Title: Novel configurations for a citrus waste based biorefinery: From solventless to simultaneous ultrasound and microwave assisted green extraction

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Abstract: Innovative green extraction configurations for the biorefining of a biomass waste (citrus peel) were developed in this work. Non-conventional energy sources, such as microwaves (MW) and ultrasound (US) irradiation, were directly irradiated to the fresh orange peel using a versatile MW coaxial dipole antenna. This particular MW configuration enabled us to build two new extraction systems: 1) a coaxial solventless MW-assisted extraction approach (SMWAE) and, 2) a simultaneous ultrasound coaxial MW-assisted hydrodistillation (US-MWHD). The yield and chemical composition of the essential oils of the orange peel obtained by the two innovative approaches were analyzed as a function of the extraction time and compared with the coaxial microwave hydrodistillation (MWHD) and conventional hydrodistillation (CH). The EOs were chemically characterized by GC and GC-MS analysis. The residue mash was then used to extract pectin by a MW-assisted procedure. Structure and thermal stability of the pectin were investigated by FTIR and TG.

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Dear Editor,

We would like you to consider the attached manuscript, "Novel configurations for a citrus waste based biorefinery: from solventless to simultaneous ultrasound and microwave assisted green extraction" for publication in Bioresource Technology.

In this work we propose innovative extraction configurations for the biorefining of a biomass waste (citrus peel) by means of microwave coaxial technology. This particular MW configuration allowed us to build up two new extraction systems: 1) A solventless MW-assisted extraction approach, which features space savings, clean (solventless) and fast processing and, 2) the simultaneous uses of ultrasound and MW energy in an integrated cavity-less device. By using our green configurations, we obtained high quality products with substantially lower energy consumption, low environmental impact (solventless) and rapid processing times.

The manuscript includes an in-depth discussion of the following aspects: i) the kinetic investigations of the percentage yield and chemical composition of orange peel essential oil (EO) obtained by four different extraction methodologies, ii) the pectin isolation from the solid residues after the EO extraction by a fast MW-assisted procedure, iii) the full characterization of the isolated value added compounds using several techniques (GC, GC-MS, FTIR, TG); and, iv) insights into the extraction mechanism by the novel MW-assisted extraction approaches.

We hope that this work will be of interest to readers of Bioresource Technology. I declare that the work presented in the manuscript is original and is not under consideration elsewhere.

Best regards,
Celia Duce

Graphical Abstract (for review)
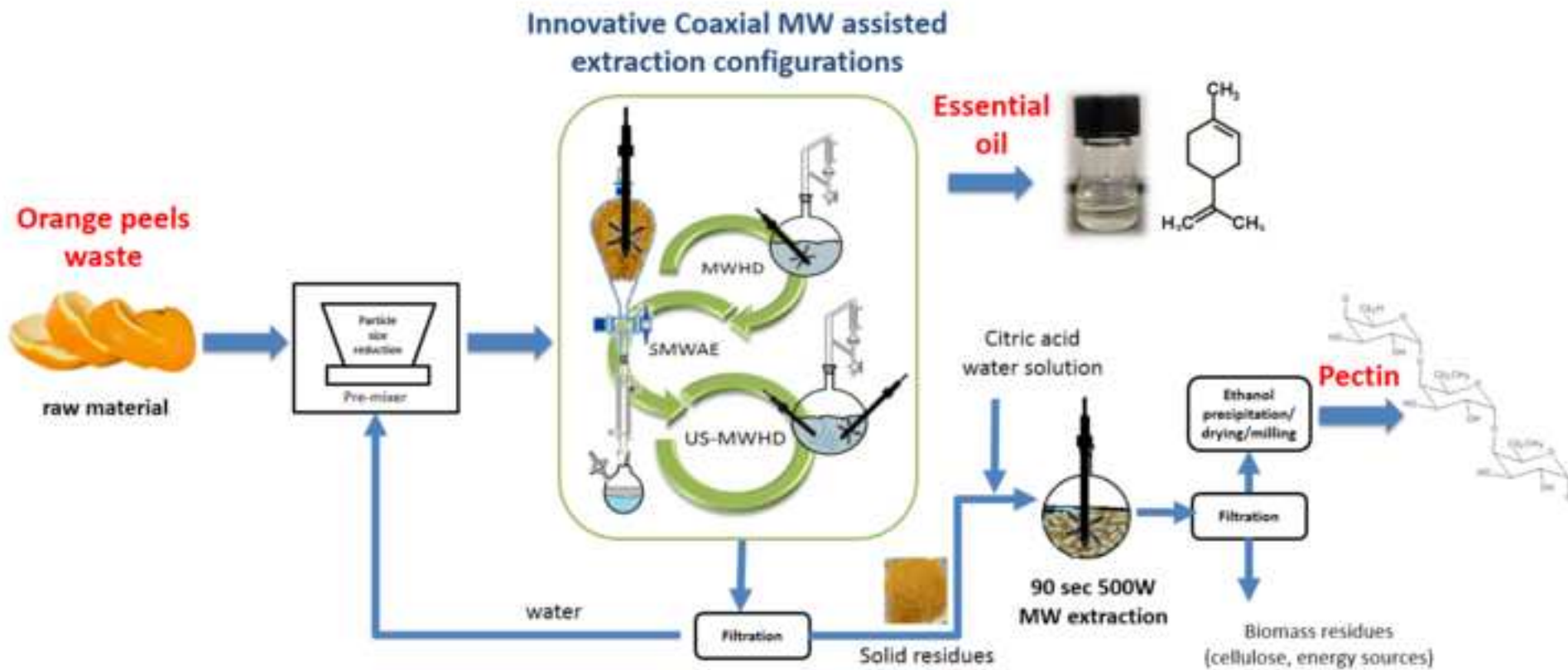
- Biorefining of EOs and pectin from citrus waste
- Innovative solventless MW-assisted extraction approach
- Simultaneous ultrasound coaxial MW-assisted hydrodistillation
- Pectin extraction by fast MW-assisted extraction
- Reductions of time, energy and solvents involved in the extraction processes

Novel configurations for a citrus waste based biorefinery: from solventless to simultaneous ultrasound and microwave assisted green extraction

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ABSTRACT

Innovative green extraction configurations for the biorefining of a biomass waste (citrus peel) were developed in this work. Non-conventional energy sources, such as microwaves (MW) and ultrasound (US) irradiation, were directly irradiated to the fresh orange peel using a versatile MW coaxial dipole antenna. This particular MW configuration enabled us to build two new extraction systems: 1) a coaxial solventless MW-assisted extraction approach (SMWAE) and, 2) a simultaneous ultrasound coaxial MW-assisted hydrodistillation (US-MWHD). The yield and chemical composition of the essential oils of the orange peel obtained by the two innovative approaches were analyzed as a function of the extraction time and compared with the coaxial microwave hydrodistillation (MWHD) and conventional hydrodistillation (CH). The EOs were chemically characterized by GC and GC-MS analysis. The residue mash was then used to extract pectin by a MW-assisted procedure. Structure and thermal stability of the pectin were investigated by FTIR and TG.

Keywords: biorefinery, orange peel waste, microwave extraction, essential oils, pectin.

1. INTRODUCTION

Novel extraction processes for separating valuable compounds from different feedstocks are guided by the principles of green chemistry (Rombaut et al., 2014). The isolation of particularly active substances such as essential oils (EOs), natural drugs, pigments and biopolymers need to be carried out within operation units where the energy consumption and processing time are minimized. The raw materials involved should preferably come from renewable, low cost and easily available sources.

To address these requirements, the use of non-conventional energies, such as microwaves (MW) or ultrasound (US) and, feedstocks such as non-food biomass or food waste, represent a feasible alternative (Lin et al., 2013).

EOs obtained from the hydrodistillation of aromatic herbs illustrate the energy savings and extraction time reductions possible when the process is assisted by MW (Li et al., 2014). In addition to MW-assisted extraction (MWAE) (Chen, S.S. and Spiro, 1994), alternative configurations such as the supercritical fluid extraction (SFE) (da Silva et al., 2016) and ultrasound assisted extraction (Tiwari, 2015) have been reported.

The ultrasound assisted extraction (UAE) method improves extraction efficiency by increasing the penetration of solvents into plant cells via cavitation, and preventing the degradation of extracts (Tekin et al., 2015). All of these novel approaches produce compounds with the same quality as those obtained by conventional techniques, but more rapidly and at a lower cost.

We have developed an innovative configuration to obtain EOs from different aromatic herbs, where classical Clevenger hydrodistillation was modified by using a coaxial dipole antenna to apply microwave energy inside the aqueous extraction medium (Flamini et al., 2007; Gonzalez Rivera et al., 2016). Coaxial MW hydrodistillation extraction (coaxial

MWHD) enabled us to reduce the heating time and energy demand, compared to the conventional hydrodistillation technique (CH). In addition, it represents a safe and easy alternative approach to scale up the process compared to the conventional closed-device MW oven type methodologies, such as microwave-assisted hydrodistillation (MWHD) (Golmakani and Rezaei, 2008), microwave steam distillation (MWSD) (Chemat et al., 2006), and microwave assisted hydrodiffusion and gravity (MWHG) (Vian et al., 2008)

The economic viability of hydrodistillation to isolate EOs depends on the chemical yield, the market price of the essential oil, the cost of equipment and the energy consumption. Hydrodistillation results in high amounts of solid residues as by-products. These solid residues are mainly biopolymers, such as polyphenols or insoluble cellulosic materials (Santana-Méridas et al., 2014), which can be used as biomass precursors to obtain energy or bio-compound derivatives. Some authors have pointed out that these by-products can be recycled in order to increase the shareholder value and maximise the profit of the new hydrodistillation processes (Gonzalez Rivera et al., 2016; Santana-Méridas et al., 2014). This is in agreement with the concept of biorefinery (Boukroufa et al., 2015; Lin et al., 2013; Rombaut et al., 2014).

This paper describes innovative green extraction configurations to transform a biomass waste (citrus peel) into valuable essential oils and biopolymers. Different approaches using non-conventional energy sources, such as MW and US irradiation, are feasible due to the use of the versatile MW coaxial dipole antenna, which is easily immersed directly into the extraction media. This particular MW configuration enabled us to build two new extraction systems: 1) the coaxial solventless MW-assisted extraction approach (SMWAE) and, 2) simultaneous ultrasound coaxial MW-assisted hydrodistillation (US-MWHD).

The SMWAE configuration was inspired by the microwave hydrodiffusion and gravity (MHG) technique (Vian et al., 2008) however with the great advantage of using the coaxial antenna directly immersed into the biomass source (non-oven method).

In addition, the versatility of the MW cavity-less technology enabled us to design an integrated extraction scheme, where MW and US can be irradiated simultaneously. To the best of our knowledge, this is the first time that an integrated MW-US device has been applied to EO isolation by hydrodistillation.

The orange peel organic waste was used to test the proposed systems given its intrinsic economic (low or null cost, available waste) and physicochemical properties (high moisture, high quantity of EOs and biopolymers). In addition, limonene, the main compound of the EOs of orange peel, has a wide market and a competitive price since it is used in the formulation of fragrances, soaps and creams, flavours, etc. (Ciriminna et al., 2014). Emerging applications of EOs as versatile green chemicals and precursors for the synthesis of nanoparticles, also make them more attractive (Kahrilas et al., 2014).

In the first step of our approach, the yield and chemical composition of the EOs of orange peel obtained by the two innovative approaches were analyzed as a function of the microwave extraction time, and were also compared with the results obtained by coaxial MWHD and conventional hydrodistillation (CH). The biorefinery concept was then explored when the solid residues after the EO extraction were used as a pectin source. Pectin is a very valuable and popular biopolymer used in many different applications, such as food packaging (Eca et al., 2015), pharmaceutical applications (Srivastava and Malviya, 2011a) and biofilms (Cavallaro et al., 2013). Pectin extraction was also assisted by MW irradiation using the coaxial configuration. The presented methodologies are environmentally friendly and perfect for use in the field of sustainable chemistry.

2. MATERIALS AND METHODS

2.1 Materials.

Fresh orange peel waste was provided by the canteen of our research campus, and was frozen until used. Before each extraction process, the frozen orange peels were ground for 10 s using a laboratory blender it. The resulting precursor was then left under static air until reaching room conditions before being loaded into the extraction device. Tap water and ethanol (Carlo Erba) were used as solvents for EO extraction and pectin precipitation, respectively. Anhydrous citric acid was used to acidify the pectin acid extract suspensions. A water to substrate (W/S) ratio of 6:1 (w/w) was used for all hydrodistillations.

2.2 Solventless microwave assisted extraction (SMWAE).

The experimental procedure and the equipment configuration are described below. A total of 100 g of ground orange peel was loaded into the MW assisted device. The SMWAE vessel is a 250 ml separatory funnel-type glassware wrapped by a metallic grid to prevent the leakage of MW out of the extraction medium and to ensure safe operating conditions. A second grid was placed inside the extractor to act as a holder of the solid sample and to allow the outflow of volatiles gases (mixture of EO and water). The extractor outlet is connected to a double surface condenser followed by a glass container to collect the aqueous extracts.

MW energy was applied by means of a coaxial dipole antenna, introduced through the top inlet of the reactor where a choke is also adapted. The antenna, protected and sealed by a closed end glass tube, is immersed in the solid sample (see Fig. 1-a).

The MW source was a magnetron oscillator which supplies up to 1200 W of continuous MW power at 2450 MHz. The solid substrate was heated using 400 W of MW power at

different extraction times. The MW energy was established in accordance with the literature for the extraction of EOs from orange peel using MHG (Boukroufa et al., 2015).

A power of 400 W was applied as the best compromise between the percentage yield obtained and to avoid damaging the sample. The temperature profiles were recorded through IRT Imaging at different extraction times. Thermographic images were obtained using the Ti9 Thermal Imager (Fluke Corporation). Images were analyzed using SmartView 3.14.52.0 (Fluke Thermography)

2.3 Simultaneous Ultrasound and Microwave irradiation hydrodistillation (US-MWHD).

We used a similar configuration already proposed and described in a previous paper (Ragaini et al., 2012). The extraction device is a simple modification of the coaxial MWHD extractor, which uses a 1 liter three-necked flask equipped with a coaxial MW antenna, an ultrasound metallic horn, and a Clevenger condenser. The US horn and the MW antenna are immersed inside the liquid phase and located perpendicularly to each other. This guarantees the best performance, safe operation conditions and no perturbation of the two energy sources used simultaneously.

The laboratory coaxial US-MWHD device is shown in Fig. 1-b. A Vibra cell sonics (VCX 750) sonicator with a frequency of 20 kHz and a tip diameter of 13 mm was used to apply the US irradiation. The MW source was the same as that for the SMWAE. In a typical experiment, 100 g of orange peel was loaded together with 600 mL of tap water in the coaxial US-MWHD modified Clevenger extractor. 40% of the maximum nominal power of both US and MW was then applied (500 W of MW power and 40% amplitude US) during the induction time (15 min). Once the hydrodistillation had started, the MW energy was

reduced to 300 W and the effect of US irradiation was explored for 60, 5, and 0 min keeping the MW power constant.

On the basis of these preliminary experiments, we concluded that US irradiation is needed only during the induction time (15min) and during the first 5 min of the hydrodistillation. These conditions were thus set to explore the yield and chemical composition as a function of the hydrodistillation time.

2.4 Coaxial microwave assisted hydrodistillation (MWHD).

The MWHD apparatus and experimental set-up are fully described in our previous work (Gonzalez Rivera et al., 2016) and shown in Fig. A1-a. Briefly, 100 g of orange peel was mixed with 600 mL of tap water. The aqueous substrate dispersion was loaded into a 1000 mL flask vessel and heated in the MW assisted device, for 16 min using 500 W of MW power while continuously stirring the mixture at 250 rpm. Once the hydrodistillation had started, the power was reduced to 300 W and kept at steady state conditions for different lengths of time, in order to study the kinetics of the process.

2.5 EO extraction by conventional hydrodistillation (CH).

Hydrodistillation using conventional heating was also performed for the orange peel waste. The extraction was thermally activated using a standard electric mantle operating at 250 W (see Fig. A1-b).

2.6 Pectin extraction by a coaxial MW assisted process.

After the EOs of the orange peels had been extracted by the four different approaches, the solid residues were separated by filtration and dried at 60°C for 12 h. The pectin extraction was carried out in a 200 mL coaxial microwave assisted glass reactor using 5 g of dry solid residues dispersed in 100 mL of 0.5 M citric acid aqueous solution and heated using 500W of power for 90 s. The MW power was stopped once the boiling point of the dispersion was

reached. The viscous dispersion obtained was centrifuged (3000 rpm, 5 min), and the decanted supernatant mixed with ethanol using a 1:2 volume ratio. The mixture was left for 12 h to allow a complete pectin precipitation. The pectin, separated by centrifugation (3000 rpm, 5 min), was washed several times with pure ethanol, dried until constant weight at 60 °C, ground, and analyzed by FTIR and TGA. The yield % (w/w) was determined by gravimetric analysis (weight of the pectin extracted/weight of the dry solid residues used). The same procedure was carried out for the solid residues obtained in each extraction procedure mentioned above.

2.7 EO Yield and chemical composition analysis.

The EO yield % (w/w) and the chemical composition were investigated as a function of the extraction time for all the various extraction approaches described above. The percentage yield was determined by gravimetric analysis (weight of the EO extracted/weight of the fresh orange peels used) on samples taken at different times and each experiment was performed at least three times. The chemical composition of the EOs was determined through gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS) analysis. For the chemical composition analysis, three time intervals were selected: the EO collected from: I) 0 to 5min, II) 5 to 30min and III) 30 to the end of the extraction process. The EOs were collected, decanted, weighed and stored at 4 °C in dark containers for further characterization.

2.8 GC and GC-MS Analysis.

GC-MS analysis was carried out using a gas chromatograph (Agilent, Model 6890N, Palo Alto CA, USA) equipped with a split-splitless injector, an Agilent model 7683 autosampler and an Agilent HP5-MS fused silica column (5% phenyl-methylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 μm). The column temperature was programmed to be heated

from 60 °C to 246 °C at a rate of 3 °C/min, and kept under isothermal conditions for 20 min. The injector was maintained at 250 °C. Helium was used as carrier gas at a flowing rate of 1.0 mL/min through the column. Samples were diluted in hexane with a ratio of 1:100 and (1 μL) were injected in the split mode (1:20). The GC was fitted with a quadrupole mass spectrometer with an Agilent model 5973 detector. The MS conditions were as follows: ionization energy, 70 eV; electronic impact ion source temperature, 200 °C; quadrupole temperature, 150 °C; scan rate, 3.2 scan/s; mass range, 30-480 u. Agilent MSD ChemStation E.01.00.237 was used to analyse the mass spectra and chromatograms. The linear retention indices (RIs) for all of the compounds were determined by injection of a hexane solution containing the C_8-$C2_6$ series of n-alkanes (Van den Dool and Dec. Kratz, 1963) and were compared with authenticated samples from our database. The constituents of the EOs were identified by comparing their retention indices and their mass spectra with a home-made library or with the literature data and the mass spectra databases, including HPCH2205 (Adams, 2007) and W8N05ST (Wiley ver. 8.0 & NIST, ver. 5.0). Analytical GC was carried out in a gas chromatograph (Agilent, Model 7890A, Palo Alto, CA), equipped with a flame ionization detector (FID), an auto-sampler (Agilent, Model 7683B), an Agilent HP5 fused silica column (5% phenyl-methylpolysiloxane), 30 m × 0.25 mm i.d., film thickness 0.25 μm, using the Agilent ChemStation software system. The oven temperature was programmed from 60 °C to 250 °C (3 °C/min), the injector temperature was set at 250 °C and the carrier selected gas was helium flowing at 1.0 mL/min. The splitting ratio was 1:10, and the temperature of the detectors was 300 °C. Percentages of individual components were calculated based on GC peak areas, without FID response factor corrections.

2.9 Thermogravimetric analysis.

The TG experiments were performed using a TA Instruments Thermobalance model Q5000IR. TG measurement were performed using Pt crucibles at a rate of 10 °C/min, from 30 °C to 700 °C under nitrogen flow (25 ml/min). The amount of sample in each measurement varied between 1 and 3 mg.

2.10 Fourier transform infrared spectroscopy.

Infrared spectra were recorded using an FT-IR Agilent Technologies Spectrophotometer model Cary 640, equipped with a universal attenuated total reflectance accessory (ATRU). A few micrograms of dry and purified pectin powder sample were used with the following spectrometer parameters; resolution: 4 cm^{-1}, spectral range: 600-4000 cm^{-1}, number of scans: 128. Agilent spectrum software was used to process FTIR spectra.

Fig. 1 about here

3. RESULTS AND DISCUSSION

Table 1 summarizes the main results obtained after the biorefining of orange peel waste to EO and the pectin isolation and purification by the four extraction methodologies: CH, MWHD, SMWAE and US-MWHD.

The total extraction time was strongly dependent on the different approaches. Maximums yield of 1.18%, 1.57%, 1.56% and 1.54% (w/w) were obtained in 5, 60, 76 and 155 min for the SMWAE, US-MWHD, MWHD and CH, respectively. While limonene (>95%) was the main compound extracted, a higher amount of valencene (up to 1.4%) was obtained using US-MWHD. The pectin isolated from the solid residues after EO extraction yielded up to 21% (w/w) in 90 s, and showed different qualities regarding the degree of esterification.

Sections 3.1 and 3.2 provide a further discussion of the main findings, a full characterization of the isolated add value compounds and insights into the extraction mechanism by the two novel extraction approaches.

Table 1 about here

3.1 Orange peel EO isolation: kinetics and chemical composition analysis

Figure 2 shows the kinetics investigations of the percentage yield of orange peel EO obtained by all the extraction configurations used (see Sections 2.2 to 2.5). Two main extraction time periods (see Fig. 2) are visible in the kinetic curve of orange peel EO extraction. The first period corresponds to the induction time (t_{ind}), where all the applied energy is absorbed and used by the extraction mixture to achieve the boiling point (section in Fig. 2 where the yield is zero). The hydrodistillation then starts and a second time period (t_{extrac}) is needed to separate all the essential oils (second period, % yield increase in Fig. 2). During the t_{ind}, the EO volatile compounds begin to be released into the extraction media from exogenous secreting glands, according to the washing and diffusion model (Milojevic et al., 2013) but without actually separating. Since the temperature is continuously increased, an increase in pressure then follows within the exogenous glands (due to the expansion of the volatile compounds), leading to the cellular wall breakage and the release of the essential oil. The EO volatile compounds are then extracted, due to their diffusion out of the endogenous sites, and separated by hydrodistillation until no more EO is obtained (end of the extraction process). The total extraction time (t_{total}) is the sum of t_{ind} and t_{extrac}. As highlighted in Fig. 2, the orange peel EOs obtained using the US-MWHD, MWHD and CH show the classical hydrodistillation behavior. When hydrodistillation is involved in the

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extraction process, a complete isolation of EOs is feasible and no significant differences in the percentage total yield were observed (maximum yield of about 1.6 %), while the SMWAE approach isolated around 75% of the maximum EO (taking the maximum % yield obtained by CH as a reference).

Fig. 2 about here

The t_{ind} was strongly dependent on the different approaches. The CH took the longest, where t_{ind}=35 min and t_{total}=155 min were needed to complete EO isolation. The use of MW accelerated both the t_{ind}: SMWAE (3 min) < US-MWHD (15-16min) ≈ MWHD (16 min) and, the t_{extrac}: SMWAE (2 min) < US-MWHD (45min) < MWHD (60 min).

The t_{ind} and t_{extrac} of the SMWAE methodology were strongly reduced since no solvents were used in this approach. All the energy applied was directly absorbed by the fresh organic matrix, leading to a superheating of the moisture and to a faster separation. Figure 3 reports the IR thermographic images of the temperature profiles of the sample at different irradiation times with the SMWAE device. A selective and uniform fast heating from inside to the outside through the fully-packed organic bed was produced by *in situ* MW irradiation using the coaxial antenna. The shape and size of the solventless device and the use of the choked coaxial antenna produced an optimal system, as demonstrated by the football-shaped specific absorption (iso-SAR) surfaces (Longo and Ricci, 2007). This particular heating approach generates a local overheating which could destroy some structures (for example the cell membrane) or increase the mobility of some chemical species rather than others.

The energy transfer to the material takes place through: i) dipolar heating, due to the permanent or induced dipole moment of the chemical species involved; ii) surface effects at

the boundary between different materials (Maxwell-Wagner effect); iii) the Joule effect, due to the electrical conduction of the material (Tombari et al., 1999). The energy transfer also depends on the amount of coordinated water of the molecules, since water shows strong absorbance of microwave radiation at 2.45 GHz. Different EO components can be selectively heated on the basis of the value of their dipole moment, electric impedance and of the coordinated water (Gonzalez Rivera et al., 2016; Tombari et al., 1999). However, since the maximum yield obtained only reached 75% (w/w) of that reached by methods involving hydrodistillation, we believe that the washing effect stops after the initial minutes of the extraction and the hydrodiffusion effect predominates.

In order to increase the total % yield, we explored longer extraction times (up to 20 min). It was necessary to adjust the MW response of the extraction media because MW absorption decreased once the moisture was separated. Small amounts of a strong MW absorber (silicon carbide) were introduced within the solid extraction mixture.

Fig. 3 about here

The simultaneous uses of US and MW also had a positive effect on the t_{total} reduction while a complete yield % isolation was feasible. In particular, a clear effect on the t_{extrac} reduction was observed. The t_{ind} was slightly affected since the same energy amount is needed in order to heat the same amount of water-substrate, as in the MWHD approach. Compared with the ultrasound assisted modified Clevenger hydrodistillation using conventional heating (Pingret et al., 2014), the US-MWHD had the advantages of the t_{total} diminutions since t_{ind} is highly reduced by the use of MW. The overall extraction process is accelerated by the synergy of the sonochemical MW-assisted process. A possible mechanism based on

the cavitation effects of the ultrasound waves has been proposed (Saleh et al., 2016), highlighting that when a bubble collapses near a surface, i.e. a plant leaf, it deforms taking on a doughnut shape leading to asymmetric collapse and creating high-speed solvent jets directed towards the solid surface. These jets are very effective in breaking cell membranes, producing a hammer type action (Saleh et al., 2016).

The MWHD approach was also faster than the conventional heating methodology saving more than 50% of the t_{total}.

The chemical composition of the orange peel EOs obtained by the four methodologies is shown in Fig. 4. A complete characterization by GC and GC-MS analysis led to the identification of 11 volatile compounds (see Table A1). The orange peel EOs isolated using the different methodologies showed similar a chemical composition in line with those reported in the literature (Farhat et al., 2011). Limonene and myrcene were the two main compounds extracted in all four cases. Limonene was homogeneously isolated during all the extraction processes and, its relative quantity did not show significant differences regarding the extraction approach (see Fig. 4-a). The same trend was shown by myrcene. Thus, high quality orange peel EOs, with a limonene content above 95%, can be obtained by all the processes investigated.

Fig. 4 about here

The effects of the extraction time and approach on the chemical composition were most evident in terms of the content of various minority compounds. Figure 4-b shows that a higher amount of valencene (up to 1.4%) can be obtained by the US-MWHD. More than limonene, the content of valencene in orange EOs usually determines its commercial value and quality, due to its value in terms of its aromatic and flavoring properties (Elston et al.,

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2005). The lowest valencene amount was obtained using the CH, SMWAE and MWHD extraction approaches (ranging from 0.2 to 0.4%). On the other hand, γ-terpinene was isolated, in a higher amount, during the first 5 min of the CH, while it was not extracted using the US-MWHD. Similarly, n-decanal, n-octanal and β-pinene were only extracted with concentrations lower than 0.2%, with the same time period as the CH (see Table A1, column 4).

3.2 Citrus waste-based biorefining: Pectin MW assisted extraction, characterization and economic projections.

The extraction and commercialization of EOs from citric waste is attractive since limonene, the main compound of EO from orange peel, has a high market price (around USD $14/Kg) and increasing demand worldwide (Ciriminna et al., 2014). Therefore, the exploitation of citrus derivative waste have focused on the optimizing EO isolation. Once a low cost and clean processing has been guaranteed, the synergy of using a cheap raw material to produce a highly value-added product can ensure the success of our new approach.

Using our novel configurations, we obtained high quality orange peel EOs, with a yield and chemical composition in line with the literature, with a substantially lower energy consumption, and clean and fast processes. In addition, we further explored the biorefining concept in order to maximise the profit of our green methodologies, focusing on the pectin isolation from the solid residues after EO extraction. Orange peel waste has around 25% (w/w) of pectin and the citrus pectin market price is around $10/kg (Ciriminna et al., 2015). Thus, the pectin and EO isolation from citrus waste is a "win win" business deal.

There are several reports on pectin isolation from citrus peel including conventional acid extractions (Srivastava and Malviya, 2011b) and MW-assisted processes (Boukroufa et al.,

2015; Guo et al., 2012; Hosseini et al., 2016; Prakash Maran et al., 2013). The effects of the mineral (HCl, H_2SO_4) and organic (citric, acetic) acids used to promote pectin extraction have also been explored (Sayah et al., 2014). While MW extraction has proved to be the greenest approach with extraction times ranging from 3 to 20 min, and yields from 15 to 30 %(w/w) (Boukroufa et al., 2015; Guo et al., 2012; Hosseini et al., 2016; Prakash Maran et al., 2013), the use of organic acids, such as citric acid, is more desirable because its involves less toxic and less hazardous operation conditions.

In the biorefining step of the orange peel waste, we explored whether the EO extraction step had an effect on the yield and chemical properties of pectin isolated, once the EO extraction and purification processes had been established. A different pectin yield was obtained and depended on the raw solid residues: CH (21.1%) > MWHD (18.7%) ≈ SMWAE (18.6%) > US-MWHD (16.1%). The lowest pectin yield was obtained using the solid residues from US-MWHD EO extraction, while CH resulted in the highest pectin amount. This suggests that fast heating (MW activation) and the cavitation effect (US) causes some surface damage during EO extraction, thus some pectin is released and lost. Besides the differences in percentage pectin yield regarding the raw solid residues, the obtained results are in agreement with the percentage yield reported in the literature (Boukroufa et al., 2015; Guo et al., 2012; Hosseini et al., 2016; Prakash Maran et al., 2013) (see Table A2).

The structural characterization and the thermal stability of the extracted pectins were investigated by FTIR and TG. The infrared spectra of the extracted pectins are shown in Fig. 5-(a). The broad band at 3200-3600 cm^{-1} is ascribed to O-H stretching vibration due to the inter- and intra-molecular hydrogen bonding of the galacturonic acid polymer (Gnanasambandam and Proctor, 2000) and O-H of moisture. Bands in the range of 2800-

300 cm^{-1} refer to C-H absorption of CH$_2$ of carbohydrates and the CH$_3$ group of galacturonic acid methyl ester. The absorption in the range 1220-1445 cm^{-1} is due to the bending of C-H overlapping with the COO$^-$ stretching. The region in the range 800- 1145 cm^{-1} contains skeletal C-O and C-C vibration bands of the glycosidic bond and pyranoid ring.

Interesting structural characteristics of the pectin biopolymer can be obtained by studying the bands that appear at about 1630 and 1730 cm^{-1}. The first is due to the symmetrical stretching vibration of the COO$^-$ group of acids, while the second is due to the carbonyl group from methyl ester (Gnanasambandam and Proctor, 2000; Manrique and Lajolo, 2002). Typically, the degree of esterification (DE) is well correlated to the ratio between the absorbance at 1730 cm^{-1} and the sum of absorbances at 1630 and 1730 cm^{-1} (Manrique and Lajolo, 2002). Using the calibration curve obtained by Manrique and Lajolo (2002), we found that the pectin samples extracted by SMWAE, US-MWHD and CH approaches showed a DE of between 55-60%, while pectin samples extracted by MWHD showed a DE close to 25%. Pectin samples with a DE in the range of 25-50% are classified as low methoxyl pectin (LM), while pectin samples with a DE in the range 50-80% are classified as high methoxyl pectin (HM).

HM pectins form gel with a pH lower than 3.6 and in the presence of a cosolute (typically sucrose) which stabilize the junction zones by promoting hydrophobic interactions between ester moieties. LM pectins form gel in the presence of Ca^{2+}, which acts as a bridge between carboxyl groups. (Thakur et al., 1997). It has been reported that the chemical characteristics of the extracted pectin depend on both the extraction conditions and the sourcing material (Fidalgo et al., 2016). We believe that the difference in the DE shown by the pectin isolated from the solid residues after MWHD is more related to the heterogeneity of the raw

material, since the same pectin extraction conditions were applied across the different solid residues.

Fig. 5 about here

The thermal behavior of the pectin samples extracted by CH, SMWAE, US-MWHD, MWHD is similar to that reported in the literature for standard pectin (Einhorn-Stoll et al., 2007; Fisher et al., 2002). DTG curves under nitrogen flow of the pectin samples are reported in Fig. 5-(b), while experimental temperatures and the percentage weight loss of thermal degradation steps are listed in Table 2. The first small loss at about 100°C is due to solvent (water and/or ethanol) loss and is lower for pectin extracted by the SMWAE methodology, and the highest for MWHD extracted pectin.

The pectin degradation starts at 150°C with the esteric bond breakage, has a maximum at 235°C and a shoulder at 333°C due to the galacturonic ring breakage (Einhorn-Stoll et al., 2007). It should be noted that the MWHD sample showed the highest solvent mass loss and the mass loss at 156°C since the esteric bonds do not break, highlighting its higher hydrophilic behavior due to the lowest DE as shown by FTIR analysis.

Table 2 about here

In summary, we have developed innovative extraction configurations which can be used to exploit food derivative biomass waste. The following features make our integrated processes highly attractive, thus guaranteeing successful applications in the chemical industry:

- MW energy can be directly irradiated to fresh orange peel, without using any chemical solvent, leading to the SMWAE technology. The combined use of the choked coaxial antenna and the funnel-like extractor configuration represents an optimal system, regarding the football-shaped specific absorption (iso-SAR) surfaces (Longo and Ricci, 2007) which facilitate a complete and nearly uniform MW heating of the sample.

- The versatility of the coaxial MW antenna facilitates, for the first time, the simultaneous use of ultrasound and MW energy in an integrated cavity-less device (US-MWHD) for essential oil isolation.

- The extraction process of pectin is also fast and green thanks to the use of the coaxial MW technology.

4. CONCLUSIONS

A food-waste feedstock was transformed into a valuable products in a fast and eco-friendly novel MW assisted extraction configuration where the energy and solvents involved in the processes are minimized. The SMWAE extraction device involves space savings, and involves clean (solventless) and fast processing. The US-MWHD cavity-less configuration was also a faster methodology compared with the use of only MW and the US-assisted CH, which also leads to the highest quality EO isolation with. The biorefining of EOs and pectin from a citrus waste maximise the profit of our proposed green methodologies, which involve safe operability, faster processing and are easily scaled-up.

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Figures captions

Figure 1. Experimental extraction configurations used in this work: (a) coaxial solventless MW assisted extraction (SMWAE) and (b) simultaneous US and coaxial MW assisted hydrodistillation (coaxial US-MWHD). Insert details: (I) MW generator, (II) coaxial antenna, (III) choke, (IV) 250 mL funnel-like glass extractor, (V) double surface condenser, (VI) extracts collected beaker, (VII) clevenger condenser, (VIII) US metallic horn, (IX) 1 L glass vessel, (X) stirrer and (XI) sonicator.

Figure 2. Kinetics investigation of the percentage yield of orange peel EO obtained by (■) CH, (○) MWHD, (*) SMWAE, (▲) US-MWHD.

Figure 3. IR thermographic images of temperature profiles at different MW irradiation times of the orange peel EO isolation using SMWAE.

Figure 4. Chemical composition of the orange peel EOs obtained by CH, SMWAE, US-MWHD and MWHD at different extraction times, (a) composition higher than 2%, (b) composition lower than 2%.

Figure 5. FTIR spectra (a) and DTG curves under nitrogen flow of extracted pectin by (━) CH, (━) MWHD, (━) SMWAE and (━) US-MWHD.

Figure 1
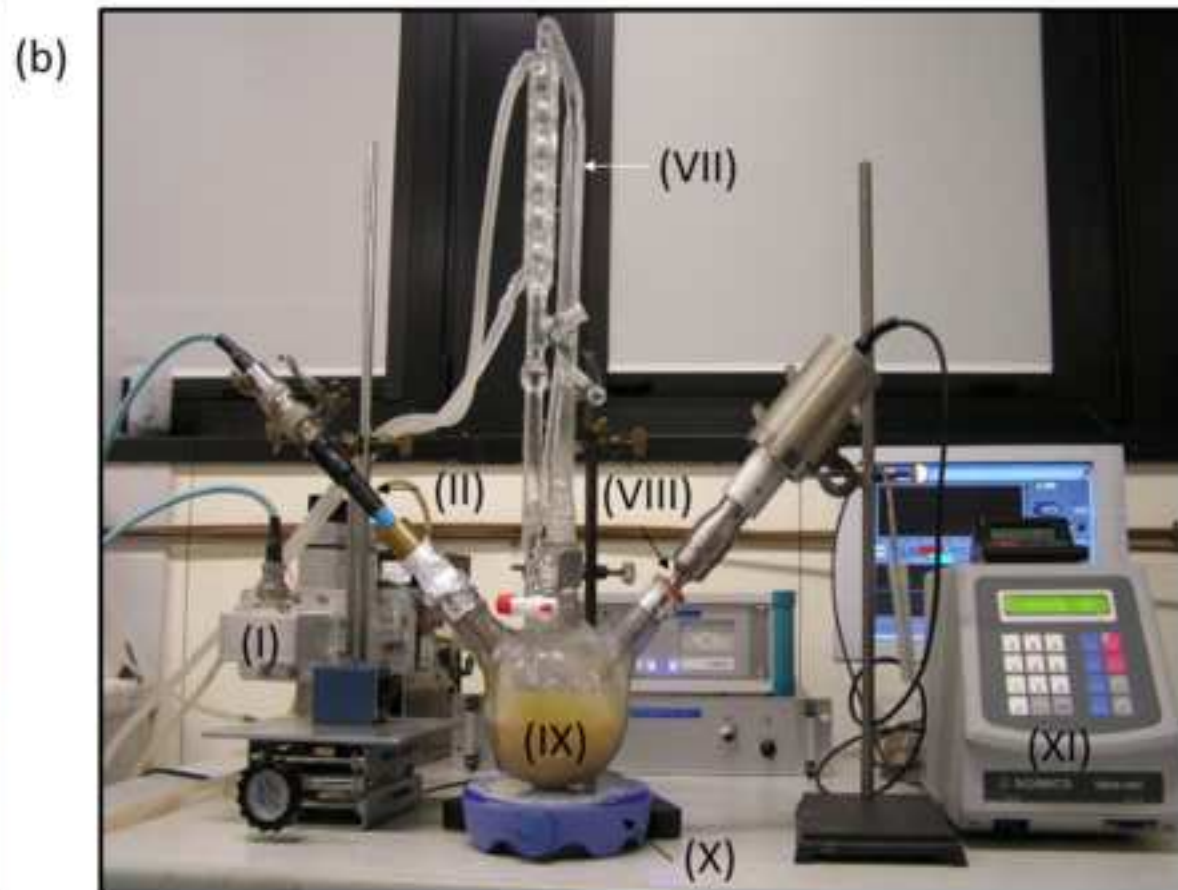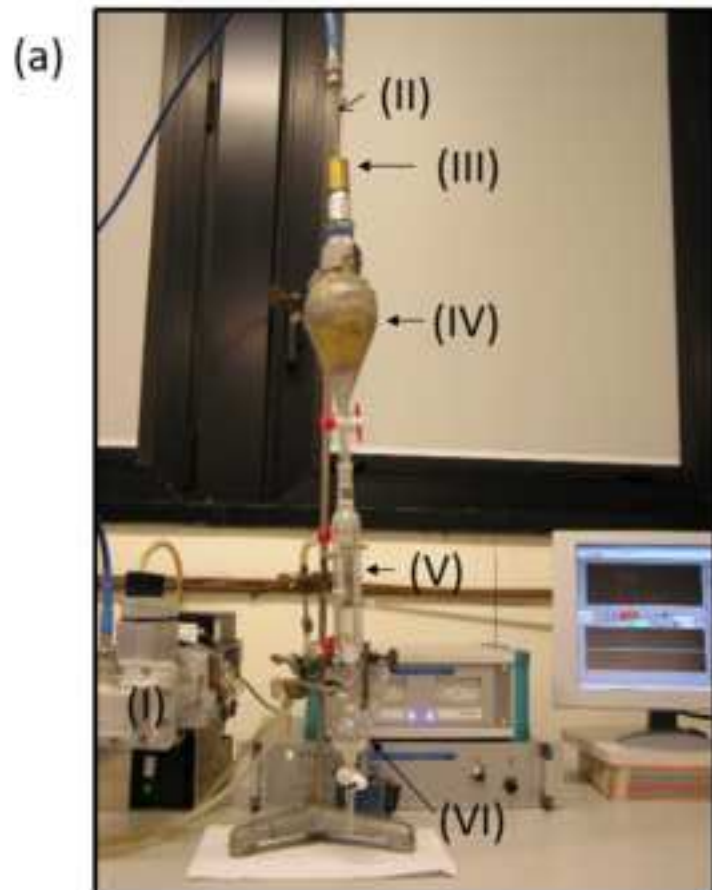
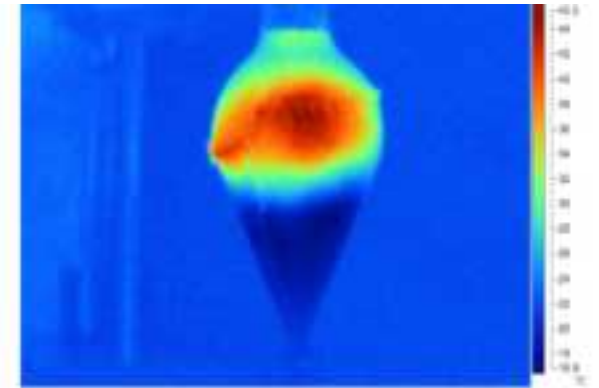**Figure 2**
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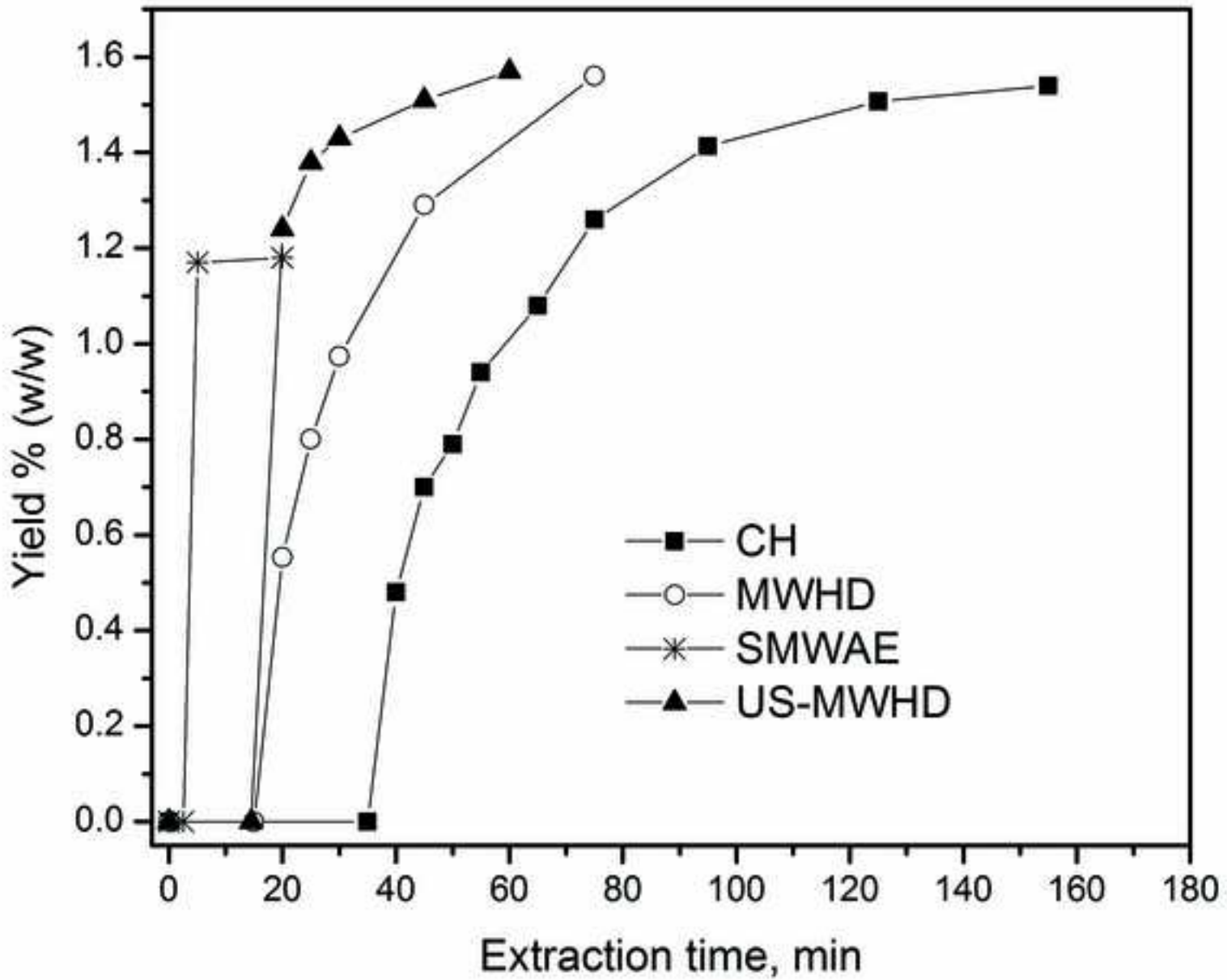
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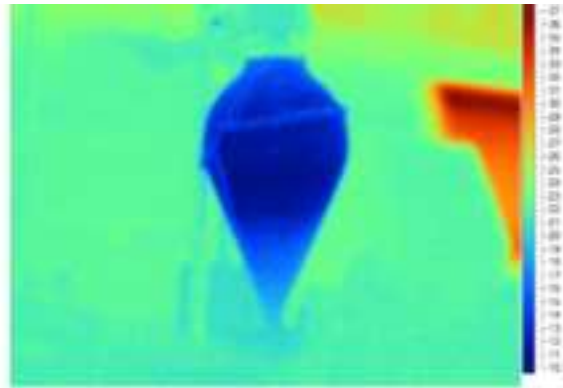
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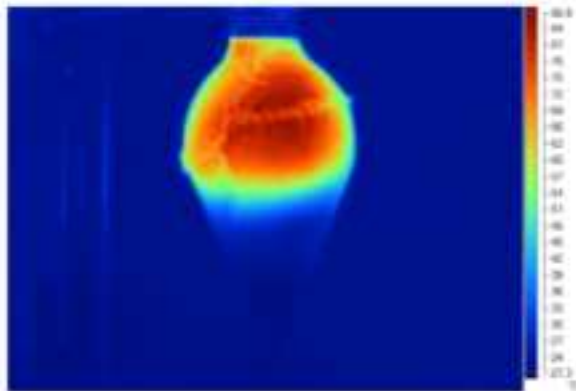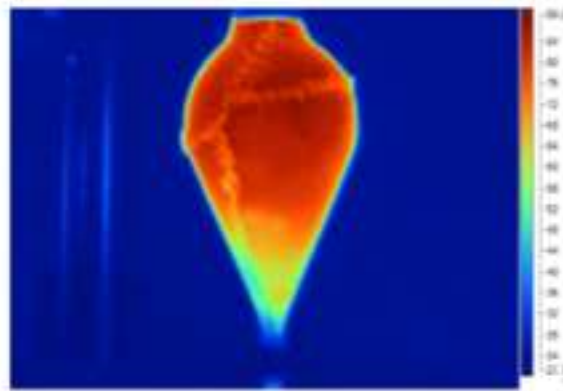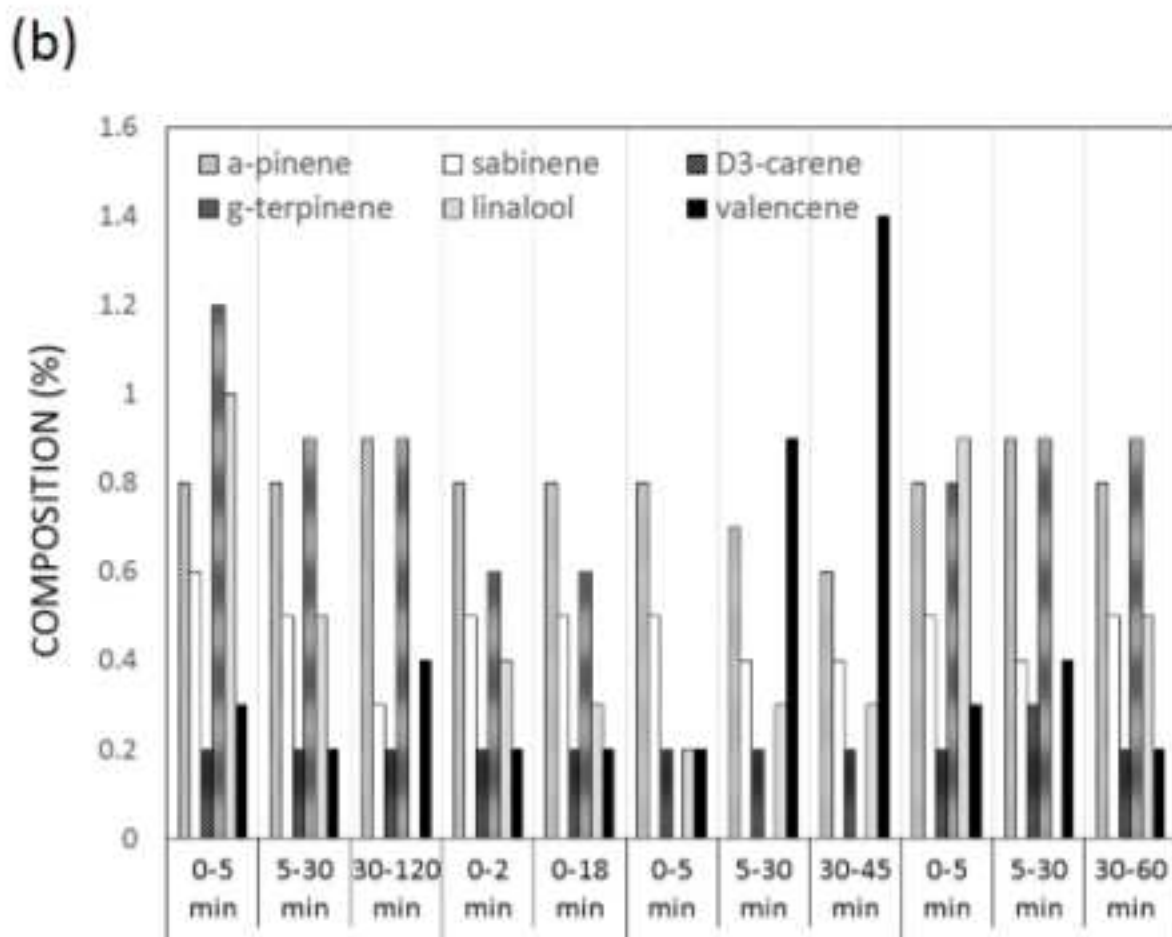
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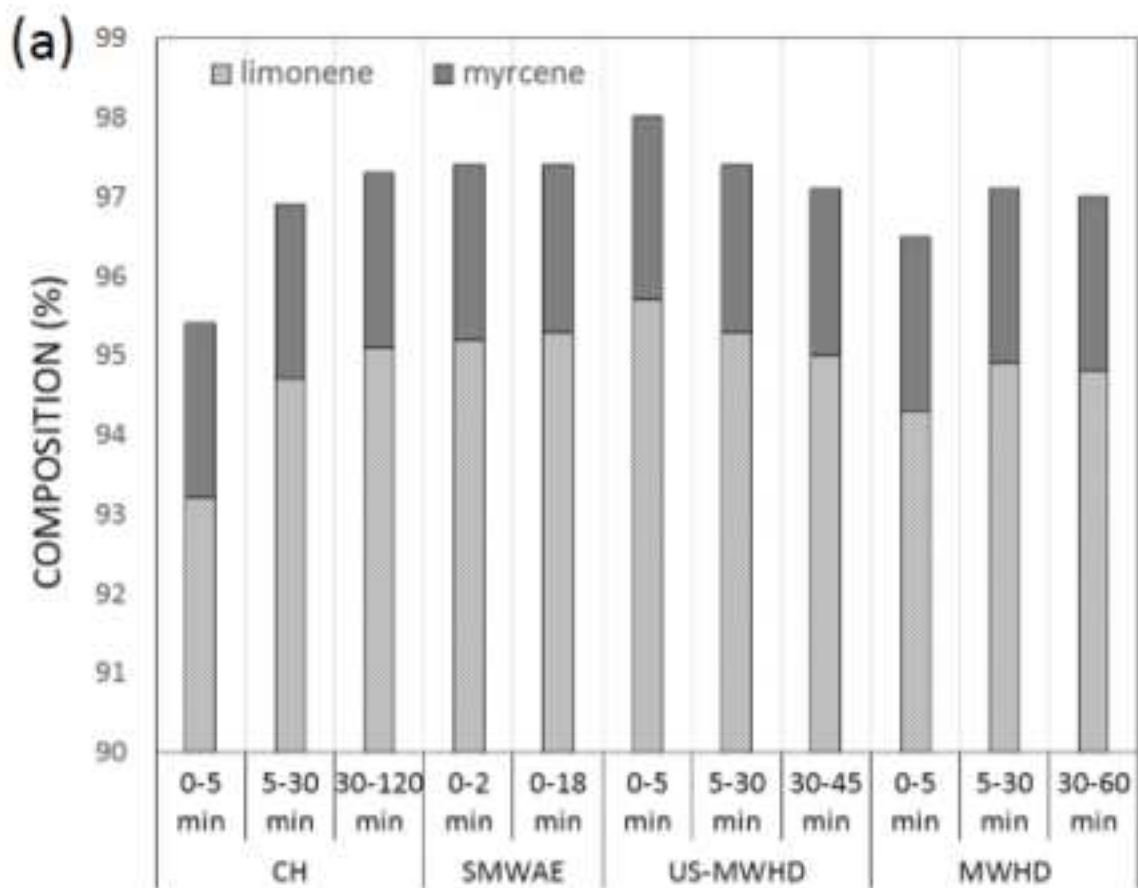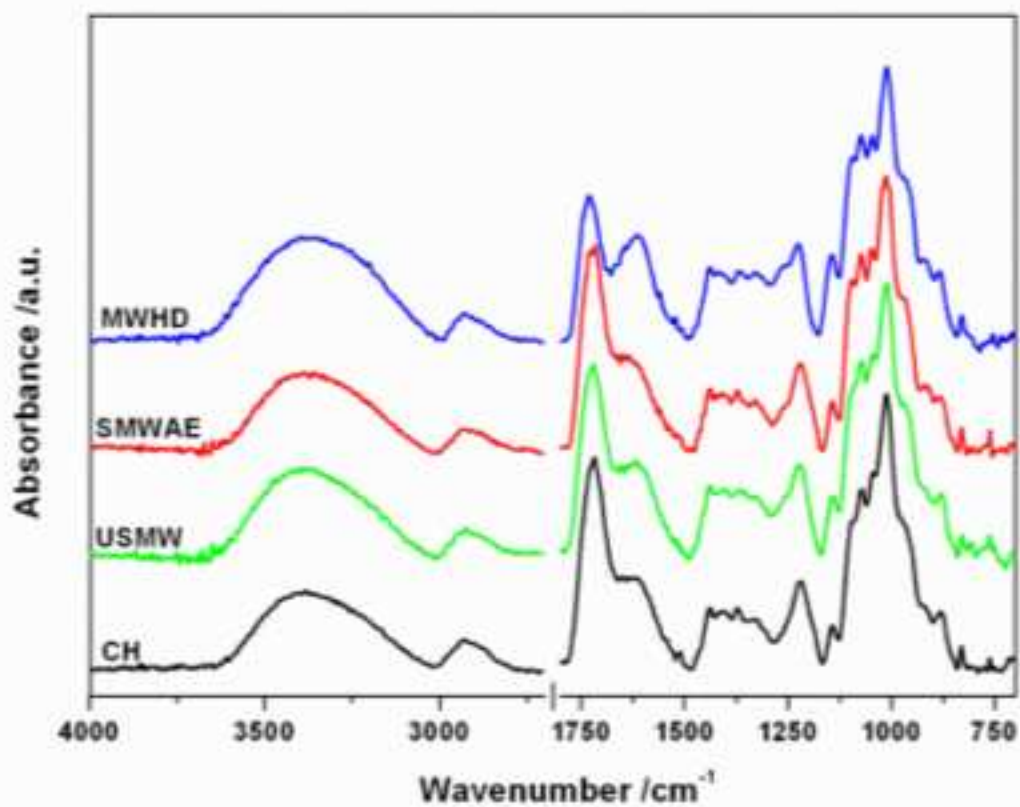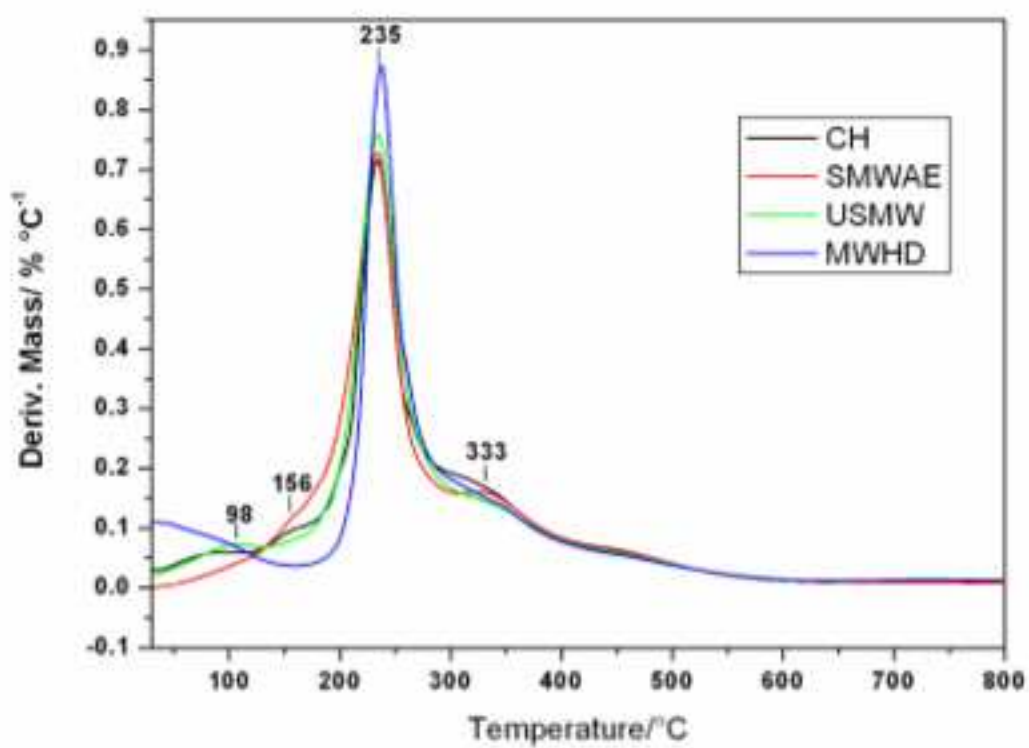
Table 1

Table 1 Main factors and process parameter values established by the different extraction methods used for biorefining of orange peels waste developed in this work.

Extractive method	Orange peels waste biorefining					
	EO extraction				Pectin extraction[+]	
	Solvent	EO extraction conditions		Amount of valuable compounds in the isolated EO		Pectin yield (wt. %)	Quality (DE)*
		t_{TOT} (min)	Yield (wt. %)	Limonene (%)	Valencene (%)		
CH	Water	155	1.54	95.1	0.4	21.1	55-60
MWHD	Water	76	1.56	94.8	0.2	18.7	<25
US-MWHD	Water	60	1.57	95.0	1.4	16.1	55-60
SMWAE	solventless	5	1.18	95.2	0.2	18.6	55-60

[+]pectin isolated in 90 seconds using 500W of MW power at 2450 MHz
*determined by FTIR spectroscopy

Table 2

Table 2: Experimental temperatures and percentage weight loss of thermal degradation steps of pectine samples extracted by CH, SMWAE, US-MWHD, MWHD

Step	CH	SMWAE	US-MWHD	MWHD
1	90-100°C (4.8%)	90-100°C (1.3%)	90-110°C (6.6%)	30-110 °C (10.4%)
2	156°C shoulder	156°C shoulder	156°C Shoulder	-
3	235°C (68.3%)*	235°C (72.0%)*	235°C (66.8%)*	235°C (62.8%)*
4	333°C* shoulder	333°C* shoulder	333°C* shoulder	333°C* shoulder
Residue at 800°C	26.9%	26.7%	26.6%	26.8%

* Total mass loss in the temperature range 150-335°C

Electronic Annex
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