Title: EXTRACTION METHOD OF A POLYESTER POLYMER OR CUTIN FROM THE WASTED TOMATO PEELS AND POLYESTER POLYMER SO EXTRACTED

Abstract: A method for extraction of a polyester polymer composed of a complex mixture of interesterified, long-chain alpha-hydroxy acid with typically a 16- or 18-carbon skeleton from the waste of tomato peels. The method provides a thermal treatment of the tomatoes peels, in which tomato peels are immerged in an alkaline solution; a filtration phase, for separating solid residue by liquid; then the liquid is kept for a step of acidification adding an inorganic acid until the solution changes its color so that the pH of the solution after the color changing is comprised in the interval 5-6. Follow a centrifugation phase, in which the solution is centrifuged at a range of 10000-14000 rpm for 15-20 minutes; after the centrifugation the supernatant is discarded or reintroduced in the process for another extraction, while the solid residue is kept and centrifuged with water DDW from 1 to 3 times in the same conditions; these further steps having the scope of washed/cleaned the solid residue.

[Continued on next page]
Declarations under Rule 4.17:

— as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))

Published:

— with international search report (Art. 21(3))
**TITLE:** EXTRACTION METHOD OF A POLYESTER POLYMER OR CUTIN FROM THE WASTED TOMATO PEELS AND POLYESTER POLYMER SO EXTRACTED

**FIELD OF THE INVENTION**

The invention relates to the development of a method for the valorization of industrial processing tomato by-products (skins), which consists in the extraction of a polyester polymer composed of a complex mixture of interesterified, long-chain ω-hydroxy acid with typically a 16- or 18-carbon skeleton, named cutin, from the waste of tomato peels.

In particular the invention relates to a method for producing monomers and oligomers of tomato cutin, extracted from tomato peels, whose main component is 10,16-dihydroxyhexadecanoic acid.

**STATE OF THE ART**

Cutin is a support biopolyester involved in waterproofing the leaves and fruits of higher plants. Cutin is the main component (between 40% and 85%, w/w) of the plant cuticle, the continuous and lipidic extracellular membrane that covers the aerial parts of leaves, fruits and non-lignified stems of plant. Associated to cutin there are cuticular waxes or lipids soluble, they are embedded within the matrix cuticular waxes intracuticulares, or deposited on the outer surface of the cuticle waxes epicuticular. Furthermore, the cuticle also contains a number of components lipid such as polysaccharides (mainly cellulose and pectin), polypeptides and phenolic compounds. Thus, the plant cuticle can be considered a polyester waxes complex associates, very small hydrophobic
nature reactivity, since most of the carboxylic groups present in the membrane are esterified with aliphatic hydroxyl groups of other fatty acids, which covers the aerial part of leaves, fruits and non-lignified stems of plant. Considering the average weight of an isolated cuticle, cutin can be considered the major lipid plant polymer (between 40 and 85% w/w of the plant cuticle).

Cutin plays an important role in cuticle as a structural component and as a defense barrier toward pathogens and the uncontrolled loss of water, as well as in transporting substances across the plant tissues.

From a chemical point of view, cutin is defined as a polymeric network of polyhydroxylated C16 and C18 fatty acids cross-linked by ester bonds, as it is reported in A. Heredia, “Biophysical and biochemical characteristics of cutin, a plant barrier biopolymer”, Biochimica et Biophysica Acta 1620 (2003) 1-7. Most of them are exclusively fatty acids C16 belonging to the family in which the 10,16-dihidroxihexadecanoic acid, and its positional isomer-9,16-dihidroxihexadecanoic acid, are the main components for tomato cutin. Only a small fraction of the cutin are formed by fatty acids belonging to the family C18, including acids 9,10-epoxy-18-hydroxyoctadecanoic and 9,10,18-trihidroxiocatadecanoic are the most abundant, although some derivatives may be present as unsaturated cutin, as reported in G. M. Lopez “Biomecanica de la epidermis y la cutícula del fruto de tomate (solanum lycopersicum l.) y su la relacion con el agrietado” (2006), Ph. Thesis, facultad de Ciencias, Departamento de Biologia molecular y Bioquimica, Universidad de Malaga.
Cutin is generally extracted from plant material with methods using enzymatic treatments, organic solvents or acid hydrolysis, as reported in literature.

All the methods reported in literature are aimed to analyse the composition of cutin and they show only studies and characterizations of cutin, but not how to use cutin as raw materials for other preparations, that is the target of this invention.

In particular in the enzymatic method the cuticular membrane is isolated by enzymatic treatment to degrade the polysaccharides. The cuticle is then extracted (solid-liquid extraction by Soxhlet with organic solvent CHCl3 and MeOH) to remove soluble material, waxes and other small molecules, yielding cutin enriched residues, as reported in R. Jarvinen, A. J.D. Silvestre, A. M. Gil, H. Kallio, “Solid state 13CP-MAS NMR and FT-IR spectroscopic analysis of cuticular fractions of berries and suberized membranes of potato” Journal of Food Composition and Analysis 24 (2011) 334-345 and in H. Kallio, R. Nieminen, S. Tuomasjukka, M. Hakala “Cutin composition of Five Finnish berries” Journal of Agricultural and Food Chemistry 54 (2006), 457-462.

With this method, it’s possible to obtain only dewaxed peels and peels without sugars, but not cutin. In this method lacks a procedure that permits to isolate from dewaxed peels cutin. Moreover this method is solvent consuming, considering that the Soxhlet extraction utilizes organic toxic solvent as chloroform (chloroform is carcinogen) and methanol and this extraction has to
be repeated to increase the yield of extraction. So the procedure
doesn’t appear very eco-friendly.

Another organic solvent used for cutin extraction is aceton, as
for the manufacture of oligo- and polyesters from a mixture of
carboxylic acid obtained from suberin and/or cutin and use
thereof”, where cutin is isolated by a solvent extraction with
aceton in a Soxhlet apparatus and then with a system of refluxing
in a basic solution.

This method has been experimented, but the final extract
didn’t present the usual aspect of cutin. In fact the IR spectrum on
this sample didn’t show the usual aspect of the cutin spectrum.
Moreover this method is solvent and time consuming.

Finally another possibility reported in literature to extract
cutin is with an acid hydrolysis, as reported in J. J. Benitez, R.
Garcia-Segura, A. Heredia, “Plant biopolyester cutin: a tough way
to its chemical synthesis”, Biochimica et Biophysica Acta 1674
(2004) 1-3. In this method cutin samples were obtained after
hydrolysis of dewaxed cuticles in a 6 M HCl solution for 12 h at
105° C to remove polar hydrolysable components and then
depolymerized in a 3% (w/v) sodium methoxide solution for 18 h
at 100° C. After extraction of the monomers of tomato fruit cutin
in an organic phase (diethyl ether), the solvent was evaporated to
quantify and identify the cutin monomers by gas chromatography-
mass spectrometry analysis.
The above method has been experimented, but the yield was very low and the extract showed a bad ability to form a new biolacquer.

OBJECT OF THE INVENTION

The invention consists in a solubilization of waste tomato skin in alkaline solution, in fact the skin is for the most part (about 60-80%) solubilized by an alkaline solution and subjected to a thermal treatment at a temperature of 100°C for around 6 hours.

Subsequently, the raw bioresins, in solution as sodic resinates, are treated with acid to reduce pH and they are separated and cleaned of impurities, first by precipitation and then by centrifugation.

In this way a raw bioresin, partially depolimerized, is obtained in a pasty mass containing around 40% of water.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is subsequently explained in more detail with reference to exemplary embodiments in conjunction with the drawings, in which the Figure 1 shows the extraction protocol of cutin, object of said invention.

DETAILED DESCRIPTION

Cutin is extracted from waste tomato peels and precisely cutin is obtained in the extraction of a polyester polymer composed of a complex mixture of interesterified, long-chain ω-hydroxy acid with typically a 16- or 18-carbon skeleton.

Some different kinds of peels can be utilized: desiccated peels, fresh frozen peels and peels from which was extracted lycopene.
At an initial stage of the claimed extraction method from wasted tomato peels, tomato skins and seeds are separated by flotation with flow water.

Then, the claimed process provides the following macro-steps:

- Thermal treatment of the tomatoes peels
- Filtration
- Acidification
- Centrifugation
- Drying phase (eventually)

So, the first step relates to the thermal treatment; tomato peels are subjected to a thermal treatment, preferably in an autoclave device, where the tomato peels are immersed in an alkaline solution (preferably NaOH) at 3% in weight, a concentration included between 0.5 M and 6 M (preferably 0.75 M); the temperature is included between 65°C and 130°C for a time more than 15 minutes and less than 6 hours (preferably around 2 hours); the ratio weight tomato peels/V NaOH can chances from 0.1 to 10.

After the above thermal treatment phase the solution is filtered so that solid residue can be separated by liquid; in this way the solid residue can be discarded or re-introduced in the process, while the liquid filtrate is kept. This liquid solution filtered is of brown colour.

Follow the acidification phase in which said filtrated liquid solution is added with an inorganic acid, and more precisely HCl
with a concentration included between 12M and 6M, until the solution changes its colour and from brown it became ochre.

Preferably it is added around 10% (preferably 5%) HCl of the total volume and the pH of the solution after the color changing is comprises in the interval 5 - 6.

After the above acidification phase, the subsequent step is the centrifugation phase in which the solution is centrifuged at a range of 10000-14000 rpm for 15-20 minutes.

After the centrifugation the supernatant was discarded or reintroduced in the process for another extraction, but in the latter case only after the pH is being adjusted to the original value, preferably around 10.

The solid residue is kept and centrifuged with water DDW from 1 to 3 times in the same conditions; these further steps having the scope of washed/cleaned the solid residue.

In this way a raw bioresin, partially depolimerized, is obtained in a pasty mass containing around 25-80% of water.

A final stage could be applied: said final phase consisting of a drying phase for evaporating the water contained in the pasty mass.

By means of GC-MS analysis, the yield in 10,16 - dihydroxyhexadecanoic acid is included between 60-80%.

The yield of extraction has been included between 10 and 30% (around 15%).

To determine the percentage of water present in the extracted raw cutin it’s necessary to heat the weighted sample of raw cutin (1 g) at 100°C for 4 hours in a specific sample pan,
previously treated at 200°C for 30 minutes (passivation's treatment). The percentage of solid residue is calculated from the weight's difference between the initial weight of sample and the weight of sample after the thermal treatment.
CLAIMS

1. Method for extraction of a polyester polymer composed of a complex mixture of interesterified, long-chain ω-hydroxy acid with typically a 16- or 18-carbon skeleton from the waste of tomato peels, characterized in that said method comprising the step of:
   a. Thermal treatment of the tomatoes peels, in which tomato peels are immerged in an alkaline solution,
   b. Filtration, for separating solid residue by liquid; then the filtrated liquid is kept for a step of
   c. Acidification adding an inorganic acid until the solution changes its color so that the pH of the solution after the color changing is comprises in the interval 5 - 6
   d. Centrifugation phase, in which the solution is centrifuged at a range of 10000-14000 rpm for 15-20 minutes; after the centrifugation the supernatant is discarded or reintroduced in the process for another extraction, while the solid residue is kept and centrifuged with water DDW from 1 to 3 times in the same conditions; these further steps having the scope of washed/cleaned the solid residue.

2. Method according to claim 1, characterized in that said thermal treatment in alkaline solution occurs with NaOH at 3% in weight, a concentration included between 0.5 M and 6 M and at a temperature included between 65°C and 130°C for a time more than 15 minutes and less than 6 hours.
3. Method according to claim 2, characterized in that said thermal treatment in alkaline solution provides a concentration of 0.75 M and at a temperature included between 65°C and 130°C for around 2 hours.

4. Method according to claim 1, characterized in that after filtration phase solid residue is re-introduced in the process.

5. Method according to claim 1, characterized in that said inorganic acid is Hydrogen chloride (HCl) with a concentration included between 12M and 6M, preferably 5% HCl of the total volume.

6. Method according to claim 1, characterized in that after centrifugation phase a supernatant is obtained and reintroduced in the process for another thermal treatment; before of this the PH of the supernatant is adjusted at the original value, around 10.

7. Method according to claim 1, characterized in that a final stage could be applied; said phase consisting of a drying phase of the pasty mass obtained with the method.

8. A raw bioresin, partially depolimerized, obtained in a pasty mass containing around 25-80% of water according the method of claim 1.

9. A raw bioresin according to claim 8 wherein monomers and oligomers of tomato cutin, whose the main component is 10,16 - dihydroxyhexadecanoic acid with a monomer yield included between 60 and 80%, are obtained.

10. A raw bioresin according to claim 8 wherein the dominant fraction of monomers and oligomers of tomato
cutin obtained has a relative molecular weight of 650 g/mol (GPC based on calibration with polystyrene standards). This dominant fraction corresponds to a dimer or trimer taking into consideration that the major monomer of cutin (10,16 dihydroxyhexadecanoic acid) has molecular weight 284g/mol.
FIG. 1

Tomato peels in NaOH

Thermal treatment

Filtration: sieves

Solution: Cutin extract (brown colour)

Discard: solid (peels)

Acidification:
Addition of HCl

Centrifugation:
Super-centrifuge and decanter

Adjusting the pH to 10

Solid residue

Discard: liquid solution (pH 5-6)

Washings of the solid residue
Addition of water DDW to the solid residue precipitated and centrifugation

To repeat from 1 to 3 times

RAW CUTIN
## INTERNATIONAL SEARCH REPORT

**International application No**

PCT/EP2014/067187

### A. CLASSIFICATION OF SUBJECT MATTER

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**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)

C08J  C11B  A61K  C07C  C08G

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, COMPENDEX, INSPEC, IBM-TDB, WPI Data

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C.

See patent family annex.

### Date of the actual completion of the international search

5 September 2014

### Date of mailing of the international search report

17/09/2014

### 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

West, Nuki

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