatochromogenes* and *Saccharomyces cerevisiae*.

Suitable cutinases can be selected from wild-types or variants of cutinases endogenous to strains of *Aspergillus*, in particular *Aspergillus oryzae*, a strain of *Alternaria*, in particular *Alternaria brassiciola*, a strain of *Fusarium*, in particular *Fusarium solani*, *Fusarium solani pisi*, *Fusarium oxysporum*, *Fusarium oxysporum cepa*, *Fusarium roseum culmorum*, or *Fusarium roseum sambucium*, a strain of *Helminthosporium*, in particular *Helminthosporium sativum*, a strain of *Humicola*, in particular *Humicola insolens*, a strain of *Pseudomonas*, in particular *Pseudomonas mendocina*, or *Pseudomonas putida*, a strain of *Rhizoctonia*, in particular *Rhizoctonia solani*, a strain of *Streptomyces*, in particular *Streptomyces scabies*, a strain of *Coprinopsis*, in particular *Coprinopsis cinerea*, a strain of *Thermobifida*, in particular *Thermobifida fusca*, a strain of *Magnaporthe*, in particular *Magnaporthe grisea*, or a strain of *Ulocladium*, in particular *Ulocladium consortiale*.

In a preferred embodiment, the cutinase is selected from variants of the *Pseudomonas mendocina* cutinase described in WO 2003/076580 (Genencor), such as the variant with three substitutions at I178M, F180V, and S205G.

In another preferred embodiment, the cutinase is a wild-type or variant of the six cutinases endogenous to *Coprinopsis cinerea* described in H. Kontkanen et al, *App. Environ. Microbiology*, 2009, p2148-2157

In another preferred embodiment, the cutinase is a wild-type or variant of the two cutinases endogenous to *Trichoderma reesei* described in WO2009007510 (VTT).

In a most preferred embodiment the cutinase is derived from a strain of *Humicola insolens*, in particular the strain *Humicola insolens* DSM 1800. *Humicola insolens* cutinase is described in WO 96/13580 which is hereby incorporated by reference. The cutinase may be a variant, such as one of the variants disclosed in WO 00/34450 and WO 01/92502.

- 5 Preferred cutinase variants include variants listed in Example 2 of WO 01/92502. Preferred commercial cutinases include Novozym 51032 (available from Novozymes, Bagsvaerd, Denmark).

Suitable sterol esterases may be derived from a strain of *Ophiostoma*, for example

- 10 *Ophiostoma piceae*, a strain of *Pseudomonas*, for example *Pseudomonas aeruginosa*, or a strain of *Melanocarpus*, for example *Melanocarpus albomyces*.

In a most preferred embodiment the sterol esterase is the *Melanocarpus albomyces* sterol esterase described in H. Kontkanen et al, *Enzyme Microb Technol.*, 39, (2006), 265-273.

15

Suitable wax-ester hydrolases may be derived from *Simmondsia chinensis*.

The lipid esterase is preferably selected from lipase enzyme in E.C. class 3.1.1.1 or 3.1.1.3 or a combination thereof, most preferably E.C.3.1.1.3.

- 20 Examples of EC 3.1.1.3 lipases include those described in WIPO publications WO 00/60063, WO 99/42566, WO 02/062973, WO 97/04078, WO 97/04079 and US 5,869,438. Preferred lipases are produced by *Absidia reflexa*, *Absidia corymbifera*, *Rhizomucor miehei*, *Rhizopus delemans* *Aspergillus niger*, *Aspergillus tubigensis*, *Fusarium oxysporum*, *Fusarium heterosporum*, *Aspergillus oryzae*, *Penicillium camembertii*, *Aspergillus foetidus*, *Aspergillus*
25 *niger*, *Thermomyces lanuginosus* (synonym: *Humicola lanuginosa*) and *Landerina penisapora*, particularly *Thermomyces lanuginosus*. Certain preferred lipases are supplied by Novozymes under the tradenames. Lipolase®, Lipolase Ultra®, Lipoprime®, Lipoclean® and Lipex® (registered tradenames of Novozymes) and LIPASE P "AMANO®" available from Areario Pharmaceutical Co. Ltd., Nagoya, Japan, AMANO-CES®, commercially
30 available from Toyo Jozo Co., Tagata, Japan; and further *Chromobacter viscosum* lipases from Amersham Pharmacia Biotech., Piscataway, New Jersey, U.S.A. and Diosynth Co., Netherlands, and other lipases such as *Pseudomonas gladioli*. Additional useful lipases are described in WIPO publications WO 02062973, WO 2004/101759, WO 2004/101760 and WO 2004/101763. In one embodiment, suitable lipases include the "first cycle lipases"
35 described in WO 00/60063 and U.S. Patent 6,939,702 B1, preferably a variant of SEQ ID No.

2, more preferably a variant of SEQ ID No. 2 having at least 90% homology to SEQ ID No. 2 comprising a substitution of an electrically neutral or negatively charged amino acid with R or K at any of positions 3, 224, 229, 231 and 233, with a most preferred variant comprising T23 IR and N233R mutations, such most preferred variant being sold under the tradename

5 Lipex® (Novozymes).

The aforementioned lipases can be used in combination (any mixture of lipases can be used). Suitable lipases can be purchased from Novozymes, Bagsvaerd, Denmark; Areario Pharmaceutical Co. Ltd., Nagoya, Japan; Toyo Jozo Co., Tagata, Japan; Amersham
10 Pharmacia Biotech., Piscataway, New Jersey, U.S.A; Diosynth Co., Oss, Netherlands and/or made in accordance with the examples contained herein.

Lipid esterase with reduced potential for odor generation and a good relative performance, are particularly preferred, as described in WO 2007/087243. These include lipoclean ®
15 (Novozyme).

Fat solubilisation

The solvents used in the fat solubilisation are preferably selected from aromatic, alcohols, alkanes, ethers, CO₂, chlorinated organic solvent. In some case it may be necessary to
20 gentle heat the solvent to 40°C to solubilise the fat. Preferably the solvent is toluene. Preferably the solvent does not dissolve the surface on which the fat is placed.

Preferably the fat is separated from the bulk of any water present before solubilisation.

MALDI Mass Spectrometer

MALDI (matrix-assisted laser desorption ionization) Mass Spectrometry is discussed in *Electrospray and MALDI Mass Spectrometry: Fundamentals, Instrumentation, Practicalities, and Biological Applications: Fundamentals, Instrumentation, and Applications* (Cole, R.B. ed, Wiley Blackwell 2010).

30 MALDI Mass Spectrometry involves placing a drop of the fat solution onto a metal sample plate, preferably stainless steel, and letting the solvent evaporate. A matrix chemical may be added to the solution before evaporation, separately deposited onto the sample, or no matrix chemical maybe added. The sample is placed into the spectrometer where it is irradiated
35 with a pulsed laser causing ablation and desorption of the sample and matrix material. The

analyte molecules are ionized in the hot plume of ablated gases, and are then analysed by a mass spectrometer. Preferably no matrix material is used

In the current description MALDI includes laser desorption ionization (LDI) and surface
5 assisted laser desorption ionization (SALDI).

For MALDI the matrix materials include 2,5-dihydroxybenzoic acid. Dithranol, 5-ethyl-2-mercaptothiazole, and α -cyano-4-hydroxycinnamic acid (CHCA) preferably with crystal sizes of 5 to 20 microns after spray deposition.

10 Any mass spectrometer may be used. Suitable detectors are preferably selected from Fourier Transform Ion Cyclotron Resonance Mass Analyzers, Triple quadrupole (QqQ) mass analyzers and Quadrupole-Time of Flights analyzers (Q-Tof) and Time of flight analyzers (Tof).

15 MALDI Mass Spectrometer may be purchased from providers such as: Bruker, Shimadzu, Waters

Examples of suitable spectrometers are:

Bruker autoflex maX; Shimadzu MALDI-8020; Waters MALDI SYNAPT G2-Si Mass

20 Spectrometer.

Mass Spectral Analysis

The mass spectra acquired should be analysed for, triglycerides and diglycerides. These are typically present as the Na salt observed in positive mode.

25

MALDI analysis of animal fats is described in G. Picariello, R Sacchi and F Addeo, *Eur. J. Lipid Sci. Technol.* **109** (2007) 511-524.

The level of triglyceride can be measured relative to a known standard of different mass to
30 the material under test. Preferably an internal standard is used where a known concentration of a di or triglyceride is added to the solvent used to dissolve the fat. The added triglyceride standard should preferably have a mass which does not significantly overlap with triglycerides of the fat. For fats which are predominately based on C16 and C18 fatty acids an internal standard triglyceride with ACN:DB of C42:0 is most preferred. For other fats an
35 internal standard triglyceride with ACN:DB of C60:0 is most preferred.

For LARD an internal standard triglyceride with ACN:DB of C42:0 is most preferred. The concentration of the fat under test is measured by comparison of the signal size to the signal size of the standard.

5 Detergent formulations

The invention will be further described with the following non-limiting examples.

Examples

COOP brand lard was purchased from the Coop (UK), stored in a domestic refrigerator and
10 used as supplied. A small portion of lard was placed in a glass test tube and the lard melted
by placing in a batch of hot water. 0.070g of the melted lard was placed in a small flat-
bottomed glass tube and allowed to solidify in a film on the bottom. 10ml of a cold aqueous
wash solution at pH=7, 24°FH with 0.5g/L sodium lauryl ether sulfate (2EO) was added, the
tube sealed and placed in an incubator set to 40°C and a shaker speed of 150rpm for 1
15 hour. After wash the sample was placed in ice for 30 minutes then the wash liquor removed
and the tube rinsed twice with 50ml of cold 24 °FH water. The experiment was done in
triplicate. The experiment was repeated with the addition of 1ppm of a lipase (lipex evity ®
ex Novozymes) to the wash liquor. The samples were left overnight (18hours) then the
residual lard in each tube dissolved in 5ml of toluene and the solution used to spot onto a
20 stainless steel MALDI plate. For each tube 6 MALDI deposition spots were made. When
the toluene had evaporated the MALDI mass spectra were measured on a Bruker Autoflex
using 100% laser power.

The mass spectra was analysed and the results shown in the table below.

25

The RA is the relative area of each glyceride peak which is calculated as:-

$$\text{RA} = \text{area of glyceride} / \text{sum of area of triglyceride (C48:1 to C54:1)}$$

The triglyceride peaks used for the sum of area of triglycerides were c48:1, c50:3, c50:2,
30 c50:1, c50:0, c51:1, c51:0, c52:4, c52:3, c52:2, c52:1, c52:0, c53:2, c53:1, c53:0, c54:6,
c54:4, c54:3, c54:2, and c54:1

95% confidence limits were calculated from the repeats.

35 The results are shown in the table below.

The SLES + lipase increases the concentration of DG34:1 but not DG35:0, indicating the lipase does not hydrolyse all the triglycerides at equal rates. The distribution of the triglycerides changes slightly with a relative increase in the triglycerides with an even ACN compared to odd ACN.

5

ACN:DB	SLES		SLES + lipase	
	RA	95%	RA	95%
DG34:1	0.027	0.005	0.151	0.008
DG35:0	0.046	0.009	0.043	0.005
c48:1	0.018	0.002	0.016	0.002
c50:3	0.013	0.001	0.015	0.001
c50:2	0.047	0.001	0.050	0.001
c50:1	0.072	0.004	0.071	0.003
c50:0	0.027	0.002	0.024	0.001
c51:1	0.036	0.011	0.016	0.002
c51:0	0.020	0.002	0.015	0.002
c52:4	0.019	0.001	0.020	0.001
c52:3	0.085	0.004	0.091	0.003
c52:2	0.250	0.005	0.265	0.005
c52:1	0.146	0.008	0.148	0.004
c52:0	0.038	0.002	0.034	0.001
c53:2	0.024	0.002	0.020	0.001
c53:1	0.044	0.004	0.038	0.003
c53:0	0.025	0.002	0.024	0.002
c54:6	0.012	0.001	0.011	0.001
c54:4	0.021	0.002	0.025	0.001
c54:3	0.044	0.002	0.051	0.002
c54:2	0.041	0.001	0.047	0.002
c54:1	0.020	0.002	0.019	0.002

SLES is Lauryl ether sulfate with 2 moles of ethoxylation

CLAIMS

1. A method of chemically identifying the di- and tri-glyceride composition of a fat stain on a surface, said method comprising:-
 - a) stain surface with fat stain;
 - 5 b) treat fat stain by washing with an aqueous solution of a laundry detergent composition dosed at a 1 to 20g/L, wherein the laundry detergent composition comprises surfactant, preferably at levels of from 1 to 50 wt.%; then remove aqueous solution and optionally rinse the surface;
 - c) optionally dry the residual stain in air;
 - 10 d) solubilise the residual fat from the surface using an organic solvent;
 - e) measure the di- and tri-glyceride content of the residual fat using MALDI (matrix-assisted laser desorption ionization) mass spectrometry.
2. A method according to claim 1, wherein the surface in step a) is selected from
15 textiles and clothing.
3. A method according to claim 1 or claim 2, wherein the stained surface is stored for from 0 to 400 hours, preferably at a temperature below 313K, more preferably 253 to 303K, most preferably 283 to 298K.
20
4. A method according to any preceding claim, wherein the solubilisation step of method step d) has a solvent to fat weight ratio of 10:1 to 5000:1, more preferably 20:1 to 100:1.
- 25 5. A method according to any preceding claim, wherein the fat is selected from lard, butter, ghee, goose fat, duck fat, chicken fat, olive oil, suet, palm oil, sunflower oil, rapeseed oil, nut oils, soya oil, fish oil, cocoa butter, shea butter, sebum, borneo tallow, Illipe butter, sesame oil, safflower oil, linseed oil, poppy oil, pumpkin seed oil, wheat germ oil, corn germ oil.
30
6. A method according to any preceding claim, wherein the fat is a solid at 30°C, most preferably at 37 °C.
7. A method according to any preceding claim, wherein the di- and tri-glycerides are
35 analysed in positive mode in the MALDI spectrometer.

8. A method according to any preceding claim, wherein the laundry detergent composition comprises an anionic surfactant.

5 9. A method according to any preceding claim wherein an internal standard is used where a known concentration of a di or triglyceride is added to the solvent used to dissolve the fat.

10

15

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/074398

A. CLASSIFICATION OF SUBJECT MATTER		
INV. C11D11/00 H01J49/00	C11D3/20	C11D7/26 C11D7/50 C11D3/43
ADD.		
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) C11D H01J		
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 practicable, search terms used) EPO-Internal, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 2 325 649 A1 (CENTRE NAT RECH SCIENT [FR]; UNIV LILLE SCIENCES TECH [FR]) 25 May 2011 (2011-05-25) paragraphs [0028], [0051], [0060], [0062]; claims 1, 9 -----	1-9
A	US 2018/073046 A1 (BOERIU CARMEN GABRIELA [NL] ET AL) 15 March 2018 (2018-03-15) paragraphs [0049], [0074], [0091] - [0115]; figures 2, 5, 6 -----	1-9
A	US 2010/311897 A1 (WILLIAMS NEAL ST JOHN [GB] ET AL) 9 December 2010 (2010-12-09) paragraph [0117] - paragraph [0121] ----- -/-	1-9
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "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 "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 7 November 2019		Date of mailing of the international search report 18/11/2019
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Cetinkaya, Murat

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/074398

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JONATAN HALL-ANDERSEN ET AL: "MALDI imaging of enzymatic degradation of glycerides by lipase on textile surface", CHEMISTRY AND PHYSICS OF LIPIDS., vol. 211, 6 November 2017 (2017-11-06), pages 100-106, XP055640124, IR ISSN: 0009-3084, DOI: 10.1016/j.chemphyslip.2017.11.004 2.2 Textile staining and wash; page 101 2.4 MALDI-MSI; page 101 - page 102 3.1 MALDI imaging of lipids on textile surfaces; page 102 - page 104; figures 1-3; table 1 -----	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 2325649	A1	25-05-2011	NONE

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			US 2018073046 A1 15-03-2018
			WO 2016151115 A1 29-09-2016

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			BR PI0819500 A2 26-05-2015
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			ZA 201005081 B 28-04-2011
