ACS Sustainable Chemistry & Engineering

Research Article

pubs.acs.org/journal/ascecg

Eco-Friendly Extraction of Pectin and Essential Oils from Orange and Lemon Peels

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Supporting Information

ABSTRACT: Pectin and *d*-limonene were extracted from waste orange and lemon peel by an innovative eco-friendly process using only water as dispersing medium and microwaves as energy source. Both bioproducts were characterized by diffuse reflectance infrared (DRIFT) spectroscopy, and the pectin samples also by scanning electron microscopy (SEM). For pectin and essential oils extracted from waste lemon peel, the results obtained at laboratory and semi-industrial scales are compared. The high yields obtained, the quality of the bioproducts and the environmental viability of the process support forthcoming commercialization of the technology.

KEYWORDS: *Pectin, Waste citrus peel, Biopolymer, Biorefinery, Bioeconomy*

INTRODUCTION

Pectin, the main heteropolysaccharide component of the primary walls of nonwoody plant cells, is a valued commercial hydrocolloid, whose excellent health[1] and functional properties justify a wide and increasing use in the food, cosmetic, medical and pharmaceutical industries.[2] Global demand for functional foods has been constantly rising for the last 2 decades, translating into a market for hydrocolloids,[3] of which pectin is the most labile and consumer friendly. In medicine, pectin is used in wound healing preparations and in special medicine adhesives, like colostomy devices. Pectin is a natural part of the human diet and its intake is highly beneficial, as it binds to cholesterol in the gastrointestinal tract (reducing blood cholesterol levels), and slows glucose absorption by trapping carbohydrates.[4] Numerous drugs are encapsulated within a pectin film to protect the gastric mucosa and to allow sustained release of the active ingredient into blood.[5] In cosmetic and in personal care products, besides being used as a natural texturizer for ointments, oils and creams, and as thickener and stabilizer for shampoos, lotions and hair tonics, pectin is now used as an effective skin antiaging agent.[6] In 2013, a leading chemical magazine reported that the global pectin market had reached $850 million.[7]

From the structural point of view, pectin belongs to a family of pectic polysaccharides containing 1,4-linked α-D-galactosyluronic residues. The most abundant are homogalacturonan (HG) and rhamnogalacturonan-I (RG-I). Usually, pectins are composed of interconnected HG and RG-I regions, also called "smooth" (HG) and "hairy" (RG-I) regions, whose relative proportions determine the flexibility and mechanical properties of the cell walls, and vary depending on the pectin source and extraction method. HG is a linear chain of α(1-4)-D-galacturonic acid residues, whereas RG-I is a branched polymer containing a repeated disaccharide backbone of α(1-4)-D-galacturonic acid and α(1-2)-L-rhamnosyl residues, [4)-α-D-GalpA-(1,2)-α-L-Rhap-(1], branched with different side chains of varying degrees of polymerization. The galacturonic acid-rich (HG) regions of pectin molecules enhance molecular interactions between cells and the polysaccharide, while the branched galactose-rich (RG) hairy regions promote the formation of entangled structures.[8,9]

Received: December 17, 2015
Revised: February 9, 2016
Published: February 11, 2016

DOI: 10.1021/acssuschemeng.5b01716
ACS Sustainable Chem. Eng. 2016, 4, 2243−2251

Pectin has been shown to inhibit the actions of galectin-3, a β-galactoside-binding protein associated with cancer progression. The discovery by Gao et al. that the side chains and backbone play distinct roles in regulating this inhibiting activity of RG-I fragments is valuable for producing highly active pectin-based galectin-3 inhibitors.[10]

Some of the carboxyl groups in the galacturonic acid residues, both in HG and RG regions, are methyl esterified or exist as uronic acid salt. The degree of esterification (DE) of pectin influences its gelation conditions, which in turn determine the specific applications: pectins with high DE (methoxyl content > 50%) tend to form gels at low pH, stabilized by hydrophobic interactions,[11] whereas pectins with low DE tend to form gels electrostatically stabilized with metal cations.[12] In general, the pectin polymer contains between 300 and up to around 1000 saccharide units (150 kDa molecular weight).

Today, pectin is mainly extracted from citrus peel via hydrolysis in hot water under tightly controlled conditions.[13] We have lately surveyed open opportunities toward larger scale production of this valued biopolymer, including efforts to broaden the sourcing raw materials and improving the extraction methods.[14]

On the other hand, the essential oils present in the rind of citrus fruits are mostly *d*-limonene, which is a cyclic terpene with exceptional functional properties. Commercially, *d*-limonene is recovered via mechanical treatment of the citrus fruit or via steam-distillation from orange and lemon peel, for numerous applications, ranging from biosolvent, fragrance, insecticide and food additive, the global production being estimated at 70 000 tons in 2014.[15]

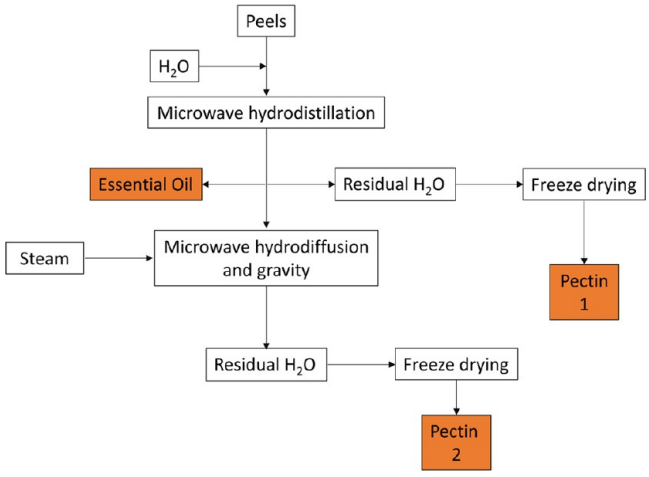
Microwave-assisted solvent-free modification of pectin was reported in 2012,[16] when the use of microwaves to concomitantly produce pectin and limonene from citrus peel was first described by Clark and co-workers, using an organic solvent as extraction medium.[17] Similarly, optimal microwave assisted extraction of pectin from orange peel in acidified water was reported in 2013.[18] According to Chemat and co-workers,[19] green and sustainable extraction methods of bioproducts rely on reduced energy consumption, using alternative solvents and renewable natural products, and ensuring a safe and high quality final product. The solvent-free microwave extraction (SFME), and microwave extraction combining hydrodiffusion and gravity (MHG) methodologies fall into this category.[20] Following these general procedures, we were able to develop an innovative process, using SFME or MHG followed by freeze-drying (FD). This allows recovering simultaneously pectin and essential oils from fresh and waste orange and lemon peel, using only water as dispersing medium and microwaves as energy source. To the best of our knowledge, this is the first reported simultaneous extraction of pectin and essential oils by an environmentally clean process.

The bioproducts extracted at laboratory and semi-industrial scales were characterized using DRIFT spectroscopy, while the pectin fibers were also characterized by scanning electron microscopy (SEM). The high yields obtained, the quality of the bioproducts and the environmental viability of the process support forthcoming commercialization of the technology in the context of the emerging bioeconomy.[21]

LABORATORY SCALE EXTRACTION AND ANALYSIS

Experimental. Pectin and essential oils were extracted from fresh and waste peel of lemon and orange via microwave hydrodistillation followed by freeze-drying (Pectin 1), or by hydrodiffusion and separation by gravity prior to freeze-drying (Pectin 2), according to the general procedure represented in Scheme 1.

Scheme 1. Extraction of Pectin and Essential Oils via Microwave Hydrodistillation Followed by Freeze Drying (Pectin 1) or by Microwave Hydrodiffusion and Gravity Followed by Freeze Drying (Pectin 2)



The extraction and separation were carried out using commercially available extractors (NEOS for hydrodistillation, NEOS-GR for the experiments in which steam was used, and MAC 75 for the scaled up process). No organic solvent was employed. The extracted samples are named in Table 1 (where FL = fresh lemon; WL = waste lemon; FO = fresh orange; WO = waste orange; P1 = pectin isolated via microwave distillation followed by freeze-drying and P2 = pectin obtained via microwave distillation, hydrodiffusion and gravity, followed by freeze-drying; EO = essential oil).

Table 1. Samples Obtained from Fresh and Waste Orange and Lemon Peels

raw material	Pectin 1	Pectin 2	essential oil
fresh lemon peel	FL-P1	FL-P2	FL-EO
waste lemon peel	WL-P1		WL-EO
fresh orange peel	FO-P1	FO-P2	FO-EO
waste orange peel		WO-P2	WO-EO

Each sample obtained was extensively analyzed: the surface morphologies of pectins were characterized by scanning electron microscopy (SEM) with a Hitachi S4100-1, operating at 25 kV. The surfaces were previously sputter-coated with a gold layer ~20 nm thick, to avoid charging effects during observation. Their structure was assessed by infrared Fourier transform spectroscopy in diffuse reflectance mode (DRIFT), using a Mattson FTIR spectrometer with a Specac Selector, in the range 4000−400 cm^{-1} (wide band MCT detector), at 2 cm^{-1} resolution. The spectra were the result of ratioing 1000 added scans for each sample against the same number of scans for the background (grinded KBr). For the essential oils, a drop was deposited on a KBr grinded bed and the spectra also obtained in diffuse reflectance.

Analysis of Pectins. Figures 1 and 2 compare the SEM microphotographs of pectin extracted from different sources and by different methods. Figure 1 shows that the pectin samples extracted from fresh peels through microwave distillation, hydrodiffusion and

DOI: 10.1021/acssuschemeng.5b01716
ACS Sustainable Chem. Eng. 2016, 4, 2243−2251

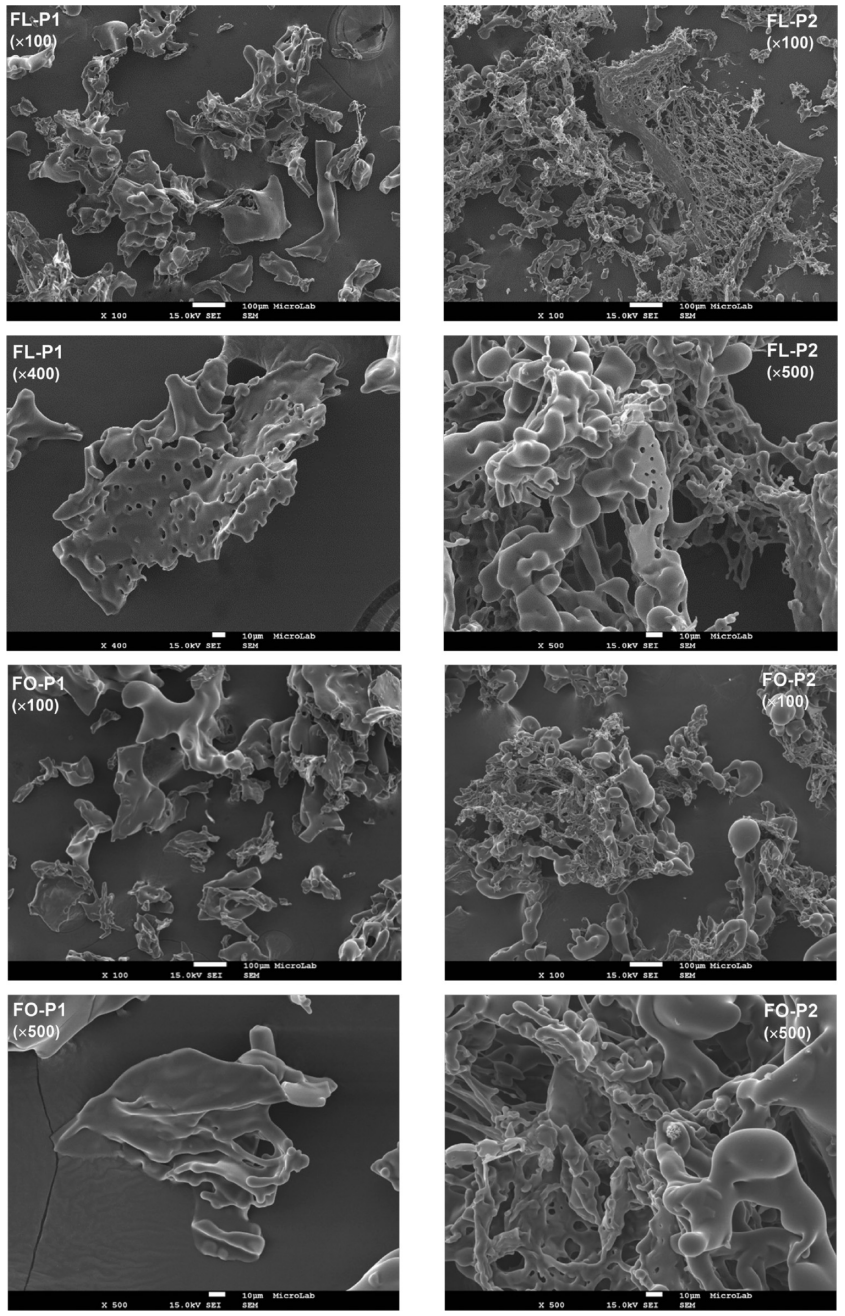


Figure 1. SEM microphotographs of Pectin 1 (left) and Pectin 2 (right) extracted from fresh lemon (FL) and fresh orange (FO) peels, at different magnifications, as indicated.

gravity followed by freeze-drying (MHG-FD, Pectin 2) have a filamentous morphology, whereas those extracted through microwave distillation followed only by freeze-drying (SFME-FD, Pectin 1) are formed essentially by aggregates with smooth surfaces.

Comparing Figures 1 and 2, we conclude that the pectin fibers obtained from waste peels are more filamentous than those obtained from fresh ones, irrespective of the extraction procedure. This observation at the micron scale must reflect the presence of different contents of hairy domains of RG-I.[22]

The DRIFT spectra of pectin samples obtained from the different sources are compared in Figure 3, and the full band assignments are given in Table S1 in the Supporting Information. The infrared spectrum of pectin has been extensively analyzed since the pioneering work by Filippov.[23−25] It is expected that the specificities of the source and extraction method result in different esterification degrees (DE), and different proportions of HG and RG-I regions.

The relative intensities of the νO—H, νC—H, νC═O and δC—H bands observed in Figure 3 depend on the pectin source (orange or lemon), suggesting a detailed analysis by spectral regions: 3800−2200 cm^{-1} and 1800−700 cm^{-1}, shown in Figures 4 and 5, respectively.

The 3800−2200 cm^{-1} region is dominated by broad bands related to the stretching vibrations of hydroxyl and CH_x groups (νO−H and νC−H). The νO−H band, with maximum at 3200−3300 cm^{-1}, is associated with hydroxyl groups of the pyranose rings and adsorbed water, interacting in different intra- and intermolecular hydrogen bonds: for orange peel pectins this band is narrower and insensitive both to the source (fresh or waste) and to the extraction procedure; in contrast, pectin from lemon peel is affected by the extraction procedure, since Pectin 1 has a larger fraction of OH groups with weaker hydrogen-bond interactions, responsible for the high wavenumber shoulder (at ∼3500 cm^{-1}).

DOI: 10.1021/acssuschemeng.5b01716
ACS Sustainable Chem. Eng. 2016, 4, 2243−2251

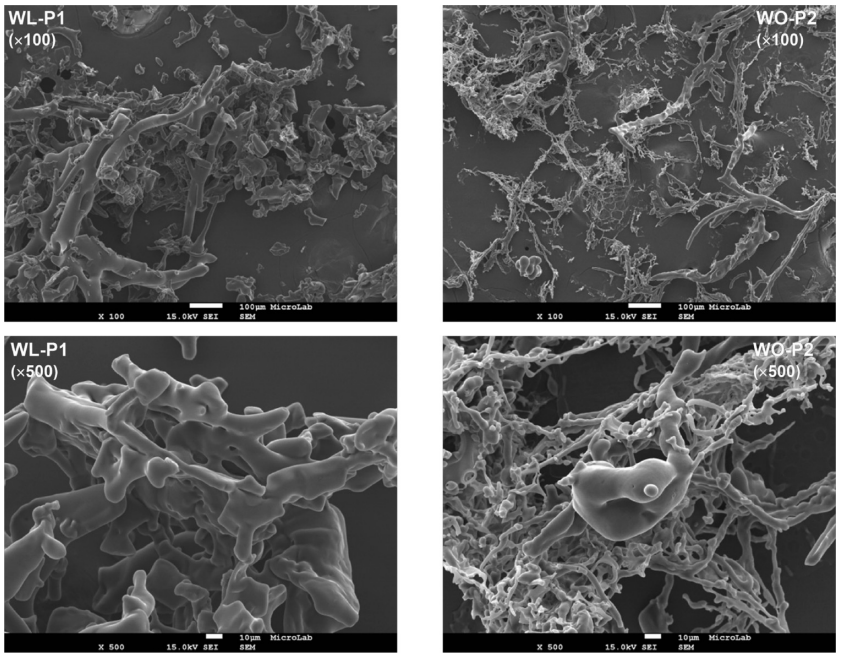


Figure 2. SEM microphotographs of Pectin 1 extracted from waste lemon (left) and Pectin 2 extracted from waste orange (right) at different magnifications, as indicated.

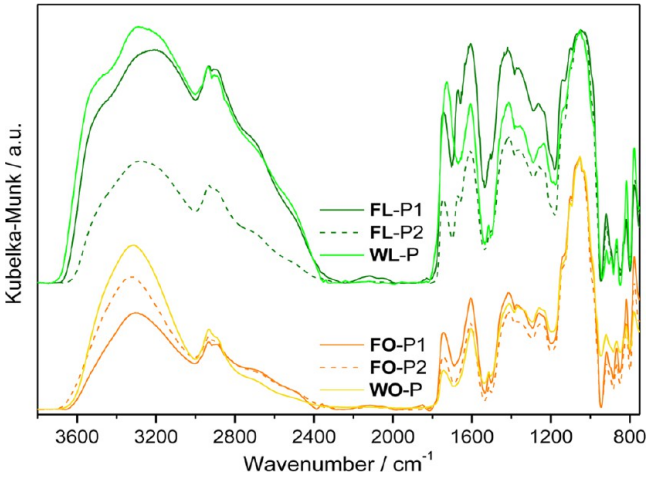


Figure 3. DRIFT spectra of lemon and orange pectin extracted from fresh peels and waste residues, as indicated. The spectra were normalized to the 1048 cm^{-1} band (νC−O mode).

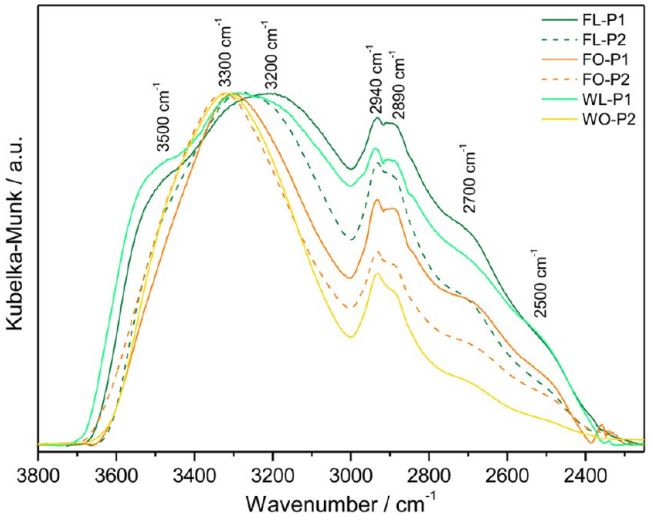


Figure 4. DRIFT spectra in the 3800−2200 cm^{-1} region, normalized to the maximum in this region (νO−H band).

The maximum at 2930−2940 cm^{-1} is assigned to νCH and $\nu_{as}CH_3$ modes of the pectin backbone, and to $\nu_{as}CH_2$ modes of galactose and arabinose rings of the "hairy" regions. The shoulder at 2860−2890 cm^{-1} correlates with the ν_sCH_3 modes of the backbone and also with different νCH modes of the pyranose rings, both in HG and RG regions; the definition of this shoulder is poorer and its relative intensity is lower for P2 samples, but the mode overlapping in this region is so extensive that this observation cannot be useful for estimating the proportions of HG and RG regions. Two shoulders located near 2700 and 2500 cm^{-1} are frequently assigned as satellite ν(CO)O−H bands of carboxylic acid dimers.[26]

The two strong bands in the 1800−1500 cm^{-1} region, with maxima at ∼1730 and ∼1610 cm^{-1}, are assigned to the stretching modes of carbonyl groups (mostly from esterified galacturonic acid, $\nu(C{=}O)_{ester}$) and of carboxylate groups ($\nu_{as}COO^-$), respectively. The weaker band at 1670 cm^{-1}, clearly observed for pectin extracted from fresh lemon, may correlate with nonesterified hydrogenated acidic carbonyl groups, $\nu(C{=}O)_{acid}$. The main CH_x and C−O−H deformation modes appear partially overlapped, in the 1500−1200 cm^{-1} region. The band at 1230 cm^{-1}, visible in all the spectra, and the one at 1330 cm^{-1}, only detected in the orange derived samples, are assigned to in-plane deformation modes of alcohol hydroxyl groups in the pyranose rings of the pectin chain, $\delta(C{-}O{-}H)_{pyranose}$.

The band at 1370 cm^{-1} is assigned to the symmetric methyl deformation mode, $\delta_s(CH_3)$, of ester methyl groups in the galacturonic rings and of rhamnose rings of the pectin backbone. The corresponding antisymmetric mode is hardly identified as a shoulder, at ∼1440 cm^{-1}. The other ester-related band in this region is the C−O−C stretching mode, $\nu(C{-}O{-}C)_{ester}$, which appears at 1265 cm^{-1}, partially overlapped with the 1230 cm^{-1} band. The band at 1410 cm^{-1} is assigned to the symmetric stretch of carboxylate groups, ν_sCOO^-, present in all the samples. The group of five intense and partially overlapped bands observed in the 1200−950 cm^{-1} region is typical of pectin. These are assigned to the skeletal and C−O−C stretching modes of the pyranose ring, $\nu(C{-}C)_{pyranose}$ and $\nu(C{-}O{-}C)_{pyranose}$, to C−O−C stretching vibrations of the glycosidic bond,

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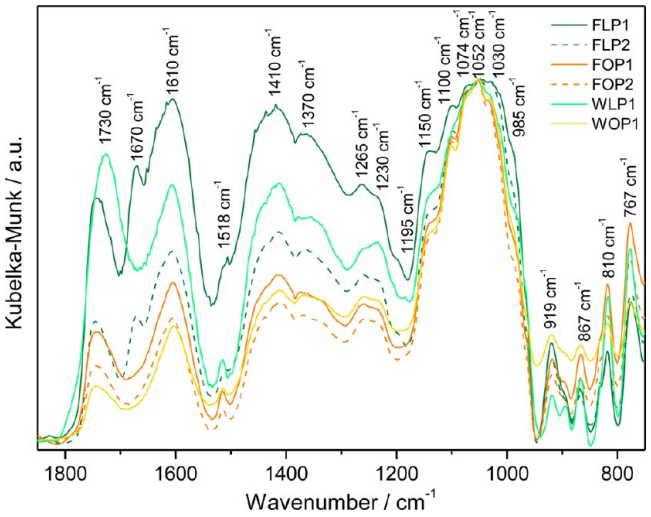

Figure 5. DRIFT spectra in the 1800−700 cm^{-1} region, normalized to the maximum in this region.

$\nu(C-O-C)_{glycoside}$, and to a combination of the $\nu C-OH$ and $\nu C-C$ modes from the pyranose rings. The 950−700 cm^{-1} region contains the bands related to the external deformation vibrations of methyl, methylene and methyne groups. The band at 919 cm^{-1} is assigned to the rocking mode of the ester methyl group, $\rho(CH_3)_{ester}$.

The degree of esterification (DE) of pectin may be represented by the percent or fraction of esterified carboxyl groups or by the methoxyl content of total pectin. DRIFT spectra have been used for this purpose, using the spectral region related to the carbonyl and carboxylate stretching modes. An empirical relationship has been derived, based on a titrimetric calibration of the DRIFT results, taking into account only the band at 1760−1745 cm^{-1}, assigned to the carbonyl of esterified groups.[27] Other authors improved this method, considering also the band at 1640−1620 cm^{-1}, assigned to carboxylate groups, and estimating the DE by a ratio of areas of the esterified carboxyl (assumed in the interval 1844−1682 cm^{-1}) and the sum of the esterified and nonesterified carboxylate (in the range 1682−1532 cm^{-1}) (eq 1):[28]

$$DE = A_{1844-1682}/(A_{1844-1682} + A_{1682-1532}) \quad (1)$$

These DE values presented better precision and better accuracy when compared to gas chromatographic (GC) results on the quantification of methanol obtained by pectin de-esterification. A similar approach was followed in this work, supported by a detailed analysis of the 1800−1500 cm^{-1} spectral region: given that the carbonyl groups may be involved in different hydrogen bonds, and therefore contribute with more than one component, the best estimates for the $\nu C{=}O$ and $\nu_{as}COO^-$ band areas were obtained by decomposing the whole 1900−950 cm^{-1} region (two consecutive absorption zeros) in a sum of Gaussian components, using a nonlinear least-squares fitting method. The areas, wavenumbers and assignments of the components are shown in Table S2 in the Supporting Information. The DE values were obtained by eq 2, and are summarized in Table 2:

$$DE = \sum A_{\nu(C=O)ester}/(\sum A_{\nu(C=O)ester} + A_{\nu(C=O)acid} + A_{\nu asCOO^-}) \quad (2)$$

The polygalacturonic acid content has also been estimated by DRIFT spectroscopy, from the total areas of the carbonyl absorption bands. Excellent correlation with pectin calibrated standards was obtained.[29] In the present work, we took into account that HG regions are richer in galacturonic residues than RG ones. Since all the pectin regions have similar contents in C−O−C bonds (pyranose rings and glycosidic), but the carbonyl and carboxylate groups are only present in the galacturonic residues, the contribution of the HG regions to the whole pectin structure will be proportional to the ratio:

$$HG \propto (\sum A_{\nu(C=O)} + A_{\nu asCOO^-})/(\sum A_{\nu(C-O-C)} + \sum A_{\nu(C=O)} + A_{\nu asCOO^-}) = [A_{1780-1590}/(A_{1780-1590} + A_{1190-950}] \quad (3)$$

This ratio yields just a trend when comparing the different samples. Yet, the results summarized in Table 2 are relevant and provide useful insight in identifying the optimal conditions and raw material source for commercial pectins obtained through the present green methodology.

The first striking result is that the degrees of esterification of all the samples are lower than 50%, which allows considering them as low methoxy (LM) pectins. It is known that pectins with DE > 50% (high methoxy or HM) gel predominantly in the "sugar-acid gelling mechanism",[30] for which a certain amount of acid is required to suppress the dissociation of the free carboxyl groups, thereby preventing repulsion of negatively charged moieties. At high sugar concentration, the pectin molecules are dehydrated, promoting chain−chain rather than chain−solvent interactions. LM pectins are also able to gel in a sugar-acid gelling mechanism. However, they may easily form gels in a broad pH range in the presence of multivalent cations (e.g., Ca^{2+} ions). The calcium concentration required for gelling depends on pH and soluble solids. With relatively small additions of calcium, a gel will form, whereas an overdosage of calcium ions will lead to irreversible calcium pectinate precipitation.

Another general remark from Table 2 is that the DE of pectin is not determined by the extraction procedure (the pairs of samples FL-P1/FL-P2 and FO-P1/FO-P2 are similar), but rather by the source material: the DE is lower for fresh lemon than for fresh orange derived pectins, inverting for waste peels. This is understandable because the two extraction methods do not differ in the process parameters that usually affect the DE. Obviously, extraction processes involving other technologies may achieve very different degrees of esterification. For instance, pectin extracted from citrange fruit by electromagnetic induction heating has a DE of ~60%.[31]

Very differently, the content in HG regions depends both on the source and on the extraction method: all lemon-derived polysaccharide samples have higher HG contents than the orange-derived ones; comparing sample WL-P1 with the corresponding FL-P1 or the pair WO-P2/FO-P2, it appear that pectins from waste peels have slightly lower HG content than the ones extracted by the same procedure from fresh peels. The influence of the method is clear: the P1 samples have higher HG content than the corresponding P2 samples. This can be attributed to an effect of the microwave assisted hydrodiffusion (in process 2) that may favor the reactivity of carboxylate groups, thus

Table 2. Degree of Esterification (DE) and Relative HG Content for the Different Pectin Samples Obtained at Laboratory Scale

sample	$\sum A_{\nu(C=O)ester}$	$A_{\nu(C=O)acid} + A_{\nu asCOO^-}$	DE (%)	$\sum A_{\nu(COC)pyranose}$	HG ∝ to (%)
FL-P1	6.97 + 30.49	10.2 + 116.07	23	145.01	53
FL-P2	4.58 + 13.71	6.0 + 52.54	24	128.31	37
FO-P1	5.39 + 16.71	2.2 + 37.70	36	136.77	31
FO-P2	3.91 + 11.76	1.6 + 27.40	35	124.28	26
WL-P1	1.48 + 58.95	12.0 + 59.16	46	155.80	46
WO-P2	2.48 + 5.46	1.1 + 18.71	29	98.67	22

DOI: 10.1021/acssuschemeng.5b01716
ACS Sustainable Chem. Eng. 2016, 4, 2243−2251

reducing the galacturonic acid contribution. The high value obtained for sample WL-P1 sums up both effects: origin and process. It is expected that HG regions (smooth) will organize more easily, resulting in aggregated structures, whereas the lateral chains of RG-I regions (hairy) will hinder aggregation, yielding more filamentous structures. These structures at molecular level may reflect in the much larger scale morphologies observed by SEM. Thus, pectin extracted from fresh peels by process 2 is expected to be more filamentous in comparison to process 1; the same is true for pectin extracted from waste versus fresh peels.

Analysis of Essential Oils. The infrared spectra of essential oils from the different sources are compared in Figure 6. Their assignment is made in Table S3, in the Supporting Information.

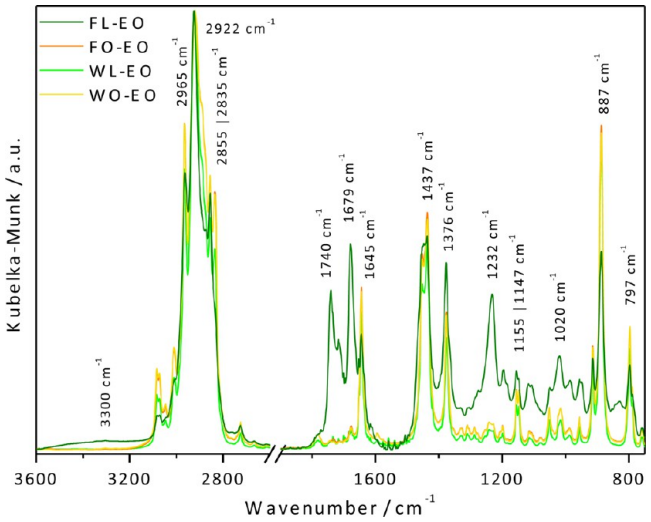

Figure 6. DRIFT spectra of lemon and orange essential oils extracted from fresh peels and waste residues, as indicated. The spectra were normalized to the 2922 cm^{-1} band.

The spectra of essential oils extracted from orange peel, both fresh and waste, and from waste lemon peel are very similar. The bands between 2800 and 3100 cm^{-1} are characteristic of CH stretching vibrations for both sp^2 and sp^3 hybridized carbon atoms. The frequency of the small sharp peak at 3100 cm^{-1} is indicative of a $=CH_2$ group. In addition, a sharp $\nu C{=}C$ mode can be seen at ~1645 cm^{-1}. The bands at 1376 and 1437 cm^{-1} are assigned to deformation modes of CH_3 groups, the one at 887 cm^{-1} to the $=CH_2$ out-of-plane bending mode of vinylidene groups, and at 797 cm^{-1} to the =CH out-of-plane mode of a trisubstituted alkene.[32] These spectra are very similar to that of limonene,[33] which is not surprising because it is expected as the main constituent of lemon peel essential oils.[34] On the other hand, the essential oil from fresh lemon peel shows other strong bands at 1740, 1679 and 1232 cm^{-1}, plus smaller components at 1690 and 1020 cm^{-1}. According to the statistical study of Lota et al., most lemon peel oils may also contain appreciable amounts of β-pinene, γ-terpinene, linalyl acetate and linalool (up to 15.8%, 18.0%, 31.2% and 23.3%, respectively).[35] The distinctive intensity ratio between the bands at 1740 and 1232 cm^{-1} (assigned to $\nu C{=}O$ and $\nu C{-}O{-}C$ modes, respectively) suggests the presence of linalyl acetate,[36] plus a small fraction of linolool (the small band at ~3480 cm^{-1} is characteristic of the alcohol), which is the main hydrolysis product of linalyl acetate.

SEMI-INDUSTRIAL EXTRACTION AND ANALYSIS

Experimental. To gain insight into the technical feasibility, at industrial scale, of the waste citrus peel extraction using the newly developed microwave-assisted process in water, we carried out the extraction of 20 kg of waste lemon peel obtained from a candy production plant based in Sicily (Canditfrucht), using a compact commercial extractor (MAC 75, Milestone, Italy) capable to process up to 30 kg of raw material in each extraction cycle. The extractor uses 4 microwave Magnetron generators each absorbing 1.5 kW of power. In detail, 20 kg of waste lemon peel was mixed with 36 L of water. The waste lemon peel (as such, not dried) was packaged into a cotton bag (Figure 7) and directly inserted into the Mac 75 Teflon-based drum, thereby avoiding costly purification or drying processes.

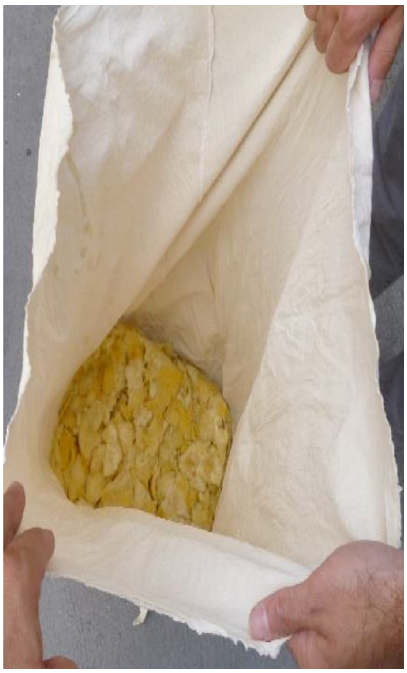

Figure 7. Cotton bag with the waste lemon peel.

The extraction cycle was carried out at 80 °C for 1 h, which should be compared to 4−5 h employed in the classical distillation process used to extract the essential oils from the waste citrus peel. Once the extraction was complete, two bioproducts were simultaneously extracted: pectin mixed with water leaving the drum from a tap at the bottom of the extractor (Figure 8A), and the essential oil floating on the water/pectin mixture from which it is easily separated.

From 20 kg of waste lemon peel, 18 kg of an organic residue (Figure 8B) devoid of essential oil and pectin was obtained, which is mainly composed of hemicellulose ready for fermentation, or cattle feeding. A subsequent liophilization step allows to isolate pectin in dried form (Figure 9A), whereas the essential oil phase is isolated upon refrigeration of the liquid mixture (Figure 9B).

From each extraction cycle, about 30 kg of liquid pectin mixture is obtained. Upon liophilization, 15% of this mixture is isolated as dried pectin. Hence, starting from 20 kg of waste lemon peel, about 3 kg of pectin is obtained, along with 10 mL of essential oil.

For comparison, when pectin is extracted using conventional hydrolysis in hot water, much of the "hairy" regions of the polymer are destroyed, leaving mainly the galacturonic acid "smooth" regions, with a few neutral sugar units attached or in the main linear chain. Common yields of pectin are ~3% of the peel weight.[37] Eventually, out of 100 g of wet peel, after drying (85 g of water are removed), typically 3 g of pectin is obtained, whereas 12 g of depectinized peel goes to cattle feeding.

Analysis of Pectins and Essential Oils from Semi-Industrial Extraction, Compared to Laboratory Scale. To evaluate the influence of a process scale-up on the quality of the bioproducts, the DRIFT spectra of pectin extracted from waste lemon peel at the two scales are compared in Figure 10.

The main differences observed in the spectrum of the semi-industrial extraction consist in the better definition of the pectin typical bands in the 1100−900 cm^{-1} region, the narrowing of the O−H stretching band, indicative of a decrease in the proportion of free OH groups and their involvement in more similar hydrogen bonds, the

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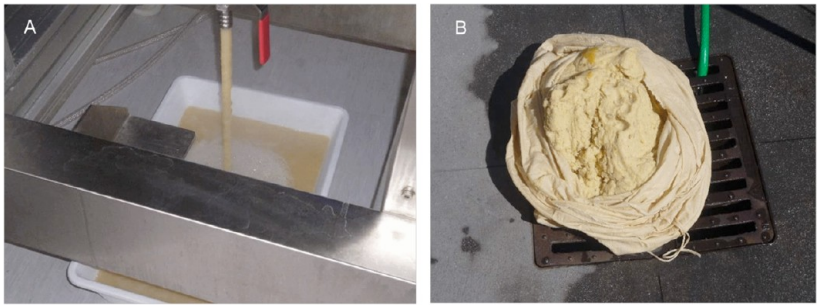

Figure 8. (A) Pectin mixed with water leaving the drum from a tap at the bottom of the extractor; (B) organic residue devoid of essential oil and pectin.

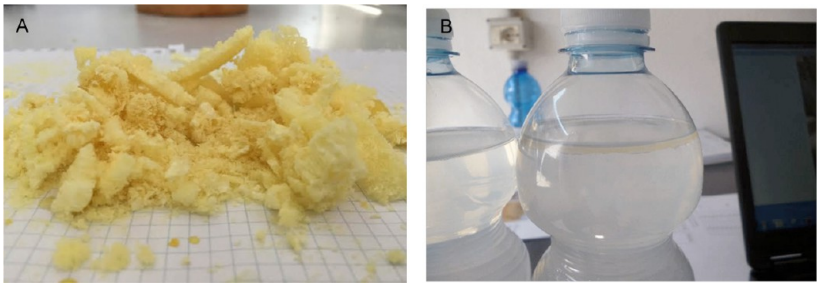

Figure 9. (A) Dried pectin; (B) supernatant essential oil obtained from the extraction of 20 kg of waste lemon peel using the Mac 75 extractor.

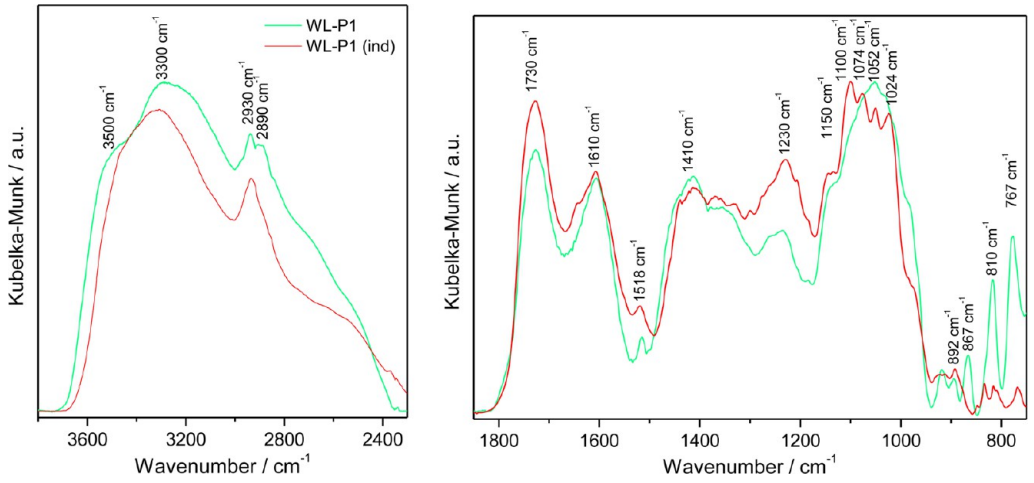

Figure 10. Comparison of the DRIFT spectra of Pectin 1 obtained from waste lemon at laboratory (WL-P1) and semi-industrial (WL-P1$_{ind}$) scales, in two regions. Spectra normalized to the maximum absorption in the 1900−750 cm^{-1} region.

poorer definition of the C−H stretching modes and the decrease in the relative intensity of the external deformation modes of methyl, methylene and methyne groups (below 900 cm^{-1}). All these changes suggest a decrease in the proportion of rhamnose, galactose and arabinose rich "hairy" regions. A quantitative treatment to assess DE and HG proportions was performed (described in the Supporting Information), and the results are summarized in Table 3:

Table 3. Comparison of the Degree of Esterification (DE) and Relative HG Content for the Pectin Samples Obtained at Laboratory and at Semi-Industrial Scales from Waste Lemon Peel

sample	DE (%)	HG ∝ to (%)
WL-P1	46	46
WL-P1$_{ind}$	45	54

In what concerns the degree of esterification, the scale-up does not introduce alterations, which is not surprising, since DE depends mostly on the pectin source. The higher proportion of HG regions is confirmed, and can be explained by an increased breaking of RG backbone units upon the semi-industrial process, similarly to what happens with the conventional pectin extraction via acid hydrolysis in hot water.[38]

The effect of scaling-up on the essential oils extracted from waste lemon was also analyzed comparing the infrared spectra (Figure 11).

The fingerprints of *d*-limonene are present in the essential oil at semi-industrial scale, but it becomes clear that scaling-up the process was responsible for increasing the content in linalyl acetate, suggested by the bands at 1713, 1268, and 1020 cm^{-1}, and, in a larger proportion, of linolool, identified by the strong band at ∼3400 cm^{-1}, associated with the change in relative intensities of the bands at 887 and ∼920 cm^{-1}.

DOI: 10.1021/acssuschemeng.5b01716
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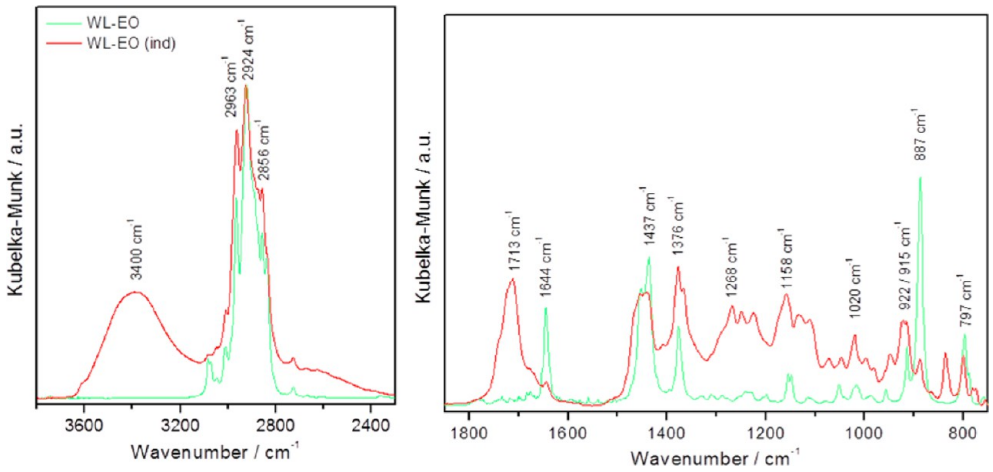

Figure 11. Comparison of the DRIFT spectra of essential oils obtained from waste lemon at laboratory (WL-EO) and semi-industrial ($WL\text{-}EO_{ind}$) scales, in two regions. Spectra normalized to the maximum absorption in the 3200−2800 cm^{-1} region.

CONCLUSIONS AND PERSPECTIVES

We have extracted pectin and essential oils from fresh and waste rind of orange and lemon grown in Sicily, both at laboratory and at semi-industrial scales, by a totally eco-friendly process run entirely in water, with microwave and heat as the only energy sources. In each case, high yields of bioproducts were obtained. The samples were extensively characterized by DRIFT spectroscopy and scanning electron microscopy. The DRIFT results have shown that the two significant characteristics of pectin, namely DE and fraction of HG regions, depend mostly on the source and on the extraction procedure, respectively. Thus, the scale-up of the extraction of pectin from waste lemon peel does not modify the DE, but the proportion of molecular smooth regions (HG) is increased, leading to a more aggregated structure.

We remind here that the chemical characteristics of the extracted pectin depend upon the extraction conditions and the sourcing material. Hence, prior to shipping, the industrial product obtained is characterized (pectin for use in food is a polymer with at least 65% galacturonic acid units) and separated into low and high methoxyl pectin.

The degree of esterification and the distribution of the carboxyl groups in the pectin polymer correlate with the gel setting rate and gel texture under otherwise similar conditions. Because of a blockwise distribution of carboxyl groups,[39] citrus pectins with the same degree of esterification will form gels with a slightly higher setting temperature and a more elastic texture when compared to apple pectins. The same blockwise carboxyl groups' distribution of high methoxyl pectins additionally provides advantages regarding protein stabilization in acidified milk drinks.

The findings from DRIFT spectroscopy are consistent with the SEM observations, although at very different scales, and are significantly relevant from an applicative viewpoint.

Pectin, the partial methyl esters of polygalacturonic acid and its salt, obtained by extraction in an aqueous medium, is an exceptional polymer whose large and increasing use as hydrocolloid by the food industry is rapidly expanding into other industrial sectors. Even in the food sector, pectin traditional usage as thickening and stabilizing agent is being complemented by its emerging utilization as fat replacer, while producing improved texture.[40]

Limonene, too, is a versatile terpene molecule whose direct and indirect uses are rapidly growing well beyond the conventional role as clean solvent, leading to a significant market shortage. The process described in this work is environmentally sound, scalable and of direct interest to citrus growing countries and companies. Remarkably, the potential of microwave technology for the recovery and manufacturing of chemicals from biowastes has been lately clearly identified.[41]

The need to expand the production of both pectin and limonene, while renewing and improving conventional extraction methods, meets the appearance of new citrus-biorefineries in citrus producing countries, where this fruit's production has grown from 78 million tons in 1990 to 123 million tons in 2010 (and has, ever since, continued to grow).[42] Work is in progress to establish one such biorefinery in Sicily.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.5b01716.

Assignments of the DRIFT spectra of pectin samples, results of the spectral deconvolution by non-linear least squares fitting, and assignments of the DRIFT spectra of essential oils extracted from fresh peels and waste residues of lemon and orange, (PDF).

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Thanks to Dr. Stefano Arvati, Renovo Bioindustry, for his valued support towards the foundation of Sicily's first biorefinery in Caltagirone. We thank Candidfrucht (Barcellona Pozzo di Gotto, Sicily) and Ortogel (Caltagirone) for generous gifts of waste lemon peel, and fresh orange and lemon peels.

DOI: 10.1021/acssuschemeng.5b01716
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DEDICATION

This work is dedicated to Professor Farid Chemat, Université d'Avignon (France), for all he has done to advance the practice of the microwave natural products extraction in the last 2 decades

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DOI: 10.1021/acssuschemeng.5b01716
ACS Sustainable Chem. Eng. 2016, 4, 2243−2251