

# Linear tuning of microwaves power for the extraction of bioactive molecules from citrus peels

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## ABSTRACT

In the last years, an alternative and convenient way to composting and/or bio-gasifying food waste is represented by the extraction of high value bioactive components from such materials. In particular, essential oils contained in matrices such as orange or lemon peels may represent high value bioactive components for the nutraceutical and pharma industry. In recent years, microwave assisted processes have been considered for use in solvent-free extraction. However, the microwave assisted extraction is often performed in very simple microwave systems, without an accurate control, if any, of the power release.

In this work, the linear tuning of microwave power for the extraction of bioactive components from citrus peels is discussed, with emphasis on the consequent process yield and extract characteristics. Chemical analysis of the extracted mixture showed the presence of quite a number of active molecules of relevant interest for pharmaceutical and nutraceutical industries, such as glycoside flavanone (Hesperidin and Eriocitrin) in lemon peels, and polymethoxylated flavones (Nobiletin and Sinensetin) in orange peels.

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## KEYWORDS

microwaves, food processing, bioactive compounds

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## INTRODUCTION

The term *citrus* refers to the cultivated plants belonging to the genus *Citrus* of the subfamily Aurantioideae (Rutaceae family) and to their fruits (oranges, lemons, mandarins, clementines, bergamot, chinotto). Even if originating in India and Far East, all citrus fruits today spontaneously grow in Indonesia, Malaysia, New Guinea, and Philippines. The species reached Europe at different times. Cedar, supposed to be the first to come, was known among the ancient Romans as the Persian apple. Citrus fruits are widely cultivated in the subtropical belt of the whole world.

Italy occupies a prominent place in the world production, with a share of around 5% of the world production, with an annual production of 4 million tons, that is a remarkable amount if referred to the Country size. The production is concentrated in the southern regions, with Sicily in the front row (about two thirds of national production), followed by Calabria, Campania, Puglia, Basilicata, and Sardinia.

The citrus processing industries generate huge amounts of waste every year: it has to be noted that only 40% of the total fruit mass is transformed in food products, while the remaining 60% becomes waste. This latter is rich in bioactive molecules such as flavonoids, carotenoids, essential oils, polyphenols, vitamins, and pectine (Petra et al., 2011) that are of a basic importance for nutraceutical and cosmetic industries. However, traditional extraction techniques present a number of significant disadvantages as they require very long processing times, typically involve use of toxic organic solvents with the relevant drawbacks of exposure of the operators, disposal and, above all, unsatisfying quality of the final extract (Ferhat et al., 2007).

Therefore, the agri-food sector has been investing, in the last years, time and money to develop new extractive procedures that could reduce use of organic solvents, energy consumption, process times, and CO<sub>2</sub> emissions, thus respecting the basic principles of *green chemistry*, with consequent money saving and reduced environmental impact (Chemat et al., 2012). Such an approach could transform waste into a real resource. The wastes can thus be seen as by-products or, even better, co-products. Recently, microwave assisted extraction (MAE) has been considered as one of the most sustainable innovative extraction techniques. With the dual mechanism of ionic conduction and dipole rotation, microwave energy can rapidly heat solvents to extract analytes from the sample matrix into the solvents (Leonelli and Villa, 2008). MAE has been successfully applied in extracting various substances from plant matrices, including flavonoids from grape pomace, anthocyanins from blackcurrant (Nora et al., 2013), caffeine from green coffee beans (Upadhyay et al., 2012), pesticides and metals.

In this work the solvent free microwave extraction technique (Lucchesi et al., 2004), as an alternative to the traditional ones, has been analyzed, based on microwave heating, to obtain essential oils, hydrolates and mixtures of polyphenols from orange and lemon peels, deriving from the manufacturing process. The technique has been applied to citrus pulp, residue from processing of lemons (variety *Sfusato Amalfitano*<sup>TM</sup>) and/or oranges (variety *Tarocco*<sup>TM</sup>), planted in Amalfi Coast. Actually, heating of the internal, native water contained in the vegetable matrix by microwaves and causes bursting of glands and oleiferous receptacles (Li et al., 2013). The process frees essential oil, which is thus extracted by the water by azeotropic distillation. The extracted vapors then pass through a condenser outside the microwave cavity.

The operating variables were studied which mostly influence the extraction process.

Aim of the work was to investigate the effect on the extraction process of the operating variables either related to the raw materials (humidity and size of matrices) or of the plant



arrangement (mode of microwaves power supply, duration of the process). Results are examined in terms of percentage yield of citrus peel oil, hydrolates and mix of flavonoids).

## MATERIALS AND METHODS

### The extraction plant

The work was carried in an extraction apparatus consisting of a microwave cavity, Whirlpool MWF 426 SL, 800 W (cavity size:  $L = 31.7$  cm;  $H = 21$  cm;  $W = 31.5$  cm), 2,450 MHz, equipped with a properly designed extraction chamber and a condenser. A 2.5 cm hole, made in the center of the cavity top side, allowed the vapors coming from the extraction chamber to be conveyed through a food-grade silicone tube to the liebig condenser (Fig. 1). A rotating paddle guarantees the microwave field homogenization, also avoiding the need for a rotating plate. The sample temperature is monitored through the use of optical fibers Fisio Tecnologías<sup>®</sup> ( $-40$  to  $300$  °C).

The apparatus has been operated in two modes of microwaves power supply: (i) in the cavity named “A” power supply is regulated by a duty-cycle, with magnetron either at maximum power (800 W) or turned off, alternating the time fraction of functioning according to the nominal mean power required (square wave) (see Fig. 2a); (ii) in the “B” cavity, a 5 kV specially designed power supplier, 1,000 W maximum power, equipped with a control feedback, allows a linear regulation of the microwaves power, that is supplied with continuity, by modulating the current intensity (Fig. 2b).

### The vegetables matrices: Lemon and orange peels

In each experiment, 150 g of peels were subjected to microwaves. Before each test, the humidity of the initial sample was measured, by a MB45 Ohaus, resulting for all tests is in the range 68–80%.

### The extraction runs

Experimental tests were carried out aiming at process intensification. Runs were performed in the power range 150–600 W, that gave the best results. Actually, operating above 600 W involves the risk of burning the vegetable matrices before distillation occurs, whereas to operate at a power below 150 W requires a too long extraction time.

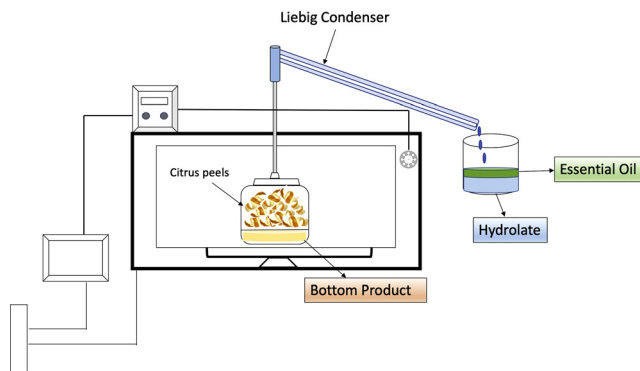


Fig. 1. Extraction Apparatus. Three products of the extraction are highlighted



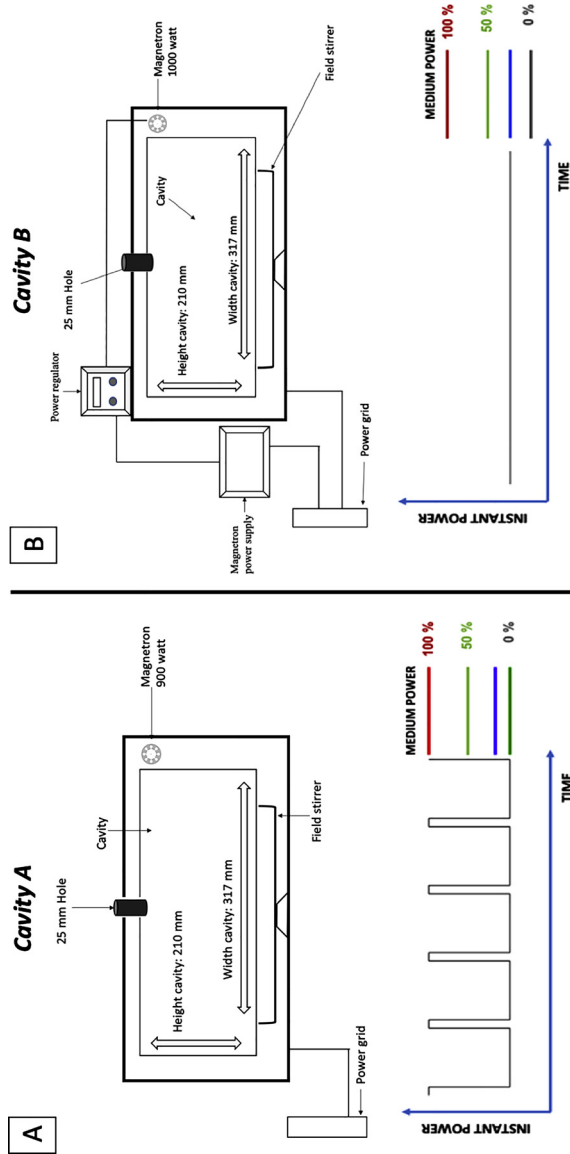


Fig. 2. (A) Duty cycle plant sketch; (B) Linear Tuning plant sketch



Table 1. Operating conditions

Level	Power (W)	Time (min)	Specific power (W/g)	Total energy supplied (kJ)
1	150	40	1	360
2	350	20	2.3	420
3	550	15	3.6	495

Experiments have been done at three power levels, namely 150, 350, and 550 W (Table 1). Times of the runs were decreased as power increased, to avoid the risk of matrix sample overheating and, consequently, bioactive molecules spoiling.

Bottom products are collected in the extraction chamber at the end of the process. Essential oils and aromatic water leaving the chamber as head products are collected and condensed at the cavity exit (Fig. 2). Fractional yield for each product is calculated in term of (mass of product)/(initial mass of vegetable matrix).

### The extraction products analysis

UPLC PDA analyses were performed on Ultra Pressure Liquid Chromatographic Acquity system (UPLC, Acquity I-Class, Milan, Italy) consisting of a Waters Acquity binary solvent manager, a sample manager (FL), a Column Manager (CM-A), PDA eLambda detector (equipped with a 500 nL detector flow cell volume), Acquity QDa detector and a degassing system. The whole configuration was driven by Empower software v3.0 from Waters Corporation. UPLC analysis was carried out on Kinetex C18 150 × 2.1 mm (100 Å), packed with 2.6 µm core shell particles, column (Phenomenex, Bologna, Italy). The optimal mobile phase consisted of H<sub>2</sub>O (A) and ACN (B) both acidified by formic acid 0.1% v/v. Analysis was performed in gradient elution as follows: 0–3.00 min, isocratic to 2% B; 3–25.00 min, 2–50% B; 25–30.00 min, 50–80% B; 30–30.01 min, isocratic to 82% B; then 5 min for column re-equilibration. Flow rate was 0.4 mL/min. Column oven temperature was set to 40 °C. Injection volume was 2 µL of extract. The following PDA parameters were applied: sampling rate, 20 points/sec; resolution, 1.2 nm. Data acquisition was set in the range 190–800 nm and chromatograms were monitored at 280 nm and 330 nm at the maximum absorbance of the compounds of interest. MS detection of polyphenol extracted was operated in both positive and negative ionization mode.

## RESULTS AND DISCUSSION

### Effect of power supply

For all products, yield in operations performed in Cavity B (linear microwaves tuning), is larger with respect to Cavity A (Table 2). It is hypothesized that duty cycle in Cavity A hinders the distillation process since water, when magnetron is turned off, keeps on evaporating taking the necessary energy from the vegetable matrix that, in turn, cools down, thus stopping the process. On the contrary, this latter is favored in Cavity B, that keeps the plant temperature constant with time by supplying a controlled power. As a consequence, yield in essential oil is almost negligible when operating with the duty-cycle of Cavity A. Hydrolate and Bottom products yields are affected too by the mode of power supply, being remarkably lower with Cavity A.

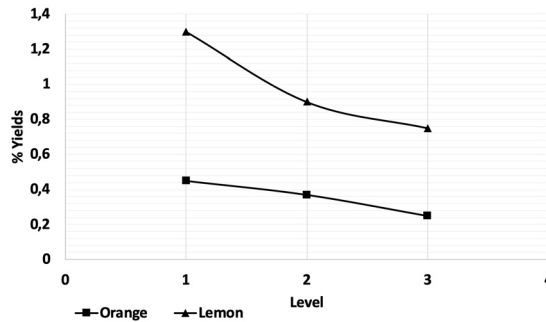


Table 2. Difference yields between Cavity A and Cavity B

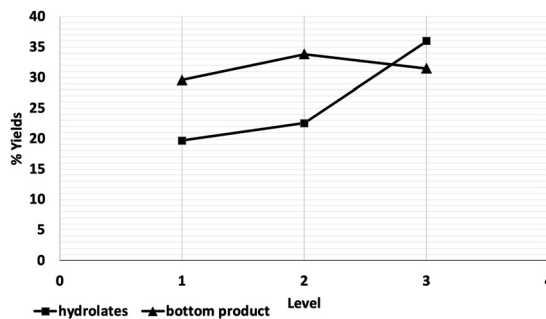
Level	Cavity A			Cavity B		
	Yields%			Yields%		
	O.E	Bottom product	Hydrolate	O.E	Bottom product	Hydrolate
1	0%	21.8%	12.21%	0.45%	29.6%	19.7%
2	0%	27.74%	14.8%	0.37%	33.82%	22.57%
3	0%	25.35%	28.23%	0.24%	31.5%	36.01%

**Effect of power**

Three levels of specific power have been used in performing the experimental runs (see Table 1). Results show that the lower power level (1 W/g) favors the essential oil extraction (Graph 1), whereas amount of bottom products and hydrolate increases when applying higher powers levels (2.3 W/g and 3.6 W/g). In particular power level 2 (2.3 W/g) favors the bottom product extraction, while power level 3 (3.6 W/g) favors hydrolate extraction (Graph 2).



Graph 1. Yields orange and lemon essential oil



Graph 2. Yields orange and lemon hydrolate and bottom product



## Effect of humidity

The best results were obtained using vegetable matrices with humidity in the range 70–80%. When the value is below 50%, the ability of sample to generate heat by water interaction with microwaves decreases. As a consequence, the amount of essential oils decreases. Conversely, if humidity exceeds 80%, it causes overheating of the plant matrix with possible degradation of the more volatile compounds.

## Analysis of bottom products

The presence of various molecules such as polyphenols, polymethoxyflavones and vitamins has been highlighted in the bottom products extracted from lemon peels. In detail, 27 polyphenols were identified, as reported in Table 3.

In particular, glycosidic flavanones such as eriocitrin (peak 18) and hesperidin (peak 25) (Fig. 3), molecules with overt antioxidant, antiphlogistic (Parhiz et al., 2015), hypotensive and hypocholesterolemic properties have been isolated (Hiramitsu et al., 2015).

Table 3. List of 27 substances identified in the bottom product extracted from lemon peel

Peak	Compound
1	Quinic acid
2	Coumaroylquinic acid
3	Coumaroylquinic acid isomer
4	Coumaroyl-glucaratc or -galactatc
5	Coumaroyl-glucarate or -galactate isomer I
6	Dihydro-caffeoyl-o-glucoside
7	Coumaroyl-glucarate or -galactate isomer II
8	Coumaroyl-glucaratc or -galactatc isomer III
9	Feruloyl-lucarate or -galactate
10	Unknown + hydroxy-methyl-glutaryl
11	Unknown + hydroxy-methyl-glutaryl
12	Dihydro-feruoyl-O-glicoside
13	Dihydro-fcruoyl-O-glicosidc isomer
14	Viccni II (Apigcniin-6,8-di-C-D-glucopyranoside)
15	Chrysoeriol-6,8-di-C-β-D-glucoside
16	Diosmetin-6,8-di-C-β-D-glucopyranoside (Lucenin-2-4'-methyl ether)
17	Dihydro-fcruloyl-glucosil-hydroxy-mcthil-glutaryl
18	Eriodictyol-7-O-rutinoside (Eriocitrin)
19	Kacmpfcrol-3-O-rutinoside
20	N äringen in-7-O-rutinoside (Narirutin, Isonaringin)
21	Hesperctin-7-O-neohesperidoside (Neohesperidin)
22	Isorhamnetin-3-O-rutinoside
23	Limocitrin-O-glucosil-O-rhamnosidc
24	Limocitrin-O-glucosil-hydroxy-methyl-glutaryl-O-glucose-ester
25	Hesperctin-7-O-rutinoside (Hesperidin)
26	Limocitrol-O-glucosil-hydroxy-methyl-glutaryl-O-glucose-ester
27	Limocitrin-O-glc-di-hydroxymcthilglutaryl-caffcoyl



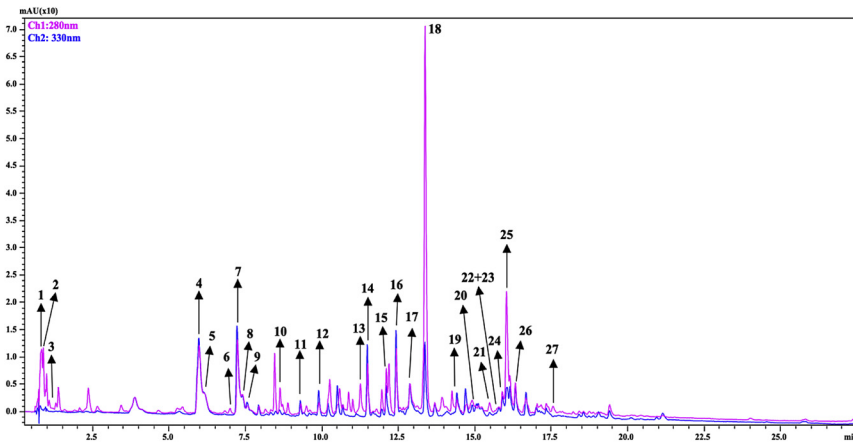


Fig. 3. LC/MS chromatogram of a bottom product extracted from lemon peels

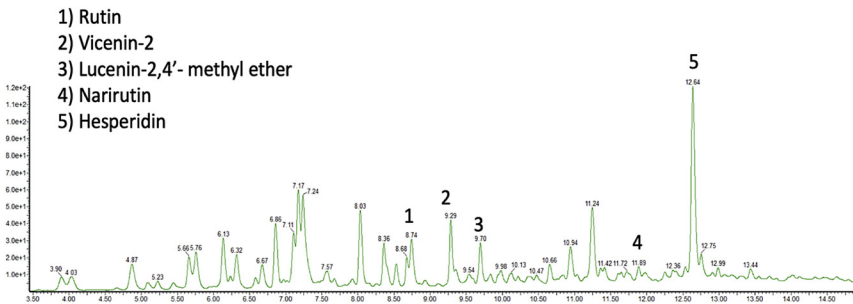


Fig. 4. LC/MS chromatogram of a bottom product extracted from orange peels – Part 1

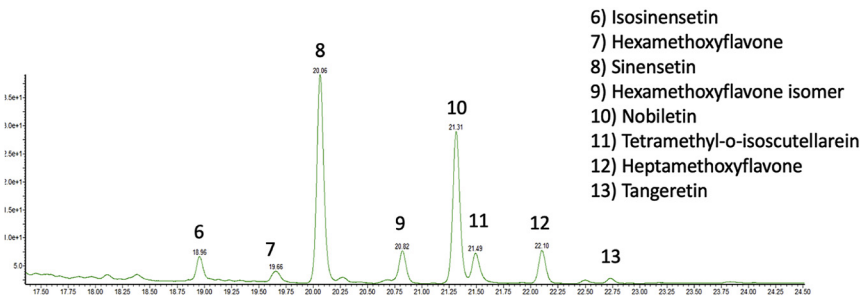


Fig. 5. LC/MS chromatogram of a bottom product extracted from orange peel – Part 2





The same kind of preliminary analysis was carried out on the bottom products extracted from orange peels. In this case, the most abundant compound was Hesperidin (peak 5) (Fig. 4).

Particularly interesting is the presence of polymethoxyflavones, nobiletin (peak 10) and sinensetin (peak 8) (Fig. 5). In fact, antitumor activity of those compounds is well recognized (Wang et al., 2014).

## CONCLUSIONS

Results of the experimental tests show that the microwave power must be supplied via a linearly variable delivery mode, as it guarantees feasibility and yields of the extraction of valuable compounds from waste derived by the citrus industries. Glycoside flavanone such as Hesperidin and Eriocitrin are actually found in the extract from lemon peels, whereas polymethoxylated flavones such as Nobiletin and Sinensetin are mostly found in oranges.

Low power operations appear to best work in the extraction of essential oils and hydrolates, while high powers are best suitable for the extraction of flavonoids.

It has been also seen that use of commercial apparatuses working with a power modulation by duty-cycle, i.e., alternating magnetron start and shutdown phases to produce a sort of pulse-width modulation, hinders the reproducibility of the extraction protocols.

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