LINEAR TUNING OF MICROWAVES POWER FOR THE EXTRACTION OF BIOACTIVE MOLECULES FROM CITRUS PEELS

GIUSEPPE D'AURIA[1]*, GIACINTO D'AMORE[2], ALDO ROMANO[1], FRANCESCO MARRA[2]

Abstract:

In the last years, an alternative and convenient way to composting and/or bio-gasifying the food waste is represented by the extraction of high value bioactive components from such wastes. Particularly, essential oils contained in matrices such as orange or lemon peels may represent high value bioactive components for the nutraceutical and pharma industry. In the recent years, microwave assisted processes where been considered for solvent free extraction. Microwave assisted extraction is often performed in simple microwave systems, with poor control of microwave 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.

The chemical analysis of extract mixture has shown the presence of quite a number of active molecules of great interest for the pharmaceutical and nutraceutical industry, such as glycoside flavanone (Hesperidin and Eriocitrin) in lemon peels and polymethoxylated flavones (Nobiletin and Sinensetin) in orange peels.

*Keyword***:** microwaves, food processing, bioactive compounds

Introduction:

The term citrus refers to the cultivated plants belonging to the genus Citrus of the subfamily Aurantioideae (Rutaceae family) and their fruits (oranges, lemons, mandarins, clementines, bergamot, chinotto). The origin of all citrus fruits is India and Far East. Today, they spontaneously grow in Indonesia, Malaysia, New Guinea and the Philippines. The various 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. The largest producer is the USA, following the Mediterranean (Europe and Africa), Asia and South America. Italy occupies a prominent place in the world production, with a share of around 5%

[1] Dipartimento di Farmacia – Università degli studi di Salerno, Fisciano SA Italy
[2] Dipartimento di Ingegneria Industriale – Università degli studi di Salerno, Fisciano SA Italy

*corresponding author: gdauria@unisa.it

(comparable to Japanese and Spanish production, but much lower than that of Brazil and the United States, reaching 25 % and 20%, respectively). In Italy, the production of citrus fruits 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. Only 40% of the total mass of the fruit is transformed in food product, while the remaining 60% becomes waste. Nevertheless, waste are rich in bioactive molecules such as flavonoids, carotenoids, essential oils, polyphenols and vitamins, that are of a basic importance for nutraceutical and cosmetic industries. However, the traditional extraction techniques present a number of significant disadvantages: require very long processing times, and typically involve the use of toxic organic solvents with the relevant drawbacks of exposure of operators, disposal and, above all, quality of the extract.

For this reason, in the last years the agri-food sector has invested in the development of new extractive procedures that could reduce use of organic solvents, energy consumption, process times and CO_2 emissions, thus respecting the basic principles of *green chemistry*, with consequent money saving and reduced environmental impact. Such an approach is able to transform the waste into a real resource. The wastes can thus be seen as by-products or, even better, co-products.

In this work an extractive technique, 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 (Limon amalphitanus), deriving from the manufacturing process. The operating variables of the matrix (i.e. humidity, size, etc.), and of the plant (mode of power supply, either with a duty cycle or linearly variable, reactor design, microwave field distribution in the oven cavity, duration of the process, etc.) were studied , which mostly influence the extraction process.

Materials and Methods

The extraction plant. The work was carried out using two microwave cavities, sketched in Figs 1-2.

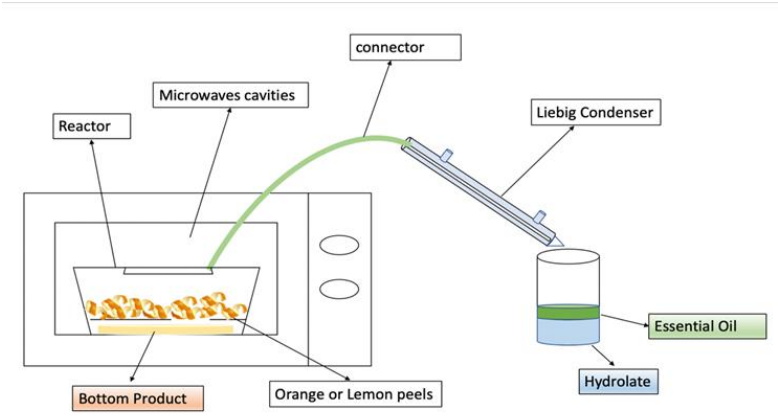

Figure 1**:** Extraction plant sketch

The cavities had different microwaves field distribution and power supply.

Both the extraction systems used consist schematically of three components: i) linear variable power microwave cavity; ii) 2: extraction chamber; iii) condenser.

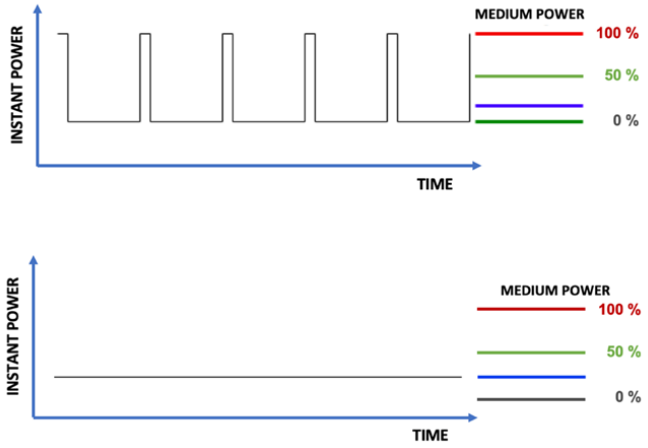

Figure 2: Schematics of duty cycle (a) and linearly variable power supply (b)

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. resulting for all tests is in the range 68-80%.

The extraction runs. Experimental tests were carried out aiming at process intensification. The power range 150 - 600 watts gave the best results, as operating above 600 watts involves the risk of burning the plant matrices before distillation occurs, whereas to operate at a power below 150 watts requires a too long extraction time. Operating conditions are reported in the followings Table.

Level	Power [W]	Time [min]	Total Energy Supplied [kJ]
Low	150	40	360
Medium	350	20	420
High	550	10	330

Table 1. Operating conditions

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_2O (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 80-2% B; then five minutes 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

From a qualitative point of view, the presence of various molecules such as polyphenols, polymethoxiflavones and vitamins has been highlighted in the bottom product extracted from lemon peels. In detail, 27 polyphenols were identified, as reported in Table 2.

Peak	Compound
1	Quinic acid
2	Coumaroylquinic acid
3	Coumaroylquinic acid isomer
4	Coumaroyl-glucarate or - galactate
5	Coumaroyl-glucarate or – galactate isomer I
6	Dihydro-caffeoyl-*O*-glucoside
7	Coumaroyl-glucarate or – galactate isomer II
8	Coumaroyl-glucarate or – galactate isomer III
9	Feruloyl - lucarate or- galactate
10	Unknown + hydroxy-methil-glutaryl
11	Unknown + hydroxy-methil-glutaryl
12	Dihydro-feruoyl-O-glicoside
13	Dihydro-feruoyl-O-glicoside isomer
14	Vicenin II (Apigenin-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-feruloyl-glucosil-hydroxy-methil-glutaryl
18	Eriodictyol-7-*O*-rutinoside (Eriocitrin)
19	Kaempferol-3-*O*-rutinoside
20	Naringenin-7-*O*-rutinoside (Narirutin, Isonaringin)
21	Hesperetin-7-*O*-neohesperidoside (Neohesperidin)
22	Isorhamnetin-3-*O*-rutinoside
23	Limocitrin-*O*-glucosil-*O*-rhamnoside
24	Limocitrin-*O*-glucosil-hydroxy-methil-glutaryl-*O*-glucose-ester
25	Hesperetin-7-*O*-rutinoside (Hesperidin)
26	Limocitrol-*O*-glucosil-hydroxy-methil-glutaryl-*O*-glucose-ester
27	Limocitrin-*O*-glc-di-hydroxymethilglutaryl-caffeoyl

Table 2: List of 27 substances identified in the bottom product extracted from lemon peel

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

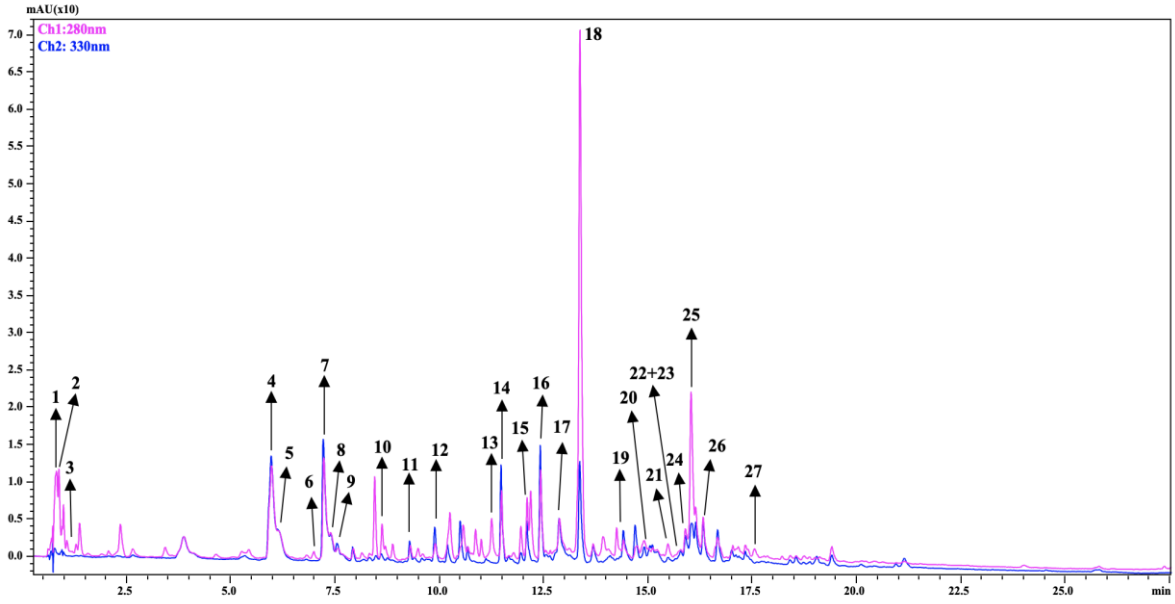

Figure 3: LC/MS chromatogram of a bottom product extracted from lemon peels

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

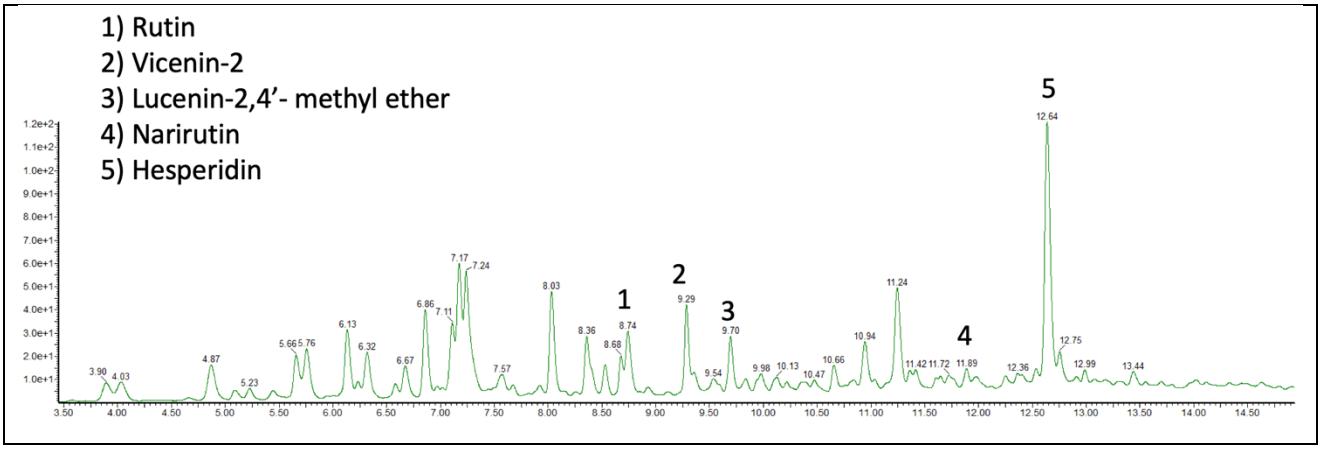

Figure 4: LC/MS chromatogram of a bottom product extracted from orange peels - Part 1.

Particularly interesting is the presence of polymethoxiflavones, nobiletin (peak 10) and sinensetin (peak 8) (Figure 5), compounds whose antitumor activity is well recognized (Wang et al., 2014).

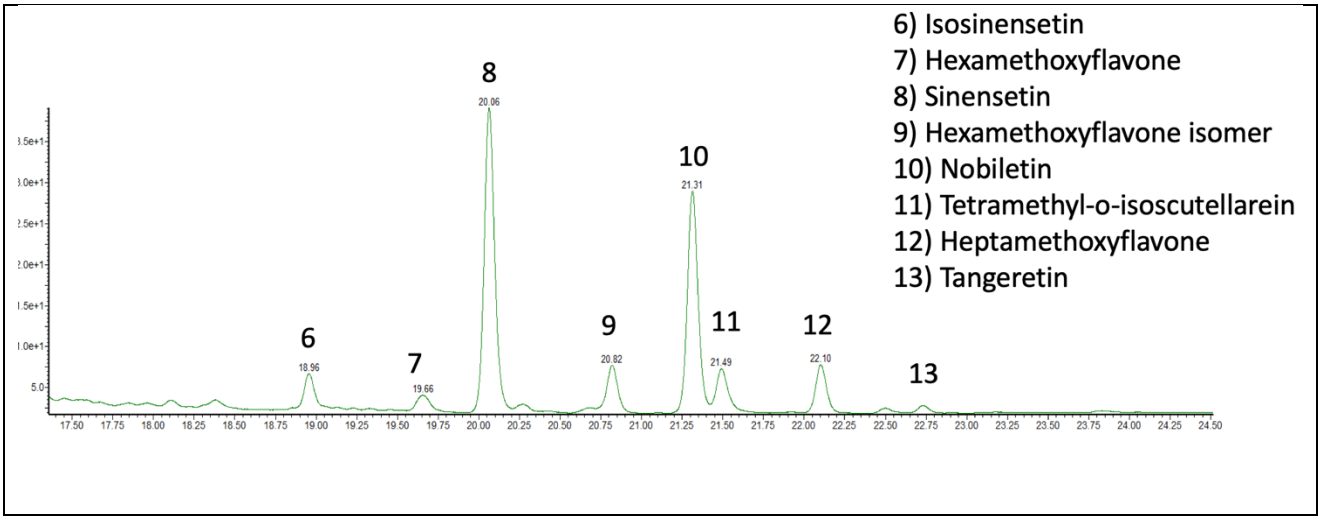

Figure 5: LC/MS chromatogram of a bottom product extracted from orange peel - Part 2.

Conclusions

The results of the experimental tests show that the microwave power must be supplied via a linearly variable delivery mode, that guarantees the feasibility of the extraction of valuable compounds from the wastes 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.

Finally, a significant role is played by the shape of the extraction chamber.

Acknowledgments

This work was done in the framework of Microexativa project, run at University of Salerno, Italy.

“Progetto cofinanziato dall’Unione Europea, dallo Stato Italiano e dalla Regione Campania, nell’ambito del POR Campania FESR 2014-2020” Microexativa - Estrazione senza solvente, mediante microonde, di principi attivi per la cosmetica e la nutraceutica da matrici di Cannabis Sativa”

References

Chemat, F., Vian, M., Cravotto, G. (2012) *Green Extraction of Natural Products: Concept and Principles.* IJMS 13, 8615–8627. https://doi.org/10.3390/ijms13078615

Ferhat, M.A., Meklati, B.Y., Chemat, F. (2007) *Comparison of different isolation methods of essential oil fromCitrus fruits: cold pressing, hydrodistillation and microwave 'dry' distillation*. Flavour Fragr. J. 22, 494–504. https://doi.org/10.1002/ffj.1829

Hiramitsu, M., Shimada, Y., Kuroyanagi, J., Inoue, T., Katagiri, T., Zang, L., Nishimura, Y., Nishimura, N., Tanaka, T. (2015) *Eriocitrin ameliorates diet-induced hepatic steatosis with activation of mitochondrial biogenesis.* Sci Rep 4, 3708. https://doi.org/10.1038/srep03708

Lucchesi, M.E., Chemat, F., Smadja, J. (2004) *Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydro-distillation.* Journal of Chromatography A 1043, 323–327. https://doi.org/10.1016/j.chroma.2004.05.083

Marra, F. (2012) *Microwave and Radio-Frequency Heating Processes for Food*, in: Ahmed, J., Shafiur Rahman, M. (Eds.), Handbook of Food Process Design. Wiley-Blackwell, Oxford, UK, pp. 1031–1056. https://doi.org/10.1002/9781444398274.ch35

Parhiz, H., Roohbakhsh, A., Soltani, F., Rezaee, R., Iranshahi, M. (2015) *Antioxidant and Anti-Inflammatory Properties of the Citrus Flavonoids Hesperidin and Hesperetin: an Updated Review of their Molecular Mechanisms and Experimental Models* Phytother. Res. 29, 323–331. https://doi.org/10.1002/ptr.5256

Wang, L., Wang, J., Fang, L., Zheng, Z., Zhi, D., Wang, S., Li, S., Ho, C.-T., Zhao, H. (2014) *Anticancer Activities of Citrus Peel Polymethoxyflavones Related to Angiogenesis and Others*. BioMed Research International 2014, 1–10. https://doi.org/10.1155/2014/453972