**Microwave assisted hydro-distillation of essential oils from wet citrus peel waste**

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**Abstract**

Over 30% of the total citrus fruits grown all over the World are processed, generating large quantities of peel waste from which essential citrus oils are often extracted by traditional methods demanding high energy costs, long extraction times and additional reagents. In this research an alternative technique based on microwave-assisted hydro-distillation (MAHD) has been studied to successfully extract the essential oil present in wet citrus peel waste whilst reducing costs, avoiding the use of additives and improving the effectiveness of the process. To achieve this goal factors affecting the scalability of the process to develop a new bio-refinery model to be used at industrial scale were considered. Optimal conditions for the essential oil MAHD involved the irradiation of a waste orange peel:water mixture (1:1.5) over two subsequent steps using different irradiation powers for a total extraction time of 20 min (keeping a constant pressure of 300 mbar through all the process). The essential oil yield obtained in *Navel Navelate* oranges using MAHD accounted for 1.8 ± 0.1% (dry basis; n=3) and was comparable to that obtained by conventional hydro-distillation (1.7 ± 0.1%; dry basis; n=3). The applicability of the suggested methodology was successfully tested in other varieties of orange and citrus fruits leading to the potential to apply MAHD of essential oils from other waste feedstocks and offering an attractive process for future extraction processes at industrial scale.

**Keywords**: essential oil, citrus waste, microwaves, extraction

**1. Introduction**

Citrus fruits including oranges, lemons, limes, grapefruits and tangerines are considered commodity products similar to coffee and tea in terms of international trade. In 2013-2014 the major citrus producing countries, such as Brazil, China, India, US, EU-27, Mexico, Egypt, Turkey and South Africa, produced around 140 Mt of these fruits, of which 60% were oranges (FAO, FAOSTAT, 2015). Worldwide, it is estimated that over 30% of citrus fruit produced (40% in the case of oranges) is processed by the food industry each year. This processing, including juicing and canning, generates large quantities of citrus peel waste (19 Mt annually) that has the potential to be used as a bio-refinery raw material.

Major components of dry citrus peel waste include sugars (23%), cellulose (22%), pectin (25%), hemicellulose (11%), flavonoids (4.5%) and up to 4% of essential oil, often referred as citrus essential oil (Marin et al., 2007). Citrus essential oils are some of the most popular fragrances in natural skin, bath, and body care products where they add an uplifting and refreshing quality. These oils comprise a mixture of volatile compounds like terpenes and oxygenated derivatives such as aldehydes (citral), alcohols and esters, their main component being D-limonene –a non-oxygenated monoterpene made from the combination of two isoprene units (Shaw, 1979). Constituting up to 90–95% in orange peel oil and 75% in lemon peel oil (Kesterson et al., 1971) D-limonene plays an important role in the field of flavour and fragrances due to its physicochemical properties (Ciriminna et al., 2014). Such properties explain its use in a large variety of consumer products, in addition to its flavour and fragrance properties (Siles Lopez et al., 2010). As demonstrated by Virot et al. (2008) it can also be used as a non-toxic solvent in oleochemical, wax, resin, paint and glue industries and as a valuable renewable bio-solvent -an alternative to hazardous petroleum solvents. Another major application of D-limonene is as a cleaning agent replacing environmental unfriendly cleaning agents like toluene, hexane and chlorinated organic solvents, one of the most important drivers for the growing interest in this compound (Toplistek and Gustafson, 1995).

The production of essential oil from waste orange peel (WOP), or any other suitable citrus, is often carried out by traditional methods like steam distillation on a commercial scale (Masango, 2005). These conventional methods, however, show some disadvantages related to high energy costs and long extraction times. Scientists have therefore been developing greener and more efficient methodologies to be used in alternative biorefinery concepts to i) increase production efficiency and ii) contribute to environmental preservation. Extractions based on hydro-distillation (HD) like those involving Clevenger and soxhlet apparatus were first reported several years ago (Wang and Weller, 2006). The extraction times required in these processes are generally achieved in several hours. More recent developments based on ultrasound extraction (Vinatru et al., 1999), supercritical fluid extraction (Baysal and Starmans, 1999), subcritical water (Gamiz-Gracia and Luque de Castro, 2000) and microwave-assisted extraction (Ferhat et al., 2007) are now being studied as faster, more efficient and cost effective alternatives. Some of these techniques, however, tend to require hazardous solvents or additives to carry out a successful extraction of essential oils. Moreover, most of the previous studies were carried out using dry orange peel waste as a raw material or without fully considering the scalability of the process (i.e. peeling the fruit by hand and/or removing the flavedo from the peel). Drying the feedstock prior to its valorisation entails large costs and it is time consuming. Thus, a methodology relying on green solvents and techniques, able to cope with wet feedstock was still necessary. Here lies the advantage of the microwave protocol compared to other techniques. Recently, microwave technology has gained increased industrial interest in the food sector since it i) can be applied directly to the desired biomass without any need of pre-treatment (i.e. drying); ii) allows a rapid and homogeneous heating; and iii) is adaptable for continuous processes and easily scalable (Filly et al., 2014). The scalability of this technology has been proved in different studies and has shown important advantages over traditional methods (Pardo and Zufia, 2012). These features allow microwave technology be applied to different systems in order to obtain more flexible processes, leading to lower energy consumption and environmental impact (Mellouk et al., 2016).

In this research a microwave-assisted hydro-distillation (MAHD) technique for the extraction of essential oil from citrus peel has been developed considering the most relevant variables for up-scaling the process including WOP:water ratio, extraction time, power and efficiency. Special attention has been paid to those factors affecting the scalability of the process such as the viscosity of the feedstock and the configuration of the reactor. Finally, in order to prove the applicability of the suggested methodology, extractions were carried out in fresh oranges, lemons, limes, satsumas and grapefruits to further study and characterise the different products.

**2. Materials and methods**

**2.1. Chemicals and reagents**

D-limonene (97% purity), α-myrcene (>95% purity), α- and ß- pinene (>98% purity), p-cymene (99% purity), α-terpineol (>90% purity) and linalool (>97% purity) were used as standards for GC-TOF analysis of essential oil samples obtained by MAHD -all were purchased from Sigma-Aldrich. De-ionised water was mixed together with the WOP during the MAHD and to clean all the glassware. Ethanol (ethanol absolute, Normapur) was also used to clean the glassware and to avoid any contamination between different experiments and batches.

**2.2. Citrus samples and pretreatment**

Experiments to find optimum conditions under the studied parameters were conducted using WOP (*Citrus sinensis* or sweet oranges; *Navel Navelate* variety) grown by Frutsol (Valencia, Spain) and supplied by Chingford Fruits (Dartford, UK). The oranges were juiced and then frozen at -18 °C within 12 h of delivery Before use, WOPs were defrosted at 4 °C and milled using a Retsch™ Knife Mill Grindomix GM300 food processor for 3 min at 2,500 rpm (<5 mm particles). The applicability of the suggested extraction process was studied using fresh peel waste (non-frozen) from different citrus materials: sweet oranges (*Valencia Late* and *Navel Powell* varieties, grown in Spain), lemons (*Primofiore* variety, grown in Spain), limes (*Tahiti* variety, grown in Brazil), satsumas (*Nihowase* variety, grown in South Africa) and grapefruits (*Star Ruby* variety, grown in South Africa) all of which were purchased from Morrisons (York, UK).

**2.3. WOP physical analysis**

To understand the process to be optimised and its overall applicability to larger scales there was a need to characterise the physical properties of WOP in presence of water. The moisture content of WOP before the extraction was determined following oven drying in a PF60 (200) oven manufactured by Carbolite. Samples were dried at a maximum temperature of 70 °C until constant weight was reached. Samples were allowed to reach room temperature inside a desiccator before weighting. The density of the biomass was studied using different solid percentages containing aqueous solutions. The viscosity of different WOP:water mixtures was measured at 20, 50 and 80 °C using a Brookfield dial viscometer.

**2.5. Extraction by hydro-distillation**

WOP was submitted to conventional hydro-distillation using a Clevenger apparatus (Council of Europe, 1996) to prove the effectiveness of the MAHD. Extractions were carried out using 100 g of WOP and 1 L of water. The extraction was carried-out for 240 min at 100 °C until no more oil was obtained. The obtained fraction was also stored at 5 °C and further characterised using GC-TOF.

**2.6. Extraction by microwave-assisted hydro-distillation (MAHD)**

The MAHD process is based on a conventional hydro-distillation system with the exception that microwave energy is used during the heating process. For that purpose, a Milestone RotoSYNTH microwave (a 45° rotative solid-phase microwave system) was used in conjunction with a 4 L Pyrex® reactor vessel to heat up the sample (a mixture of de-ionised water and WOP). The vessel is then connected to a condenser (4 °C) placed in line outside the microwave cavity to cool the gas phase obtained during the extraction as shown in Fig. 1. Liquid fractions were collected in a two-neck round-bottom flask fitted at the end of the condenser. A second condenser (4 °C) was connected between the aforementioned round bottom flask and the vacuum pump (CVC2, Vacuubrand®) as a security measure to make sure no volatile compounds were lost through the vacuum line. The addition of a vacuum line on the system was done to study the effect of lower pressures on the system as well as to lower the boiling point of the mixture and therefore the time and microwave energy required for the heating process. The process vacuum and energy consumption was monitored at all times and energy consumption measurements were carried out using a plug-in Power and Energy Monitor Prodigit 2000MU. In order to optimise the MAHD process, different extractions were carried out placing the appropriate mass of WOP inside the microwave reactor together with its corresponding volume of de-ionised water (total weight of 1.5 L). The WOP and de-ionised water mixture was then irradiated with microwaves using different irradiation powers, vacuum and extraction times. The gas phase produced was condensed, collected and further separated using a separating funnel. The obtained fraction was stored at 5 °C and further characterised by means of GC-TOF. Yields of essential oil were calculated based on wet WOP.

**2.7. Chemical analysis**

The resulting oil was characterised and quantified by GC-TOF analysis on an Agilent 6890 GC coupled to a Pegaus IV TOF mass spectrometer (Leco). The GC was fitted with a 30 m x 0.25 mm Rxi-5Sil MS column (Thames Restek). The film thickness was of 0.25 μm. The carrier gas was He. It was used in constant flow mode at 1 mL min-1. A 1 μL sample was injected at a 100:1 split ratio. The oven program was isothermal at 40 °C for 2 min then ramped at 5 °C min-1 to 300 °C and held for 2 min. Electron impact mass spectra were generated at 230 °C and 70 eV. Data was collected at unit mass resolution over the range 20 - 450 m/z and 20 scan s-1. Data was analysed using the ChromaTof 4.5 software (Leco). Compounds were identified by reference to authentic standards or by spectral matches to the NIST 05 and Wiley 7 libraries.

**3. Results and discussion**

**3.1. Biomass characterisation**

To understand the process to be optimised and its overall applicability to larger scales there was a need to characterise the properties of WOP in presence of water. As a first approach, the moisture content and solid particle percentage was determined in all studied citrus peels (Table 1). The moisture content in citrus varied considerably (78.6-87.1%) not only between different citrus, but also between different varieties of oranges. Satsumas and grapefruits were the fruits in which the measured water content was the highest. The reason behind this observation might be linked to the juicing step, in which the processing for these two fruits was not as efficient as for the rest of the samples due to their size (grapefruits were too large for the machine to appropriately handle them whilst the satsumas were too small).

Table 1. Moisture and solid particle content in studied citrus fruits.

|  |  |  |  |
| --- | --- | --- | --- |
| Citrus | Variety | Moisture (%) | Solid particles (%) |
| Oranges | Navel Navelate | 78.6 | 22.4 |
| Oranges | Midknight | 82 | 18 |
| Oranges | Valencia Late | 84.2 | 15.8 |
| Lemons | Verna | 85.3 | 14.7 |
| Limes | Persian | 84.9 | 15.1 |
| Satsumas | Nihowase | 88 | 12 |
| Grapefruits | Star Ruby | 87.1 | 12.9 |

At larger scale set-ups, and in order to work in a continuous way, pumping the material through the system becomes essential. For this purpose, as reported by Gardas and Coutinho (2008), lowering the inherent viscosity of the feedstock is required. As it can be observed in Fig. 2, the viscosity measured in mixtures containing different percentages of WOP solid particles can be explained using power regression models not only at 20 °C (y = 0.0039x2.9908;R² = 0.9855) but also at 50 °C (y = 0.0015x2.7354; R² = 0.9598) and 80 °C (y = 0.001x3.1604;R² = 0.9795). In liquids, viscosity is a parameter dependant on temperature and in most cases it tends to decrease as the temperature increases (Lazzus and Pulgar-Villarroel, 2015). In this study, the highest viscosities were always measured at 20 °C. When comparing values obtained for mixtures at 50 and 80 °C, higher viscosities were measured in all mixtures at 80 °C. The reason behind this increase could be linked to the partial extraction of some components present in orange peel such as sugars and pectin; known for their thickening and/or gelling properties (Liu et al., 2006).

Coulson et al. (1983) reported that viscosities lower than 10 Pa s allow high flexibility when working with solid containing liquids using large scale equipment. Thus, according to results in Fig. 2, the percentage of solid particles present in a mixture of WOP and water should not exceed 13%. This result was taken into consideration when establishing the range of WOP:water ratio to be studied in the MAHD optimisation.

Finally, the presence of WOP related solid particles in different WOP:water ratios was measured (Fig. 3). Results for solid particle percentages ≤ 13% showed no significant difference in density compared to that of water for the pumping of the feedstock at larger scales. Thus, in this study, density was not a critical parameter to be further considered.

**3.2. MAHD of citrus essential oil**

**3.2.1. Preliminary work**

A multivariable design based on linear regression was chosen to study how the different parameters influenced the essential oil extraction yield using MAHD and its efficiency. Multiple linear regression attempts to model the relationship of all studied variables with the response variable by fitting a linear equation as shown in Eq. 1. Here the coefficients (*b0*, *b1*, *b2*, *b3* and *bn*) determine the influence of the different independent variables (x1, x2, x3 and xn) on the outcome of the response variable (*Yn*). This approach is convenient when the number of experiments and parameters to be studied is not too high.

*Yn = bo + b1*x1 *+ b2*x2 *+ b3*x3 *+ ... + bn*xn Equation 1

The parameters studied were: irradiation power (300-1,000 W), pressure (100-1,013 mbar), extraction time (14-45 min) and WOP:water ratio (1:1.5-1:5). The total volume of WOP and water mixture was kept constant (1.5 kg) through the different experiments. This was done to maintain sufficient headspace within the 4 L reactor, preventing sample loss during the extraction process. All experiments (n=17) were done in replicate depending on the WOP:water ratio to ensure that enough essential oil could be collected: 1:1.5 (WOP:water), n=2; 1:2.5 (WOP:water), n=3; 1:3.5 (WOP:water), n=4; and 1:5 (WOP:water), n=5. Table 2 shows the parameters used in each one of the experimental runs together with their corresponding essential oil extraction yield (on dry matter). Each row represents a single experiment. All yields are expressed as percentage of dry matter.

Table 2. Experiments including studied parameters and their corresponding essential oil yields (%, dry basis).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Exp # | Power (W) | Pressure (mbar) | Time (min) | Ratio (WOP:water) | Yield (%, dry basis) |
| 1 | 600 | 1,013 | 30 | 1:1.5 | 1.402 |
| 2 | 800 | 400 | 45 | 1:1.5 | 1.822 |
| 3 | 600 | 700 | 18 | 1:2.5 | 1.121 |
| 4 | 400 | 100 | 19 | 1:2.5 | 0.140 |
| 5 | 1,000 | 1,013 | 14 | 1:5 | 0.514 |
| 6 | 600 | 500 | 15 | 1:5 | 0.467 |
| 7 | 700 | 600 | 18 | 1:2.5 | 1.042 |
| 8 | 900 | 800 | 15 | 1:3.5 | 1.636 |
| 9 | 500 | 500 | 30 | 1:3.5 | 1.168 |
| 10 | 675 | 475 | 43 | 1:1.5 | 1.509 |
| 11 | 400 | 400 | 44 | 1:2.5 | 1.542 |
| 12 | 300 | 300 | 43 | 1:5 | 1.168 |
| 13 | 600 | 400 | 35 | 1:2.5 | 1.523 |
| 14 | 600 | 1,013 | 24 | 1:5 | 1.449 |
| 15 | 700 | 900 | 19 | 1:3.5 | 0.607 |
| 16 | 400 | 250 | 35 | 1:3.5 | 0.748 |
| 17 | 600 | 800 | 25 | 1:2.5 | 1.626 |

The results of the statistical analysis based on a multiple linear regression are shown in Table 3. At a first glance, the relatively low R2 value (0.4712) would lead us to conclude that no clear linear correlation exists between the different parameters and the yield of essential oil; only the extraction time was a significant variable to be considered (95% confidence level; p = 0.0064). Looking at its corresponding positive coefficient (0.0329), it suggests that longer extraction times will lead to higher essential oil yields, as was initially expected. In this first approach pressure and power showed no significant influence on the yield. One explanation could be that pressure and power only influence the time necessary to reach (and maintain) the temperature at which the MAHD takes place and that a more vigorous boiling due to i) a higher microwave power or ii) a lower pressure does not significantly affect the amount of extracted essential oil.

Table 3. Statistical analysis of one-step MAHD results

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Intercept | Power | Pressure | Time | Ratio | Statistics |
| Coefficient | b0 | b1 | b2 | b3 | b4 | Adjusted R-Square |
| Yield | -0.2384 | 0.00045 | 0.00079 | 0.0329 | -0.0894 | 0.4712 |

With the aim of producing additional, more valuable information from the aforementioned experiments, all the data was once again studied from a different point of view. These results were used to make a calibration curve for the efficiency of the microwave radiation energy vs time (Figure 4). Efficiency is here defined as the effective percentage of the applied microwave energy (depending on the chosen power and the irradiation time) considering the total energy needed to heat up the microwave cavity and carry out the evaporation of the water and essential oil mixture as shown in Eq. 2.

*Energy efficiency (%) = 100 [Total Energy (kJ)/Applied Energy (kJ)]* Equation 2

As it can be observed, the results can be easily fitted to a linear trend or regression (y = 106.68 -0.6435x; R² = 0.9032) and show a decrease in the efficiency of the applied microwave energy as the extraction time increases. Thus, in order to develop an efficient extraction process using microwave energy, short irradiation times should be chosen. However, in the studied system, it was previously observed that higher essential oil yields were obtained following longer extraction times. This leads us to believe that instead of applying a constant power over the extraction time (leading to a less efficient procedure), it would be more beneficial for the extraction process to apply the microwave energy in (at least) two different steps to i) save energy costs and ii) increase the essential oil yield.

**3.2.2. MAHD optimisation**

Based on the aforementioned preliminary conclusions a new series of experiments was proposed. In this case the MAHD was carried out following a two-step irradiation process: i) a first step using a higher power to start the biomass boiling process as quickly as possible (while making use of its higher efficiency) and ii) a second step at a lower power yet sufficient enough to maintain the biomass at its boiling point (Table 4). Experiments (n=9) were divided in 3 groups (A, B and C) to study the effect of different combinations of power and pressure for both steps based on the results obtained after the analysis of the microwave energy efficiency. The pressure was kept constant in both steps at 800, 500 and 300 mbar for groups A, B and C, respectively. In all experiments the length of the first irradiation step was set at 5 min whereas during the second step the system was irradiated for 5, 15 and 30 min (resulting in a total irradiation time of 10, 20 and 35 min). In this new series the WOP:water ratio was left constant as, from previous experiments, it was not a critical variable. Considering its negative contribution to the multiple linear regression model and in order to have the maximum amount of WOP within the system in each experiment, it was decided to set the WOP:water ratio at its minimum (1:1.5).

Table 4. Second set of experiments (n=9) considering different combinations of power (W) and pressure (mbar) during 2 subsequent irradiation steps together with their corresponding essential oil yields (%, dry basis) and irradiation times (min).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Exp # | Step 1 | | | Step 2 | | | Total time (min) | Yield (%, dry basis) |
| Power (W) | Pressure (mbar) | Time (min) | Power (W) | Pressure (mbar) | Time (min) |
| A1 | 1,200 | 800 | 5 | 400 | 800 | 5 | 10 | 0.88 |
| A2 | 1,200 | 800 | 5 | 400 | 800 | 15 | 20 | 1.40 |
| A3 | 1,200 | 800 | 5 | 400 | 800 | 30 | 35 | 1.73 |
| B1 | 982 | 500 | 5 | 250 | 500 | 5 | 10 | 1.36 |
| B2 | 982 | 500 | 5 | 250 | 500 | 15 | 20 | 1.79 |
| B3 | 982 | 500 | 5 | 250 | 500 | 30 | 35 | 1.87 |
| C1 | 785 | 300 | 5 | 250 | 300 | 5 | 10 | 1.21 |
| C2 | 785 | 300 | 5 | 250 | 300 | 15 | 20 | 1.78 |
| C3 | 785 | 300 | 5 | 250 | 300 | 30 | 35 | 1.82 |

The yields obtained after carrying out all the experiments reveal that the combination of highest power and pressure selected for group A result in the lowest yields at an early stage of the irradiation process, and only the yield after 35 min of irradiation is comparable to that of groups B and C. This might be due to the higher temperature (93 °C) the system has to reach to allow the initiation of the boiling process in comparison to groups B and C (80 and 69 °C, respectively) and/or the lower vacuum (800 mbar). No significant difference in yield was observed between experiments B and C. Thus, in order to keep the power consumption and boiling temperature as low as possible to reduce costs and potential long-term product degradation, it was decided to use the conditions as stated for group C (step 1, 785 W, 300 mbar; step 2, 250 W, 300 mbar) and further study the effect of the irradiation time through the second step of the process.

Four additional experiments were carried out at total irradiation times of 15, 25, 30 and 45 min on replicates (n=3). Results for the obtained essential oil yields (%, dry basis) together with their standard deviations and the consumed power (kWh) in each experiment are shown in Fig. 5. As it can be observed, the yield for the essential oil yield reaches a plateau after 20 min of total extraction time in which yields obtained after longer irradiation times show no significant difference (α=0.05) from that at 20 min. Good reproducibility (RSD=6.5-7.3%; n=3) was obtained in every experiment regardless of the yield. As expected, the power consumed during the irradiation process shows a constant increase in time (R2=0.9972), meaning that shorter extraction times are more cost efficient. Therefore, and considering the different yields for essential oil and the economic costs involved in the process, the optimal conditions for the essential oil MAHD in this study were determined as follows: a first step where the biomass consisting on a 1:1.5 (WOP:water) mixture is irradiated using 785 W for 5 min, followed by a second step in which the system is irradiated using 250 W for 15 min (keeping a constant pressure of 300 mbar through all the irradiation process).

**3.3. MAHD versus HD**

The essential oil yield obtained in this study from *Navel Navelate* oranges using MAHD under the optimum conditions was 1.8 ± 0.1% (dry basis; n=3) and was comparable to that obtained by HD (1.7 ± 0.1%; dry basis; n=3) and also within the range of those reported in the literature using traditional extraction methods (Lopresto et al., 2014) and those based on microwave technology (Ferhat et al., 2006). A significant reduction in energy consumption was also observed comparing the suggested extraction technique in this study and the conventional HD process (0.5 and 3.2 kWh, respectively), resulting in a great advance in terms of time and cost efficiency. In order to know the composition of the oil obtained by MAHD and assess the similarities/differences between that extracted by HD, extracts were analysed by means of GC-TOF (Table 5).

Table 5. Chemical composition (%) of essential oil samples extracted from *Navel Navelate* variety oranges by HD and MAHD.

|  |  |  |
| --- | --- | --- |
| **Technique** | **HD1** | **MAHD2** |
| **Citrus** | **Orange** | **Orange** |
| **Variety** | **Navel Navelate** | **Navel Navelate** |
| **Monoterpenes** | **98.56** | **99.34** |
| D-Limonene | 96.75 | 97.38 |
| γ-Terpinene | - | 0.04 |
| β Pinene | 0.05 | 0.06 |
| α Pinene | 0.32 | 0.39 |
| R-β-Myrcene | 0.74 | 0.79 |
| Sabinene | 0.49 | 0.50 |
| α-Terpinolene | 0.20 | 0.18 |
| **Oxygenated Monoterpenes** | **0.14** | **0.14** |
| Linalool | 0.05 | 0.05 |
| Terpinene-4-ol | 0.01 | 0.01 |
| Terpineol | 0.01 | 0.01 |
| Eucalyptol | 0.07 | 0.06 |
| **Sesquiterpenes** | **0.00** | **0.01** |
| *Trans* α-bergamotene | - | 0.01 |
| **TOTAL** | **98.70** | **99.49** |

HD1: Hydro-distillation

MAHD2: Microwave assisted hydro-distillation

Comparing the essential oil samples extracted by means of HD and MAHD no remarkable difference was observed with regards to the identified compounds in this study. D-limonene was the most abundant chemical in both samples (96.75 and 97.38% for HD and MAHD, respectively). In all samples extracted by HD yields were similar to those obtained by MAHD. γ-Terpinene and trans α-bergamotene were also detected and quantified in the oil obtained by MAHD, proving that our alternative extraction technique is not only able to recover the same compounds present in orange oil extracted but also some additional chemicals of interest, adding extra value to the obtained product.

**3.4. Applicability to other citrus fruits**

The optimised method was applied to different citrus material for the extraction of essential oils to prove its applicability. Results are shown in Fig. 7 together with their corresponding standard deviation (n=3).

As expected from literature (Bousbia et al., 2009), differences in yield were obtained not only between the studied citrus (oranges, lemons, limes, satsumas and grapefruits), but also within the different varieties of oranges. Comparable yields of essential oils were obtained from oranges (*Valencia Late* variety) limes, satsumas and grapefruits (α=0.05), whereas lower yields were observed in the case of *Navel Navelate* and *Midknight* oranges, and lemons.

Almost all the components detected in the studied essential oils were non-oxygenated monoterpenes; accounting for >96.1% in all samples (Table 6). D-limonene was once again the most abundant compound within the oils extracted from the studied citrus, ranging from 61.93% in the case of lemons to >96.3% in the case of oranges. γ-terpinene, α-pinene and β-pinene were also detected in considerable amounts in the case of lemons, limes and grapefruits and their yields are in agreement with those found in the literature and extracted by different methodologies (Lota et al., 2002). Regardless of the differences in yields, similar compositions were determined after analysing the essential oils extracted by MAHD from the three studied varieties of orange (*Navel Navelate*, *Midknight* and *Valencia Late*). It should also be highlighted that fewer compounds were detected within the essential oils extracted from oranges compared to the other citrus in this study, particularly in the case of oxygenated terpenes. This is reflected in the price of these products in the global market (International Trade Centre, 2015).

Table 6. Chemical composition (%) of the essential oil samples extracted from oranges (*Navel Navelate,* *Valencia Late* and *Navel Powell* varieties), lemons (*Primofiore* variety), limes (*Tahiti* variety), satsumas (*Nihowase* variety) and grapefruits (*Star Ruby* variety) by MAHD.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Citrus** | **Orange** | **Orange** | **Orange** | **Lemon** | **Lime** | **Satsuma** | **Grapefruit** |
| **Variety** | **Navel Navelate** | **Midknight** | **Valencia Late** | **Verna** | **Persian** | **Satsuma** | **Grapefruit** |
| **Monoterpenes** | **99.34** | **98.54** | **98.51** | **96.84** | **96.10** | **97.36** | **98.39** |
| D-Limonene | 97.38 | 96.36 | 96.54 | 68.42 | 61.93 | 92.98 | 89.20 |
| γ-Terpinene | 0.04 | 0.04 | 0.04 | 11.35 | 16.93 | 0.08 | 4.82 |
| β Pinene | 0.06 | 0.07 | 0.07 | 12.31 | 11.36 | 0.28 | 1.59 |
| α Pinene | 0.39 | 0.41 | 0.35 | 1.89 | 2.35 | 0.79 | 0.96 |
| R-β-Myrcene | 0.79 | 0.90 | 0.84 | 0.83 | 0.90 | 2.01 | 0.93 |
| M-3-Carene | - | 0.09 | - | 0.12 | 0.17 | 0.16 | - |
| Sabinene | 0.50 | 0.46 | 0.53 | 1.00 | 0.77 | 0.57 | 0.23 |
| α-Thujene | - | - | - | 0.33 | 0.46 | 0.22 | 0.17 |
| p-Cymene | - | - | - | 0.19 | 0.21 | - | 0.10 |
| Terpinene | - | - | - | 0.13 | 0.29 | 0.02 | 0.05 |
| α-Terpinolene | 0.18 | 0.22 | 0.13 | 0.27 | 0.74 | 0.25 | 0.33 |
| **Oxygenated Monoterpenes** | **0.14** | **0.16** | **0.16** | **1.36** | **1.19** | **0.46** | **0.19** |
| Linalool | 0.05 | 0.08 | 0.07 | 0.10 | 0.14 | 0.20 | 0.02 |
| Terpinene-4-ol | 0.01 | 0.01 | 0.01 | 0.42 | 0.39 | 0.02 | 0.04 |
| Geraniol acetate | - | - | - | 0.41 | 0.10 | 0.09 | 0.02 |
| Terpineol | 0.01 | 0.01 | 0.01 | 0.32 | 0.39 | 0.10 | 0.04 |
| Eucalyptol | 0.06 | 0.07 | 0.06 | 0.12 | 0.16 | 0.05 | 0.06 |
| **Sesquiterpenes** | **0.01** | **0.51** | **0.52** | **0.10** | **1.04** | **0.06** | **0.09** |
| Valencene | - | - | 0.08 | 0.02 | 0.01 | 0.05 | 0.04 |
| β-Elemene | - | - | - | - | - | - | 0.01 |
| Trans α-bergamotene | 0.01 | - | 0.01 | 0.08 | 0.16 | 0.01 | 0.04 |
| β-Bisabolene | - | 0.51 | 0.43 | - | 0.87 | - | - |
| **Oxygenated Sesquiterpenes** | **0.00** | **0.00** | **0.00** | **0.24** | **0.66** | **0.13** | **0.01** |
| Z-Citral | - | - | - | 0.16 | 0.29 | - | 0.01 |
| E-Citral | - | - | - | 0.07 | 0.38 | 0.13 | - |
| **Other oxygenated compounds** | **0.00** | **0.01** | **0.00** | **0.13** | **0.23** | **0.02** | **0.04** |
| Neryl acetate | - | 0.01 | - | 0.13 | 0.23 | 0.02 | 0.04 |
| **TOTAL** | **99.49** | **99.22** | **99.19** | **98.54** | **99.00** | **98.01** | **98.67** |

Kamal et al. (2011) reported that in addition to the variation between different citrus fruits, essential oil content and composition is also highly dependent on factors such as storage, transportation and pre-treatment. It is possible that the differences observed in this study could be attributed to these additional variables. It should be noted, however, that most of the results in this study were comparable, if not higher, than those reported in the literature, confirming the good applicability of the MAHD process. Moreover, it has to be highlighted that one of the highest yields obtained was that in the case of *Valencia Late* oranges (2.3 ± 0.2%; dry basis; n=3). *Valencia Late* oranges are grown worldwide and their well-known characteristics make them one of the most profitable varieties for citrus processing industries (Reuter et al., 1967-1989). The ability of the MAHD process to obtain a high yield of essential oil using this particular variety of oranges results in it being an extremely attractive alternative at industrial scale.

**4. Conclusions**

MAHD of fresh waste citrus peel has been proved to be an effective alternative method for the extraction of essential oils from the low value by-products of the fruit processing industry. It has been demonstrated that MAHD presents distinct advantages for the fast and reproducible production process of essential oil whilst significantly reducing the energy and solvent consumption related to conventional production methods. During its development different features affecting the upscaling process have been considered to offer an attractive process for extractions at industrial scale: i) no drying of the feedstock is necessary, ii) no additives are required, and iii) extraction conditions could be chosen based on the energy efficiency of the microwave technology. As a result, a short irradiation time at a high power, using the minimum amount of additional water, was found to be required for an efficient extraction of the oil. Under these conditions the yields and composition of oils extracted from different citrus fruits were comparable to those reported in the literature using different approaches. Although a detailed cost-benefit analysis is required to further assess the economic practicability of the suggested methodology, these results could be of great significance to maximise process profitability. In any case, the applicability of the MAHD technology for citrus highlights its potential to be used to process other waste feedstocks and could help establish reliable guidelines for the development of an industrial scale facility.

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