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(54) PROCESS FOR THE SELECTIVE PREPARATION OF DERIVATIVES OF MONOSACCHARIDES AND POLYOLS WHICH ARE PARTIALLY ACYLATED

(57) The process refers to highly selective enzymatic production of monosaccharides and mono- polyols and/or disubstitued with fatty acids. The procedure is based on the simple mixture of polyol or monosaccharide with the fatty acid, the addition of solvent, and the use of lipases that permit the highly selective obtainment of these products without catalysts or toxic agents. The water produced during the process must be continuously removed throughout the same. The invention belongs to the alimentary, pharmaceutical, cosmetic and chemical sectors. The products obtained are excellent biodegradable and biocompatible non-ionic tensioactive agents which are used as additives for their emulsifying, oily, thickening and moisturising properties.

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Description

Sector of the technique

[0001] The invention belongs to the alimentary, pharmaceutical, cosmetic and chemical sectors. The products obtained arc excellent biodegradable and biocompatible non-ionic tensioactive agents which are used as additives for their emulsifying, oily, thickening and moisturising properties.

State of the technique

[0002] The employment of enzymes in organic media is an attractive alternative to classical organic synthesis. The main drawbacks of conventional chemical techniques are their high energy consumption (high temperatures and pressures), the low selectivity of the processes and/or the employment of inorganic catalysts which produce coloured impurities of variable toxicity. In contrast, enzymatic catalysts can achieve selective synthesis of a given product under less extreme pressures and temperatures and are less environmentally toxic.

[0003] Lipases are the hydrolytic enzymes most effective at catalytic processes involving long chain fatty acids. These form covalent bonds with alcohols in media with a low degree of hydration. Because of their versatility and wide availability these make very valuable industrial biocatalysts. Besides, lipases are biodegradable. When several hydroxyl groups are present in the same molecule, because of their extreme selectivity lipases can replace long synthetic pathways (with phases of protection /exposure) with one enzymatic step. This increases yields and reduces the cost of the process itself and also that associated with treatment of the waste products [Biocatalysts for industry (1991) Plenum Press, N.Y. Ed. J. Dordick] compared to alternative chemical processes. Moreover, the chemo- and enantioselectivity of lipases can give rise to highly competitive synthetic processes.

[0004] More than 80% of the world market of tensioactive agents are molecules formed by condensation between polyols or monosaccharides and long chain fatty acids (glycerides, spanes, tweenes etc.). Esterified sorbitan derivatives with long chain fatty acids are biocompatible glycolipid surfactants commonly used in a variety of industries. The commercial name for esterified sorbitan derivatives is Spanes. These are highly lipophilic and are precursors of Tweenes. At present, Tweenes and Spanes are prepared industrially by non-enzymatic procedures at 180-150ºC. Gluco and glycolipidic surfactants are additives used in cosmetic and pharmaceutical products for their different properties (moisturizing and lubricative capacities etc.). The employment of many of these emulsifiers, which are themselves biocompatible, for certain applications (e.g. in the alimentary sector) is often problematic. These problems are related with the conventional chemical method used in their preparation. The presence of certain synthetic agents in the chemical processes used to manufacture or purify the additive constitute one of the main drawbacks of the preparation methods used. Selective obtainment of emulsifiers by enzymes can solve this problem. European and American legislation consider substances produced or modified by enzymes as natural products.

[0005] The enzymatic production of glucolipidic tensioagents derived from monosaccharides and polyols has been Studied in the literature although only low yields were obtained [Khaled, N., Montet, D. Pina, M. and Graille, J. Biotech. Let., 13, 167-172 (1991) and Schlotterbeck, A. Lang, S., Wray, v. and Wagner, F. Biotechnol. Lett. 15, 61-64 (1993)] and/or these require preliminary non-enzymatic steps [Fregapane, G., Sarney, D.B. and Vulfson, E.N. Enz. Microb. Technol.13, 796-800 (1991) and Bjorklong, F., Godtfredsen, S.E. and Kirk, O.J.Am. Soc. chem. Commun. 934-935 (1989)]. One enzymatic method based on an aqueous mixture of reagents at 20-60ºC [offenlengugsschrift DE-B3430 944 (SEINO)] and the later improved version of the process using high pressure water evaporation [Japanese patent J62195-292-A] were patented in Germany and Japan respectively. In both cases the yields obtained are relatively low due to displacement of the thermodynamic equilibrium of the aqueous process towards hydrolysis. The sugars recommended in these patents are glucose, saccharose, raffinose, dextrin, mannan, cellulose, sorbitol and xylitol. Yields have been increased using almost anhydrous organic media and increasing the solubility of the starting sugar in the medium by previous chemical modification. This was also the aim of a number of international patents. The Japanese patent from 1988 [Japanese patent published on the 18th of May 1988, nº63-112993] includes a preliminary step that consists of acetylating the sugar and the European patent published in 1989 [European patent published on the 27th of September, 1989, publication nº. 0334 498 A1; application nº. 89301994.3] alkylates the glucoside before carrying out the enzymatic acylation. These patents all include a previous non-enzymatic step to decrease the polarity of the substrate followed by enzymatic processes with high yields. However, the tensioactive molecules obtained are more complex than those obtained with the previous methods.

Brief Description of the Invention

[0006] This invention consists of an enzymatic procedure of selective synthesis of partially acylated polyol and monosaccharide esters with fatty acids in almost anhydrous media. Quantitative or almost quantitative yields are obtained in only one step.

Detailed description of the invention

[0007] The invention refers to the highly selective enzymatic production of monosaccharides and mono polyols and/or disubstituted with fatty acids. The procedure is based on a simple mixture of polyol or monosaccharide with the fatty acid, the addition of solvent and the use of lipases that permit the highly selective obtainment of these products without catalysts or toxic agents. The water produced during the process must be continually removed throughout the process.

[0008] The tensioactive agents synthesized present a higher biodegradability than ethoxylated alkyl phenols commonly used in washing powders etc.

[0009] Moreover, these enzymatic reactions permit the recovery and reassessment of the waste products. Lipases can act with pure fatty acids and also with mixtures of these derived from natural oils and fats (olive, coconut or palm oil etc.) The European Union produces large surpluses of oils and fats. Minimization of the waste products and the recovery of by-products (oils and fats) and their industrial reassessment and recycling is one of the priority objectives in Europe and elsewhere.

[0010] The chemical procedures currently used for the industrial preparation of these products involve high energy consumption and the corresponding installations for conditions of high pressure and temperature. Because of the low selectivity of these chemical procedures [American patent: n° 2 759 922], unlike enzymatic processes these chemical procedures can not obtain products with a specific and constant structure. Previously patented enzymatic procedures [Offenlengugsschrift DE-B3439 944 (SEINO) and Japanese patent J62195-292-A] which use aqueous medium, achieve very low yields, limiting their industrial use. The present enzymatic process can achieve almost quantitative yields by direct esterification of the polyol and/or monosaccharide in an almost anhydrous organic medium. Our invention can directly use the polyol or sugar without the prior chemical modification described in previous patents [Japanese patent published on the 18th May 1988, nº63-112993] and the [European patent published the 27th of September, 1989, publication nº 0334 498 A1; application nº. 89301994.3] which obtain different final products than those obtained here. In both these procedures the previous non-enzymatic step and also the possible use of solvents with a high boiling point make the patented processes more expensive than the present procedure. The present method preferentially uses cheaper solvents of easy elimination and low toxicity.

[0011] The invention is applicable to linear polyols such as sorbitol. Also, monosaccharides such as glucose, fructose, mannose etc. can be selectively transformed. The reaction with galactose is less selective and results in a greater mixture of mono and diesters.

[0012] With the patented procedure, different fatty acids of variable chain length, preferably from C8 to C22 can be used. The acid chain can be saturated or unsaturated, linear or branched (i.e. octanoic acid, hexadecanoic acid, oleic acid etc.). Also, a mixture of fatty acids can be used (i.e. industrial acids from coconut, soya, corn, cotton seeds, etc.). Direct use of ethylic esters of fatty acids is much less effective in these processes.

[0013] High temperatures are not required although they can be used in some cases. Milder temperatures (0-60ºC) are generally preferred.

[0014] The process can be catalyzed by lipolytic enzymes of yeast or animal, vegetable, bacterial or fungal origin. This can be in a native form, immobilized in an inert support and/or modified chemically or can be obtained by genetic engineering. Thus, high yields have been obtained with immobilized lipases of *Candida antartica* (Novozim 435) and *Mucor miehie* (Lipazyme IM) prepared by Novo Industria A/S, with Novozim 435 proving very effective.

[0015] Selective esterification of primary hydroxyls is produced and the secondary hydroxyls remain unaltered.

[0016] The process can be carried out in different solvents or relatively polar organic media in order to, at least partially, solubilize the polyol (or the sugar), preferentially ones with a low boiling point to facilitate their elimination.

[0017] The solvent and the reagents should have a low water content, preferentially below 5% w/w and if possible in the range of 0.01 to 1% w/w.

[0018] The fatty acid content should preferentially be above 100 mM and have a molar excess of 1.5 times that of the sugar.

[0019] The process should be carried out in a continuously agitated tank. The amount of enzyme required depends on the specific activity of the enzyme preparation used.

[0020] The invention will be specifically described hereinafter by the following examples:

Example 1

Synthesis of glucose 6-monolaurate

Example A

[0021] 2 ml of acetone were added to the reactor which contained 100 mg of Novozim 435, 60 mg of glucose and 200 mg of lauric acid. The reaction was carried out under mild continual agitation at 40ºC and water was continually removed from the reaction medium. 121 mg of glucose 6-monolaurate were obtained after 3 days of reaction corresponding to a yield of 99%.

Example B

[0022] 8g of Novozim 435, 5g of glucose and 16.7g of

lauric acid were added to 160 ml of acetone. After 5 days of mild agitation at 40º with constant removal of water from the reaction mixture, 9.6g of glucose 6-monolaurate (98% yield) were obtained.

Example C

[0023] 80 g of Novozim 435,50 g of glucose and 167g of lauric acid were added to 1.61 of acetone. After 6 days of mild agitation at 40ºC and with continuous removal of water from the reaction medium 90 g of glucose 6-mono-laurate were obtained (90% yield).

Example 2

Reaction between sorbitol and the fatty acids derived from hydrolysis of olive oil

[0024] 60 mg of sorbitol, 480 mg of a mixture of fatty acids and 100 mg of Novozim 435 were added to 2 ml of acetone. The reaction was carried out under continuous mild agitation and water was continually removed from the medium that was maintained at 40ºC for 6h and later at 10ºC. 234 mg of 1,6 sorbitol diester and 8 mg of monoester corresponding to yields of 94% and 2% respectively were obtained.

Example 3

Reaction between mannose and lauric acid

[0025] This reaction used 60 mg of mannose and 200 mg of lauric acid in 2 ml of acetone in the presence of 100 mg of Novozim 435, the reaction mixture was maintained at 60ºC and under continuous mild agitation and water was continually removed from the reaction mixture. 107 mg of monoester and 18 mg of diester were obtained after 15 hours. The yields were 90% and 10%, respectively.

Example 4

Reaction between sorbitol and lauric acid

[0026] 100 mg of sorbitol, 330 mg of lauric acid and 100 mg of Novozim 435 were added to 2 ml of acetonitrile. After 4 days of reaction at 40ºC with the continual removal of water from the reaction mixture a quantitative conversion of sorbitol was obtained in the 1,6-diester (302 mg, 100% yield).

Claims

1. **PROCEDURE FOR THE SELECTIVE PREPARATION OF PARTIALLY ACYLATED MONOSACCHARIDE AND POLYOL DERIVATIVES** characterized by the mixture of polyol or monosaccharide with the fatty acid, addition of a solvent, employment of lipases, the water produced during the process being removed during the process.

2. A procedure according to claim 1 characterized by the lipase being *Candida antartica* or *Mucor miehie.*

3. A procedure according to claim 1 characterized by the employment of a reaction medium whose water content is controlled in the range of 0.001 and 5% w/w.

4. A procedure according to claims 1 and 3 characterized because it is performed between 0 and 60ºC.

5. A procedure according to claims 1,3 and 4 characterized in that the concentration of fatty acids exceeds 100 mM and are in a molar excess of at least 1.5 fold the concentration of the sugar.

6. A procedure according to claims 1,3,4, and 5 characterized by the employment of different fatty acids of variable chain length between C8 and C22 in which the acid chain can be saturated or unsaturated, linear or branched. The procedure can use a combination of fatty acids from different sources.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/ES 97/00240

A. CLASSIFICATION OF SUBJECT MATTER

IPC[6] C12P7/62, C12P19/44

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[6] C12P, C07C

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)

CIBEPAT, EPODOC, WPIL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0506159 A (UNICHEMA CHEMIE BV) 30 September 1992 (30.09.92), Column 1, Lines 29-49; Column 2, Lines 32-58; Column 3, Lines 1-39; Column 5, Example II; Claims	1-4,6
X	EP 0274798 A (UNILEVER NV) 20 July 1988 (20.07.88), Page 2, Lines 16-37	1,2,4,6
X	Data base WPIL en Questel, week 9210, London, Derwent Publications Ltd., AN 87-280944 & JP 62195292 A (DAIICHI KOGYO SEIYAKU), 28 August 1978 (28.08.78), Abstract	1,2,3,6
X	Data base WPIL en Questel, week 8805, London, Derwent Publications Ltd., AN 88-031288 & JP 62289190 A (DAICHI KOGYO SEIYAKU CO. LTD.) 16 December 1987 (16.12.87), Abstract	1,3,4,6

☐ 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 document but published on or after the international filing date

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"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	Date of mailing of the international search report
10 December 1997 (10.12.97)	15 December 1997 (15.12.97)
Name and mailing address of the ISA/ S.P.T.O Facsimile No.	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/ ES 97/00240

Patent document cited in search report	Publication date	Patent familiy member(s)	Publication date
EP 0506159 A	30.09.92	CA 2062935 A JP 5115288 A	20.09.88 14.05.93
EP 0274798 A	20.07.88	DK 671387 A US 4956286 A JP 631923393 A	20.06.88 11.09.90 09.08.88
JP 62195292 A	20.08.87	NONE	
JP 62289190 A	16.12.87	NONE	

Form PCT/ISA/210 (patent family annex) (July 1992)