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Lemon Essential Oil of Variable Composition by Changing the Conditions of the Extraction from Lemon Peel via Microwave Hydrodiffusion and Gravity

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Microwave hydrodiffusion and gravity extraction of waste lemon peel carried out on semi industrial scale, enabled not only to extract high quality essential oil via an entirely green process, but also to selectively adjust the oil composition by varying the hydrodiffusion time.

Essential oils (EOs) from citrus peel are valued natural flavor and fragrance materials of exquisite taste and smell, widely employed in beverages, confectioneries, pharmaceuticals, cosmetics, and perfumes.^[1] Citrus oil is generally extracted from the fruit rind in a continuous mechanical process, in which the EO is released when the citrus fruits placed on steel rollers, partially submerged in a water bath, are rolled over numerous sharp points. Driven by a strong and global consumer preference for natural products in flavor and fragrance, food and beverage, cosmetic, personal care, biopesticide and therapeutic formulations,^[2] the global citrus essential oil market is anticipated to increase 1.6 times in terms of value between 2016 and 2026, to reach and surpass \$4.3 billion in revenues by 2026.^[3]

Such high and sustained demand is translated in considerable rise in price of all main citrus oils (orange, lemon, lime, mandarin, tangerine and grapefruit), making the production of EOs an ever more significant part (up to 50 per cent) of the revenues of today's citrus juice producers. With the supply of citrus oil increasingly tighter, the enhanced extraction of EOs

from waste citrus peel has become an important challenge for today's applied chemical research.

The research groups led by Clark^[4] in the United Kingdom and Chemat^[5] in France have proposed the concept of a waste orange peel (WOP) biorefinery based on integrated green extraction processes using, respectively, microwave and ultrasound and microwave techniques to obtain essential oil, mesoporous cellulose, polyphenols and pectin. In this context in which numerous other green processing techniques are rapidly emerging for the extraction of valued products from food residues,^[6] we have recently reported an eco-friendly process based on microwave hydrodiffusion and gravity (MHG) for the simultaneous extraction of high-quality EOs and pectin from lemon, orange and grapefruit peel.^[7] Pectin is the most valued natural hydrocolloid,^[8] and its growing demand at fast rate in the course of the last decade has caused availability problems and increased price similar to what happened with citrus EOs.^[9] Compared to conventional methods such as cold pressing and hydrodistillation, MHG^[10] not only affords complete extraction of the whole EO bouquet, but it also broadens the extraction possibilities. For example, it remarkably allows to extract EOs showing significant differences in composition, depending on the citrus fruit and fruit region used as source: namely, outer skin (exocarp), peel (exo- and mesocarp), and waste (exo-, meso-, and endocarp).^[11]

Now, we report the outcomes of the optimization of lemon essential oil extraction via microwave hydrodiffusion carried out on semi industrial scale, which enables to extract high quality essential oil via an entirely green process, and also to change the profile of the extract by accurately varying the conditions of the extraction. Lemon oil has become particularly valued, having an even higher market value than orange and grapefruit oil,^[12] due to its antimicrobial, antifungal, anticarcinogenic, and lipid antioxidant properties.^[13] The EO is mainly comprised of *d*-limonene, γ -terpinene and β -pinene, followed by four other monoterpene hydrocarbons (myrcene, sabinene, α -pinene and *p*-cimene) and some 40 other compounds in five different classes (sesquiterpene hydrocarbons, carbonyl compounds, alcohols, esters and oxides).^[12]

All the experiments were performed using a semi industrial microwave extractor MAC75 (Miletone, Italy). The extractor was loaded with a defined amount of waste lemon peel (Figure 1) followed by water added as extracting medium, as well as to promote the steam generation for EO hydrodiffusion.

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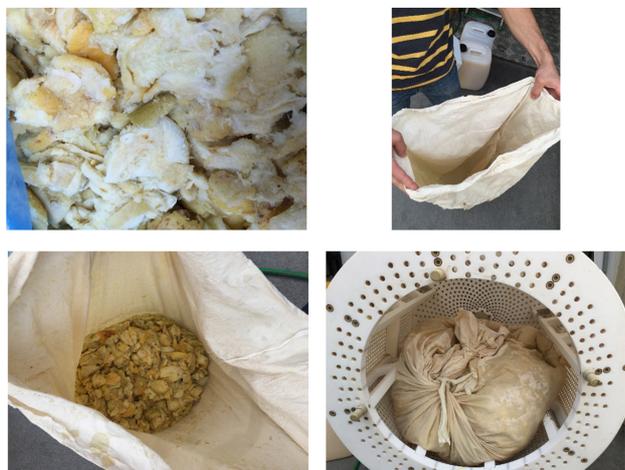


Figure 1. Frozen waste lemon peel and cotton bag (top), prior to insertion in the MAC75 drum (bottom).

Two extraction procedures were followed, differing in the water temperature, and in the way the waste lemon was introduced in the drum, as described in detail in the Electronic Supporting Information. Tables S1 and S2 summarize the extraction conditions for the two procedures.

EO samples were collected at subsequent times, affording an overall EO mass of 2.96 g when following Procedure 1 (Table 1) and of 4.93 g when following Procedure 2 (Table 2), starting from 20 kg of waste lemon.

Table 1. EO distillate fractions at subsequent extraction times in "Procedure 1" ^a		
Extract	Time (min)	EO (g)
1.1	95	0.49
1.2	100	2.06
1.3	105	0.15
1.4	110	0.17
1.5	130	0.085
Total		2.96

^aExtraction conditions: 20 kg waste lemon peel (no bag) + 36 L water at room temperature

Table 2. EO distillate fractions at subsequent extraction times in "Procedure 2" ^a		
Extract	Time (min)	EO (g)
2.1	60	3.71
2.2	70	0.83
2.3	80	0.19
2.4	90	0.20
Total		4.93

^aExtraction conditions: 20 kg waste lemon peel (with bag) + 36 L water at 60 °C

To check the reproducibility of the method, a repetition of "Procedure 2" was carried out. To our delight, it yielded a

Table 3. EO distillate fractions at subsequent extraction times in repeated "Procedure 2" ^a		
Extract	Time (min)	EO (g)
2'.1	80	2.83
2'.2	90	0.93
2'.3	100	0.78
2'.4	105	0.33
Total		4.87

^aExtraction conditions: 20 kg waste lemon peel (with bag) + 36 L water at 60 °C

practically unvaried amount of EO (Table 3), even though distillation now started after 70 min (Table S3).

"Procedure 1" was useful to obtain the heating profile using room temperature water, and the amount of water (36 L) was chosen to allow the drum to properly work for the whole duration of the extraction. Yet, during the extraction process, some waste lemon peel leaked from the extractor drum to the cavity (Figure 2), rendering more difficult the recovery of all solid extract.

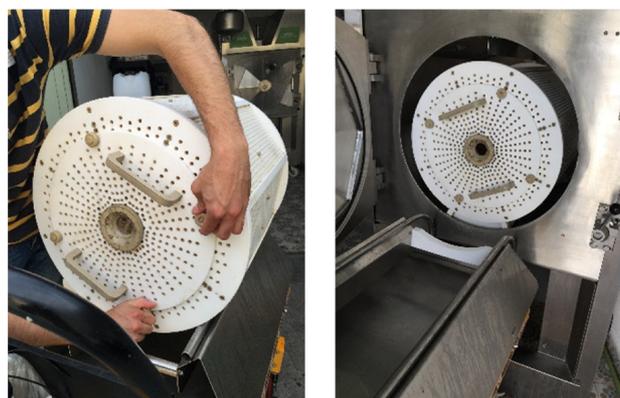


Figure 2. The drum with packaged waste lemon peel prior (left) and after (right) insertion in the MAC75 extraction cavity.

Inserting the waste citrus peel into a cotton bag ("Procedure 2") was enough to prevent any material leaks, while the use of water at 60 °C in place instead of room temperature water translates into halving the time for reaching the hydrodiffusion temperature (Figure 3).

The advantages of "Procedure 2" are also apparent from the EO yield, which increases from 0.015% ("Procedure 1") to 0.024%.

In order to assess any changes in the composition of the extracted essential oils at different hydrodiffusion times, we analyzed via diffuse reflectance infrared (DRIFT) spectroscopy the four EO distillate samples of Table 3, obtained after 80, 90, 100 and 105 min. The spectra in Figure 4 show indeed striking differences with time.

The detailed band assignments are included in a previous paper,^[11] where the infrared spectra of EOs extracted at a

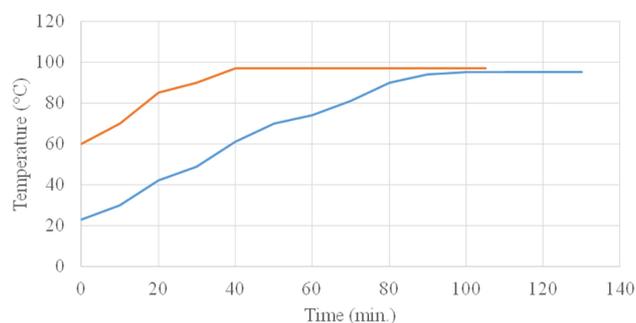


Figure 3. Time needed under microwave irradiation to reach the EO hydrodistillation temperature from waste lemon peel starting with water at room temperature ("Procedure 1", blue line) and at 60 °C ("Procedure 2", red line).

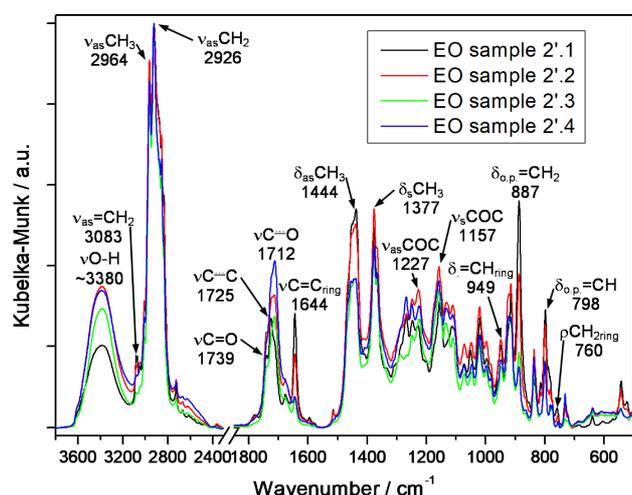


Figure 4. DRIFT spectra of EO samples obtained at subsequent hydrodiffusion times from waste lemon peel with the MAC75 under the conditions of Table S3. The spectra are normalized to the band at 2926 cm^{-1} .

laboratory scale from different citrus and different fruit regions (waste, peel and outer skin) were analyzed. Normalizing the DRIFT spectra of the different extracts to the strongest band (at 2926 cm^{-1}), assigned to the $\nu_{\text{as}}\text{CH}_2$ mode of sp_3 carbon atoms, there are general trends in the relative intensities of some bands as a function of the extraction time: the νOH band (broad due to hydrogen bonding of the hydroxyl groups) tends to increase for longer extraction times, indicating that the fraction of alcohols is becoming more significant. It is also clear that the bands related to $=\text{CH}$ and $=\text{CH}_2$ groups, namely at 3083 cm^{-1} ($\nu_{\text{as}}=\text{CH}_2$), 3009 cm^{-1} ($\nu=\text{CH}$), 887 cm^{-1} ($\delta_{\text{o.p.}}=\text{CH}_2$) and 798 cm^{-1} ($\delta_{\text{o.p.}}=\text{CH}$) tend to decrease, showing that the fraction of components with vinyl or substituted vinyl groups (present in most monoterpenes and terpenoids) decreases. Also the bands at 2964 ($\nu_{\text{as}}\text{CH}_3$) and 2857 ($\nu_{\text{s}}\text{CH}_3$) cm^{-1} and the corresponding deformations, at 1444 and 1377 cm^{-1} , respectively, decrease for longer extraction times, indicating that the proportion of terminal CH_3 groups is decreasing. The band at 1644 cm^{-1} (assigned to the $\nu\text{C}=\text{C}$ mode of rings in limonene or γ -terpinene, for instance) visibly decreases for longer extraction

times. This is accompanied by a decrease in the bands at 949 cm^{-1} ($\delta\text{CH}_{\text{ring}}$) and 760 cm^{-1} ($\rho\text{CH}_{2\text{ring}}$). Also the band at 1739 cm^{-1} ($\nu\text{C}=\text{O}$) is well defined for the first extracted fractions, but becomes just a shoulder for the subsequent ones. The relative intensities of the doublet at 1725/1712 cm^{-1} invert: the one at 1725 cm^{-1} ($\nu\text{C}=\text{O}$ of, for instance, isoimperatorin) clearly predominates in the first extraction, in the second one the two bands are equivalent, and from then on the one at 1712 cm^{-1} ($\nu\text{C}=\text{O}$ of citral isomers) becomes the main component. The bands related to C–O–C groups (ν_{as} and ν_{s} at 1227 and 1157 cm^{-1} , respectively) do not follow a clear trend. Regarding the doublet at 922/915 cm^{-1} , in the first extract there is only one band at 915 cm^{-1} ($\rho(\text{O})\text{CH}_3$), then a shoulder starts to appear at 922 cm^{-1} (ρCH_3), and the two components become equivalent in the last extracts, indicating that they are relatively poorer in methoxyl groups.

In summary, for longer extraction times, the essential oils become richer in alcohols and oxygenated monoterpenes, and poorer in monoterpenes and polymethoxylated flavones. The composition of the EOs at semi industrial scale may thus be optimized by adjusting the extraction time, even though the yields significantly decrease for subsequent extracts.

A comparison with the spectra of lemon EOs extracted at laboratory scale from different fruit regions is made in Figure 5.

Even the first extracted fractions have a relatively stronger OH stretching band ($\sim 3380 \text{ cm}^{-1}$) than the EOs extracted at laboratory scale from any fruit region (Figure 5, top). This band could be assigned to residual water, given the much higher proportion (water volume:peel mass) used in the semi industrial process: 1.8 L/kg versus 0.8 L/kg in the laboratory scale process (400 mL/500 g).^[11] However, the band at $\sim 1644 \text{ cm}^{-1}$, which contains a contribution from the water HOH deformation (Figure 5, bottom), has a similar relative intensity in both extraction scales and, furthermore, decreases for longer extraction times, contrarily to the OH stretching band.

Thus, we may conclude that the semi industrial EOs are in fact richer in alcohols, such as oxygenated monoterpenes (linalool, borneol or geraniol) or hydroxylated polymethoxyflavones (tangeretin or limocitriol). Nor the temperature at which the processes take place ($\sim 100 \text{ }^\circ\text{C}$) neither the extraction time (also $\sim 80 \text{ min}$ for lemon peel at laboratory scale) may be the cause for this variation in composition, which is instead due to the more effective extraction of hydrophilic molecules from the rapidly turning waste lemon peel in the MAC75 under more effective outwardly dielectric heating^[14] produced by the four opposing magnetrons, when compared to the single MW generator of the laboratory extractor.

The spectral region between 1640 and 1750 cm^{-1} (Figure 5, bottom) is the most different when comparing EOs extracted at laboratory and semi industrial scales, since there is a striking inversion of the band intensities: the main band for the EOs extracted at semi industrial scale is at 1712 cm^{-1} , showing that the carbonyl related bands predominate, but are shifted to lower wavenumbers due to hydrogen bonding or conjugation with resonant C=C bonds. This is another hint for the predominance of citral isomers or polymethoxyflavones in

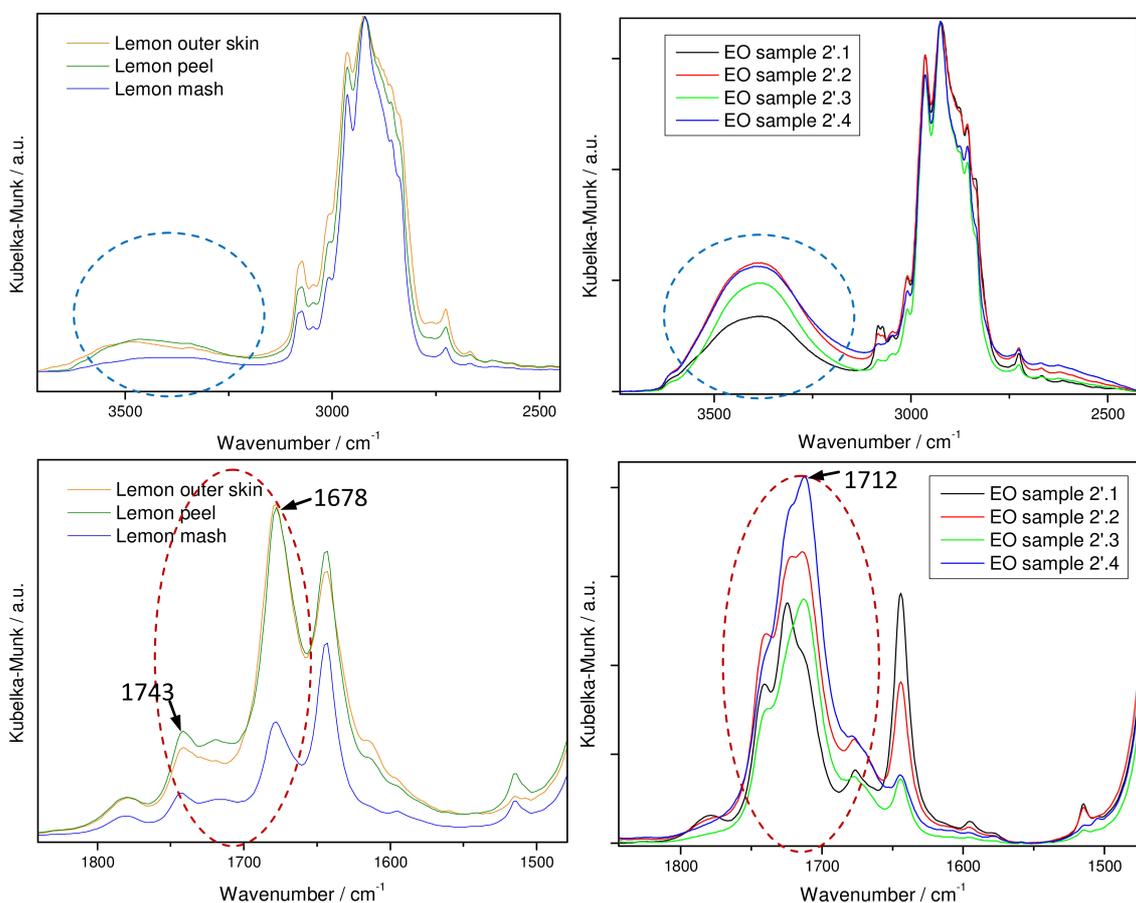


Figure 5. Comparison of the DRIFT spectra (in two wavenumber regions) of lemon essential oils obtained at laboratory scale from different fruit regions (*left*) and those extracted at semi industrial scale at different extraction times, in the present work (*right*).

these EOs, in detriment of monoterpenes, as their characteristic band at $\sim 1680\text{ cm}^{-1}$ is comparatively much reduced.

In general, higher oxygenates content, especially the citral isomers (geranial and neral), as well as esters such as neryl acetate and alcohols increase the quality of lemon EO, making it also more stable on storage.^[15,16] A practical outcome of these findings, adding to the advantages of microwave-assisted extraction in light of extraction processes at industrial scale,^[17] is that it becomes possible to tune the composition of the desired citrus essential oil by selecting the hydrodiffusion time. Industrial upscaling would require optimization of the extraction conditions to minimize energy and water utilization (for example using response surface methodology).^[18]

These findings, in conclusion, further advance the practical feasibility of producing EO (and pectin) of nutraceutical grade with tuneable composition, directly from wet citrus peel waste via microwave-assisted hydrodiffusion and gravity.

Supporting Information Summary

Full experimental details can be found in the Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: citrus · essential oil · hydrodiffusion · microwave extraction

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