C. R. Chimie 17 (2014) 252–260

Contents lists available at ScienceDirect

Comptes Rendus Chimie

www.sciencedirect.com

Mémoire

Towards a comprehensive exploitation of agrofood residues: Olive tree – olive oil as example

María Dolores Luque de Castro

Department of Analytical Chemistry, Annex Marie Curie Building, Maimónides Institute of Biomedical Research (IMIBIC), Reina Sofía University Hospital, Campus of Rabanales, University of Córdoba, 14071 Córdoba, Spain

ARTICLE INFO

Article history:
Received 17 July 2013
Accepted after revision 21 November 2013
Available online 11 January 2014

Keywords:
Residues
Agrofood industry
Olive oil pomace
Antioxidants
Phenols

ABSTRACT

A comprehensive use of residues from the agrofood industry requires development of a number of steps – discussed in this article taking as a model the olive tree – olive oil binomial – consisting of: (i) extraction, identification and quantitation of valuable products (with isolation of individual compounds, if required); (ii) assessment of beneficial effects (which could be made mainly through metabolomics); (iii) improvement of cultivation varieties (through cross-breeding or other agricultural resources); (iv) commercialization as nutraceuticals, food supplements, pharmaceuticals or even as antibacterial and antifoam agents in water treatment plants; (v) use of the final residues to produce compost, foodstuffs, biogas or heat (after checking the characteristics of these residues which can be very different from those of the original residue). This working scheme may be applied to any other system to break the traditional one cultivation–one commercial product scheme.

1. Introduction

A characteristic aspect of the Mediterranean agriculture and the derivate industries is that each cultivation results in only one valuable product: that is, olive trees produce olive oil (only a small part of the fruit is used as table olives). Nevertheless, residues such as leaves, branches and stems are non-valuable. On the other hand, a very undesirable waste from oil production is oil pomace; that is, alperujo (if a two-phase system is used for oil production), or alpechín plus orujo (when the production system consists of three phases). In both cases the antioxidant nature of the waste makes it very polluting.

Concerning vineyards, they also give non-valuable residues such as vine shoots and the leaves (the former are a source of valuable antioxidants and leaves contain a wide variety of colorants, the nature of which depends on the type of cultivar). Grapes are used in a small proportion as fruit or to produce juice, but a major part is used for wine production, thus giving place to non-valuable residues such as grape skin and grape seeds (the sum of which constitutes the wine pomace), and also to lees of different particle size depending on the vinification step. Finally, wine orujo is transported to ethanol distillation industries from which a more degraded waste is formed [1].

There are, in the Mediterranean basin, other cultivars with lower production than olive tree or vineyards (e.g. oranges, lemons, apples, tiger nuts) whose manufactured products (e.g. juices, cider, horchata) give place to low-priced residues or undesirable waste. All deserve appropriate treatment to be converted into more or less valuable products by extracting those components, which can be of commercial interest. Most times, when the extracted components are antioxidants, the solid material remaining after extraction has lost its pollutant character (mainly due to the presence of antioxidants) and can be used for purposes for which it was prohibited before removal of antioxidants.

A rational use of unwanted, useless or low valuable residues would create a source of additional wealth, which in some cases, could have a value even higher than that

Email address: qa1lucam@uco.es.

1631-0748/$ – see front matter © 2013 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.crci.2013.11.010

traditionally given to the only reason of the target crop, thus providing interesting perspectives to implement new industries.

Nevertheless, the easy-to-plan implementation of an industry (e.g. an extraction plant to obtain antioxidants from any of the residues of the Mediterranean agrofood industry: vine shoots, olive tree leaves, wine lees or olive oil pomace) requires a series of subsequent steps to obtain the desired profit from the target raw material(s). Therefore, comprehensive exploitation of these residues requires the following steps to be developed (taking as a model the most characteristic case in the Mediterranean basin: olive tree – olive oil), consisting of:

- extraction, identification and quantitation of valuable products (isolation of individual compounds, if required);
- assessment of beneficial effects (mainly through metabolomics);
- improvement of traditional cultivars;
- commercialization as nutraceuticals, food supplements or pharmaceuticals;
- use of the final residues to produce compost, foodstuffs, biogas, or heat, as the most important.

2. Extraction, identification and quantitation of valuable products

This multistep procedure requires a first development at the laboratory scale, which can be divided into different analytical steps. Fig. 1 shows examples of raw materials, types of possible energies to be used to improve, accelerate, and/or automate extraction, comparison with conventional methods, and analytical equipment for identification–quantitation of target compounds in the extracts.

2.1. Extraction of the target compounds

Fulfillment of present trends on green processes require to have in mind some key principles:

- use of non-toxic, non-pollutant extractants;
- reduction of the extractant volume if the extracted compounds are finally used in the solid state (thus decreasing energy consumption to remove the extractant).

A short extraction time is also desirable to increase production, particularly when dealing with stational crops that require treatment of huge amounts of the target raw material in short times to avoid drying and storage steps. Decrease of the extraction time and an increased efficiency is achieved by application of technologies based on the use of auxiliary energies. After surpassing the boom of supercritical fluid extraction (SFE), which elevated the use of supercritical CO_2 at the panacea category [2], this technology has not been forgotten, but it is almost devoted to the sole extraction of non-polar compounds (mainly fats and oils), as it corresponds to the non-polar nature of the extractant [3]. Other more versatile technologies, with lower costs and easy to scale to pilot plants and then to industrial plants, are gaining popularity. Among the auxiliary energies that apply these new technologies, are ultrasound (US) [4], microwaves (MW) [5] and high temperature + pressure, this last giving place to superheated liquid extraction (SHLE) [6]. Another name given to SHLE is pressurized liquid extraction (PLE), which does not express the effect of the two physical parameters involved in the technique: increased pressure is not the variable that provide to the technique their properties as overpressure is only required to maintain the liquid (extractant) in this state at temperature above its boiling point. High temperature of the extractant is the key for the success of the methods based on SHLE; while the increase of pressure has a negligible effect once the liquid is maintained as such. Fig. 2 shows laboratory extractors based on the use of the three types of energy.

The SHL extractor in Fig. 2A allows both static and dynamic steps. The former is achieved by switching off valves V1 and V2, then applying the pressure–temperature program. The dynamic step starts by switching on valve V1, and regulating V2 to the optimum flow rate and the optimum working pressure at the selected temperature [7].

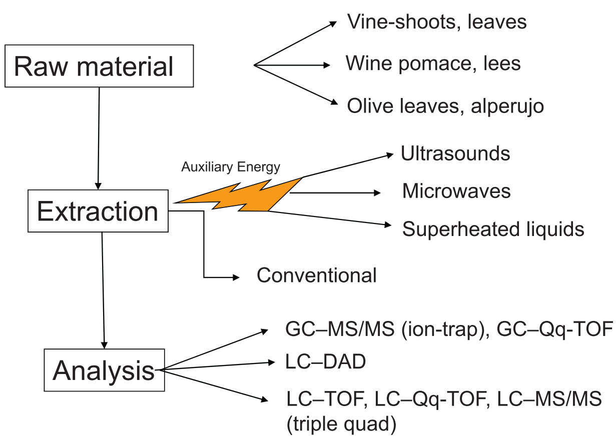


Fig. 1. Analytical steps to be followed to characterize components from unwanted, useless or low valuable residues from the agrofood industry typical of the Mediterranean basin. Gas chromatography–mass spectrometry in tandem-ion trap (GC–MS/MS-IT); GC–quadrupole and time-of-flight (GC–Qq-TOF); liquid chromatography–diode array detection (LC–DAD); LC–time-of-flight (LC–TOF); LC–Qq-TOF, LC–MS/MS (triple quad).

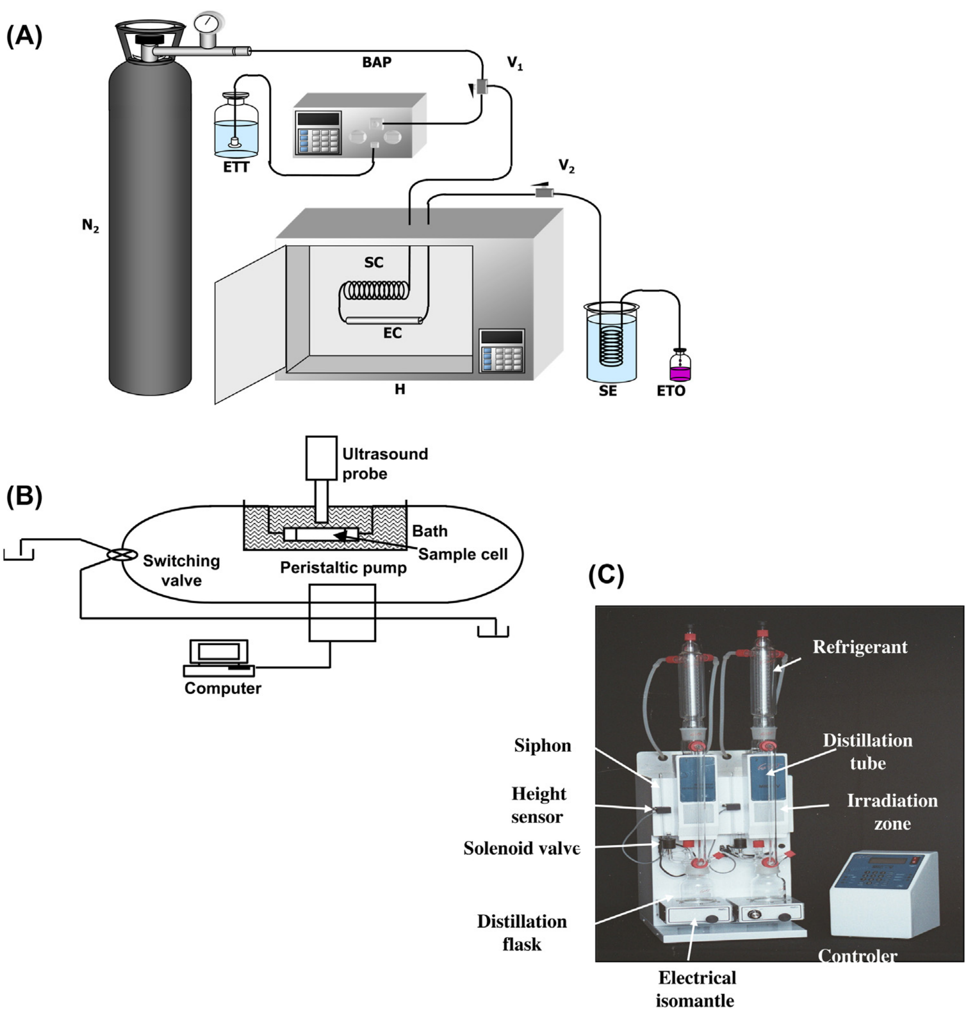


Fig. 2. Typical laboratory extractors based on (A) superheated liquids. BAP: high pressure pump; EC: extraction chamber; ETT: extractant; ETO: extract; H: oven; SC: heating coil; SE: cooling coil; V1: switching valve; V2: restrictor. (B) Application of ultrasound in a dynamic system. (C) Microwave irradiation in a Soxhlet-based system.

US-assisted extraction (USAE) can also be developed in a static and/or a dynamic mode. Fig. 2B shows a dynamic extractor in which the extractant is recirculated to minimize dilution of the extract. With this aim, the flow direction is changed at preset intervals thus also avoiding increased compactness of the solid in the extraction chamber [8]. Easy automation of the extraction step is a common characteristic of these extractors that are endowed with reproducibility when the US device is a probe or a bath designed for analytical purposes [4].

MW has demonstrated to be an excellent energy to improve extraction, especially when dealing with polar compounds. Devices for MW irradiation to favor extraction range from domestic ovens to dedicated extractors with strict control of temperature, pressure, power and time for application. Also Soxhlet extraction has been accelerated by using MW with different degrees of similarity to the original technique and with also different success. One of these devices is the focused microwave-assisted Soxhlet extractor (FMASE) designed by the author's team, a dual version of which (with control of the extractant volume and the time for sample–extractant content together with continuous or discontinuous MW irradiation) is shown in Fig. 2C [9].

It could be said that these three technologies cover all the possible needs of matrix–extracted compounds: polar and non-polar compounds, thermostable and thermounstable compounds (US can work at very low temperatures), and compounds resistant to the action of free radicals, as those formed in polar media subjected to high-power US (frequencies in the range 20–40 kHz).

An example of the use of devices based on the three types of energy for the extraction of phenols from olive leaves by using ethanol–water mixtures (Table 1) shows that the superheated liquid extractor provides the most concentrated extract; US-assisted extraction requires the lowest ethanol percentage (probably due to the formation of free radicals which compensate for the lower ethanol content in the extractant); while the MW-assisted extraction is the fastest as it requires only 8 min for quantitative

Table 1
Comparison of the types of energy-assisted extraction of phenols from olive leaves.

Variable	Extraction with superheated liquids	Ultrasound-assisted extraction	Microwave-assisted extraction
Extraction time (min)	13	25	8
Ethanol–water ratio in the extractant	70:30	60:40	80:20
Extractant volume (mL)	11	15	24

extraction [10]. Therefore, the most appropriate to the given application can be selected.

Formation of very fine emulsions between two immiscible extractants increases the surface contact between both and with the solid subjected to extraction (in this case a more correct name of the step is leaching). Emulsion formation is favored either by US (through the cavitation phenomenon) [11], or by boiling of the extractant with lower boiling point, in the case of MW [12], thus making possible simultaneous extraction of polar and non-polar compounds from plants, as shown in Fig. 3.

2.2. Identification and quantitation of valuable products in the extracts

Extraction is usually followed by one or several of the metabolomics strategies in Fig. 4 to obtain information on the vegetal metabolites extracted from each raw material or to know their effects on living organisms (particularly on humans).

The preferred metabolomics approach to classify the samples is fingerprinting, thus establishing the differences and similarities between cultivars, ripening state, crops, etc., while untargeted analysis is used to obtain a comprehensive profile of the metabolome of the target raw sample. Once the compounds of interest have been identified in the extract, targeted analysis allows quantitation of the given metabolites.

Examples of comprehensive identification of compounds in alperujo – more than 50 compounds – [13] and olive tree leaves – 26 compounds – [14], as well as in lees [15] or volatile fraction of vine shoots (more than 80 volatile compounds, and 18 studied cultivars) and oak ships (more than 50 volatile compounds, and 5 types of wood) [16] appears in recent literature on these subjects.

Once the compounds of interest have been identified, quantitation requires, most times, separation of the target compounds from the rest of the extract and sometimes also preconcentration. An excellent technique for both aims is solid-phase extraction (SPE), which is properly

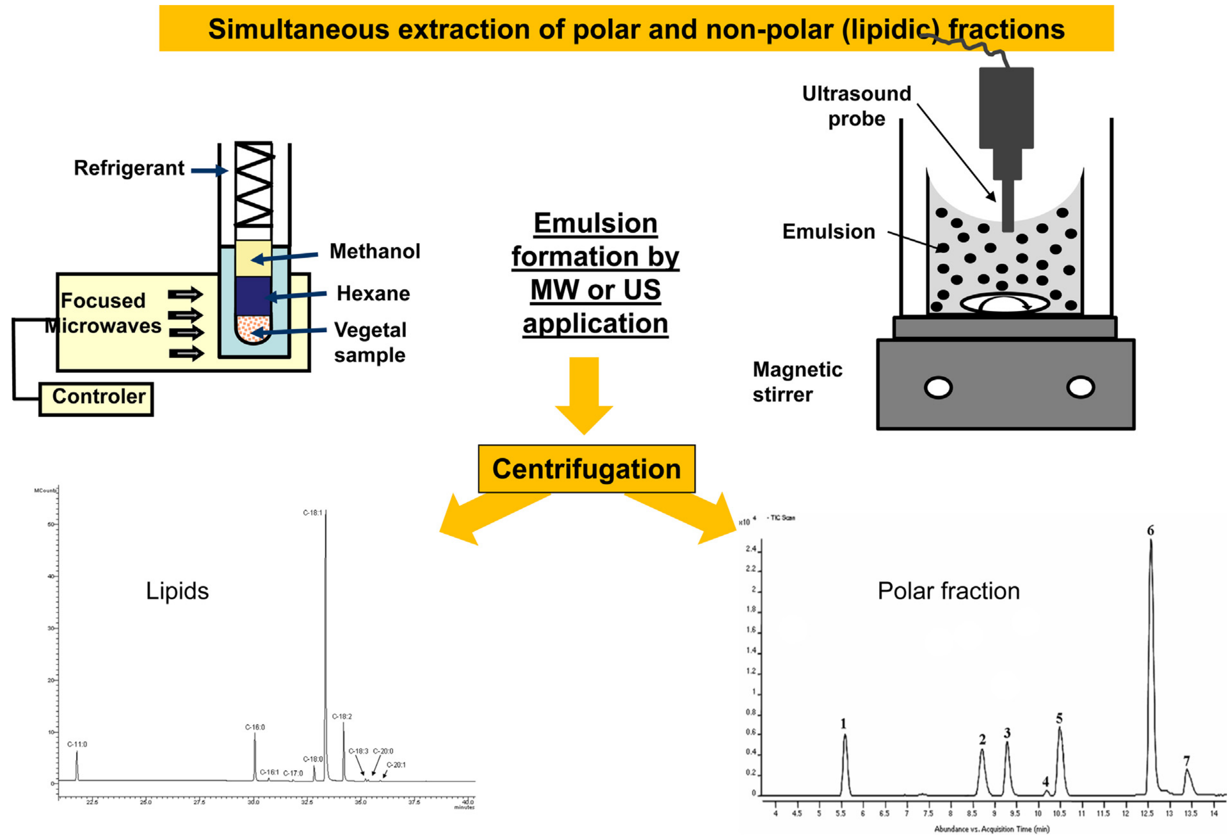
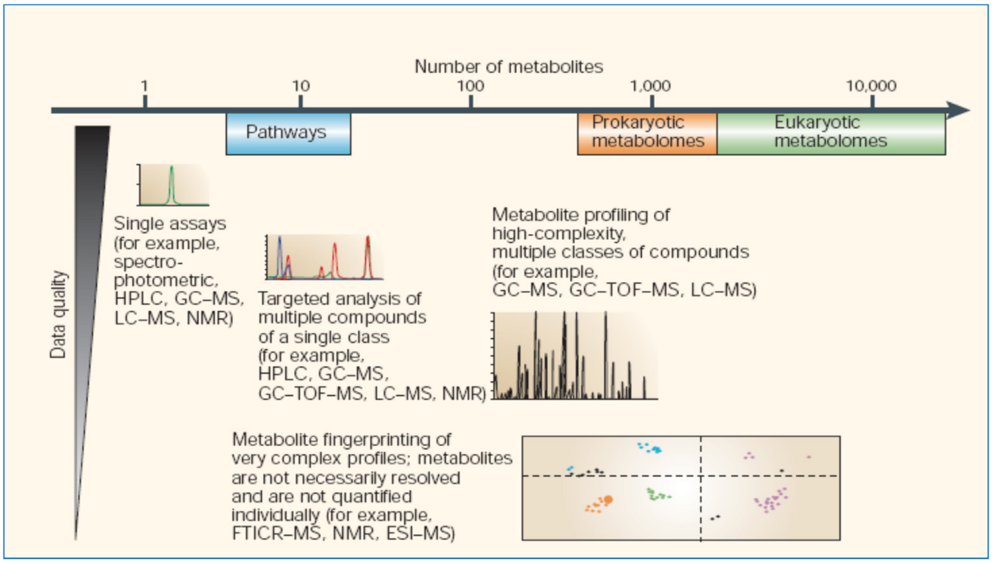


Fig. 3. Formation of a fine emulsion between immiscible extractants (favored by either high-power US or boiling of one of the extractants) makes possible the simultaneous extraction of polar and non-polar compounds.



Targeted analysis

Qualitative and quantitative study of one (single assay) or, more frequently, a small group of chemically related meta-bolites.

Fingerprinting analysis

High-throughput, rapid analy-sis of biological samples that provides patterns for sample classification and screening.

Untargeted global analysis

Detection of a broad range of metabolites to obtain a comprehensive profile of the metabolome.

Fig. 4. Strategies for metabolomics analysis.

implemented on line with subsequent individual separa-tion, generally by gas or liquid chromatography, and less commonly by capillary electrophoresis (CE). This coupling allows elution of the retained compounds by the chromatographic mobile phase. In this way the total amount of retained compounds is driven to the chromato-graphic column and maximum sensitivity is obtained as a result. Quantitation by MS is preferred because of its well-known characteristics. Precise quantitation is achieved only if standards of the target metabolites are available to run the corresponding calibration curve; otherwise, only semiquantitative determination is possible by interpola-tion of the signal provided by the given compound with no available standard within the calibration curve of the compounds with higher similarity.

3. Assessment of beneficial effects (mainly through metabolomics)

There is in the literature a wide number of proved beneficial effects from plants and/or their extracts; nevertheless, only a few of them have been assessed in such a way that the corresponding organism has found enough proofs of the effect to accept the corresponding principle as the cause. This is the case with hydroxytyrosol, for which the corresponding Panel of the European Food Safety Authority (EFSA) has concluded that "a cause and effect relationship has been established between the consumption of olive oil polyphenols (standardized by the content of hydroxytyrosol and its derivatives)" [17]. Therefore, the step subsequent to extraction, identification and quantitation is assessment. In the case of olive compounds, the author's team has contributed by proving the beneficial effect of extracts to improve food quality and favor essential human metabolic pathways.

3.1. Improvement of food quality

An example of how metabolites in the extracts from olive leaves or alperujo can improve the stability of oils is the research on refined oils [18]. These oils lose polar antioxidants in the extraction process that involves liquid–liquid extraction with hexane; therefore, the oils were enriched with extracts from olive leaves or alperujo at different concentrations, as shown in Fig. 5. Maize soy, high-oleic sunflower, sunflower, olive and rapeseed oils were enriched, and lowercase or capital letters were used to name the oils enriched with low (200 μg/mL) or high concentrations (400 μg/mL) of antioxidants in the extracts; "a" and "l" were used to express the type of extract (from alperujo or leaves). The stability of each oil, studied by the Rancimat method [19], is shown in Fig. 6. As can be seen in the figure, all oils improved their stability that, in some cases – as that of refined maize oil enriched with 400 μg/mL of alperujo extract (ROO-A) – became even higher than that of extra virgin olive oil (EVOO) with 400 μg/mL of original content of phenols.

3.2. Improvement of human welfare

A study which supports the effect of olive phenols on heated oils and their impact on the pathways of key,

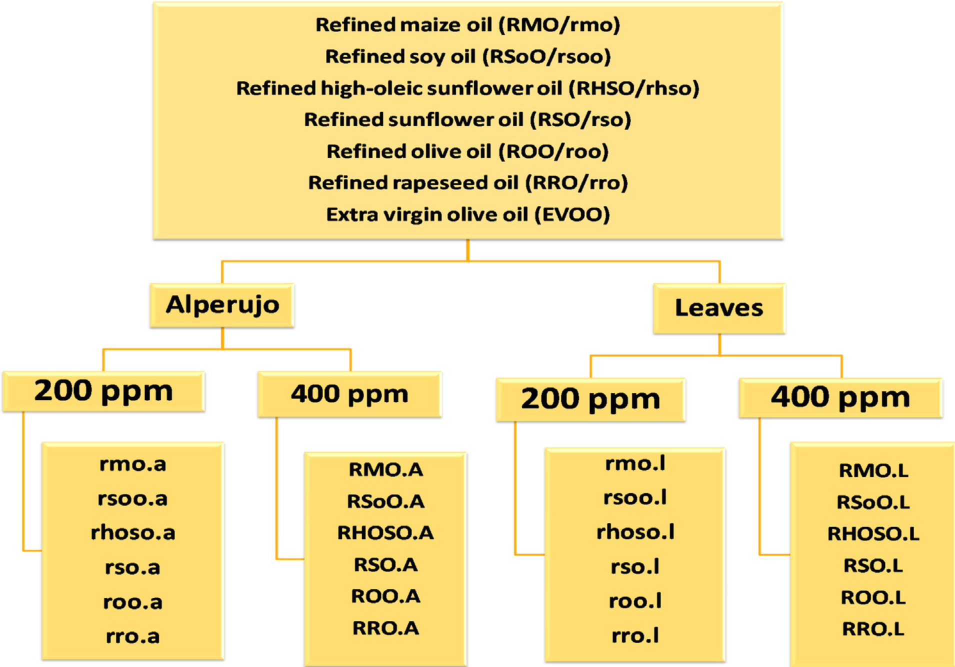


Fig. 5. Nomenclature of oils enriched with extracts from olive leaves or alperujo at different concentrations. Extra virgin olive oil (EVOO) had a natural phenol content of 400 μg/mL.

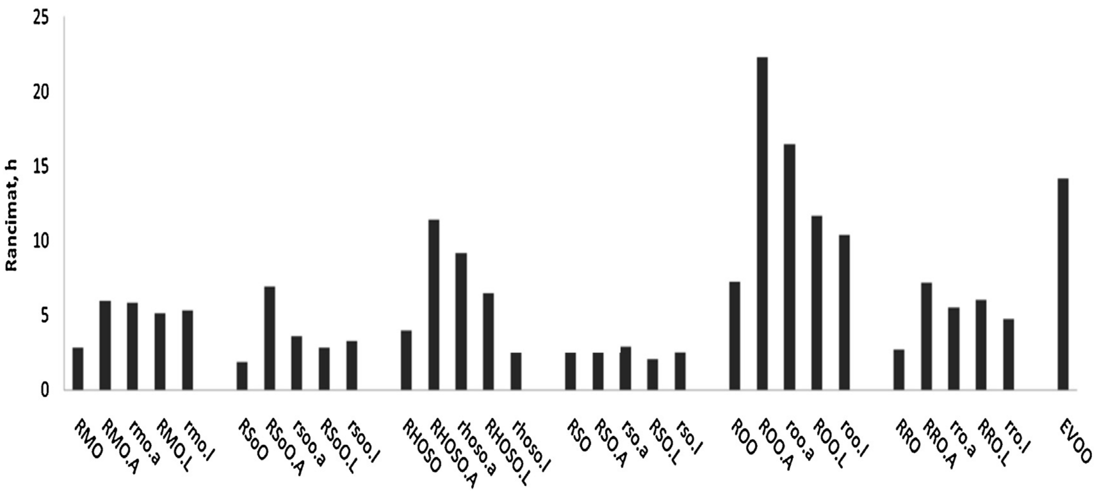


Fig. 6. Rancimat stability of enriched and non-enriched oils as compared with that of EVOO. (With permission of Springer, [18]).

essential fatty acids is the following: virgin olive oil (VOO) containing 400 μg/mL of total phenols and sunflower oil enriched with either an artificial antioxidant (dimethylsiloxane, DMSO) or with an olive phenols extract (in both cases at 400 μg/mL) with the respective names of DSO and ASO were used, together with non-enriched sunflower oil (SO). All four oils were subjected to a simulated frying process consisting of twenty heating cycles of five min at 180 °C; then, the oils were used to prepare breakfasts to be randomly ingested by 26 obese individuals (men and women). Serum was sampled before and after breakfast intake. The effect of the intake through the long-chain alcohols, sterified and non-sterified fatty acids was clearly demonstrated [20], but the most crucial effect on human organisms was on the pathways catalyzed by lipo-oxygenases, cyclo-oxygenases and cytochrome P-450, which give place to prostanoids, including prostaglandins and thromboxanes [21]. These metabolites are involved in pro- and anti-inflammatory processes, and possess pro-aggregating, vasoconstriction and immunosuppressive properties [22], among others. Fig. 7 shows the results of the analyses of serum samples and the effect produced by the intake of the simulated fried oils containing different antioxidants on the changes in the pathways of

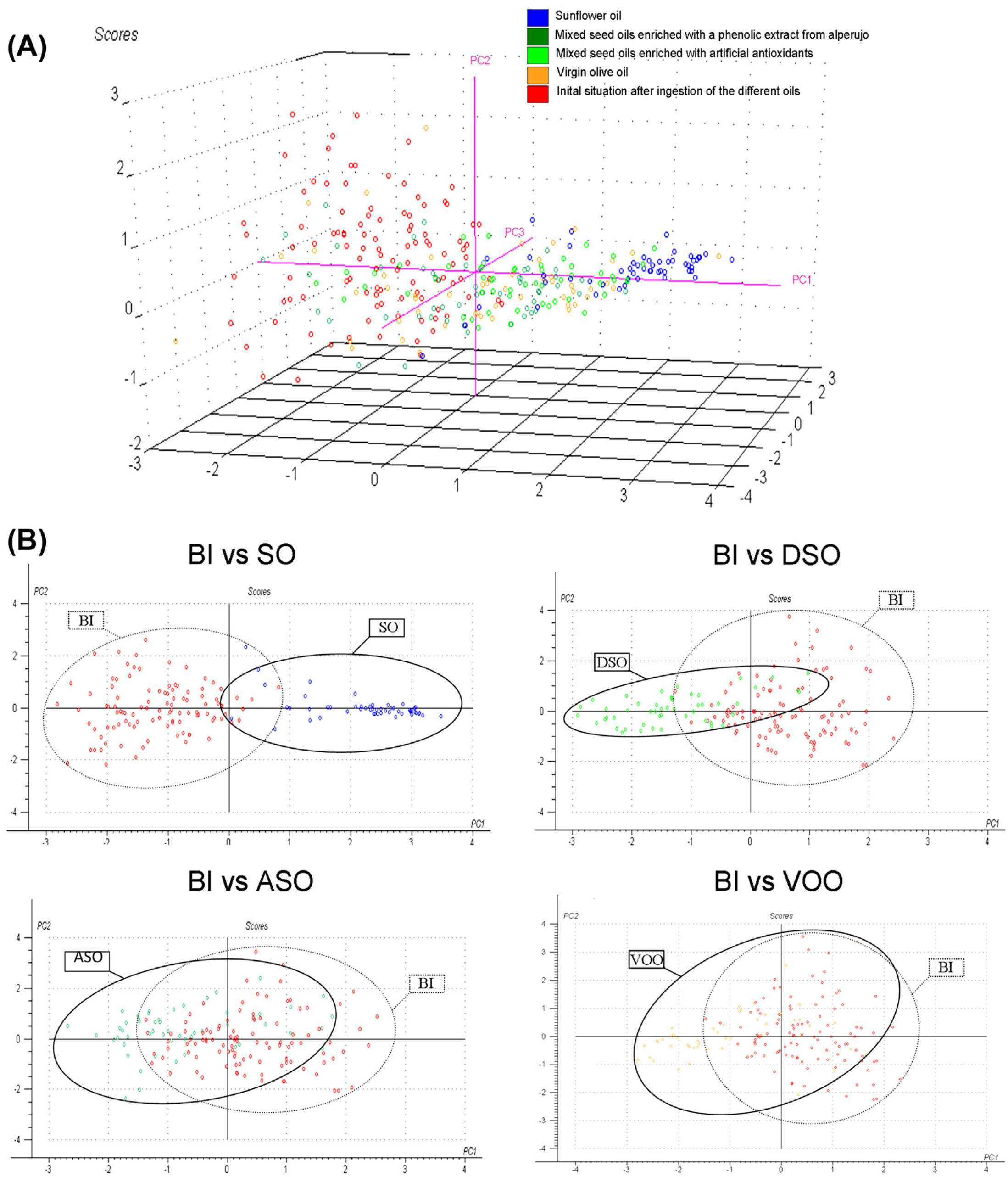


Fig. 7. Effect of the intake of fried oils on fatty acids metabolism. Scores graphs from PCA of: (A) individuals after intake of prepared breakfast versus control individuals attending to serum levels of monitored eicosanoids. (B) Individuals after intake of each prepared breakfast versus individuals before intake (BI) attending to serum levels of monitored eicosanoids. (With permission of Elsevier, [21]).

essential oils (eicosanoids): Fig. 7A illustrates the scores graph by representing the three principal components with higher contribution to explain the observed variability (91%), thus demonstrating that the less affected by the fried oils were the individuals who ingested the fried VOO (closer to the profile of eicosanoids before intake). The most affected individuals were those who ingested the non-enriched oil. Closer to the behavior of VOO was ASO and then that of DSO. Fig. 7B confirms this behavior, showing the scores graph corresponding to each pair control/intervention breakfast, in which the situation more similar to that before intake corresponds to the intake of VOO, then to ASO and DSO and, finally, to the oil without any type of antioxidants.

4. Improvement of traditional cultivars

After checking the beneficial effect of compounds from an agrofood residue or waste and proving a high added value of compounds in it, a desirable step is to increase their content (e.g. by modifying the cultivars by crosses between the appropriate genitors). This has been the case with the varietal cross-breeding program developed at the University of Córdoba to study production of new olive tree cultivars with higher phenols content to obtain VOO of better quality as a result. With this aim, the following objectives were addressed:

- optimize the methods for quantitation of phenols present in VOO;
- know the influence of the variability associated with the harvesting time and to genotypes on the phenols content in VOO to determine the optimum time for comparison between genotypes;
- study the variability of VOO phenol composition obtained from varieties adapted to high density olive hedgerows;
- monitor the evolution along ripening of the phenol content of VOOs from "Sikitita", the first registered cultivar from the Córdoba olive breeding program in two planting systems: intensive and high density hedgerow system;
- study the phenotypic variability of phenols in VOO and its components to understand the genetic basis and estimate the heritability for the assessed traits [23,24].

5. Commercialization as nutraceuticals, food supplements or pharmaceuticals

The final aim of the study is commercialization of extracts endowed with a quality as good as possible. Some actions to be developed at the University level are as follows:

- create technology-based enterprises, for which economical support is offered in most countries;
- contact with industries either food, pharmaceutical or cosmetics industries;
- use the excedent extracts as antibacterial and antifoam agents in water treatment plants;
- present projects in the calls for proposals of the local, regional, national government or in the frame programs of the European Community to look for funding to continue research and create new applications.

6. Use of the final residues as compost, foodstuffs, biogas production, heating

Despite the final solid residues resulting from extraction of valuable compounds could seem a material less useful than the original residues, the former require an in-depth research to know the new potential resulting from either the removal of antioxidants or the treatment to which the residue has been subjected. Some examples of this behavior are as follows:

- alperujo or orujo are not useful for gas production as their high content in antioxidants hinders enzyme catalysis. After lowering the antioxidant content to a certain level causing no deactivation of biocatalyzers, the residue can be successfully used for gas production;
- compost production from alperujo or orujo is a time consuming task as micro-organisms are destroyed by the enormous antioxidants content of these materials. After phenols extraction compost is obtained from alperujo or orujo in a short time;
- use of these materials as foodstuffs is also easy after the high charge of phenols in alperujo is decreased.

Other materials such as wine pomace result in different composition after being subjected to ethanol production as the drastic working conditions (mainly high temperature) of this process gives place to smaller compounds resulting from hydrolysis of polymers such as condensated tannins, cellulose, hemicellulose, etc. [25].

7. Conclusions

Implementation of the above commented steps in a rational way can yield interesting results from almost all raw materials presently unexploited.

The working scheme as applied to the olive tree–olive oil binomial can be implemented to any other case to break the traditional one cultivation–one commercial product, also shown by some given examples.

Acknowledgement

The author thanks to the Spanish Ministerio de Economía y Competitividad (MEC) and FEDER program for financial support through Project CTQ2012-37428.

References

[1] A. Peralbo-Molina, M.D. Luque de Castro, Trends Food Sci. Technol. 32 (2013) 16–24.
[2] M.D. Luque de Castro, M. Valcárcel, M.T. Tena, Analytical Supercritical Fluid Extraction, Springer, Verlag, 1994.
[3] J.R. Williams, A.S. Clifford (Eds.), Supercritical fluid. Methods and protocols, Humana Press, New Jersey, 2000.
[4] M.D. Luque de Castro, F. Priego-Capote, Analytical uses of ultrasound, Elsevier, Amsterdam, 2006.
[5] F. Chemat, G. Cravotto (Eds.), Microwave-assisted extraction of bioactive compounds, Springer, Heidelberg, 2013.
[6] J.L. Luque-García, M.D. Luque de Castro, J. Chromatogr. A. 959 (2002) 25–35.
[7] J.M. Luque-Rodríguez, P. Pérez-Juan, M.D. Luque de Castro, J. Agric. Food Chem. 54 (2006) 8775–8781.
[8] R. Japón-Luján, P. Janeiro, M.D. Luque de Castro, J. Agric. Food Chem 56 (2008) 7231–7235.
[9] R.C. Prados-Rosales, J.L. Luque-García, M.D. Luque de Castro, J. Chromatogr. A 993 (2003) 121–129.
[10] R. Japón-Luján, Avances tecnológicos y metodológicos para el aprovechamiento de los residuos del olivo y de la industria aceitera y para la mejora de los aceites comestibles, PhD Thesis, University of Córdoba, 2008.
[11] J.A. Pérez-Serradilla, F. Priego-Capote, M.D. Luque de Castro, Anal. Chem. 79 (2007) 6767–6774.
[12] J.A. Pérez-Serradilla, R. Japón-Luján, M.D. Luque de Castro, Anal. Chim. Acta. 602 (2007) 82–88.
[13] A. Peralbo-Molina, F. Priego-Capote, M.D. Luque de Castro, J. Agric. Food Chem 60 (2013) 11542–11550.
[14] M. Herrero, T.N. Temirzoda, A. Segura-Carretero, R. Quirantes, M. Plaza, E. Ibáñez, J. Chromatogr. A 1218 (2011) 7511–7520.
[15] J.A. Pérez-Serradilla, M.D. Luque de Castro, Food Chem. 124 (2011) 1652–1659.
[16] M.P. Delgado de la Torre, F. Priego-Capote, M.D.J. Luque de Castro, Agric. Food Chem. 60 (2012) 3409–3417.

[17] European Food Safety Authority, EFSA J. 9 (2011) 2033.
[18] V. Sánchez de Medina, F. Priego-Capote, C. Jiménez-Ot, M.D. Luque de Castro, J. Agric. Food Chem. 59 (2011) 11432–11441.
[19] Official Methods of Analysis, AOCS, Champaign, IL (1997), Oil stability index, Cd 12b-92.
[20] M.I. Orozco-Solano, F. Priego-Capote, M.D. Luque de Castro, Anal. Bioanal. Chem. 405 (2013) 6117–6129.
[21] C. Ferreiro-Vera, F. Priego-Capote, J.M. Mata-Granados, M.D. Luque de Castro, Food Chem. 136 (2013) 576–584.
[22] D. Wang, R.N. Dubois, Methods Enzymol. 433 (2010) 27–50.
[23] M. El Riachy, Genetic variability and inheritance of virgin olive oil phenolic compounds, PhD Thesis, University of Córdoba, 2012.
[24] M. El Riachy, F. Priego-Capote, L. Rallo, M.D. Luque de Castro, L. León, Eur. J. Lipid Sci. Technol. 114 (2012) 542–551.
[25] A. Peralbo-Molina, F. Priego-Capote, M.D. Luque de Castro, Talanta 101 (2012) 292–298.