Cutin isolated from tomato processing by-products: extraction methods and characterization.

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Keywords: tomato processing by-products, cutin, saponification, FT-IR spectroscopy.
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Currently the cultivation and processing of tomatoes is the largest agricultural business in Southern European countries. Tomato processing by-products represent 5-13% of the whole tomato production [1]. At the present time, tomato industrial solid waste, in particular tomato peels, is currently disposed in the zoo-technical sector for animal feed or, once it is dried, as the substrate for the production of fertilizer and lately for the production of biogas. Numerous studies have looked at the extraction of food additives or of ingredients for cosmetics (lycopene, carotenoids, essential oils, dietary fibres, etc.).
This study regards as promising aim the tomato cuticle valorization, for the extraction of added-value products, not commercial available, such as 10, 16- dihydroxyhexadecanoic acid (and its olygomers), for the formulation of a lacquer to use as internal protective coating for metal food packaging.
This study investigates the possibility of extracting cutin from tomato peels using a green chemistry protocols devoid organic solvent. In particular, three different cutin extraction methods are compared, in order to enhance the purity and the reproducibility of the extracted products.
The first studied method consists of a saponification reaction [2], the second depth procedure is a hydrogen peroxide-assisted alkaline hydrolysis reaction ($NaOH/H_2O_2$ route), while the last method involves hydrocloridric acid free-selective precipitation of sodium carboxylate.
Cutin has been successfully isolated from tomato peels using all proposed methods. Indeed the GC-MS analysis shows that the main monomeric unit of cutin extracted is, whatever the followed route, the 10,16 – dihydroxyhexadecanoic acid, the principal component of tomato cutin [3], with a percentage between 83 and 96%. The extracted products, by different routes, are different for apperance, solubility, crosslinking and molecular weight, as shown by GPC analysis. In addition, the distinctive reactivity of the cutin samples was studied by a real-time homopolymerization monitoring. Besides, the thermogravimetric study (TGA) has been carried out.
Although the products obtained by $NaOH/H_2O_2$ and carboxylate route, have higher purity, as suggested by FTIR analysis; preliminary tests have shown that the gummy mass obtained by simple alkaline hydrolysis, is the best raw material for the bio-resin preparation.

1.Introduction

The total tomato production in the EU-28 was estimated in about 14 million tonnes in 2013 [4], while in all Europe it was estimated about 27 million metric tons. Tomato processing by-products represent 5-13% of the whole tomato production according Ventura et al. [1]. For instance, each year in the EU-27 more than 200000 tons of solid tomato residues (peels and seeds) are produced to generate a variety of tomato products including peeled tomatoes, tomato purée, crushed tomato, tomato concentrate, tomato juice [5]. At the present time, tomato industrial solid waste, in particular tomato peels, is currently disposed or used partly in the zoo-technical sector for animal feed or, once it is dried, as the substrate for the production of fertilizer and lately for the production of biogas. Anyway such disposal has a cost estimated at a current value of 0.04 euro for Kg due to transport. However, tomato peels are a cheap and abundant raw material from which it is possible to obtain very interesting chemicals with high commercial value. In addition, in recent years, an efficient management and reuse of agro-industrial wastes have become a priority [6]. The food industry produces large volumes of by-products resulting from the production, preparation, and consumption of food. These by-products pose increasing disposal and potentially severe pollution problems and represent a loss of valuable biomass and nutrients. Over the last few years, new methods and policies for by-products handling and treatment have been introduced in the recovery, bioconversion, and utilization of valuable constituents from food processing by-products.
The valorization of agro-industrial waste is a key technology that contributes to the development of sustainable processes [7], to the generation of value-added products and to the realization of a circular economy. In fact, the circular economies represent one of the most promising goal in terms of environmental sustainability with measures covering the whole cycle: from production and consumption to waste management and the market for secondary raw materials. In this direction also the European Union moves from 2014 with EU Action Plan for the Circular Economy.
The tomato peel tissue, which is a highly structured material, can be considered a polyester waxes complex associates, with a very small hydrophobic nature reactivity, containing cutin, cuticular waxes or lipids soluble, polysaccharides (mainly cellulose and pectin), polypeptides, phenolic compounds, lycopene and several minor carotenoids, ash, minerals (the major elements are K, Ca, Mg and Na), fatty acids. The cuticle is the external layer covering the epidermis of the aerial parts of plants. Thus, considering the average weight of an isolated cuticle (around 600 g/cm^2), cutin is the

main component (between 40% and 85%, w/w) of the plant cuticle. 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 [8]. Cutin plays an important role in cuticle as a structural component, as a defense barrier against pathogens [9], as protection against the uncontrolled loss of water together with waxes [10], as well as in transporting substances across plant tissues [11].
An interesting opportunity is to use tomato peels for the extraction of value –added products not commercial available as standardized tomato extracts, such as 10,16- dihydroxyesadecanoic acid (and its olygomers). In fact, the long chain hydroxy acids, following their peculiar reactivity, (being tri-functional compounds, with the 10,16-dihydroxyesadecanoic acid example), are very interesting starting material for many applications, such as in perfume and pharmaceutical industry and as excellent monomers for the synthesis of plastics with good adhesive properties, by the condensation with pthalic andydride and the glycerol [12], for the preparation of lacquer and fibres [13]. Even previous methods for cutin extraction exist [14,15], organic solvent-free polyhydroxy acid extraction methods and with efficient yield are very few or missing, [16], in particular using tomato by-products as raw material.
In addition, in order to realize the cutin extraction process at industrial o semi- industrial scale, and to have a commercially feasible product simple process, efficient and sustainable in terms of environmental and economic impact (inexpensive and environmentally friendly reactants) should be selected. This study was aimed to identify methods, which, without the use of organic solvents and under mild conditions, provides effective depolimerization of the involved species, the degradation of the compact ligno-cellulosic skeleton of tomato peel tissue [17], and able to obtain high purity products. Previous studies [18] showed that the action of hydrogen peroxide at pH higher than 7 is able to generate an extremely nucleophilic species, mainly hydroperoxide ion (HOO^-), and oxidizing radicals which dominate under alkaline conditions (pH=11.6). Considering the previous literature [19], we suppose that the addition of peroxide to the reaction mixture significantly enhances cell walls disruption, cutin depolimerization, and the lignin hydrolysis due to cleavage of α-aryl ether linkage of a guaiacyl-type lignin according to Dakin-like mechanism in alkaline solution [20]. Therefore, this study has the objective to make a comparison between a cutin extraction method already known [2] and, to our knowledge, innovative process able to realize, different depolymerization degrees of polyhydroxylated fatty acids cross-linked polyester network and to degrade interfering compounds, in order to find a more selective 16-dihydroxyesadecanoic acid extraction method. Cutin is first isolated by means of a hydrolysis with NaOH, studying the effect of the saponification reaction conditions on the characteristics of the product. Also a treatment with 2-4% H_2O_2 under basic condition is deepen. An alternative peroxide-free route involving cutin recovery at basic pH is proposed. Finally the products obtained are fully characterized in terms of chemical structure, solubility, thermal properties, molecular weigh.

2 Materials and methods

2.1 Samples and reagents

Tomato peels (TP) from tomato industrial processing were collected from local tomato industry in Parma, Italy. Reagents and solvents were used as received without further purification. Sodium hydroxide (NaOH, beads, VWR), Hydrochloric acid (HCl, 37%, ACS GR, , VWR), hydrogen peroxide (H_2O_2, 40% w/v solution, GR ACS, Carlo Erba), Tetrahydrofuran (THF, anhydrous, ≥99.9%, Sima-Aldrich), ethanol (≥98.9%, Fluka), Dowanol PMA (≥99.5%, Sigma-Aldrich), Methyl isobutyl ketone (MIBK, ACS reagent 98.5% Sigma-Aldrich), Butyl Glycole (≥99.5%, Sigma-Aldrich), tert-butyl methyl ether (for gas chromatography, SupraSolv), dichlormethan Pestanal[@] stabilized with amylene (approx. 25 mg/L), dichloromethane, CH_2Cl_2 for analysis, Riedel- deHaën), methanol anhydrous (≥99.8%, Sigma-Aldrich), sodium sulfate anhydrous (≥99.0 %, Sigma-Aldrich), N,O-bis (trimethylsilyl)trifluoro-acetamide (BSTFA, 99%) with trimethylchlorosilane (TMCS, 1%) (Sigma-Aldrich), sodium cube in mineral oil (99.9%, Aldrich), pyridine RPE-ACS (≥ 99.6%, Carlo Erba), sulfuric acid ACS reagent (95.0-98.0%, Sigma-Aldrich).

2.2 Cutin isolation

Cutin was isolated from tomato peels (TP) by different extraction procedures, an alkaline hydrolysis (method A, A1 and A2), an alternative route that involving cutin recovery as sodium carboxylate (method B) and, finally, sodium hydroxide/ hydrogen peroxide ($NaOH/H_2O_2$) route (method C).
All investigated procedures involved a first step that consisted of a thermal treatment of tomato peels (100g) with 3 % NaOH (400mL) at high temperature, elimination of exhausted skins, filtration of the dark brown solution to eliminate the residual pulp and suspended solids.
The thermal treatment was carried out at 100°C for 6h (method A1), at 130°C for 2h (method A2), at 130°C for 15 minutes in methods A, B and C. The second step of methods A, A1 and A2 involved the solution acidification with HCl fuming 37% up to pH 4-4.5. Then, the obtained residue gummy was washed till acid free.
In alternative method B the cutin was separate out as sodium carboxylate (Figure 1) from alkaline solution by filtration or centrifugation. The $NaOH/H_2O_2$ route (method C) requested a second step in which the addition of peroxide H_2O_2 (4% V/V) to the reaction mixture (pH 12.7) cooled at 90°C was performed. The reacted solution was filtered, 5 % p/p HCl at room temperature was added, as before, and the pale yellow solid is collected.
The visual analysis of extracted cutin samples was carried out and the reaction yield was calculated for all methods.

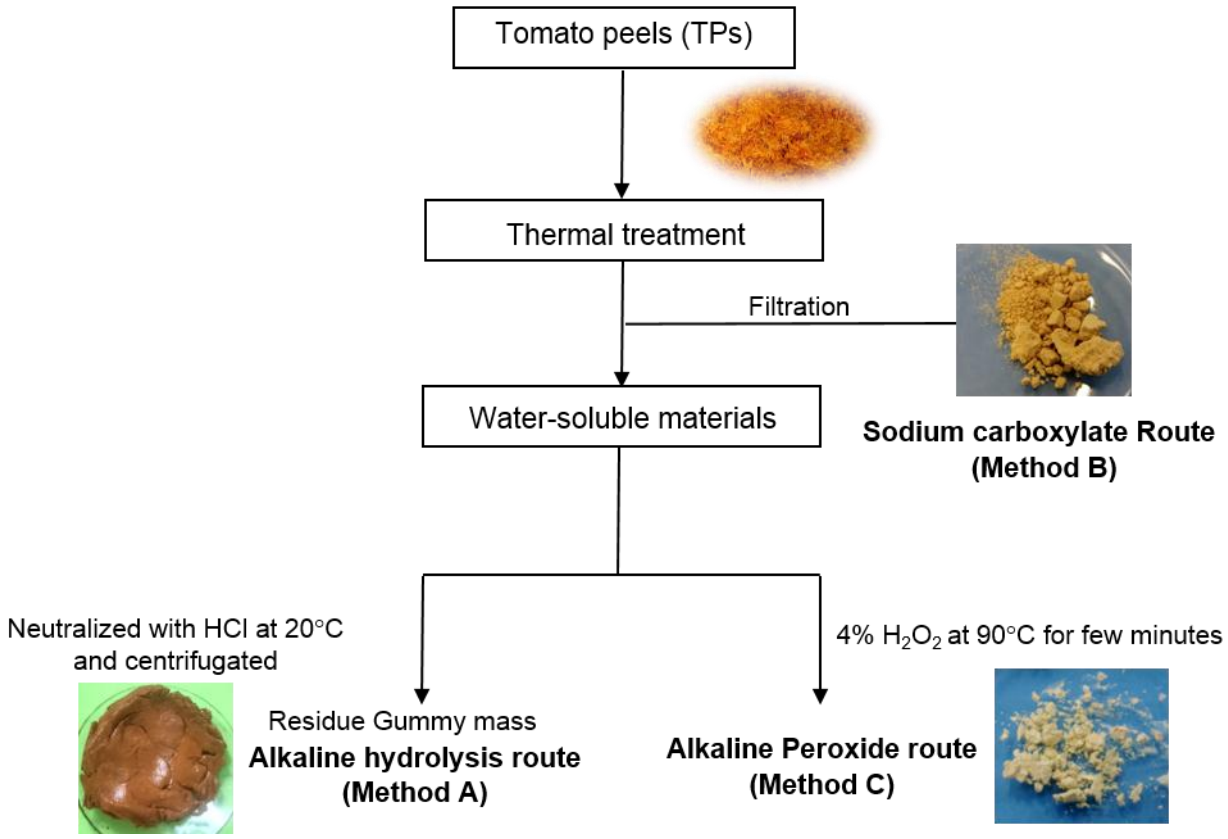

Figura 1 Flow chart of tested extraction methods of tomato cutin.

2.3 Spectroscopy FT-IR studies

The Fourier transform infrared spectroscopy (FT-IR) spectra were recorded on a Perkin-Elmer FT-IR Spectrum One spectrometer, equipped with a Spectrum v5.3.1 software. The FT-IR spectra were recorded in the 4000–450 cm^{-1} range, with a resolution of 4 cm^{-1} and accumulating 16 scans for each spectrum, spectrum average was calculated considering at least three replicates.

2.4 GC-MS Analysis.

The composition of cutin monomers of each sample was determined by GC-MS analysis either as methyl esters or as TMSi esters by $NaOCH_3$-catalyzed methanolysis, with OH groups derivatized to TMSi ethers according to the procedure previously reported in literature [3, 25, 26].

GC-MS analysis on methanolysates cutin samples was performed by Varian 450 GC gas chromatograph (Walnut Creek, CA, USA) coupled with Varian 300 MS mass spectrometry (Walnut Creek, CA, USA). The compounds were separated on a ZB-Semivolatiles, Celona, PA, USA) (30 m X 0.25 mm Ø, 0.25 µm film thickness, Phenomenex), with the GC following conditions: with an injector temperature of 300°C, a mass transfer line temperature of 300°C, oven temperature program, 125°C (iniziale temperature), rate of 5°C/min up to 205°C (10 min), subsequent rate of 5°C/min up to 300°C (5 min), flux 1 mL/min. The MS source temperature was 330°C. The analysis was carried out under full-scan acquisition within 50-600 a.m.u range. The software Bruker MS Workstation version 7.0 was used. The eluted compounds were identified with the Wiley or NIST library, if with the first one no matching is found. The certainty of identification was given by a probability factor and by the Match Factor (F.M.), (showing the goodness of the match between the spectrum of the sample and the spectrum identified by library).

Cutin composition was calculated from integrated areas of the corresponding peaks in the GC-MS ion chromatograms.

The cutin monomer yield was determined gravimetrically as the $CHCl_2$ –soluble material taking into account the ratio expressed in percentage, of the weight of the initial sample and the sample obtained after NaOMe methanolysis, addition of H_2SO_4 and extraction with CH_2Cl_2 (addition of water and solvent in ratio 1:1 p/p), filtration, drying and evaporation of the solvent. At least triplicate chromatographic analyses of the products obtained by different extraction, for each method, were carried out. Mean ± SD (standard deviation) values for monomers and the cutin monomer yield were calculated. The abundance of each compound was referenced against the sum of the chromatographic peak areas in total ion chromatograms.

2.5 Gel permeation chromatography (GPC) (materiali e metodi)

The GPC system was composed by a WATERS 1525 pump equipped with a WATERS 2414 IR detector, a chromatographic SEC column 4.6 X 300 mm particle size 5 μm (Phenomenex, USA). The solvent used was tetrahydrofuran. GC-MS analysis was performed with the following conditions: flow 0.3 ml/min, 1600 PSI pression, temperature 35°C. A Unipoint Gilson Software was used to record and elaborate chromatograms. The column calibration was obtained with the following polystyrene standards of known molecular masses: 17500, 9580, 63902, 980, 947, 510 and 486 Da. Weight averaged (Mw) and number-averaged (Mn) molecular weights, and polydispersity (P ¼ Mw·Mn^{-1}) were calculated as described elsewhere [21]. Only fully dissolved part of sample was analyzed by GPC; some parts of all samples are retained non-dissolved, cutin solution in THF showed a slight turbidity (about 0.30 g of cutin in 1 mL di THF). At least triplicate GPC analyses of the products obtained by different extraction, for each method, were carried out.

2.6 Thermogravimetric analysis (TGA)

Thermogravimetric analysis TGA was performed on samples (6-8 mg) using a Perkin–Elmer thermograimetric analyzer Pyris,1 TGA instrument using a temperature program of 25 to 600 °C at a heating rate of 10 °c min^{-1} under N_2 (24 mL min^{-1}). Onset temperature (T onset), maximum decomposition temperature (Td, max), weight of the solid residue remaining at 600°C, weight loss (ΔW) and derivatives thermograms (DTG) were analyzed using Pyris v8 softwar. At least triplicate TGA analyses of the products obtained by different extraction, for each method, were carried out.

2.7 Solubility assay

The solubility of each cutin sample (10g) was evaluated in single solvent. For tests at ambient/room temperatures (20 ° C) an ultrasonic agitation for at least 30 minutes to facilitate the solid aggregate dissolution was used. The solid residue remaining after solubilization, was separated by centrifugation (4,500 RPM X 30 min at 20°C), dried con N_2 flow and weighted. The amount of soluble material was obtained by subtracting the weight of residue from that of initial cutin (data corrected for the dry residue). At least triplicate solubility assays of the products obtained by different extraction, for each method, were carried out.

2.8 Viscosity analysis

Viscosity of each cutin sample (at least triplicate of three different extractions for each solvent tested) was examined at 20 °C, using a Dynamic Rotational Viscometers (Haake Rheo Stress 600, Thermo Electron Cooperation) manufactured and supplied by the Thermo Electron Corporation, equipped with a a Peltier temperature control unit parallel plate (60mm) geometry for routine testing, i.e. The rheometer runs were settled in CR (constant rate) on a shear rate of 50 s^{-1}, a gap was setted of 1.000 mm. Data were elaborate with Thermo Scientific HAAKE RheoWin 3 Software.

2.9 Noncatalyzed Melt-Condensation of cutin

Polyester films from each cutin extraction method were prepared by noncatalyzed melt-polycondensation at moderate temperature (150°C) directly in air, placing about 300 mg of different extracted products in an open glass petri dish (30 X 30 mm^2 and 0.1 mm deep) and heated inside an oven at 150°C for different periods of time (from 2h up to 8h). The films were used for TGA analysis. At least triplicate analyses of the products obtained by different extraction, for each method, were carried out.

2.10 Real-Time Omopolymerization Monitoring of cutin.

The Real-Time kinetic study for each cutin sample was performed using a Perkin-Elmer FT-IR Spectrum One spectrometer coupled with a high temperature transmission cell (Hot-One CIC Photonics), equipped with a mould with cavity dimensions of 0.50 cm^2. For analysis, each sample, about 7 (±1)g m^{-2} , was laid out on thin layer. The Time-based spectroscopy software was used to collect infrared spectra at 20 seconds intervals, using 16 scans at 4 cm^{-1} resolution, and using the temperature controller. Isothermal or temperature ramps from 30 to 160 ° C were set, with a scan rate of 2°C min^{-1}. At least triplicate analyses of the products obtained by different extraction, for each method, were carried out.

3. Results and discussion

3.1 Cutin isolation

Many conditions of thermal treatment for saponification reaction were tested, in particular, considering extraction yield and physical-chemical properties of products obtained, the time/temperature combinations shown in table 1 (A, A1 and A2 methods) were deemed optimal for cutin extraction.

The properties of products isolated from A, A1 and A2 methods, thus changing only the thermal treatment parameters (table 1) were, in particular in terms of appearance, fairly similar. In the hydrolysis reaction at 130 ° C for 2h the obtained process yields were slightly better than the treatment at 100°C for 6h and 130°C for 15 minutes, which were not significantly different. On the basis of these considerations, the most feasible and simple treatment, that was selected to compare the methods A, B and C was at 130°C-15'.

The extraction procedures investigated (A, B and C methods) showed significant differences in terms of reaction yield and appearance of the extract, as shown in table 1. The reaction yield in method A is about twice then yield in method B; this experimental evidence could be explained considering that the method A, involved the co-precipitation in HCl of all insoluble components extracted at alkaline pH, while the method B requested that the solid residue separated out from supersaturated alkaline solution is selectively collected. In the first case a solution color change occurred, while in the second method the supernatant retained a dark brown color. The reaction yield of the method C is about 4%, significantly lower than the method A, this may be due to both the hydrogen peroxide reaction [20] (valuable by color shift of alkaline solution from dark brown to light brown), and the water solubility of cutin C, even at acid pH; therefore, the product C collection by filtration could not be complete. Also the appearance of products obtained by different routes employed (methods A, B and C), is very different (figure1 and table 1). In fact the gummy mass, obtained by method A, is a sticky mass at room temperature, presentes property to take the shape of the container it is kept, it does not dry at room temperature, and the sticky and clinging film produced laying the product on thin layer, has a natural colour yellow-dorè and also does not dry at room temperature. Instead the products obtained by methods B and C are easily dried as crystalline solid with yellow and pale yellow color respectively and are not able to form film by water evaporation.

Table 1
Cutin isolation from tomato peels: reaction, appearance and yields.

Method	**Reaction condition**	Reaction parameters (time-temperature)	**Appearance**	**Yields[c] (%)**
A	3% NaOH	15'130°C	dark brown paste	18 ± 3
A1	3% NaOH	6h 100°C	dark brown paste	20 ±5
A2	3% NaOH	2h 130°C	dark brown paste	28 ± 4
B	3% NaOH[a]	15'130°C	yellow crystalline solid	9 ± 2
C	3% NaOH + 4% H_2O_2[b]	15'130°C	pale yellow crystalline solid	4 ± 1

[a] cutin separated out as sodium carboxylate
[b] 4% H_2O (pH12.7, 90°C for 5 minutes)
[c]All yield values were based on original TP in % and were corrected for the dry residue value.

3.2 FT-IR spectra

The FT-IR spectra of cutins A, A1 and A2 derived from alkaline hydrolysis method were fairly equal in the range of standard deviations; thereby we focused on the FT-IR analysis of cutins A, B and C.
FT- IR spectra analysis of cutin samples extracted by methods B and C (Figure 2), allowed the identification of bands characteristic of the hydroxy acids. The main bands (table 1) were ascribed to hydrogen-bonded hydroxyl (ν (OH) at 3320 cm^{-1}), a sharp intense band at 1702 cm^{-1} and shoulders at 1705 and 1690 cm^{-1} (Figure 1b and 1c) were assigned to carbonyl stretch of carboxylic acid, methylene (mainly ν_a (CH_2) at 2925, $\nu s(CH_2)$ at 2851 cm^{-1}, $\delta(CH_2)$ scissoring at 1463 cm^{-1} and $\delta(CH_2)$ rocking at 720 cm^{-1}, CH_2 bending at 723 cm^{-1} indicated long-chain compound). [22].
The infrared spectra of cutin extracted by method A (figure 1a), showed significant differences from samples B e C FT-IR spectra. Considering the frequencies of carbonyl stretch, the main peak at 1711 cm^{-1} assigned to carbonyls in a loosely packed polyester phase as well as to ester groups interacting with residual hydroxyls and to loosely associated carboxyls [22], shifted about 10 cm^{-1} compared to B and C samples, showed several shoulders: at 1690 and 1704 cm^{-1} ascribed to carbonyl stretch of carboxylic acid, at 1727 cm^{-1} (ν(C=O)) to ester. Other bands ascribed to ester are νa(C-O-C) band at 1169 cm^{-1} and νs(C-O-C) at 1104 cm^{-1}.
The C=O stretching at 1711 cm^{-1} was assigned by different authors to interactions by H-bonding of ester group and COOH or residual hydroxyls [15]. Therefore, the FT-IR analysis showed that the sample A consists of a mixture of acids and esters, has a certain esterification degree, missing in the samples B and C.
In addition to bands already explained above, FT-IR spectrum of sample A showed double bonds (ν(C=C) at 1640 cm^{-1}), ν(C=C) of phenolic acids (1632 cm^{-1}), ν(C-C) aromatic (1605 cm^{-1}) and (C-H) and (C-C) out of plane bending (835 cm^{-1}) from aromatic, and three ν(C-C) aromatic conjugated with C=C (1556, 1515and 1450 cm^{-1}). Others minor bands could be from proteins (ν(C=O) at 1660cm^{-1} and δ(N-H) at1550cm^{-1} from the amide groups, and between 1100 and 1500 cm^{-1} [17]. Moreover, shoulders on the ν(C=O) band, usually about 1715 and ester (ν(C=O) at 1729cm^{-1}, νa(C-O-C) at 1169cm^{-1} and νs(C-O-C) at 1106 cm^{-1}) were identified. In addition, the FT-IR spectrum of cutin showed at about 1250 cm^{-1} an overlapping zone of different bands, common in compounds with some heterogeneity [23].
Moreover, for all samples, the couple of bands at 1042 and 1060 cm^{-1} could be from bending of hydroxyl (O-H) primary and secondary or stretching ν(C-O) in polysaccharides, a certain amount of glycosidic bonds could not be excluded.

Table 2. Assignments of the most relevant signals in the FTIR spectra of cutin derived from methods A, B and C.

Peaks[b]	FTIR /cm^{-1}			Assignment[a]
	Method A	**Method B**	**Method C**	
1	3307 ± 20	3300 ± 40	3300.53 ± 30	str (O-H----O)

2	2933 ± 4	2917 ± 4	2922 ± 8	str asymmetric (CH_2)
3	2856 ± 1	2848 ± 2	2850 ± 1	str symmetric (CH_2)
4			1468 ± 1	bend (CH_2)
5	1463.7 ± 0.3	1462.8 ± 0.2	1463 ± 2	
6		1433 ± 1		
7	1374 ± 2		1374.62 ± 3	
8		1323 ± 1	1323 ± 1	
9	724.23 ± 0.2	723 ± 2	721 ± 1	
10	in overlapping zone	1219 ± 1	1221.06	str (C-C) chain
11	1728 ± 7	-	-	str (C=O) ester group
12	1712 ± 1	-	-	str(C=O----H) H-bonded ester groups
13	1702.9 ± 0.2	1702.0 ± 0.3	1703 ± 2	str (C=O) free acid group
14	1693 ± 1	1692.9 ± 0.2	1693 ± 1	shoulders on band of str(C=O----H) H-bonded acid groups
15	1697.9 ± 0.4		1698.4 ± 0.4	
16		1706	1707 ± 2	
17	1169.9 ± 0.1	-	-	str asymmetric and symmetric (C-O-C) ester
18	1105.9 ± 0.5			
19	1632.35 ± 0.03	-	-	str (C=C phenolic acid)
20	1277± 1 In overlapping zone	1282.9 ± 0.1	1286 ± 2	(C-O) stretching asymmetric (carboxylic acid)
21	1410.8 ± 0.5	1411 ± 1	1411 ± 1	bend O-H (carboxylic acid)
22	936.6 ± 0.3	936.1 ± 0.5	935 ± 2	
23	1606 ± 1	-	-	str (C-C aromatic)
24	1588 ± 1			
25	1454 ± 1			
26	1639.80 ± 1	-	-	str (C=C) double bonds
27	1559 ± 2	-	-	str (C-C aromatic conjugated with C=C)
28	1541 ± 1			
29	1515.8 ± 0.2	-	-	str (C-C aromatic in phenolic compounds
30	835.45 ± 0.05	-	-	bend oop (C-H and C-C) aromatic
31	1070 ± 1	1060.1 ± 0.3	1065 ± 7	bend (O-H) primary and secondary and possible str (C-O) polysaccharides
32	1058 ± 1	1042 ± 1	1042 ± 2	
33	1254 ± 4 In overlapping zone	1246.3 ± 0.1	1248 ± 1	

[a] Heredia-Guerrero et al. (2014). [b] peak name in figure 2.

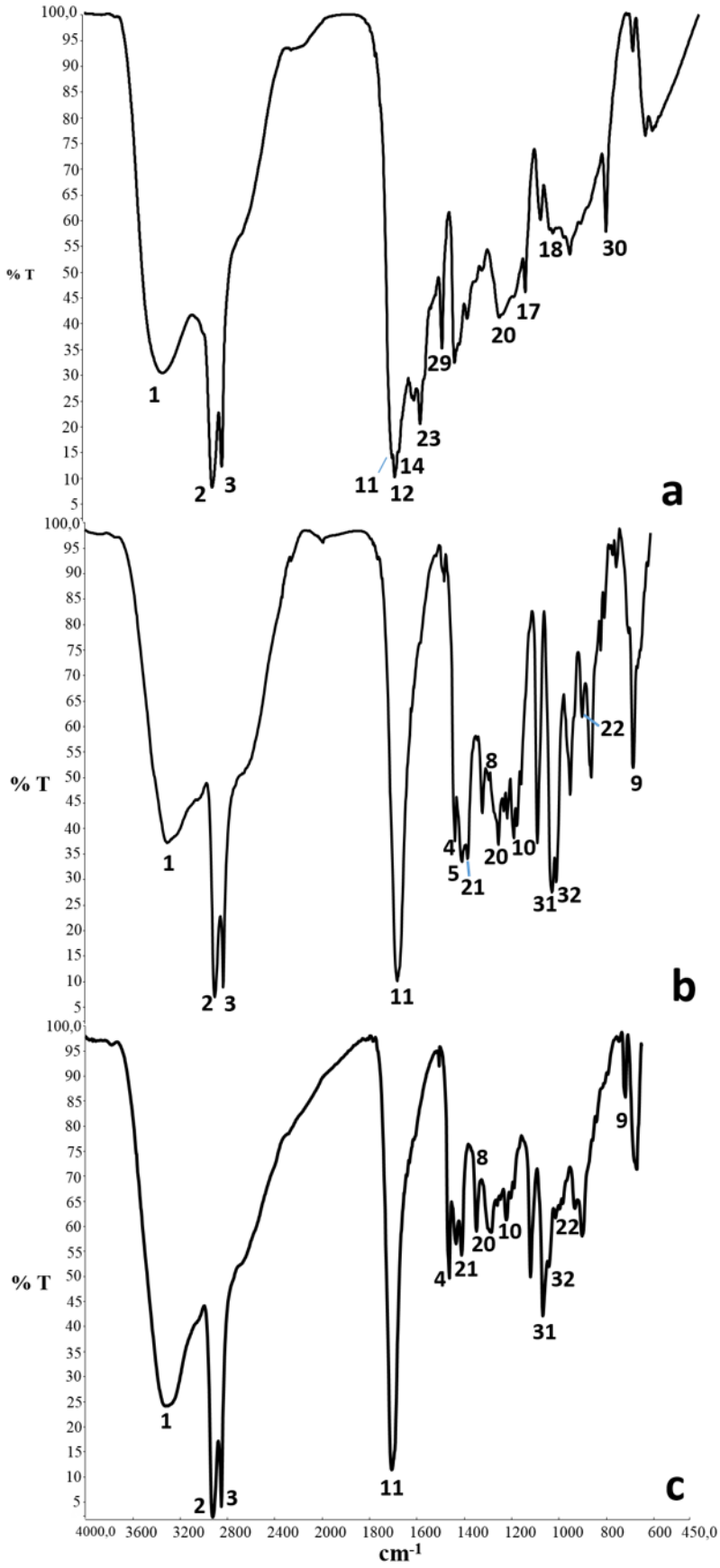

Figure 2 . FTIR spectra of cutin derived from methods A **(a)**, B **(b)** and C **(c).**

3.3 Cutin Monomer Composition by GC-MS analysis.

Identification of $CHCl_2$ soluble cutin monomers of the tomato peels was performed by means of GC-MS.

The monomer profiles of cutins A, A1 and A2 derived from alkaline hydrolysis process were fairly equal in the range of standard deviations; thereby in table 3 were compared the monomer composition of cutins A, B and C. The major monomers in all cutins studied (methods A, B and C) were 10,16-dihydroxy hexadecanoic acid isomers known to commonly exist in cutin polymers of tomato [3]. The principal peak in GC-MS spectra chromatograms of cutin samples obtained (figure 3), which corresponded to the 10,16-dihydroxy hexadecanoic acid, showed a retention time of 22.23 (±0.04) minutes, and its mass spectrum (figure 4) had diagnostic ions of long-chain methyl ester of 10,16-diOH-16:0, majior cleavage ions was at 275 m/z and 171 m/z, as the main fragmentation in *beta* to the oxygen atom was known to be [24] when the hydroxy group is in a central part of the molecule, and e.g. rearrangement ions at 169 m/z and 273 m/z, a further loss at 129 m/z are also possible (figure 4). In this study, several isomers of 10,16-dihydroxyhexadecanoic acid were also found in all studied samples. Several different methy and/or TMS derivatives, especially in the case of hydroxyl acids were identified also in previous studies [25].The major isomers was the 9,16-dihydroxyhexadecanoic acid, while the isomers 8,16 and 7,16 occurred in smaller amount [26]. Hydroxy acids comprised 93.3 +/- SD and for all cutin samples. In particular, mainly long-chain ω-hydroxy acids (table 3, mainly 16-OH-16:0) also with mid-chain functionalities. In addition even tri-hydroxy acids in minor amount (<0.4%) have been identified. Furthermore, compounds with chain lengths C18, epoxy-hydroxy monomers were identified. Suberin-like α,ω-diacids were found in all the cutins in minor amounts. Saturated acids and some unsaturated acid (e.g. oleic acid, peak **m** in figure 3a), probably coming from residue seeds of tomato waste that was used as raw material for cutin extraction. In fact, for these compounds, a quite large abundance variability (SD 1-3%) was observed. Besides, mono or diunsaturated monomeric compounds were mainly identified in chromatograms of cutins derived from method A in approximately 5 % amount and in very low amount 1.3 (± 0.6)%, and only in few cutin samples obtained from method B (in fact, the FT-IR spectrum did not show bands from ν(C=C) double bonds), while unsaturated compounds were not found in cutins derivated from H_2O_2 –route. Aromatic compounds were found in quite large amount in cutins extracted by method A (in approximately 6 %), and in very low amount (< 0.2%) and only in few cutin samples obtained from method B, while aromatic compounds were fully missing in cutins derivated from hydrogen peroxide –route. The most commonly-found aromatic monomeric compounds in chromatographic monomer profiles of cutin was fenolic compounds, derivatives of cinnamic acid: esterified cinnamic acids (e.g. octyl 4-methoxycinnamate), p-coumaric acid and its isomers; and derivatives of hydroxybenzoic acid; known to generally exist esterified to the cutin polyesters in tomato cuticle [27]. The chromatographic profile of the cutin obtained from the alkaline hydrolysis method showed, at retention time of (22.26, 26.23 and 28.47 minutes, peaks **n**, **o, p** and **q**, in figure 3a) ascribed to acylglycerols at low molecular weight (between 2-10 % of total composition).

Table 3 Main monomers obtained from TMS derivatized and methanolysates cutins derived from methods A, B and C, identified by GC-MS analysis.

Method	**Identification**	[a] *peak name*	**Retention time (min)**	**Certainty of identification**		**Composition (%)**
				F.M[b]	probability (%)	
	Hydroxyacids					
A	10,16-dihydroxyhexadecanoic acid	a	22.36	836	95	62 ± 15
B			22.23	824	93	82 ± 4
C			22.23	751	88	82 ± 6
B	Isomers of 10,16-dihydroxyhexadecanoic acid	a' a''	21.70 23.76	721 717	84 81	2 ± 1 1.5 ± 0.4
C		a' a''	21.24 23.75	658 715	48 81	1.7 ± 0.1 1.3 ± 0.8
C	9,10-dihydroxyoctadecanoic acid	b	21.97	557	10	0.8 ± 0.3
A	16-Hydroxyhexadecanoic acid	c	20.52	871	96	2.2 ± 0.5
B			20.41	901	98	6 ± 1
C			20.41	897	98	5 ± 2
A	9,10-epoxy-18-hydroxyoctadecanoic acid, (or 18-hydroxyoctadec-9-enoic acid)		23.15	590	18	2 ± 1
B			22.34	615	7	2 ± 1
C			22.35	579	10	0.9 ± 0.3
	Alkanoic acids					
A	Hexadecanoic acid	d	15.53	861	44	2 ± 1
B			15.32	908	68	1.0 ± 0.5
C			15.32	888	65	2 ± 1
C	Octadecanoic acid	e	18.53	893	70	0.77 ± 0.05
A	Decanedioic acid	f	23.13	608	24	2 ± 1
B	Alternative name:		23.02	625	40	4 ± 3
C	sebacic acid		23.02	609	35	4 ± 3
C	Isomer of sebacic acid	f'	21.51	573	8	0.6 ± 0.1
A	Docosanedioic acid		20.26	791	26	0.70 ± 0.04
	Unsaturated acids					

A	18-hydroxyoctadec-9-enoic acid	g	21.11	578	15	2 ± 1
A	*9,12-octadecandienoic acid*	h	18.18	920	37	3 ± 2
	Aromatics					
A	(E)-3-(4-hydroxyphenyl)-2-propenoic acid Alternative names: 4-hydroxycinnamic acid, *p-coumaric acid*	i	12.97	930	91	2.0 ± 0.5
A	*Isomer of p-coumaric acid*	i'	15.75	897	88	3 ± 1
A	*1,4-Benzenedicarboxylic acid*	l	10.29	860	97	0.44 ± 0.01

[a]peak name in figure 3
[b]Match Factor (F.M.)

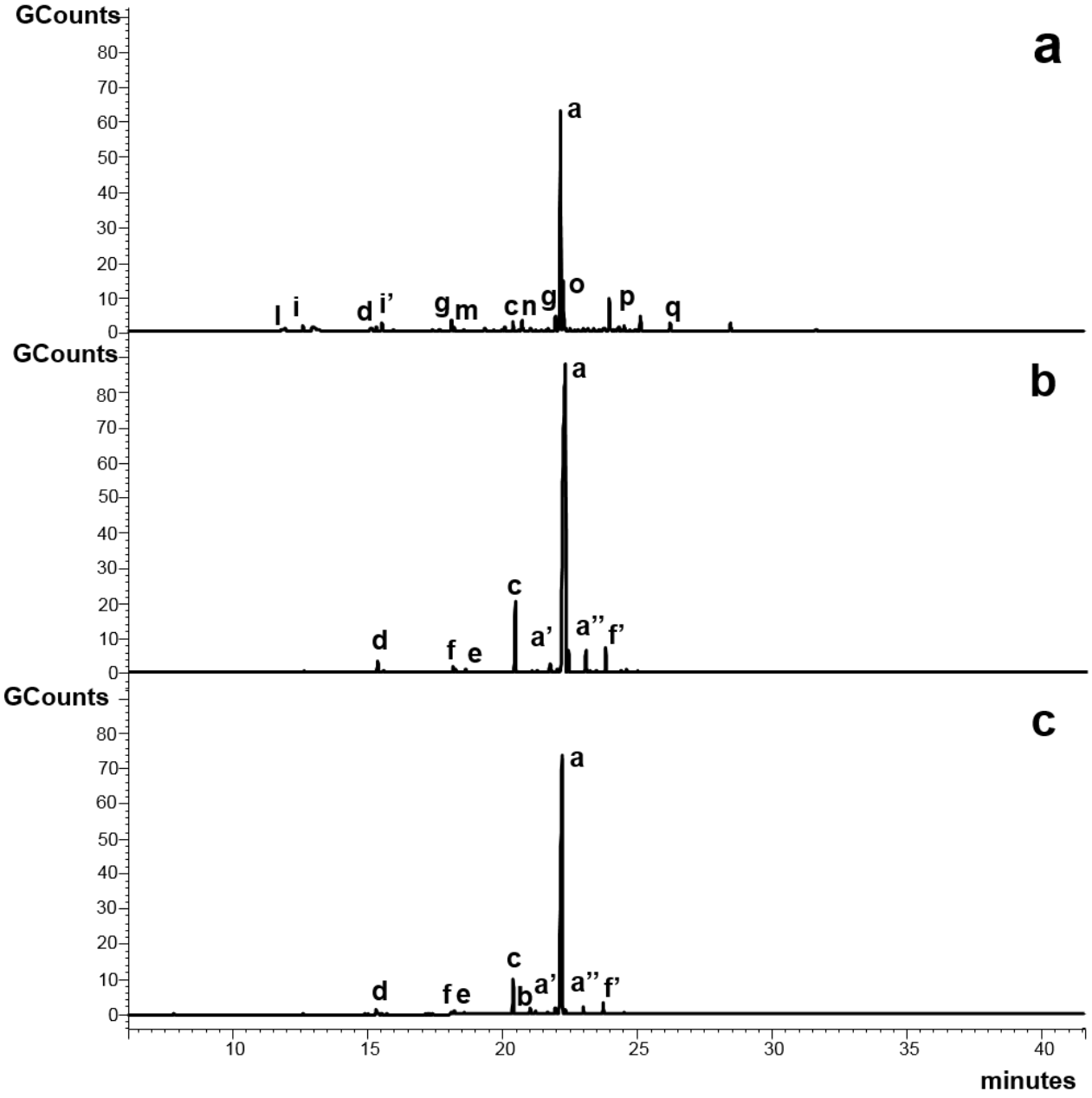

Figure 3. Total ion chromatograms of TMS methyl esters derivatives of the $CH2Cl_2$ extract of cutins obtained from methods **(a)** alkaline hydrolysis, **(b)** selective precipitation route and **(c)** (H_2O_2 route). Peak names are given in Table 3.

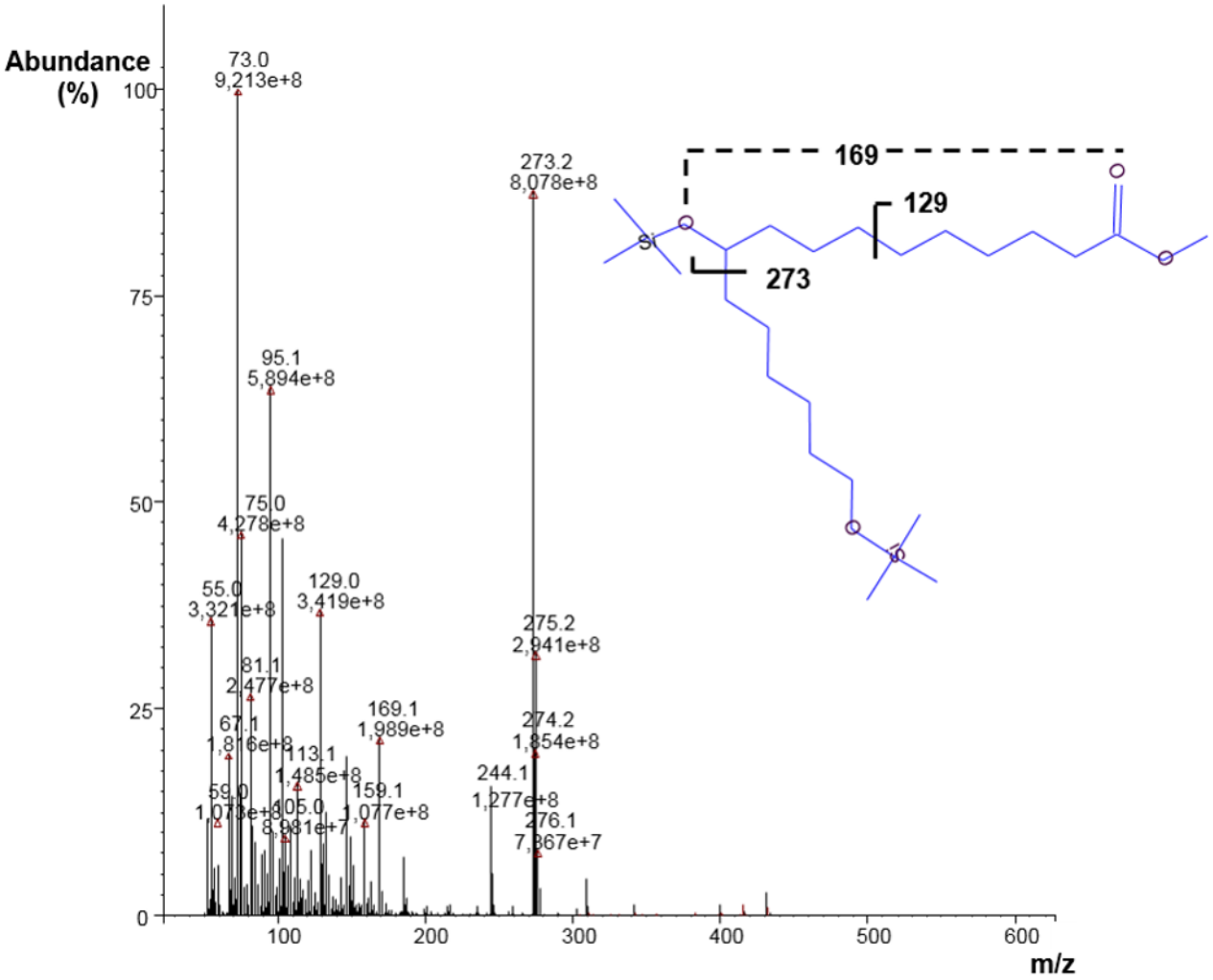

Figure 4 MS spectrum of 10,16-dihydroxyhexadecanoic acid (at 22.12 min retention time).

Table 4. Cutin Monomer Yields as Percentage of the Extractive-free Raw Cutin by Methanolysis

Method	*cutin monomer yield* [a]	*cutin monomer yield* [a, b]
A	65 ± 9	83 ± 6
B	68 ± 6	92 ± 6
C	67 ± 2	96 ± 4

[a]determined gravimetrically of $CHCl_2$ –soluble material from NaOMe methanolysis.
[b]corrected for dray residue

3.4 GPC analysis

The cutins obtained from each studied method were characterized by means of GPC analysis. The sample A showed the largest nominal (M_w) and (M_n), and the P values resulted well above 1 for all samples, indicating a great degree of heterogeneity amoung molecules (Table 5).

The cutins obtained from method B and C presented similar chromatograms, with a sharp and intense peak at 75 minutes corresponding to molecular weight of 10,16- dihydroxyhexadecanoic acid dimeric form, and poorly intense signal at 70 minutes. The GPC chromatogram of cutin A highlighted, in addition to the peak already mentioned for the cutins B e C, a broad region between 52 and 65 minutes; integrating the chromatograms in this first elution region, a large hydrodynamic size was found ($M_w \approx 23000$ Da and $M_n \approx 19000$ Da).

Table 5 Weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (P) calculated from GPC chromatograms for each cutin samples.

Method	M_n (Daltons)	M_w (Daltons)	Polidispersity
*A	800	5000	7. 6
**B	384	384	1.0
**C	370	371	1.0

*SD= 10 %, **SD < 1%

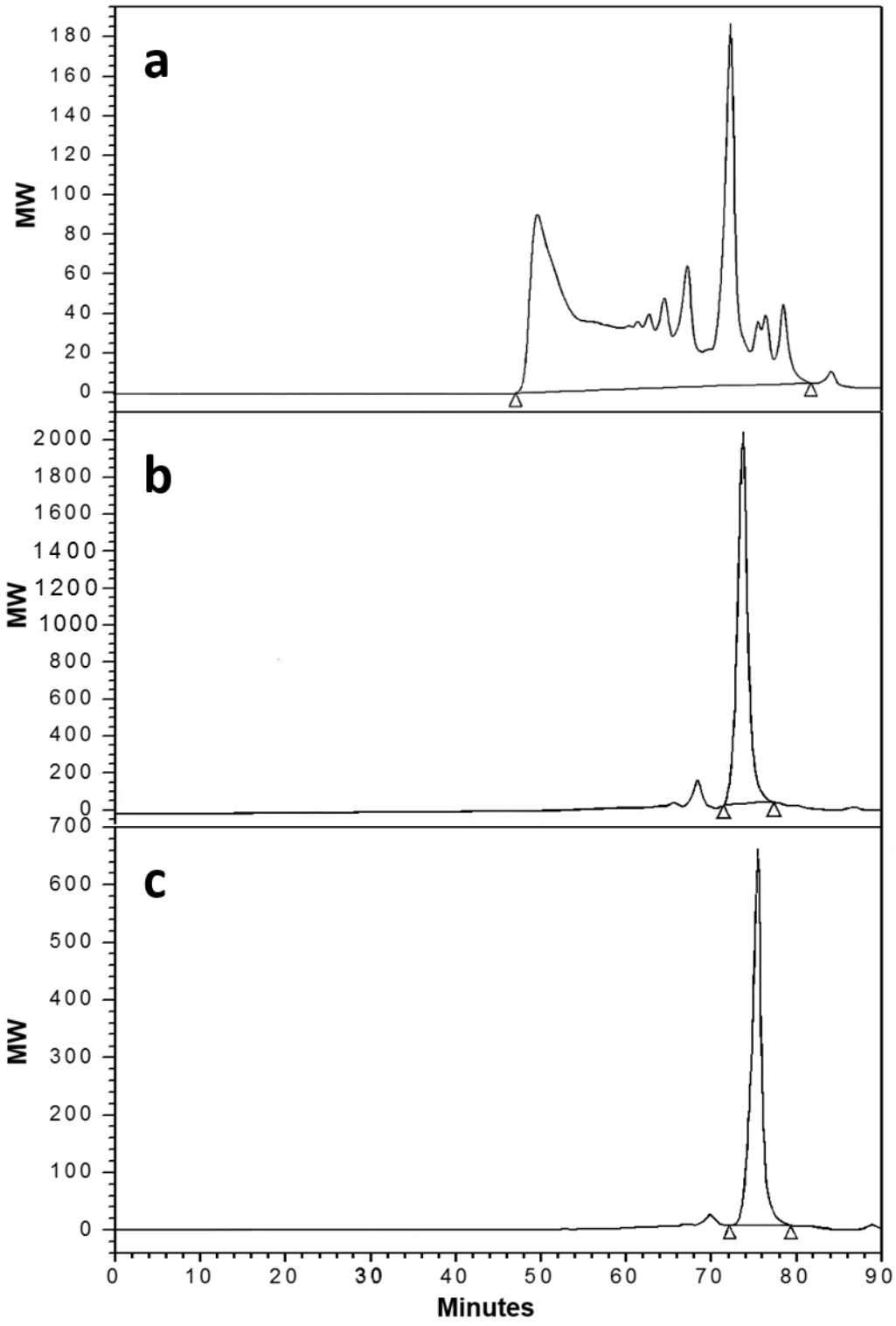

Figure 5 GPC chromatograms for cutins derived from method A **(a)**, method B **(b)**, and method C **(c)**.

3.5 Thermogravimetric analysis (TGA)

TGA analysis was performed to examine the main thermal degradation steps of cutin samples because the thermal stability of a polymer was found to be structure sensitive [28].

The figures 6b and 6c showed TGA curves of cutins derived from B and C processes with a dry residue (DR) of 98 (± 1)%, (conditioned in a controlled humidity chamber). The TG curve of cutin isolated from B and C methods showed a two-stage weight loss as commonly shown in TGA patterns of fatty acids and hydroxyacid [29]. Cutins B and C were thermally stable up to 220-230°C (peak maximum in DTG at 268 (± 6)°C and 263 (± 4)°C respectively) beyond which they showed a second decomposition multisteps stage. The low temperature peak is mostly associated to the dehydration and decarboxylation processes of the hydroxyacids [28], while the second TG weight loss, can be attributed to the decomposition of aliphatic chain [30, 31] and accounting a total loss of approximately 73%.

The TG curve of cutin isolated from process A was quite different from others cutins and showed a weight loss in different stages (at least 5). The devolatilization process of secondary components of cutin A, which was identified by means of FT-IR and GC-MS analysis, occurs in the investigated temperature range (between 200-350°C, the cellulose depolymerization reactions occurs, and at temperatures above 400 °C, CO and CO_2 are released by depolymerization reactions from the lignin-rich aromatic carbonaceous matrix [32,33]). We focus on the principal weight losses, due to hydroxyacids, the principal components of cutin.

The first weight loss in TG profile of cutin A (peak 1 in DTG curve, figure 6a) corresponds to the weight loss of water adsorbed in equilibrium with atmosphere. In order to show the water uptake of the gummy-mass product obtained from method A, the TGA profiles of cutin A at different humidity degrees was investigated (DR of 98 (±1)%, 78 (± 2)% and 65 (± 3)% (de-watering in a controlled humidity chamber). The TGA profiles underlined that increasing DR of cutin A sample, the mass loss at 113 ± 3°C decreased (table 6a), suggesting that this weight loss was firstly due to the water uptake by the several hydroxyl groups (as confirmed hydrogen bonded hydroxyls FTIR bands, figure 2) of cutin, that are able to acting as water coordination sites. On the basis of the plasticizing effect of adsorbed water, the characteristic appearance of cutin A could been explained.

However it was not possible exclude that this weight loss (2) could be due to polycondensation process; because the real-time FT-IR monitoring of omopolymerization of cutin A (figure 7b) showed that a significant increase of the ν(C=O) ester band intensity occured already at 110°C, while the reaction progression in sample B and C was lower.

In cutin A with DR of 98 (±1)%, that looks like a very cohesive mass, this weight loss (about 2%) occurs at 132 ± 3 °C. Differently, peaks 3, 4 and 5 of DTG curves do not depend on humidity degrees of sample (figure 6a).

In the TGA profile of cutin A appeared, unlike the cutins B and C, another weight loss (4 ± 1%) at 195 (±8)°C, could be attributed to the devolatilization of volatile compounds. Indeed, any thermal treatment at high temperature of gummy mass produces caramel-like flavors.

All samples showed a gradual multi-step weight loss, that starts at around 400°C, with three main peak temperatures at approximately 400, 450 and 490°C typical of complex biomass based samples [34], in fact the GC-MS analysis (table 3) pointed out that the studied samples are complex mixtures of different hydroxyl acids. This last decomposition stage (stage 5, table 6b), likewise the stepwise decomposition pattern of estolides derived from acid oils [29] could be due to fragmentation of the aliphatic chain of fatty acids with different long chain or their oligomers.

The samples B and C showed a weight loss at about 260°C (peak 4 of DTG curves) greater (10 ± 1% and 14 ± 3 % respectively) than sample A (5 ± 2%). As stated above, this step was due to dehydration and decarboxylation processes of the hydroxyacids [28], and therefore indicates their polymerization and crosslinking degree. In order to confirm the interpretation above reported, the TGA and FT-IR analysis of treated cutin samples in oven on thin layer for 4 and 6 hours was performed. The treated samples reached a certain polymerization degree, as confirmed by the ν(C=O) ester band at 1730 cm^{-1} in the FT-IR spectra (figure 8). Indeed, the TGA profiles of treated samples in oven (figure 6) showed a lower weight loss (about 2-3 % at most) at about 260°C (peak 4 of DTG curves) than untreated samples (table 6).

The solid residue remaining at 600°C is about the same (between 13-15%) for all samples.

Table 6a Thermal analysis of cutin samples derived from method A with different dry residue (DR). Namely Onset temperature (T onset), maximum decomposition temperature (Td, max), weight of the solid residue remaining at 600°C, weight loss (ΔW).

Peaks[a]						
	1			2		
Sample[b]	**ΔW** (%)	**DTG** T_{MAX} (°C)	T_{ONSET} (°C)	**ΔY** (%)	**DTG** T_{MAX} (°C)	T_{ONSET} (°C)
A (DS 65 ± 3%)	5 ± 2	51± 9	51± 9	20 ± 1	113 ± 3	92 ± 4
A (DS 78 ± 2%)	0.6 ± 0.3	47 ± 7	36 ± 9	8 ± 1		
A (DS 98 ± 1%)	NF	NF	NF	1.92 ± 0.01	132 ± 3	121 ± 2

[a]Peaks showed in DTG curves (figure 6)

[b]Peaks 1 and 2 were not found in DTG profiles of cutins B and C.

Table 6b Thermal analysis of cutin samples derived from method A, B and C, namely Onset temperature (T onset), maximum decomposition temperature (Td, max), weight of the solid residue remaining at 600°C, weight loss (ΔW).

Sample	**Treatment on thin layer in oven**	**Peaks**[a]						Multi-step stage (5) **Decomposition range** 396-494 °C			**Residue (%)**
		3			4						
		ΔW (%)	**DTG T_{MAX}** (°C)	**T_{ONSET}** (°C)	**ΔY** (%)	**DTG $T_{d,MAX}$** (°C)	**T_{ONSET}** (°C)	**ΔY** (%)	**DTG $T_{d,MAX}$** (°C)	**T_{ONSET}** (°C)	
A		4 ± 1	195 ± 8	183 ± 5	5 ± 2	254 ± 10	233 ± 12	64 ± 2	401 ± 8 454 ± 5 494 ± 9	N.C.[c] 456 ± 6 N.C.	15 ± 2
B		NF.[b]	NF	NF	10 ± 1	263 ± 4	235 ± 4	73 ± 4	422 ± 13 436 ± 11 480 ± 9	399 ± 11 N.C. N.C.	17 ± 4
C		NF	NF	NF	14 ± 3	268 ± 6	206 ± 1	73 ± 5	424 ± 11 451 ± 11 486 ± 10	397 ± 8 N.C. N.C	13 ± 5
A	150°C-4h	NF	NF	NF	2 ± 1	245 ± 8	220 ± 4	76 ± 5	400 ± 1 503 ±10	N.C. N.C	14 ± 1
A	150°C-6h	NF	NF	NF	NF	NF	NF	83± 4	404 ± 1 495 ±16	N.C. 457 ± 3	10 ± 5
B	150°C-6h	NF	NF	NF	2.5 ± 0.2	290 ± 7	258 ± 6	83± 2	453.5 ± 0.1 495 ± 7	418 ± 3 N.C.	14 ± 2
C	150°C-4h	NF	NF	NF	3.1 ± 3	292 ± 7	258 ±13	85 ± 2	435 ±1 448 ± 10 492 ±1	415 ± 5 N.C. N.C	12 ± 4

[a]Peaks showed in DTG curves (figure 6)
[b] N.F. not found, missing weight loss
[c] N.C. not calculable

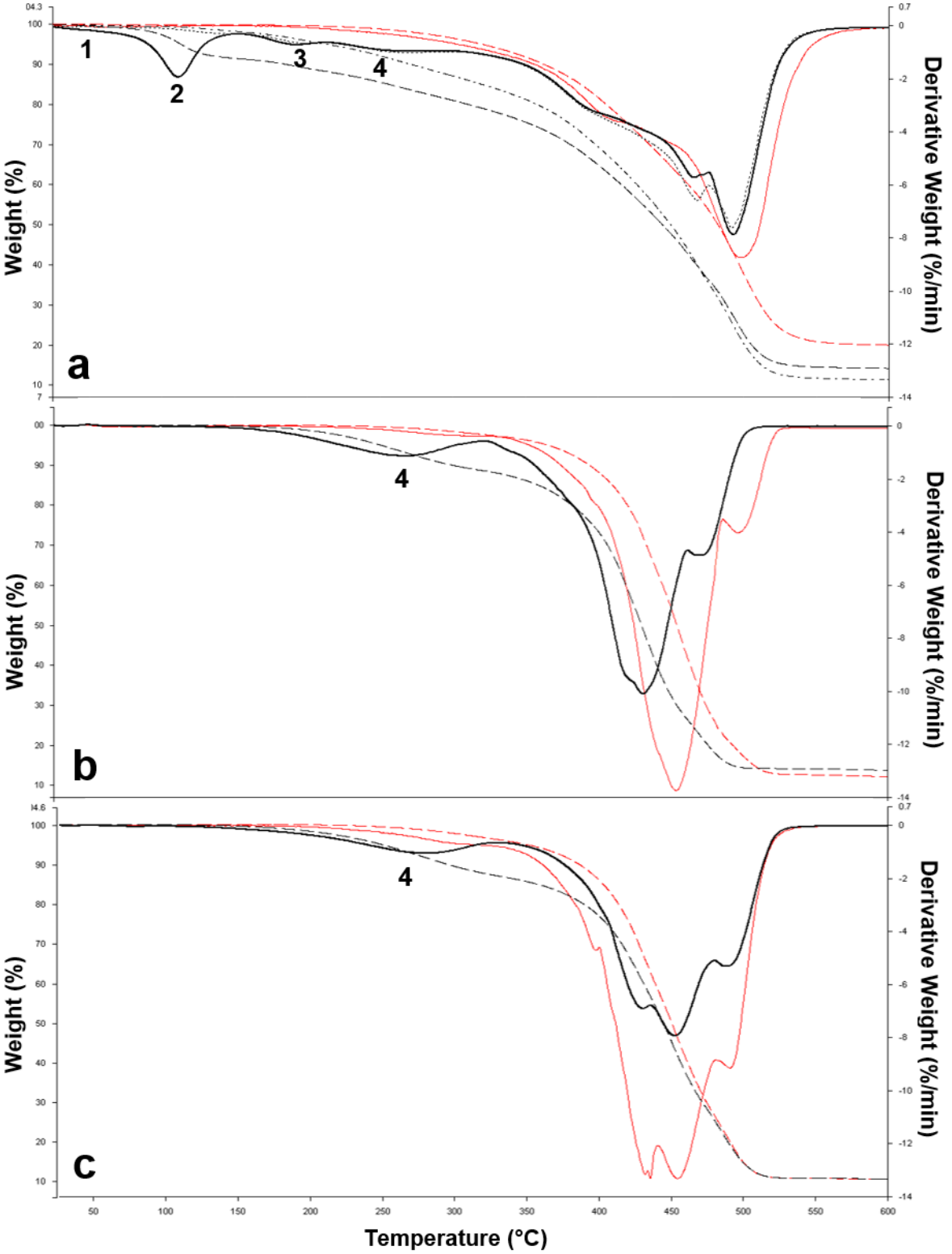

Figure 6 (a) TGA curve (dashed black line) and DTG curve (solid black line) of cutin A with DR 87%; TGA curve (black dash dotted line) and DTG curve (dotted black line) of cutin A with DR 98% ; TGA curve (dashed red line) and DTG curve (solid red line) of treated cutin A at150°C-6h**; (b)** TGA curve (dashed black line) and DTG curve (solid black line) of cutin B (DR 98%); TGA curve (dashed red line) and DTG curve (solid red line) of treated cutin B at150°C-6h**; (c)** TGA curve (dashed black line) and DTG curve (solid black line) of cutin C (DR 98%), TGA curve (dashed red line) and DTG curve (solid red line) of treated cutin C at 150°C-4h**;** under N_2 flow.

3.6 Real-Time Omopolymerization Monitoring of cutin.

The real time monitoring of non-catalyzed omopolymerization of cutins on thin layer and in air, was performed in order to point up the possible differences from reactivity point of view among the cutins extracted by different processes. FTIR data confirmed for all studied samples the formation of the polyester by progressive increase of the characteristic ν(C=O) band at 1730 ± 2 cm^{-1}, ν(OC–O–C) bands at 1254 ± 4 cm^{-1} and 1170 ± 1 cm^{-1}) from ester group; and by the progressive reduction of population of free hydroxyl groups. Moreover, the FT-IR spectra showed an intensity decrease and shift (from 3310 ± 30 cm^{-1} to 3330 ± 20 cm^{-1}) of hydroxyls band due to thermal treatment (4°C/ min until160°C) (figure 7a), according to previous studies [28, 36].

The figure 7b focus on ν(C=O) band assigned to carbonyls in cutin A, that already when is raw and untreated appears as a mixture of ester and acid (table 2), and then compared to other cutins, represented the more complex study-case. FT-IR monitoring underlined a progressive decrease and shift of shoulders at lower wavenumber side (1702.9 ± 0.2 cm^{-1}, 1697.9 ± 0.4 cm^{-1} and 1693 ± 1 cm^{-1} (figure 7b, peaks a and b), that were ascribed to the high concentration of free acid. Besides, FT-IR monitoring showed a progressive decrease of the shoulder at 1712 (± 1)cm^{-1} (figure 7b, peak c) assigned to loosely packed polyester interacting with residual acid groups [22], and an intensity progressive increase of bands at 1722.4 (± 0.2)cm^{-1}, 1728 (± 2)cm^{-1}, 1737.1 (± 0.4)cm^{-1} (figure 7b, peaks d, e and f) that indicated the reaction progression and the perturbation of carbonyl vibration.

The figure 8 shows FT-IR spectra of treated cutin samples in an oven at 150°C for 4 and 6h, and analyzed by means of TGA. The FT-IR spectrum of cutin A indicated the nearly full conversion of ν(c=o) band of the carboxylic acid into characteristic band of ester. Unlike, the FT-IR spectrum of cutin B, which was treated in the same conditions, highlighted several shoulders of ν(c=o) ester band at lower wavenumber side, that indicated a lower reaction progression. This result appeared according to GPC analysis of untreated samples, which showed that the investigated products were obtained from distinct methods with different molecular weights; therefore the reaction progression in the same condition was different for the samples A, B and C. Considering the frequencies of carbonyl stretch, the FT-IR spectrum of cutin that was derived from method C and then treated at 150°C for 4h showed a main peak at 1709 (± 1) cm^{-1} and several shoulders, that indicated the incomplete conversion ν(c=o) band of the carboxylic acid into characteristic band of ester.

The thermal treatment of sample C on thin layer at 150°C for 6h produced a large degradation of product, evidenced by the onset in the FT-IR spectrum of a broad band from 1600 to 1650 cm^{-1}, probably due to a dehydration process that involves the secondary free-hydroxyls of the 10,16-dihydroxyesadecanoic acid, the main component of cutin C, as showed by GC-MS analysis (table 3).

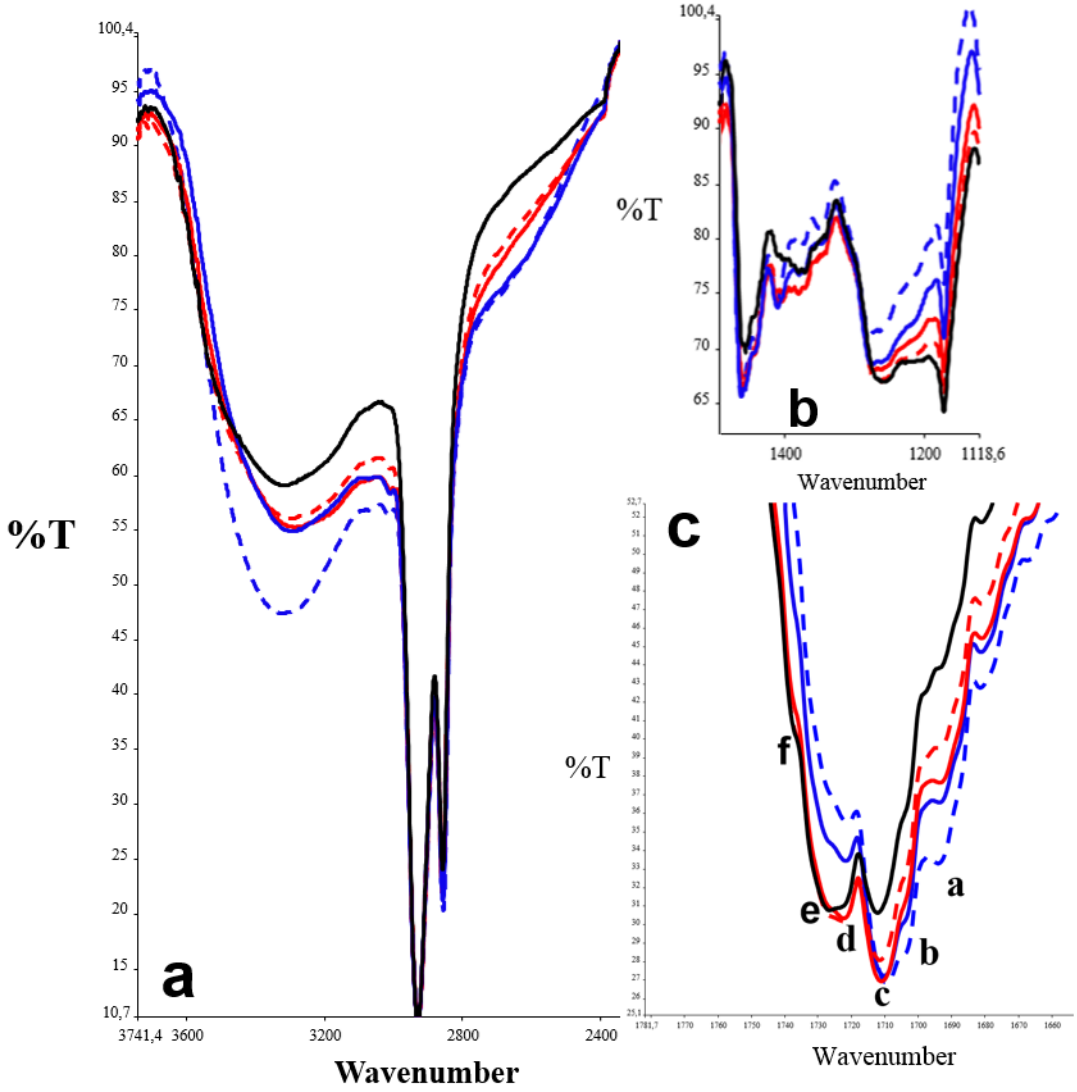

Figure 7 Real-Time FT-IR monitoring of cutin obtained by method A, temperature ramp from 30 to 160 °C, rate scan 2°C min^{-1}. FT-IR spectrum at 30°C (blu dashed curve), at 60°C (blu solid curve), at 110°C (red solid curve), at 130°C (red dashed curve), at160°C(black curve). FTIR spectra normalized respect to the $\upsilon_a(CH_2)$ asymmetric stretching band.

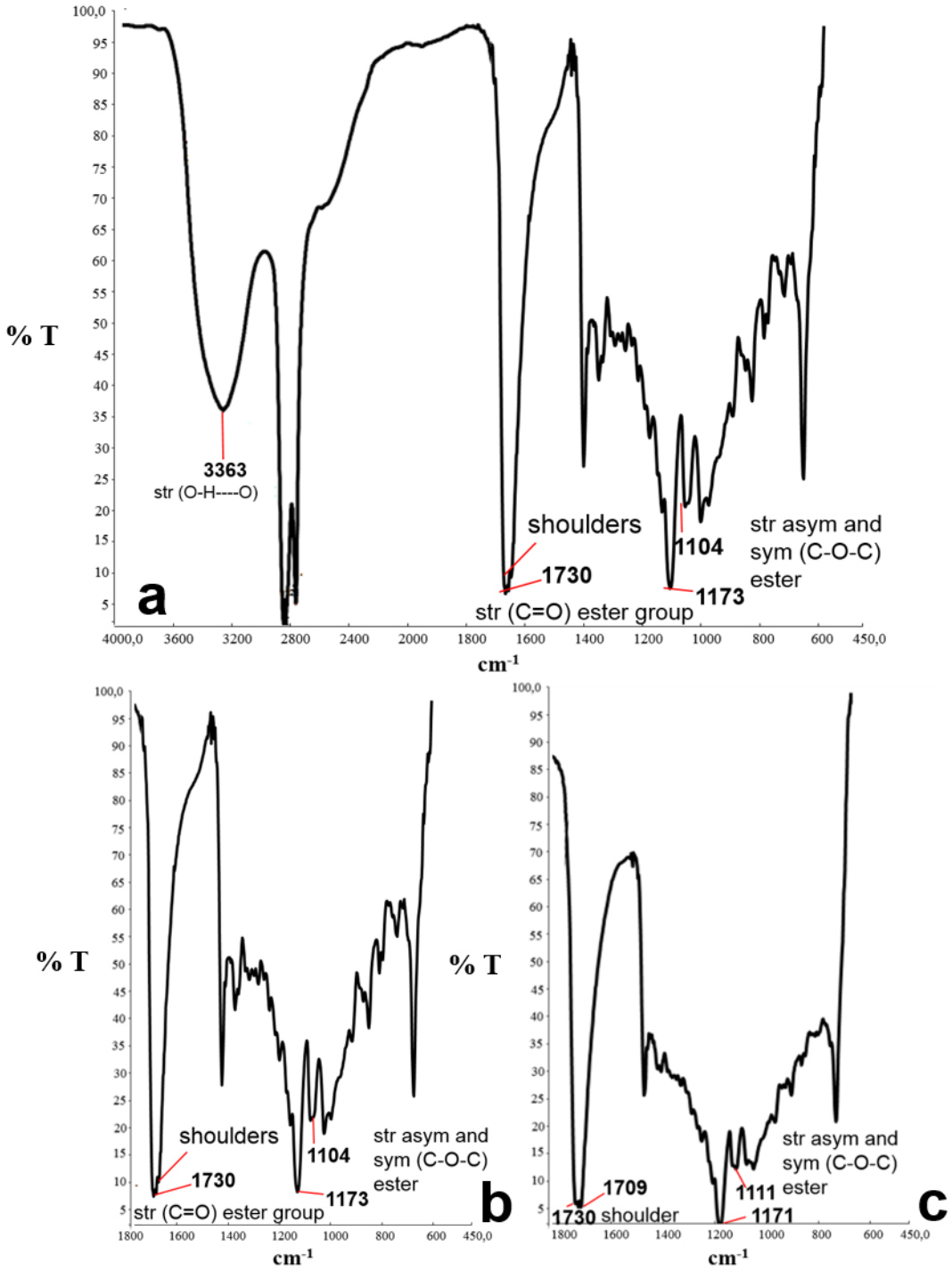

Figure 8. FTIR spectra of **(a)** treated cutin A in oven at 150°C for 6h; **(b)** treated cutin B in oven at 150°C for 6h; **(c)** treated cutin C in oven at 150°C for 4h**.** FTIR spectra normalized respect to the υ_a (CH_2) asymmetric stretching band.

3.7 Solution properties evaluation.

The solubility of each cutin sample was evaluated in alcoholic medium (ethanol), in glycol (buthy gluycole), acetate (Dowanol PMA), ketone (MIBK), ether (THF) and chloroform to underline the different properties of studied products. The three cutins (A, B and C) showed different compatibility with organic solvent, in the tested conditions.

According to previous studies regarding the solubility of long chain hydroxyacids [35], the products derived from method C appeared soluble, in hot water and ethanol, and sparingly soluble in THF. The cutin obtained from method B appeared soluble in ethanol, buthyl glycole and THF. The sample A at temperature lower than 60°C in buthy glycole, Dowanol PMA and MIBK looked like a solid mass sparsely compatible with the solvent, but at about 60°C melts, and in these conditions, was solubilized also in 1:1 w/w ratio with the solvent, obtaining a clear resin, at 60°C in buthy gluycole and Dowanol PMA and at 90°C in MIBK, stirring the solution for 15 minutes (table 7).

The table 7 shows the solutions viscosity increase, when the temperature or the duration of the solubilization thermal treatment were increased, probably due to molecular weight growing. All studied cutins (A, B and C) are practically insoluble in chloroform.

Table 7 Solution properties of cutins extracted by method A, B and C in different solvents.

Method	Solvent	Solubility*,[a]	Solution properties at 20°C (10 g cutin in 10 mL solvent) Viscosity (mPa· s)[b]	Treatment parameters	Appearance
A	Ethanol	soluble (39 g/L)	N.C.[c]	25°C	clear solution
B		soluble (48 g/L)		25°C	
C		soluble (36 g/L)		25°C	
A	THF	soluble (134 g/L)		25°C	clear solution
B		soluble (72 g/L)			
C		sparingly soluble (15 g/L)			
A	Dowanol PMA	freely soluble	1190	60°C for 15 minutes	clear resin
B		slightly soluble (10 g/L)	N.C.	60°C for 15 minutes	clear solution
C		very slightly soluble		Not changes with temperature increase	
A	Buthyl glycole	freely soluble	530	60°C for few minutes	clear resin
B		soluble (100 g/L)	N.C.	60°C for 15 minutes	clear solution
C		sparingly soluble (16 g/L)		60°C for 15 minutes	clear solution
A	MIBK	freely soluble	1320	90°C for 15 minutes	clear resin
			4590	distillation at 90°C for 2 hours[d]	
B		sparingly soluble (25 g/L)	N.C.	90°C for 15 minutes	clear solution
C		very slightly soluble		Non cambia /non aumenta changes with temperature increase	

*SD = 2-10%
[b] flow flux index closed to 1
[c] Not calculated viscosity (N.C).

4. Conclusions

Effective isolation of cutin from tomato peels has been demonstrated by an alkaline hydrolysis [2], a hydrogen peroxide-assisted alkaline hydrolysis reaction ($NaOH/H_2O_2$ route), and by hydrocloridric acid free-selective precipitation of sodium carboxylate process. Indeed the GC-MS analysis established that the main monomeric unit of cutin extracted, is, whatever the exploited method, the 10,16 – dihydroxyhexadecanoic acid, the principal component of tomato cutin, with a percentage between 83 and 96%.

The extracted products by different methods showed very different characteristics, first of all, as appearance and solubility.

Indeed, the gummy mass, that was obtained from method A, is a sticky mass, probably as a result of intermolecular hydrogen bonding and to higher molecular weight, according to GPC analysis. The GPC chromatogram of cutin A showed a broad band, missing in the GPC chromatograms of cutins B and C, that matched a large hydrodynamic size ($M_w \approx 23000$ Da and $M_n \approx 19000$ Da) missing in GPC chromatograms of cutins B and C.

FT-IR, GPC and GC-MS analysis established that by hydrocloridric acid free process (method B) and, in particular, by hydrogen peroxide-assisted hydrolysis reaction (method C) is possible to obtain a higher purity product and a larger degree of depolimerization of tomato cutin than method A, although the reaction yields are lower.

The method B could be proposed as a simple and cheap alternative to hydrogen peroxide-assisted route, although some differences between the two obtained products were emerged, as illustrated by solubility assay, GC-MS analysis and by omopolymerization reaction FT-IR monitoring. Indeed, a secondary dehydration reaction with -OH-free loss was highlighted in cutin C on thin layer, by means of FT-IR analysis, unlike other cutins. The FT-IR analysis of cutin melt-condensation reaction showed a higher reaction progression in sample A than other cutins. Besides, the unsaturated compounds and aromatics were not found in cutin C chromatograms, in very low amount (approximately 1% and <0.2% respectively) in cutin B samples, and in significant amount (approximately 5% and 6% respectively) in cutin A.

The solubility assay showed that all studied samples (A, B and C) were insoluble in chloroform, and were soluble in ethanol. The sample B appeared soluble at 60°C even in buthyl glycole, and in THF at room temperature, while the sample C is sparingly soluble in glycol and THF in the same conditions. The sample A appeared soluble in ethanol and THF and freely soluble in dowanol PMA and butyl glycole at 60°C, and in MIBK at 90°C. The cutins B and C didn't show a good solubility in dowanol PMA at 60°C and in MIBK at 90°C.

Although the products derived from $NaOH/H_2O_2$ and carboxylate route, have higher purity, and a lower polydispersity; considering the best reaction yields and the good compatibility with organic solvents in tested conditions, the gummy mass obtained from a simple alkaline hydrolysis could be the best product obtained, among those studied, with a good chance to use as raw material for bio-resin preparation.

Acknowledgements
Research activities carried out within the BIOCOPACPLUS project, funded under LIFE+ Environment Policy and Governance project application (Grant Agreement No. LIFE13 ENV/IT/000590).

We are indebted to Mirella Baldini for technical assistance in GC-MS analysis.

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