DEVELOPMENT OF A MICROWAVE PRETREATMENT FOR THE EXTRACTION OF ACTIVE COMPOUNDS FROM NATURAL BYPRODUCTS

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PhD thesis

Universidad de Valladolid

Escuela de Doctorado Universidad de Valladolid

PROGRAMA DE DOCTORADO EN INGENIERÍA QUÍMICA Y AMBIENTAL

TESIS DOCTORAL:

DESARROLLO DE UN PRETRATAMIENTO CON MICROONDAS PARA LA EXTRACCIÓN DE COMPUESTOS ACTIVOS A PARTIR DE RESIDUOS AGRÍCOLAS

Presentada por Ana Álvarez Martín para optar al grado
de
Doctora por la Universidad de Valladolid

Dirigida por:
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Universidad de Valladolid

Escuela de Doctorado Universidad de Valladolid

DOCTORAL PROGRAM IN CHEMICAL AND ENVIRONMENTAL ENGINNEERING

PhD THESIS:

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Memoria para optar al grado de Doctor,
con Mención Doctor Internacional,
presentada por
Ana Álvarez Martín

Siendo los tutores en la Universidad de Valladolid
Prof. Rafael B. Mato
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Y en *Green Chemistry Centre of Excellence, University of York*
(Reino Unido)
Dr. Vitali Budarin

Valladolid, Septiembre 2018

UNIVERSIDAD DE VALLADOLID

ESCUELA DE INGENIERÍAS INDUSTRIALES

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La presente tesis doctoral queda registrada en el folio número _______ del correspondiente libro de registro número _______.

Valladolid, a _______ de _______________ de 2018

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Certifican que:

Ana Álvarez Martín ha realizado bajo su dirección la presente memoria titulada "*Development of a microwave pretreatment for the extracton of active compounds from natural byproducts*", en el departamento de Ingeniería Química y Tecnología del Medio Ambiente de la Escuela de Ingenierías Industriales de la Universidad de Valladolid. Considerando que dicho trabajo reúne los requisitos para optar al título de Doctor en Ingeniería Química y Ambiental, expresan su conformidad con su presentación.

Valladolid, a 24 de Septiembre de 2018

Fdo. Rafael B. Mato   Fdo. María José Cocero

Reunido el tribunal que ha juzgado la tesis doctoral titulada "*Development of a microwave pretreatment for the extraction of active compounds from natural byproduct*" presentada por la Ingeniera Química Ana Álvarez y en cumplimiento con lo establecido por el Real Decreto 99/2011 de 28 de Enero de 2011 acuerda conceder por __________________________ la calificación de ____________________.

Valladolid, a _____ de _________________ de 2018.

Presidente Secretario Vocal

TABLE OF CONTENTS

TABLA DE CONTENIDOS

— Sección I —

— Sección II —

— Sección III —

ABSTRACT

ABSTRACT

During the last decades, industry has paid an increasing attention to intensification techniques due to its potential to save time, space, energy and material agents, like solvents. In particular, the extraction of active compounds from natural products has thrived because of these techniques. Microwave technology is one of those intensification methods whose advantages have been widely proved, although mainly just at laboratory scale. Thus, the aim of this thesis is to develop a microwave intensification process feasible to be implemented at industrial scale. For this purpose, an innovative approach to facilitate its scale up was proposed: in this work, microwaves have been used as a pretreatment prior to the conventional extraction. The main feature of this step was its short duration. Not only was this brief radiation time beneficial for the stability of the active compounds (thermal degradation), but it also allowed the use of a compact microwave oven, feasible at a larger scale to uniformly irradiate the material. Three abundant agricultural wastes like grape pomace, olive pomace and saffron flowers have been used to assess the convenience of the proposed microwave pretreatment for the extraction of polyphenols.

This thesis is divided into three sections. In the first one, grape pomace valorisation has been developed. In the second part, an analysis of the possible interaction of microwave on the solid-liquid interface has been studied. And finally, the knowledge developed has been proved with other agricultural wastes (olive pomace and saffron flowers) to check if the pretreatment was suitable for other products too. The assessment of the microwave pretreatment was performed regarding a compromise between yield enhancement (an indication of the raw material exhaustion grade) and product richness (crucial parameter that determines the proportion of polyphenols in the final dry product).

In the first section, it was observed that grape pomace polyphenol extraction, and especially anthocyanin release, was substantially enhanced by the addition of the microwave pretreatment due to a differentiated extraction acceleration among polyphenols and other substances. A high quality product in terms of polyphenol richness (32% richer) and antioxidant activity (up to 133% greater cellular antioxidant activity) was obtained. Such dramatic improvement gave grounds for the study of its implementation at larger scale. Nonetheless, it was necessary first to determine the dielectric properties of the extraction media to perform an efficient oven design. Thus, a model able to predict the permittivity of the complex mixture grape pomace-solvent from mixture constituents was developed from experimental measurements. It enabled to estimate the dielectric constant and the loss factor with an accuracy of 8% and 25%, respectively. These measurements were crucial, since they allowed to identify a boost in the loss factor due to the appearance of ionic losses, ranging between 9-83%. Therefore, they could be accounted during the design to reduce the likely

formation of hot spots inside the oven and thus, promoting a uniform treatment. A compact oven with a diameter of 0.035 m and a 0.560 m length was designed to treat a flow of 19.5 kg/h absorbing a specific energy of 324 kJ/mL which would cause a 100ºC temperature increase. Its continuous operation proved its effectiveness, with a high energy absorbance efficiency of 97%, and its viability, since similar yield than the ones observed at laboratory scale were observed.

Additionally, grape pomace exploitation was further analysed by performing a subsequent microwave assisted hydrolysis and a pyrolysis to achieve a total valorisation of the residue. The microwave benefits were also observed in these processes. A substantial temperature and time reductions (from 180ºC to 155ºC and from more than 30 minutes to 7.5 minutes) were found. However, it must also be considered that, despite attaining a similar yield to that of the hydrothermal process, microwave hydrolysis presented a lower sugar selectivity. The batch reactor disposition was blamed for that.

The substantial improvement found in polyphenols microwave extraction and the lack of a scientific reasoning able to explain it led to consider that the radiation might be influencing the solid-liquid equilibrium, what motivated the development of the second section of this thesis. The previous hypothesis was supported by an ongoing scientific discussion about the effect of microwaves on liquid-vapour equilibrium. The solubilities of gallic acid and sodium sulphate in water, ethyl acetate, isopropyl alcohol and ethanol were measured in a dynamic system with two flows of energy: the heat released by radiation and the refrigeration needed to maintain a constant temperature. Due to inhomogeneities, temperature deviations of around 0.5ºC caused an average solubility error of 4.3%. An unsound uncertainty for a solubility report, but low enough to assure that, for the systems analysed, no significant influence of the microwave radiation on the solid-liquid equilibrium was found.

Finally, the convenience of the microwave pretreatment with other residues (olive pomace and saffron flowers) was appraised to check if it could be employed as a multi-feedstock facility in the third section.

Olive pomace presented a harder structure with the polyphenols highly bounded, thus, severe conditions were required. A solvent free microwave pretreatment was employed to concentrate the radiation in the solid. The pretreatment resulted to be not so effective in terms of yield improvement as in the case of grape pomace, although polyphenol richness and individual polyphenol richness were substantially enhanced. Specifically, final product polyphenol composition in the commercial dry extract was increased a 40%, and hydroxytyrosol and oleuropein content was enhanced a 35%

and a 181%, respectively. Polyphenol olive pomace extraction was also tested in the continuous oven. Its dielectric properties were measured and modelled with an accuracy of 9% and 29% for the dielectric constant and the loss factor, respectively. For the continuous operation, the oven stub tuners were attuned to minimize the reflected power, so an efficient absorbance of the microwave energy was assured. The complex flow behaviour of the material associated to the reduced scale of the plant impeded a stable continuous operation. For this reason, 500-gramme batches were employed instead. Like in the grape pomace case, olive pomace also presented a great energy absorbance (97%) and provided excellent extraction results.

On the other hand, the addition of the pretreatment to the polyphenols and anthocyanins extraction process from saffron flowers considerably reduced extraction time up to 12 times, as well as produced a 25% polyphenol richness increase. It was noteworthy the high content of delphinidin in the extract, which supposed the 80% of the total anthocyanins. Such enhancement was also reflected in the colour of the extract: with the greater concentration of anthocyanins a more cyanic extract was obtained.

In sum, it can be concluded that the microwave pretreatment proposed, developed and implemented at pilot-plant scale in this thesis is a suitable alternative for the industrial intensification of extraction processes. Its feasibility has been proved with three different agricultural wastes, obtaining, in all the cases, promising results. Additionally, this work has been combined with a study of the material-microwaves interaction with the aim of getting a deeper understanding of the effect of microwaves in natural products extraction processes.

INTRODUCTION

1. FOUNDATIONS AND MOTIVATION

Nowadays, assuring the efficiency and sustainability of an industrial process is key to guarantee its success. Through the valorisation of wastes and low value sub-products, chemical industry can find a potential source of high added value compounds (Clark et al., 2006). Among all the residues produced, those that come from a natural source (known as biomass) are one of the most preferred options since they constitute a renewable source. Nonetheless, since the valorisation of these waste feedstocks responds to an ecological concern, its production route has to be in accordance too. In order to address this challenge, process intensification arose in the 1970s (Reay et al., 2008). New multidisciplinary techniques were developed to drastically reduce the size or volume of process plants, without compromising the final output (Stankiewicz and Moulijn, 2000). But soon other advantages (like safety, environmental impact or emission reduction) also appeared. Such plant volume reductions allow the spread of small decentralized plants, which plays a crucial role when the raw material is a solid (like biomass) since it substantially reduces mobilization costs. Additionally, the fact that the plants can be located close to the biomass source would also impact the economy of agricultural areas. This new production conception responds to the biorefinery philosophy, in which the manufacture of commodities such as energy, fuel and chemicals is sought (Audsley and Annetts, 2003).

Inside the chemicals latter group, nutraceuticals have gained plenty of attention during the last decades. The interest in them comes from their potential to substitute synthetic preservatives together with their health promoting benefits (Serra et al., 2008). This group encloses proteins, amino acids, fatty acids, fiber and antioxidants obtained from natural resources (Moldes et al., 2017). The applications for nutraceuticals are in the fields of food, pharmaceutic and cosmetic industry (Babbar et al., 2015), as preservatives to extend the food shelf life, nutritional supplements, natural colourants, and cosmetic additives. In sum, they are usually employed as bioactive ingredients. To this point, it must be added that the global market for health ingredients grew a 6.75% between 2013-2014, and it is expected to continuously increase up to a 300 billion USD in 2020 according to a Euromonitor International study (Pathak, 2017). Besides, since between the 20%-30% of global food and agricultural production is discarded as waste (Ajila et al., 2012, Kibler et al., 2018), the raw material availability is guarantee. However, although their properties make them suitable to be used as food additives, nutraceuticals are still not regulated. Its legal status is not clear since they fall between food (nutrition) and drug (pharmaceutic) definitions (Orlando, 2018). Nevertheless, there is a new agency in the EU, the EFSA (European Food Safety Authority), whose objective is to control false claims respect to this kind of products and provide European Commissions with a sound foundation for legislation (Regulation(EC)1924, 2006).

On a whole, it can be concluded that the study of the extraction of polyphenols from natural wastes is a field of high economic and environmental interest, even taking into account the legal concerns. For this reason, hundreds of works related to this topic can be found in literature and several approaches have been proposed. Likewise, the aim of this thesis is to contribute to the sustainable development of agricultural and food industries by the valorisation of their residues. In particular, it is focused on the extraction of antioxidants (also known as polyphenols) from wine, olive and saffron industrial by-products. These wastes are a common sub-product from the Spanish countryside and they have been obtained from the agricultural activity developed in the region of Castilla y León. An area devoted to agricultural activities but still with a conspicuous pronounced intra-regional economic imbalance (CES, 2016). A contrast that has increased over the recent years with the gradual decrease of the agricultural trade figures (IRS and CSIL, 2007). So, actual policies advocate for championing these activities as an attempt to lessen inequalities.

2. AGRICULTURAL BYPRODUCTS AS A SOURCE OF POLYPHENOLS

The term polyphenol encompasses a great amount of phytochemicals (compounds produced by plants) with a common structure that consists of a single or more phenol rings, like the one shown in Figure 1 (Ignat et al., 2011, Tsakona et al., 2012). The hydroxyl groups of this structure provide polyphenols with an outstanding property: its antioxidant capacity. In an oxidant environment, polyphenols easily yield hydronium ions to quench reactive oxygen species (usually known as ROS). This capacity makes polyphenols able to subdue free-radical induced diseases. That is why polyphenols are commonly said to have anti-diabetic, anti-inflamatory, anti-cancer, anti-bacterial, cardiovascular and skin protective properties (Jakobek, 2015, Saura-Calixto, 2011, Nowshehri et al., 2015, Roleira et al., 2015, Lutterodt et al., 2011, Chatthongpisut et al., 2015, Wittenauer et al., 2015, Serra et al., 2012, Shrikhande, 2000). Polyphenols applications are also derived from their antioxidant activity and the current trend to minimize the use of synthetic chemicals.

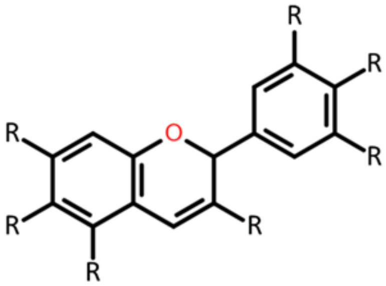

Figure 1. Typical polyphenol structure.

Among all the polyphenols types, anthocyanins are a renowned subgroup due to their specific properties. They are natural pigments with a great antioxidant activity. Their colour can vary from orange to violet, depending on the number of hydroxyl groups in the molecule (Ananga et al., 2013). The most abundant anthocyanins, ordered from orange to violet, are: pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin; although several derived compounds, such as glycoside forms, also exit (López-Gutiérrez et al., 2016). These hues give anthocyanin an important distinction over other active compounds since cyanic colorants are quite scarce in nature (Araújo et al., 2018). Regarding anthocyanin extraction, they are easily leaked as they are accumulated in accessible vacuoles (located in the skin in the case of grapes and in the saffron petals) (Yang et al., 2009). However, during their extraction it must also be taken into account that anthocyanins are highly sensitive compounds, as they readily degrade due to changes in pH, temperature and light (Ananga et al., 2013).

Three different agricultural wastes have been employed as raw materials in this work: grape pomace, olive pomace and saffron flowers. They have been selected because of economic and environmental considerations. Specifically, wine and olive industries generate a large amount of solid wastes whose jettisoning supposes an economic and ecological issue (Llobera and Cañellas, 2007, Beres et al., 2017). Businesses have to pay to get rid of them since they are not allowed to directly dispose them. Nevertheless, it has been proved that they can be a profitable source of high value compounds, like antioxidants. Thus, their revalorisation not only would produce and economic income, but also a lessening of the costs associated to their current treatment. In the case of saffron flower, the production of the specie is quite laborious and so, the incorporation of an extra value from the flower residue exploitation will highly benefit the sustainability of this industry.

3. PROCESS INTENSIFICATION

The traditional method used to obtain a polyphenol extract is maceration, which consists in putting the biomass in contact with the solvent at a certain mild temperature during a long extraction time. Maceration main disadvantage, apart from the duration of the process, is the large consumption of toxic solvents (Albahari et al., 2018). Extraction yield is enhanced by the use of noxious solvents, like methanol and benzene. Therefore, there needs to be a method based on green solvents (for instance ethanol and water, recognised as safe (Putnik et al., 2017)) that can be used for a sustainable polyphenol extraction. This can be accomplished by an intensification step. There are several intensification techniques available and developed for each kind of raw material (Barba et al., 2016). The most well-known methods are ultrasounds, electric pulse discharge, pulse electric fields and pressurize solvent. However, this study is focused on microwaves.

3.1. MICROWAVE FUNDAMENTALS

Microwaves are a type of electromagnetic radiation with frequencies ranging between 300 MHz and 300 GHz. However, not all the frequencies are allowed to be freely used. The International Telecommunication Union (ITU) assigned certain frequency bands for scientific, medical and industrial applications (ITU-R, 1994). The renowned frequency of 2.45 GHz is the one worldwide allowed for microwave heating. It can be considered as a standard for microwave ovens, since it is extensively employed in commercial equipment (Spigno and De Faveri, 2009).

Microwave systems are mainly composed of three parts: the magnetron, the waveguide and the applicator. The magnetron is the piece of equipment where the electromagnetic field is created with a certain frequency and power. Due to its nature, waves are propagated in all directions, what makes necessary to implement a waveguide to confine and convey the radiation to the applicator. This latter part is where the radiation gets in contact with the material. Different types of applicators can be used, although there are two that outstand. Those are single-mode and multi-mode applicators. A single-mode microwave utilises a solution to the Maxwell equation, creating a single resonant mode (Thostenson and Chou, 1999). It provides a well-defined standing wave pattern easier to be controlled, and thus, recommendable for industrial applications (Galan et al., 2017). On the other hand, commercial ovens and heating applications units usually employ multi-mode applicators. This type of applicator is characterized for the formation of several nodes, resulting in an uneven electric field distribution that makes the radiation difficult to predict (Sturm et al., 2012).

The above mentioned Maxwell's equations are used to describe the steady-state, sinusoidal time-varying propagation of these waves (Meredith, 1998, Metaxas and Meredith, 1988). This set of partial differential equations considers the interactions between the electric and magnetic field, as well as the interaction of the material with the radiation. Depending on this latter interaction, materials can be divided into opaque or transparent (lossy) to electromagnetic radiation. Permittivity is the magnitude that characterises this interaction. Permittivity (ε*) is a complex number formed by the dielectric constant (ε') as the real part, and the loss factor (ε'') as the imaginary term (equation [1]). The dielectric constant is proportional to the material's ability to storage energy; whereas the loss factor represents the losses due to energy dissipation into heat (Navarrete et al., 2011). However, a relation between these two parameters, the loss tangent (equation [2]), is commonly employed. It represents the ability of the solvent to dissipate and to absorb microwave energy (Leonelli et al., 2013). The higher this factor is, the faster the radiated media would heat up.

$$\varepsilon^* = \varepsilon' - j\varepsilon'' \tag{1}$$

$$tan\delta = \frac{\varepsilon''}{\varepsilon'} \tag{2}$$

Therefore, there is a transmission of power from the electromagnetic field to the material exposed to radiation. This value is given by equation [3] and it is computed as a function of the angular frequency (calculated from the frequency used as $\omega=2\pi f$, dimensionless), free space permittivity (ε_0 = 8.84· 10^{-14} F/m), loss factor (ε'', dimensionless) and electric field strength (*E* in V/m). It must be noticed that, since microwave heating takes place volumetrically (Kappe, 2013), this power is calculated in W/m^3 (ratio between the absorbed power, Q_{MW}, and the sample volume, *V*).

$$P_d = \frac{Q_{MW}}{V} = \frac{1}{2}\omega\varepsilon_0\varepsilon''|E| \tag{3}$$

Another important parameter to consider during microwave heating is penetration depth. As it has been said, materials vary from transparent to opaque when exposed to microwaves. This means that radiation will penetrate the material with different ranges. The depth at which the transmitted power is reduced to *1/e* of its value at the external surface (37%) is taken as reference to compute the penetration depth (d_P, measure in meters) (Wang et al., 2003). It can be calculated according to equation [4]. A simplification can be added if lossy materials (tanδ<<1) are studied.

$$d_p = \frac{c}{2\sqrt{2}f\sqrt{\varepsilon'}\left[\sqrt{1+\tan^2\delta}-1\right]^{1/2}} \simeq \frac{\lambda_0\sqrt{\varepsilon'}}{2\pi\varepsilon''} \qquad [4]$$

In this latter equation, *c* stands for the speed of light (m/s) and λ_0 for the wave length in vacuum.

Moving forward to microwave implementation at an industrial scale, safety and scale-up are the main factors to be considered.

Regarding microwave safety, Li et al. (2013) conducted a HACCP (hazard analysis and critical control points) and a HAZOP (hazard and operability analysis). It was finally concluded that radiation time, temperature gradients and unsafe applications are the critical points; whereas materials, overradiation and equipment damages are crucial for a safety operation. In sum, as long as the equipment is correctly design, so as to be controllable and not to allow radiation leakages (safety exposure limit of 137 V/m (NTP698, 2012)) as well as a good maintenance is performed, there are no safety hazards to discourage its use.

On the other hand, scale-up is the main issue that deters microwave expansion into industry applications. Energetic efficiency determines the convenience of microwave implementation at large scale. It is conditioned by the two energy transformations that take place. First, from electricity to electromagnetic radiation, which has a typical yield of 60% (Wang et al., 2015). And second, its absorbance that depends of the interaction between the material and the electromagnetic field. It can vary from 40-90%. Here is why an optimal oven design is crucial. Not only to maximize energy efficiency, but to avoid the inhomogeneities so characteristic of this technology. A good oven design enables a uniform field distribution which is translated in minimizing the formation of hot-spots.

3.2. MICROWAVE ASSISTED EXTRACTION

One of the most extended application of microwaves, and the one that this thesis pursues, is the extraction of active compounds from natural by-products. Microwave assisted extraction (MAE) consists in the radiation of a solid matrix to boost the release of active compounds to a liquid phase. However, there is also a variation (worth to consider) that does not use any external liquid phase: the solvent free microwave extraction, found to present excellent yield for the extraction of essential oils. MAE processes have been widely studied at laboratory scale where its outstanding advantages have been widely proved. Nonetheless, a scientific reasoning able to explain the microwave enhancement has not been found. Although there is an exception: the extraction of essential oils from trichomes, where micrographs have shown the disruption of this oil containing vegetal structures due to its explosion (Navarrete et al., 2011, Lucchesi et al., 2007).

Several hypotheses to explain the microwave improvement have been suggested. Most of them are focused on the temperature profile. It must be taken into account that microwave heating presents an inverted temperature profile respect to the conventional heating (Kappe, 2013). In this latter case, heat is transferred from the walls into the media, whereas microwaves are directly absorbed by the material (Luque et al., 2012). In addition, microwave radiation is selectively absorbed in a miscellaneous mixture by those points with higher dielectric points. This temperature gradient settles the grounds for the enhancement discussion. Galan et al. (2017), as many other authors, argues that even a small temperature gradient (like 1ºC) can contribute to enhance the driving force. As a consequence of such changes in the thermodynamics, the equilibrium could also be affected. This hypothesis is partly supported by the ongoing discussion to address if microwaves can influence vapor-liquid equilibrium (Altman et al., 2010, Gao et al., 2013, Werth et al., 2015, Li et al., 2017).

Another reasoning line to try to explain the microwave advantages focuses on a possible disruption of the vegetal structure due to radiation, like in the case of trichomes above mentioned. However, the micrographs used to support this argument (for instance the ones provided by Yedhu and Rajan (2016)) are not clear enough to lead to a convincing conclusion.

Up to this point, MAE technology is in a standstill. Few attempts have been done to further exploit the advantages of microwave technology at industrial scale (Galan et al., 2017). Watson (2012) undertook a survey among chemical industries to discern major deterrents. Despite being aware of the advantages of microwave processing, industries perceive scale-up as the main difficulty to surmount. It is a sound conclusion considering that actual works do not take into account that the penetration depth of microwaves in a system is constant, independently of the oven scale. For instance, a microwave radiation of 2.45 GHz has a penetration depth of 2.32 cm in water, whether it is irradiated in a small laboratory equipment or in a large industrial tank. Therefore, it is not suitable to irradiate a large vessel since only the external parts are going to be affected. There is also an alternative to increase penetration depth and it is to use a lower frequency, such as 915 MHz, but it implies a counterproductive reduction of the thermal effect.

The limitation of the penetration depth considered together with the power profile that is usually employed during a MAE led to the idea of using a microwave pretreatment instead of a MAE, with the aim of reducing the exposition time and the volume of the microwave applicator. Regarding this latter fact, Galan et al. (2017) reviewed that during MAE processes the highest power available is used to rapidly achieved the extraction condition, whereas during the rest of the process a very low power is provided to offset the heat losses. Therefore, in practice, MAE processes are close to the pretreatment concept. So, there is no point in investing in a large bulky equipment as no effective treatment is going to be achieved, but in a microwave pretreatment.

3.3. MICROWAVE PRETREATMENT

The addition of a microwave pretreatment to the conventional solid-liquid extraction processes is proposed as an alternative to MAE. It has been devised to overcome microwave scale up limitations, namely the use of a compact oven and the uniform radiation of the material.

The pretreatment consists in irradiating the extraction media with a short but intense peak of energy, causing a sharp temperature increase. After the pretreatment is finished, temperature is rapidly cooled down to milder conditions to minimize degradation reactions, and the process continues with a conventional solid-liquid extraction. Figure 2 gathers the temperature profile followed by the pretreatment, as well as a contrast with the conventional and the MAE processes. As it can be seen, microwave pretreatment is a combination of these two latter methods. Mild conventional conditions are employed for the majority of the extraction, but extraction time is considerably reduced by the microwave enhancement. The rapid temperature increase contributes to the acceleration of the extraction of active compounds, but due to its short duration, thermal degradation is minimized. As Sólyom et al. (2014) proved, anthocyanins from grape pomace at high temperature were degraded in a small fraction (less than 10%), which is admissible considering the overall improvement.

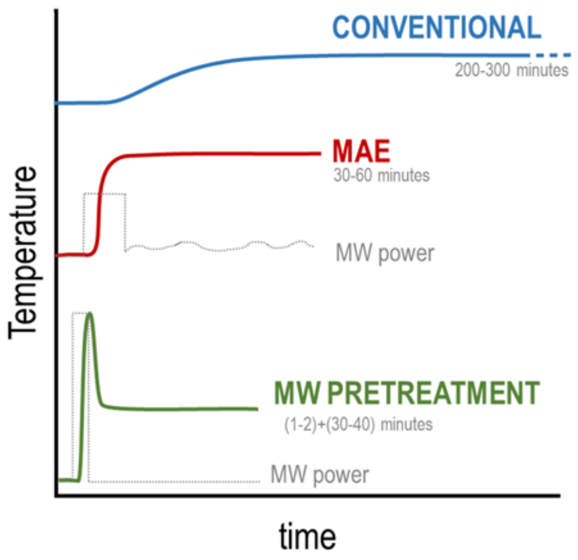

Figure 2. Comparison of the temperature profiles for a conventional, a microwave assisted (MAE) and a microwave pretreatment extraction.

Therefore, a microwave pretreatment is suitable for industrial implementation since a compact oven is required as a consequence of the radiation time reduction, and thus, a homogeneous irradiation of the material can be easily achieved. Besides, despite reducing the irradiation time, the energy absorbed during the pretreatment is of the same order as in a MAE process, so the benefits associated with the use of microwaves are maintained. For these reasons, the microwave pretreatment has been proposed and studied in this work as an intensification complement for the extraction of active compounds from natural products. More details about the methodology followed can be found in section 5.

4. THESIS OUTLINE

This thesis is focused on the development of a microwave pretreatment easy to be implemented at industrial scale as a complement of a conventional solid-liquid extraction. The objective has been addressed in eight chapters divided into three sections.

In the first section, grape pomace has been studied as the raw material for a microwave assisted biorefinery. Three consecutive steps to tackle the complete depletion of the pomace have been followed. First, the extraction of polyphenols (chapter 1). The outstanding results of chapter 1 led to propose its scale-up to a pilot plant. In order to attain an efficient microwave oven design, the dielectric properties of the irradiated media had to be known. So, in chapter 2, a model to estimate the complex permittivity of the multiphase mixture solvent-grape pomace was carried out. This model was used in chapter 3 for the design of an efficient microwave oven able to work continuously at a pilot plant scale. Finally, the complete exhaustion of the grape pomace was studied in a microwave hydrolysis to obtain a sugar rich hydrolysate and, in a subsequent pyrolysis (chapter 4).

Due to the significant yield enhancement that the implementation of microwaves supposed, an explanation able to justify it was sought in section II (chapter 5), where a study of the radiation influence on solid-liquid equilibrium was carried out.

And last but not least, the knowledge developed was applied to other by-products (olive pomace and saffron flowers) in section III. Polyphenol extraction was optimised for both residues (chapter 6 and 8). Olive pomace dielectric properties were also measured to use this raw material in a pilot plant (chapter 7).

5. METHOGOLOGY

5.1. STUDY OF THE EXTRACTION PROCESS

Extraction conditions and their ranges were selected based on a literature review, although also considering industrial constrains. EU edible allowed solvents were preferred over organic ones (EU Directive 2009/32/EC, 2009), such as water and ethanol over benzene or methanol. Another important limitation that has been contemplated was the solvent consumption. The industrial use of organic solvent is quite restricted and the plants that need them are subjected to high costs due to expensive solvent stock and taxes. So, a reduction either in the solvent consumption or in the organic proportion employed can be regarded as a satisfactory breakthrough.

The extraction variables analysed have been solvent composition, solvent to feed ratio and absorbed energy. A statistical approach has been followed to identify the significant effects and the possible interaction between variables.

In terms of process optimization, conventional solid-liquid extraction was first optimised to then implement the microwave pretreatment. The conventional solid-liquid process was taken as a control reference to assess the influence of the microwave pretreatment. Figure 3 gathers a scheme of the experimental procedure followed. First, the raw material and the solvent were mixed and homogenised to be latter irradiated. After the pretreatment was finished, extraction media was rapidly cooled down and the process continued as a short conventional extraction.

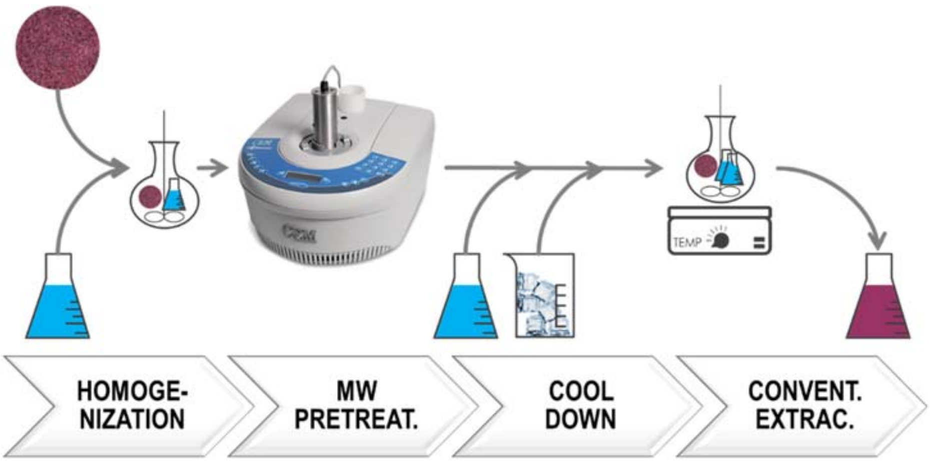

Figure 3. Outline of the experimental approach followed.

The advantages of microwave pretreatment implementation were quantified by means of a kinetic analysis. Experimental results were fitted to the first order kinetic equation presented in equation [5] and this curve was used to compute initial extraction rate (equation [6]) and final yield (equation [7]). Figure 4 presents the meaning of these variables with respect to the conventional curve. The higher the initial extraction rate is, the faster the final yield will be achieved. Final yield (C_∞) represents the concentration obtained at an infinite extraction time. That means the complete exploitation of the raw material at the operational conditions. For industrial exploitation, a process yield of 95% can be considered a reasonable objective. The addition of the microwave can sharply reduce the time required to achieve this yield, as a result of the microwave acceleration of the initial extraction rate.

$$\mathrm{C_{cal}} = \mathrm{C_0} + \mathrm{C_f}[1 - \exp(-\mathrm{k}\mathrm{t})] \qquad [5]$$

$$\mathrm{u_0} = \left.\frac{\partial \mathrm{C}}{\partial \mathrm{t}}\right|_{\mathrm{t}=0} = \mathrm{C_f}\mathrm{k} \qquad [6]$$

$$\mathrm{C_\infty} = \lim_{\mathrm{t}\to\infty} \mathrm{C} = \mathrm{C_0} + \mathrm{C_f} \qquad [7]$$

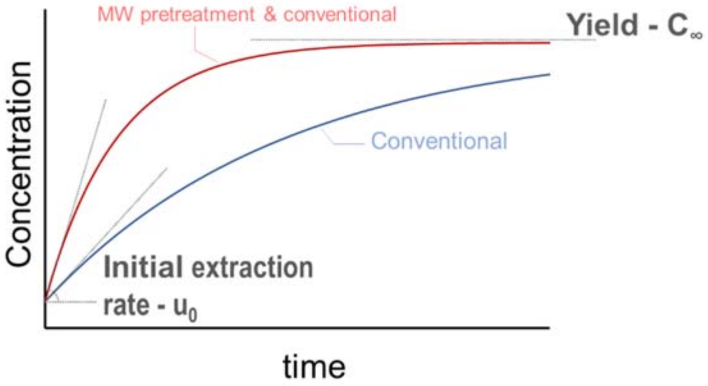

Figure 4. Example of the kinetic curve and the two derived parameters, yield and initial extraction rate, used to characterise the extraction.

5.2. CHARACTERIZATION OF THE FINAL PRODUCT

Extraction studies tend to tackle yield (extraction efficiency) as the only important respond of the system. However, it can not be analysed isolated, improvements on the final product quality are also crucial and they have to been appraised jointly. Product quality can be determined by several parameter, like richness, product composition or antioxidant activity. Product richness stands out as a novel parameter since it has not been previously evaluated nor reported. It indicates the amount of polyphenols obtained respect to the total amount of substances extracted. Since the polyphenolic extract is normally commercialised in a solid state after its formulation, the proportion of polyphenols in the final dried product is of vital importance. An increase in the polyphenol richness boosts its quality and, in consequence, the market price of the final product. Product value is also conditioned by its individual composition and antioxidant activity. Individual polyphenols (like quercetin, gallic acid or oleuropein) or anthocyanins (malvidin or delphinidin) are greatly appreciated due to its antioxidant activity, so the larger their concentration the better.

6. CONCLUDING REMARK

In this work, the valorisation of natural by-products from the region of Castilla y León has been studied through the development of a microwave pretreatment to the conventional solid-liquid extraction, which has been selected because of its advantages towards industrial implementation. The intensification step proposed takes place before the conventional solid-liquid and its effects on process yield and product quality have been assessed to evaluate its convenience. The main aspects to be improved have been the reduction of the extraction time and the improvement on the product quality, enhancements that support the viability of the microwave pretreatment to be scaled-up.

NOMENCLATURE

Acronyms

MAE	Microwave assisted extraction
MW	Microwave

Symbols

c	Speed of light (m/s)
C_0	Initial concentration (mg/g)
C_∞	Polyphenol yield (mg/g)

C_{cal}	Regressed polyphenol concentration (mg/g)
C_f	Pre-exponential regression factor (mg/g)
d_P	Penetration depth (m)
E	Electric field intensity (V/m)
f	Frequency (Hz)
k	Extraction rate constant (min^{-1})
P_d	Power density (W/m^3)
Q_{MW}	Absorbed microwave energy
t	time (min)
$\tan\delta$	Loss tangent (dimensionless)
u_0	Initial extraction rate (mg/g·min)
V	Volume (m^3)
ε'	Dielectric constant (dimensionless)
ε''	Loss factor (dimensionless)
ε^*	Complex permittivity (dimensionless)
ε_0	Free space permittivity (dimensionless)
λ_0	Vacuum wavelenght (Hz)
ω	Angular frequency (rad/s)

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SCOPE AND AIMS

Ensuring process ***sustainability*** and enhancing ***product quality*** by the addition of microwaves to an extraction process have been pursued in this thesis.

Agricultural residues without actual use but with a large potential have been valorised with the main objective of extracting active compounds (polyphenols and anthocyanins) through a microwave step, what constitutes the overall aim of this thesis. Moreover, one of the key features sought for this microwave extraction intensification was to be devised in a way that the limitations of the microwave technology were surmounted. Thus, the development of a microwave process feasible to be implemented at industrial scale is the major scope of this work.

Regarding the effects that the microwaves have on extraction processes, this thesis has delved into the following specific objectives:

» *Increase polyphenol extract quality.*
Boost the polyphenol content as well as enhance the concentration of specific high added value compounds.

» *Study of the extraction conditions.*
Performance of a kinetic assay to evaluate the influence of extraction operational conditions. A reduction on the extraction time has been sought in order to avoid the need of bulky equipment.

» *Microwave extraction fundamentals.*
A contribution to understand why the addition of the microwaves to an extraction process provides such great improvements has been aimed.

» *Usefulness for other by-products.*
Study if the knowledge developed could be extrapolated to other residues.

On the other hand, the utility of the proposed process for a future industrial application has been considered of vital importance. For this purpose, these additional aims have been tackled too:

» *Efficient design of a pilot plant.*
Dielectric properties of complex mixtures have been studied in order to have reliable data for the microwave oven design and simulation.

» *Construction of a pilot plant.*
The construction of a microwave continuous oven has been pursued to demonstrate the convenience of the suggested process.

SECTION I

GRAPE POMACE VALORISATION

CHAPTER 1

Microwave pretreatment to improve extraction efficiency and polyphenol extract richness from grape pomace. Effect on antioxidant bioactivity

MICROWAVE PRETREATMENT TO IMPROVE EXTRACTION EFFICIENCY AND POLYPHENOL EXTRACT RICHNESS FROM GRAPE POMACE. EFFECT ON ANTIOXIDANT BIOACTIVITY

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ABSTRACT

Microwave assisted extraction advantages are widely recognised. However, its implementation at industrial scale is restricted due to microwave limitations. In this work, a microwave pretreatment is proposed as an easy scale-up alternative for grape pomace polyphenol extraction, especially for anthocyanins. The double effect of this pretreatment on extraction yield and on product richness is assessed. Microwaves accelerate the extraction kinetics of most compounds, but their effect on polyphenols is more pronounced than in other substances (like sugars and fibres). These differentiated rates are exploited to improve the polyphenol richness of the final dry product. By selecting the appropriate operating conditions, polyphenol yield was increased by 57% and, simultaneously, dry product richness was enhanced by 32%. Also, anthocyanin extraction boost was remarkable. Its content in the final dry product was 85% higher than the one obtained without the microwave pretreatment. The cellular bioactivity of these extracts was improved by 83% and 133%.

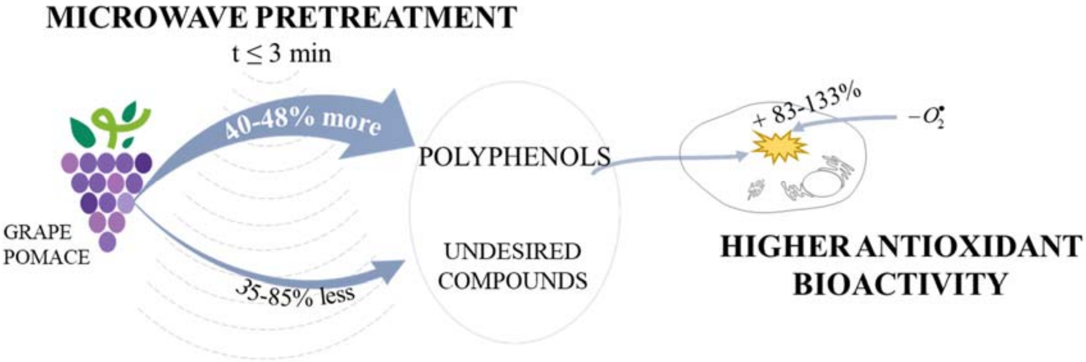
KEYWORDS

Microwave-pressure pretreatment; polyphenols; anthocyanins; extraction yield; richness; antioxidant bioactivity

HIGHLIGHTS

- Microwave intensification pretreatment for grape pomace polyphenol extraction
- Simultaneous high polyphenol yield and richness are obtained
- The differentiated extraction rates lead to a polyphenol richness optimum
- Anthocyanin product richness is boosted by microwaves
- Polyphenol and anthocyanin rich extracts have higher cellular antioxidant activity

GRAPHICAL ABSTRACT



1. INTRODUCTION

Nutraceutical's demand is growing every year as a consequence of the society concern for chemical preservatives (Frost and Sullivan, 2011; Serra et al., 2008). Since grape pomace is a propitious source of polyphenols, it can be used as a suitable resource to cover this demand of natural preservatives (Lutterodt et al., 2011; Shrikhande, 2000). Polyphenols are widely known for having beneficial health effects due to their ability to subdue free radical induced diseases (Nowshehri et al., 2015). This property makes them highly valuable for applications in cosmetic and food industry; for example, as supplements, food preservatives and natural colourants (Ignat et al., 2011). In particular, polyphenols provide a skin protection that prevents aging and other skin conditions (Yamakoshi et al., 2004). For instance, Wittenauer, et al. (2015) demonstrated the large effectiveness of catechin and procyanidins (polyphenols found in grapes) in the inhibition of collagen-elastase (cause of skin premature aging). In sum, there is a promising niche market for antioxidant products from natural extracts.

Grape pomace is not only a notable source of polyphenols, but also its use as raw material contributes to solve the ecological problem that this residue means (Fontana et al., 2013; Llobera and Cañellas, 2007). According to FAO (2016) and Rombaut (2014), in 2014 winemaking industry produced 5.8 million tonnes of what until recently was considered a valueless residue. However, it has now been appraised as a valuable material that can be used to improve wine industry sustainability. On the other hand, industrial sustainability comes through an effective process, what can be achieved by process intensification. In the case of the extraction of polyphenols from grape pomace, the main drawback to overcome is the bulky equipment and the long extraction times. Several novel intensification techniques have been studied at laboratory scale, such as ultrasound, high voltage or pressurized extraction (Duba et al., 2015; Goula et al., 2016; Puértolas and Barba, 2016). Between these novel processes, microwave assisted extraction (MAE) has been found to provide very good results (Casazza et al., 2010). Its main advantages are the shortening of the extraction time and the reduction in the amount of solvent used (Li et al., 2013). In addition, Yedhu Krishnan and Rajan (2016) have shown how microwave radiation disrupts the solid structure of the raw material *Terminalia bellerica*, facilitating the leakage of active compounds, what leads to the yield enhancement achieved with MAE. Regarding grape polyphenol extraction, MAE allows to obtain just in 10 minutes twice the amount of polyphenols that is extracted in 3 hours by a conventional solid-liquid extraction (Brahim et al., 2014). Also, the anthocyanin boost is remarkable: 17.6% more of these active compounds are obtained by microwave extraction (Liazid et al., 2011). However, this new extraction technique presents severe scale-up limitations. MAE main disadvantage is its low penetration depth, which for a frequency of 2.45 GHz and the mixture grape pomace-solvent is about 2 cm (Álvarez et al., 2017; Thostenson and Chou, 1999). Therefore, it is not possible to uniformly irradiate a large vessel, as only the external parts will be irradiated. The alternative to increase penetration depth is to use a frequency of 9.15 MHz, which will imply the counterproductive reduction of the thermal effect. That is why, in this work, a short microwave pretreatment is proposed as a preliminary stage to the conventional extraction, instead of a full time MAE process. A low residence time pretreatment will allow the material to be homogeneously irradiated in a size-reasonable microwave oven. In addition, by using an intense but short microwave pretreatment, the material will be able to absorb an amount of energy equivalent to MAE milder radiation conditions. In fact, Yuan Li et al. (2012) pointed out that the total energy absorbed by the material was more relevant than the power irradiated. Thus, both intensification processes can be considered equivalent in terms of matrix structure disruption. The peak of energy during the pretreatment causes a sharp temperature increase. But, because of its short duration, the degradation of active compounds is avoided (Sólyom et al., 2014), while the microwave thermal effect that accelerates the extraction is maintained. Once the disruption of the cell wall is accomplished, extraction continues as a conventional solid-liquid extraction to assure the leakage of the remaining active compounds.

Another important aspect to consider is the quality of the extract. The improvement on the extraction has to be in quantity (process yield) and in quality (product richness), understanding as product richness the concentration of active compounds in the final product. This value should be reported on a dry basis, since this is the state in which the final product is commercialised in order to assure its stability (de Souza et al., 2015). Final product polyphenol richness is of crucial relevance for its commercialization since the higher the polyphenol richness, the more valuable the product is. Undesired substances, like sugars and fibres that are also obtained in the extraction, depreciate the final product, and also interfere in its biofunctionality. These interactions may sometimes be positive, whereas in other cases they hinder polyphenol antioxidant capacity. For instance, Benayad et al. (2014) reported that polyphenol concentration is not proportional to antioxidant activity due to structure difficulties during oxidation patterns because of macromolecule steric hindrances. On the other hand, Serra et al. (2008) found that grape extracts present a larger antimicrobial activity than single polyphenol solutions with the same equivalent concentration. A synergetic effect between polyphenol and other substances was reported.

In sum, polyphenol's bioactivity not only depends on its concentration, but also on its accessibility (Benlloch-Tinoco et al., 2015). Polyphenols are associated with cell wall molecules, such as lipids, proteins, and sugars (Pérez-Jiménez et al., 2014). So, these compounds are the most likely to interfere in polyphenol activity. Lipid interaction is important regarding digestion, as it restrains lipid absorption (Jakobek, 2015). Several articles have been published about this. An example is the prevention of weight increase in mice with a fat dietary supplemented with black tea polyphenols (Uchiyama et al., 2011). In the case of proteins, the interaction conceals the polyphenol properties. Hasni et al. (2011) demonstrated that the binding between tea polyphenols and milk proteins makes both molecules unable to perform their functions. Sugars, instead, are able to encapsulate polyphenols and deliver them where their ability to quench free radicals is more useful (Jakobek, 2015). For instance, sugars can protect polyphenols through the gastrointestinal parts and release them in the colon, creating a positive antioxidant growth medium for microflora (Jakobek, 2015; Saura-Calixto, 2011). However, hampering effects have also been described, such as the bioactivity reduction caused by ferulic acid and arabinoxylands bonding (Adam et al., 2002).

The objective of this work is to develop an intensification step easy to be implemented at industrial scale to enhance the conventional solid-liquid extraction process. Extraction conditions are studied in order to fulfil industrial requirements, like solvent consumption reduction and the production of a polyphenol rich extract. Process yield and product richness are analysed together to optimise the extraction conditions. In addition, both chemical and cellular antioxidant activity assays are performed to assess whether the microwave pretreatment has any effect on final product antioxidant capacity.

2. MATERIALS AND METHODS

2.1. MATERIALS

Grape pomace from red *Tempranillo* grape was kindly given by Bodega Matarromera (Spain). It corresponded to 2014 vintage. Grape pomace was stored at -18ºC to preserve its antioxidant activity. Samples were thawed overnight at 4ºC before use. A mixed solvent of 50% ethanol and acid water was employed for extraction. Water was acidified with sulphuric acid to pH=1 in order to improve polyphenol stability.

Chemical reagents Folin-Ciocalteu, potassium chloride, sodium acetate, and sodium carbonate were purchased from Panreac Química (Barcelona, Spain). HPLC standards were bought in Extrasynthese (Lyon, France). Cell culture media and supplements, namely RPMI 1640-medium, fetal bovine serum (FBS), penicillin-streptomycin and trypsin/ethylenediamine tetraacetic acid (EDTA) were purchased from Invitrogen (Gibco, Invitrogen Corporation, Paisley, UK). For cytotoxicity and antioxidant activity assays, phosphate buffer solution (PBS) was prepared using the phosphate buffered saline powder, 3-(4,5-dimetylthiazol-2-yl)-5-(3-carboxylmethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS), 2´,7´-dichlorofluorescin diacetate (≥ 97%), quercetin (≥ 97%) and 2,2'-azobis (2-methyl-propionamide) dihydrochloride (AAPH, 97%) were provided by Sigma-Aldrich (St Louis, MO, USA).

2.2. EXTRACTION PROCEDURE

An industrial process currently running was used as a control and secondary process to assess the effectiveness of a microwave pretreatment (Moro González, 2009). Laboratory conditions to reproduce industrial conventional solid-liquid extraction were as follow. The amounts of 30 grams of grape pomace and 60 mL of mixed solvent were poured in a 100 mL round bottom flask. A 5 minute homogenization (stirring at ambient temperature) was performed before the extraction. The extraction was set to begin when the flask was introduced in a water bath at 60ºC with vigorous shaken (750 rpm). The solid-liquid extraction was carried out for 3 hours.

For the extractions were microwaves were used, the microwave pretreatment was implemented between the homogenization and the extraction stages. A commercial monomode oven working at a frequency of 2.45 GHz, CEM Discover One microwave (CEM Corp.), was used. A different solid-liquid ratio than in the conventional extraction was employed. For the pretreatment, 30 grams of grape pomace were mixed with 40 mL of solvent. A preliminary analysis of the solid-liquid ratio during the pretreatment suggested the convenience of using a higher ratio during the microwave pretreatment than in the subsequent conventional extraction (0.75 and 0.50 g/mL, respectively). It was found that a ratio of 0.75 g/mL during the microwave pretreatment fulfils a balance between mass transfer and energy efficiency. This is owing to the fact that this ratio provides enough solvent for the diffusion forces to continue to impel the leakage of polyphenols, but the media is not so diluted that the microwave effect is attenuated. After being homogenised, the mixture was irradiated with 300 W of power for 30, 60, 120 and 150 seconds, so pretreatment temperatures of approximately 60, 80, 100 and 120ºC were achieved. These energy densities have been labelled as MP60, MP80, MP100 and MP120, respectively. Temperature was continuously recorded with an optic fibre thermometer (FoTEMP 4, OPTPcon GmbH), calibrated in an ice water bath. For the MP100 and MP120 pretreatments, a glass pressure vessel (QianCap, QLabtech) was used to maintain the solvent in a liquid phase under pressure. Figure 1 represents the energy absorbed during each pretreatment. It includes a Control experiment that represents the conventional solid-liquid extraction, in which no pretreatment took place. Energy absorbed was computed by the sum of the sensible and latent heat. Heat losses to the environment were dismissed, since Sólyom et al. (2011) proved its low contribution. The efficiency between the emitted and the absorbed energy was found to be 58%.

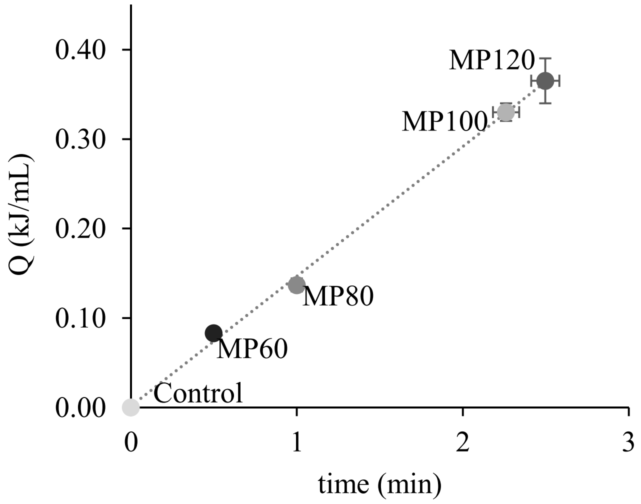


Figure 1. Specific energy absorbed during the microwave extraction pretreatment.

When irradiation was over, 20 mL of solvent were added to reach the same ratio used in the conventional solid-liquid extraction process. The grape pomace-solvent mixture was quickly cooled down to 60ºC in an ice bath, and it continued with the conventional solid-liquid extraction in a water bath.

Liquid samples were taken during the extraction at different times to perform a kinetic study. The start of the extraction was set after the 5-minute homogenization (t=0). The initial concentration (C_0) represents the released of non-bound polyphenols. After the homogenization, samples were taken after the pretreatment and during the subsequent conventional process. Extraction kinetics followed a first order equation, like shown in expression [1]; where C_{cal} stands for the calculated concentration in mg/g at a time t (minutes), C_0 represents initial concentration (mg/g), C_f is a pre-exponential factor (mg/g) that together with C_0 (C_0+C_f) corresponds to extraction yield (t = ∞), and k is the rate extraction constant (min^{-1}).

$$C_{cal} = C_0 + C_f\left(1 - exp(-kt)\right) \qquad [1]$$

Concentrations have been expressed in two different ways: as mg/g of dry pomace and as mg/g of dry extract. The first one, mg/$g_{\text{Dry Pomace}}$, represents the extraction yield: the amount of polyphenols extracted from the raw material. The second, mg/$g_{\text{Dry Extract}}$, describes the final dry product richness, as it indicates the ratio between polyphenols and the total extracted substances.

Expression [1] correlation parameters C_0, C_f and k have been adjusted by minimizing the average relative deviation (ARD) between the experimental concentration and the calculated value, as equation [2] shows. Regressions have been performed for yield and for richness results. In the case of extract richness, the extraction of other compounds must be also considered. That is why richness regression curve has to be computed as the ratio between the concentration of active compounds and the total amount of substances extracted (dry extract residue).

$$ARD = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{C_{\exp} - C_{cal}}{C_{\exp}}\right| \qquad [2]$$

Experiments were performed in triplicate to assure the reproducibility of the process. Results have been expressed as mean ± standard deviation.

2.3. SOLID RESIDUE

The dry extract residue was measured gravimetrically by drying extracts at 85ºC during 48 hours.

2.4. POLYPHENOL CHARACTERIZATION

2.4.1. TOTAL POLYPHENOL CONTENT

Total polyphenol content was measured by Folin-Ciocalteu method (Waterhouse, 2001). Briefly, 40 μL of diluted sample were mixed with 3000 μL of distillate water and 200 μL of Folin-Ciocalteu reagent. After 5-minute shakening, 600 μL of 20% sodium carbonate were added. Samples were left 30 minutes at 40ºC. Then, absorbance was measured at 765 nm (Shimadzu UV/VIS Spectrophotometer). Results were expressed in gallic acid equivalents (mg_{GAE}/g).

2.4.2. ANTHOCYANIN CONTENT

Anthocyanin content was determined by the pH differential method (AOAC, 2005). Samples were diluted in two buffers (potassium chloride 0.025 M pH=1 and sodium acetate 0.4 M pH=4.5). Absorbance was calculated as the increment in absorbance at 520 minus the increment at 700 nm between the two buffers. An extinction coefficient of 26900 L/mol/cm was used. Results were expressed as cyanidin-glucoside equivalents (mg_{CGE}/g).

2.4.3. IDENTIFICATION AND QUANTIFICATION OF POLYPHENOLS (HPLC)

Gallic acid, catechin, procyanidin B2, epicatechin, myrecetin, quercetin and malvidin were quantified on a Waters e2695 Separation Module equipped with a quaternary pump and a Waters 2998 photodiode array detector (DAD). A method for grape and wine phenols was used (Gómez-Alonso et al., 2007). A C18 guard colum OptiGuard 1 mm (Sigma-Aldrich, Spain) and a C18 column Mediterranea Sea 5 μm 250x4.6 mm (Teknokrima, Spain) were employed. Temperature was kept at 30ºC and 1 mL/min of the ternary eluent was pumped. Eluent's gradient is specified in Table 1. Samples were filtered with 0.45 μm regenerated cellulose syringe filters and a sample volume of 20 μL was injected in the system. DAD signals were recorded at 280, 320, 360 and 520 nm. UV/VIS spectra was also registered. Waters Empower 3 software acquired the data and allowed the processing. Compound identification was made by comparing retention time and UV/VIS spectra of each polyphenol with standards.

Table 1. Ternary mobile phase gradient.

time (min)	%Eluent A[a]	%Eluent B[b]	% Eluent C[c]
0	100	0	0
2	100	0	0
5	92	8	0
17	0	14	86
22	0	18	82
29.5	0	21	79
55	0	33	67
70	0	50	50
75	0	50	50
78	20	80	0
81	20	80	0
86	100	0	0

[a] Eluent A: $NH_4H_2PO_4$ 50 mM, pH=2.6

[b] Eluent B: 20% eluent A and 80% acetonitrile

[c] Eluent C: H_3PO_4 200 mM, pH=1.5

2.5. OTHER SUBSTANCES DETERMINATION

2.5.1. NON-POLYPHENOL CONTENT

Non-polyphenol content was computed by subtracting the concentration of polyphenols to the total amount of substances extracted, determined by the dry solid residue.

2.5.2. SUGAR CONTENT

Sugars were determined by the anthrone method. In a 70% (v/v) sulfuric acid solvent 2 g/L of anthrone reagent were dissolved. A volume of 5 mL of this solution was mixed with 1 mL of sample, cooled down in an ice bath, and thorough shaked for 15 s. Then, the samples were put 10 minutes in a boiling water bath. After cooling them down, absorbance at 625 nm was recorded. Results were quantified by comparison with a glucose calibration curve, and so, results were expressed as glucose equivalents (mg_{GE}/g).

2.5.3. FIBRE CONTENT

Dietary fibre was computed spectrophotometrically at 320 nm, using an absorptivity coefficient of 5.7 L/g/cm (Prozil et al., 2014). This value was corroborated by an external laboratory assay (Agrolab, Spain).

2.6. CHEMICAL ANTIOXIDANT ACTIVITY

Chemical antioxidant activity was measured by the oxygen radical absorbance capacity assay (Ou et al., 2001). It measured the sample capacity to quench peroxyl radicals from α,α'-azodiisobutyramidine dihydrochloride (AAPH) by inhibiting fluorescein sodium oxidation. Phosphate buffer (10 mM, pH=7.4) was used as solvent for all the solutions. Trolox standards were employed to quantify the antioxidant capacity. A 96-well plate was filled with 25 µL sample (trolox standard or diluted extract) and 150 µL fluorescein sodium salt (100 nM). The plate was incubated at 37ºC for 30 minutes. Reaction was started by the addition of 25 µL of AAPH 240 mM. Fluorescence was recorded by a BMG LABTECH Fluostar OPTIMA fluorescent reader at the emission wavelength of 530±25 nm and excitation wavelength of 485±20 nm, during 90 minutes. Each sample was measured at least six times. Antioxidant capacity was computed by a calibration obtained from the area under the oxidation curve of trolox standard solutions. Results were expressed as trolox equivalents per gram of dry extract ($\mu mol_{Trolox}/g_{Dry\ Extract}$).

2.7. CELL ASSAYS

2.7.1. CELL CULTURE

Human colon cancer cell line, Caco-2, was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Barunshweig, Germany). The cell line was grown in RPMI 1640 medium supplemented with 10% (v/v) of FBS and 5000 units of penicillin-streptomycin. Stock cells were maintained as monolayers in 75 cm^2 culture flasks and subcultured at a split ratio of 1:4 by treatment with 0.1% trypsin/0.02% EDTA and incubated at 37 ºC in a 5% CO_2 humidified atmosphere.

2.7.2. CYTOTOXICITY ASSAY

Toxicity assays were performed as previously described by Serra et al. (2013) using completely confluents Caco-2 cells which are a good model of the intestinal barrier (Sambuy et al., 2005). Briefly, Caco-2 cells were seeded at a density of 2×10^4 cells/well in 96-well plates and the medium was changed every 48 h. After achieving confluence (5-7 days), cells were exposed with different concentrations of the grape pomace extracts, dissolved in culture medium (RPMI 1640-medium with 0.5% (v/v) FBS) and incubated during 4 hours at 37 ºC and 5% CO_2. After the incubation time, the medium with different sample concentrations was removed and cells were washed with PBS twice. Then, 100 µL of MTS dissolution in RPMI 1640-medium with 0.5% FBS (16.6% (v/v) of a stock solution of 1:10 (pure MTS: RPMI 1640-medium with 0.5%FBS) was added to each well and the 96-

well plate was incubated during 2 hours. The MTS assay is based on the conversion of a tetrazolium salt into a coloured, aqueous soluble formazan product by mitochondrial activity of viable cells at 37°C. The amount of formazan produced by dehydrogenase enzymes is directly proportional to the number of living cells in culture and can be measured at 492 nm (Malich et al., 1997). The amount of formazan produced was quantified spectrophotometrically at 492 nm in a microplate reader (EPOCH, Bio-Tek, USA). Results were expressed as percentage of cellular viability relative to the untreated control (cells with RPMI 1640-medium with 0.5% (v/v) FBS). In order to achieve reproducibility in the results, experiments were performed in triplicate in three independent assays.

2.7.3. CELLULAR ANTIOXIDANT ACTIVITY

In order to evaluate the cellular antioxidant activity of the grape pomace extracts, Caco-2 cells were seeded at a density of 2×10^4 cells/well in 96-well plates and the medium was changed every 48 h. The experiments were performed using completely confluents cells (5-7 days). Intracellular antioxidant activity of the different grape pomace extracts was evaluated following the formation of reactive oxygen species in Caco-2 cells after treatment with a chemical stress inducer (AAPH). The formation of intracellular reactive oxygen species was monitored using the fluorescent probe, DCFH-DA, as described by Wang and Joseph (1999) and Serra et al. (2010). Briefly, confluent Caco-2 cells were washed with PBS twice. Then, cells were exposed with different concentrations of the different extracts dissolved in PBS (50 µL/well) and with 50 µM DCFH-DA (50 µL/well), to be later incubated during 1 hour at 37 ºC in a 5% CO_2 humidified atmosphere. Quercetin was used as standard. After the incubation time, the medium was removed and the AAPH solution with a concentration of 0.6 mM was added (100 µL/well). Fluorescence was measured for each sample between 0 and 60 min in a FLx800 BioTek fluorescence microplate reader. Cellular antioxidant activity of the extracts was quantified according to Wolfe and Liu (2007) and expressed as quercetin equivalents per gram of dry extract ($\mu mol_{Quercetin}/g_{Dry\ Extract}$). In order to achieve reproducibility in the results, experiments were performed in quadruplicate in four independent assays.

3. RESULTS AND DISCUSSION

3.1. YIELD AND RICHNESS IMPROVEMENT

Current running conventional solid-liquid extraction processes are carried out during long extraction times to deplete the raw material and so, obtain a high polyphenol yield. However, it also compromises polyphenol richness, as Figure 2(A) proves. Long times entail the extraction of undesired substances that reduce product richness. This is clearly evident in anthocyanin extraction, as shown in Figure 3(A). The maximum anthocyanin richness was reached at 60 minutes, where only the 79% of total anthocyanins and 86% of total polyphenols had been extracted. By continuing the extraction to 180 minutes, all the available polyphenols were obtained, but anthocyanin richness decreased a 31% (from 10.4±2.6 $mg_{CGE}/g_{Dry\ Extract}$ at 60 minutes to 7.2±0.7 $mg_{CGE}/g_{Dry\ Extract}$ at 180 minutes). Therefore, conventional extraction did not allow to obtain maximum yield and richness simultaneously at any operation conditions, neither for polyphenols nor for anthocyanins.

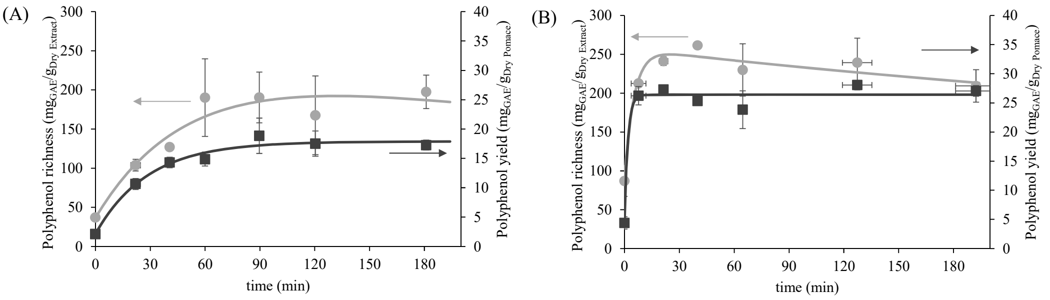


Figure 2. Polyphenol extraction richness and yield for (A) conventional extraction and (B) MP100 pretreatment. ● Polyphenol richness; ■ Polyphenol yield.

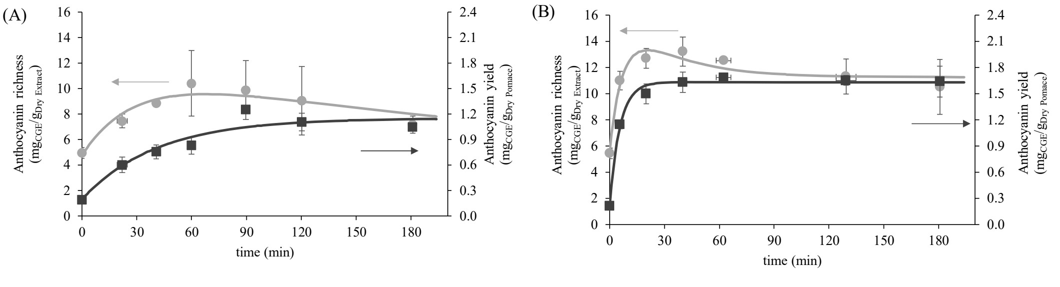


Figure 3. Anthocyanin extraction richness and yield for (A) conventional extraction and (B) MP80 pretreatment. ● Anthocyanin richness; ■ Anthocyanin yield.

The addition of a microwave-pressure pretreatment to the conventional solid-liquid extraction accelerated polyphenol extraction. Higher yields were obtained at shorter times: between 42-66% more polyphenols were extracted per gram of raw material. Taking into account product richness, the extraction of other undesired compounds was also affected by the radiation, but not in such a significant way as polyphenols did. These two characteristics, the polyphenol extraction acceleration and the different extraction rate of other compounds, made possible to find an optimal time where both yield and richness were maximum.

Extraction kinetic parameters for each pretreatment studied are detailed in Table 2. Richness regression curve must be computed as the fraction between the concentration of active compounds and the total extracted substances (total polyphenols and non-polyphenols present in the dry residue). This is due to the necessity of taking into account the extraction kinetics of the active compounds and of the non-polyphenols.

MP120, MP100 and MP80 pretreatments allowed to obtain the maximum yield in shorter extraction times. The lowest energy pretreatment tested, MP60, did not show a large microwave improvement for total polyphenol content. It presented similar kinetics to the control experiment, in which yield increased with extraction time. This difference between MP60 with the rest of the pretreatments can be clearly seen in the values of the extraction rate constant (k). MP60 and Control extraction rate constant are one order of magnitude lower than the constants of the rest of the pretreatments. Regarding product richness, the same distinction between high energy pretreatments (MP120, MP100, MP80), and MP60 and Control experiments was found. In the first group, extract polyphenol richness soared, achieving its maximum after 10-30 minutes of extraction. Then, it slightly decreased when extraction carried on. The combination of pressure and microwave radiation up to a temperature of 100ºC (MP100 pretreatment) provided the best results in terms of total polyphenol. Figure 2 shows the extraction kinetics of MP100 pretreatment compared to conventional extraction. As it can be observed, all the extractable polyphenols were obtained after the pretreatment, there was no further yield improvement in the ensuing conventional extraction. A 48% higher yield was obtained as well as a 7-fold extraction time reduction. In addition, by stopping the extraction after 40 minutes, it was possible to increase product polyphenol richness without sacrificing yield. At this time, the richness was maximum (261.5±2.5 $mg_{GAE}/g_{Dry\ Extract}$) and it was 1.3 times higher than the conventional extraction process. If the extraction continued, no more polyphenols were extracted. Instead, richness steadily decreased.

Table 2. Extraction kinetic parameters for equation [1].

		YIELD (mg/g$_{DryPomace}$)					RICHNESS (mg/L)				
		MWP120	MWP100	MW80	MW60	Control	MWP120	MWP100	MW80	MW60	Control
TPC	C_0	2.4	4.4	2.3	2.0	2.1	640	1129	706	820	520
	C_f	21.8	22.0	20.0	25.8	15.8	3.62E+03	3.58E+03	3.19E+03	3.97E+03	4.13E+03
	k	2.47E-01	5.50E-01	1.15E-01	2.91E-02	3.51E-02	1.98E-01	2.66E-01	9.80E-02	2.72E-02	1.43E-02
	ARD	4.0E-02	3.9E-02	7.4E-02	9.2E-02	3.1E-02	4.8E-02	5.6E-02	9.1E-02	1.2E-01	7.1E-02
AC	C_0	0.2	0.2	0.2	0.2	0.2	61	61	70	55	69
	C_f	0.8	0.7	1.4	0.9	1.0	114	111	224	142	118
	k	2.47E+00	6.24E-01	1.80E-01	1.58E-01	2.45E-02	3.32E-01	2.28E-01	9.26E-02	1.61E-01	2.95E-02
	ARD	6.5E-02	1.3E-01	1.9E-02	5.3E-02	6.5E-02	6.9E-02	1.2E-01	7.8E-02	5.2E-02	6.5E-02
Non-polyphenols	C_0	33.8	56.8	39.0	50.8	62.7	1.32E+04	1.11E+04	1.19E+04	1.24E+04	1.33E+04
	C_f	34.1	44.1	96.7	84.2	46.4	8.47E+03	2.97E+05	1.06E+04	1.09E+04	5.38E+05
	k	1.18E-01	7.11E-03	2.70E-02	7.30E-02	1.34E-02	1.55E-02	8.73E-05	2.72E-02	5.42E-02	5.25E-05
	ARD	4.1E-02	1.0E-01	1.3E-01	4.2E-02	7.7E-02	5.9E-01	6.1E-02	3.5E-01	4.3E-02	8.6E-02
Sugars	C_0	0.3	1.7	1.6	1.1	1.7	67	429	413	301	606
	C_f	11.7	11.5	9.7	13.1	26.8	2.08E+03	1.86E+03	1.62E+03	2.07E+03	9.96E+03
	k	2.33E-01	1.88E-01	1.69E-01	4.29E-02	5.28E-03	2.01E-01	1.79E-01	1.40E-01	4.25E-02	1.50E-03
	ARD	7.5E-02	1.3E-01	6.1E-02	9.4E-02	1.1E-01	8.6E-02	1.3E-01	7.5E-02	8.0E-02	1.4E-01
Fibres	C_0	3.8	3.6	3.2	7.2	7.8	1.01E+03	9.33E+02	9.59E+02	1.44E+03	1.76E+03
	C_f	27.1	21.8	21.9	23.3	34.7	4.32E+03	3.53E+03	3.52E+03	3.76E+03	9.34E+03
	k	4.69E-01	6.74E-02	7.86E-02	2.27E-02	6.09E-03	3.92E-01	5.71E-02	6.23E-02	2.20E-02	2.91E-03
	ARD	2.3E-07	7.5E-09	6.7E-12	1.1E-07	1.8E-09	1.2E-08	8.6E-09	2.2E-09	6.6E-08	2.2E-08

[d] Initial concentration.

[e] Pre-exponential factor.

[f] Rate extraction constant.

[g] Average relative deviation.

Particular attention deserves anthocyanin extraction. The dilution effect due to undesired extracted compounds was more noteworthy in this group. Anthocyanins are mainly located in grape skin, so, its extraction does not have great mass transfer limitations to overcome (Pinelo et al., 2006). This led to a fast anthocyanin extraction during both conventional and pretreatment processes. As in polyphenols extraction, this faster release enables to stop the processes when yield and richness are at their maximums. The most favourable time to obtain rich extracts was placed at the early stages of the process for pretreated extractions (20-30 minutes), and at the middle of the process for the conventional extraction (60 minutes). In the case of the combination of pressure and microwave pretreatment (MP120 and MP100), the decrease in anthocyanin richness was quite significant. Reductions of 66% and 50% were found by no stopping the extraction in the early stages, and letting it continue for longer. These high energetic pretreatments are not recommendable for anthocyanin extraction, even though no fruitless effects, such as degradation, has been observed (yield remained steady at its maximum along the extraction). In contrast, the lowest energy pretreatment, MP60, also provided low anthocyanin richness, although this result was due to the low efficiency that this pretreatment has been proved to have. To obtain an anthocyanin rich product, the MP80 pretreatment is recommendable. It was possible to get an 85% more concentrated extract than the one obtained by conventional extraction by stopping the process at 30 minutes, as Figure 3(B) demonstrates.

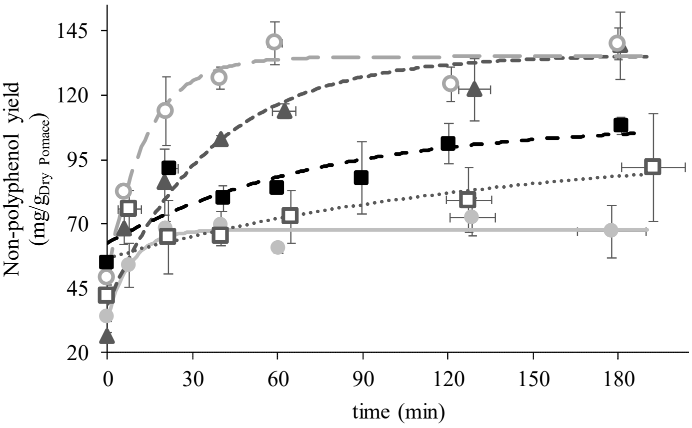


Figure 4. Non-polyphenol extraction kinetics. ● MP120, □ MP100, ▲ MP80, ○ MP60, ■ Control.

The richness peaks found during the extractions corroborated the importance of stopping the process at a proper time. Figure 4 attests that the dilution effect is due to the leakage of other substances. The addition of the pretreatment accelerated the release of non-active compounds. However, this enhancement had a lower pronounced effect than the acceleration observed in polyphenol extraction.

This differentiated effect of microwaves between each group led to find an optimal time for richness, in which polyphenols were completely extracted, but undesired substances were still being released. The inefficiency of the combined pretreatment of microwaves and pressure (MP120 and MP100) for non-polyphenols was conspicuous. While only microwave pretreatments (MP80 and MP60) improved the yield of non-active compounds over conventional extraction, microwave-pressure pretreatments presented a low efficiency for these undesired compounds. Of these non-active compounds, sugars and fibre extraction have also been quantified. The kinetic parameters obtained are gathered in Table 2. A maximum of 127.8±27.8 $mg_{GE}/g_{Dry\ Extract}$ and 256.9±13.3 $mg_{Fibre}/g_{Dry\ Extract}$ were obtained for sugars and fibres, respectively.

3.2. SELECTION OF THE OPTIMAL EXTRACTION CONDITIONS AND REPRESENTIVE EXTRACTS

In the previous section, the importance of knowing the extraction kinetics in order to select the optimal conditions to enhance the process was exposed. In the present section, a representative sample of each extraction process has been selected to assess its functionality. Selection was performed attending to product quantity and quality. Table 3 summarizes the characterization of each sample further analysed.

Chromatography analysis revealed the same pattern found for the extraction of total polyphenols and anthocyanin for each key polyphenol analysed. As Figure 5 shows, MP100 and MP80 are the pretreatments that greatly enhance the output.

Table 3. Representative samples selected from each extraction process.

Extraction process	time (min)	Polyphenols ($mg_{GAE}/g_{Dry\ Extract}$)	Anthocyanins ($mg_{CGE}/g_{Dry\ Extract}$)	Non-polyphenol ($mg_{Dry\ Extrac}/L_{Extract}$)
MP120	8	186.6 ± 6.5	8.3 ± 0.2	1.92E+04 ± 2.33E+03
MP100	21	241.6 ± 6.8	11.2 ± 0.3	1.58E+04 ± 2.43E+03
MP80	40	165.5 ± 5.0	14.0 ± 0.3	2.14E+04 ± 3.91E+02
MP60	21	115.5 ± 4.4	8.7 ± 0.1	2.22E+04 ± 2.47E+03
Control	60	190.4 ± 9.4	10.8 ± 0.4	1.52E+04 ± 5.18E+03

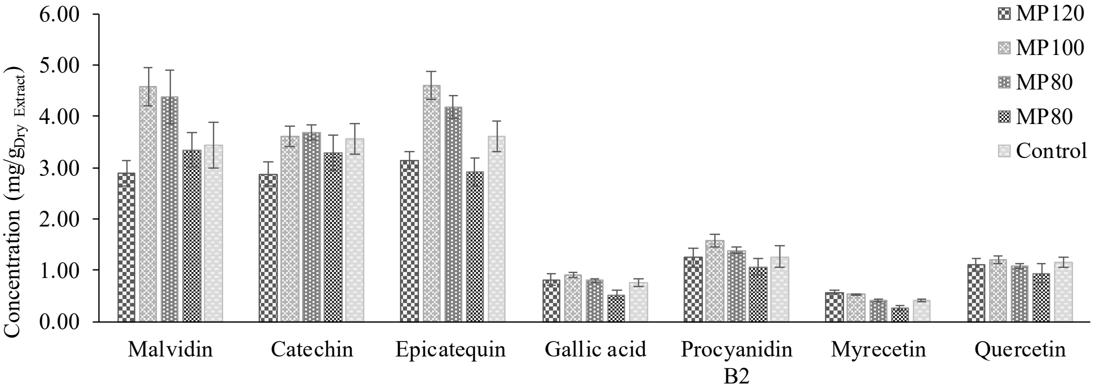


Figure 5. HPLC analysis of the representative extraction samples according to Table 3.

3.3. CELL VIABILITY

Cell viability was not affected up to doses of 10 mg$_{GAE}$/g$_{Dry\ Extract}$ in any of the extracts tested. However, Figure 6 clearly shows the dose dependent toxic effect of the high dose extracts, except for MP80 whose effect on cell viability stood for longer. It may be due to the large concentration of anthocyanins, particularly malvidin, which may play a role as a cytoprotective agent. Especial attention deserved MP120 and MP100, since cell proliferation was improved by small doses of these extracts. This could be explained by their lower content in non-polyphenols, which can be associated to cellular toxicity. A more prolonged exposure of the matrix at higher temperature might increase the extract concentration in toxic degradation products and also affect the cytoprotective action of specific polyphenols such as anthocyanins (Choi et al., 2007).

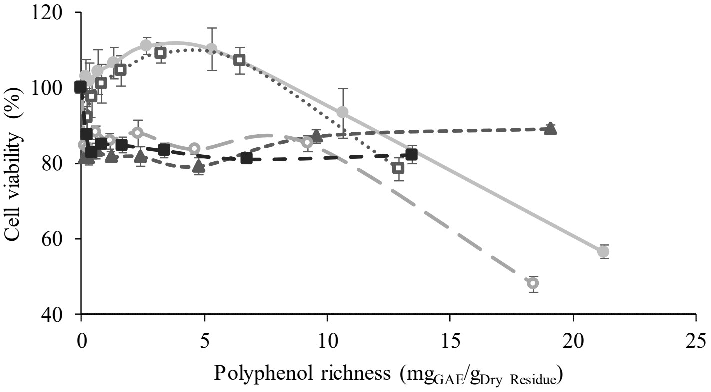


Figure 6. Cell viability. ● MP120, □ MP100, ▲ MP80, ○ MP60, ■ Control.

3.4. CHEMICAL AND CELLULAR ANTIOXIDANT ACTIVITY

Antioxidant activity is the main feature of this kind of extracts. The extracts capacity regarding scaveging of peroxyl radicals were evaluated by two distinct assays. First, a chemical assay was performed and the results compared with the extracts activities at cellular level. Results can be seen in Figure 7.

Chemical antioxidant activity was proportional to the concentration of polyphenols, but cellular antioxidant capacity did not present this direct relation. Regarding cellular antioxidant activity, MP120, MP60 and Control provided similar antioxidant capacities. In contrast, MP100 and MP80, the richest polyphenol and anthocyanin extracts, had the highest antioxidant bioactivity: 133% and 83% more, respectively, than the conventional extract. MP100 had the lowest proportion of non-polyphenol substances, so, the interference that these compounds may cause in the absorption of the active compounds may be subdued. On the other hand, MP80 enhancement can be attributed to the high antioxidant activity of anthocyanins. Their large capacity may help to overcome limitations that restrict other samples.

Therefore, MP100 and MP80 pretreatments not only improved the extraction process and the product quality, but also its bioactivity. A characteristic that is considered an asset in the development of commercial products.

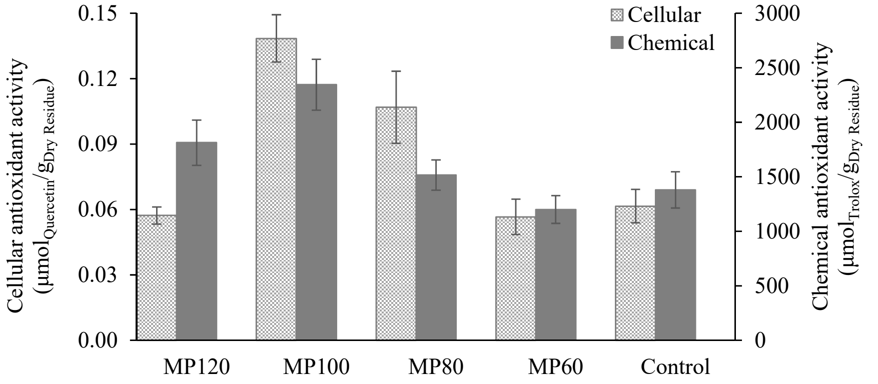


Figure 7. Chemical and cellular antioxidant activity against peroxyl radical.

4. CONCLUSIONS

In terms of process efficiency, the addition of the here proposed microwave pretreatment allowed to improve extraction yield and polyphenol richness, both at the same time. The acceleration effect of microwaves on extraction kinetics was more pronounced on polyphenols than on other compounds, such as sugar and fibres. This result led to an optimal extraction operation time when polyphenols had already been fully extracted, but those other substances that reduce polyphenol richness were still being released. At that time, the maximum yield and richness were achieved simultaneously. Conversely, in conventional extraction maximum yield and maximum richness were achieved at different extraction operation times, which made it impossible to achieve both objectives concurrently.

Two different pretreatments have been selected to optimise polyphenol or anthocyanin extraction. Regarding total polyphenol content, a 120 seconds pretreatment (MP100) was found to provide the highest polyphenol yield and richness, by a combination of microwave and pressure up to a temperature of 100ºC. The extract yield was improved by 48% and the final dry product richness by 35%, using only one seventh of the extraction time required in the conventional process. If the extraction objective were anthocyanins recovery, the optimal microwave pretreatment time would be 60 seconds (MP80), and the extraction media would be heated up to 80ºC. Microwaves provided high efficiency to the anthocyanin extraction process, what significantly soared final dry product richness, and allowed to obtain an 85% richer anthocyanin extract.

Both pretreatments (MP100 and MP80) not only improved process efficiency, but also product bioactivity. It was found that these polyphenol and anthocyanin rich extracts presented a more pronounced cellular antioxidant activity against peroxyl radical damage. The final product bioactivity is doubled by the addition of the microwave pretreatment.

In sum, the microwave pretreatments here proposed improved product quality and quantity, as well as extract biofunctionality.

ACKNOWLEDGEMENTS

This work was supported by the European project WineSense (FP7-386 MC-IAPP), and by Junta de Castilla y León regional project VA330U13 and project VA040U16. Ana Álvarez would like to thank Spanish Ministry of Education for her fellowship (FPU13/04678). Ana Matias is grateful to Fundação para a Ciência e a Tecnologia by her Investigator FCT grant fellowship. A. Crespo is gratefully acknowledged for her technical assistance.

NOMENCLATURE

Acronyms

ARD	Average relative deviation
CGE	Cyainidin glucoside equivalents
GAE	Gallic acid equivalents
MP	Microwave pretreatment

Symbols

C_0	Initial concentration (mg/$g_{Dry\ Pomace}$)
C_{cal}	Estimated concentration (mg/$g_{Dry\ Pomace}$)
C_f	Pre-exponential factor (mg/$g_{Dry\ Pomace}$)
k	Extraction rate constant (min^{-1})
n	Number of experiments considered (adimensional)
Q	Absorbed energy (kJ/mL)
t	time (min)

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CHAPTER 2

Measurement and correlation of the dielectric properties of a grape pomace extraction media. Effect of temperature and composition

MEASUREMENT AND CORRELATION OF THE DIELECTRIC PROPERTIES OF A GRAPE POMACE EXTRACTION MEDIA. EFFECT OF TEMPERATURE AND COMPOSITION

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ABSTRACT

Microwave assisted extraction has been proved to be a useful tool for the recovery of valuable compounds from natural products. However, little is known about the dielectric properties for mixtures of natural products and solvents. In this work, a semi-empirical model is proposed to calculate the dielectric properties of these systems. The variables considered were temperature, solvent composition and solid-liquid ratio. Grape pomace was used as the solid matrix and ethanol-water mixtures as the solvent. The experimental dielectric constant and loss factor of these multiphase solid-solvent samples were measured and compared to the proposed model. Loss factor values were taken under special consideration, since they were boosted by the release of ions from the solid (an increase between 9-83%). The model proposed here reproduces the dielectric properties of this multiphase media with an average deviation of 8.1% for the dielectric constant and of 24.8% for the loss factor.

KEYWORDS

Dielectric constant; loss factor; mixed solvent; multiphase mixture; temperature; composition.

HIGHLIGHTS

- Measurements of multiphase grape pomace ethanol-water mixtures permittivities
- An empirical model is proposed to calculate multiphase system dielectric properties
- Model variables are temperature, solvent composition and solid-liquid ratio
- Grape pomace boosts ionic conductivity by releasing ions into the solvent
- Solvent composition revealed as the most influential variable

1. INTRODUCTION

In the last decades, microwave technology has gained a great deal of attention due to the advantages that it presents. Many conventional extraction processes have been redeveloped to include this technology, as it allows to reduce extraction time and the amount of solvent required. Examples of the remodelled processes are the solvent-free extractions or the addition of microwave pretreatments to enhance anaerobic digestions (Appels et al., 2013; Filly et al., 2014). However, despite the fact that this technology has been widely studied, little attention has been paid to dielectric properties. Scarce dielectric data or correlations at temperatures above ambient are available. In addition, there are many discrepancies in dielectric loss temperature dependence for many common solvents. Even though there are few frequencies allowed for commercial and industrial applications (Heileman et al., 2013), most studies have thoroughly analysed permittivity changes along the frequency spectrum at close to ambient temperature. Thus, this knowledge results to be insufficient to simulate a microwave process from an engineering point of view, since few measures at the set frequency and temperature are actually available. Moreover, temperature dependence is critical for reliable modelling and simulation studies. For instance, water permittivity variation with temperature is highly significant: dielectric constant decreases a 34% and loss factor an 87% by increasing temperature from 0ºC to 90ºC (Franco et al., 2015; Malmberg and Maryot, 1956). A temperature and composition characterization of the medium will allow 1) the assessment of the effectiveness and efficiency of the microwaves in the heating process, and 2) the proper simulation of evolution in time of the heating of the material. This will lead to a conscious cavity design, and eventually, to a higher quality final product.

Electric and magnetic fields are described by Maxwell's equations together with the constitutive equations (Sosa-Morales et al., 2010). Those equations require the knowledge of both complex permittivity and permeability of the involved materials. Most natural materials are not affected by a magnetic field, so, magnetic permeability can be dismissed in these cases (Muley and Boldor, 2013). The interaction of the electric field and a dielectric material depends on the frequency and it is described by means of its complex permittivity (ε^*) (Datta and Anantheswaran, 2001). For polar liquids like water, this frequency dependence can be successfully computed by Debye's model (Stuerga, 2008). For non-polar substances or complex molecules (e.g. polymers), some modifications are reported based on relaxation time distributions. The most extended ones are the Cole-Cole and the Cole-Davidson models (Stuerga, 2008). However, in the case of ethanol, the dielectric behaviour can be accurately described by Debye's equation due to its polarity and structure. Gregory and Clark (2006) defined a range of permittivity values inside which substances can be considered as polar, and so, characterized by Debye's equation. These limits are ε'=10-100 and ε''=1-80. Ethanol permittivity is in this range (ε'=16-22 and ε''=1-10), hence, Debye's model is suitable for ethanol dielectric characterization.

Debye's permittivity is shown in equation [1], where ε_S and ε_∞ stand for static (frequency tending to cero) and high-frequency permittivity; *j* corresponds to the imaginary unit; *f* is the frequency in Hz; and τ the relaxation time in seconds.

$$\varepsilon^* = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + j2\pi f\tau} \tag*{[1]}$$

Permittivity can also be expressed as the sum of the dielectric constant (ε') and the loss factor (ε''). These terms correspond, respectively, to the real and imaginary parts of the complex permittivity, as expressed in equation [2]. The dielectric constant is proportional to the material's ability to storage energy; whereas the loss factor represents the losses due to dielectric relaxation and conductivity (Navarrete et al., 2011). They can also be directly computed from relaxation time, static and high-frequency permittivities, as it is shown in equations [3] and [4].

$$\varepsilon^* = \varepsilon' - j\varepsilon'' \tag*{[2]}$$

$$\varepsilon' = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + (2\pi f)^2 \tau^2} \tag*{[3]}$$

$$\varepsilon' = \omega\tau \cdot \frac{\varepsilon_s - \varepsilon_\infty}{1 + (2\pi f)^2 \tau^2} \tag*{[4]}$$

When ions are present in the medium, its conductivity also contributes to the loss factor term as it is expressed in equation [5]. Ionic losses are a function of conductivity (σ, in S/m), and they are referred to the free space permittivity (ε_0=8.854·10^{-14} F/m).

$$\varepsilon_{\sigma} = \frac{\sigma}{j2\pi f\varepsilon_0} \qquad [5]$$

The most critical factors that affect permittivity are frequency, temperature and moisture (Guo et al., 2011). Food composition (mainly ash and salt content) can have an important role too (Liu et al., 2009). Microwave heating processes for scientific, medical and industrial applications are forced to use some specific frequency bands assigned by the International Telecommunication Union (ITU) (ITU-R, 1994). The well-known frequency of 2450 MHz is worldwide allowed for microwave heating and employed in commercial ovens (Spigno and Faveri, 2009). Thus, it may be considered as a heating standard technology for microwave ovens. Permittivity dependence on temperature is quite complex, since many other important variables are highly influenced by temperature. A general behaviour pattern cannot be obtained without a thoughtful analysis of the system components. So, temperature is always a crucial parameter to take into account. Moisture effects are more evident: dielectric constant and loss factor tend to approach water dielectric behaviour as moisture content increases (Zhu et al., 2012).

Debye’s law is only valid for pure substances. In order to characterize homogeneous and multiphase mixtures, various mixing rules have been previously proposed. They are summarized in Table 1. There are many other semi-empirical expressions. For example, the one suggested by Jouyban et al. (2004), where binary interaction coefficients and a temperature correction term are added to the weighted permittivity average. As it can be deduced from Table 1, there is not a universal mixing rule, and each mixture has to be individually analysed in order to choose the most appropriate expression, especially with heterogeneous materials (Sihvola, 2002). In the case of complex natural materials, it is unlikely to find an accurate formula to predict their behaviour. Instead, a mean permittivity that enables an acceptable estimation is usually preferred.

Hitherto, microwave assisted extraction processes have been widely studied at bench scale (Chemat and Cravotto, 2013). Next step is its implementation into industrial applications. To accomplish an optimal microwave applicator design, it is highly important to have an estimation of the medium’s permittivity. This knowledge will allow the characterization of the process (heating rate, penetration depth or temperature distribution among others), and will help to understand what happens during the process. For instance, from the knowledge of oven dimensions and sample dielectric properties, computing simulations may be performed to optimize the design of the microwave cavity in order to achieve a uniform electric field, which also entails a homogeneous temperature distribution (Dominguez-Tortajada et al., 2007). So, the formation of overheated regions or hot spots can be avoided (Zhu et al., 2012). There are different methods to achieve this uniformity, such as turning trays (Kopyt and Celuch-Marcysiak, 2003), multiple feeding and slotted waveguide feed cavities (Chan and Reader, 2000) or mode stirrers (Plaza-Gonzalez et al., 2004).

This work is focused on the measurement and calculation of dielectric properties of a multiphase mixture to be used in the design of a microwave cavity for the extraction of active compounds from a natural matrix. In this case, the extraction of polyphenols from grape pomace by an ethanol-water solvent, where microwaves have been attested to provide favourable intensification results (Casazza et al., 2010). It must be noticed that grape pomace presents a great variability due to the vintage and winemaking process, so a high accurate prediction is not feasible. Nonetheless, the argument proposed here can be applied to characterize any medium involving microwaves. The variables considered are temperature, solvent composition and solid-liquid ratio.

Table 1. Mixing rules and average deviations obtained for the mixed solvent and the multiphase mixture.

Mixing rules	Equations	ARD (%)			
		EtOH+H_2O		Grape pomace + EtOH+H_2O	
		ε'_{ARD}	ε''_{ARD}	ε'_{ARD}	ε''_{ARD}(*)
Böttcher-Bordewijk (a)	$X_1 \frac{3\varepsilon_1^*}{\varepsilon_1^* + 2\varepsilon^*} + X_2 \frac{3\varepsilon_2^*}{\varepsilon_2^* + 2\varepsilon^*} = 1$	9.5%	13.7%	23.0%	25.7%
Bruggeman (b)	$X_1 \frac{\varepsilon_1^* - \varepsilon^*}{\varepsilon_1^* + 2\varepsilon^*} + X_2 \frac{\varepsilon_2^* - \varepsilon^*}{\varepsilon_2^* + 2\varepsilon^*} = 0$	9.5%	13.7%	23.0%	25.7%
Ideal mixture (c)	$\varepsilon^* = X_1\varepsilon_1^* + X_2\varepsilon_2^*$	12.3%	19.0%	24.1%	24.8%
Kraszewski (d)	$\varepsilon^{*1/2} = X_1\varepsilon_1^{*1/2} + X_2\varepsilon_2^{*1/2}$	9.4%	9.6%	23.3%	25.5%
Lichtenecker-Rother (e)	$\varepsilon^* = \varepsilon_1^{*X_1} + \varepsilon_1^{*X_2}$	9.3%	24.7%	22.5%	26.1%
Looyenga (d)	$\varepsilon^{*1/3} = X_1\varepsilon_1^{*1/3} + X_2\varepsilon_2^{*1/3}$	9.4%	12.7%	23.0%	25.7%
Maxwell Garnet (b)	$\varepsilon^* = \varepsilon_1^* + 3X_2\varepsilon_2^* \frac{\varepsilon_1^* - \varepsilon_2^*}{\varepsilon_1^* + 2\varepsilon_2^* - X_2(\varepsilon_1^* - \varepsilon_2^*)}$	100.6%	70.4%	17.6%	25.4%
Peon-Iglesias (c)	$\varepsilon^* = (X_1\varepsilon_1^* + X_2\varepsilon_2^*)\left[1 - \frac{2}{3}\ln\left(\frac{1 + X_1(\varepsilon_1^*/\varepsilon_2^* - 1)}{(\varepsilon_1^*/\varepsilon_2^*)^{X_1}}\right)\right]$	9.4%	16.6%	23.0%	25.7%
Power law (b) (1)α=0.442; (2)α=-16.451	$\varepsilon^{*^\alpha} = X_1\varepsilon_1^{*^\alpha} + X_2\varepsilon_2^{*^\alpha}$	9.3%(1)	10.4%(1)	15.7%(2)	25.9%(2)

(a) (Liu et al., 2009); (b) (Sihvola, 2013); (c) (Amooey, 2013); (d) (Sólyom et al., 2013); (e) (Gilani et al., 2012).

(*) ε'' includes conductivity losses.

2. MATERIALS AND METHOD

2.1. MATERIALS

Red wine grape pomace from *Tempranillos* Grape was kindly provided by Matarromera winery in November 2013. It consisted of a mixture of grape seeds, skins and some stems, obtained after wine fermentation. In order to preserve its antioxidant activity, potassium metabisulfite (0.1 wt.%) was added, and samples were storage at -18ºC. Samples were thawed at 4ºC overnight, before being used. Table 2 gathers the moisture characterization of the raw material. A thermogravimetric analysis was employed to determine grape pomace moisture (105ºC during 24 hours).

Table 2. Grape pomace characterization

	Constituents (% w/w)	Moisture (% w/w)
Pomace	-	60±1%
Seeds	42±5%	51±1%
Skin	58±5%	66±1%

A mixture of ethanol and acidified water was used as solvent for the extraction process so as to use the same mixed solvent that has been previously reported for this process (Sólyom et al., 2014). Degasified water was acidified to pH=1 with sulphuric acid (96% v/v, Panreac) and then mixed with ethanol (96% v/v, Panreac). The studied ethanol concentrations were 25, 50 and 75% (v/v). The conductivity of the sulphuric electrolytes was dismissed (only 3 mL of sulphuric acid were added to 1 L of water). Their contribution to final conductivity, in comparison with the effect produced by the ions released by the grape pomace, is negible.

2.2. DIELECTIC PROPERTIES MEASUREMENT

A dielectric kit for vials, developed by the Microwave Division of the ITACA Institute (Universitat Politècnica de València, Spain), was used to determine the permittivity of the solvent-grape pomace mixture. The basis of this unit is the shift of resonant frequency and the decrease of the quality factor when a dielectric material is placed in the resonant cavity (Sheen, 2009). The frequency used was around 2.45 GHz. The coaxial resonant structure of the dielectric kit allows the introduction of a standard 8 mL polypropylene vial (12 mm diameter and 75 mm length) whereas the equipment dimensions are 190x230x85 mm. Even though the dielectric kit does not require a previous calibration but leaving the resonant structure without vial, this equipment is highly convenient and provides accurate measurements: 1% error in dielectric constant and 2-5% in loss factor determination.

Three solid-liquid ratios were analysed: 0.50, 0.75 and 1.00 g/mL. These concentrations correspond to volume fractions of 0.35, 0.44 and 0.51% (v/v). First, the proper amount of pomace was weighted in a vial. The corresponding solvent volume was then poured into the vial. After homogenization, samples were heated to the target temperature within the range 20-70º C by microwave radiation. A maximum temperature of 70ºC was selected in order to avoid solvent composition changes (a crucial parameter for the measurement's correlation), because of solvent losses. Temperature was recorded by an optic fibre with a diameter of 0.120 mm (TempSens, Opsens and OTG-A fibers). The advantage of using microwave energy instead of conventional heating methods is to achieve a similar medium to the one created during a microwave assisted extraction. Yedhu Krishnan and Rajan (2016) have demonstrated that microwave radiation causes a greater structure disruption in the raw material than the conventional extraction does. Thus, mass transfer limitations are different in each case. If the extracts composition is not the same (active substances, fibres, sugars, proteins, salts…), the dielectric behaviour may not be equivalent.

Once the vial was prepared, it was placed inside the dielectric kit and permittivity was measured. Temperature was continuously monitored during the dielectric characterization by a TempSens optical fibre. The temperature probe was immersed the same depth into the upper volume of the sample during the measurements, in order to correlate the dielectric and temperature measurements. Both temperature and relative permittivity values were synchronously recorded. Matlab software was used to process the temperature dependent measurements.

Optic fibre position in the cross sectional plane was not possible to be controlled. Since the insertion of the temperature probe during the dielectric properties measurement might distort the procedure, several measurements with and without the optical fibre probe were carried out and negligible differences were observed due to its presence. The differences detected were within the manufacturer uncertainty in all cases. This might be due to the small volume of the optical fibre introduced in the sample and the big losses of the measured samples that do not allow the electric field to interact with the optical fibre at the upper part of the samples.

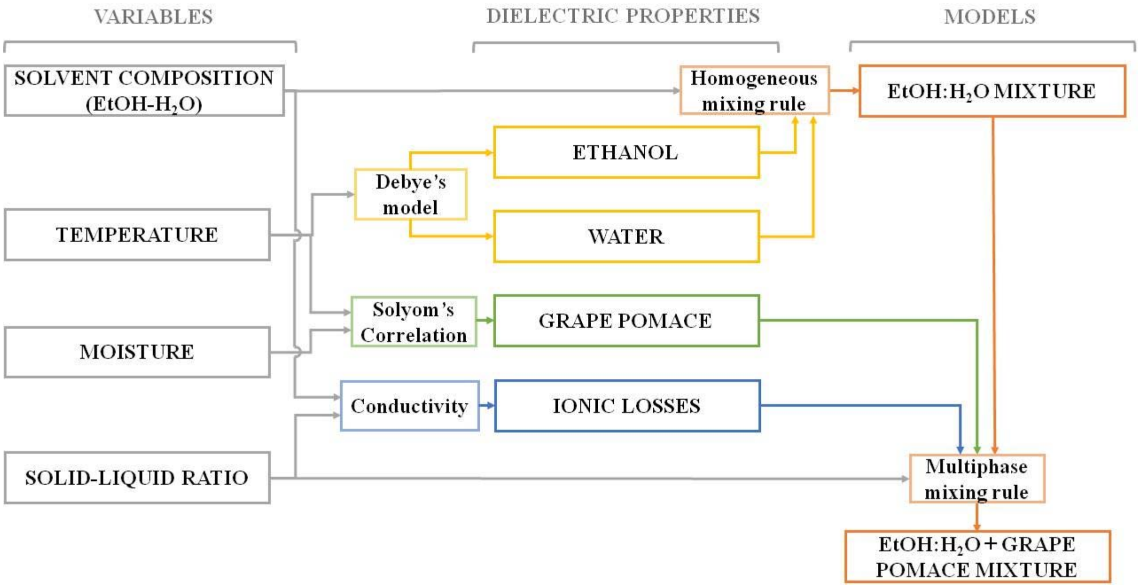

Figure 1. Calculation sequence of the multiphase mixture of ethanol-water-grape pomace.

3. DIELECTRIC PROPERTIES CALCULATION

Apart from measuring the dielectric properties of the multiphase grape pomace-solvent mixtures, the final aim of this work is to obtain a model capable of calculating their permittivities in order to be used in electromagnetic simulations to accomplish an optimal microwave applicator design. A model based on the mixture components and the system variables is required for this purpose. These variables are temperature, solvent composition and solid-liquid ratio. The multiphase mixture studied consists of a natural matrix (grape pomace) and a mixed solvent (a mixture of ethanol and water). Bibliographic data have been used to evaluate pure components and grape pomace dielectric properties as a function of temperature and moisture content. In this work, only the dielectric properties of the multiphase mixture of grape pomace and solvent have been measured. The model calculates the dielectric properties of the mixture using different mixing rules (Table 1). These mixing equations consist in the combination of the individual constituents' contributions on a volume-weighted average. The multiphase permittivities are correlated using the experimental values here reported. Figure 1 shows the calculation sequence followed.

Pure ethanol and water permittivities were computed with Debye's law. The corresponding parameters (ε_S, ε_∞, and τ) were adjusted to temperature functions, using literature values. ε_S and ε_∞ were fitted to linear models (eq. [6] and [7]). In the case of τ, an Erying equation type (eq.[8]) was preferred. The adjustable parameters of this latter expression were a pre-exponential factor (τ_0) and an activation energy (E). Regression coefficients for all equations (a, b, c, d, τ_0, E) were obtained by minimizing the absolute deviations between experimental and calculated values.

$$\varepsilon_S = a \cdot T + b \tag{6}$$

$$\varepsilon_\infty = c \cdot T + d \tag{7}$$

$$\tau = \tau_0 \exp\left(E/T\right) \tag{8}$$

Grape pomace's dielectric constant and loss factor were calculated by the correlation given by Solyom et. al (2013), as a function of temperature and original moisture content. Equations are reported in the final appendix. It must be added that grape pomace contains a low amount of residual ethanol from the fermentation (usually less than 1.5%). However, this contribution has been dismissed due to the small fraction that it represents in the multiphase mixture.

Mixed solvent (ethanol and water) and multiphase mixture (grape pomace and ethanol-water solvent) dielectric properties were computed using different mixing rules. Table 1 shows the different mixing equations tested in this work.

The contribution of ionic conductivity losses to the loss factor (eq.[5]) was only taken into account in the multiphase mixture, when the grape and the solvent are placed in contact. Electrical conductivity (σ) was measured in a Crison Basic 30 Conductivimeter. Experimental values were correlated as a function of solvent concentration (X_{EtOH}) and solid-liquid ratio (X_{Pomace}) by means of a quadratic regression model (eq [9]).

$$\sigma = 4131 - 8611 \cdot X_{EtOH} - 3370 \cdot X_{Pomace} + 2939 \cdot X_{EtOH}^{\;2} + 4625 \cdot X_{EtOH} \cdot X_{Pomace} \tag{9}$$

The different mixing rules used in the correlation were assessed in terms of the resulting average relative deviation, ARD (eq.[10]), between calculated (ε_i^{cal}) and literature or experimental values (ε_i^{exp}), for solvent and multiphase mixtures, respectively.

$$ARD = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{\varepsilon_i^{\exp} - \varepsilon_i^{cal}}{\varepsilon_i^{\exp}}\right| \tag{10}$$

4. RESULTS AND DISCUSSION

4.1. CORRELATION OF PURE WATER PERMITTIVITY

Debye's water parameters ε_S, ε_∞ and τ were adjusted to temperature regressions according to equations [6], [7] and [8]. ε_S and ε_∞ experimental data was obtained from Buchner et al.(1999), Kaatze (1989), Meissner and Wentz (2004), Nelson and Travelsi (2012), and Petong et al. (2000). Equations [11] and [12] were obtained for ε_S and ε_∞, with a mean relative error of 0.4% and 6.2%, respectively. In the case of relaxation time, Meissner and Wentz (2004) values were fitted to the exponential curve shown in equation [13]. Relaxation time can be calculated with this relation with a 6.8% mean deviation error.

$$\varepsilon_S = -0.35 \cdot T + 183.68 \quad [11]$$

$$\varepsilon_\infty = -0.03 \cdot T + 14.40 \quad [12]$$

$$\tau = 1.61 \cdot 10^{-3} \exp(2470/T) \quad [13]$$

Once the temperature relations have been established, it is possible to complete Debye's equation [1], and compute the water permittivity values as a function of temperature. Figure 2 compares the calculated permittivities of water with experimental values reported in other works (Dean, 1985; Franco et al., 2015; Goodwin, 2000; Hui et al., 2006; Kaatze, 1989; Liao et al., 2001; Malmberg and Maryot, 1956; Meissner and Wentz, 2004). The relations propose to calculate water permittivity allow to accurately predict its dielectric constant (ARD=1.6%), whereas larger errors were found for loss factor (ARD=17.0%), due to divergences in literature experimental values.

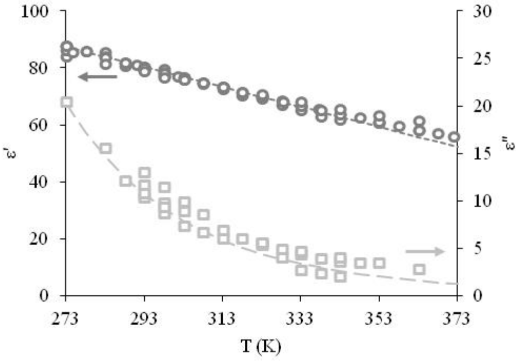

Figure 2. Experimental and calculated water permittivity as a function of temperature at a frequency of 2450 MHz. ○ Experimental ε'; □ Experimental ε''; - - - - Calculated ε'; — — — Calculated ε''.

4.2. CORRELATION OF PURE ETHANOL PERMITTIVITY

In the case of ethanol, less data is available. Indeed, great discrepancies were found between different literature data sets. Figure 3 shows this discrepancy. In Figure 3a, dielectric constant presents two opposite trends. Some authors report an increasing behaviour of dielectric constant with temperature, while others a decreasing one. This is also held for loss factor (Figure 3b). Authors that claim a similar dielectric behaviour for water and ethanol used resonant cavity (Henke et al., 2010; Petong et al., 2000) and time-domain measuring techniques (DDBST, 2014). The opposite behaviour was obtained with coaxial probes (Campos et al., 2014; Muley and Boldor, 2013) and admittance cells (Gregory and Clarke, 2012). These latter methods do not provide so accurate measurements. Coaxial probes and admittance cells are employed to measure dielectric constants over the entire frequency spectra. Furthermore, they are not recommended for low-loss factor materials (Venkatesh and Raghavan, 2005). Resonant cavity and time-domain reflectometry methods measure at one single frequency and they present a larger precision. This makes these latter techniques more reliable to obtain reference data (Clarke et al., 2003). This reasoning supports the election of values measured with resonant cavity and time-domain measurements as more precise. In consequence, Petong et al. (2000), Henke et al. (2010), and DDBST (2014) data sets were chosen here for establishing reference relations. It is also important to take into account that these data sets temperature dependence is in accordance with the most accepted theory to describe temperature influence (Franco et al., 2015). Dielectric constant decrease with temperature is explained by a viscosity reduction due to the temperature increase that favours the alignment of the molecules in the electric field. In addition, the model so obtained for pure ethanol by correlation of these experimental sets, when used together with water's model and proper mixing rules, also provides a better estimation for permittivity values of water-ethanol mixtures, as reported below, in section 4.3.

Pure ethanol parameters for Debye's equation were obtained by correlating ε_S, ε_∞ and τ experimental values versus temperature (Khirade et al., 1999; Lone et al., 2008; Petong et al., 2000; Shirke et al., 2001). Equations [14], [15], and [16] show these relations. Fitting deviations for ethanol's Debye parameters ε_S, ε_∞ and τ were 1.3%, 1.2% and 11.9%, when compared to reported values.

$$\varepsilon_S = -0.15 \cdot T + 69.50 \quad [14]$$

$$\varepsilon_\infty = -6.50 \cdot 10^{-3} \cdot T + 5.30 \quad [15]$$

$$\tau = 3.34 \cdot 10^{-2} \exp\left(2494/T\right) \quad [16]$$

Ethanol dielectric properties calculated with Debye's model using the previous relationships were compared to the selected experimental data sets (DDBST, 2014; Henke et al., 2010; Petong et al., 2000). The average relative deviations between experimental values and Debye's calculation were 8.3% for *ε'* and 4.6% for *ε"*. This correlation is represented by the dashed line in Figures 3a and 3b.

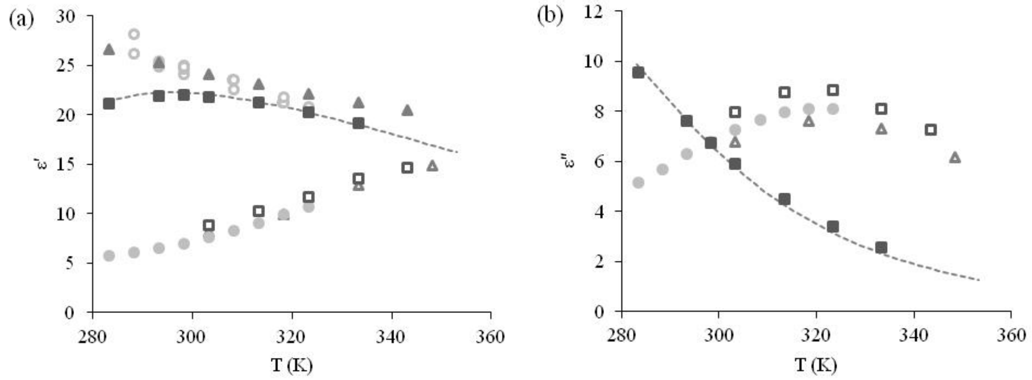

Figure 3. Calculated and experimental values for pure ethanol (a) dielectric constant and (b) loss factor, at a frequency of 2450 MHz. △ Campos et al., 2014; ○ DDBST, 2014; ● Gregory and Clarke, 2012; ▲ Henke et al., 2010; □ Muley and Boldor, 2013; ■ Petong et al., 2000; ---- Calculated permittivity.

4.3. ETHANOL-WATER MIXTURE PERMITTIVITY

Microwave extractions usually employ mixed solvents in order to combine the properties of interest to reach the highest yield. In most cases, this combination consists of water and an organic solvent. Water provides high dielectric properties, while the organic solvent assures the solubilisation of the extracted compounds. In this section, the dielectric behaviour of ethanol-water mixtures is studied. An analysis of different mixing equations is proposed. The goodness of the prediction is assessed in terms of the mean average deviation between bibliographic and calculated data. Ethanol-water experimental permittivities were obtained from Akerlöf's (1932) and Petong's (2000) works.

Prediction results are presented in Table 1. The Maxwell-Garnett's equation provides the worst prediction. This large error is due to the fact that this equation is used for the prediction of the dielectric properties of a two-phase mixture (Sihvola, 2013). The errors obtained with other mixing rules are much lower (9.3-12.3% for *ε'* and 9.6-24.7% for *ε"*). It has been found that the ethanol-water mixture follows a simple relation, and a rough characterization gives the same accuracy than more complex relations, which are more aware of structural inhomogeneities (like Böttcher-Bordewijk, Bruggeman or Peon-Iglesias models). Moreover, power laws (exponential modifications over ideal equation) provide the best fittings. These equations are Kraszewski (α=0.50), power law with adjustable exponent (α=0.44) and Looyenga (α=1/3) equations. Dielectric constant deviations are approximately the same for most mixing rules, but loss factor errors with power equations are the smallest ones. Compared to Kraszewski, the optimization of the exponent to α=0.44 provides a slightly lower error for *ε'* (9.3% vs. 9.4%). However, loss factor deviation is almost a point higher (10.4% vs. 9.6%). Because of this, and in order to reduce the number of adjustable parameters in the model, Kraszewski mixing equation was selected to predict ethanol-water permittivity from solvent composition and pure constituent's permittivity.

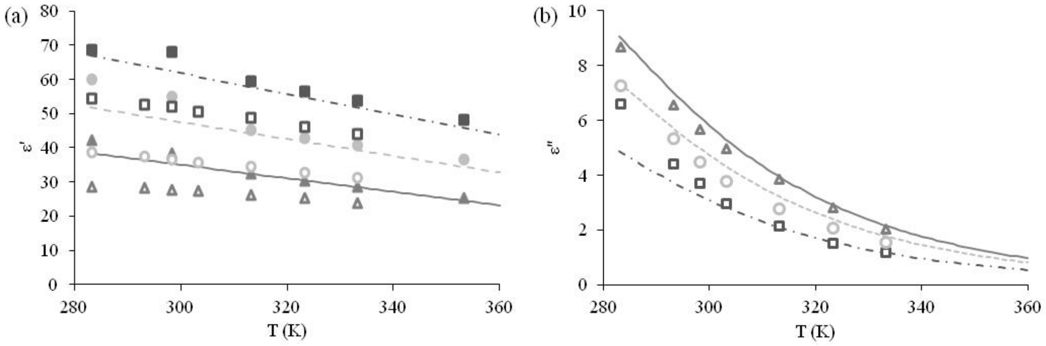

Figure 4. Ethanol-water mixture complex permittivity at 2450 MHz obtained by Kraszewski's mixing rule: (a) dielectric constant, (b) loss factor. ■ $X_{\mathrm{EtOH,Akerlöf}}=0.25$;● $X_{\mathrm{EtOH,Akerlöf}}=0.50$; ▲ $X_{\mathrm{EtOH,Akerlöf}}=0.75$; □ $X_{\mathrm{EtOH,Petong}}=0.25$; ○ $X_{\mathrm{EtOH,Petong}}=0.50$; △ $X_{\mathrm{EtOH,Petong}}=0.75$; —·— $X_{\mathrm{EtOH,Calculated}}=0.25$; - - - - $X_{\mathrm{EtOH,Calculated}}=0.50$; —— $X_{\mathrm{EtOH,Calculated}}=0.75$.

Figure 4 shows experimental and calculated permittivities. For an ease comparison, Akerlöf values have been interpolated to the studied solvent concentrations. Average deviations for dielectric constant and loss factor are 9.4% and 9.6%, respectively.

In summary, once Debye's equation is known for pure ethanol and water at different temperatures, it is possible to compute the dielectric behaviour of the mixtures under the same conditions by applying a simple square root mixing rule. In this way, ethanol-water mixtures are estimated with an error lower than 10%.

4.4. ETHANOL-WATER AND GRAPE POMACE MIXTURE PERMITTIVITTY

A multiphase solid-liquid media is characterized in this section. The formerly ethanol-water mixture is employed as the solvent, and grape pomace as the solid phase. Dielectric behaviour of grape pomace has been determined by Sólyom et al. (2013) by means of a temperature and moisture function. The permittivities of the multiphase solid-liquid samples were calculated using mixing rules with the solvent and solid matrix permittivity values.

Moreover, an additional term was also required to account for ionic contribution to permittivity (eq. [5]). Conductivity measurements showed that a significant amount of ions were released from the solid matrix during microwave extraction. Ionic losses contribution requires a model that includes this phenomenon. Conductivity (*σ*) was measured for each initial condition (Table 3). Measurements varied between 127 µS/cm and 2.33 mS/cm, depending on solvent concentration and solid-liquid ratio (eq.[9]). A high correlation coefficient relates calculated and measured conductivities (R=0.994).

Ionic losses were computed and added to the resulting solid-liquid mixture loss factor term of the model obtained by the corresponding mixing rule. The dielectric constant is not changed by the presence of ions, but loss factor is. Ionic losses must be added to the mixing rule loss factor as an additional term, as the one shown in equation [17], in view of the fact that they cannot be assigned to any other component's contribution. Although ions come from the grape pomace, Solyom's correlation is not able to compute grape pomace conductivity losses since electrolytes are bounded to the structure and cannot interfere with the electromagnetic field. However, in the multiphase mixture ions are released to the solvent and so, they are able to interact with the field. The result is the appearance of the conductivity losses. For this multiphase system, ionic losses represent a significant fraction (9-83%) of the total loss factor term.

Table 1 gathers the results from multiphase mixing rules (*ε"* including ionic losses). Deviations were quite uniform: about 22% for *ε'* and 26% for *ε"*. Maxwell Garnet equation provides the best results for the complex permittivity, *ε** (17.6% and 25.4%), while power law and ideal mixture equations predict each complex permittivity component, *ε'* and *ε"*, with a smaller error (15.7% for *ε'* and 24.8% for *ε"*). The analysis of the results obtained with each expression shows that temperature was the most dominant factor, and in consequence, the influence of the other variables was concealed. Modifications to these equations were studied in order to improve composition contribution. Power law was modified to obtain equation [17].

$$\varepsilon_{Mix}^{*} = \left[\left(X_{Pomace} \cdot \varepsilon_{Pomace}^{*} \right)^{1.425} + \left(X_{Solvent} \cdot \varepsilon_{Solvent}^{*} \right)^{1.425} \right]^{1/1.425} + \frac{\sigma}{j\omega\varepsilon_0} \qquad [17]$$

By introducing the volumetric fraction under the power exponent, the solid-liquid ratio presents a higher influence on the outcome, as required by the experimental evidence. The exponent of the power was also optimized to a value of 1.425. The deviations obtained by this new expression were 8.1% and 24.8% for ε' and ε", respectively. A considerable reduction in the dielectric constant error was achieved. For the loss factor error, no additional reduction was obtained. This apparently high error (24.8%) is however considered acceptable for cavity modelling purposes, as the loss factor value shows a very large variation range (5.41-15.71). The full range of experimental values (ε’=27.72-47.10 and ε’’=5.41-15.71) reasonably agrees with that of the calculated ones (ε’=28.57-49.54 and ε’’=3.37-13.03). Experimental and calculated values are reported in Table 3.

Table 3. Experimental and calculated (eq [17]) permittivity values for the multiphase solvent-grape pomace mixture at 2450 MHz.

Conditions				Experimental		Calculated		ARD	
T (K)	X_{EtOH} (v/v)	X_{Pomace} (v/v)	σ (S/m)	ε'_{Exp}	ε''_{Exp}	ε'_{Model}	ε''_{Model}	ε'_{ARD}	ε''_{ARD}
293	25%	0.35	0.2010	46.87±0.02	12.23±0.23	49.54	13.03	6%	7%
343	25%	0.35	0.2330	43.82±0.35	9.57±0.04	41.22	12.88	6%	35%
323	50%	0.35	0.0976	42.25±0.08	8.48±0.08	36.62	7.47	13%	12%
293	75%	0.35	0.0127	27.72±0.25	13.47±0.13	33.26	6.35	20%	53%
343	75%	0.35	0.0136	31.07±0.22	5.41±0.14	28.57	3.37	8%	38%
323	25%	0.44	0.1912	47.10±0.11	9.37±0.03	43.04	11.67	9%	25%
293	50%	0.44	0.0862	35.73±0.31	15.71±0.43	39.97	9.01	12%	43%
323	50%	0.44	0.0919	38.19±0.05	7.48±0.06	36.86	7.58	3%	1%
343	50%	0.44	0.0865	33.70±0.44	6.43±0.11	35.25	7.03	5%	9%
323	75%	0.44	0.0156	31.42±0.08	7.09±0.12	31.81	4.35	1%	39%
293	25%	0.51	0.1714	46.87±0.04	11.67±0.28	45.72	12.15	2%	4%
343	25%	0.51	0.1892	40.22±0.45	8.51±0.07	40.87	11.92	2%	40%
323	50%	0.51	0.0674	39.70±0.14	6.89±0.08	37.42	6.76	6%	2%
293	75%	0.51	0.0230	28.86±0.19	12.68±0.13	34.95	6.66	21%	47%
342	75%	0.51	0.0227	30.62±0.11	5.72±0.05	32.81	4.71	7%	18%

Once the model has been developed, it is possible to assess the influence of model variables. Table 4 shows how permittivity changes with these variables in the studied ranges. Solvent concentration is the most influential factor, as it makes dielectric constant to vary between 19-32%, and loss factor between 45-75%. Solvent concentration is directly related with moisture, a crucial variable whose effects are clearly shown here. It must be also taken into account that as water concentration increases, also electrolyte release does. This explains the great variation in loss factor. Temperature dependence is also considerable. Temperature increments between 293 and 343 K make dielectric constant to decrease a 6-17%, and loss factor a 3-42%. Solid-liquid ratio presents the lowest influence, just 2-12% for *ε'* and 8-25% for *ε"*. However, loss factor dependence on solid-liquid ratio is greater: the solid matrix contributes to increase ionic losses by the release of electrolytes, but this effect is characterized and accounted in the solvent influence.

Table 4. Temperature, composition and solid-liquid ratio influence on complex permittivity of multiphase solvent-grape pomace mixtures.

Variable	Range	Influence	
		ε'	ε"
T	293-343 K	6-17%	3-42%
X_{EtOH}	25-75% (v/v)	19-32%	45-75%
X_{Pomace}	0.35-0.51 (v/v)	2-12%	8-25%

5. CONCLUSIONS

Complex permittivity of a multiphase mixture (solid grape pomace and ethanol-water solvent) has been characterized as a function of temperature (293-343 K), solvent composition (25-75%) and solid-liquid ratio (0.50-1.00 g/mL). Experimental values were measured, and used to develop a model to calculate the complex permittivity of the multiphase mixture from pure component parameters.

Pure solvents permittivities, ethanol and water, were computed by Debye's equation with temperature dependent parameters. Static and low-frequency permittivities were fitted to linear temperature equations, while relaxation times were adjusted to exponential curves. Results were compared to experimental values. In the case of ethanol, a large discrepancy was found between bibliographic data sets, showing opposite temperature trends. These discrepancies arise from the use of different measurement techniques. Experimental values measured with resonant cavity and time-domain measurements were considered more reliable, and used for pure ethanol parameters correlation. Ethanol dielectric properties were calculated using Debye's law with an accuracy of 8.3% for the dielectric constant, and 4.6% for the loss factor. Water's permittivity errors were 1.6% and 17.0%.

Ethanol-water mixture dielectric properties were predicted from pure component values with a simple mixing rule. A square root relation (Kraszewski model) can describe the behaviour of the mixture at different temperatures and compositions with an average deviations of 9.4% and 9.6% for each component of the complex permittivity.

When developing the final stage of the model for the multiphase mixture, it was highly necessary to include the conductivity losses. Electrolytes were released from the solid matrix during the heating process, and they contributed to boost the loss factor (between 9-83%). Conductivity losses were added to the resulting multiphase mixing rule as an additional term, as they contribute to the mixture but cannot be attributed to any of the mixture components. The use of the same mixing rules previously employed for the mixed solvents led to larger errors in the calculation of the permittivity than in that case. This was due to the large temperature influence that hid composition dependence. To override this problem, the standard power law mixing rule was modified in order to obtain a higher dependence on composition. This was done by including the solid-liquid ratio under the power exponent. Lower deviations were so obtained (8.1% and 24.8%).

The relative influence of the variables on the permittivity of the multiphase mixtures was assessed with the developed model. In the range of operation, the solvent concentration (directly related with water content) was the most influential parameter. Nonetheless, solid-liquid ratio had to be taken into special consideration, since it was responsible for the release of ions that boost ionic conductivity, and therefore loss factor.

It is worth highlighting that, despite the numerous parameters used to characterize pure constituents, only one adjustable parameter has been used to correlate the multiphase mixture. The final error is quite significant (8.1% for ε' and 24.8% for ε''). However, it is acceptable taking into account that an inhomogeneous natural material is used, and that the permittivity variation in the analysed range is large (the dielectric constant value almost doubles, 27.72-47.10 and the loss factor triples, 5.41-15.71). This permittivity calculation method may be useful to develop an effective microwave cavity design, which leads to an efficient microwave assisted extraction process of active compounds from grape pomace at industrial scale.

ACKNOWLEDGEMENTS

The authors thank the European project WineSense (FP7-MC-IAPP) and regional government project EDU/592/2013 Ref: VA330U13 for the funding. A.A. thanks the Spanish Ministry of Education for her FPU fellowship (FPU13/04678).

NOMENCLATURE

f	Frequency (Hz)
Mc	Grape pomace moisture content ($g·g^{-1}$)
T	Temperature (K)
X_{EtOH}	Ethanol concentration in the mixed solvent ($m^3·m^{-3}$)
X_{H2O}	Water concentration in the mixed solvent ($m^3·m^{-3}$)
X_{Pomace}	Grape pomace fraction in the multiphase mixture ($m^3·m^{-3}$)
$X_{Solvent}$	Mixed solvent fraction in the multiphase mixture ($m^3·m^{-3}$)
ε*	Complex dielectric permittivity ($F·m^{-1}$)/ ($F·m^{-1}$)
ε’	Dielectric constant ($F·m^{-1}$)/ ($F·m^{-1}$)
ε”	Loss factor ($F·m^{-1}$)/ ($F·m^{-1}$)
$ε_0$	Free space permittivity ($F·m^{-1}$)
$ε_S$	Static permittivity ($F·m^{-1}$)/ ($F·m^{-1}$)
$ε_\infty$	High-frequency permittivity ($F·m^{-1}$)/ ($F·m^{-1}$)
$ε_σ$	Ionic losses ($F·m^{-1}$)/ ($F·m^{-1}$)
σ	Electrical conductivity ($S·m^{-1}$)
τ	Relaxation time (s)

APPENDIX: Summary of the equations used in the dielectric properties calculations

Pure solvents

Debye’s model

$$\varepsilon^* = \varepsilon' - j\varepsilon'' = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + j2\pi f\tau} \tag{A.1}$$

Water Debye’s parameters

$$\varepsilon_{S,H_2O} = -0.35 \cdot T + 183.68 \tag{A.2}$$

$$\varepsilon_{\infty,H_2O} = -0.03 \cdot T + 14.40 \tag{A.3}$$

$$\tau_{H_2O} = 1.61 \cdot 10^{-3} \exp(2470/T) \tag{A.4}$$

Ethanol Debye’s parameters

$$\varepsilon_{S,EtOH} = -0.15 \cdot T + 69.50 \tag{A.5}$$

$$\varepsilon_{\infty,EtOH} = -6.50 \cdot 10^{-3} \cdot T + 5.30 \quad \text{(A.6)}$$

$$\tau_{EtOH} = 3.34 \cdot 10^{-2} \exp\left(2494/T\right) \quad \text{(A.7)}$$

Mixed solvent permittivity

$$\varepsilon^{*\,1/2}_{Solvent} = X_{EtOH}\,\varepsilon^{*\,1/2}_{EtOH} + X_{H_2O}\,\varepsilon^{*\,1/2}_{H_2O} \quad \text{(A.8)}$$

Grape pomace

Solyom’s correlation

$$\varepsilon'_{Pomace} = 231.80 - 0.108 \cdot T - 9.596 \cdot Mc + 0.107 \cdot Mc^2 \quad \text{(A.9)}$$

$$\varepsilon''_{Pomace} = 7.79 - 0.361 \cdot Mc + 0.006 \cdot Mc^2 \quad \text{(A.10)}$$

Multiphase ethanol-water-grape pomace mixture

Conductivity

$$\sigma = 4131 - 8611 \cdot X_{EtOH} - 3370 \cdot X_{Pomace} + 2939 \cdot X_{EtOH}^{2} + 4625 \cdot X_{EtOH} \cdot X_{Pomace} \quad \text{(A.11)}$$

Mixture equation

$$\varepsilon^{*}_{Mix} = \left[\left(X_{Pomace} \cdot \varepsilon^{*}_{Pomace}\right)^{1.425} + \left(X_{Solvent} \cdot \varepsilon^{*}_{Solvent}\right)^{1.425}\right]^{1/1.425} + \frac{\sigma}{j2\pi f \varepsilon_0} \quad \text{(A.12)}$$

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CHAPTER 3

Development of a continuous microwave pretreatment pilot plant for the extraction of active compounds

DEVELOPMENT OF A CONTINUOUS MICROWAVE PRETREATMENT PILOT PLANT FOR THE EXTRACTION OF ACTIVE COMPOUNDS

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ABSTRACT

Microwave industrial implementation is deterred by the limitations of this technology. The difficulty to scale up a microwave process from laboratory to a larger scale is one of the main hindrances to overcome. A microwave pretreatment has been proposed as an easily scalable alternative, since it allows the uniform irradiation of the material in a feasible configuration. To fulfil this purpose, a microwave oven has been designed, simulated and constructed to operate at a pilot plant scale. A quasi-cylindrical applicator has been developed to treat 19.5 kg/h of extraction media so that 324 J/mL are absorbed in 90 seconds, achieving a maximum temperature increment of 100ºC.The effectiveness of the pilot plant was validated by comparing the results with those from a laboratory scale extraction of polyphenols from grape pomace.

KEY WORDS

Microwave pretratment, scale-up, industrial implementation, continuous pilot plant, quasy-cylindrical applicator.

HIGHLIGHTS

- » Construction of a microwave pretreatment pilot plant
- » Design and simulation of a quasi-cylindrical microwave applicator
- » Uniform temperature distribution achieved
- » Scan of the different impulsion alternatives available
- » Pilot plant effectiveness verified with the extraction of polyphenols from grape pomace

1. INTRODUCTION

The advantages of microwave application in extraction processes (usually known as MAE, microwave assisted extraction) have been widely demonstrated (Vinatoru et al., 2017). Compared to the conventional solid-liquid extraction, MAE processes are able to boost yield, while reducing the extraction time from hours to minutes (Llompart et al., 2018). However, the vast majority of published works deal with laboratory scale conditions that are not feasible at industrial scale (Chemat and Cravotto, 2013). For instance, the use of very low solid-liquid ratios or still too long extraction times that entails bulky equipment. These make microwave breakthroughs difficult to come to fruition. A statement corroborated by the fact that a reduced number of industrial microwave trade companies dealing with extraction equipment from natural products have been found: Advance Microwave Technologies (http://www.advancedmicrowavetechnologies.com/), Sairem (https://www.sairem.com/) and Radient (http://radientinc.com/index.php). Nonetheless, if the limitations perceived by industry as implementation milestones are overcome (detailed in the survey provided by Watson (2012)), the spread of this technology will be approachable. Specifically, the main three limitations identified are the short penetration depth, the high cost of electrical energy, and the investment cost.

Regarding penetration depth, it must be taken into account that microwaves interact with the material irradiated, which can vary from opaque to transparent depending on the media permittivity (Chan and Reader, 2000). For example, if water is irradiated with microwaves at a frequency of 2.45 GHz, radiation will only have an effective penetration (up to the 37% of the power at the surface) of 2.32 cm, no matter the size of the volume irradiated. This means that, results from laboratory irradiation of a flask are not equivalent to the irradiation of a larger tank, since the same part (that 2.32 cm layer closer to the boundary) will be heated by the microwaves. Here a crucial factor to consider arises: the homogeneity of the radiation. There is no sense in investing in a large microwave cavity since it will not be able to uniformly treat the material as only the external parts would be affected. In the case of solid-liquid extraction processes, the alternative proposed here to enable an efficient treatment and improve yield, is to use a microwave pretreatment. It consists in a peak of microwave energy added before the conventional extraction where the extraction media temperature is rapidly increased. After the pretreatment, the media is cooled down to continue with the conventional solid-liquid extraction. Compared to a MAE process, the pretreatment presents a substantial reduction of the irradiation time: it only requires irradiation during times of the order of seconds-minutes. So, the treatment of the material can be attained in a size-reasonable microwave oven that also allows a homogeneous treatment. Therefore, the pretreatment can take place in a tubular reactor with a diameter low enough to ensure the homogeneous irradiation of the material. This novel configuration entails a reduction in the size of the equipment, the energy consumption and consequently also in the investment costs.

Additionally, in terms of electricity consumption, the cavity design will determine the energy absorption efficiency. Thus, an optimal oven design is paramount, since it will assure not only a high energy efficiency, but also a uniform temperature distribution, and so the formation of hot spots will be reduced (Dominguez-Tortajada et al., 2007, Zhu et al., 2012). Additionally, the oven can have auxiliary components such as turning trays, slotted feed cavities or mode stirrers to improve the homogeneity of the electromagnetic field (Chan and Reader, 2000, Kopyt and Celuch-Marcysiak, 2003, Dominguez-Tortajada et al., 2007).

In sum, this work aims to achieve an effective scaling up of a microwave pretreatment to intensify a solid-liquid extraction process. To achieve this objective, the optimization of the polyphenol extraction from grape pomace has been taken as a reference (Álvarez et al., 2017b). In addition, the dielectric properties of the extraction media are known (Álvarez et al., 2017a), what enables a specific oven design which will allow to optimize its efficiency.

2. DESIGN AND SIMULATION OF THE CONTINUOUS MICROWAVE OVEN

2.1. DESIGN BASIS

The optimal extraction conditions presented by Álvarez et al. (2017b) were selected for the oven design as they determined the operational conditions where microwave extraction showed the most promising results. The ranges of these parameters were:

i. A solvent composition close to 50% (v/v) ethanol:water.
ii. A solid-liquid ratio of around 0.75 g/mL.

iii. A mean absorbed energy of 0.40 kJ/mL. This high energy entailed a substantial temperature increment above the boiling point, so the system required to operate under pressure (4 bar, 120ºC) to maintain the solvent in the liquid state.
iv. A residence time of approximately 90 seconds.
v. A treatment capacity of 20 kg/h of the mixture grape pomace-solvent.

An energy balance was performed to estimate the oven requirements. First, interval boundaries were given to the specified process variables. Then, its influence on dependent process variables was analysed to finally select the most suitable oven specifications.

Microwave power, tube diameter, residence time and outlet temperature values were varied inside the following ranges. Oven power determines the global size of the equipment (and therefore its cost) and the processing capacity. Commercial magnetrons can be found to provide powers between 300 W to 30 kW (Muegge, 2018). However, considering the design basis of 20 kg/h, magnetron power was specidied around 3 kW. Thus, 2-3 kW was the power range analysed. Tube diameter was also taken as a critical variable, since it was crucial to assure a homogeneous irradiation of the material. That meant that it could not be larger than twice the penetration depth. Tube diameter values were studied between 0.020 and 0.040 m. Residence time and outlet temperature variation range were fixed to 45-120 s and 100-120ºC, respectively, accordingly to a previous work (Álvarez et al., 2017b).

The influence of the above variables on mass flow, velocity, length of the oven, power density and energy density was also studied. Mass flow was dependent on the microwave power (*P* in W) and the specified outlet temperature (T_{out}, K). It was calculated according to equation [1] and expressed in kg/h, assuming no solvent vaporization takes place during the pretreatment, since operation runs bellow the boiling point.

$$Mass\ flow = \frac{P \cdot \eta}{C_P(T_{out} - T_{in})} \qquad [1]$$

In the above expression, η is the power conversion efficiency (%), C_P the material specific heat (J/gK), and T_{in} the inlet temperature.

Power density (the average heat power absorbed by volume unit of material inside of the oven) was computed by equation [2], as a function of the material residence time (t_R) and the outlet temperature. It was expressed in kW/L.

$$Power\ density = \frac{P \cdot \eta}{V_{oven}} = \frac{\rho \cdot C_P(T_{out} - T_{in})}{t_R} \qquad [2]$$

On the other hand, the energy density (the total energy absorbed by volume unit of material passing through the oven, in J/mL) only depends on the outlet temperature, as it is shown in equation [3]. It must satisfy the optimal pretreatment value previously optimized in laboratory studies.

$$Energy\ density = \frac{P \cdot \eta}{Q} = \rho \cdot C_P(T_{out} - T_{in}) \quad [3]$$

The length of the tube inside the cavity was calculated with equation [4], in meters.

$$Length = \frac{4 \cdot t_R \cdot P \cdot \eta}{C_P(T_{out} - T_{in})\pi \cdot D^2 \cdot \rho} \quad [4]$$

And finally, the average velocity of the extraction material across the tube was computed as the quotient between the volumetric flow and the tube section (expression [5]). It was measured in cm/s.

$$Velocity = \frac{4 \cdot mass\ flow}{\pi \cdot D^2 \cdot \rho} \quad [5]$$

The meaning of all these variables as well as their units can be found in the nomenclature section.

It must be noticed out that, in all these calculations, the magnetron's consumed power is always followed by an efficiency conversion coefficient of electrical to microwave power, *η*, which has been assumed to be of 65%. A value that agrees with previous reported conversions (Wang et al., 2015).

Table 1 presents the analysis with the above defined variables. This data was used to select the most suitable oven specifications. Regarding oven consumed power, 3.0 kW was preferred over 2.0 kW since it provided mass flows closer to the design basis. A low diameter entailed a long (and hence a costly) equipment. However, 0.35 m was selected instead of 0.40 m to assure that, if materials with lower penetration depth were irradiated in a future, there would be a uniform energy distribution. And finally, a residence time of 90 seconds and an outlet temperature of 120ºC were chosen accordingly to initial conditions. The value of the dependant variables associated to this selection can also be found in Table 1. In sum, a mass flow of 19.5 kg/h can be treated in a 0.56x0.035 m tube during 90 seconds achieving an energy density of 324 J/mL.

Table 1. Analysis of the specified process variables influence on calculated process variables and selected values (denotated with *).

Specified process variables				Calculated process variables				
P (kW)	D (m)	t_R (s)	T_{out} (ºC)	Mass Flow (kg/h)	Power density (kW/L)	Energy density (J/mL)	Length (m)	Velocity (cm/s)
2.0	0.020	45	100	16.3	5.8	259	0.72	0.55
2.0	0.020	45	120	13.0	7.2	324	0.57	0.44
2.0	0.020	120	100	16.3	2.2	259	1.92	0.55
2.0	0.020	120	120	13.0	2.7	324	1.53	0.44
2.0	0.040	45	100	16.3	5.8	259	0.18	0.55
2.0	0.040	45	120	13.0	7.2	324	0.14	0.44
2.0	0.040	120	100	16.3	2.2	259	0.48	0.55
2.0	0.040	120	120	13.0	2.7	324	0.38	0.44
3.0	0.020	45	100	24.4	5.8	259	1.08	0.83
3.0	0.020	45	120	19.5	7.2	324	0.86	0.66
3.0	0.020	120	100	24.4	2.2	259	2.87	0.83
3.0	0.020	120	120	19.5	2.7	324	2.30	0.66
3.0	0.040	45	100	24.4	5.8	259	0.27	0.83
3.0	0.040	45	120	19.5	7.2	324	0.22	0.66
3.0	0.040	120	100	24.4	2.2	259	0.72	0.83
3.0	0.040	120	120	19.5	2.7	324	0.57	0.66
*3.0	*0.035	*90	*120	*19.5	*3.6	*324	*0.56	*0.63

Once the oven specifications were settled, the oven cavity was designed.

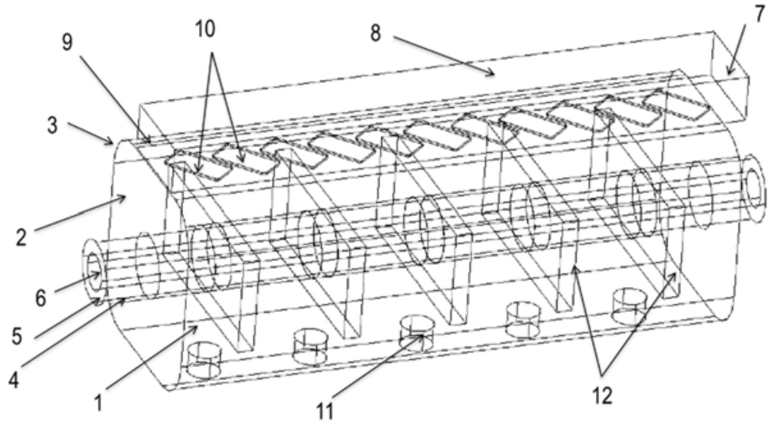
2.2. ALTERNATIVES AND PROCESS SELECTION

A single mode applicator is advisable for industrial applications since it provides a well-defined standing wave easy to be controlled (Thostenson and Chou, 1999). Regarding its shape, a cylindrical applicator is quite common and its use has been widely patented (for instance, some highlighted patens are: WO2011048349 (Zadyraka et al., 2011), MX2016006872 (Wilber and Shuping, 2016), JP2016091636 (Motoharu et al., 2016), US201213688465 (Milicevic et al., 2013)). However, in this work, a quasi-cylindrical applicator was selected instead because of its better advantages, which are detailed in the next section.

The design and simulation of the oven have been performed by Dr. J. Monzó Cabrera and Dr. J. Fayos Fernández, from the Universidad Politécnica de Cartagena (Spain).

2.3. DESCRIPTION

The quasi-cylindrical applicator proposed here consisted of an applicator with a rectangular section and circular edges. The combination of a circular and a rectangular section allows to have flat walls in the central parts of the section where slotted antennas can be included. This multi-feed cavity enables to achieve a greater efficiency and stability during the oven operation. The cavity is crossed by a Teflon (PTFE) pipe, where the extraction media is pumped through. A schematic diagram with its main parts can be found in Figure 1.



1) Microwave cavity.
2) Quasi-cylindrical section.
3) Rectangular edges substituted by curve lines.
4) Tube rail.
5) PTFE tube
6) Grape pomace and solvent to be irradiated
7) Waveguide port.
8) Waveguide.
9) Isolation port.
10) Slotted waveguide.
11) Dielectric tuners.
12) PTFE tube supports.

Figure 1. Main parts of the microwave oven

Multiple attempts have been carried out until a satisfactory design was reached. Table 2 gathers the final dimensions selected for the microwave oven parts detailed in Figure 2.

Table 2. Microwave cavity dimensions for Figure 2 expressed in millimetres.

t_x	t_{x2}	t_{x3}	t_{x4}	$desplaza_x$	$desplaza_y$	$R_{\_PTFE}$	r_{iris}
45	44	45	45	5	10	32	33

Guide width	d_1	d_{1_cav2}	d_{1_cav3}	d_{1_cav4}
32	216	247	155	212

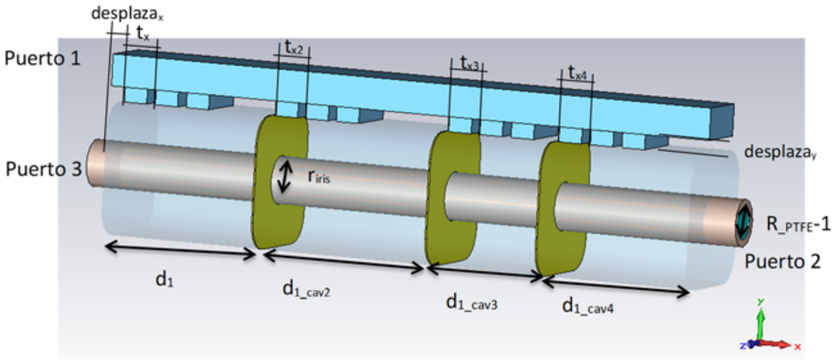


Figure 2. Microwave cavity specifications.

This cavity presented a reflection coefficient lower than -15 dB in the range of 2.45 to 2.49 GHz and -10 dB under reflections through the useful frequency range of the magnetron. Such absorption efficiency and radiation reflection were checked for a wide range of permittivities and loss factors. In particular, dielectric constants between 28 and 54, and loss factors between 4 and 17 were analysed. These results can be observed in Figure 3. Therefore, an efficiency above the 90% can be expected, independently of the material irradiated.

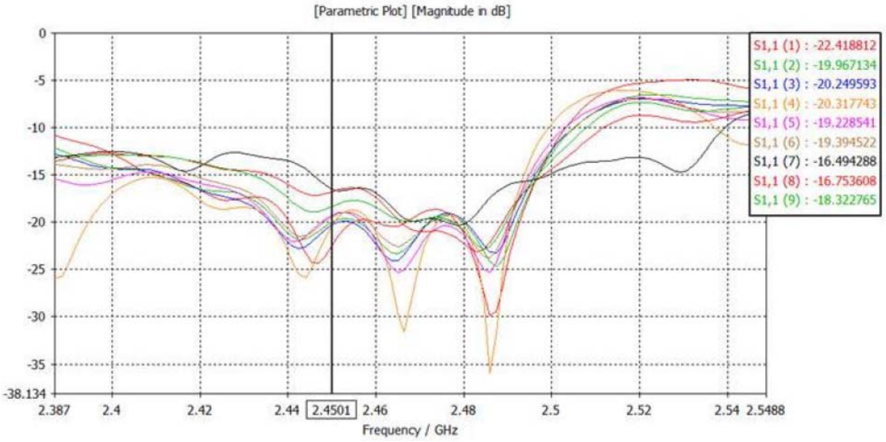


Figure 3. Radiation reflection with frequency.

A crucial factor to be considered for this design is the heating distribution of the grape pomace-solvent mixture passing through the cavity. The software CST Microwave Studio 2014 was employed for the simulations. As it can be seen in Figure 4, the heating uniformity was good, despite the fact that the bottom of the tube was less heated. A fact that could have been solved by implementing a stirring system but, due to the laminar plug flow configuration, it was not possible. Nonetheless, the continuous movement of the extraction material favours a homogeneous treatment since it is exposed to the different zones as it passes through the oven.

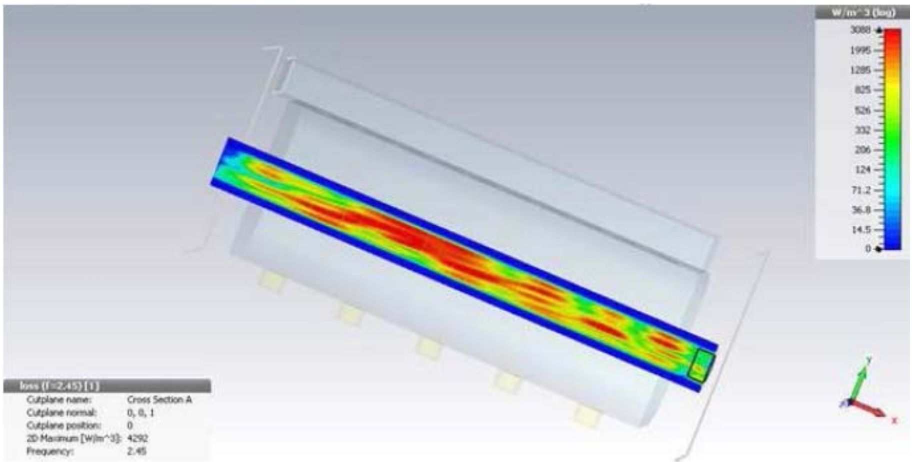


Figure 4. Heat distribution simulation.

This microwave oven design was constructed by the company Sistemas RBT (Valencia, Spain). It incorporates a MX300D-151KL microwave generator of 3 kW, from Muegge. The integrated software ME0200V-007AB allows to measure the reflected power by a CAN BUS interface with the magnetron. The reflected energy is dissipated in this equipment by a refrigerated water circuit, to avoid damaging the magnetron. It also counts with stub tuners in the waveguide and dielectric tuners in the cavity to adapt the electromagnetic field for different applications with other extraction mediums and conditions.

3. PILOT PLANT SET UP

Initially, the pilot plant was devised to be able to work under pressure. To achieve this purpose, PTFE pipe had a thickness able to support the design pressure (safety limit for a tube with an inner diameter of 20 mm and a thickness of 2 mm of 150ºC or 5.3 bar (Adtech, 2014)) and the addition of a control gate valve was considered. By operating the valve's wedge in open/close pulses, it was expected to achieve an average overpressure. A pneumatic system was also planned to balance such pressure variations. However, because of the circulation difficulties later found (detailed below), this idea was postponed until a reliable pumping system is developed.

The connection between the oven and the feeding system was performed by means of a flange, as it is depicted in Figure 5. Its main feature is that it is directly connected to a conduction to which the feed/impulsion system is attached. Both flanges must be electrically connected to avoid possible electric arcs.

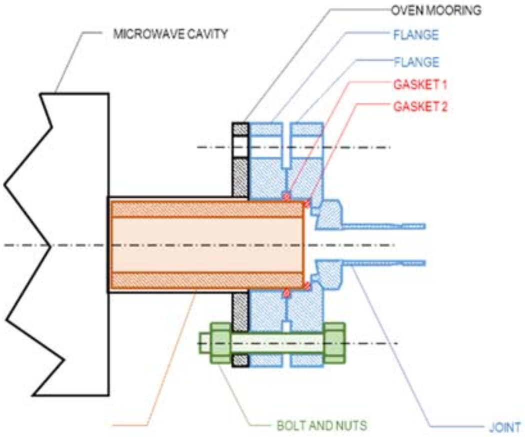


Figure 5. Schematic diagram of the connection between the microwave oven and the conduction.

Regarding the circulation of the solid-liquid mixture, severe difficulties have been found because of the grape pomace-solvent mixture flow characteristics. Commercial equipment available were not suitable for such low flow (19.5 kg/h) with a high proportion of solids (0.75 kg/L) and with a large particle diameter. Also, its rheological behaviour was crucial for the selection of a proper pumping system. It was observed that, as soon as this non-homogeneous mixture was exposed to a strain, the solid became compact and the liquid was drawn out through the filtration cake that the pomace formed, producing the obstruction of the circulation tube.

Several pumping trials were performed with the following equipment:

- Centrifugal pump. It was discarded because it crushed the pomace, what made that undesired compounds were easily leaked, reducing so the quality of the final product (it caused a decreased in the polyphenol richness).
- Progressive cavity (also known as eccentric) pump. This model was discarded for not being able to maintain the proportion of pomace-solvent, and not ensuring prolonged operation.
- Peristaltic pump. It presented clogs that hindered a long operation.
- Pressurize feed tank. Compressed air was used to produce an overpressure in the feed tank so that pomace could be propeled along the system. It was also discarded because the impulsion was not enough to overcome the pressure drop of the system caused by clogs. The attempts to avoid clogging with a vigorous stirring and the addition of a scraper were not enough to favour the flow.
- Screw pump. This set up provided the most promising results, although its operation for a long time always raised problems.

Thus, after this thoughtful check of the available alternatives, screw pump was selected to carry out the impulsion. A prototype for the pilot plant with some modifications were performed in order to minimize its malfunction (mainly due to the formation of clogs after long operation time). The inner diameter was increased from 3.5 cm to 4.0 with special attention paid to lessen roughness inside the PTFE pipe. Also, an adjustable speed motor was coupled to the screw so that the impel was constant and could be tuned to the system requirements. These improvements enable the use of the screw pump for the impulsion of the pomace-solvent through the microwave oven.

4. MICROWAVE OVEN OPERATION

Two experiments were performed in the microwave oven with grape pomace and solvent for a total operation time of 40 minutes (60 times the material residence time). A mass flow of 20 kg/h and a final temperature of 80ºC were used. These conditions corresponded to a previous optimization for the extraction of polyphenols from grape pomace (Álvarez et al., 2017b). Different power levels were tested, obtaining in all the cases absorption efficiencies greater than 97%. To reach this high efficiency value and get maximum power transfer it was necessary to adjust the 3-stub tuner located at the waveguide, between the magnetron head and the cavity oven. This operation was performed attending at the percentage of reflected microwave energy measured in the magnetron isolator, which was reported by the control software Muegge ME0220V-007AB. It must be added that no burnt spots were found in the treated grape pomace, so it can be inferred that the heat distribution was uniform and no hotspots took place.

Table 3. Comparison of the results obtained with the conventional solid-liquid and the microwave pretreatment at laboratory and pilot plant scale.

	Yield ($mg_{GAE}/g_{Dry\ pomace}$)	Richness ($mg_{GAE}/g_{Dry\ extract}$)
Conventional extraction	12.54±0.39	216±46
Pretreatment at laboratory scale	14.00±0.30	166±5
Pretreatment in the continuous oven	9.86±3.16	273±44

Table 3 gathers the results obtained with the pilot plant as well as those from the conventional solid-liquid extraction and with the pretreatment al laboratory scale. As it can be seen, there are slight differences regarding yield and richness. A smaller yield was obtained with the pilot plant but also a larger richness, compared to the other processes. These differences may have arisen from the difficulties to control the stability of the continuous flow. Both the mass flow and the solid-ratio were kept as constant as possible during operation. However, small deviations took place, what can explain these variations. Nonetheless, since they were in the same range and considering also the inhomogeneities of the raw material, it can be said that the pilot plant was able to successfully reproduce the advantages of the microwave pretreatment found at laboratory scale.

CONCLUDING REMARKS

A continuous pilot plant has been designed and built for the scale-up of a microwave pretreatment aimed at the extraction of polyphenols from grape pomace.

A quasi-cylindrical microwave applicator has been proposed as the most suitable alternative. Among its advantages, it stands out its versatility (the oven can be used to treat different materials without modifying the design), its high efficiency and its compact volume. The oven design achieved and presented in this work stands out because of the homogeneous treatment that it provides to the extraction media, as well as its high energy absorption efficiency. Since there are no commercial equipment or published works regarding this invention, the design is in the process of being patented.

Nonetheless, the operation with the pilot plant has presented severe difficulties to surmount, mainly due to the unstable circulation of the pomace and solvent through the pipe, because of their complex flow behaviour. This forced to make some changes in the original design. It was not possible to simultaneously control the total flow and the solid-liquid ratio. The solution chosen to solve this problem was to fix the pomace mass flow and then dispense the solvent to the pomace conduction to achieve the desire solid-liquid ratio. However, how to improve the homogeneity (solvent uniformly damping the pomace) is still an issue to be optimized. Such difficult circulation also made to temporarily discard operation under pressure.

In sum, it can be said that the alternative suggested, the microwave pretreatment, has been able to overcome microwave industrial implementation limitations. A laboratory scale extraction process has been scaled up into a pilot plant able to treat 19.5 kg/h of the mixture grape pomace-solvent for the extraction of active compounds with similar yields. So, the convenience of this equipment has been successfully proved.

NOMENCLATURE

C_P	Heat capacity of the extraction media (J/gK)
D	Tube inner diameter (m)

P	Microwave power consumed by the magnetron head (W)
Q	Volumetric flow of extraction media (m^3/s)
T_{in}	Microwave inlet temperature (ºC)
T_{out}	Microwave outlet temperature (ºC)
t_R	Residence time inside the microwave oven (s)
V_{oven}	Volume of extraction media inside the microwave oven (m^3)
η	Electrical to microwave conversion power efficiency in the magnetron (%)
ρ	Extraction media density (kg/m^3)

ACKNOWLEDGEMENTS

This work was funded by the European Proyect WineSense (FP7-386 MC-IAPP) and by the Spanish regional government Junta de Castilla y León projects VA330U13 and VA04U16. A.A. would like to thank Spanish Ministry of Education for her PhD grant (FPU13/04678).

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CHAPTER 4

Intensification of grape pomace hydrolysis by microwave radiation

INTENSIFICATION OF GRAPE POMACE HYDROLYSIS BY MICROWAVE RADIATION

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ABSTRACT

This work studies the microwave assisted hydrolysis of grape pomace, examining the effects of temperature (140-200ºC), time (2-16 min) and solid-liquid ratio (0.10-0.30 g/mL) on the process. Their influence on the hydrolysate as well as on the remaining solid fraction was determined. A considerable reduction of temperature (from 200ºC in the conventional process to 170ºC with microwaves) and time (from more than 30 minutes to 16 minutes) was attained with the implementation of microwave technology. Concentrated solid-liquid mediums did not provide a yield improvement in contrast to what was expected based on previous evidences. Optimal operational conditions (155ºC, 7.5 minutes and 0.10 g/mL) were set to provide the best results in terms of maximum sugar selectivity and yield, in addition to the lower formation of degradation products. Additionally, the thermal behaviour of the residual solid was analysed by a thermogravimetic assess and compared to the original material. It was concluded that the remaining solid after the hydrolysis was more difficult to volatilise since the weakest biomass parts have been already extracted.

KEYWORDS

Microwave hydrolysis; solid-liquid ratio; sugar selectivity; thermogravimetric analysis; pyrolysis kinetics.

HIGHLIGHTS

- Microwave assisted hydrolysis of grape pomace was compared to hydrothermal
- Temperature and time were reduced by microwave implementation
- No microwave effect on concentrated solid-liquid mediums was found
- Conditions were optimised for a sugar rich product in an efficient process
- Thermal degradation kinetics were analysed for the pyrolysis of the residual solid

1. INTRODUCTION

In the last decades, research has found in microwaves a convenient tool for process intensification. Despite the fact that the extraction of active compounds from natural byproducts have been widely analysed, little further steps have been taken towards the complete exploitation of biomass employing microwaves. Albeit, it still remains a great potential as biorefinery feedstock. A biorefinery is defined as a facility able to transform different biological feeds into high value chemicals and fuels in such a greener way that residue generation is minimized (Clark et al., 2006, Demirbas, 2010, Bozell, 2008). Biorefinery's objective is to fractionate biomass so that different building or platform molecules can be isolated. Some examples of such commodities are sugars, which can be converted in new sources of energy such as bioethanol (Toribio and Morales, 2016, Rabelo et al., 2011); lactic acid, valuable for the production of biopolymers (Corma et al., 2007); or hydroxymethylfurfural (HMF), used in fuel and pharmaceutical industry (De bruyn et al., 2016), among others (Gallina et al., 2018, Cherubini, 2010). These platform blocks can be obtained from the hydrolysis of the three main biomass components: hemicellulose, cellulose and lignin. The latter is an amorphous and complex structure of phenols units that envelopes the whole matrix. Cellulose and hemicellulose consist of polysaccharides, counting hundreds or thousands of monomers. The differences between these both fractions, cellulose and hemicellulose, are related to their structure and repetitive unit. Hemicellulose is branched, amorphous and mainly constituted by pentoses, whereas cellulose has a high degree of crystallinity and is composed of hexoses (Bobleter, 1994).

Several biomass fractionation techniques have been deeply studied. The most extended ones are acid or alkaline hydrolysis, pyrolysis, enzymatic hydrolysis, gasification, heterogeneous and hydrothermal hydrolysis (Tekin et al., 2014, Demirbas, 2010, Sánchez-Bastardo et al., 2017). Among all, hydrothermal fractionation stands out, since it just requires water and temperature to degrade lignocellulosic biomass. By employing different temperature intervals, biomass can be specifically fractionated. Hemicellulose can be recovered between 120ºC and 180-215ºC, depending on the biomass. However, its optimal temperature is usually around 180ºC (Cocero et al., 2018). For cellulose hydrolysis, higher temperatures (above 240ºC) are required. In the case of lignin, even more extreme conditions are needed, which are around 350-400ºC.

In this work, the addition of microwave radiation to the hydrothermal hydrolysis has been studied to assess its influence on operating conditions. Not only has the microwave intensification due to the fast heating been proved, but also an athermal effect on cellulose crystallinity has been reported. Microwave rapid heating allows to obtain a richer sugar product, since side reactions that consume sugars and generate degradation products are minimised because of the shorter extraction time (Fan et al., 2013b, Prado et al., 2016). On the other hand, Budarin et al. (2010) described an athermal effect of radiation affecting cellulose structure during microwave assisted pyrolysis. It was found that cellulose began to decompose at a lower temperature, 180ºC, than the conventional process. A cellulose crystallinity change was reported to be responsible for such variation. This conclusion is also corroborated by the fact that cellulose and hemicellulose are more polar than lignin, what makes them be selectively microwave heated (Rojo et al., 2015, Wolfgang et al., 2015). When microwave assisted hydrolysis was tested, the same enhancement was found at 180ºC (Fan et al., 2013b). In addition, a preference for glucose depolymerization was observed. Other authors have also found fruitful results when microwaves are implemented into hydrolysis processes (Zhou et al., 2017b, Zhou et al., 2017a, Li et al., 2018, Carnaroglio et al., 2015).

The variables chosen for analysis in this work have been temperature, time and solid-liquid ratio. Temperature and time are expected to decrease according to the above mentioned. The inclusion of solid-liquid ratio is a novelty among this kind of works. Since it has been proved that only the cellulose that is in direct constant with the radiation is affected (Budarin et al., 2010), it is presumed that if a more concentrated solid-liquid medium is employed, the energy will be concentrated in the solid. Therefore, better results can be achieved. Besides, high ratios entail a decrease in the energy consumption, a vital parameter to bear in mind in these processes. Additionally, the solid fraction remaining after the microwave assisted hydrolysis has been further submitted to thermogravimetric analysis, and a subsequent kinetic degradation study to determine its viability for further pyrolysis treatment.

Grape pomace has been used as raw material. In order to complete the total exploitation of this biomass, an extractive-free grape pomace was used first in a microwave assisted hydrolysis, followed by a pyrolyisis. In consequence, this work would contribute to bring to fruition a microwave intensificated biorefinery. Among the previous works published about grape hydrothermal hydrolysis, a great disparity of results has been observed. Yields range from 6.4% to 23.1wt%, for hydrolysis at 258ºC-30 min and for 180-250-340ºC in 45 minute intervals respectively in semicontinuous reactors (Prado et al., 2014, Yedro et al., 2015). A low generation of degradation products (mainly HMF) was found in both processes. Yedro et al. (2015) also reported a large degree of fractionation. Cellulose and hemicellulose were mostly hydrolysed, rendering a more than 90 wt% lignin rich solid. Further treatment at severe conditions was also studied to produce bio-oil with again great efficiency (Yedro et al., 2014). Thus, it is clear that grape pomace is a promising raw material, not only for the extraction of extractives, but also as a biorefinery feedstock.

In sum, the objective of this work is to assess the operational conditions temperature, time and solid-liquid ratio of a microwave hydrolysis process in terms of liquid and solid efficiency. Liquid yield is evaluated in terms of overall yield and sugar selectivity, whereas a thermogravimetric and kinetic analysis lead to appraise the convenience of the remaining solid fraction to be used in a subsequent pyrolysis step.

2. MATERIALS AND METHODS

2.1. RAW MATERIAL AND COMPOSITION

Grape pomace without extractives was used. Grape pomace from *Tempranillos* grape and 2015 vintage was given by Bodega Matarromera (Spain). Extractives were removed by a conventional solid-liquid extraction (Álvarez et al., 2017). Ethanol-water (50:50% v/v) was used as solvent in a solid-liquid ratio of 0.50 g/mL. The mixture was left for 3 hours at 60ºC with vigorous stirring. When the extraction was finished, pomace was separated by filtration, dried until constant weight, and ground in a Retsch ball mill.

Table 1 gathers the CHN characterization of the raw material.

Table 1.Grape pomace composition

Elementary analysis	
C (wt.%)	55.103±0.156
H (wt.%)	5.885±0.057
N (wt.%)	1.879±0.184

Distillate water was used as solvent. The rest of chemical reagents were HPLC or analytical grade.

2.2. EXPERIMENTAL PROCEDURE

A CEM Discover microwave (CEM Corp) was employed for the microwave hydrolysis of grape pomace. This unit was equipped with a pressure cap and an infrared thermometer. This thermometer was daily calibrated at 200ºC with glycerol before the experimental work. For each experimental point, vials were filled with the proper solid-liquid ratio up to 20 mL and, after being homogenized for 5 minutes at ambient temperature, placed in the microwave. Since high power has been proved to provide better results (Lam et al., 2015), a constant power of 300 W was fixed. The control temperature of the equipment turned the power on and off to achieve the set temperature. When the hydrolysis time was finished, irradiation was turned off, and the sample was cooled down with air to a temperature of 70ºC.

As a control, a hydrothermal treatment of the pomace in a Berghof 25 mL stainless steel pressure reactor was also performed. The experimental procedure was equal to the microwave hydrolysis one. Berghof reactor was equipped with a thermocouple to control the temperature. The cool down after the experiment was also carried out with air.

After the hydrolysis, the solid and liquid phases were separated by centrifugation at 3500 rpm during 20 minutes at 12ºC. Solid pomace was dried overnight in an oven, and liquid phase was storage for further analysis.

2.3. LIQUID CHARACTERISATION

Hydrolysate characterisation has been performed in terms of yield and selectivity regarding the overall process, and the formation of sugars and degradation products. Yield accounts for the hydrolysis efficiency (results expressed in relation to the amount of pomace employed, mg_C/g_{Pomace} for the overall yield and $mg_C/g_{C\ Pomace}$ for sugar and degradation products yield), whereas selectivity indicates the sugar richness in the liquid products (results expressed in relation to the hydrolysate, $mg_C/g_{C\ hydrolysate}$).

2.3.1 TOTAL ORGANIC CARBON

Total organic carbon analyses were conducted in a Elementar Vario TOC Select. Measurements have been transformed to be expressed in mg_C/g_{Pomace} (overall yield).

2.3.2. LIQUID CHROMATOGRAPHY

Cellobiose, fructose, furfural, glucose, HMF, rhamnose, and the acetic, formic, lactic and levulinic acids were quantified in an Agilent 1260 HPLC with a DAD and RI detector.

Glucose, fructose, xylose, cellobiose, rhamnose and the organic acids were analysed in a Agilent Hi-Plex H column (300x7.7 mm, 8 µm) at 60ºC after a sample injection of 5 µL. Sulfuric acid 5mM with a flow of 0.4 mL/min was used as eluent. Individual compounds were detected and quantified by the refractive index detector at 55ºC for a total run time of 35 minutes.

Furfural and HMF were measured in an ACE C18 column (250x4.6 mm, 5 µm) at 30ºC using a mixture of acetonitrile:water (25/75 v/v%) with a flow rate of 0.8 mL/min as mobile phase. The injection volume was 5 µL. DAD detector was set to record for 22 minutes absorbance at 220 nm.

The results have been transformed to be expressed in $mg_C/g_{C\ pomace}$ by means of the total organic carbon, since this conversion allowed to have all the results in the same scale. In order to facilitate the interpretation, results were assembled in two gropus: sugars and degradation products. Sugar group consisted in cellobiose, glucose, xylose, fructose and rhamnose. They were studied as sugar richness so their results were expressed as $mg_C/g_{C\ hydrolysate}$. Formic, lactic, levulinic acid, furfural and HMF were considered degradation products. In this case, degradation product yield was considered and results have been used in $mg_C/g_{C\ Pomace}$.

2.4. SOLID CHARACTERISATION

2.4.1. SOLID COMPOSITION

Hemicellulose, cellulose and lignin composition of the pomace was quantified according to the standard method provided by the National Renewable Energy Laboratory (Hames et al., 2005, Sluiter et al., 2008a, Sluiter et al., 2008b, Sluiter et al., 2008c). Firstly, any remaining extractives were removed in two consecutive Soxhlet extraction with water and ethanol. Then, 300 mg of the pomace was mixed with 3 mL of sulphuric acid solution 72% (wt.) for 60 minutes at 30ºC. After the acid hydrolysis, the solid was separated from the liquid by vacuum filtration. The solid fraction was dried in a muffle at 550ºC overnight to quantify lignin and ashes. The liquid samples were neutralized with calcium carbonate and analysed in a Water HPLC, with a SUGAR SH-1011 Shodex column at 55ºC, volume injection of 20 µL and a flow rate of 0.01 N sulphuric acid of 0.8 mL/min. Cellobiose, glucose, xylose, galactose, arabinose and fructose were quantified in a Waters 2414 IR detector. Cellulose composition was computed considering C6 sugars and hemicellulose C5s.

2.4.2. THERMOGRAVIMETRIC ANALYSIS

A Rheometric Scientific Simultaneous Thermal Analyzer STA 625 was employed for the measurements. An initial weight of around 10 mg was treated with a nitrogen flow of 60 NmL/min from ambient temperature to 625ºC with a heating rate of 10ºC/min.

2.4.3. THEMOGRAVIMETRIC MODEL

The model used to fit the experimental data was previously developed by Cabeza et al. (2015). This model followed an autocatalytic kinetics and it was demonstrated to be a suitable option to reproduce the thermal degradation of biomass. It can be found in the appendix. This model was based on a reaction pathway which was a modification of the Waterloo's mechanism. It includes the degradation of each biomass fraction into volatiles and charcoal. The gas production from char and the boiling of the liquid phases (water and oils) were also considered. Figure 1 presents the mechanism proposed.

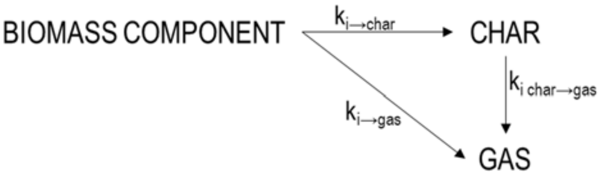

Figure 1. Reaction pathway for each component of the biomass (hemicellulose, cellulose and lignin)

Once the experimental data was fitted to minimize the absolute average deviation (equation [1]) between the experimental weights ($W_{exp,i}$) and the simulated ones ($W_{model,i}$). The calculated kinetics was used to perform a deeper study of the effect of the pretreatment on the biomass pyrolysis. The objective was to evaluate the change in the kinetic constant of the reaming solid after the hydrolysis (K_i) compared to the one of the raw material (K_{pomace}). This comparison was performed according to equation [2].

$$\mathrm{AAD}\ (\%) = \frac{1}{n} \cdot \sum \left| \frac{W_{exp,i} - W_{model,i}}{W_{exp,i}} \right| \cdot 100 \qquad [1]$$

$$\Delta K\ (\%) = \frac{K_i - K_{pomace}}{K_{pomace}} \cdot 100 \qquad [2]$$

2.5. STATISTICAL ANALYSIS

The experimental variables chosen for this work were temperature (140-200ºC), time (2-30 min) and solid liquid ratio (0.1-0.3 g/mL). The responses analysed have been yield and selectivity, in terms of the overall hydrolysis process (microwave and hydrothermal yield), the formation of sugars (microwave sugars yield and selectivity) and the generation of degradation products (microwave degradation products yield).

A face central composite design (α=1) was followed. Table 2 gathers the experimental conditions for each experiment. ANOVA tables, with a confidence of 95% (p-value of 0.05) have been used to identify significant effects. These have been discussed according to the main effect and interaction diagrams provided by the software Design Expert. A desirability function has been employed for the global optimization of the process.

Table 2. Experimental results.

Exp	T (ºC)	time (min)	S:L (g/mL)	MW yield (mg_C/g_{Pomace})	HY yield (mg_C/g_{Pomace})	MW sugars yield ($mg_C/g_{C\ Pomace}$)	MW sugars Selectivity ($mg_C/g_{C\ hydrolysate}$)	MW degradation products ($mg_C/g_{C\ Pomace}$)
1	140	30	0.30	74.18	70.10	14.54	203.73	10.90
2	170	16	0.30	62.29	49.77	20.93	188.82	12.11
3	140	30	0.10	66.49	49.38	39.10	288.39	11.96
4	170	16	0.20	81.38	61.87	24.26	190.06	16.12
5	200	2	0.10	64.76	54.10	31.13	218.68	20.80
6	170	16	0.20	83.57	81.54	23.17	193.81	16.20
7	170	2	0.20	66.94	48.62	19.14	193.21	6.81
8	170	16	0.10	51.61	52.33	44.13	282.55	20.26
9	200	30	0.10	66.74	66.34	37.44	233.42	59.75
10	200	2	0.30	31.38	25.69	13.26	109.12	14.11
11	170	16	0.20	63.33	49.73	25.01	194.68	14.98
12	200	30	0.30	48.42	30.89	14.68	128.23	20.42
13	170	30	0.20	59.65	51.86	21.22	170.75	17.21
14	140	2	0.30	57.76	45.25	12.01	199.35	20.85
15	140	2	0.10	37.19	35.43	31.64	350.23	13.75
16	200	16	0.20	47.07	31.82	16.96	132.41	24.94
17	140	16	0.20	70.64	39.61	16.50	177.58	6.30

3. RESULTS AND DISCUSSION

3.1. HYDROLYSIS PRODUCTS

3.1.1. OVERALL LIQUID YIELD

Total organic carbon measurements have been taken as reference to compute the liquid yield of the hydrolysis. Microwave and hydrothermal results have been compared. The ANOVA tables for both processes are presented in Table 3. In the case of microwave hydrolysis, temperature, time and solid-liquid ratio showed significance, what is more, a quadratic influence took place for the three variables. For the hydrothermal hydrolysis, only temperature and ratio were significant in the range analysed. Figure 2 presents the main effect graphs for the two processes.

In the case of temperature, hydrothermal hydrolysis followed a linear relation with temperature whereas microwave yield began rendering a higher yield at low temperature due to a quadratic influence. Nonetheless, as long as temperature increased, hydrothermal and microwave hydrolysis achieved the same yield. This is thought to may be due to a change in the cellulose structure. As it has been already mentioned, Budarin (2010) and Fan et al. (2013a) reported a transformation into the amorphous part of cellulose at lower temperatures (180ºC) what can explain the yield improvement. Thus, microwaves allowed to obtain higher yields at lower hydrolysis temperature.

Table 3. Overall liquid yield analysis of variance for microwave and hydrothermal hydrolysis.

	Microwave hydrolysis					Hydrothermal hydrolysis				
Source	Sum of squares	DF	Mean square	F-value	p-value	Sum of squares	DF	Mean square	F-value	p-value
Model	3190	13	245	42.4	0.0052	3436	13	264	30.5	0.0084
A-Temperature	168	1	168	29.0	0.0126	628	1	628	72.5	0.0034
B-time	87	1	87	15.0	0.0306	1.6	1	1.6	0.2	0.6987
C-S:L	279	1	279	48.2	0.0061	138	1	138	15.9	0.0281
AB	70	1	70	12.1	0.0401	2.0	1	2.0	0.2	0.6674
AC	26	1	26	4.5	0.1253	198	1	198	22.8	0.0174
BC	119	1	119	20.5	0.0201	6.8	1	6.8	0.8	0.4417
A^2	105	1	105	18.2	0.0236	44	1	44	5.1	0.1095
B^2	86	1	86	14.9	0.0309	0.8	1	0.8	0.1	0.7816
C^2	88	1	88	15.1	0.0301	2.1	1	2.1	0.2	0.6544
ABC	2.7	1	2.7	0.5	0.5409	16	1	16	1.8	0.2693
A^2B	7.7	1	7.7	1.3	0.3325	14	1	14	1.7	0.2878
A^2C	2.8	1	2.8	0.5	0.5367	0.9	1	0.9	0.1	0.7640
ABÂ^2	11	1	11	1.9	0.2584	11	1	11	1.3	0.3392
Residual	17	3	5.8			26	3	8.7		
Lack of Fit	4.2	1	4.2	0.6	0.5080	25	1	25	73.5	0.0133
Pure Error	13	2	6.6			0.7	2	0.3		
Cor Total	3207	16				3462	16			

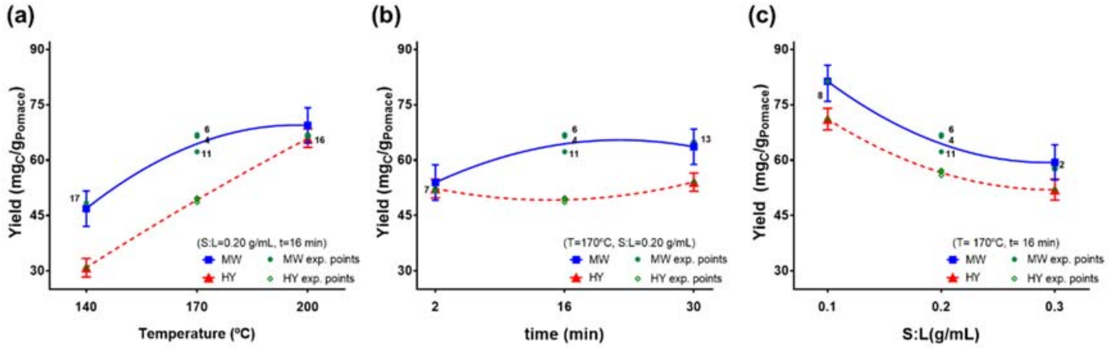

Figure 2. Comparison between microwave (■) and hydrothermal (▲) hydrolysis effect of (a) temperature, (b) time and (c) solid-liquid ratio on the overall liquid yield.

Time was not found as a significant variable in the hydrothermal process. It is obviously an important factor, but due to hydrothermal presenting a slowest heating and cooling ramp, its effect may be hindered. On the other hand, microwave hydrolysis presented similar yields to the hydrothermal at a low time, but an enhancement when a longer time was employed. However, at a time of 16 minutes, hydrolysis yield seemed to have reached an optimum, since no enhancement was observed at even longer times (30 minutes). This and the improvement over hydrothermal hydrolysis can be also connected to the change in the cellulose crystallinity due to microwave radiation. A longer of exposure to the radiation (from 2 to 16 minutes) may favour a wider disruption of the structure, making the biomass more accessible for the hydrolysis, and therefore, boosting yield. However, such disruption was completely accomplished at 16 minutes, since no further enhancement was found at a longer time.

This work was also aimed to study if a more concentrated media could be employed. The use of less solvent would have concentrated the absorbed energy closer to the solid-liquid interphase, maximizing a likely athermal effect. Unfortunately, as figure Figure 2(c) proves, there was not such effect. Indeed, both presented the same tendency. Although a slight difference was observed, it can not be attributed to radiation. The error bars overlapped, preventing so from coming to any sound conclusion.

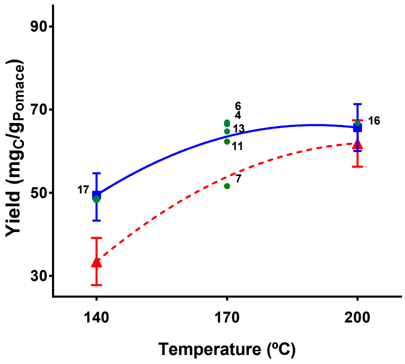

Figure 3. Temperature and time interaction during microwave hydrolysis. ■ t=30 min, ▲ t=2 min, S:L=0.20 g/mL .

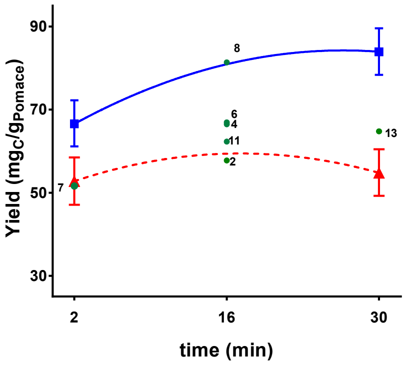

Figure 4. Time and solid-liquid interaction during microwave hydrolysis. ■ S:L=0.10 g/mL, ▲ S:L=0.30 g/mL, T=170ºC.

Regarding variables interaction, the significant couples in each process were opposed. While for microwave hydrolysis temperature-time and time-ratio were noteworthy, hydrothermal temperature-ratio was significant. In the latter case, time was absence from any interaction, according to its no main effect significance. In contrast, time was involved in the two interaction pairs for microwave hydrolysis. In the interaction temperature-time, time had a larger effect at low temperatures. However, a temperature increase concealed any time effect, since similar yields were reached at high temperature independently of time, as it can be found in Figure 3. A behaviour that agrees with the concept of severity factor (Pedersen and Meyer, 2010). Figure 4 gathers the second interaction, time-ratio. Mass transfer was represented in this combination. At short times, there was no solid-liquid ratio influence because of the short exposure. However, at longer times, low ratios boosted the yield while higher ratios did not present an improvement. Again, here it is corroborated that water is a crucial factor, and decreasing its concentration in favour of a higher energy concentrated media is futile.

3.1.2. SUGAR YIELD AND DEGRADATION PRODUCTS

The objective of this section was to assess the hydrolysis conditions to optimise the final liquid product. Cellobiose, glucose, xylose, fructose and rhamnose were grouped as ‘*sugars*’, whereas formic, lactic, levulinic acid, furfural and HMF as ‘*degradation products*’. A rich sugar extract (high sugar selectivity, $mg_C/g_{C\ hydrolysate}$) as well as a low degradation products formation were sought, together with an efficient process in terms of overall yield and sugar yield. Table 4 presents these operational conditions. Thus, temperature, time and solid-liquid ratio have been varied to maximize overall yield, sugar yield and sugar selectivity, and to minimize degradation products. These two latter responses were attributed a greater importance than the first ones in the desirability function, which resulted to be of 0.832. The conditions of 155ºC, 7.5 minutes, 0.10 g/mL were found to provide the best responses.

Table 4. Optimal conditions for an efficient and sugar selective microwave hydrolysis.

Variable	Objective	Interval of Variation	Relative importance	Theoretical optimum
Temperature (ºC)	None	140-200		155
time (min)	None	2-16		7.5
S:L (g/mL)	None	0.10-0.30		0.10
MW yield (mg_C/g_{Pomace})	Maximize	31.38-83.57	++	54.25
MW sugar yield ($mg_C/g_{C\ Pomace}$)	Maximize	12.01-44.13	+++	39.51
MW sugar selectivity ($mg_C/g_{C\ hydrolysate}$)	Maximize	109.12-350.23	+++++	321.51
MW degradation products yield ($mg_C/g_{C\ Pomace}$)	Minimize	6.30-59.75	+++++	12.76

Regarding temperature, it had a positive effect in the overall yield and in the reduced formation of degradation products, but a detrimental influence on sugar yield and selectivity. Sugar's hydrolysation (yield and selectivity) was maximum at 165ºC, but higher temperatures decreased it in favour of degradation reaction. This behaviour was in agreement with the data reported by other authors, since oligomers directly degrade into smaller oligomers and sugars (monomers) which would further degrade into several compounds, like organic acids or furfural (Piqueras et al., 2017). So, an optimal temperature of 155ºC represents a balance between these clashing tendencies.

Similar tendencies were found in the case of time. Time promoted overall yield and degradation products, but its influence on sugar release was not so significant. Sugar's yield presented a slight maximum at 16 minutes, and selectivity was nearly unaffected. Thus, a short time, like 7.5 minutes, represented a suitable value to obtain a sugar selective hydrolysate, with a low proportion of degradation products in an efficient process.

In the case of solid-liquid ratio, overall yield and sugar responses were favoured by low ratios. Degradation products did not have a ratio dependence, since their production mainly took place in the liquid phase and the ratio focused their action on the mass transfer between the pomace and the water. Thus, it was clear that a low solid-liquid ratio, like 0.10 g/mL, favours the effectiveness of the process.

The graphs describing the above behaviour are presented in Figure 5.

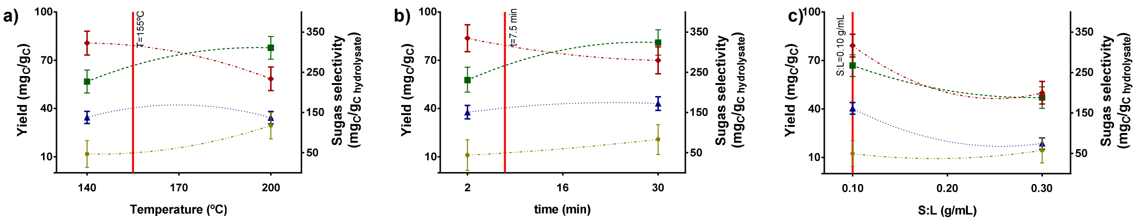

Figure 5. Selection of the microwave hydrolysis optimal conditions (T=155ºC, t=7.5 min, S:L=0.10 g/mL) for (a) temperature, (b) time and (c) solid-liquid ratio for overall yield (■), sugar yield (▲), sugar selectivity (♦) and degradation product yield (●).

Yedro et al. (2015) claimed an overall yield of 48.40 mg_C/g_{Pomace} (value computed from the reported data with a mean carbon fraction of 0.40) obtained at 180ºC and 45 minutes. While the optimal yield estimated in this work was 54.25 mg_C/g_{Pomace} at 155ºC and 7.5 minutes. The enhancement with the microwave implementation is clear. Not so much for the yield improvement (which can be considered inside the experimental error), but because of the substantial operation conditions reduction. The microwaves disservice was the great formation of degradation products. However, this is thought to be not because of the radiation, but due to the reactor selection. A batch reactor was used for the microwave hydrolysis, whereas semi-continuous systems for the hydrothermal hydrolysis were used in bibliography. The main advantage of this latter configuration is that the liquid phase has a low residence time inside the reactor what prevents degradation reactions to take place. Unlike batch reactors, in which the liquid has the same reaction time than the solid phase. In conclusion, it would be necessary to develop a semi-continuous microwave hydrolysis system to utterly optimise the hydrolysis.

3.2. REMAINING SOLID THERMOGRAVIMETRIC KINETIC ANALYSIS

The influence of the experimental microwave hydrolysis conditions on the thermal degradation behaviour of the remaining solid has also been studied. A thermal decomposition kinetic analysis has been performed using the data gathered in Table 5. These kinetic parameters have been computed by modelling the thermogravimetric assay. The average absolute deviation of these fittings was around 1.92%.

3.2.1. HEMICELLULOSE

As it was exposed in the introduction, hemicellulose consists of the easiest biomass part to be affected during a hydrothermal treatment. It can be extracted or transformed into hydrochar (Carpenter et al., 2014), what agrees with the results gathered in Table 5: a decrease in the hemicellulose content up to a complete fading (like, for instance, in Exp 16 at T=200ºC). Concerning thermal degradation, hemicellulose volatilization and charring were the most affected decomposition reactions. The release of volatiles during an onforward pyrolyisis after the treatment requested more activation energy than the original biomass ($\Delta K_{HC\rightarrow gas}<0$, according to equation [2]). As the pretreatment had already removed the weakest fractions, only the parts that required higher energy remained in the solid, explaining so this behaviour. Consequently, lower kinetic constants were found, as it is proved in Figure 6. In the case of hemicellulose second degradation reaction, char production (Figure 7), the reaction was favoured by the pretretament ($\Delta K_{HC\rightarrow char}>0$). A possible reason to justify this promotion could be, again, the fact that the remaining hemicellulose would be composed of the harder parts, which would be easier to transform into char.

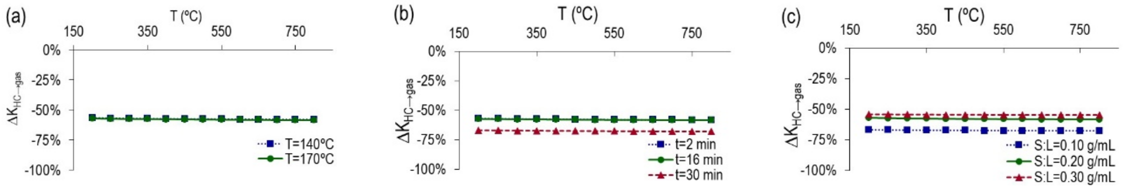

Figure 6. Hemicellulose to gas kinetic constant variation depending on the (a) temperature, (b) time and (c) solid-liquid ratio.

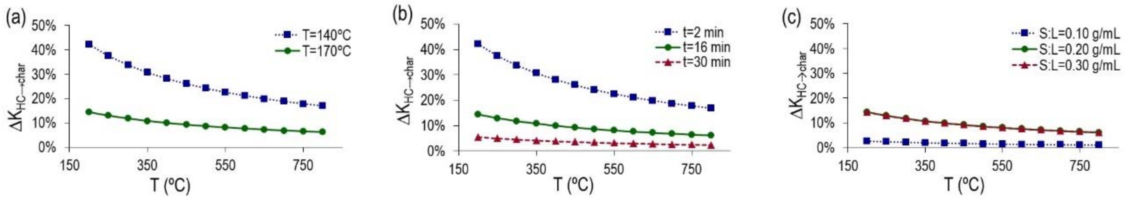

Figure 7 . Hemicellulose to char kinetic constant variation depending on the (a) temperature, (b) time and (c) solid-liquid ratio.

Regarding the specific effect of the pretreatment variables, it can be checked that all of them (temperature, time and solid-liquid ratio) had a similar role for both reactions, worsening the volatilisation and promoting the charring. However, there were some differences that should be explained more in detailed. Temperature and time showed the same kinetic variation in the volatilisation, independently of their value. Which could be explained by the fact that any of the operational conditions was enough severe to affect the hemicellulose fraction. In contrast, for charring, the higher these variables were, the lower kinetic modification. A result that was expected, since the more severe the operational conditions are, the lowest char is produced. On the other hand, the solid ratio influence always depended on its specific value. And, the higher it was, the lower kinetic modification. A result that could be related to its role on the mass transfer between the solid and the water, as a high solid-liquid ratio would imply a lower driving force.

3.2.2. CELLULOSE

Cellulose's thermal behaviour can be observed in Figure 8. The main finding was that its volatilization was greatly affected by the pretreatment, being promoted at hydrothermal temperatures lower than 200ºC. This enhancement at mild pretreatment temperatures could be due to either the cellulose weakest part extraction, the likeable structural changes (Cabeza et al., 2017), or the loose of hemicellulose (which has been demonstrated to behave as a shield for cellulose thermal degradation (Cabeza et al., 2015)). At higher temperatures, the kinetics for the volatilization would be worsened since cellulose extraction would be significant at that condition. The same idea would explain the effect of the solid-liquid ratio and time. Concerning char production and its volatilization, they were not modified by the pretreatment, probably because of the mild conditions used. These results have been compared with the ones reported by Cabeza et al. (2015) in which grape seeds were hydrolysed at 250-340ºC, and with a work dealing with Brazilian gingseng treated with pressurized water at 60ºC(Cabeza et al., 2017). Despite the fact that the raw materials were not utterly equivalent, and neither the pretreatments, the conclusions were similar to the ones obtained in this work. The only difference found was regarding grape seed's char production, which was worsened. This variation was attributed to the higher temperature and time employed.

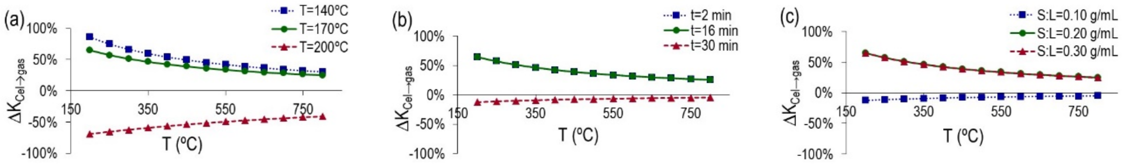

Figure 8. Cellulose to gas kinetic constant variation depending on the (a) temperature, (b) time and (c) solid-liquid ratio.

3.2.3. LIGNIN

For lignin thermal degradation (Figure 9), a decrement in the volatilisation was always observed, being this decrease higher at lower temperature and time. On the other hand, char production and its degradation were unaffected. The grounds of this behaviour could be the extraction of the weakest part of lignin, which would lead to a residue more difficult to be degraded. Such decrease in the lignin volatilization was far lower when higher temperature or time were used during the pretreatment. A tendency that could be explained by a degradation of the lignin hard fractions. Solid-liquid ratio presented the same response. Likewise cellulose, the mild conditions could explain why the char reactions were not modified by the previous hydrolysis. Again, similar results for lignin were obtained by other authors (Cabeza et al., 2015, Cabeza et al., 2017).

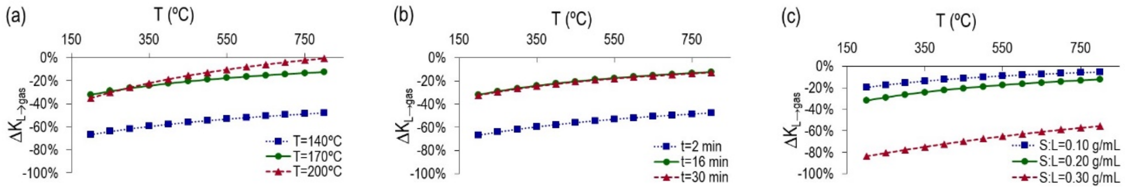

Figure 9. Lignin to gas kinetic constant variation depending on the (a) temperature, (b) time and (c) solid-liquid ratio.

Table 5. Pretreatment conditions and fitted mass transfer and kinetic parameters of remaining solid thermogravimetric analysis

Exp	Untreated grape pomace	Central Point(*)	#17	#16	#7	#13	#8	#2
T (ºC)	-	170	140	200	170	170	170	170
time (min)	-	16	16	16	2	30	16	16
S:L (g/mL)	-	0.20	0.20	0.20	0.20	0.20	0.10	0.30
Water (wt.%)	0.032	0.024	0.019	0.019	0.015	0.019	0.032	0.027
Extractives (wt.%)	0.267	0.183	0.129	0.242	0.184	0.212	0.187	0.233
Hemicellulose (wt.%)	0.109	0.103	0.142	0.000	0.156	0.083	0.091	0.095
Cellulose (wt.%)	0.174	0.142	0.197	0.178	0.170	0.163	0.161	0.201
Lignin (wt.%)	0.416	0.544	0.512	0.561	0.474	0.522	0.529	0.444
Ashes (wt.%)	0.001	0.002	0.002	0.000	0.001	0.002	0.000	0.001
k_W	4297	4297	4297	4297	4297	4297	4297	4297
n_W	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
k_O	27	27	27	27	27	27	27	27
n_O	1.11	1.11	1.11	1.11	1.11	1.11	1.11	1.11
$k_{HC\to gas}$	26977	10981	11005	0	10963	8501	8502	12000
$k_{HC\to char}$	29926	29932	29942	0	29679	29876	29873	29932
$k_{HC\ char\to gas}$	0.64	0.64	0.64	0.00	0.46	0.64	0.64	0.64
$k_{Cel\to gas}$	15117	15250	14891	15250	14999	15317	15250	15250
$k_{Cel\to char}$	22877	22779	22848	22865	22872	22833	22779	22779
$k_{Cel\ char\to gas}$	0.14	0.14	0.14	0.14	0.16	0.14	0.14	0.14
$k_{L\to gas}$	7401	7908	5515	10348	8934	7865	8000	7272
$k_{L\to char}$	31267	31266	31266	31266	30504	31200	31266	31266
$k_{L\ char\to gas}$	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.03

Table 5 (continued). Pretreatment conditions and fitted mass transfer and kinetic parameters of remaining solid thermogravimetric analysis

Exp	Untreated grape pomace	Central Point(*)	#17	#16	#7	#13	#8	#2
$Ea_{HC\rightarrow gas}/R$	8913	8889	8885	8889	8886	8890	8889	8900
$Ea_{HC\rightarrow char}/R$	7413	7350	7247	7615	7058	7357	7400	7350
$Ea_{HC\ char\rightarrow gas}/R$	101	100	101	101	100	100	100	100
$Ea_{Cel\rightarrow gas}/R$	13312	13080	13012	13880	13571	13379	13379	13080
$Ea_{Cel\rightarrow char}/R$	8006	8006	8006	8006	7291	7805	8006	8006
$Ea_{Cel\ char\rightarrow gas}/R$	423	423	423	423	423	423	423	423
$Ea_{L\rightarrow gas}/R$	8312	8526	8694	8677	8473	8582	8454	9164
$Ea_{L\rightarrow char}/R$	8302	8302	8302	8302	8302	8302	8302	8302
$Ea_{L\ char\rightarrow gas}/R$	300	300	300	300	295	300	300	300
AAD (%)	2.60	2.53	2.16	1.27	0.95	1.06	0.97	2.37

(*) Central point computed as the mean of experiments 4, 6 and 11.

4. CONCLUSIONS

Microwave assisted hydrolysis of extractive-free grape pomace has been studied in this work. It has been proved that the addition of microwaves to the hydrothermal process allows to reduce operational time and temperature. The proportion solvent-solid was also tested. Considering the previously reported athermal effect affecting the biomass structure, it was expected that microwaves would allow to decrease the solvent requirements. Presumably, if the radiation was concentrated on the solid, the improvement would still be evident despite the solvent shortage. However, the microwave boost that allowed a temperature and time reduction was not maintained for solvent consumption. Still low solid to liquid proportions were required. A reduction in the solvent consumption would have been a substantial contribution and an undeniable justification for microwave implementation. However, it seems that the microwave enhancement is only due to its fast heating, which represses degradation reactions.

Hydrolysis conditions have been optimised in order to obtain an efficient sugar selective process. It was found that 155ºC, 7.5 minutes and 0.10 g/mL provided the maximum sugar selectivity, sugar yield and overall yield, while keeping degradation products formation to a minimum. Compared to other reported values, no substantial yield increase was obtained. However, the reduction on the operational conditions was noteworthy.

Regarding a further valorisation of the remaining solid fraction by a pyrolysis, a thermal kinetic degradation analysis was performed. Hemicellulose was the most affected biomass fraction by the pretreatment due to the mild operational conditions. Cellulose and lignin were also modified, but in a lower extent. During the microwave pretreatment, the easiest or more accessible fractions were hydrolysed. In terms of temperature, time and solid-liquid ratio, their influence on the thermal decomposition kinetics were in agreement with their role in the hydrothermal extraction.

In sum, the exploitation of grape pomace by means of microwave intensification is assessed in this work. A rich sugar hydrolysate has been obtained and the influence of the hydrolysis conditions on the subsequent pyrolysis of the solid residual has been determined.

ACKNOWLEDGEMENTS

A.A. thanks the Spanish Ministry of Education for her grants (FPU13/04678 and EST16/00177).
A.C. also thanks the Ministry of Education for his grant FPU13/01516.

NOMENCLATURE

Acronyms

AAD	Absolute average deviation (%)
Cel	Cellulose
HC	Hemicellulose
L	Lignin
HY	Hydrothermal
MW	Microwave

Symbols

C	Total organic carbon
Ea_i/R	Activation energy for the reaction *i* (K)
$k_{i\ char \rightarrow gas}$	Preexponential factor for the reaction *i char* to gas (min^{-1})
$k_{i \rightarrow char}$	Preexponential factor for the reaction *i* to char (min^{-1})
$k_{i \rightarrow gas}$	Preexponential factor for the reaction *i* to gas (min^{-1})
k_o	Mass transfer coefficient for the extractives ($kg \cdot m^3 \cdot min^{-1} \cdot m^{-2} \cdot kmol^{-1}$)
k_w	Mass transfer coefficient for water ($kg \cdot m^3 \cdot min^{-1} \cdot m^{-2} \cdot kmol^{-1}$)
n_o	Extratives mass transfer order (dimensionless)
n_w	Water mass transfer order (dimensionless)

S:L	Solid-liquid ratio (g/mL)
T	Temperature (ºC)
t	Time (min)
w_{exp}	Weight measured during the thermogravimetric analysis (mg)
w_{model}	Weight estimated by the model for the thermogravimetric analysis (mg)
ΔK	Kinetic constant increment of pretreated samples respect non-pretreated ones (dimensionless)

APPENDIX. Thermogravimetric kinetic model

1/ Mass balances

Individual mass balance

$$\frac{dm_j}{dt} = r_j = \sum_{i=1}^{N_r} g_{ij} \cdot r_i \tag{S1}$$

Global mass balance

$$\frac{dM}{dt} = \sum_{j=1}^{N} \frac{dm_j}{dt} \tag{S2}$$

2/ Kinetics

Boiling liquids (water and oils)

$$r_i = h \cdot \left(C_j^*\right) \cdot m_j^{nl_i} \tag{S3}$$

3/ Solid fractions

Cellulose

$$r_i = k_{o_i} \cdot e^{-\frac{E_{a_i}}{R\cdot T}+c\cdot T+\ln(T)} \cdot m_j^{n_i} \cdot \left(1-\alpha_i \cdot m_j\right)^{\beta_i} \tag{S4}$$

Hemicellulose and lignin

$$r_i = k_{o_i} \cdot e^{-\frac{E_{a_i}}{R\cdot T}} \cdot m_j^{n_i} \cdot \left(1-\alpha_i \cdot m_j\right)^{\beta_i} \tag{S5}$$

4/ Nomenclature

C	Correction factor for the effect of the heating rate on the kinetics (dimensionless)
C_j^*	Equilibrium concnetration of the liquid j (kmol·m^{-3})
E_a	Activation energy (J·mol^{-1})
g_{ij}	Stoichometric coefficient (g·g^{-1})
h	Partial mass transfer coefficient (kg$_j$·m^3·min^{-1}·m^{-2}·kmol$_j$$^{-1}$)
k_{oi}	Preexponential factor for the *i* reaction (min^{-1})

m_j	Mass fraction of the *j* compound ($g \cdot g^{-1}$)
N	Total number of compounds (dimensionless)
n_i	Reaction order (dimensionless)
n_{li}	Mass transfer order (dimensionless)
N_r	Total number of reactions (dimensionless)
R	Ideal gass constant ($J \cdot mol^{-1} \cdot K^{-1}$)
r_i	Reaction rate ($g \cdot min^{-1} \cdot g^{-1}$)
r_j	Reaction rate for the compound *j* ($g \cdot min^{-1} \cdot g^{-1}$)
t	Time (min)
T	Temperature (K)
α_i	First autocatilytic factor (dimensionless)
β_i	Acceleration factor (dimensionless)

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SECTION II

FURTHER MICROWAVE CONSIDERATIONS

CHAPTER 5

Study of the possible influence of microwaves on solid-liquid equilibrium

CHAPTER 5

A STUDY OF THE POSSIBLE INFLUENCE OF MICROWAVES ON SOLID-LIQUID EQUILIBRIUM

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ABSTRACT

The influence of microwaves on solid-liquid equilibrium has been assessed. Gallic acid and sodium sulfate solubilities in water, ethanol, ethyl acetate and isopropyl alcohol were measured. Solid-liquid systems were selected according to their dielectric properties. The resulting microwave field was characterised in terms of absorbed power density and electric field intensity. To maintain constant temperature, the heat released by the absorbed microwave energy was continuously removed by external air-cooling. Although significant temperature variations were found, solubility errors caused by these deviations (an average of 4.3%) were proved not to be significant for the purpose of this work. Since none of the cases studied provide any significant improvement over the conventional results, it was concluded that no appreciable enhancement on the solid-liquid equilibrium took place because of microwave radiation.

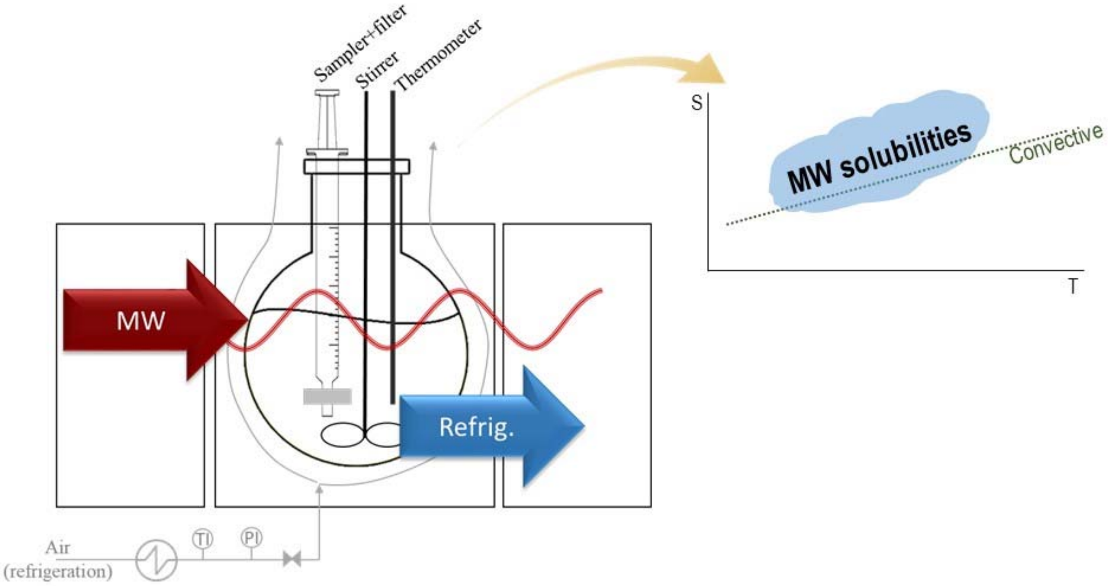
KEYWORDS

Solid-liquid equilibrium; solubility; microwave; dielectric properties.

HIGHLIGHTS

- » No appreciable microwave effect on solid-liquid equilibrium was found
- » Solvent dielectric properties did not affect solubility under microwave radiation
- » The dynamic system used led to temperature deviations but with no influence on conclusions

GRAPHICAL ABSTRACT



1. INTRODUCTION

The use of microwaves in extraction processes has been proved to be an asset, as it improves process efficiency and product quality (Álvarez et al., 2017b). Despite the fact that several studies have reported microwave assisted extraction (*MAE*) yield boosts (Dorantes-Alvarez et al., 2017, Vinatoru et al., 2017), in comparison to conventional extraction processes, only in a reduced number of cases a scientific reasoning able to explain such effect has been provided. This is the case of essential oil extraction by microwave assisted distillation. Trichomes are cellular structures where essential oils are stored. During microwave extraction, water inside trichomes wall cells increase its temperature above boiling point, causing an increase of pressure that entails the disruption of the cell wall (Navarrete et al., 2011, Lucchesi et al., 2007). In the case of most other active compounds (one of the most widely applications of microwave extraction), such evidences have not been yet reported. SEM images have been widely used in literature (Yedhu Krishnan et al., 2016), and they can be considered as indication of a possible damage in cell structure, but the identified yield enhancement cannot be explained in a conclusive way by image differences between microphotographs, as it was in the case of exploded trichomes.

Therefore, there is not a definitive understanding of fundamental changes caused by the use of microwaves in extraction processes. The aim of this work is to check if solid-liquid equilibrium may be affected by radiation, as an attempt to find a contribution to explain yield and kinetics increases observed in MAE. The assessment of this hypothesis is supported by the already ongoing discussion on the possible effect of microwaves on vapor-liquid equilibrium. Altman et al. (2010) found a change in the vapor-liquid equilibrium diagram of binary mixtures of propionic acid, 1-propanol, and n-propyl propionate when the system was microwave irradiated. The main claim was that the equilibrium shift was only found when the vapor-liquid interface was subjected to microwave radiation. In contrast, no alter took place when only the bulk liquid was heated by microwaves. Later, Gao et al. (2013) designed a device to measure vapor-liquid equilibrium (VLE) under microwave radiation to corroborate the results. Binary systems with different dielectric properties were tested to conclude that permittivity was a key variable for microwave effect on VLE. It is then clear that dielectric properties perform a crucial contribution, since the lower the loss tangent, the more energy is absorbed and so, superheating and hot spots take place. This can lead to the formation of an oversaturated vapor rich in the volatile compound (Werth et al., 2015, Li et al., 2017). Electric field intensity is another parameter important to consider. It characterises the strength of the radiation inside the media. Lossy materials (with a low dielectric loss) are inefficient absorbing and converting microwave energy into heat, so their electric intensity is enhanced.

Regarding solid-liquid equilibrium (SLE), no works related with microwaves have been yet published. The assumption that microwaves can also have an effect on SLE is supported by the likely interaction of the electromagnetic field with the solvent and solute molecules. The radiation may cause molecule re-orientation, both for molecular and for ionic species, what would affect interactions among them. Such interactions could infer a change in the value of the activity coefficients (γ_i^{sat}) and consequently, also in solubility $x_i^{sat}(s)$, as equation [1] (which describes the aqueous solubility of a compound *i* (Schwarzenbach et al., 2005)) proves.

$$x_i^{sat}(s) = \frac{1}{\gamma_i^{sat}} exp\left(-\Delta_{fus} G_i / RT\right) \qquad [1]$$

Therefore, the selection of the solvent is crucial since its permittivity will determine the energy absorption and so, the interactions between the solute and the solvent (Luque de Castro and Castillo-Peinado, 2016). As it can be seen in equation [2], permittivity (*ε**) consists of two terms: the dielectric constant (*ε'*), and an imaginary part, the loss factor (*ε''*). The dielectric constant is proportional to the solvent's ability to storage energy, whereas the loss factor represents the dissipation of energy in the form of heat (Ulaby et al., 2010). However, it is more common to use the ratio between these terms, which is known as the loss tangent (*tan δ*, equation [3]). It represents the ability of the solvent to dissipate and to absorb microwave energy (Leonelli et al., 2013).

$$\varepsilon^* = \varepsilon' - j\varepsilon'' \qquad [2]$$

$$tan\delta = \frac{\varepsilon''}{\varepsilon'} \qquad [3]$$

The higher this factor is, the faster the solvent will heat up. In this work, water, ethanol, ethyl acetate and isopropanol have been employed. Their selection was based on their dielectric properties, which are summarized in Table 1. These values have been measured in a Dielectric Kit facility (Catalcá Civera et al., 2011) and will be published in a near future. As it can be seen, dielectric constant, loss factor and loss tangent varied from efficient radiation converters to energy dissipaters (Mello et al., 2014, Veggi et al., 2013).

Table 1. Solvent dielectric properties at 25ºC and 2.45 GHz.

Solvent	$T_{boiling}$ (ºC)	Dielectric constant (ε')	Dielectric loss (ε'')	Loss tangent (tan δ)	Penetration depth (cm)
Water	99.61	79.02±0.24	7.464±0.094	0.09	2.32
Ethyl acetate	77.05	5.18±0.01	0.757±0.004	0.15	5.86
Isopropyl alcohol	82.65	4.14±0.02	3.069±0.034	0.92	1.29
Ethanol	78.09	7.95±0.02	7.229±0.204	1.28	0.76

Gallic acid (3,4,5-trihydroxybenzoic acid) and sodium sulphate (Na_2SO_4) were chosen as solutes. The first one is a representative compound for polyphenols and it is used as a reference in the majority of works dealing with antioxidant extraction from natural products, whereas the latter is an ionic specie, which will allow to assess if ionic conductivity (a relevant contributor to dielectric loss) has a major role.

In order to uphold solubility measurements, the homogeneity and stability of the system has to be assured for a period long enough to achieve phase equilibrium. In a system exposed to microwave radiation, such uniformity has to be achieved in terms of electric field and temperature. A previous study regarding electric homogeneity has proved that the heating of the unit employed in this work (a CEM Microwave) is not uniform, but a vigorous stirring greatly ameliorates it (Sturm et al., 2012). On the other hand, temperature stability is harder to attain, since it is a dynamic system. In conventional solubility measurements, thermal stability is easily achieved just by placing the media in a constant temperature bath. The system reaches the equilibrium temperature by itself, and no or negligible heat flow or temperature gradient are present in the system once equilibrium is achieved. In contrast, the addition of microwaves highly complicates the operation. Equilibrium cells irradiated with microwaves behave as dynamic systems, where two energy flows are involved: the heat released by microwave absorption, and the refrigeration required to remove that heat flow. In the case of vapour-liquid equilibrium, a volumetric refrigeration is spontaneously performed by liquid boiling, which keeps constant temperature since this value is determined by pressure and equilibrium. The heat is finally removed through the vapour exiting the cell, which must be condensed outside and returned to the liquid phase. This behaviour ensures a global constant temperature in the liquid. In contrast, the case of solid-liquid equilibrium operation is definitely more complex, since both heat flowrates have to be keep equal independently to achieve a constant cell temperature. Heating is controlled by microwave absorption, which depends on irradiated power and cell conditions, while cooling is performed by external air-cooling, determined by air flowrate and temperature. Vigorous stirring is also essential to achieve uniform temperature and minimize temperature gradients, mainly in the boundary layer close to the cell wall. Microwave power fluctuations, due to the inherent instability of magnetrons, and difficulties to keep constant refrigeration in a small microwave cavity, pose a serious problem to maintain constant temperature conditions in solubility cells which must be operated at temperatures certainly above ambient conditions.

In sum, the objective of this study is to asses if microwaves can have any appreciable effect on solid-liquid equilibrium. Should be clear that the aim of this work is not to obtain high precision solubility data, as it is the usual case of solid-liquid equilibrium papers were microwaves are not used, but only to elucidate if the effect of radiation could be relevant enough to be considered as one of the contributions to kinetics and yield improvement in microwave assisted extractions. The availability of high precision results would require a high cost method, only needed for a deeper analysis. Additionally, this work also provides conventional solubility measurements at high temperature, which are quite scarce in literature.

2. MATERIALS AND METHODS

2.1. MATERIALS

Gallic acid (97.5-102.5% - titration) was purchased from Sigma, and sodium sulfate, absolute ethanol (not denatured), ethyl acetate, isopropyl alcohol and methanol from Panreac. Ultrapure water was obtained from a MilliQ unit (Millipore).

2.2. EXPERIMENTAL PROCEDURE

A 50% oversaturated solution was prepared in a round-bottom flask. A such concentrated system was selected to maximize the interface area, which is the region where according to literature the possible microwave effect could take place. A thermometer, a stirrer and a sampler were also placed inside the flask from the beginning of the experiment, to ensure constant temperature in all elements. Temperature was continuously recorded by an optical fibre (TempSens, Opsens and OTG-A fiber). The stirrer consisted in two baffles connected to an external motor. Rotation speed was set around 1000 rpm. The sampler was a syringe (PP/PE, without a rubber lock) with a 0.22 µm PVDF filter (Jet Biofil). The syringe was immersed into the solvent from the beginning of the experiment, but an air chamber was maintained in the intake of the syringe to keep the filter separated from the solution until sampling. The presence of all these elements was proved to have little effect on the electromagnetic field (Álvarez et al., 2017a, Lehndorff and Schilling, 1993, Shigeyoshi, 1995).

The flask was placed in the microwave cavity of a CEM Discover ONE unit. The microwave was set to irradiate a constant power (30, 50, 70 or 90 W). In order to use the higher power values without achieving boiling temperature, a high flow of constant temperature chilled air was continuously blown over the external surface of the flask. Since air is transparent to microwave radiation, it allowed refrigerating without interfering with the electromagnetic field. Air was previously cooled down by passing through an ethylene glycol bath. Its flow was regulated according to the system requirements.

The system was operated using constant operation variables until temperature was maintained below a certain fluctuation for a minimum period of three hours. Then, a sample was taken into the syringe through the filter; and saved for further characterization.

In order to achieve constant temperature operation, the heating power released by radiation (Q_{MW}) must be identical to the air-cooled refrigeration flow (Q_{ref}). This means that equation [4] has to be accomplished.

$$Q_{MW} = Q_{ref} \qquad [4]$$

Proper characterization of microwave heating flow must be reported in terms of absorbed power density (*heating power*), since the use of emitted power may be confusing as absorption efficiency is a function of many parameters (microwave cavity, sample location, sample amount…), and so difficult to reproduce. The conversion from consumed electric power to emitted microwave power in the magnetron has a usual efficiency around 70%, but efficiency conversion from emitted to absorbed power can vary in a wide range from 40 to 90% (Wang et al., 2015). The former depends on the magnetron efficiency, and the second on system permittivity. In this work, the absorbed power (Q_{MW}) is reported. It has been evaluated by calculating the air-cooled refrigeration heat flow, according to equation [4]. For this purpose, once the solubility measurement was finished, microwave radiation was stopped, but the rest of the system (stirring and air cooling) remained unaltered. The equilibrium cell temperature (T_i) cooling evolution was then recorded, and the resulting curve was adjusted to the equilibrium cell non-stationary energy balance, gathered in equation [5].

$$mC_P \frac{dT_i}{dt} = Q_{ref} \qquad [5]$$

By integrating this equation together with Newton's Law (equation [6]), the expression [7] was finally obtained.

$$Q_{ref} = US \cdot \left(T_i - T_{ref}\right) \qquad [6]$$

$$ln\left(\frac{T_i - T_{ref}}{T_{eq} - T_{ref}}\right) = \frac{US}{mC_P} \cdot t \qquad [7]$$

The refrigeration heat transfer coefficient value (US) was calculated by regressing the experimental temperature evolution data (t, T_i) to equation [7], starting at t=0 with the equilibrium temperature (T_{eq}). Then, this US value was used in equations [4] and [6] to obtain the constant power absorbed by the system during the equilibrium experiment (Q_{MW}), using for T_i the constant equilibrium cell temperature value (T_{eq}).

In the above expressions, C_P represents the heat capacity in J/gK, m the mass of the sample in the equilibrium cell in grams, Q energy in watts, T_i the temperature of the sample in Celsius degrees, T_{ref} the temperature of the cooling air in Celsius degrees, and US the heat transfer coefficient for the exchange surface in W/K.

The average effective value of the electric field strength (E) is also an important parameter to characterise the media. It can be calculated with the equation [8], where E stands for the electric field intensity in V/m, f is the frequency (2.45 GHz), P''' the power density measured in W/m^3, ε_0 the free space permittivity in F/m, and ε_r'' the dielectric loss factor of the sample. The value of P''' is obtained dividing Q_{MW} by the volume of the liquid sample (V).

$$P''' = \frac{Q_{MW}}{V} = 2\pi f \varepsilon_0 \varepsilon'' E^2 \qquad [8]$$

To assess any possible microwave effect, solubility results were compared with bibliographic data and measurements obtained by conventional convective heating in a thermostatic bath. These measurements were performed in an ethylene glycol thermostatic bath, with vigorous stirring and continuous temperature control. The experimental procedure followed was alike the one described above: a syringe with a filter was initially introduced in the media and after at least three hours of thermal stability a sample was taken.

2.3. CONCENTRATION ANALYSIS

The concentration of the saturated solutions was determined by gravimetry. First, the empty syringe and the syringe with the sampled solution were weighted. In order to avoid solvent vaporisation, a lid was always used to keep the syringe closed. The sample was poured in a crystal vial, and methanol or water were used to consciously wash the syringe so that any possible remaining crystallized solute was dragged. The vial with the sample and the washing liquid was dried at 105ºC overnight and weighted. Equation [9] was used to compute the solubility concentration. Results were expressed in $mg_{Solute}/100\ g_{Solvent}$.

$$S = \frac{m_{vial+solute} - m_{vial}}{m_{syringe+solute+solvent} + m_{vial} - m_{syringe} - m_{vial+solute}} \cdot \frac{1}{100} \qquad [9]$$

2.4. EXPERIMENTAL ERROR AND PROPAGATION

Temperature uncertainty was computed as the maximum deviation of the recorded temperature (measured with an accuracy of 0.1ºC) during the thermal stability period of each experiment (3 hours). A mean variation of 0.5ºC was found among all the experiments. Although this value is unsound for a conventional solubility report, its influence on the final measurement will be proved to be low enough for the aim of this study.

Solubility uncertainty (ΔS) is calculated in this paper from the two main contribution terms to this value: gravimetric determination uncertainty (u_S) and temperature fluctuation uncertainty ($u_{\Delta T}$); accordingly to equation [10].

$$\Delta S^2 = \mathrm{u}_{\mathrm{S}}^2 + \mathrm{u}_{\Delta T}^2 \qquad [10]$$

Contribution to solubility uncertainty due to gravimetry (u_S) was calculated as an indirect measurement from the weights used to compute it, as it is detailed in equation [11]. The derivative of S calculated from expression [11] was used in equation [9] to calculate the u_s value due to weight measurements uncertainty (u_m).

$$u_S = \left(\frac{dS}{dm}\right) \cdot u_m \qquad [11]$$

On the other hand, solubility deviations caused by temperature fluctuations ($u_{\Delta T}$) were worth to be also considered. Maximum and minimum values of the temperature fluctuation range were transformed into corresponding solubilities by means of the temperature-solubility curve obtained by regression of the solubility measurements in the thermostatic bath. The value $u_{\Delta T}$ is calculated by subtracting these two values.

Solubility uncertainty computed thereby was considered along with the experimental measurement as the solubility measurement ± the uncertainty.

3. RESULTS AND DISCUSSION

3.1. GALLIC ACID SOLUBILITY IN DIFFERENT SOLVENTS

Water, ethanol, ethyl acetate and isopropyl alcohol were chosen because of their dielectric properties (Table 1) to analyse the effect of microwave radiation on the solid-liquid interface and its impact on solubility. Figure 1 represents the results obtained for gallic acid solubility in water, whereas Figure 2, Figure 3 and Figure 4 gather solubilities of gallic acid in ethanol, ethyl acetate and isopropyl alcohol, respectively. Empty squares stand for bibliographic data. The rest corresponds to experimental results of this work. Gallic acid water solubility was comprehensively measured by other authors in previous works (Lu and Lu, 2007, Srinivas et al., 2010, Daneshfar et al., 2008, Grünewald, 1964). For the other solvents, not so many bibliographic data was available. Daneshfar (2008) solubilities were included for ethyl acetate. Ethanol reported data were excluded since it was below the temperature range of this study. No measurements regarding isopropyl alcohol were found.

As it can be seen in these figures, temperature error bars associated to microwave experiments are much larger than those obtained with a constant temperature bath (imperceptible). These errors were mainly due to temperature fluctuations caused by the dynamic behaviour of the microwave equilibrium cell, as it was explained in the introduction section. The contribution of the uncertainty associated to the gravimetric analysis was negligible (0.01%). However, the associated errors on solubility caused by these temperature deviations were not so high to hinder the identification of a possible microwave athermal effect, which was the aim of this work. For instance, in the case of water, uncertainties due to temperature were in the range of ±0.3-1.2ºC, which implied solubility errors of 2.3-9.9%, with an average of 5.3% (errors detailed in Table 2). Such large variations are unsuitable for a solubility assessment. However, for the purpose of this study, they can be considered not that unreasonable in comparison to the error of the correlated convective solubility values, which was intimately related to the accuracy of the measurement. Continuing with the case of water, since the same tendency was observed for the microwave experiments, a common model for both sets (microwave and conventional) could be developed. This model was obtained by a regression based on the conventional solubilities. It was able to reproduce conventional results with 1.8% error and microwave solubilities with 3.0%. So, it can be checked that the solubility error due to the temperature uncertainty is quite similar to the error between the correlated and the experimental values. Thus, it can be concluded that the effect of the temperature fluctuations was not significant. The same deduction was achieved when the rest of the solvents studied were considered. Ethanol, ethyl acetate and isopropyl alcohol presented average solubility errors due to temperature instabilities (3.6%, 6.3% and 3.9%) slightly larger than the error between the conventional correlation and the microwave results (2.9%, 4.7% and 3.4%, respectively).

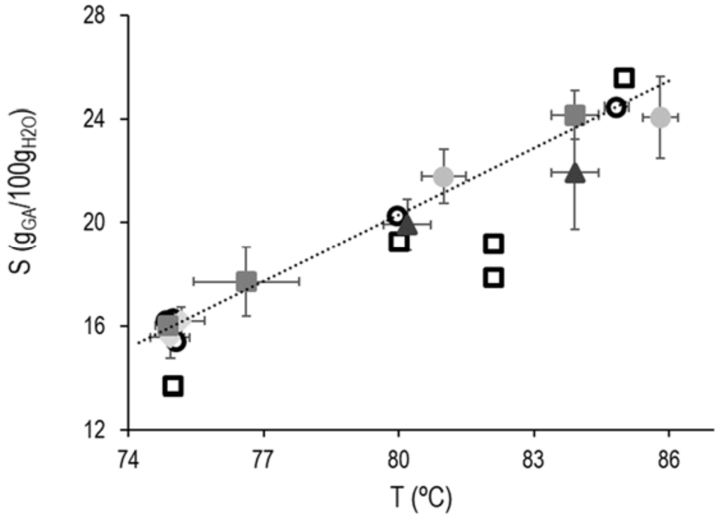


Figure 1. Water gallic acid solubility measured by conventional (□ Bibliographic data, ○ Experimental convective heating,⋯⋯ regression of Experimental convective heating) and microwave heating (♦30 W, ■50 W, ▲ 70 W, ● 90 W).

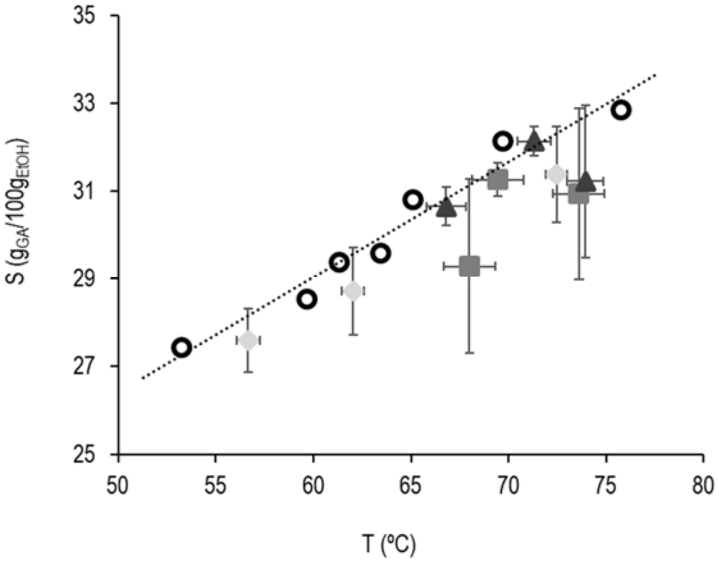


Figure 2. Ethanol gallic acid solubility measured by conventional (○ convective heating, ,······ regression) and microwave heating (♦30 W, ■ 50 W, ▲ 70 W).

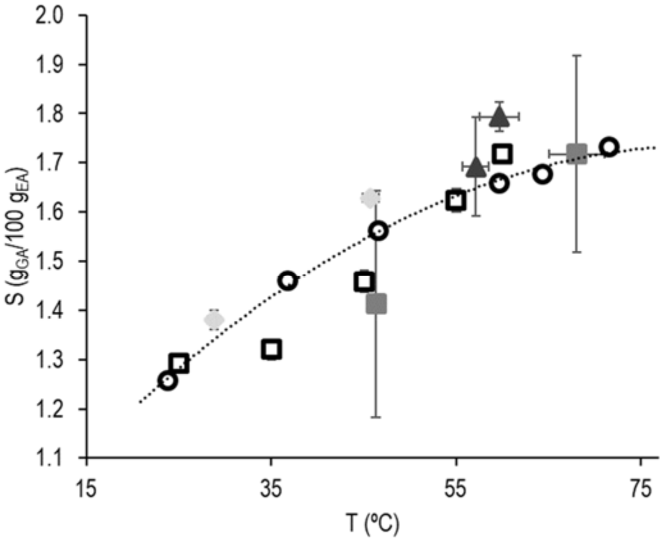


Figure 3. Ethyl acetate gallic acid solubility measured by conventional (□ Bibliographic data, ○ convective heating,······ regression) and microwave heating (♦30 W, ■ 50 W, ▲ 70 W).

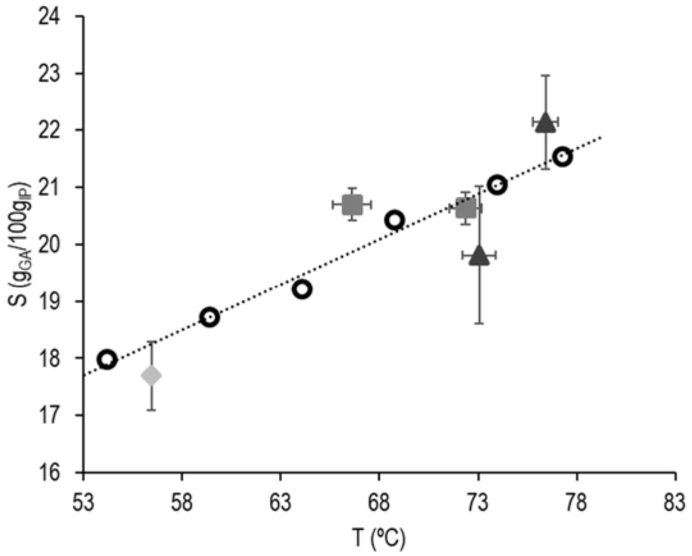


Figure 4. Isopropil alcohol gallic acid solubility measured by conventional (○ convective heating,⋯⋯ regression) and microwave heating (♦30 W, ■50 W, ▲ 70 W).

As it can be seen in the previous figures, microwave and conventional solubilities were in the same range in all the studied cases. Neither of the solvents tested provided a microwave improvement over the convective results. If there was to be a microwave athermal enhancement, it would be expected to be enough prominent so as to be able to explain MAE yield boost. Considering that MAE processes usually present yield improvements over 20% (Vinatoru et al., 2017), solubility errors of around 5% would still allow to identify such effect. However, results did not corroborate this hypothesis. Therefore, no evidences that microwaves influence equilibrium, and thus, no contribution to boost MAE yield were observed.

Regarding solvent dielectric properties, none of the different microwave-solvent interaction grades tested provided any impact on equilibrium. Although for VLE dielectric properties were found to be crucial (Altman et al., 2010), SLE was independent of the solvent permittivity.

Table 2. Gallic acid solubility measurements in water, ethanol, ethyl acetate and isopropyl alcohol.

Solvent	Irradiated power (W)	T (ºC)	S $(\mathrm{mg_{GA}}/100\mathrm{g_{solvent}})$	Power density (kW/m^3)	Electric field (kV/m)
Water	convective heating	74.8 ± 0.1	16.22 ± 0.00	-	-
		75.0 ± 0.1	16.29 ± 0.00	-	-
		75.1 ± 0.1	15.37 ± 0.00	-	-
		80.0 ± 0.1	20.27 ± 0.00	-	-
		84.8 ± 0.2	24.47 ± 0.00	-	-
	30 W	74.9 ± 0.4	15.54 ± 0.78	431	19.89
		75.2 ± 0.5	16.20 ± 0.51	433	19.94
	50 W	74.9 ± 0.3	16.02 ± 0.37	637	24.19
		76.6 ± 1.2	17.71 ± 1.33	645	24.34
		83.9 ± 0.5	24.16 ± 0.93	736	25.99
	70 W	80.2 ± 0.5	19.92 ± 0.99	761	26.44
		83.9 ± 0.5	21.94 ± 2.18	758	26.38
	90 W	81.0 ± 0.5	21.80 ± 1.05	738	26.02
		85.8 ± 0.4	24.07 ± 1.58	768	26.55
Ethanol	convective heating	53.3 ± 0.1	27.43 ± 0.00	-	-
		59.7 ± 0.1	28.53 ± 0.00	-	-
		61.3 ± 0.1	29.39 ± 0.00	-	-
		63.5 ± 0.1	29.59 ± 0.00	-	-
		65.1 ± 0.1	30.81 ± 0.00	-	-
		69.7 ± 0.1	32.14 ± 0.00	-	-
		75.8 ± 0.2	32.85 ± 0.00	-	-
	30 W	56.7 ± 0.6	27.60 ± 0.72	293	16.66
		62.0 ± 0.6	28.72 ± 1.01	340	17.95
		72.5 ± 0.5	31.38 ± 1.08	406	19.62
	50 W	68.0 ± 0.5	29.29 ± 1.99	324	17.51
		69.4 ± 0.4	31.26 ± 0.38	338	17.89
		73.6 ± 1.0	30.94 ± 1.95	361	18.50
	70 W	66.8 ± 1.0	30.65 ± 0.44	394	19.33
		71.3 ± 0.9	32.14 ± 0.35	368	18.68
		73.9 ± 0.9	31.23 ± 1.73	409	19.68

Table 2 (continued). Gallic acid solubility measurements in water, ethanol, ethyl acetate and isopropyl alcohol.

Solvent	Irradiated power (W)	T (ºC)	S ($\mathrm{mg_{GA}/100g_{solvent}}$)	Power density (kW/m^3)	Electric field (kV/m)
Ethyl acetate	convective heating	23.8 ± 0.1	1.26 ± 0.00	-	-
		36.8 ± 0.1	1.46 ± 0.00	-	-
		46.6 ± 0.1	1.56 ± 0.00	-	-
		59.7 ± 0.1	1.66 ± 0.00	-	-
		64.3 ± 0.2	1.68 ± 0.00	-	-
		71.6 ± 0.2	1.73 ± 0.00	-	-
	30 W	28.8 ± 0.7	1.38 ± 0.02	307	52.70
		45.8 ± 0.9	1.63 ± 0.01	281	50.44
	50 W	46.3 ± 0.6	1.41 ± 0.23	323	54.07
		68.1 ± 3.0	1.72 ± 0.20	312	53.16
	70 W	57.2 ± 1.4	1.69 ± 0.10	531	69.33
		59.7 ± 2.2	1.79 ± 0.03	496	67.03
Isoporpyl alcohol	convective heating	36.8 ± 0.1	15.91 ± 0.00	-	-
		54.2 ± 0.1	17.97 ± 0.00	-	-
		59.4 ± 0.2	18.71 ± 0.00	-	-
		64.1 ± 0.1	19.21 ± 0.00	-	-
		68.8 ± 0.2	20.43 ± 0.00	-	-
		73.9 ± 0.3	21.05 ± 0.00	-	-
		77.3 ± 0.3	21.54 ± 0.00	-	-
	30 W	56.4 ± 0.3	17.70 ± 0.60	515	33.91
	50 W	66.6 ± 1.0	20.70 ± 0.98	641	37.84
		72.4 ± 0.8	20.63 ± 0.28	738	40.58
	70 W	73.1 ± 0.8	19.81 ± 1.21	771	41.49
		76.4 ± 0.7	22.15 ± 0.82	801	42.27

Table 3. Average global heat coefficient US (W/K)

	H2O	EtOH	EA	IP
US (W/K)	0.77 ± 0.13	0.40 ± 0.03	0.55 ± 0.10	0.87 ± 0.02

In order to characterise each equilibrium media, total absorbed power and electric field intensity have been computed. Results can be found in Table 2. Absorbed power has been computed by means of the global heat coefficient, which its estimation is presented in Table 3 for each solvent. These values presented deviations of around 10%. Although it can be thought to be excessive, they are in agreement with the error of well stablished correlations (Sinnot, 2000). So, these values were taken as suitable to estimate the total absorbed power. In regard to power efficiency, it was noticed that the absorbance efficiency decreased when power was increased, from 92.6% at 30 W to 61.2% at 70 W. This absorbance efficiency was calculated as the ratio between the absorbed power and the nominal emitted power. The average efficiency was 71%. Concerning electric intensity, it is a parameter that, although describes the power density distribution inside the media, the great majority of microwave works do not include it. The intensity values provided by Yi-Chung et al. (2005) for microwave heating (10^4-$5 \cdot 10^4$ V/m) were in the same range of the intensities found in this work. Thus, it can be said that a sufficient intense electromagnetic field has been employed to validate the conclusions of this work. Regarding these two parameters, absorbed power and electric field intensity, results agreed with solvent dielectric properties. Water power absorbance (around 656 kW/m^3) was in accordance with its high dielectric constant, whereas ethyl acetate low dielectric loss determined its low efficiency to transform radiation, and hence, its large electric strength (around 49.35 kV/m). Ethanol presented the lowest absorbed power and electric intensity (a mean of 359 kW/m^3 and 18.42 kV/m, respectively). Isopropyl alcohol presented an intermediate behaviour where high power was absorbed as well as transferred (a mean absorbed power of 693 kW/m^3 and an intensity of 39.22 kV/m). This discussion can also be observed in Figure 5, where a comparison between power density and electric field intensity is presented.

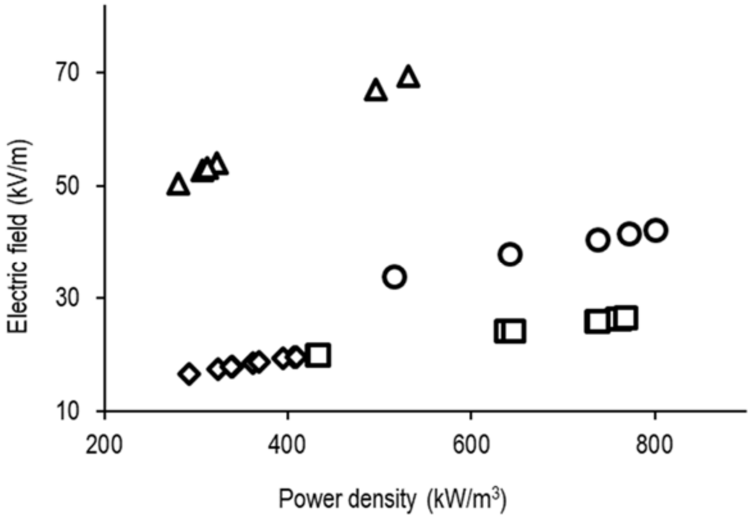


Figure 5. Power density and electric field distribution for the gallic acid solid-liquid equilibrium mediums with water (□), ethanol (◊), ethyl acetate (Δ) and isopropyl alcohol (○).

So, as it can be seen, a wide range of conditions was tested. It can be then concluded that, neither the absorbed power, the transparency to radiation nor the electric strength had a significant effect on solid-liquid equilibrium.

3.2. SODIUM SULFATE SOLUBILITY

Sodium sulfate solubility in water was analysed in order to elucidate if the presence of an ionic specie could imply a different behaviour. The loss factor of the medium was boosted by the presence of electrolytes. Such increase was estimated by means of the electrical conductivity since no experimental dielectric measurements were available. At ambient temperature and for a 20% (w/w) solution (equilibrium), sodium sulfate electrical conductivity is 109 mS/cm (Haynes et al., 2017) . This value entailed a 54% rise of the loss factor. Thus, the new medium (water and the electrolytes) maintained the high dielectric constant of water and doubled the loss factor, and so the loss tangent, what could lead to a different tendency.

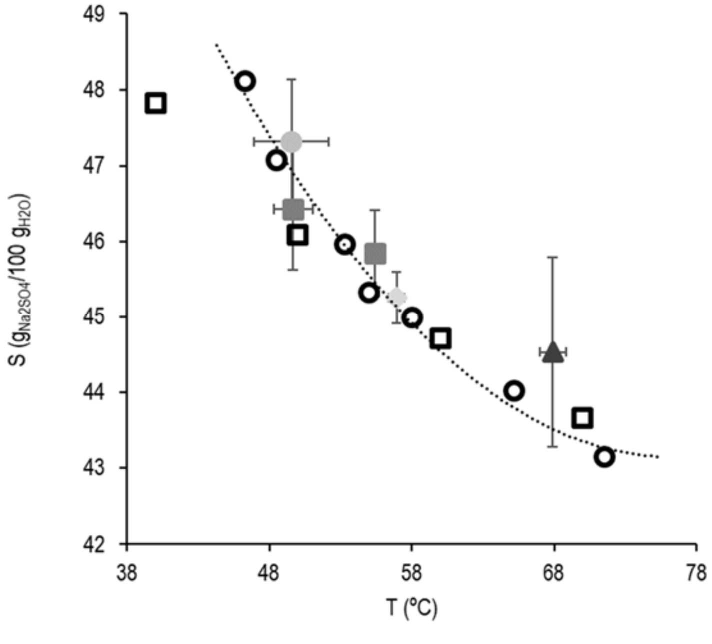


Figure 6. Sodium sulfate solubility in water measured by conventional measurement (□ Bibliographic data, ○ convective heating, ······ regression) and microwave heating (♦30 W, ■50 W, ▲ 70 W, ●90 W).

Figure 6 depicts the results obtained, which are also gathered in Table 4. Bibliographic data was used to compared the results (Haynes et al., 2017). Previously reported data presented a substantial sharp change in the solubility tendency at 40ºC. However, since literature data showed a continuous temperature behaviour in agreement with the experimental measurements, bibliographic solubilities in the studied temperature interval (40-70ºC) were taken as suitable.

Table 4. Sodium sulfate solubility experimental data.

Irradiated power (W)	T (ºC)	S ($mg_{Na2SO4}/100g_{water}$)	Power density (kW/m^3)
convective heating	46.3 ± 0.1	48.12 ± 0.00	-
	48.5 ± 0.2	47.08 ± 0.00	-
	55.0 ± 0.1	45.33 ± 0.00	-
	58.0 ± 0.1	44.99 ± 0.00	-
	53.3 ± 0.2	45.96 ± 0.00	-
	65.2 ± 0.2	44.02 ± 0.00	-
	71.5 ± 0.2	43.15 ± 0.00	-
30 W	56.9 ± 0.5	45.25 ± 0.34	543
50 W	49.7 ± 1.4	46.42 ± 0.81	525
	55.4 ± 0.4	45.84 ± 0.57	595
70 W	67.9 ± 0.9	44.53 ± 1.25	778
90 W	48.2 ± 2.6	47.31 ± 0.82	710

Microwave experiments presented similar results to convective heating solubilities. So, no microwave effect was again observed. In this case, the solubility errors due to thermal deviations resulted to be between 0.75% and 2.81%. But again, as in the previous cases, these errors were estimated low enough to discard any microwave effect on sodium sulfate solubility.

Regarding medium characterization, absorbed power was computed with the global heat transfer coefficient US=1.03±0.12 W/K (as expected, very similar to the water coefficient of Table 3). High power densities were attained, varying between 525 and 778 kW/m^3 (an outstanding high average power efficiency of 98%). Field intensity could not be computed since the dielectric properties of the medium were not available at the temperature range employed in this work. However, a great electric field intensity was presumed due to the contribution of ionic losses to the dielectric loss.

4. CONCLUSIONS

The advantages of implementing microwaves on extraction processes have been widely reported. However, there is still no clear understanding of the role of microwaves in these processes. Evident proofs able to explain the yield and kinetics enhancements promoted by microwaves have not been yet provided, except for the case of trichomes explosion of essential oils. This work tried to elucidate the possible contribution to the explanation of these MAE enhancements by analysing if solid-liquid equilibrium can be altered by microwave radiation.

Gallic acid and sodium sulphate were chosen as solutes, to consider molecular and ionic species. Water, ethanol, ethyl acetate and isopropyl alcohol were the solvents tested. They were selected according to their wide dielectric properties ranges, which varied from efficient microwave energy converters to energy dissipaters. Water presented a high absorbed power with a mean value of 656 kW/m^3, ethyl acetate the largest electric field intensity (an average of 57.79 kV/m), and isopropyl alcohol a combination of high power (693 kW/m^3) and high electric field intensity (18.42 kV/m). Ethanol had the lowest power absorbance (359 kW/m^3) and electric intensity (18.42 kV/m). The mixture sodium sulfate-water was estimated to present a large power absorbance and a great electric intensity, boosted by the presence of electrolytes. However, due to the lack of dielectric measurements for this combination, electric field intensity could not be computed.

A dynamic system with two continuous compensated flows (microwave heating and refrigeration) was employed to perform the measurements. Since the dynamic equilibrium was not stable enough, a certain temperature fluctuation was found. These fluctuations led to average gallic acid solubility errors of 5.3%, 3.5%, 6.3% and 3.9% for water, ethanol, ethyl acetate and isopropyl alcohol, respectively. Sodium sulfate solubility average error was estimated to be 1.7%. Despite the fact that these errors are large for solubility reported data, they were acceptable for the objective of this study.

It was concluded that microwaves did not have any significant effect on the solid-liquid equilibria systems studied. Therefore, no contribution to the explanation for the MAE yield boost was found in a possible solid-liquid equilibrium alteration caused by microwaves.

ACKNOWLEDGEMENTS

This work was funded by Junta de Castilla y León regional project VA040U16. A.A. thanks the Spanish Ministry of Education for her grant FPU13/04678.

CHAPTER 5

NOMENCLATURE

Acronyms

EA	Ethyl acetate
EtOH	Ethanol
H2O	Water
IP	Isopropyl alcohol
MW	microwave
Ref	Refrigeration

Symbols

C_P	Heat capacity (J·g^{-1}·K^{-1})
E	Electric field intensity (V·m^{-1})
f	Frequency (Hz)
m	Mass (g)
P'''	Power density (W·m^{-3})
Q_{MW}	Absorbed power (W)
Q_{ref}	Air cooled refrigeration Flow (W)
R	Ideal gas constant (J·mol^{-1}·K^{-1})
S	Solubility (g/100g$_{solvent}$
T	Temperature (ºC)
T_{eq}	Equilibrium temperature (ºC)
T_i	Temperature evolution of the system during cooling after equilibrium measurement (ºC)
T_{ref}	Temperature of refrigeration air (ºC)
tan δ	Dissipation factor (dimensionless)
US	Global heat transfer coefficient (W·K^{-1})
x_i^{sat}	Concentration of compound *i* at saturation (mol·mol^{-1})
g_i^{sat}	activity coefficient of compound *i* at saturation (dimensionless)
$\Delta_{fus}G_i$	Free energy of fusion of the compound *i* (J·mol^{-1})
ε'	Dielectric constant (F·m^{-1})/(F·m^{-1})
ε''	Loss factor (F·m^{-1})/(F·m^{-1})
ε_0	Free space permittivity (F·m^{-1})

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SECTION III

UTILITY OF THE KNOWLEDGE DEVELOPED FOR OTHER BYPRODUCTS

CHAPTER 6

Microwave pretreatment optimization for the extraction of olive pomace polyphenols

MICROWAVE PRETREATMENT OPTIMIZATION FOR THE EXTRACTION OF OLIVE POMACE POLYPHENOLS

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ABSTRACT

Industrial constrains regarding polyphenol extraction from olive pomace has been considered to develop an efficient and easy scalable microwave pretreatment process, able to intensify the conventional solid-liquid extraction stage. So, a short but intense microwave pretreatment has been optimised, together with the conventional extraction process. The analysed operating variables for the conventional solid-liquid extraction stage were temperature (40-70ºC), solvent composition (ethanol-water 25-75% v/v) and solid:liquid ratio (0.05-0.50 g/mL). For the intensification step, the variable studied was the absorbed specific energy (0.06-1.26 kJ/g), in the presence and absence (solvent free) of solvent. The extraction kinetics were analysed to determine the efficiency of the process, and the composition of the extract was measured to estimate the quality of the final product. Solvent free microwave pretreatment resulted to provide the best results in terms of 1) initial extraction rate (extraction time was considerably reduced up to 6.5 times), and 2) dry product richness (a 92% more concentrated extract was obtained). In particular, the concentration of hydroxytyrosol and oleuropein were boosted a 35% and a 181%, respectively. The disadvantage of this pretreatment was the production of hydroxymethyl furfural, an undesirable compound. To conclude, it can be said that, although the implementation of the pretreatment did not significantly enhanced the extraction, the product quality was substantially improved.

KEY WORDS

Microwave pretreatment; olive pomace; polyphenol extraction; process efficiency; product quality.

HIGHLIGHTS

- Industrial limitations, mainly regarding solvent consumption, have been considered
- Microwave pretreatment optimization for the extraction of polyphenols from olive pomace
- Solvent free microwave pretreatment provided a richer extract in a shorter extraction time
- The composition of hydroxytyrosol and oleuropein was boosted by the pretreatment
- The undesired compound hydroxymethyl furfural appeared in the solvent free microwave pretreatment

1. INTRODUCTION

Olive oil consumption has gained a lot of popularity as a consequence of the Mediterranean diet expansion (Dinu et al., 2017). According to such demand, world olive oil production has increased by 14% since the beginning of the new millennium (FAOSTAT-FAO, 2015). However, this industry also entails the generation of large quantities of residues, namely olive pomace, which have to be treated before their disposal because of environmental sustainability concerns (Albahari et al., 2018). If the olive pomace generation rate of 35-40 kg/100 kg of olives is considered (Akay et al., 2015), in 2014 more than 1.4 million tonnes of olive pomace were produced worldwide. This entails a severe environmental problem regarding its management (Clemente et al., 1997, Lesage-Meessen et al., 2001). On the other hand, due to its great content in active compounds, olive pomace has recently become a potential source of polyphenols. The further valorisation of this second generation biomass would be beneficial for the oil industries, since waste-managing services would be reduced as well as new incomes would appear from polyphenol trading (Roselló-Soto et al., 2015, Putnik et al., 2017).

The polyphenolic extract obtained from this by-product would be interesting for its antioxidant activity and its consequent ability to subdue free radical induced diseases (Nowshehri et al., 2015). In particular, olive pomace is rich in four high-valuable compounds: tyrosol, hydroxytyrosol, oleuropein and oleocanthal (Adhami et al., 2015, Wani et al., 2018). This latter compound, oleocanthal, has recently drawn a lot of attention because of its outstanding health promoting benefits (Batarseh et al., 2017, Ayoub et al., 2017, Polini et al., 2018). However, it is not yet widely commercially available and it has to be synthesised by complex routes (Smith et al., 2005) or isolated from olive oils (Impellizzeri and Lin, 2006). It must be noticed that a natural extract containing a mix of active compounds presents a better biofunctionality than the individual components, as it was proved for similar extracts, obtained from olive leaves (Frankel et al., 2013).

Concerning the extraction process used to release the polyphenols from the olive pomace, severe conditions are required. Maceration (solid-liquid extraction at a mild temperature) has been found not to provide high extraction yields, despite its large solvent consumption, the long extraction time or the addition of acids to enhance it (Albahari et al., 2018, Alu'datt et al., 2010). Instead, better efficiencies have been achieved with pressurized solid-liquid extractions, although still long extraction time and large solvent consumption are required. For instance, Aliakbarian et al (2011) optimized the extraction by using 0.10 g/mL of pretreated pomace with methanol at 180ºC for 90 minutes. The optimal temperature of 180ºC was found after also studying 100ºC and 150ºC. The kinetic analysis at these temperatures proved that there was no yield enhancement at the lower temperatures. The yield was only boosted for the extraction conditions of 180ºC and 90 minutes, to fall down again after 120 minutes, probably due to polyphenol degradation. Such temperature was also pointed out by Moubarik et al. (2015) when the thermogravimetric analysis of the olive kernel (a main constituent of the pomace) proved that this structure was stable until 180ºC. These results lead to the conclusion that the olive pomace is difficult to disrupt, and its polyphenols are highly bounded in the structure. Therefore, severe conditions able to distort the solid matrix are needed to achieve an effective extraction. Thus, it is sound to consider the implementation of an intensification step in order to improve the extraction efficiency. Microwave, ultrasound and high voltage assisted extraction are the three most extended techniques. They have been thoroughly studied not only for olive pomace, but for the other olive residues too (Roselló-Soto et al., 2015, Albahari et al., 2018). Nonetheless, it stands out that, when microwaves have been studied for the extraction of polyphenols from the pomace, non-edible solvents like hexane and methanol have been the only ones tested (Yanık, 2017, Pérez-Serradilla et al., 2007). These solvents imply strong restriction when used for foodstuff (EU Directive 2009/32/EC, 2009), what makes them unsuitable for the main application of this product. So, there is a lack of knowledge here that this work aims to fulfil.

Microwaves are proposed here as a pretreatment prior to a conventional solid-liquid extraction. The main feature of this pretreatment is that it is a short but intense step easy to be scaled-up for a future industrial implementation. Due to the drastic reduction of the residence time inside the microwave in comparison to microwave assisted extraction processes (MAE), the oven size is also substantially shrunk. Thus, a reasonable size microwave oven with higher power density can be employed. During the pretreatment, the extraction media is rapidly heated up to a high temperature, which causes an acceleration in the extraction of the active compounds. After reaching that desired temperature, the media is quickly cooled down to avoid the thermal degradation of the polyphenols (Sólyom et al., 2014). The extraction is continued as a conventional solid-liquid process to allow the leaching of the remaining compounds. This microwave pretreatment approach has already been proved, and with good results, during the extraction of polyphenols from grape pomace (Álvarez et al., 2017a).

Regarding the extraction conditions, special attention has been paid to industrial limitations, namely the type and the amount of solvent needed. From a green chemistry point of view, there is a trend to substitute organic solvents for benign options (Anastas and Waner, 1998), a policy that is also supported by legal restrictions and taxes. Ethanol can be a good alternative since it is considered a GRASS (generally recognized as safe) solvent by the FDA (Putnik et al., 2017). Additionally, it has been found to provide similar extraction yields than methanol for the case studied here (Aliakbarian et al., 2011, Paini et al., 2015). However, it must be also pointed out that ethanol extracts have a slightly inferior quality as they present a lower antioxidant activity (Paini et al., 2015). Nonetheless, a mixture of polar and non-polar solvents, like water and ethanol, is generally employed in this kind of processes in order to solubilize all types of compounds. The other important fact to consider is the solvent consumption. Many works deal with extremely low solid-liquid ratios (like 0.020 g/mL (Albahari et al., 2018)) that are feasible at laboratory scale but completely unrealistic at industrial scale. So, in this work higher solid-liquid ratios have been tested.

In sum, this work seeks the optimization of the polyphenol extraction from olive pomace considering industrial concerns. A microwave pretreatment prior to the conventional solid-liquid extraction is studied as an alternative for the intensification of the process.

2. MATERIALS AND METHODS

2.1. RAW MATERIAL

Olive pomace was kindly given by *Oliduero* olive mill (Medina del Campo, Spain). It corresponded to 2017 crop. It was freshly collected and stored at -20ºC. Before being used, olive pomace was thawed at 4ºC overnight. One conventional extraction experiment was repeated with fresh and thaw material to ascertain that the freezing of the olive pomace did not alter the results of the extraction. Table 1 gathers the proximate composition of the raw material employed in this work. Moisture was assessed gravimetrically by drying the pomace at 105ºC until constant weight. Extractives and fat were determined by a Soxhlet extraction with hexane, ethanol and water respectively. The protein content was computed by Kjeldahl method (with a conversion factor of 6.25). The amount of ashes corresponded to the char formed at 550ºC. All yield compositions are expressed by gram of dry olive pomage ($g_{dry\ OP}$).

Table 1. Olive pomace proximate composition.

Moisture ($g_{H2O}/g_{Dry\ OP}$)	Fat ($mg/g_{Dry\ OP}$)	Ash ($mg/g_{Dry\ OP}$)	Protein ($mg/g_{Dry\ OP}$)	Extractives ($mg/g_{Dry\ OP}$)
2.20±0.3	111.52±6.86	32.59±1.66	139.99±6.82	237.77±9.07

Olive pomace heat capacity has also been measured in a Setaram Micro DSC II microcalorimeter (Páramo et al., 2002) from 20ºC to 85ºC resulting to be of 3.51±0.04 J/gK.

2.2. CHEMICAL REAGENTS

MilliQ water obtained from a Millipore unit, and ethanol and chlorhydric acid purchased from Panreac, were used to prepare the extraction solvent. For polyphenol analysis, gallic acid from Sigma, Folin-Ciocalteu reagent and sodium carbonate from Panreac were employed. HPLC standards hydroxytyrosol, tyrosol and oleuropein were bought from Extrasynthese, whereas hydroxymethyl furfural was obtained from Sigma-Aldrich. Methanol, phosphoric acid, and sulfuric acid from Panreac were used as HPLC eluents.

2.3. EXTRACTION PROCEDURE

2.3.1 SOLID-LIQUID EXTRACTION

A preliminary study with the objective of finding the optimal conditions for the solid-liquid extraction was performed. Solid-liquid ratio, temperature and solvent composition were the variables studied in a statistical experimental designed. To perform these experiments, the corresponding amount of olive pomace was weighted in a round bottom flask and mixed with 70 mL of solvent. First, a homogenization step was carried out by stirring the media at ambient temperature for 5 min. The extraction was set to begin when the flask was placed in a thermostatic bath. Liquid samples along the extraction were taken in order to perform a kinetic analysis.

2.3.2. MICROWAVE PRETREATMENT

The influence of the solvent and the absorbed specific energy during the microwave pretreatment has been assessed as a complement to the conventional solid-liquid extraction. Regarding solvent, a mixture of ethanol:water and solvent free microwave pretreatments have been tested. A CEM Discover One has been employed for the solvent free and the pretreatments with a solvent temperature bellow boiling point. In the cases where the temperature was above boiling point, an Anton Paar microwave was used to assure the liquid phase of the solvent.

Since the microwaves are here proposed as a pretreatment to the conventional process, the pretreatment was conducted after the homogenization step and before placing the extraction media in the thermostatic bath. Briefly, an amount of 30 grams of olive pomace was weighted and mixed with the corresponding amount of solvent. It must be added that, whereas for the CEM experiments 100 mL flasks were employed, Anton Paar's were conducted in 30 mL vials. So, these latter experiments contained a third of the amounts used in the CEM pretreatments. The mixture was initially stirred for 5 minutes at ambient temperature to homogenize it. Then, the media was placed in the microwave, where it was irradiated at 300 W and 100 W, with the CEM and the Anton Paar microwave, respectively. To obtain comparable results, microwave irradiation was measured in terms of absorbed specific energy. The media was irradiated between 30 and 180 seconds. Temperature was recorded along the extraction by means of an optic fibre (FoTEMP 4). Especial attention was paid during solvent free pretreatments to the stirring so that the formation of hot spots was avoided. When the pretreatment was finished, the media was rapidly cooled down, and weighted to evaluate solvent evaporation. Then, solvent was added if requested (solvent free pretreatments), and placed in the thermostatic bath to continue with the conventional extraction process. Liquid samples were also taken along the extraction for the kinetic analysis.

As it has been previously said, the intensity of the pretreatment has been quantified in terms of absorbed specific energy ($Q_{absorbed}$ in J/kg). It has been computed considering the sensible and the latent heat, as it is expressed in equation [1].

$$\mathrm{Q_{absorbed}} = \mathrm{Q_{sensible}} + \mathrm{Q_{latent}} \qquad [1]$$

Sensible heat was calculated as the energy required to increase pomace and solvent temperature from ambient temperature to the pretreatment temperature in the case of the pomace, and to the boiling point in the case of the solvent. Equation [2] was employed.

$$\mathrm{Q_{sensible}} = \sum \mathrm{C_P \Delta T} \qquad [2]$$

Especial attention deserves experiments were pressure was required, since in these cases the evaporated solvent accumulates in the gas phase of the closed vessel. To compute their latent heat, an ideal gas behaviour was assumed, in order to estimate the number of moles of solvent evaporated ($n_{evaporated}$) (equation [3]). This value, and final temperature, were calculated from the final pressure value.

$$P\left(V_0 + \frac{n_{evaporated}}{\rho_{mol}}\right) = \left(n_0 + n_{evaporated}\right)RT \qquad [3]$$

Once the mass of evaporated solvent (*m*) was known, it was used in equation [4], together with solvent vaporization enthalpy (*λ*).

$$\mathrm{Q_{latent}} = \frac{\mathrm{n}_{evaporated}\,\lambda}{m} \qquad [4]$$

In the above expressions *Q* stands for specific energy in J/g, C_P for heat capacity in J/gK, *m* represents the mass of pomace and solvent in grams, $n_{evaporated}$ the moles of solvent evaporated from the liquid phase, ρ_{mol} the liquid molar density of the solvent in mol/m^3, *T* is the temperature in K, ΔT the temperature increase in K, *λ* the solvent vaporisation heat in J/g, *P* the final pressure in Pa, V_0 the initial gas volume in the closed vessel in m^3, n_0 the initial amount of gas moles in the closed vessel, and *R* the gas constant 8.31 J/molK.

2.3.3. KINETIC ANALYSIS

The evolution of the extraction has been analysed in terms of polyphenol extraction yield and product richness. The former refers to the amount of polyphenols extracted per gram of dried olive pomace ($\mathrm{mg_{GAE}/g_{Dry\ OP}}$), whereas the latter refers to the concentration of polyphenols in the final dry extract product ($\mathrm{mg_{GAE}/g_{Dry\ extract}}$) These values along the extraction has been assessed by means of liquid samples. It must be added that the extraction was set to begin (t=0 min) after the 5-minute homogenization step. This *t=0* concentration corresponded to the release of non-bounded compounds.

The evolution of the extraction yield followed a first order kinetic expression, like the one shown in equation [5].

$$\mathrm{C_{cal}} = \mathrm{C_0} + \mathrm{C_f}[1 - \exp(-\mathrm{kt})] \qquad [5]$$

This expression was used to fit the experimental data to a kinetic curve. The value of the three parameters C_0, C_f and *k* were adjusted to minimize the average relative deviation (equation [6]).

$$\mathrm{ARD} = \frac{1}{\mathrm{n}}\sum\left|\frac{\mathrm{C_{exp}} - \mathrm{C_{cal}}}{\mathrm{C_{cal}}}\right| \qquad [6]$$

However, for an easy interpretation of the data, the kinetic derived parameters, initial extraction rate (u_0) and final yield (C_∞), were the factors analysed. The comparison of the initial extraction rates (computed by equation [7]) indicates the acceleration that the extraction suffers for each condition.

On the other hand, final yield (calculated by equation [8]) represents the maximum amount of polyphenols that can be obtained at certain extraction conditions.

$$u_0 = \left.\frac{\partial C}{\partial t}\right|_{t=0} = C_f k \qquad [7]$$

$$C_\infty = \lim_{t\to\infty} C = C_0 + C_f \qquad [8]$$

In these expressions, *C* stands for polyphenol concentration ($mg_{GAE}/g_{Dry\ OP}$), C_0 is the initial concentration ($mg_{GAE}/g_{Dry\ OP}$), C_f is a pre-exponential factor ($mg_{GAE}/g_{Dry\ OP}$), *k* is the extraction rate in min^{-1}, *t* the time in minutes, u_0 the initial extraction rate in $mg_{GAE}/g_{Dry\ OP}\cdot min$, and C_∞ the total yield in $mg_{GAE}/g_{Dry\ OP}$.

2.4. EXTRACT CHARACTERIZATION

2.4.1. TOTAL POLYPHENOL CONTENT

Folin Ciocalteu method was followed to determine the total polyphenol content. Briefly, a volume of 40 µL of diluted samples were mixed with 3000 µL of water and 200 µL of Folin Ciocalteu reagent. After 5 minutes, 600 µL of 20% sodium carbonate were added. Samples were left in a water bath at 40ºC during 30 minutes before measuring its absorbance at 765 nm (Shimadzu UV/vis Spectrophotometer). Results were expressed in gallic acid equivalents (mg_{GAE}/g).

2.4.2. INDIVIDUAL COMPOUNDS QUANTIFICATION

Hydroxytyrosol, tyrosol, oleuropein and hydroxymethyl furfural were measured by HPLC.

Hydroxytyrosol, tyrosol and oleuropein were quantified using a previous method with little modifications (Brenes et al., 1999). A Waters e2695 Separation module with an autosampler (20 µL of inyection volume) and a quaternary pump were employed. As eluents, acidified water to pH=3 with phosphoric acid and methanol were employed. A flow of 1 mL/min was used. Eluents' gradient was varied according to Table 2. Separation took place in a C18 Mediterranea Sea column (250x4.6 mm, 5 µm) at 35ºC. A OptiGuard 1 mm guard column (Sigma-Aldrich) was also employed. Polyphenols were identified in a Waters 2998 photodiode array detector set at 280 nm. Active compound identification was performed by means of the retention times and the UV/vis spectra, which was also recorded. Water Empower 3 software allowed the processing of the results.

Table 2. HPLC eluent gradient.

time (min)	Eluent A (%)	Eluent B (%)
0	90	10
10	70	30
30	70	30
40	60	40
45	60	40
50	50	50
55	40	60
60	30	70
65	0	100
80	90	10

Eluent A: acidified water

Eluent B: methanol

Hydroxymethyl furfural was determined in a Water HPLC. A 0.01 N sulfuric acid solution was used as eluent with a 0.8 mL/min flow. Sample injections were of 20 μL. Separation took place in a SH-1011 Shodex column at 55ºC. Hydroxymethyl furfural was quantified in a 2414 IR detector.

2.4.3. DRY EXTRACT PRODUCT

The total amount of substances extracted was determined gravimetrically by drying the extract at 105ºC overnight.

2.5. STATISTICAL ANALYSIS

Conventional solid-liquid extraction was studied by means of a statistical experimental design. A face central composite design (α=1) was employed. Temperature (40-70ºC), solid-liquid ratio (0.05-0.50 g/mL) and solvent composition (25-75% (v/v) of ethanol) were the variables studied. Industrial scale-up concerns were considered when the variables range was selected, specifically regarding solid-liquid ratio and solvent composition. Table 3 gathers the conditions of the experiments performed.

Data analysis has been performed with the software Design Expert by means of the ANOVA table (with a confidence of 95%) to identify the significant variables. The main effect and interaction diagrams were used to interpret variables influence.

Table 3. Conventional solid-liquid extraction statistical experiment design and results

Exp	T (ºC)	S:L (g/mL)	Solvent composition (EtOH%)	Yield ($mg_{GAE}/g_{Dry\ OP}$)	Richness ($mg_{GAE}/g_{Dry\ extract}$)
1	55	0.275	50%	20.18	80.93
2	70	0.275	50%	21.28	78.75
3	40	0.050	25%	25.29	78.76
4	55	0.500	50%	15.79	60.40
5	70	0.500	75%	16.63	76.39
6	70	0.050	75%	26.55	61.73
7	40	0.050	75%	24.37	63.30
8	55	0.275	25%	17.41	73.47
9	40	0.275	50%	17.70	67.23
10	55	0.275	50%	19.81	80.06
11	40	0.500	75%	16.25	75.47
12	55	0.275	50%	21.14	88.06
13	55	0.050	50%	28.22	146.51
14	55	0.275	75%	20.66	88.72
15	70	0.500	25%	13.74	73.48
16	70	0.050	25%	25.79	102.96
17	40	0.500	25%	9.27	46.88

3. RESULTS AND DISCUSSION

3.1. CONVENTIONAL SOLID-LIQUID EXTRACTION

Conventional solid-liquid extraction was optimised in terms of process yield and product richness, assessing the influence of temperature, solid-liquid ratio and solvent composition. As it can be seen in the analysis of variance presented in Table 4, the three studied variables as well as the binary interaction solid-liquid ratio and solvent composition were found significant.

Table 4. Analysis of variance for yield and richness of the conventional solid-liquid extraction.

	Yield					Richness				
Source	Sum of squares	DF	Mean square	F-value	p-value	Sum of squares	DF	Mean square	F-value	p-value
Model	403	13	31	99	0.0015	7292	13	561	10.4	0.0394
A-Temperature	6.4	1	6.4	20	0.0203	66	1	66	1.2	0.3489
B-S:L	77	1	77	246	0.0006	3707	1	3707	68.5	0.0037
C-Solvent	5.3	1	5.3	17	0.0263	116	1	116	2.1	0.2389
AB	0.6	1	0.6	1.9	0.2647	3.0	1	3.0	0.1	0.8293
AC	0.7	1	0.7	2.3	0.2259	331	1	331	6.1	0.0898
BC	13	1	13	40	0.0080	972	1	972	18.0	0.0240
A^2	2.2	1	2.2	6.9	0.0780	827	1	827	15.3	0.0297
B^2	7.0	1	7.0	22	0.0181	445	1	445	8.2	0.0641
C^2	4.9	1	4.9	16	0.0287	240	1	240	4.4	0.1257
ABC	4.2	1	4.2	13	0.0358	0.0	1	0.0	0.0	0.9968
Residual	0.9	3	0.3			162	3	54		
Lack of Fit	0.0	1	0.0	0.0	0.9758	124	1	124	6.4	0.1267
Pure Error	0.9	2	0.5			39	2	19		
Cor Total	404	16				7454	16			

Figure 1 represents the main effect that the variables had on process yield. It presented a slight improvement when temperature and solvent composition was increased. However, the most significant effect was found when solid-liquid ratio was decreased. Yield was drastically reduced as a consequence of mass transfer. Since long extraction time was required to achieve final yield, internal mass transfer resistance was inferred predominant. Regarding the ratios' role on the mass transfer, when high solid-liquid ratios were used, the leaked polyphenols were rapidly accumulated in the small amount of volume employed, reducing the concentration gradient and so the internal mass transfer. Thus, a higher concentration in the bulk was achieved. On the contrary, when low solid-liquid ratios were used, extracted polyphenols were diluted in the larger amount of solvent employed, and as a result, a much faster internal mass transfer was maintained. This reasoning led to the consideration of polyphenol concentration as the main target, rather than yield. An assumption that came supported by the fact that olive pomace was not an expensive raw material and so, its complete depletion is not justified beyond achieving an efficient process. Around 6 times more concentrated extract was obtained with a ratio of 0.50 g/mL compared to an extraction at 0.05 g/mL. Therefore, it is industrially more convenient to employ a high ratio since a much more concentrated liquid extract can be obtained, and therefore, the industrial expenses of solvent recovery and extract dying would be much lower.

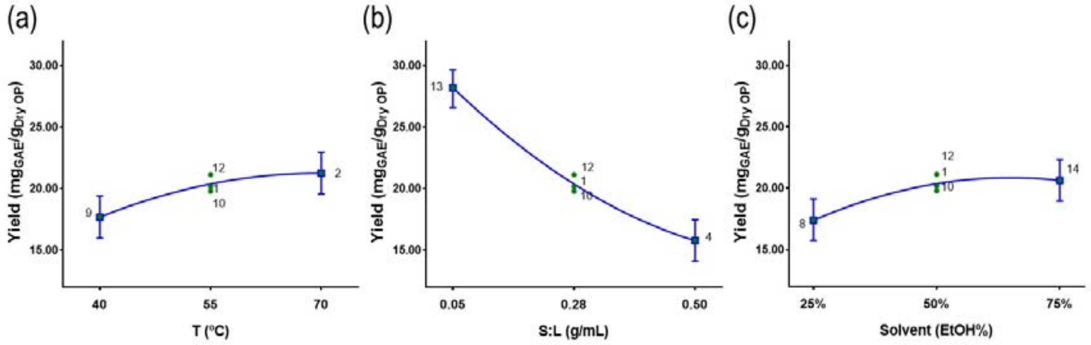

Figure 1. Temperature, solid-liquid ratio and solvent composition main effects on extract yield (T=55ºC, S:L=0.275 g/mL, 50% EtOH)

The above reasoning, together with the effect of the organic solvent composition, could be observed if the interaction between ratio and solvent (Figure 2) was considered. In terms of solvent composition, no appreciable difference was observed at low solid-liquid ratios. However, a significant solvent influence was found in extractions at high solid-liquid ratios. In this case, the amount of solvent available was so low that the equilibrium concentration had a noticeable influence. A fact that would explained why a lower yield was obtained for 25% ethanol solvent compared to 75% (polyphenols had a higher solubility in organic solvents).

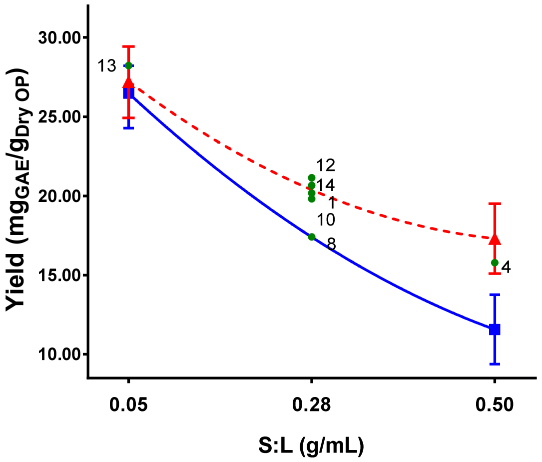

Figure 2. Interaction between solid-liquid ratio and solvent (■ 25% ethanol, ▲ 75% ethanol)

These same tendencies were found when extract richness was regarded. Temperature and solvent were not significant, but solid-liquid ratio and the interaction solvent-ratio maintained their influence on the process. Since the extraction of non-polyphenol compounds was considered inside the richness analysis (ratio of extracted polyphenols over total extracted compounds), the fact that this latter parameter presented the same tendencies than yield (extraction of polyphenols from the olive pomace) allowed to assure that the extraction of both, polyphenols and undesired substances, took place at the same rate. And thus, the leakage of all the extracted compounds were affected in the same way by the process conditions. So, the same conclusions previously attained for polyphenols can be applied to the total amount of extracted compounds.

The analysis of the extraction efficiency led to find that an average increment of only 25% respect to the initial polyphenol composition was achieved. This, together with the comparison of the total amount of extractives (237.77±9.07 mg/$g_{Dry\ OP}$, Table 1) respect to the maximum yield reached during the extraction (28.22 mg_{GAE}/$g_{Dry\ OP}$, Table 3), suggested that only non-bounded polyphenols were isolated. Therefore, there might be still plenty of room to improve the process efficiency. A fact that could be accomplished by an intensification step. A microwave pretreatment was proposed as an alternative that could help to disrupt the solid matrix, facilitating so the leaching of active compounds. In order to study the effects of the microwave pretreatment, a reference conventional extraction performed at 70ºC, with a ratio of 0.50 g/mL and a 50% ethanol:water solvent was selected. These conditions have been chosen regarding industrial constrains for a possible future application (namely related with solvent consumption), despite the fact that they did not correspond to the mathematical optimum. Table 5 gathers a comparison between these two sets of conditions. The main difference lay in the solid-liquid ratio. Whereas the mathematical optimum requires ten times more solvent than the industrial set, the enhancement in yield and richness was only of the around 22%.Such massive solvent consumption, in comparison to the improvement, did not sustain the use of the mathematical optimum over the industrial considerations. In addition, as it has been previously detailed, a higher ratio entailed a more concentrated extract that also implied a reduction in the solvent recovery costs. Thus, a ratio of 0.50 g/mL was selected. In the case of temperature, since 70ºC provided a slightly better yield and no thermal degradation was observed, it was selected as the extraction temperature.

Table 5. Conventional solid-liquid extraction conditions selected as reference for the microwave pretreatment analysis.

	T (ºC)	S:L (g/mL)	Solvent composition (EtOH%)	Yield (mg_{GAE}/$g_{Dry\ OP}$)	Richness (mg_{GAE}/$g_{Dry\ extract}$)
Considering industrial constrains	70	0.50	50%	17.90±1.50	53.21±3.99
Mathematical yield optimum	56	0.05	55%	22.60±1.34	68.52±3.61

3.2. MICROWAVE PRETREATMENT EXTRACTION

Conventional solid-liquid extraction was intensified by the addition of a previous microwave pretreatment. It consisted of a short but intense step in which the extraction medium temperature was rapidly increased, and subsequently cooled down to continue with the conventional solid-liquid extraction. The conditions of this latter post extraction corresponded to the industrial optimization detailed in Table 5.

A screening of the microwave pretreatment conditions have been firstly performed. Microwaves (MW) and the combination of microwaves and pressure (MW&P) were evaluated. Also the type of solvent, a mixture of ethanol:water or no solvent (solvent free), were assesed.

3.2.1. EXTRACTION YIELD

Results regarding extraction yield can be found in Figure 3.

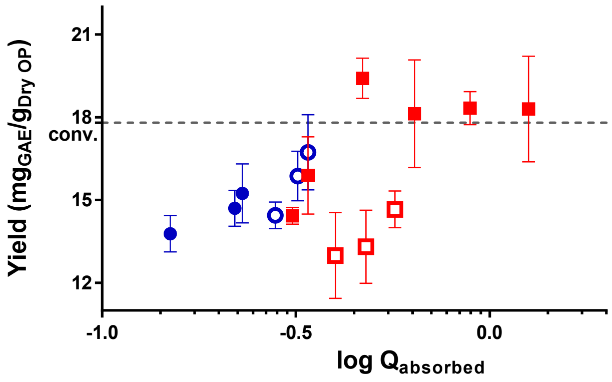

Figure 3. Relation between pretreatment specific energy ($Q_{absorbed}$ in kJ/g) and final yield for the different pretreatments tested. ● MW ethanol:water, ○ MW&P ethanol:water, ■ MW solvent free, □ MW&P solvent free and - - - - conventional solid-liquid extraction (17.90±1.50 mg$_{GAE}$/g$_{Dry\ OP}$).

It was concluded that none of the pretreatements tested was able to substantially enhance yield respect to the conventional process, although solvent free showed a slight increase. This enhancement was obtained when the extraction media absorbed a specific energy of 0.47 kJ/g. This value is in the same range than the one provided by Roselló-Soto et al. (2015) when other intensification techniques were used in this process. In the case of the microwave pretreatment with an absorbed specific energy of 0.47 kJ/g, such energy was employed in the evaporation of the olive pomace humidity, which was reduced a 20%. It must be added that higher energies did not entail a proportional yield improvement. The solvent free microwave pretreatment enhancement was attributed to the disruption of the olive pomace structure caused by the vaporisation of the inner water, resulting so in the leaching of the active compounds. This disruption improved the polyphenol extraction kinetics, but the absorbed specific energy was not high enough to release other more strongly linked polyphenols. Similar conclusions were achieved when the extraction of essential oils by microwave extraction was studied (Navarrete et al., 2011). Regarding the other pretreatments tested, none of them provided any advantage. Nonetheless, it stood out that the solvent free microwave-pressure pretreatment, despite combining two intensification techniques, did not improved the process efficiency at all, but it even reduced it. This was thought to be due to the distribution of the absorbed specific energy. Whereas for microwave-pressure most of the energy was employed in heating up the sample (only a small amount of vapour was produced to increase pressure), in the case of solvent-free microwave pretreatment a considerable fraction of the energy (above 29%) was used in the vaporisation of the inner moisture water.

3.2.2. INITIAL EXTRACTION RATE

Contrary to the previous section, the implementation of the pretreatment had a significant fruitful effect on initial extraction rate, at least for solvent free and pressurized solvent free microwave pretreatments, as Figure 4 shows. Initial extraction rate was accelerated between 1.4 and 6.3 times for the former pretreatment, and between 2.5 and 3.6 for the latter. Both pretreatments presented a proportional acceleration to the absorbed specific energy of the pretreatment. Such acceleration enabled to obtain the final extraction yield much faster. For instance, to reach the 95% of the final extraction yield, the required extraction time was reduced from 39 minutes in conventional extraction to 6 minutes with the microwave solvent free pretreatment, and to 9 minutes if the combination with pressure was employed (considering the most energetic pretreatments). On the other hand, the use of ethanol:water as a solvent during the pretreatment did not present an improvement over the conventional solid-liquid extraction.

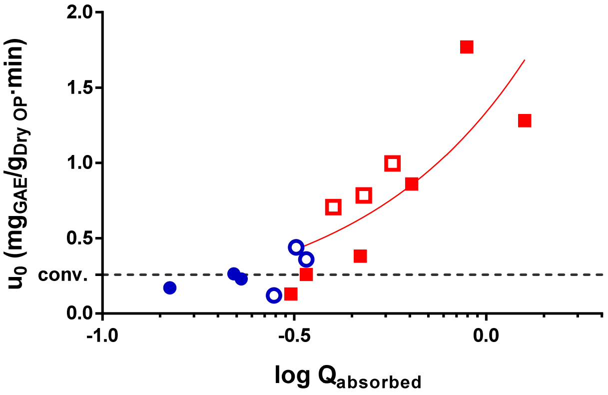

Figure 4. Initial extraction rate for each microwave pretreatment studied: ● MW ethanol:water, ○ MW&P ethanol:water, ■ MW solvent free, □ MW&P solvent free and - - - - conventional solid-liquid extraction (0.26±0.02 $mg_{GAE}/g_{Dry\ OP}\cdot min$).

3.2.3. EXTRACT RICHNESS AND COMPOSITION

Final product polyphenol richness (the proportion of active compounds in the final dried commercial product) was also considered as it represents a vital quality of the final product. Total polyphenol and high-value compounds richness have been considered. Nonetheless, polyphenol richness has been studied only for microwave solvent free pretreatments, since these were the only extractions that provided an improvement over the conventional process. These latter results are presented in Figure 5. As it can be seen, there was a substantial enhancement of extract richness by the addition of the pretreatment. In particular, richness increased between a 25% and a 40% respect to the conventional extraction. A maximum can be found when a pretreatment with an absorbed specific energy around 0.55 kJ/g is employed. It later decayed while pretreatment energy rose. Since no significant polyphenol degradation was observed (extraction yield was almost constant for the more energetic pretreatments), it can be deduced that absorbed specific energies up to 0.55 kJ/g were employed for polyphenol extraction, whereas higher energies strayed from the objective, causing the release of undesired substances that decreased polyphenol richness. So, it is advisable to select a solvent free microwave pretreatment energetic enough to extract polyphenols, without any surplus that may result in a polyphenol richness shrinking.

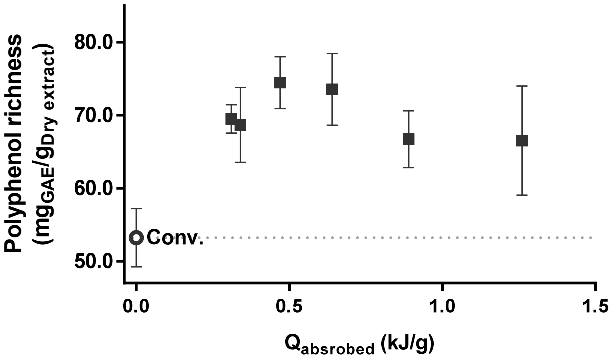

Figure 5. Polyphenol richness obtained by solvent free microwave pretreatment (■) compared to the conventional extraction (○).

Since these pretreatments were performed without solvent, radiation tended to concentrate in hot spots causing the burning of the raw material and the subsequent formation of Maillard degradation products such as hydroxymethyl furfural. So, that previous surplus of energy was found also crucial not only to enhance richness but also to avoid the formation of undesired products. As it can be seen in Figure 6, the greater the pretreatment energy, the higher the production of hydroxymethyl furfural. Since this product is suspected to be carcinogenic (Klaus et al., 2011, Sachse et al., 2016, Oral et al., 2014), a low concentration will deter the potential applications of the final extract. It must be added that the production of hydroxymethyl furfural by the other pretreatments was negligible.

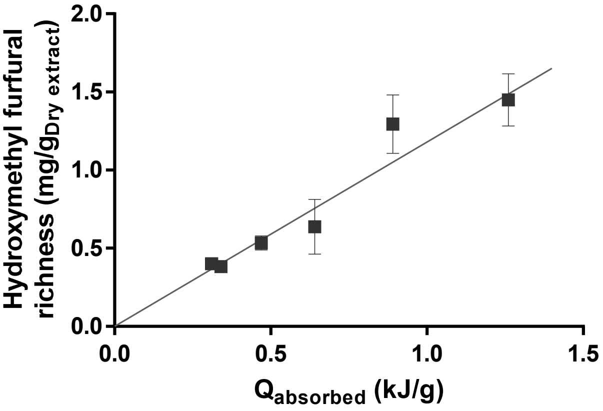

Figure 6. Hydroxymethyl extract richness obtained by solvent free microwave pretreatments as a function of the pretreatment energy.

Regarding high-value compounds, hydroxytyrosol, tyrosol and oleuropein richness was analysed. Results can be found in Figure 7. The different pretreatments tested provided variations in the phenolic concentration profile respect to the conventional extraction. Tyrosol richness was worsened by the addition of the pretreatment (an average decrease of 33%), whereas hydroxytyrosol and oleuropein were boosted. It may be thought that tyrosol and hydroxytyrosol can be in equilibrium so that, if one decreases, the other increases. However, due to the molecular geometry and the acid medium, the addition of a hydroxy group is unlikely. What is more, complex bioconversions have been extensively studied in the recent years to transform one form into the other (Wani et al., 2018, Chung et al., 2017). Concerning hydroxytyrosol, pretreatments combining pressure and microwaves, as well as solvent free pretreatment, rendered better efficiencies for this compound. This improvement varied from a 25% increase to a 60%. In the case of oleuropein, the pretreatment enhancement was massive. Coupled with hydroxytyrosol, microwave-pressure and solvent free pretreatments allowed to obtain an extract between 2.7 and 5.0 times richer in oleuropein.

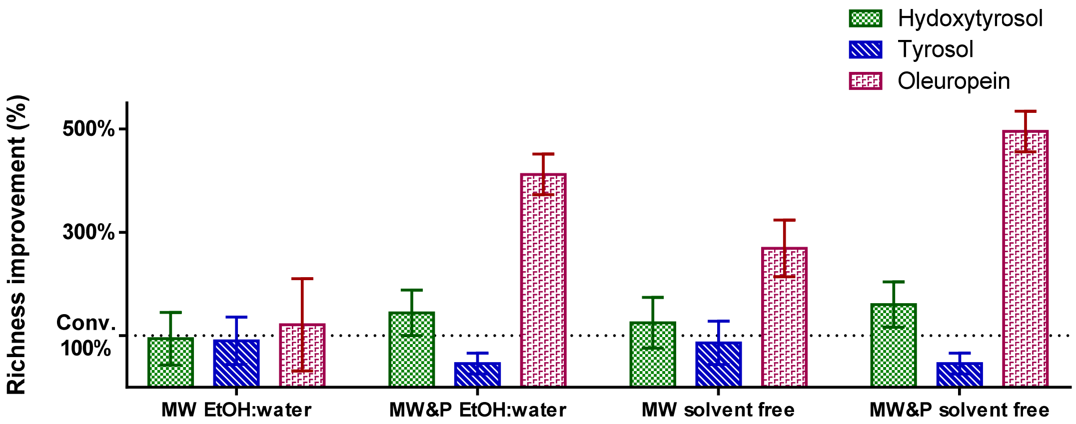

Figure 7. Hydroxytyrosol, tyrosol and oleuropein richness improvement in comparison to the conventional solid-liquid extraction.

In sum, microwave solvent free pretreatment is the most advisable one since it is able to provide high polyphenolic richness and presents a great efficiency towards hydroxytyrosol and oleuropein.

4. CONCLUSIONS

The novelty of this works lies on the approach, from an industrial point of view, to the extraction of polyphenols from olive pomace. Industrial limitations, namely solvent consumption, process efficiency and product quality have been taken into account when selecting the optimal conditions. In addition, a microwave pretreatment to the conventional solid-liquid extraction has been analysed in order to intensify the extraction by a low-cost and easy scale-up microwave step. With this purpose, firstly, conventional solid-liquid extraction conditions were optimized, and next, these results were used as a reference to assess the convenience of the microwave pretreatment.

Concerning conventional solid-liquid extraction, it was proved that low solid-liquid ratios provided a slightly better extraction efficiency than higher ratios (20%). However, due to the large solvent consumption, polyphenol liquid extract was greatly diluted, what contributed to boost product drying and solvent recovery expenses. Thus, 0.50 g/mL was preferred over lower ratios. Finally, the set of extraction conditions 0.50 g/mL, 50% (v/v) ethanol:water solvent and 70ºC was selected as a control reference to assess the influence of the microwave pretreatment.

Four types of pretreatments were tested in regards to the solvent employed (ethanol:water and solvent free) and the intensification techniques (microwaves and the combination of microwaves and pressure). Four parameters were considered to evaluate the convenience of the pretreatment:

i. Final extraction yield (or the efficiency to extract the maximum amount of polyphenols from the olive pomace). It was found not to be substantially affected by the pretreatment. What is more, some pretreatments even provided a poorer yield than the conventional extraction. Only the solvent free microwave pretreatment was able to slightly increase it.
ii. Initial extraction rate. This parameter indicated how quickly extraction yield is achieved. The higher it is, the less extraction time will be required. A substantial acceleration of up to 6.3 times that of the conventional initial rate was found for the solvent free microwave pretreatment. So, the extraction time is drastically reduced from the 39 minutes required in the conventional extraction up to 6 minutes with the addition of the solvent free pretreatment.
iii. Dry product richness. This value reports the concentration and quality of the final polyphenolic dry product extract. Like in the previous case, solvent-free microwave pretreatment provided a significant increase up to 40%. In addition, it was also found that the absorbed specific energy was crucial, since a maximum was presented at 0.55 kJ/g. Higher energy absorptions did not enhance polyphenol richness, but it was employed in the release of undesired substances that decrease richness.
iv. Product composition. Four compounds have been tracked: hydroxymethyl furfural, tyrosol, hydroxytyrosol and oleuropein. Hydroxymethyl furfural, undesirable for the future potential applications of the extract, was only detected when solvent free microwave pretreatments were used. Its composition was proportional to the absorbed specific energy used in the pretreatment. Regarding high-value compounds, no influence of the intensification step was observed for tyrosol extraction, whereas hydroxytyrosol and oleuropein were greatly improved. In particular, an enhancement of around 35% and 181% was founded for the solvent free microwave pretreatment, respectively. Therefore, the addition of the pretreatment resulted to be highly specific for the extraction of these compounds.

In sum, an exhaustive analysis of the advantages and disadvantages of the addition of a microwave pretreatment to the conventional solid-liquid extraction of polyphenols from olive pomace has been carried out. It was concluded that a solvent free microwave pretreatment provided the best results in terms of process efficiency and product quality. Although it also presented the inconvenient of the formation of hydroxymethyl furfural, that must be carefully controlled.

ACKNOWLEDGEMENTS

This work was funded by Spanish regional government Junta de Castilla y León (VA040U16). Dr Páramo is kindly thanked for the measurements of the specific heat of olive pomace. A. Álvarez is grateful for her PhD grant FPU13/04678.

NOMENCLATURE

Acronyms

ARD	Average relative deviation
EtOH	Ethanol
GAE	Gallic acid equivalents
MW	Microwave
MW&P	Microwave and pressure
OP	Olive pomace
S:L	Solid-liquid ratio

Symbols

C	Concentration ($mg_{GAE}/g_{Dry\ OP}$)
C_0	Initial polyphenols concentration ($mg_{GAE}/g_{Dry\ OP}$)
C_∞	Polyphenol final yield ($mg_{GAE}/g_{Dry\ OP}$)
C_f	Pre-exponential factor ($mg_{GAE}/g_{Dry\ OP}$)
C_P	Heat capacity (J/gK)
k	Extraction rate constant (min^{-1})
m	mass (g)
$n_{evaporated}$	Number of moles of evaporated solvent (mole)
P	Pressure (Pa)
Q	Specific energy (J/kg)
R	Gas constant (J/molK)
ΔT	Temperature increase in microwave pretreatment (K)
t	time (min)
u_0	Initial extraction rate ($mg_{GAE}/g_{Dry\ OP}/min$)
V	Volume of the gas phase in the closed vessel (m^3)
λ	Enthalpy of vaporization (J/g)

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CHAPTER 7

Scale up of a microwave pretreatment for the extraction of polyphenols from olive pomace

SCALE UP OF A MICROWAVE PRETREATMENT FOR THE EXTRACTION OF POLYPHENOLS FROM OLIVE POMACE

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ABSTRACT

The widely proved advantages of microwaves in the extraction of bioactive compounds from natural products firmly support its implementation at industrial scale. However, the long extraction time required by MAE (microwave assisted extraction) hinders its scale up. A microwave pretreatment is studied in here as a feasible alternative to be implemented at larger scale. To achieve an efficient process, olive pomace dielectric properties have been measured under microwave radiation as a function of temperature and moisture content. The measurements have been correlated to a regression that allows to estimate the dielectric constant with an average relative deviation of 9%, and the loss factor of 29%. This data has been used to tune and operate a microwave oven at a pilot plant scale (able to treat 500 grams each batch). A previous optimization for the conventional extraction of polyphenols from olive pomace has been taken as reference and reproduced at a larger scale. The escalation of the process can be considered satisfactory since similar (or even better) yield than in laboratory scale has been attained.

KEY WORDS

Olive pomace, dielectric properties, permittivity correlation, microwave pretreatment, pilot plant.

HIGHLIGHTS

- Olive pomace permittivity has been measured and correlated to temperature and composition.
- Additional measurements considering mixtures with solvents are also provided.
- A novel equipment to determine the dielectric properties in a microwave field has been used.
- Scale up of a microwave pretreatment for the extraction of olive pomace polyphenols.
- Efficient pilot plant designed to reproduce a laboratory optimization.

1. INTRODUCTION

Microwave scaling up and industrial implementation of microwave extraction processes is still a pending issue. Although it has been proved to be highly convenient for thermal processes such as pasteurization or microbial inactivation processes, especially for the processing of sensible organoleptic products (Franco et al., 2017, Bozkurt-Cekmer and Davidson, 2017), a step toward its application has not taken place yet. In particular, this work is focused on the efficient scale up of the extraction of polyphenols from olive pomace by means of a microwave pretreatment.

Olive pomace is an olive-mill residue obtained after pressing and decanting the olives. Its disposal supposes a nuisance for this industry, since businesses have to pay to get rid of it (Albahari et al., 2018, Clemente et al., 1997, Lesage-Meessen et al., 2001). However, the high content of active compounds in this residue makes of it a potential source of polyphenols. Its applications as a health promoting ingredient is included in the nutraceutical growing trade, which is actually gaining a lot of popularity (Roselló-Soto et al., 2015, Putnik et al., 2017). Thus, the valorisation of this biomass can help not only to improve the sustainability of the olive oil industry, but also it responds to the actual demand for active ingredients.

Polyphenols have been found to be highly bounded in the kernel structure of the olive pomace. So, its isolation requires the use of severe conditions. A fact that gives grounds for the use of intensification techniques to aid its extraction, such as microwave technology. A microwave pretreatment of the olive pomace prior its extraction with solvent has been considered as an interesting alternative to perform the scaling up of the process. The preference for a microwave pretreatment over what is usually known as MAE (microwave assisted extraction) is a consequence of the extraction conditions. While a MAE process entails high temperature and extraction time, the microwave pretreatment takes place at higher temperature, but at a dramatically shorter time. This innovative application of microwaves has been found to provide excellent results for the extraction of polyphenols from grape pomace (Álvarez et al., 2017b) at mild microwave conditions (temperature increased to 100ºC in 2.3 minutes). So, such improvement led to consider its application for the extraction of olive pomace polyphenols.

In addition, MAE long extraction time makes this process difficult to be scaled up as it requires large oven volumes. The main limitations to surmount in terms of microwave industrial implementation are its energy efficiency and its homogeneity. Energy efficiency is given by an optimal oven design, which provides a high fraction of absorbed radiation, and a homogeneous irradiation of the material. Regarding this latter factor, the penetration depth of the microwaves has a major role. Microwave energy, as a consequence of its interaction with the material, decays its power while it pierces the material (Martín and Navarrete, 2018). There is a specific parameter to estimate this effect: the penetration depth. It is defined as the distance in which microwave energy power is reduced to *1/e* of the initial value (Wang et al., 2003). Penetration depth can be computed by means of equation [1], as a function of permittivity (which determines the interaction between the field and the material).

$$d_P = \frac{\lambda_0 \sqrt{\varepsilon'}}{2\pi\varepsilon''} \qquad [1]$$

Since penetration depth has values of only centimetres for the common solvents used in microwave extraction of natural products, the irradiation of a large volume is not suitable, as it will not be homogenously treated (Estel et al., 2017). On the other hand, microwave pretreatment requires of a short residence time inside the microwave oven, thus, a compact unit, where the uniform irradiation of the material is assured, is feasible.

As it has been previously suggested, how the electromagnetic field interacts with the irradiated material is of vital importance for an efficient microwave process. The dielectric properties (or permittivity) rule this interaction. Permittivity is a complex number formed by the dielectric constant (real part) and the loss factor (imaginary part). Expression [2] relates permittivity (ε^*) with the dielectric constant (ε') and the loss factor (ε''). Dielectric constant addresses the ability of the material to storage energy, whereas the loss factor represents the losses due to energy dissipation into heat (Navarrete et al., 2011).

$$\varepsilon^* = \varepsilon' - j\varepsilon'' \qquad [2]$$

There is an important contributor to this latter term: the ionic losses (eq. [3]). They are a function of the material conductivity (σ, in S/m), microwaves frequency (*f*) and the free-space permittivity (ε_0=8.854·10^{-14} F/m). Ionic losses have been proved to greatly contribute to the loss factor of solvent-biomass extraction media (Álvarez et al., 2017a). This is due to the release of the electrolytes present in the biomass to the solvent and their free movement. The ionic losses consideration is crucial, since they can boost loss factor and therefore, substantially increase the likelihood of hot-spots formation.

$$\varepsilon_{\sigma} = \frac{\sigma}{j2\pi f \varepsilon_0} \qquad [3]$$

An accurate knowledge of the dielectric properties is paramount to design an efficient microwave oven. Dielectric properties allow to comprehend what happens during the process as well as determine its main features, such as heating rate or temperature distribution. For instance, permittivity can be used in an oven simulation to optimise the waveguide and oven dimensions so that a homogeneous temperature distribution is attained and hot-spots are avoided (Dominguez-Tortajada et al., 2007, Zhu et al., 2012). However, the actual knowledge of dielectric properties is quite limited. Although there are techniques available for each application (frequency, nature of the material, accuracy…) (Nelson and Trabelsi, 2012), Vaucher et al. (2006) attributed the incomplete understanding to a lack of advanced instrumentation. Nonetheless, in this work, a cutting-edge equipment has been employed to measure the real olive pomace permittivity under an electromagnetic field (Catalá-Civera et al., 2015). Since dielectric properties depend on temperature and on the heating system (Binnen et al., 1994, Arai et al., 1992), an electromagnetic field and the dielectric heating produced interacts with the material varying its permittivity. Therefore, to obtain accurate data these interactions must be counted to obtain accurate data. A fact that is commonly obviated when dielectric data is reported.

Thus, this work aims to efficiently scale up a microwave pretreatment for the intensification of polyphenol extraction from olive pomace. The dielectric properties of the olive pomace have been accurately measured to assure an effective and efficient microwave oven able to work at a pilot scale plant.

2. MATERIALS AND METHODS

2.1. RAW MATERIAL

Olive pomace from 2017 crop was kindly given by *Oliduero* olive mill (Medina del Campo, Spain). It was collected right after decanting the olive oil from the solid by-product. Olive pomace was packed in individual bags and stored at -20ºC.

The dielectric properties measurement required to asses the moisture of the pomace so, partially freeze-dried samples were obtained from a Telstar LyoQuest unit.

The raw material used for the extraction of active compounds did not undergo any previous treatment. It was just defrosted overnight before being used at 4ºC. The extraction kinetics of a fresh sample were compared to those of a defrosted sample to check that the freezing of the samples for their preservation did not introduce any difference in the results.

As solvent, a mixture of 50% (v/v) ethanol:water (water acidified to pH=1 with chloridric acid) was employed.

2.2. CHEMICAL REAGENTS

Ethanol, Folin-Ciocalteu reagent, hydrochloric acid and sodium carbonate were purchased from Panreac, whereas gallic acid from Sigma. MilliQ water obtained from a MilliPore purification system.

2.3. DIELECTRIC PROPERTIES MEASUREMENT

A dual-mode cylindrical cavity developed by ITACA Institute (Universitat Politècnica de València, Spain) was used to determine the complex permittivity of the olive pomace. Additional measurements of solvents and solid-liquid measurements were also performed to provide novel permittivity data. The variables studied were temperature and composition.

The novelty of the unit employed in this work was that it allowed to simultaneously irradiate an electromagnetic field and to measure its permittivity. Since dielectric properties vary in the presence of a microwave radiation, this equipment enabled to determine the real behaviour of the sample while it is being heated in a microwave oven. Nonetheless, it must be added that no interaction between heating and measurement took place due to the cavity modes isolation provided by a cross-coupling filter. This equipment was successfully validated with a 95% accuracy (Catalá-Civera et al., 2015).

The equipment was previously calibrated with the empty tube before each test. For the measurement, a volume of 1.5 mL of sample material was placed in a quartz vial (15x9.8 mm), which was introduced in the microwave cavity. The microwave cavity dimensions were 85 mm high and 104.92 mm diameter. Temperature was continuously recorded by an IR thermometer.

Once dielectric constants were measured, they were correlated as a polynomial function of temperature and moisture. Coefficients were adjusted to minimize the difference between the experimental and the model value. Excel Solver tool was used to estimate these coefficients by minimizing the objective function described in equation [4]. The goodness of the fitting was quantified by means of the average relative deviation (equation [5]).

$$O.F. = \sum \left(\varepsilon_{\mathrm{exp}} - \varepsilon_{\mathrm{model}}\right)^2 \qquad [4]$$

$$\mathrm{ARD} = \frac{1}{\mathrm{n}} \sum \left| \frac{\varepsilon_{\mathrm{exp}} - \varepsilon_{\mathrm{model}}}{\varepsilon_{\mathrm{model}}} \right| \qquad [5]$$

2.4. EXTRACTION PROCEDURE

A previous laboratory optimization was taken as a reference to assess the efficiency of the scale up. The experimental procedure consisted of two steps: a microwave pretreatment and a subsequent conventional solid-liquid extraction.

The microwave pretreatment was performed in an in-house designed microwave oven, in collaboration with the Departamento de Tecnologías de la Información y las Comunicaciones of the Universidad Politécnica de Cartagena. The oven was equipped with a Muegge MX3000D-151KL microwave generator, and was specifically designed to carry out this kind of processes. It was composed of a PTFE tube (diameter of 4.0 cm and 56.0 cm of length) that passed through a quasi-cylindrical applicator. This latter part was designed to assure a uniform irradiation of the material. The magnetron had a maximum power of 3.0 kW. Reflected power was measured by the control software ME0200V-007AB, which was coupled to the magnetron through a CAN BUS interface. This reflected power was dissipated by an external refrigerated water circuit to avoid damaging the microwave source.

A known amount of olive pomace (between 100 and 500 g) was placed in a carrier and placed inside the PTFE tube. Its initial temperature was recorded and then, it was irradiated. Different combinations of mass and power were tested to achieve the set absorbed energy fixed in previous laboratory experiments (which was of 0.47 kJ/g). When the pretreatment was finished, two solid samples were taken. One of them was used to compute the absorbed energy, and the second one was subjected to subsequent solid-liquid extraction.

This second sample of the pretreated pomace (30 grams) continued with a solid-liquid extraction at 70ºC, a ratio of 0.50 g/mL and using a 50% (v/v) mixture of ethanol:water (acidified to pH=1 with clorhidric acid). Liquid samples were taken at different times during the extraction for the subsequent kinetic analysis detailed in section 2.6.

Regarding the calculations of the energy absorbed during the pretreatment, since a temperature of 100ºC were achieved and moisture evaporation took place, it was calculated gravimetrically. Two contributions were considered: the sensible heat and the latent heat, as it is detailed in equation [6]. Heat losses to the environment were dismissed according to Solyom's conclusions (Sólyom et al., 2011).

$$Q_{absorbed} = Q_{sensible} + Q_{latent} \qquad [6]$$

The sensible heat was computed as the energy increment from the initial temperature (*T_{in}*) and the final temperature (*T_{out}*). Olive pomace heat capacity (*C_P*) was measured in a Setaram Micro DSC II microcalorimeter (Páramo et al., 2002) from 20ºC to 85ºC, resulting to be of 3.51±0.04 J/gK. Expression [7] gathers the equation employed.

$$Q_{sensible} = mC_P(T_{out} - T_{in}) \qquad [7]$$

To calculate the latent heat, the amount of moisture evaporated had to be known, which was possible by a gravimetric analysis. The moisture of untreated and pretreated olive pomace were calculated by drying samples at 105ºC during a day. The comparison between these two values allowed to estimate the amount of water evaporated (*m* in grams), which was further used in equation [8], together with water enthalpy (*λ*=2254 J/g), to compute latent heat contribution.

$$Q_{latent} = m\lambda \qquad [8]$$

2.5. EXTRACT CHARACTERIZATION

Total polyphenol content was characterised by the Folin-Ciocalteu assay. Briefly, 40 μL of sample were diluted in 3000 μL of water. A volume of 200 μL of Folin-Ciocalteu reagent was added. The mixture was gently stirred for five minutes before pouring 600 μL of 20% oversaturated sodium carbonate. It was left to react for 30 minutes at 40ºC. Then, absorbance at 765 nm was measured (Shimadzu UV/vis Spectrophotometer) and compared with a gallic acid calibration curve. Therefore, results were expressed in gallic acid equivalents (mg_{GAE}/g).

It must be added that this measurement has been transformed into two different parameters: process yield ($mg_{GAE}/g_{Dry\ OP}$) and final product richness ($mg_{GAE}/g_{Dry\ extract}$). The first one indicated the efficiency of the extraction, which is the amount of polyphenols that were obtained per gram of dry olive pomace, whereas the latter determines the quality of the final product. Since this kind of extracts are usually commercialised as a dry product, it is crucial for its selling price the concentration of polyphenol that it contains. So, the richer the extract, the more valuable it will be.

2.6. KINETIC ANALYSIS

The liquid samples taken during the subsequent conventional solid-liquid extraction to the microwave pretreatment allowed to assess the kinetic variations of the extraction yield. Experimental results were fitted to the first order kinetic equation gathered in expression [9], where the parameters C_0, C_f and k were adjusted by minimizing the difference between the experimental and the model value (equation [5] applied to concentrations). C_{cal} represented the estimated concentration ($mg_{GAE}/g_{Dry\ OP}$) at an extraction time t (min), C_0 stood for the initial concentration ($mg_{GAE}/g_{Dry\ OP}$), C_f was a pre-exponential factor ($mg_{GAE}/g_{Dry\ OP}$), k was the extraction rate (min^{-1}).

$$\mathrm{C_{cal} = C_0 + C_f[1 - \exp(-kt)]} \qquad [9]$$

However, this kinetic equation did not enable an easy comparison between the conventional extraction and the pretreatment. Instead, two derived parameters were employed. These were the initial extraction rate (u_0 in equation [10] measured in $mg_{GAE}/g_{Dry\ OP}\cdot min$) and total yield ($C_\infty$ in equation [11] measured in $mg_{GAE}/g_{Dry\ OP}$). The former assessed how quickly active compounds were leaked to the solvent. The higher this rate was, the faster the final yield was attained, what entailed a reduction of the extraction time. Total yield corresponded to the yield obtained at an infinite extraction time.

$$\mathrm{u_0 = \left.\frac{\partial C}{\partial t}\right|_{t=0} = C_f k} \qquad [10]$$

$$\mathrm{C_\infty = \lim_{t\to\infty} C = C_0 + C_f} \qquad [11]$$

Another important factor to consider was the polyphenol richness, which denotated the proportion of polyphenols respect to the total amount of substances extracted. It must be taken into account that, the larger the polyphenol richness, the more valuable the final product will be. It was computed by comparing the polyphenol concentration with the total amount of substances extracted. This value was assesed by gravimetry, drying a known volume of extract at 105ºC overnight. Polyphenol richness was expressed in $mg_{GAE}/g_{Dry\ extract}$.

3. RESULTS AND DISCUSSION

3.1. OLIVE POMACE DIELECTRIC PROPERTIES

Olive pomace dielectric properties have been measured as a function of temperature and moisture content. The temperature range used in this study covered from ambient temperature to 99ºC, whereas the moisture content analysed varied from 69% (w/w) to 4%. This latter value corresponds to the inner water which was not removed by freeze drying. Figure 1 gathers the results obtained as well as the correlation proposed to estimate olive pomace dielectric properties.

Moisture content was found the most significant variable for both permittivity components. It made dielectric constant to vary from 54.28 to 2.85 and loss factor from 36.50 to 0.37 at 80ºC. So, it can be said that moisture content caused an upsurge of olive pomace permittivity, multiplying dielectric constant by 18 and loss factor by 98. On the other hand, temperature did not have a large influence on the dielectric constant. An average increase of 30% was found when temperature was raised. However, in the case of loss factor, temperature and the interaction temperature—moisture were crucial. Whereas for the dry material (4% moisture) the loss factor varied from 0.30 to 0.40, with its initial moisture (69%) a temperature increase boosted loss factor a 69% (from 15.06 to 49.34).

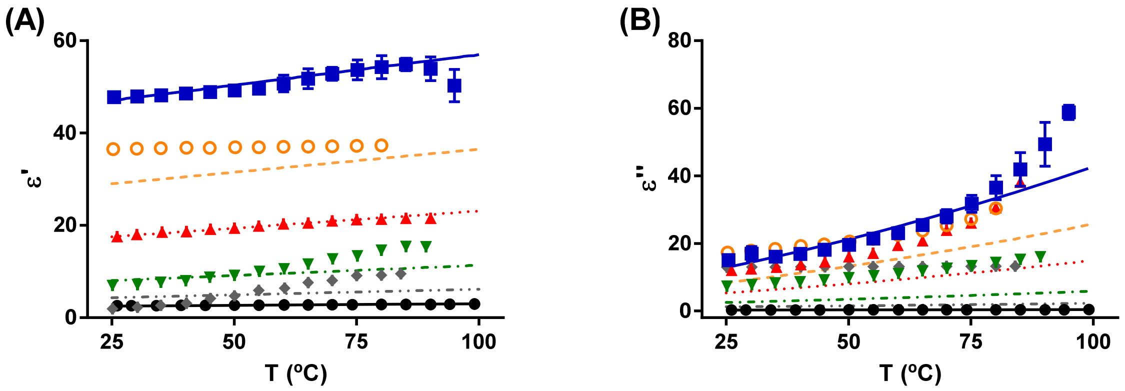

Figure 1. Olive pomace dielectric constant (A) and loss factor (B) as a function of temperature and moisture (■ H=69%, ○ H=53%, ▲ H=39%, ▼ H=23%, ♦ H=13%, ● H=4%). Lines correspond to the model proposed in equations [12] for the dielecrict constant (A) and [13] for the loss factor (B).

The model proposed to estimate olive pomace dielectric properties is detailed in equations [12] and [13]. This model provided a good fitting for the dielectric constant, since an average relative deviation of 9% was attained. However, dielectric loss presented a worse estimation: an average relative deviation of 29% was obtained. However, this error is in accordance to other loss factor estimations for natural products (Álvarez et al., 2017a).

$$\varepsilon' = 2.16 + 0.61H + 86.39H^2 + 0.19HT + 3.13 \cdot 10^{-3}TH^2 \qquad [12]$$

$$\varepsilon'' = 7.12H + 2.53H^2 + 0.50TH^2 + 2.66T^2H^2 \qquad [13]$$

3.2. MICROWAVE PRETREATMENT SCALE-UP AND POLYPHENOL EXTRACTION

Once olive pomace dielectric properties were measured, the pilot plant design was tuned to maximize the microwave energy absorbance. The energy efficiency attained was of the 97%.

To test the scale up of the process, the laboratory optimization presented in a previous work was used as a reference. This work concluded that a 2-minute pretreatment, in which an absorbed energy of 0.47 kJ/g with a 20% reduction of the initial moisture content, was advisable. Four independent experiments were performed in the pilot plant aiming to get these conditions. However, it was not possible to achieve them in a pretreatment time of 2 minutes. Instead, an absorbed energy of 0.40±0.07 kJ/g and a moisture decreased of 17% were obtained in the pilot plant. So, in order to compare similar results, laboratory scale pretreatments of 0.34 kJ/g and 0.47 kJ/g (a mean value of 0.40 kJ/g) were considered, as well as the conventional extraction without pretreatment. Nonetheless, the assessment of the pretreatment has been done based on the kinetic parameters obtained in the subsequent solid-liquid extraction. The three parameters analysed were yield, initial extraction rate and polyphenol yield.

As it was found during laboratory optimization, the microwave pretreatment did not present an improvement of yield. Similar yields were attained in all the processes considered: an average of 24.73±2.05 $mg_{GAE}/g_{Dry\ OP}$. On the other hand, initial extraction rate and polyphenol richness were highly enhanced by the pretreatment. In the case of the extraction rate, it was accelerated from 0.25 $mg_{GAE}/g_{Dry\ OP}\cdot min$ (conventional extraction) to 0.62 $mg_{GAE}/g_{Dry\ OP}\cdot min$ with the addition of microwaves. That supposed an 2.5 times acceleration that allowed to substantially reduce extraction time.

It was noteworthy the polyphenol richness boost found with the microwave pilot plant. It was enhanced from 53.21±2.84 $mg_{GAE}/g_{Dry\ extract}$ to 105.71±7.85 $mg_{GAE}/g_{Dry\ extract}$. This means a 56% improvement that was not observed at laboratory scale (74.17±4.25 $mg_{GAE}/g_{Dry\ extract}$). No explanation has been found apart from the likely inhomogeneities of the raw material.

Nonetheless, it can be said that the scaling up of the microwave pretreatment has been successfully performed, since similar or better results than in laboratory scale have been obtained.

4. CONCLUSIONS

Microwave intensification of extraction processes is widely spread at laboratory scale. However, this knowledge has not yet been implemented at industrial scale due to the difficulties to reproduce the extraction conditions that the majority of MAE processes used. So, in this work, the scale up of a short and intense microwave pretreatment, as an alternative to usual MAE, has been studied.

To obtain an efficient scale up, the dielectric properties of the olive pomace have been assesed. Measurements were performed on olive pomace samples at different temperatures and moisture contents. This data has also been adjusted by regression so that olive permittivity can be estimated with an average relative deviation of 9% for the dielectric constant and of 29% for the loss factor, as a function of temperature and moisture. Nevertheless, the novelty of these measurements lies on the way they have been obtained. An innovative equipment that allows to determine permittivity under an electromagnetic field has been employed. Thus, the actual dielectric properties of the olive pomace under irradiation have been measured.

A microwave pilot plant was used to perform the extraction pretreatment at a larger scale. The results obtained were similar, or even better, to those obtained during the laboratory optimization. So, the scale up the process was considered to be successfully fulfil.

Nonetheless, since a batch system has been employed and the pilot plant allowed to operate in a stationary state, future work has to be focused on developing an impulsion system that enables the continuous operation.

APPENDIX. Additional permittivity measurements

Due to the current interest in dielectric properties, additional permittivity measurements were performed including the solvents and the multiphase mixture of olive pomace-solvent. Although these results were not used in this work, they represent a common extraction media which can be of interest in future works. Additionally, a model to estimate these properties from the individual mixture components has been proposed.

» ETHANOL AND WATER DIELECTRIC PROPERTIES

Pure solvent dielectric constants were computed as a function of temperature by regression of experimental data. Water permittivity was fitted to second degree polynomials, corresponding to the expressions gathered in equations [14] and [15] for dielectric constant and loss factor, respectively. The average relative deviation found for water dielectric constant was 4%, and for loss factor 17%. In the case of ethanol, linear equations [16] and [17] were satisfactorily used to estimate its permittivity, with average relative deviations of 1% and 5%, respectively.

$$\varepsilon' = 0.069T^2 - 4.857T + 162.2 \quad [14]$$

$$\varepsilon'' = 0.020T^2 - 1.563T + 35.62 \quad [15]$$

$$\varepsilon' = 0.167T + 4.402 \quad [16]$$

$$\varepsilon'' = 0.054T + 7.486 \quad [17]$$

It is important to highlight that water values did not agree with other bibliographic data (Franco et al., 2015, Hui et al., 2006, Meissner and Wentz, 2004, Liao et al., 2001, Anderson et al., 2000, Kaatze, 1989, Dean, 1990, Malmberg and Maryott, 1956). This fact contributes to the ethanol quandary outlined by Álvarez et al. (2017a) after a bibliographic review (Campos et al., 2014, DDBST, 2015, Muley and Boldor, 2013, Gregory and Clarke, 2012, Henke et al., 2010, Petong et al., 2000). Reported data for ethanol permittivity (presented in Figure 2) showed two different tendencies for permittivity variation with temperature. The analysis concluded that, since different measuring techniques were employed, accuracy could explain the variations. However, these new results shed light to such dispute. It was corroborated that permittivity does not seem to be an independent physical property, since it depends on the measurement technique and the experimental conditions, as it is the presence or absence of an electromagnetic field. Hypothesis that agrees with the differentiation made between the techniques and their measuring principles.

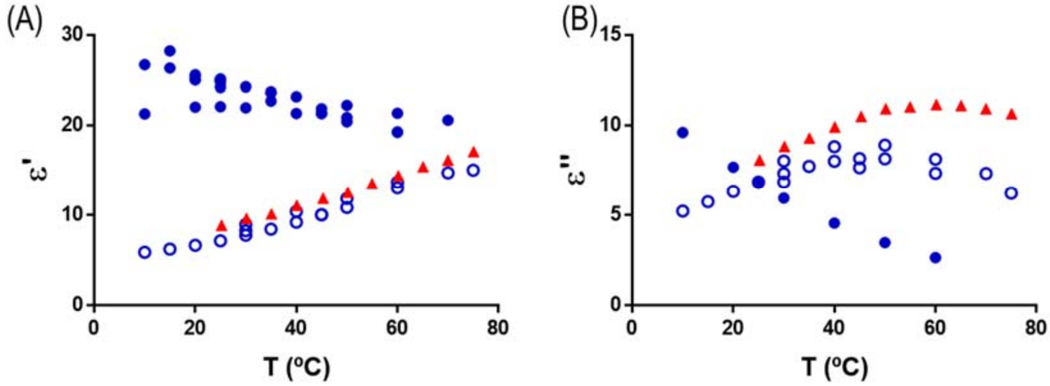

Figure 2. Ethanol permittivity. Experimental measurement (▲) and bibliographic data (● for resonant cavity and time domain measuring techniques, ○ for coaxial probes and admittance cells) (Campos et al., 2014, DDBST, 2015, Muley and Boldor, 2013, Gregory and Clarke, 2012, Henke et al., 2010, Petong et al., 2000).

» *ETHANOL:WATER MIXTURES DIELECTRIC PROPERTIES*

Ethanol:water mixtures of 25%, 50% and 75% (v/v) have also been measured. Results are presented in Figure 3 together with the model proposed for its estimation. This model has been developed applying mixing rules to the mixture pure constituents, ethanol and water.

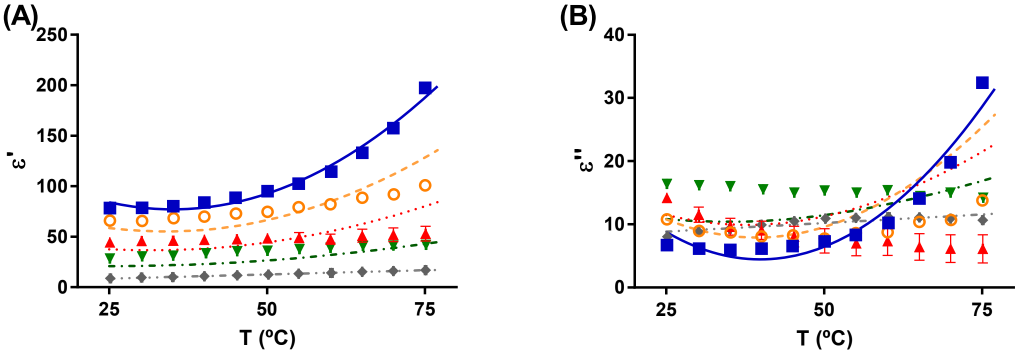

Figure 3. Water-ethanol dielectric properties as a function of temperature (■ water, ○ 25:75 (v/v) ethanol:water, ▲ 50:50 (v/v) ethanol:water, ▼ 75:25 (v/v) ethanol:water, ♦ ethanol).

Table 1. Mixing rules and average relative deviations obtained for the solvent mixture and the multiphase olive pomace solvent-mixture.

		Average relative deviations			
Mixing rule(1)	Equation	EtOH:H_2O		Olive pomace-EtOH:H_2O	
		ARD ε'	ARD ε''	ARD ε'	ARD ε''
Ideal mixture	$\varepsilon^* = X_1\varepsilon_1^* + X_2\varepsilon_2^*$	19%	45%	19%	41%
LichteneckerRother	$\varepsilon^* = \varepsilon_1^{*X_1} + \varepsilon_2^{*X_2}$	64%	61%	46%	74%
Kraszewski	$\varepsilon^{*1/2} = X_1\varepsilon_1^{*1/2} + X_2\varepsilon_2^{*1/2}$	19%	46%	19%	41%
Looyenga	$\varepsilon^{*1/3} = X_1\varepsilon_1^{*1/3} + X_2\varepsilon_2^{*1/3}$	21%	52%	20%	41%
Power law	$\varepsilon^{*\alpha} = X_1\varepsilon_1^{*\alpha} + X_2\varepsilon_2^{*\alpha}$	18%(2)	44%(2)	19%(3)	41%(3)
Álvarez	$\varepsilon^{*\alpha} = (X_1\varepsilon_1^*)^\alpha + (X_2\varepsilon_2^*)^\alpha$	19%(4)	45%(4)	18%(5)	38%(5)

(1) (Sólyom et al., 2013, Amooey, 2013, Ghanadzadeh Gilani et al., 2012)

(2) $\alpha=1.31$; (3)$\alpha=0.82$; (4)$\alpha=1.09$; (5)$\alpha=1.19$.

Table 1 shows the mixing equations tested, as well as the average relative deviation for the both permittivity parts. The estimations provided by these expressions were not accurate since deviations of around 20% and 46% were obtained for the dielectric constant and the loss factor, respectively. The minimum errors were provided by the ideal mixture law and the power law. It must be considered that the power law, despite adding a new parameter, was not able to predict permittivity with a larger accuracy than the ideal mixture. Therefore, ideal mixture was preferred to estimate the permittivity of ethanol:water mixtures. Kraszewski power law, proposed in a previous work to estimate this same mixture (Álvarez et al., 2017a), can be also considered but it provided a slight worse prediction than the ideal mixture.

» *DIELECTRIC PROPERTIES OF MULTIPHASE MIXTURES OF OLIVE POMACE AND SOLVENT*

The same mixing rules previously tested have been applied to estimate the dielectric properties of the multiphase mixture olive pomace-solvent. Olive pomace proportion has been varied from 10% to 70% (v/v) and solvent from ethanol to water (0%, 25%, 50%, 75% and 100% v/v mixtures of ethanol:water).

Table 1 gathers the average relative deviation. As well as in the case of solvents (discussed in the previous section), a good estimation could not be achieved by the combination of the mixture constituents' permittivity. Average relative deviations varied from 18% to 46% for dielectric constant and from 38% to 74% for the loss factor. The largest discrepancy between the experimental data and the model took place at high permittivity values. It was identified to correspond to high water concentrations for the dielectric constant, and high olive pomace proportion in water for the dielectric loss.

The contribution of ionic species to the loss factor was also considered in an attempt to ameliorate the model. The electrolytes inside the olive pomace could be released when the raw material and the solvent were put in contact. So, they could freely move through the extraction media, boosting the loss factor. However, if conductivity was contemplated, the average relative deviation of the loss factor soared extremely far from those measured, making the deviation raised to errors greater than the 600%. Therefore, the behaviour observed in a previous work regarding olive pomace (Álvarez et al., 2017a) can not be extrapolated to this biomass.

Thus, in order to have a model able to accurately estimate the dielectric properties of this multiphasic mixture, a polynomial equation has been proposed for each permittivity component. Expression [18] can be used to predict olive pomace-solvent mixtures with an average relative deviation of 8%, whereas expression [19] enables the calculation of the loss factor with a 22% deviation.

$$\varepsilon' = 12.55 + 48.62X_{H_2O} + 0.36TX_{OP} + 111.77X_{OP}X_{H_2O} + 47.73X_{OP}X_{EtOH} + 79.76X_{H_2O}X_{EtOH} \qquad [18]$$

$$\varepsilon'' = 0.333 + 0.86X_{H_2O} + 0.10X_{EtOH} + 9.64(X_{EtOH})^2 + 0.15TX_{OP} + 58.82X_{OP}X_{H_2O} + 29.99X_{OP}X_{EtOH} + 0.50X_{H_2O}X_{EtOH} \quad [19]$$

As it can be seen in these equations, there are important interactions between the different variables considered (T temperature (ºC), X_{OP} olive pomace composition (v/v), X_{H2O} water composition (v/v) and X_{EtOH} ethanol composition (v/v)). In particular, water composition had the greatest influence on the dielectric constant (an 86%), whereas loss factor showed a balance dependence (an average variation of 73%) for each of the three mixture components. Temperature was the less influential factor since it only made dielectric constant to vary a 11% from its initial value and loss factor a 19%.

Although this set of equations has no physical meaning like the mixing rules, it can still be used instead of them to estimate the dielectric properties of a mixture with the accuracy needed for an optimal microwave cavity design.

NOMENCLATURE

Acronyms

ARD	Average relative deviation
GAE	Gallic acid equivalents
MAE	Microwave assisted extraction
O.F.	Objective function
OP	Olive pomace

Symbols

C	Polyphenol concentration ($mg_{GAE}/g_{Dry\ OP}$)
C_P	Heat capacity (J/gK)
d_P	Penetration depth (m)
H	Moisture content (% w/w)
j	Imaginary unit
k	Extraction rate (min^{-1})
m	Mass (g)
n	Number of experiments considered (adimensional)
Q	Heat (W)
T	Temperature (°C)
t	time (min)
u_0	Initial extraction rate ($mg_{GAE}/g_{Dry\ OP}\cdot min$)
X_i	Composition of the compoenent *i* (% v/v)
α	Regression factor (adimensional)
ε'	Dielectric constant (dimensionless)

ε''	Loss factor (dimensionless)
ε^*	Complex permittivity (dimensionless)
ε_0	Free-space permittivity (F/m)
ε_σ	Ionic losses (dimensionless)
ε_0	Microwave wavelength in vacuum (m)
λ	Vaporisation enthalpy (J/g)
σ	Conductivity (S/m)

ACKNOWLEDGEMENTS

This work was funded by Spanish reginional government Junta de Castilla y León (VA040U16). A. Álvarez is grateful for her PhD grant FPU13/04678.

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CHAPTER 8

Microwave pretreatment for anthocyanin extraction from saffron flowers. Assessment of product quality

CHAPTER 8

MICROWAVE PRETREATMENT FOR ANTHOCYANIN EXTRACTION FROM SAFFRON FLOWERS. ASSESSMENT OF PRODUCT QUALITY

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ABSTRACT

The potential of saffron flowers as a source of polyphenols, and in particular of anthocyanins, was analysed for the extraction of bioactive compounds and the production of a cyanic colorant. A microwave pretreatment, previous to the conventional solid-liquid extraction process, was proposed as a feasible intensification step. The effectiveness of the microwave pretreatment was assessed considering yield enhancement and final product quality uplift. The operational variables studied were the temperature achieved during pretreatment (60-120ºC) and the solid liquid ratio (0.30-0.50 g/mL), what entailed energy densities of 0.16-0.54 kJ/mL. It was found that the addition of the microwave pretreatment to the conventional process allowed to reduce extraction time up to 12 times, and to greatly enhance final product characteristics. The extract quality evaluation was performed in terms of polyphenol richness (25% increase), product composition (80% of the anthocyanins was delphinidin), antioxidant capacity (boosted by the pretreatment) and colour (variations in red and blue hues depending on the conditions). To conclude, a microwave pretreatment where the material is heated up to a temperature of 65ºC with a solvent ratio of 0.30 g/mL was selected as the optimal to maximize process efficiency and product quality.

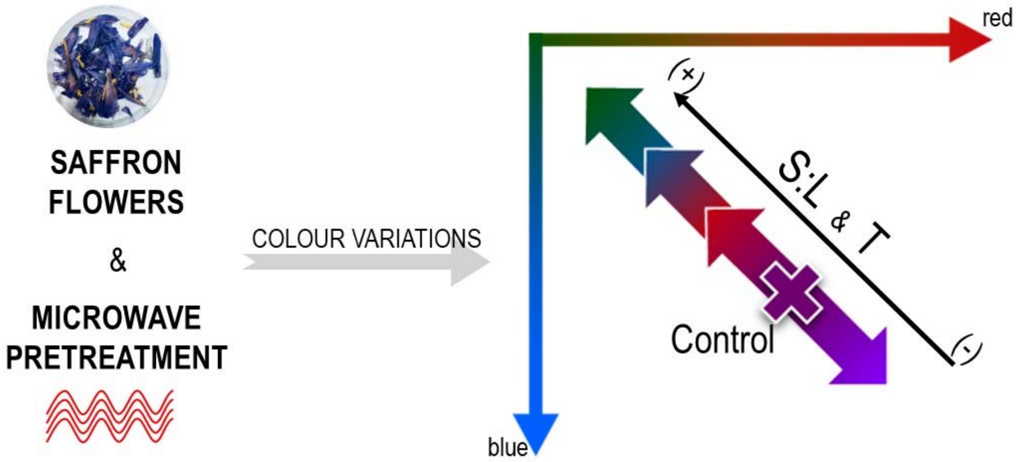
KEY WORDS

Saffron flowers, microwave pretreatment, solid-liquid ratio, kinetic analysis, colour.

HIGHLIGHTS

- Microwave pretreatment to enhance the extraction of polyphenols and anthocyanins.
- Substantial reduction of the extraction time by the addition of microwaves.
- Polyphenol richness highly improved by the microwave pretreatment.
- Delphinidin substantial richness enhancement.
- Analysis of the colour variation with the extraction conditions.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Extracts from natural products have drawn the attention of food industry as an alternative to synthetic complements due to the antioxidant properties and health benefits of their main components: the polyphenols (Shukla et al., 2009, Castro-López et al., 2017). Saffron bio-floral residues have been recently nominated as an outstanding source of polyphenols, especially of anthocyanins, due to its great potential and availability (Goleroudbary and Ghoreishi, 2016, Ahmad et al., 2018). The production of the spice entails a large generation of residues. In particular, 68 kg of flowers are needed to produced 1 kg of spice (Putnik et al., 2017). The petals and the stigmas constitute this by-product that actually has no use, but it can be further valorised in order to enhance the sustainability of the process.

A rich polyphenolic extract stemming from saffron flowers would be highly valued in food industry as a natural colourant. Actual market's trends show a predilection for such tints instead of synthetic ones because of their health benefits (Mahdavi et al., 2014, Araújo et al., 2018). In the case of saffron flowers, not only their high content in antioxidant compounds makes it a convenient food ingredient, but also their composition in minerals, dietary fibre, sugars, anions and organic acids (Serrano-Díaz et al., 2013a). Nonetheless, anthocyanins are the phytochemical that will carry the major role as food additives, as a consequence of their superoxide-radical scavenging capacity (Ongkowijoyo et al., 2018) and the cyanic hues that they bear (Xiong et al., 2006). Blue colorants are rare in nature (Araújo et al., 2018). Few examples have been described in literature, like the blue pigments isolated from Port red wine (Mateus et al., 2003). However, most of the natural blue colourants are now under research (Martins et al., 2016). Among the anthocyanins, the most abundant, delphinidin and petunidin derived, have been reported to provide the cyanic colours (purple, lilac, mauve and blue) in saffron flowers. Malvidin has also been found, but in a lower percentage (Nørbæk et al., 2002, Goupy et al., 2013, Serrano-Díaz et al., 2014b).

According to previous works, there is a correlation between the concentration of active compounds and colour (Sant'Anna et al., 2013). So, colour is an important attribute to consider too. In order to characterise hues for industrial application, the use of the CIELAB space is recognised as an international standard (Sant'Anna et al., 2013). An sphere is defined where each axis corresponds to lightness (*L**), green-vs-redness (*a**) and yellow-vs-bluish (*b**) (Ahmad et al., 2018). Table 1 specifies the range of values that these variables can have and their meaning.

Table 1. CIELAB parameters meaning.

Lightness		Hue			
L=0	black	a*>0	red	b*>0	yellow
L=100	white	a*<0	green	b*<0	blue

Most of the published works about the valorisation of saffron flower residues are focused on their *in vivo* properties, demonstrating so the potential of extracts as chemo preventive agents (Zheng et al., 2011, Sánchez-Vioque et al., 2016, Tuberoso et al., 2016, Favot et al., 2003, Meiers et al., 2001). However, extraction conditions have not been so extensively analysed, and the few results available present a conspicuous disparity. Table 2 presents a review of such studies. As it can be seen, optimum yields are not in accordance with the extraction conditions. Whereas in some works the maximum yield is obtained with water as solvent and ambient temperature (Serrano-Díaz et al., 2014a), others hold that a mixture of organic and water solvents at high temperature provides a better efficiency (Ahmadian-Kouchaksaraie et al., 2016, Ongkowijoyo et al., 2018). Beside this, they all agree that an acidified solvent enhances the extraction and the stability of the active compounds, especially of anthocyanins (Mateus and de Freitas, 2009).

Table 2. Conventional solid-liquid extraction optimal conditions and yields reported in literature.

Raw material	Solvent	S:L ratio (g/mL)	T (ºC)	t (min)	Yield	Reference
Freeze-dried and milled saffron flowers	Water:HCl 100:1 (v/v)	1/30	25	60	13.57±1.16 mg_{GAE}/g_{DrySF}	(Serrano-Díaz et al., 2014a)
Saffron flowers	Ethanol:Water 59% (v/v)	1/3	66	104	11.34±3.00 mg_{GAE}/g_{DrySF}	(Ahmadian Kouchaksaraie et al., 2016)
Dried and milled saffron flowers	Ethanol:acidified water 50% (v/v)	1/15	25	1440	1712 mg_{CGE}/L	(Jafari et al., 2016)
Dried and milled saffron flowers	Ethanol:Water 25% (v/v)	1/20	25	1440	1609 mg_{CGE}/L	(Khazaei et al., 2016)

Regarding the extraction conditions, conventional extraction works share two common traits: the use of very low solid-liquid ratios and long extraction times. Low solid-liquid ratios entail a large solvent consumption, which is against the *Green Chemistry Principles* (Anastas and Waner, 1998). Extraction times (prolonged for more than one hour) seem quite excessive for the extraction of polyphenols from petals, since the structure is not that complex to represent a large mass transfer resistance. These two features support the implementation of intensification techniques. Some of them have already been studied. High pressure extractions (subcritical water and supercritical carbon dioxide) allow to obtain a slight yield improvement (Ahmadian-Kouchaksaraie et al., 2016, Ahmadian-Kouchaksaraie and Niazmand, 2017). As well as enzymatic extraction, that also contributes to enhance the colour stability of the final extract (Lotfi et al., 2015). Da Porto and Natolino (2018) assessed the influence of solid-liquid ratio on ultrasound (UAE) and microwave assisted extraction (MAE). Although Ahmadian-Kouchaksaraie et al. (2016) conditions are used by Da Porto as a control, 4-fold higher yields, compared to other authors average results, were obtained in the three studied extractions (conventional, MAE and UAE). This, together with the use of extremely high solid liquid ratios (1/30 and 1/50 g/mL for UAE and MAE, respectively) did not allow to take these conclusions as a reference for such intensification techniques.

Since microwaves have been found to provide better yields than other intensification techniques (Castro-López et al., 2017), this work focused on the optimization of microwave extraction. However, in contrast to usual MAE processes, in this study radiation has been used as a previous step to a conventional solid-liquid extraction. MAE bench-scale experiments usually employ a temperature control that entails an initial peak of power, to rapidly achieve the temperature set conditions, and an intermittent short power radiation to maintain such conditions (Galan et al., 2017). This, together with the difficulties to scale up a large cavity, have led to consider microwaves only as a pretreatment. By providing the extraction media with a peak of microwave energy, a high temperature in a short time can be achieved. So, the possible disruption of the matrix and the rapid extraction of the compounds can take place (Yedhu Krishnan and Rajan, 2016). Also, because of its short duration, thermosensible compounds degradation is very low if the pretreatment is followed by a rapid cooling (Sólyom et al., 2014). Regarding the scale-up for the industrial application, the use of a low residence time in the microwave oven allows the use of a compact oven that can be easily designed to be implemented at a larger scale. In addition, a previous work demonstrated that a microwave pretreatment can enhance not only process yield, but also final product polyphenol richness (Álvarez et al., 2017).

Thus, this work is aimed to develop an efficient and feasible scale-up process to obtain a functional antioxidant colourant.

2. MATERIALS AND METHODS

2.1. RAW MATERIAL

Saffron flowers were donated by a local farmer. Saffron was collected before sunlight in October 2017 and manually processed. Flower by-products were immediately freezed and stored at -20ºC. Before used, flowers were thawed for one hour at 4ºC. Table 3 gathers the characterization of the raw material employed in this work. Moisture was determined gravimetrically by drying the sample until constant weight at 105ºC. Fat and extractives were quantified by a soxhlet extraction with hexane, and ethanol and water, respectively. The protein content was computed by the Kjeldahl method using a conversion factor of 6.25. Ashes were measured by means of the char formed at 550ºC. Fiber content is assumed to be 173.61 mg/$g_{Dry\ SF}$, which is the remaining not analysed. This value is in accordance with bibliographic data (Serrano-Díaz et al., 2013b).

Table 3. Saffron flowers proximate composition.

Moisture ($g_{H2O}/g_{Dry\ SF}$)	Fat (mg/$g_{Dry\ SF}$)	Ash (mg/$g_{Dry\ SF}$)	Protein (mg/$g_{Dry\ SF}$)	Extractives (mg/$g_{Dry\ SF}$)
5.096±0.001	17.69±0.70	36.28±3.46	45.66±0.30	726.76±10.18

Saffron heat capacity was measured in a Setaram Micro DSC II microcalorimeter (Páramo et al., 2002) from 20ºC to 85ºC, resulting to be of 3.89±0.02 J/g·K.

2.2. CHEMICAL REAGENTS

AAPH (α,α'-azodiisobutyramidine dihydrochloride), gallic acid, sodium fluorescein and trolox were purchased from Sigma. Acetonitrile, ammonium phosphate monobasic, chlorhydric acid, ethanol, Folin-Ciocalteu reagent, phosphate salts ($NaH_2PO_4 \cdot 2H_2O$ and $Na_2HPO_4 \cdot 12H_2O$), phosphoric acid, potassium chloride, sodium acetate and sodium carbonate were obtained from Panreac. HPLC standards catechin, delphinidin, epicatechin, gallic acid, malvidin and quercetin were bought from Extrasynthese, except for kaempferol which were purchased from TCI. A Millipore unit was used to purify the water used as solvent.

2.3. EXTRACTION PROCEDURE

A conventional solid-liquid extraction process was used as a control reference to assess the influence of the microwave pretreatment. Extraction conditions reported in bibliography were compared in order to find the optimal ones. In all the experiments, an amount of 30 grams of saffron flowers was weight and mixed with the correspondent volume of solvent to achieve the desired solid-liquid ratio. An ethanol-water mixture was used as solvent. Ethanol was chosen as the organic solvent because of its GRAS (Generally Recognise As Safe by the FDA) certification (Putnik et al., 2017). In order to enhance anthocyanin yield and stability, acidified water to pH=2 with hydrochloric acid was used (Ongkowijoyo et al., 2018).

Once the saffron flowers were mixed with the solvent, a 5-minute homogenization was carried out at ambient temperature and gentle stirred. The proper extraction was set to begin when the flask was place in a thermostatic bath at the set temperature and with vigorous stirring.

For the experiments in which a microwave pretreatment was added to the process, it was integrated after the homogenization and before the thermostatic and stirring bath. The microwave pretreatment was carried out in a CEM Discover One unit (CEM Corp). It has been devised as an intense and short pretreatment, easy to be scaled up for a future industrial application. So, the maximum power, 300 W, was irradiated for shorter than traditional MAE times, from 40 to 145 seconds. Temperatures of 60, 80, 100 and 120ºC were achieved. Such temperatures were measured with an optical fibre (FOTEMP4, OPTcon GmbH), weekly calibrated at 0ºC with an ice bath of distilled water. The pretreatments at 60 and 80ºC were conducted in an open round bottom flask, whereas for the 100 and 120ºC a pressure cell was required to guarantee the liquid phase of the solvent. A 100 mL QianCap glass pressure vessel (QLabtech) was employed.

Unlike the majority of published MAE works, the microwave pretreatment has been quantified in terms of absorbed energy instead of radiation conditions. The absorbed energy ($Q_{absorbed}$) was calculated by an energy balance from the two main contributions: the sensible and the latent heats, as it is expressed in equation [1]. Losses to the environment have been dismissed in accordance to Sólyom's conclusions (Sólyom et al., 2011).

$$\mathrm{Q_{absorbed} = Q_{sensible} + Q_{latent}} \qquad [1]$$

Sensible heat has been computed by means of equation [2], considering the energy required to increase the extraction media (solvent and saffron flower) from the initial to the pretreatment temperature.

$$\mathrm{Q_{sensible}} = \sum \mathrm{mC_P\Delta T} \qquad [2]$$

Regarding latent heat calculations, equation [3] was used. The moles of evaporated solvent ($n_{evaporated}$) were estimated by two different procedures, depending if the pretreatment was performed in an open vessel or in a pressure cell. For the open vessel experiments (pretreatments at 60 and 80ºC), solvent evaporation was calculated by weight loss. However, when the temperature in the pretreatment went above the normal boiling point of the solvent (100 and 120ºC), the pretreatment was performed in a closed vessel. In this case, the evaporated solvent was accumulated in the gas space, increasing the pressure in the vessel. The number of evaporated moles was then calculated from the maximum pressure value reached during the pretreatment, assuming ideal gas behaviour (equation [4]).

$$\mathrm{Q_{latent}} = \mathrm{n}_{evaporated}\lambda \qquad [3]$$

$$\mathrm{n_{evaporated}} = \frac{\mathrm{PV_0 - RTn_0}}{\mathrm{RT - P/\rho_{mol}}} \qquad [4]$$

In the above expression, Q stands for heat in J, m is the mass of flowers plus solvent in grams, C_P is the average heat capacity of the mixture in J/gK, ΔT represents the temperature increment in K, λ is the solvent vaporisation heat in J/g, P is the maximum final pressure in Pa, n_0 is the number of air moles in the initial gas space, T is the maximum final temperature, V_0 is the initial volume of the gas space in m^3, R is the ideal gas constant (8.314 J/mol·K) and ρ_{mol} the molar density of the solvent in mol/m^3. Liquid composition changes due to evaporation were neglected since only a very low fraction of the solvent was evaporated (between the 0.18% and 0.35%).

Once the pretreatment was finished, the media was rapidly cooled down using an ice bath. In those cases when the solid-liquid ratio used in the pretreatment was different from the one used in the subsequent extraction stage, the extra cold solvent was added just after the pretreatment to accelerate the cooling setp. Different solid-liquid ratios have already been used in previous works proving to be advantageous for the extraction efficiency (Álvarez et al., 2017). Finally, the media was placed in the thermostatic bath and continued as the conventional solid-liquid extraction. Liquid samples were taken along the process to determine the extraction kinetics. This experimental data was adjusted to the first order kinetic equation described in [5] by minimizing the average relative deviation (equation [6]).

$$\mathrm{C_{cal}} = \mathrm{C_0} + \mathrm{C_f}[1 - \exp(-\mathrm{kt})] \qquad [5]$$

$$\mathrm{ARD} = \frac{1}{\mathrm{n}}\sum\left|\frac{\mathrm{C_{exp}} - \mathrm{C_{cal}}}{\mathrm{C_{cal}}}\right| \qquad [6]$$

The regressed parameters were the initial concentration C_0 in mg/g$_{\mathrm{Dry\ SF}}$, C_f a pre-exponential factor also in mg/g$_{\mathrm{Dry\ SF}}$, and the rate constant k in min^{-1}.

This model allows to assess the extraction efficiency by means of the two derived parameters initial rate and yield. Initial extraction rate (u_0, calculated by equation [7]) indicates the extraction pace, which will determine the time required to exhaust the raw material. The higher this parameter is, the briefer will take to achieve the maximum yield. Initial extraction rate is measured in mg/g$_{\mathrm{Dry\ SF}}$/min.

$$\mathrm{u_0} = \left.\frac{\partial \mathrm{C}}{\partial \mathrm{t}}\right|_{\mathrm{t}=0} = \mathrm{C_f k} \qquad [7]$$

Yield (C_∞, equation [8]) determines the maximum amount of polyphenols that can be obtained per gram of dry saffron flowers at the extraction conditions. It is measured in mg/g$_{\mathrm{Dry\ SF}}$.

$$\mathrm{C_\infty} = \lim_{\mathrm{t}\to\infty} \mathrm{C} = \mathrm{C_0} + \mathrm{C_f} \qquad [8]$$

Polyphenol and anthocyanin extract richness were also computed to characterise the extract. This parameter indicates the proportion of polyphenols or anthocyanin against the total amount of other extracted substances (sugar, fibres…). Since extracts are commercialised as a solid product, their richness is crucial as it will boost or worsen the quality and price of the final product. Richness was expressed in mg/g$_{\mathrm{Dry\ Extract}}$, and was calculated from polyphenols or anthocyanin concentration (mg/g$_{\mathrm{Extract}}$) and the solid extract residue (g$_{\mathrm{Dry\ Extract}}$/g$_{\mathrm{Extract}}$).

2.4. pH MEASUREMENT

pH along the extraction was recorded by a Jenway 3505 pH meter.

2.5. EXTRACT CHARACTERIZATION

2.5.1. TOTAL POLYPHENOL CONTENT

Folin-Ciocalteu method was employed for total polyphenol content determination (TPC). Briefly, a volume of 40 µL of sample was mixed with 3000 µL of distillate water and 200 µL of Folin-Ciocalteu reagent. After 5 minutes, 600 µL of sodium carbonate were poured. The mixtures were left 30 minutes at 40ºC before their absorbance at 765 nm was recorded (Shimadzu UV/VIS Spectrophotometer). Results were expressed in gallic acid equivalents (mg_{GAE}/g).

2.5.2. ANTHOCYANIN CONTENT

The pH differential method was used to quantify the anthocyanin content (AC). Samples were diluted in two bufferes: one with pH=1 of potassium chloride 0.025 M and another of pH=4.5 of sodium acetate 0.4 M. The increment in absorbance between 520 and 700 nm was used together with an extinction coefficient of 26,900 L/mol·cm to work out the anthocyanin content, expressed in cyaniding-glucoside equivalents (mg_{CGE}/g).

2.5.3. SOLID RESIDUE

The solid residue was gravimetrically determined by drying the extracts at 105ºC overnight.

2.5.4. ANTIOXIDANT CAPACITY

Antioxidant activity (AAO) was computed as the ability of the extracts to quench oxygen radicals from the decompostition of AAPH (240 mM). Sodium fluorescein (100 nM) was used as probe and Trolox as the standard (200 µM). Phosphate buffer (10mM, pH=7.4) was employ as solvent during these assays.

In a 96-well plate, 150 µL of fluorescein and 25 µL of sample (standard or diluted extract) were poured. After 30-minute incubation at 37ºC, 25 µL of AAPH was added. Fluorescence was then recorded in a Fluorstar Optima (BMG Labtech) at an emission wavelength of 530±25 nm and excitation wavelength 485±20 nm, until the signal variation was null. Trolox calibration was repeated in every assay and samples were measured at least six times to minimise experimental error. Results were expressed as trolox equivalents ($mmol_{TE}/g$).

2.5.5. HPLC

Catechin, delphinidin, epicatechin, gallic acid, kaempferol, malvidin and quercetin were quantified. A Waters e2695 Separation module and a Waters 2998 photodiode array detector (DAD) were employed. A previous method was used as reference (Gómez-Alonso et al., 2007). A volume of 20 µL of sample was injected into the 1 mL/min eluent flow and passed through a Teknokrima C18 (250x4.6 mm, 5 µm) column dotted with an OptiGuard pre-column at 35ºC. The eluents used were (A) ammonium phosphate monobasic 50mM and pH=2.6, (B) a mixture of 80% acetonitrile and 20% of eluent A, and (C) acidified water with phosphoric acid to pH=1.5. The eluent gradient is detailed in Table 4. DAD signals were recorded at 360 and 520 nm as well as the UV/VIS spectra. Compound identification was performed by comparing retention time and UV/VIS spectra with the standards.

Table 4. HPLC eluents gradient.

time (min)	Eluent A[1] (%)	Eluent B[2] (%)	Eluent C[3] (%)
0	100	0	0
2	100	0	0
5	92	8	0
17	0	14	86
22	0	18	82
29.5	0	21	79
55	0	33	67
70	0	50	50
75	0	50	50
78	20	80	0
81	20	80	0
86	100	0	0

[1] $NH_4H_2PO_4$ 50 mM pH=2.6;

[2] 80% acetonitrile + 20% eluent A;

[3] H_3PO_4 200 mM pH=1.5.

2.6. COLOUR MEASUREMENT

An spectrophotometric method provided by Eaton et al. (1995) was used to determine extract colours. This method allowed to compute colour coordinates in the XYZ space by applying equations [9], [10] and [11] with the measured transmittances (T) at the selected wavelengths (see the Appendix). These measurements correspond to an illuminant C and a 2º standard observer.

$$X = 0.03269 \cdot \Sigma T_X \qquad [9]$$

$$Y = 0.03333 \cdot \Sigma T_Y \quad [10]$$

$$Z = 0.03938 \cdot \Sigma T_Z \quad [11]$$

In order to prevent errors due to extracts different dilutions, samples were dried and rediluted to have a similar concentration. Liquid samples were concentrated in a rotary evaporator (Heidolph) to remove the ethanol, and freeze dried at -50ºC and 0.1 bar (Telstar LyoQuest). Then, dried extracts were rediluted up to a concentration of around 500 ppm. This final solution was placed in a ShimadzuUV/vis Spectrophotometer where a transmittance scan from 700 to 400 nm was performed.

Since CIELAB is the most extended colour space in food industry, XYZ coordinates were transformed into the CIELAB ones (*L*, a*, b**) by means of equations [12], [13] and [14]. In these expressions, X_n, Y_n and Z_n represent the illuminat C tristimulus, which for the conditions used they have a value of X_n=98.0681, Y_n=100 and Z_n=118.2313.

$$L^* = 116 \cdot (Y/Y_n)^{1/3} - 16 \quad [12]$$

$$a^* = 500 \cdot \left[(X/X_n)^{1/3} - (Y/Y_n)^{1/3}\right] \quad [13]$$

$$b^* = 200 \cdot \left[(Y/Y_n)^{1/3} - (Z/Z_n)^{1/3}\right] \quad [14]$$

It must be noticed that, since no direct measurement of the final product was done (but a dilution instead), these measurements only allow to compare changes in colour. For an ease comparison, the total colour difference (Δ*E* in expression [15]) were used. In this equation the subindex *i* denotates the sample analysed whereas *0* stands for the control. The influence of the microwave pretreatment on product colour was assessed in this work so, the conventional solid-liquid extraction (without microwaves) was taken as the control.

$$\text{Total color difference } (\Delta E) = \sqrt{(L_i^* - L_0^*)^2 + (a_i^* - a_0^*)^2 + (b_i^* - b_0^*)^2} \quad [15]$$

2.7. EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

Solid-liquid ratio (0.30-0.70 g/ml) and microwave irradiated energy to reach temperatures between 60 and 120ºC were the pretreatment variables studied. Experimental conditions are gathered in Table 5. A factorial experimental design has been employed. This display allows to identify the effects of each variable (linear and quadratic) as well as possible interactions. ANOVA tables with a confidence of 95% (p-value of 0.05) have been used to determine significant effects. In order to compare the influence of the variables in a similar range, codified variables have been employed. Design Expert software has been used for this analysis.

For an easy CIELAB parameters interpretation, the software JMP has been employed to estimate ellipses with a 90% coverage of the experimental dispersion.

Table 5. Experimental conditions and results obtained.

Run	Pretreatment conditions			Total polyphenols extraction		Anthocyanins extraction	
	S:L (g/mL)	T_{MW} (°C)	Energy (kJ/mL)	C_∞ ($mg_{GAE}/g_{Dry\ SF}$)	u_0 ($mg_{GAE}/g_{Dry\ SF}$/min)	C_∞ ($mg_{CGE}/g_{Dry\ SF}$)	u_0 ($mg_{CGE}/g_{Dry\ SF}$/min)
1	0.50 (0)	80 (0)	0.39	26.62	2.85	6.97	0.58
2	0.50 (0)	80 (0)	0.40	29.00	2.56	8.00	0.58
3	0.50 (0)	100 (+1)	0.51	24.99	2.45	6.97	0.58
4	0.50 (0)	80 (0)	0.37	27.68	3.00	8.43	0.72
5	0.70 (+1)	60 (-1)	0.31	21.05	1.96	5.00	0.56
6	0.30 (-1)	80 (0)	0.35	28.12	10.45	7.94	2.51
7	0.50 (0)	80 (0)	0.36	26.62	1.77	6.70	0.49
8	0.30 (-1)	100 (+1)	0.51	29.53	9.25	8.05	2.38
9	0.70 (+1)	100 (+1)	0.51	24.17	2.57	7.17	0.68
10	0.50 (0)	60 (-1)	0.29	23.01	3.52	9.34	0.97
11	0.30 (-1)	60 (-1)	0.23	23.82	1.62	7.62	0.42
12	0.70 (+1)	80 (0)	0.36	27.64	1.14	6.93	0.34
13	0.70 (+1)	80 (0)	0.40	26.01	7.11	6.52	1.52
14	0.30 (-1)	120 (+2)	0.58	28.62	10.98	5.51	1.83
15	0.70 (+1)	120 (+2)	0.58	25.64	1.03	4.55	0.15
16	0.50 (0)	120 (+2)	0.64	26.68	0.98	5.06	0.18
Control	--	--		26.05	1.23	6.80	0.20

Table 5. (continued) Experimental conditions and results obtained.

Run	Final product							
	TPC richness ($mg_{GAE}/g_{Dry\ Extract}$)	AC richness ($mg_{CGE}/g_{Dry\ Extract}$)	Delphinidin richness ($mg/g_{Dry\ extract}$)	AAO ($mmol_{TE}/g_{Dry\ Extract}$)	L*	a*	b*	ΔE
1	45.51	11.71	7.43	1.09±0.06	87.54	29.18	-11.23	3.82
2	43.69	11.76	7.71	1.96±0.22	86.97	29.04	-9.46	4.35
3	44.40	9.88	6.04	2.30±0.08	88.49	24.77	-8.78	8.75
4	47.50	13.36	7.58	1.42±0.08	85.78	31.08	-11.53	1.47
5	41.52	10.58	5.99	2.04±0.23	88.80	24.15	-8.83	9.38
6	42.08	11.74	6.37	1.61±0.15	82.07	37.81	-13.43	6.67
7	41.95	10.23	5.49	1.56±0.11	87.96	25.14	-8.98	8.20
8	45.94	12.52	7.34	1.43±0.10	86.44	29.13	-10.78	3.61
9	44.02	12.37	7.89	1.11±0.08	86.98	29.61	-10.72	3.33
10	42.80	14.18	6.12	1.32±0.08	82.79	39.24	-14.82	7.95
11	44.78	12.01	5.03	0.74±0.04	84.82	34.06	-12.58	1.98
12	42.27	10.89	6.28	1.04±0.10	88.94	22.46	-4.46	12.85
13	45.18	11.51	5.38	1.32±0.08	87.89	28.04	-11.36	4.97
14	52.21	10.06	6.64	1.70±0.11	88.63	26.67	-10.43	6.64
15	45.35	8.28	6.49	0.77±0.09	87.81	28.31	-11.34	4.69
16	53.34	9.88	5.98	1.27±0.07	88.41	26.75	-9.94	6.61
Control	41.82	12.00	6.51	1.04±0.04	85.80	32.53	-11.80	0.00

3. RESULTS AND DISCUSSION

3.1. CONVENTIONAL SOLID-LIQUID EXTRACTION

Preliminary studies based on the extraction conditions reported in Table 2 stablished the range of process variables to look for the optimal extraction conditions. Industrial and scale-up concerns, like solvent consumption or the use of the saffron flowers without any conditioning (milling or drying), were taking into account.

Regarding extraction temperature, previous authors suggested the convenience of employing extraction temperatures of 30ºC and 60ºC. Similar polyphenol yields and richness were achieved in both cases. However, an anthocyanin yield decreased was found at 60ºC due to the thermal degradation of these compounds after 40 minutes of extraction. Therefore, 30ºC was selected as the extraction temperature.

In terms of solvent, water was found to greatly enhance anthocyanin extraction, but not polyphenols in such extent. So, it was discarded in favour of a 50% ethanol:water (water acidified to pH=2) solution since this mixture allowed to achieve a compromise between polyphenol and anthocyanin yield.

Solid-liquid ratio was the most influential factor, so a thoughtful analysis was performed. Ratios ranging from 0.10 to 0.75 g/mL were studied. Results can be found in Figure 1.

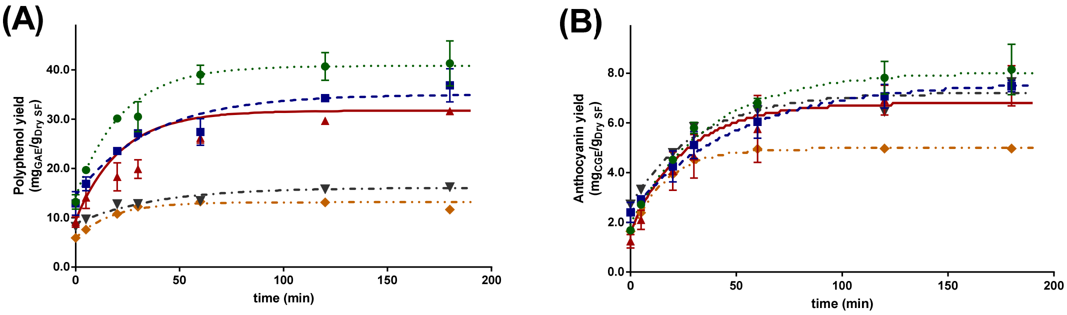


Figure 1. Solid-liquid ratio influence on conventional solid-liquid extraction for (A) polyphenol and (B) anthocyanin extraction. ●S:L=0.10 g/mL, ■ S:L=0.20 g/mL, ▲ S:L=0.30 g/mL, ▼ S:L=0.50 g/mL, ♦ S:L=0.75 g/mL.

It stood out that different final yields were obtained in the case of total polyphenols. What is more, polyphenol yields were found to decrease when the ratio was increased. This led to infer that mass transfer could be influenced by the solvent. This solvent effect could be a consequence of the amount used or the acid hydrolysis that it caused (acidified solvent). Regarding solid-liquid ratio, the proportion of solvent employed determined if either the saffron flowers were completely exhausted (large solvent consumption in low solid-liquid ratios) or the obtaining of a concentrated extract (low solvent proportion by high-solid liquid ratios). In the first case, the depletion of the saffron took place as a consequence of the great concentration gradient that arose between the solid and the large solvent amount. However, these leaked polyphenols were also diluted in the solvent, so a low concentrated liquid product was finally obtained. On the contrary, if less solvent was used, fewer polyphenols from the solid matrix were released, but they accumulated in the liquid phase, providing a more concentrated extract.

Another aspect considered was the likely influence of the acidified solvent. Since acidified water was employed as a solvent constituent, the presence of these ions may interfere with the saffron structure, reducing polyphenols linkage and enhancing the release of polyphenols. Figure 2 represents the pH evolution during the extraction at different solid-liquid ratios. Lower ratios entailed a larger solvent consumption and so, its pH was also closer to that of the fresh solvent (pH=2). Thus, a greater concentration of the hydronium ion was present in the extraction media, contributing to the extraction of compounds by aiding their released by means of acid hydrolysis.

On the other hand, unlike total polyphenols, anthocyanin extraction attained almost the same final yield independently of the solvent (except in the case of 0.75 g/mL, which was proved to be limited by solubility). This indicated that anthocyanin released from saffron flowers occurred easily.

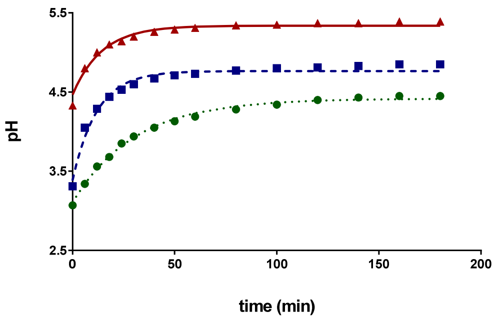


Figure 2. pH evolution during the extraction for a solid liquid ratio of
●S:L=0.10 g/mL, ■ S:L=0.20 g/mL, and ▲ S:L=0.30 g/mL.

In sum, for the subsequent study, a solid-liquid ratio of 0.30 g/mL was selected as the optimum. Despite the fact that this ratio did not achieved the highest polyphenol yield (a 23% less was attained), it allowed to obtain a much more concentrated extract. In particular, 230% more polyphenols were obtained per litre of solvent, and 276% if anthocyanins were considered. This increase is crucial for the sustainability of the process since more product can be obtained. It has to be also taken into account that less solvent needs to be recovered, so operational costs can be significantly foreshortened. Thus, the ratio of 0.30 g/mL represented a propitious balance between solvent consumption, bioactive compounds extraction and process efficiency.

3.2. MICROWAVE PRETREATMENT EXTRACTION

This section analyses the effect on kinetics caused by adding a microwave pretreatment to the conventional solid-liquid extraction. The influence of the solid-liquid ratio and the temperature reached in the pretreatment were the variables studied. Both factors influenced the total energy absorbed by the extraction media, which has been found crucial for this kind of pretreatments (Álvarez et al., 2017). However, in this work the analysis has been done in terms of the former variables due to the impossibility to clearly discern the absorbed energy influence (dependent on solid-liquid ratio and pretreatment temperature). Nonetheless, energies ranging from 0.23 to 0.64 kJ/mL were absorbed by the extraction media. It must be added that absorbed energy is a vital variable to bear if the pretreatment is to be reproduced.

A conventional solid-liquid extraction (without a microwave pretreatment) was taken as the control reference to assess the efficiency of the pretreatment.

3.2.1. INITIAL EXTRACTION RATE

The most relevant effect of implementing a microwave pretreatment was the acceleration of the initial extraction rate. This boost was found in the extraction of polyphenols and of anthocyanins, presenting both similar tendencies. The acceleration exerted by the pretreatment ranged from 2 to 12 times the conventional value. How solid-liquid ratio and pretreatment temperature influenced this acceleration can be observed in Figure 3 and Figure 4, respectively.

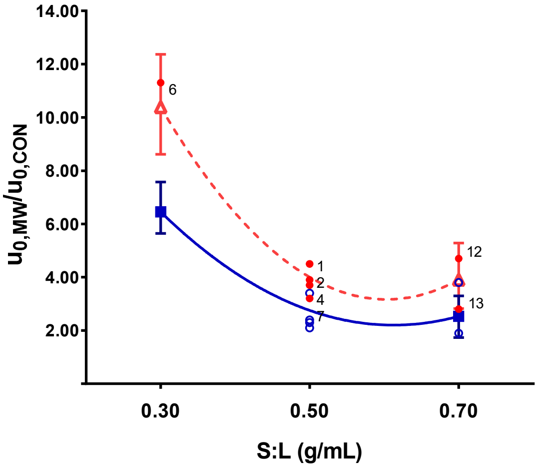


Figure 3. Solid-liquid ratio influence on initial extraction rate respect to the conventional extraction for a pretreatment temperature of 80ºC (■ polyphenol extraction, Δ anthocyanin extraction, ○ and ● experimental points for polyphenols and anthocyanins, respectively)

In the case of solid-liquid ratio, the lower the ratio, the faster the extraction. This same behaviour was found for the conventional solid-liquid extraction too (kinetic curves shown in Figure 1), but with a less pronounced tendency. It was concluded that low ratios presented higher initial extraction rates than high ratios, due to large concentration gradients. Since low ratios entailed a larger solvent consumption, the leaked active compounds were diluted in the liquid phase, which was translated in a larger driving force maintained for longer. A fact that was observed as an acceleration of the initial extraction rate. On the other hand, high ratios employed less solvent volume, so the liquid phase was more concentrated in polyphenols, what decreased the concentration gradient and so the polyphenol extraction rate. Nevertheless, the implementation of the pretreatment was able to enhance the conventional initial extraction rate, so an advantageous improvement of the mass transfer can be inferred due to the microwaves.

Regarding pretreatment temperature, initial extraction rate was accelerated by temperatures up to 100ºC. For a higher temperature (120ºC) polyphenol content showed no additional improvement whereas anthocyanin content seemed to present a decrease, despite the experimental error. If the anthocyanin richness was taken into account (results gathered in Table 5), a 17% anthocyanin richness decrease was observed from 60-100ºC to 120ºC, what corroborated the thermal degradation assumption. Thus, it can be said that while pretreatment temperature was increased up to 100ºC, polyphenol and anthocyanin extraction were rapidly accelerated, compensating their thermal degradation rate. However, at higher pretreatment temperatures the influence of degradation exerted a greater weight in the balance with extraction yield. This led to the anthocyanin concentration reduction, which became evident in the initial extraction rate. This degradation conclusion is in agreement with Serrano-Diaz et al. (2013a) that held that 70-90ºC was the best temperature range to maintain saffron flowers antioxidant properties while drying, over temperatures of 110ºC and 125ºC. On the other hand, it contrasts with Solyom's (2014) results about grape pomace anthocyanins stability, where a 10% degradation after 3 minutes at 120ºC was reported. In this work, the extraction media was exposed to that temperature during 2.4 minutes. Almost double thermal degradation was observed in this case respect to the value observed by Solyom, what led to the conclusion that saffron anthocyanins presented a lower stability when they were enclosed in this matrix.

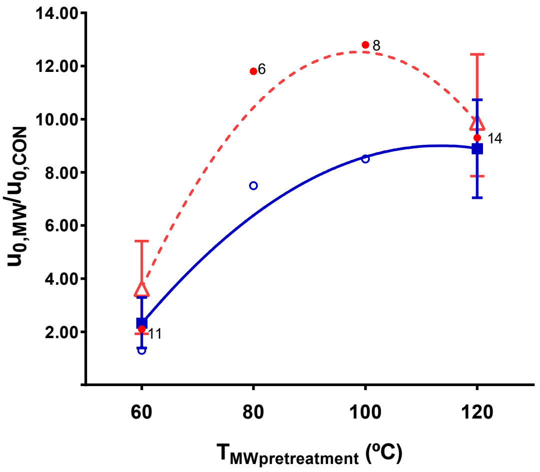


Figure 4. Pretreatment temperature influence on initial extraction rate respect to the conventional extraction for a solid-liquid ratio of 0.30 g/mL (■ polyphenol extraction, Δ anthocyanin extraction, ○ and ● experimental points for polyphenols and anthocyanins, respectively)

It is worth considering the interaction between solid-liquid ratio and pretreatment temperature for initial extraction rate. It was found significant for the extraction of polyphenols. However, for anthocyanin's it was not that crucial (a significance of 10%), although it presented the same behaviour than polyphenols. As it can be seen in Figure 5, no difference was found in initial extraction rate when a low pretreatment temperature was employed independently of the solid-liquid ratio. However, if a higher pretreatment temperature was used, a substantial acceleration was observed for low ratios. In this case, the two variables met the conditions that improve extractions: a higher temperature accelerates the rate, whereas the low solid liquid assured a large driving force for the leaching of active compounds. Thus, the microwave pretreatment at low temperature was not able to substantially overcome the common limitations of normal extraction process.

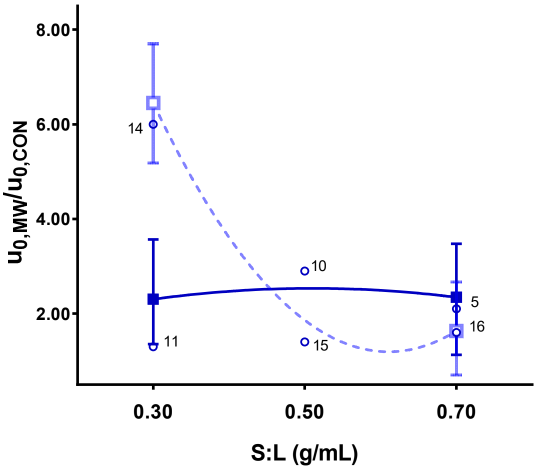


Figure 5. Solid-liquid and energy interaction influence on total polyphenol initial extraction rate respect to the conventional extraction for pretreatment temperatures of 60ºC (■) and 120ºC (□).

Thus, the implementation of the microwave pretreatment allowed to considerably reduce extraction time. A temperature of 105ºC was found optimal for the fastest total polyphenol extraction, and 87ºC for anthocyanin. Such improvement allowed to obtain a high polyphenol yield in considerably less time. For instance, considering experiment #8 for polyphenols (0.30 g/mL and 100ºC) and #6 for anthocyanins (0.30 g/mL and 80ºC), to achieve a 90% of the final yield, extraction time was reduced from 29 minutes and 53 minutes in the conventional extraction to 5 minutes with the pretreatment, respectively. That was a substantial 5 and 10 times reduction. Therefore, the addition of the pretreatment enabled to attain the highest extraction yield in the early subsequent stages after the microwave treatment.

3.2.2. EXTRACTION YIELD

Extraction yield (polyphenols extracted from the saffron flowers) was not affected by the addition of the microwave pretreatment. The same yield than in the conventional solid-liquid extraction was obtained for both polyphenols (around 26.19±2.26 $mg_{GAE}/g_{Dry\ SF}$) and anthocyanins (around 6.92±1.30 $mg_{CGE}/g_{Dry\ SF}$). So, it can be concluded that saffron flowers were completely exhausted by the extraction.

3.2.3. PRODUCT RICHNESS

Polyphenol and anthocyanin richness (the concentration of active compounds in the dry product obtained by solvent evaporation of the extract) were found constant along the extraction. This meant that the extraction of active compounds and undesired compounds (sugars, fibres…) took place always at the same kinetics ratio. The pretreatment exerted the same acceleration in both desired and non-desired compounds, unlike in the case of grape pomace (Álvarez et al., 2017). In this latter case, polyphenol leakage was substantially enhanced by the pretreatment but no other compounds that decayed polyphenol richness. What allowed to find an optimal time at which polyphenols were extracted but other compounds that decay polyphenol richness were not. Therefore, in the case of saffron flowers, it was not possible to find an optimal time to improve polyphenol richness. Thus, in order to ease the analysis of the experimental conditions influence, the average product richness along the extraction was employed. Pretreatment temperature was found significant according to the analysis of variance. However, an exhaustive analysis of this variable cannot be performed due to the large experimental error, especially for anthocyanins, as it can be seen in Figure 6. Nonetheless, it can be said that polyphenol richness was improved by higher microwave pretreatment temperatures. Considering that the conventional extraction provides a richness of 41.82 mg$_{GAE}$/g$_{Dry\ extract}$, pretreatment polyphenol richness was especially enhanced when temperatures above the solvent boiling point (combination of microwaves and pressure) were employed. A 9.8% and a 24.8% rich improvement stood out at 100ºC and 120ºC, respectively. On the other hand, no anthocyanin enhancement took place due to the microwave pretreatment. What is more, a slight decrease of 8.8% because of thermal degradation was found at higher temperatures.

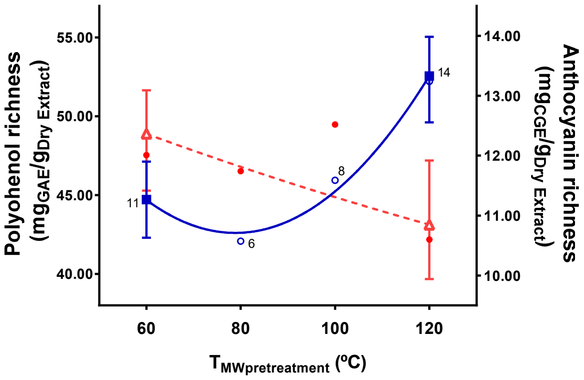


Figure 6. Polyphenol (■) and anthocyanin (Δ) richness for the extraction with microwave pretreatment and a 0.30 g/mL ratio (○ polyphenol and ● anthocyanin experimental points).

3.3. FINAL PRODUCT CHARACTERIZATION

Apart from product richness, other features also determine final product quality. Namely its main polyphenols, antioxidant capacity and colour, which has been studied in this section.

3.3.1. PRODUCT COMPOSITION

Regarding individual polyphenol identification, gallic acid, catechin, epicatechin, quercetin kaempferol and maldivin have been reported in similar extracts (Menghini et al., 2018, Serrano-Díaz et al., 2014a), but they have not been detected in this study. On the other hand, delphinidin was found in a very large concentration. It represented the 80% of the total anthocyanins detected by the HPLC (absorbance at 520 nm (Ongkowijoyo et al., 2018)) and the 15% of the total polyphenols. Delphinidin concentrations obtained in microwave pretreatment varied from 5.03 to 7.89 mg/g$_{Dry\ Extract}$. These concentrations represent an average 28% improvement respect to the conventional extract. It must be added that extraction conditions to maximize delphinidin richness agreed with the previous described tendencies to optimise anthocyanin extraction. In essence, it can be said that, although extract richness can be judged low (an average of 5% for polyphenols and 1%

for anthocyanins), the fact that a high-value compound like delphinidin was present in such large concentrations greatly uplift the quality of the final product. The addition of a purification step would allow not only to obtain a polyphenol rich extract, but a specifically rich product in delphinidin.

Composition assays were concluded with an analysis of the extract's antioxidant capacity. Most of the saffron flower extract antioxidant activities reported in other works are expressed as the ability to inhibit DPPH (Ahmadian Kouchaksaraie et al., 2016, Tuberoso et al., 2016). However, this kind of radical is scarce in biological systems (Kameya et al., 2014) so, the capacity to quench alkoxyl radicals was assessed instead. Improvements due to the addition of the microwave pretreatment could be observed. An average enhancement of 36% was found, although antioxidant activity could be boosted to more than a 100% respect to conventional when mild pretreatment conditions were employed (low pretreatment temperature and low solid-liquid ratio).

3.3.2. COLOUR

One of the likely applications of this extract is as food colorant, so variations in colour have been analysed. The three CIELAB parameters considered for this purpose (L^*, a^* and b^*) are gathered in Table 5. It must be noticed that colour was measured in a solution prepared with the dried extract, so these values can only be used to identify differences, not to characterise final product colour. In rough, the implementation of the microwave pretreatment slightly influenced the extract colour. An average total colour difference of 5.96 was found when microwaves were employed compared to the conventional solid-liquid extraction. The main difference was found in chroma, while hue (L^*) was almost constant. Nonetheless, it is worth considering each CIELAB parameters. L^*, a^* and b^* showed a relation with the anthocyanin content, as other authors have already observed (Jiménez-Aguilar et al., 2011). With the concentration of anthocyanins, luminosity was decreased (darker extracts were obtained), red hue was improved (a^* tended to higher values) as well as blue (b^* tended to negative). According to anthocyanin best extraction conditions, experiments performed with a low ratio and low pretreatment temperature (0.30 g/mL and 60ºC, which entailed an absorbed energy density of 0.23 kJ/mL) provided a great anthocyanin richness and in consequence the largest colour increment in terms of red and blue. An intense violet extract was obtained. If more pronounced conditions were employed (80-100ºC and 0.30-0.50 g/mL, absorbed energy densities between 0.39 and 0.58 kJ/mL), a similar blue hue was found than in conventional extraction, but a lower a^* parameter was obtained, indicating so a tendency to green (results gathered in Figure 7). The most severe conditions (120ºC and 0.50-0.70 g/mL, absorbed energy densities of 0.58 and 0.64 kJ/mL) caused a deviation from the colour sought. A brighter and a green-yellower extract was obtained compared to the conventional extraction. A tendency to yellow was also observed in saffron flower extracts by other authors, like Ahmad et al. (2018) while the extract was encapsulated or by Serrano-Díaz et al. (Serrano-Díaz et al., 2013a) when saffron flowers were dried. The latter attributed the colour change due to thermal degradation of the anthocyanins, which is a reasonable justification. So, if a bluer extract is sought, a mild pretreatment (in accordance to anthocyanin optimal extraction conditions) has to be employed.

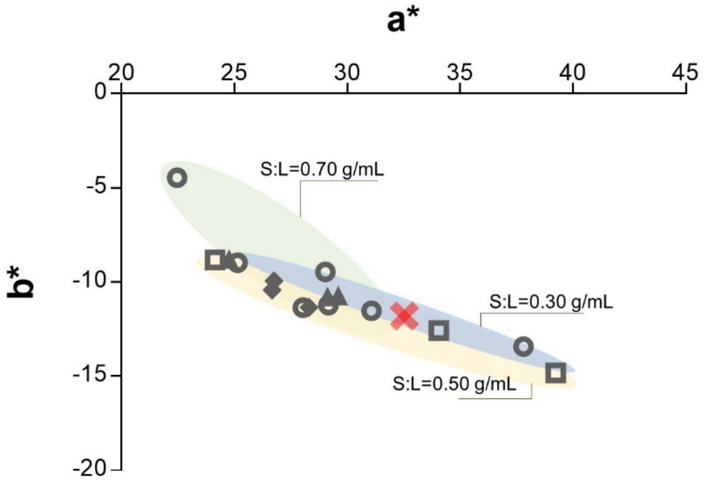


Figure 7. CIELAB parameters *a** and *b** as a function of the pretreatment temperature: x conventional solid-liquid extraction, □ T_{MW}=60ºC, ○ T_{MW}=80ºC, ▲ T_{MW}=100ºC, and ♦ T_{MW}=120ºC.

3.4. EXTRACTION OPTIMIZATION

The optimization considering all the variables has been performed. Results are presented in Table 6. Solid-liquid ratio and pretreatment temperature have been varied to maximize extraction kinetics and to improve the characteristics of the final product (richness and colour). Different importance grades have been assigned in the desirability function used for the optimization, which resulted to have a value of 0.641. A larger importance has been attributed to polyphenol richness and colour (blue and red) since these factors are intimately related with the final application of the product. Kinetics improvements (in particular extraction rate more than yield) were considered with a medium importance.

Table 6. Global process optimization and experimental values.

Variable	Objective	Interval of variation	Relative importance	Theoretical optimum	Experimental optimum
Solid-liquid ratio (g/mL)		0.30-0.70		0.30	0.30±0.00
Pretreatment temperature (°C)		60-120		65	66±0
Energy (kJ/mL)		0.16-0.54		0.24	0.24±0.01
Polyphenol yield ($mg_{GAE}/g_{Dry\ SF}$)	maximize	21.05-29.53	3	29.52	31.33±3.50
Polyphenol initial extraction rate ($mg_{GAE}/g_{Dry\ SF}$/min)	maximize	0.98-10.98	4	10.98	15.24±6.70
Anthocyanin yield ($mg_{CGE}/g_{Dry\ SF}$)	maximize	4.55-9.34	3	6.92	10.19±1.26
Anthocyanin initial extraction rate ($mg_{CGE}/g_{Dry\ SF}$/min)	maximize	0.15-2.51	4	2.38	3.25±1.43
Polyphenol richness ($mg_{GAE}/g_{Dry\ Extract}$)	maximize	41.52-53.34	5	47.18	54.25±3.06
Anthocyanin richness ($mg_{CGE}/g_{Dry\ Extract}$)	maximize	8.28-14.18	5	11.14	12.32±0.87
Antioxidant activity ($mmol_{TE}/g_{Dry\ Extract}$)	maximize	1.26-2.53	3	1.96	2.40±0.16
L*	minimize	82.07-88.94	5	87.38	90.56±0.93
a*	maximize	22.46-39.24	5	29.63	27.60±1.49
b*	minimize	-14.82-(-4-46)	5	-11.18	-16.22±0.45
ΔE	none	1.47-12.85	0	-	8.15

Experimental results obtained from this optimisation corroborated the overall convenience of adding a microwave pretreatment to the conventional extraction. Extraction efficiency and final product quality were boosted. Initial extraction rate was the most pronounced enhancement. It was accelerated 12 and 16 times for polyphenols and anthocyanins extraction, respectively. A final product upgrade also took place. A 30% polyphenol richer and specifically a 50% more anthocyanin concentrated extract was attained. Such enhancement in polyphenol concentration was also reflected in the CIELAB colour parameters: a darker and bluer extract was obtained.

4. CONCLUSIONS

The valorisation of saffron flower byproduct has been studied in order to obtain a polyphenol and especially an anthocyanin rich natural cyanic colourant. A microwave pretreatment was proposed to intensify the process. It consisted of a short but intense stage, prior to the conventional solid-liquid extraction, where the extraction media was rapidly heated to a high temperature and then cooled down to continue with the extraction at more favourable conditions for polyphenol stability. In this way, the extraction acceleration due to the temperature increase was exploited, but the thermal degradation that a process at that conditions entails was minimized. The variables analysed were the pretreatment temperature and the solid-liquid ratio during the pretreatment. The effect of the pretreatment was compared to a conventional extraction (without microwave pretreatment), whose conditions were also optimised in this work.

Regarding conventional solid-liquid extraction, saffron flowers to solvent ratio was found of vital importance. Solvent amount determined whether if a concentrated liquid product was obtained (high solid-liquid ratios) or the saffron flowers polyphenol content was completely depleted (low ratios). The acidity of the solvent employed was also inferred significant since it could have contributed to the hydrolysis of the petal structure, facilitating so the leaching of active compounds. Finally, a ratio of 0.30 g/mL was selected as it implied a balance between solvent consumption, polyphenol extraction, and liquid extract concentration. It also assured the maximum anthocyanin yield, which were the compounds of interest. This extraction media, at a temperature of 30ºC, was taken as a control reference to assess the convenience of the microwave pretreatment.

The main advantages achieved with the implementation of the microwave pretreatment were the reduction of the extraction time and the improvement of the final product quality. Initial extraction rate was the most affected parameter. It was greatly accelerated when moderately high temperatures and low solid-liquid ratios were employed. Such enhancement allowed to obtain a high yield in 5 minutes, instead of almost one hour, as the conventional process required. On the other hand, the product quality boost took place in terms of richness, composition, antioxidant activity and colour. Polyphenol and anthocyanin richness (proportion of active compounds in the final dried product) were found to present contrary tendencies. The former was increased by higher pretreatment temperatures up to a 25% at 100ºC, whereas anthocyanin richness decayed a 9% with temperature due to thermal degradation. So, anthocyanin optimal extraction conditions were set at low pretreatment temperature and also low ratio. These conditions also maximised the extract antioxidant activity (a 130%) and the final product colour. Respect to this latter feature, mild conditions enhanced red and blue hues, while severe conditions led the extract to draw away from the desired cyanic tone to a greener and yellower product, probably due to thermal degradation. Finally, in regard to extract composition, delphinidin was the major compound detected. It represented the 80% of the total anthocyanins and 15% of the total polyphenols. Such specificity raised the extract value, as it can be easily turned into a delphinidin extract by the simple addition of a purification step.

In sum, it was concluded that a mild microwave pretreatment (with a ratio of 0.30 g/mL and irradiating the media up to a temperature of 65ºC) was very convenient as it enabled to obtain a high-quality product in an efficient extraction process.

ACKNOWLEDGEMENTS

This work was funded by Spanish reginional government Junta de Castilla y León (VA040U16). A. Sahagun is kindly thanked for providing the saffron flowers, Prof. F. Roda for the colour assay guidance, and Dr. R. Páramo for the measuments of the specific heat of saffron flowers. A. Álvarez is grateful for her PhD grant FPU13/04678.

NOMENCLATURE

Acronyms

AAO	Antioxidant activity
AC	Anthocyanin content
ARD	Average relative deviation
CGE	Cyanidin glucoside equivalents
GAE	Gallic acid equivalents
MW	Microwave
S:L	Solid liquid ratio (g/mL)
SF	Saffron flower
TPC	Total polyphenol content

Symbols

a^*	CIELAB parameter indicating green-vs-redness (dimensionless)
b^*	CIELAB parameter indicating yellow-vs-bluish (dimensionless)
C	Polyphenol or anthocyanin concentration (mg/g)
C_0	Concentration at t=0 min (mg/g)
C_∞	Extraction yield (mg/g)
C_f	Pre-exponential factor (mg/g)
C_P	Heat capacity (J/gK)
k	Extraction rate constant (min^{-1})
L	CIELAB parameter indicating lightness (dimensionless)
m	Mass (g)
n	Number of experiments considered (adimensional)
n_0	Initial air moles in the pressure cell (mol)
$n_{evaporated}$	Evaporated solvent (mol)
P	Pressure (Pa)
Q	Heat (J)
R	Gas constant (J/molK)
T	Temperature (°C)
t	time (min)
$T_{MW\ pretreatment}$	Temperature achieved during the pretreatment (ºC)
T_X	Transmittance X for the colour coordinate XYZ (%)
T_Y	Transmittance Y for the colour coordinate XYZ (%)
T_Z	Transmittance Z for the colour coordinate XYZ (%)
u_0	Initial extraction rate (mg/g·min)
V_0	Initial air volume in the pressure cell (m^3)
X	Component X of the colour coordinate XYZ (dimensionless)
X_n	Component X (XYZ coordnate) for the illuminant C (dimensionless)
Y	Component Y of the colour coordinate XYZ (dimensionless)
Y_n	Component Y (XYZ coordnate) for the illuminant C (dimensionless)
Z	Component Z of the colour coordinate XYZ (dimensionless)
Z_n	Component Z (XYZ coordnate) for the illuminant C (dimensionless)

ΔE	Total colour difference
ρ_{mol}	Molar density (mol/m^3)

Appendix. Wavelengths for colour determination

	Wavelength (nm)						
#	X	Y	Z	#	X	Y	Z
1	424.4	465.9	414.1	16	585	558.5	454
2	435.5	489.5	422.2	17	588.7	561.9	455.9
3	443.9	500.4	426.3	18	592.4	565.3	457.9
4	452.1	508.7	429.4	19	596	568.9	459.9
5	431.2	515.2	432	20	599.6	572.5	462
6	474	520.6	434.3	21	603.3	576.4	646.1
7	531.2	525.4	436.5	22	607	580.4	466.3
8	544.3	529.8	438.6	23	610.9	584.8	468.7
9	552.4	533.9	440.6	24	615	589.6	471.4
10	558.7	537.7	442.5	25	619.4	594.8	474.3
11	564.1	541.4	444.4	26	624.2	600.8	477.7
12	568.9	544.9	446.3	27	629.8	607.7	481.8
13	573.2	548.4	448.2	28	636.6	616.1	487.2
14	577.4	551.8	450.1	29	645.9	627.3	495.2
15	581.3	555.1	452.1	30	663	647.4	511.2

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CONCLUDING REMARKS

CONCLUDING REMARKS

This thesis was aimed at the development of a **sustainable intensification** microwave pretreatment for the extraction of **active compounds** from three different agricultural wastes (grape pomace, olive pomace and saffron flowers) in order to obtain a high **quality final product**. In addition, the role of the **interaction of microwaves** with materials in the process performance has also been studied.

On the whole, the main achievements of this work can be summarized in the following four points:

» *Proposal of a novel microwave pretreatment*

An innovative microwave configuration has been proposed in the form of a microwave pretreatment added before the conventional solid-liquid extraction. Unlike most of the works employing microwaves, this pretreatment has been initially devised to overcome the microwave implementation limitations, which are, essentially, a non-homogeneous treatment in a bulky equipment. Thus, by employing a very short microwave residence time (up to 2 minutes), a compact oven able to assure the uniform treatment of the material can be attained.

This new configuration is suitable for its larger scale implementation, not only because of its efficiency but also because of its efficacy. In contrast to microwave assisted extractions, the pretreatment only irradiates a small volume where all the power delivered by the magnetron is concentrated. So, the radiation is focused where it can be more advantageous, instead of at the whole process. A fact that leads to the substantial boost of the pretreatment severity, which can result in a yield enhancement too, due to the higher power densities achieved.

» *Operational conditions influence*

The variables studied have been the organic proportion of the solvent (mixtures of ethanol:water have been always employed), solid-liquid ratio, and the temperature reached during the pretreatment. These three variables determined the absorbed specific energy of the pretreatment, which was a crucial parameter to characterize it.

Especial attention was paid to solid-liquid ratio so as to attain a balance between solvent consumption, process yield and extract concentration. Such quandary appeared as a consequence of the fact that the amount of solvent employed determines if either the material is completely exhausted or if a concentrated extract is obtained. In the first case, the complete depletion of the material can take place with the use of large volumes of solvent (low solid-liquid ratios). However, it also implies the production of a substantially diluted extract. On the other hand, if high ratios are employed instead, the solid is slightly drained, but the leaked polyphenols accumulate in the liquid phase, providing a concentrated extract. It must be considered that the use of large solvent amounts also entails significant solvent recovery costs and that the raw material are valueless byproducts so its depletion beyond an efficient process is not justified. Therefore, the highest solid-liquid ratio that provided a reasonable extraction yield was always selected as the optimum in this work.

» *Agricultural wastes upgrading by polyphenol microwave pretreatment extraction*

Grape pomace, olive pomace and saffron flowers have been used to assess the convenience of the microwave pretreatment for the extraction of polyphenols. Special attention was paid to anthocyanin release in the case of grape pomace and saffron flowers.

The addition of the microwave pretreatment to the polyphenol extraction process of these materials was found highly convenient, since its implementation allowed not only to improve process efficiency, but also product quality. Process efficiency was boosted by a considerable reduction of the extraction time. It was reduced from the hours required in the conventional solid-liquid extraction to less than 30 minutes with the addition of the pretreatment. This was due to the significant acceleration that the initial extraction rate experimented (up to 6.3 and 12 times depending of the raw material), what enabled to obtain a large polyphenol yield in the first stages of the subsequent conventional extraction. It was noteworthy the great polyphenol acceleration produced by the microwave pretreatment in the polyphenol extraction from grape pomace compared to non-polyphenol compounds. This differentiated kinetic effect allowed to identify an optimal extraction time at which the polyphenols were completely extracted (maximum yield) but not the undesired substances that decay polyphenol richness. A 32% polyphenol and 85% anthocyanin richer extract were obtained by stopping the extraction at each optimal time. On the other hand, olive pomace richness was increased a 40% and saffron a 25% by the addition of the pretreatment.

The heed to this new parameter, richness, was enclosed in the study of the product quality. A variable that, despite its importance, is not often taken into account. Other extract important features studied and enhanced by the microwave pretreatment have been: (1) individual compound richness (like malvidin richness in a grape pomace extract, or delphinidin in saffron flower extract, both enhanced approximately a 120%), (2) extract colour variations and (3) antioxidant activity. In particular, the enhancement found in cellular antioxidant capacity of grape pomace microwave extracts was remarkable: bioactivity was increased a maximum of 133% respect to the conventional extract.

Regarding the different raw materials tested, the analysis of the extraction efficiencies led to the conclusion that the microwave pretreatment was highly suitable for the isolation of thermosensible compounds, like anthocyanins from grape pomace and saffron flower. Olive pomace polyphenols were highly bounded to the kernel structure and it was inferred that the pretreatment was not able to greatly release them. Nonetheless, in this latter case, it has to be also considered the great specificity that the pretreatment presented towards key compounds, like hydroxytyrosol richness, which was enhanced a 35% and oleuropein improved a 181%.

Grape pomace further fractionated valorisation was also studied in a microwave assisted hydrolysis and a subsequent pyrolysis. A considerable reduction of the operational time and temperature was found with the addition of microwaves to the hydrolysis. A possible explanation for such significant reductions could be the likely change in cellulose crystallinity previously reported under microwave radiation. However, this justification was not fully accepted since its effect was not observed in the solid-liquid ratio where it was supposed to be more prominent. Hydrolysis remaining solid was subjected to a further pyrolysis, where it was proved that only the weakest pomace fractions were hydrolysed, making the residue more difficult to be volatilized.

» *Attempt to contribute to the understanding of the microwave extraction enhancement*

No scientific reasoning has been found to explain the improvement found when microwaves are added to the extraction process, except vague explanations about the alterations of the plant matrix. An ongoing discussion about the influence of radiation on vapor-liquid equilibrium led to consider that solid-liquid equilibrium could also be affected. The molecular compound gallic acid and the ionic specie sodium sulfate were chosen as solutes. The solvents water, ethyl acetate, isopropyl alcohol and ethanol were selected because of its dielectric properties ranging from efficient radiation absorbers to transparent. Although the solubility reported data presented a large experimental error due to temperature deviations (an average of 4.3%), the accuracy was high enough to assure that experimental results did not corroborate the hypothesis of an athermal microwave effect. So, no contribution of a possible solid-liquid equilibrium alteration is expected to the understanding of the performance enhancement in microwave extraction.

» *Efficient pilot plant*

To achieve the efficient design of a dedicated pilot-plant scale microwave oven, the dielectric properties of the extraction media were measured. A model able to estimate the permittivity of complex solid-liquid mixtures was developed for grape and olive pomace based on individual mixture constituents. The obtained model was able to predict the permittivity components of grape pomace-solvent and olive pomace with an accuracy of 8%-25% and 9%-29% for the dielectric constant and the loss factor, respectively. It stood out the difference between these two systems regarding ionic losses. Whereas in the case of grape pomace ionic conductivity was increased between a 9%-83%, olive pomace loss factor was not boosted by the release of ions.

Additionally, the comparison of the different bibliographic data led to the conclusion that permittivity was not an independent variable, since it was subjected to the measurement conditions (the presence or not of an electromagnetic field).

The reliable dielectric measurements enabled the design and construction of an oven able to work continuously with an energy absorption efficiency over the 97%. However, several difficulties were found with the pumping system, what hindered its exploitation. Nevertheless, it was possible to continuously operate with grape pomace for 40 minutes at a flow of 20 kg/h and with 500-gramme batches of olive pomace. Results from both sets of experiments proved that the pilot plant was able to reproduce the advantageous results obtained at laboratory scale.

In general, it can be said that a promising intensification configuration has been proposed, whose efficacy has been thoroughly demonstrated with three different agricultural residues. The comprehensive analysis of the microwave pretreatment impact on process efficiency and the product quality can corroborate its suitability. What it is more, the knowledge developed in here can be found useful not only for the scientific community, but also by industry as a useful and feasible tool for process intensification has been developed.

FUTURE WORK

These four points have been identified as the key milestones to further conclude this work:

» Olive pomace polyphenol extraction still offers plenty of room for improvement. A richer polyphenol extraction is suspected to be likely. In addition, more detailed analytical work can be performed to fully characterise the extract. Particularly, there is one compound, oleocanthal, that is gaining a lot of attention due to its health promoting properties, whose extraction would be very interesting to be studied.

» A lack of permittivity data has been detected at the conditions in which they are normally needed (2.45 GHz and high temperatures). Therefore, it would be useful to have more reliable measurements, not only for natural products, but also for the common employed solvents, at different temperatures and concentrations.

» A continuous oven at a pilot plant has been developed but due to pumping malfunctions it has not been fully operate. So, a trustworthy pumping system is required to enable its fully operation.

» An economical balance would be of highly utility to completely endorse the convenience of the pretreatment. However, the great difficulty of this point lies in estimating how changes in product quality influence the final price. A fact unclear even for the companies trading with these goods. Additionally, a techno-economic analysis regarding the microwave oven as well as a life cycle assessment study of the product and the process environmental impact would be of great interest to further uphold the selection of the process proposed in here.

» This work was focused on the extraction optimization. In order to complete the production line, it is necessary to formulate the extract for its application. It would be also advisable to add a purification step. Formulation can be carried out using techniques such as SAS or SPSS since they are recommendable for thermosensible compounds. Purification by means of resins can also be considered, although their unviable industrial application deters its use. How to implement an efficient resin purification at a larger scale is still an issue to appraise.

SPANISH SUMMARY

RESUMEN EN ESPAÑOL

DESARROLLO DE UN PROCESO DE EXTRACCIÓN ASISTIDO POR MICROONDAS PARA LA RECUPERACIÓN DE POLIFENOLES DEL ORUJO DE UVA

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ABSTRACT

Las ventajas de la adición de microondas a procesos de extracción son ampliamente conocidas. Sin embargo, su aplicación a escala industrial se encuentra restringida debido a las limitaciones de esta tecnología. A fin de vencer los inconvenientes que dificultan su implementación, se ha propuesto la utilización de las microondas en un pretratamiento previo a la extracción sólido-líquido. Dicho pretratamiento se ha probado con orujo de uva, alperujo y flores de azafrán, para la extracción de polifenoles (aunque también se ha puesto especial interés en la obtención de antocianinas). Las condiciones de extracción (energía del pretratamiento, tiempo, ratio sólido-líquido, temperatura y disolvente) se han optimizado de manera que se maximizase tanto el rendimiento del proceso (aumento de la eficiencia hasta el 57% y considerable reducción de hasta 1/12 del tiempo de extracción) como la riqueza del producto final (destacando la sustancial mejora de la riqueza en compuestos de interés como la malvidina (122%), oleuropeína (181%) o delfinidina (121%)). Los buenos resultados obtenidos respaldaron el estudio a mayor escala para la futura implementación del pretratamiento a nivel industrial. Se diseñó y construyó un horno en continuo que permitiese una alta eficiencia energética, para lo cual fue necesario contar con un modelo para estimar las propiedades dieléctricas de los medios de extracción (precisión media del 17%). La eficiencia del horno a mayor escala fue validada con la reproducción de las condiciones óptimas obtenidas previamente, encontrándose resultados similares tanto para el orujo como para el alperujo.

Estudios adicionales para la completa explotación del orujo de uva (mediante una hidrólisis asistida por microondas y el estudio de la degradación térmica del subproducto en una pirólisis), así como la

posible influencia de la radiación en el equilibrio sólido-líquido también fueron también llevados a cabo.

En definitiva, se ha realizado un estudio exhaustivo del pretratamiento con microondas sugerido a fin de demostrar las ventajas de su implementación.

PALABRAS CLAVE

Polifenoles, antocianinas, pretratamiento con microondas, interacción radiación-materia, escalado.

HITOS

» Desarrollo de un pretratamiento con microondas como complemento a la extracción convencional.
» Estudio de la extracción de polifenoles del orujo, alperujo y flores de azafrán.
» Optimización de las condiciones para maximizar la eficiencia del proceso y la calidad del producto.
» Diseño y construcción de un horno a mayor escala capaz de operar en continuo.
» Estudio de la interacción radiación-materia: medida de la permitividad e influencia en el equilibrio sólido-líquido.

1. INTRODUCTION

Actualmente, garantizar la eficiencia y sostenibilidad de un proceso industrial es clave para garantizar su éxito. En el caso de las industrias agrícolas, el tratamiento de los residuos para la obtención de compuestos activos (como polifenoles y antocianinas) supone la revalorización de un desecho y la producción de un extracto de gran valor. La principal característica de este tipo de productos es su gran actividad antioxidante, lo que les proporciona propiedades beneficiosas para la salud, tales como efectos anticancerígenos, antiinflamatorios, antidiabéticos, etc. Estas características convierten a los polifenoles en compuestos muy apreciados, tanto por la industria alimentaria como por la farmacéutica y la cosmética, puesto que responden a la creciente demanda de ingredientes naturales capaces de remplazar el uso de aditivos sintéticos. Por tanto, su producción queda justificada no solo por la aportación a la economía circular (reutilización de un residuo), sino también por la obtención de un producto de alto valor añadido.

La vía tradicional para la obtención de un extracto rico en polifenoles es la extracción sólido-líquido. Proceso durante el cual, la materia prima se pone en contacto con un disolvente a una temperatura media, produciéndose así la lixiviación de los compuestos activos del sólido a la fase líquida. No obstante, este proceso conlleva importantes inconvenientes, como son el uso de grandes cantidades de disolvente o largos tiempos de operación. A fin de salvarlos, en las últimas décadas se han desarrollado técnicas de intensificación que permiten aumentar considerablemente la eficacia del proceso. La extracción asistida por microondas destaca para la mejora de este tipo de extracciones. Numerosos trabajos a escala de laboratorio avalan sus ventajas. Sin embargo, a pesar de los probados beneficios, el uso de las microondas en procesos de extracción a nivel industrial no está tan extendido debido a las limitaciones de esta tecnología a la hora de ser escalada. La baja eficiencia energética y un tratamiento no uniforme son las principales objeciones que frenan su implementación. A este hecho cabe añadir que, tomando las medidas apropiadas, es posible vencer estos inconvenientes. La eficiencia energética puede mejorarse mediante un diseño del microondas específico para cada aplicación, y el tratamiento uniforme puede asegurarse si se utiliza un horno compacto. Para resolver estos dos hitos es necesario conocer cómo interacciona la radiación con el medio de extracción, hecho que mide la permitividad (ε^*). La permitividad es un número complejo, como recoge la expresión [1], y está compuesta por la constante dieléctrica (ε') y el factor de pérdidas (ε''). La constante dieléctrica corresponde a la habilidad del material para almacenar energía microondas, mientras que el factor de pérdidas representa la disipación de energía microondas en forma de calor.

$$\varepsilon^* = \varepsilon' - j\varepsilon'' \qquad [1]$$

La interacción de la materia con la radiación electromagnética dependerá del valor de estas propiedades dieléctricas, variando desde opacas hasta transparentes a la radiación electromagnética. A fin de caracterizar dicha relación, se ha definido la variable longitud de penetración. Esta determina la profundidad a la cual la potencia de la radiación se ha reducido a *1/e* (37%) el valor en la superficie. Los disolventes comúnmente utilizados presentan tan solo una longitud de penetración de centímetros, lo cual contribuye a las dificultades de escalado. Debe tenerse en cuenta que la longitud de penetración es independiente de la escala, es decir, el mismo espesor es siempre afectado, tanto si la radiación se aplica sobre un matraz de laboratorio como en un tanque industrial. Es por ello que, cómo se ha apuntado anteriormente, un horno compacto permite un tratamiento uniforme del medio de extracción, al poder tenerse en cuenta la longitud de penetración.

Este razonamiento condujo a la proposición de un pretratamiento con microondas como alternativa a los procesos de extracción asistidos por microondas (MAE). Dicho pretratamiento tiene lugar antes de la extracción convencional sólido-líquido y consiste en un corto pero intenso pulso de energía electromagnética. Este provoca que la temperatura del medio de extracción aumente rápidamente, favoreciendo la extracción de compuestos. Además, puesto que se emplean energías similares a los procesos MAE, se mantienen las ventajas de la adición de microondas a pesar de la reducción del tiempo de radiación. Una vez que el tratamiento ha acabado, se enfría el medio de extracción para minimizar las reacciones de degradación, y se continua con una extracción sólido-líquido, pero durante un tiempo menor al del proceso convencional.

Este pretratamiento ha sido probado para la extracción de polifenoles con tres residuos agrícolas de la región de Castilla y León (España). Estos han sido orujo de uva, alperujo y flores de azafrán. Todos ellos se caracterizan por no tener ningún valor económico, pero si un gran potencial para ser objeto de una valorización que permita aumentar la sostenibilidad de las industrias asociadas.

En definitiva, en este trabajo se ha propuesto un pretratamiento con microondas como complemento a la extracción convencional sólido-líquido de manera que se mantuviesen las ventajas de la adición de microondas, pero en una configuración que permitiese su fácil implementación a mayor escala.

1.1. OBJETIVOS

Se persigue la intensificación de la extracción de polifenoles de productos naturales por medio de un pretratamiento con microondas cuyo escalado sea factible, y que se mejore tanto la eficiencia del proceso como la calidad del producto final. Por ello, los objetivos específicos fijados para este trabajo han sido:

i. Aumentar la calidad del extracto polifenólico.
ii. Estudio de la influencia de las condiciones de extracción.
iii. Comprender la interacción de la radiación con la materia.
iv. Comprobar si el conocimiento desarrollado puede ser extrapolado a otros materiales.
v. Llevar a cabo el escalado del proceso.

2. MATERIALES Y MÉTODOS

2.1. MATERIALES

En el desarrollo de este trabajo se han empleado tres residuos agrícolas: el orujo de uva, el alperujo y las flores de azafrán. En todos los casos, tras la recepción de la materia prima se procedió a su congelado a -18ºC para mantener sus propiedades antioxidantes. Antes de ser usada, la materia prima se descongeló a 4ºC durante una noche.

En cuanto a los reactivos empleados, todos tenían la clasificación de grado analítico o de HPLC.

2.2. EXTRACCIÓN DE POLIFENOLES

Primeramente se estudió la extracción convencional sólido-líquido, siendo el ratio sólido-líquido, el disolvente y la temperatura las tres variables estudiadas. Este proceso sirvió como base para valorar la conveniencia de la adición de un pretratamiento con microondas.

Para el proceso convencional, se pesaron las cantidades de materia prima utilizada y se añadió la cantidad correspondiente de disolvente. Tras 5 minutos agitándose a temperatura ambiente para asegurar un contacto sólido-líquido homogéneo, se colocó el matraz en un baño termostático con agitación. El comienzo de la extracción se fijó tras este último punto (t=0 min). Diferentes muestras de líquido se fueron tomando a diferentes tiempos, para el posterior estudio de la eficiencia de las condiciones de extracción utilizadas.

En el caso del pretratamiento con microondas, este se realizó en el equipo CEM Discover (puntualmente un microondas Anton Paar fue utilizado durante la extracción de polifenoles del alperujo). El pretratamiento se realizó después de la homogenización y antes de colocar el matraz en el baño termostático. Se irradió la muestra a máxima potencia (300 W) durante un tiempo conocido, de manera que se aumentó su temperatura rápidamente hasta un valor fijado previamente (temperatura medida con una fibra óptica FoTEMP 4). Cabe destacar que, cuando la temperatura del pretratamiento estuvo por encima del punto de ebullición del disolvente, se utilizó una celda de presión QianCap. Una vez alcanzada esta temperatura, se paró la irradicación y se enfrió rápidamente el medio de extracción, finalizándose así el pretratamiento con microondas. Si durante el pretratamiento se utilizó un ratio diferente al convencional, en este punto se añadió el disolvente necesario para alcanzar este valor. A continuación, se continuo con el proceso de extracción convencional, colocando el matraz en el baño termostático y tomando alícuotas para el análisis cinético de la extracción.

2.3. CARACTERIZACIÓN DE LA ENERGÍA ABSORBIDA DURANTE EL PRETRATAMIENTO CON MICROONDAS

A diferencia de la mayoría de los trabajos de extracción con microondas, en este trabajo se ha cuantificado la energía absorbida y no la emitida. Para ellose ha considerado el calor sensible y el latente. Estas energías han sido dadas siempre por unidad de volumen o de masa (según el tipo de pretratamiento) a fin de que puedan ser fácilmente extrapolables.

El calor sensible se ha calculado como la energía necesaria para aumentar la temperatura de la materia prima hasta la del pretratamiento y la del disolvente hasta su punto de ebullición.

En el caso del calor latente, se diferenciaron dos casos: en los que se utilizó un matraz abierto y un en los que se empleó una celda de presión. En el primer caso, el disolvente evaporado se determinó gravimétricamente. Por otro lado, para la celda de presión, fue necesario estimar los moles de disolvente evaporados y acumulados en la fase gas que hicieron aumentar la presión. Para ello se asumió un comportamiento ideal y se utilizó la ecuación de los gases ideales para calcular los moles de disolvente evaporados.

2.4. ANÁLISIS CINÉTICO DE LA EXTRACCIÓN DE POLIFENOLES

Las curvas de extracción obtenidas siguieron una cinética de primer orden, como la presentada en la ecuación [2]. Los datos experimentales se ajustaron a esta expresión variando los valores de los parámetros C_0, C_f y k, a fin de que el error relativo medio fuese mínimo.

$$C_{cal} = C_0 + C_f[1 - \exp(-kt)] \qquad [2]$$

No obstante, esta curva no se utilizó para interpretar los datos, sino que para ello se emplearon los parámetros derivados velocidad de extracción inicial (u_0) y rendimiento total (C_∞). La velocidad inicial de extracción, calculada según la ecuación [3], indica la rapidez con la que se extraen los polifenoles. Cuanto mayor sea, menor será el tiempo de extracción necesario. Tiene unidades de $mg/g_{materia\ prima}\cdot min$.

$$u_0 = \left.\frac{\partial C}{\partial t}\right|_{t=0} = C_f k \qquad [3]$$

El rendimiento total indica la eficiencia máxima que puede obtenerse en las condiciones de extracción a un tiempo infinito. Se calcula por medio de la expresión [4] y tiene unidades de $mg/g_{materia\ prima}$.

$$C_\infty = \lim_{t\to\infty} C = C_0 + C_f \qquad [4]$$

2.5. CARACTERIZACIÓN DE LOS EXTRACTOS POLIFENÓLICOS

2.5.1. CONTENIDO EN POLIFENOLES TOTALES

El contenido total en polifenoles fue determinado por el método espectrofotométrico de Folin-Ciocalteu. Los resultados fueron expresados como equivalentes de ácido gálico (mg_{AGE}/g).

2.5.2. CONTENIDO EN ANTOCIANINAS

Las antocianinas fueron medidas por el método diferencial de pH y expresadas en equivalentes de glucósido de cianidina (mg_{GCE}/g).

2.5.3. DETERMINACIÓN DE COMPUESTOS POR CROMATOGRAFÍA.

Ácido gálico, catequina, procianidina B2, epicatequina, kaempferol, mirecitina, quercitina, malvidina, delfinidina, tirosol, hidroxitirosol y oleuropeína fueron separados en una columna C18 y cuantificados en un detector fotodiodo. Se emplearon diferentes métodos y eluyentes dependiendo de los compuestos que se buscaba identificar, aunque en todos los casos dicha identificación se realizó comparando el tiempo de retención en la columna y el espectro UV/VIS de cada compuesto con los estándares.

2.5.4. CAPACIDAD ANTIOXIDANTE

Se determinó tanto la actividad antioxidante química como la celular frente a radicales peróxido. La actividad antioxidante química se midió como la capacidad del extracto de frenar la oxidación de una sal de fluoresceína. Mientras que para la bioactividad antioxidante se analizó dicha capacidad dentro de células Caco-2. Para este último caso, fue necesario un análisis previo de la citotoxicidad del extracto en las células. Trolox y quercitina fueron utilizados como estándares para cuantificar la capacidad antioxidante.

2.5.5. DETERMINACIÓN DE COMPUESTOS NO-POLIFENÓLICOS

El contenido total de compuestos extraídos (polifenoles y no-polifenoles) fue determinado gravimétricamente secando un volumen conocido de extracto a 80ºC hasta pesada constante. Dicha medida fue completada con la determinación de los azúcares totales (método de la antrona) y de fibra (medida a través de su coeficiente de absortividad).

2.5.6. MEDIDA DEL COLOR

Se utilizó un método espectrofotométrico en el espacio XYZ, que fue posteriormente transformado al CIELAB (dado que este es el comúnmente utilizado). No obstante, la valoración del color se realizó evaluando los cambios que el pretratamiento con microondas conllevó respecto al extracto de proceso convencional.

2.6. HIDRÓLISIS ASISTIDA POR MICROONDAS DEL ORUJO DE UVA

A fin de agotar el potencial del orujo de uva, se estudió su completo agotamiento mediante una hidrólisis y una posterior pirólisis. Para ello se utilizó orujo sin extractivos, a fin de aproximarse a una valorización fraccionada según el concepto de biorrefinería.

La temperatura (140-200ºC), el tiempo (2-16 minutos) y el ratio sólido-líquido (0.10-0.30 g/mL) fueron las variables analizadas. Para la hidrólisis asistida por microondas se utilizó el equipo CEM Discover Microwave, donde el medio de hidrólisis se irradió a las condiciones establecidas con una potencia en pulsos de 300 W. Una vez hubo acabado el proceso, se enfrió con aire hasta los 70ºC. Posteriormente, la fase líquida y sólida fue separada por centrifugación y reservada para su análisis.

A fin de identificar el efecto de las microondas en el proceso, los resultados se compararon con los obtenidos en las mismas condiciones en un reactor a presión Berghof. En este último caso se siguió el mismo procedimiento experimental anteriormente descrito para el proceso con microondas.

2.7. CARACTERIZACIÓN DE LA HIDRÓLISIS DEL ORUJO DE UVA

2.7.1. CARACTERIZACIÓN DEL HIDROLIZADO

La eficiencia de la hidrolisis en relación al hidrolizado obtenido (fase líquida) se caracterizó por medio del rendimiento global y a la selectividad en la producción tanto de azúcares como de productos de degradación. El rendimiento global fue calculado como la cantidad de carbono obtenido por gramo de orujo seco empleado (mg_C/g_{Orujo}). Por otra parte, la selectividad en azúcares y productos de degradación fue calculada como la proporción de estos compuestos en el hidrolizado ($mg_C/g_{C\ hidrolizado}$). Los azúcares celobiosa, fructosa, xilosa, fructosa ramnosa fueron agrupados para el cálculo de la selectividad en azúcares, mientras que el ácido fórmico, láctico, levulínico, furfural y hidroximetilfurfural fueron considerados como productos de degradación. Todos estos compuestos individuales fueron medidos por cromatografía de líquidos.

2.7.2. CARACTERIZACIÓN DEL SÓLIDO TRAS LA HIDRÓLISIS

Del sólido resultante de la hidrólisis se midió su composición en hemicelulosa, celulosa y lignina. Estas concentraciones se combinaron con un análisis termogravimétrico (Rheometric Scientific Simultaneous Thermal Analyzer STA 625) para desarrollar un modelo que permitiese estimar las cinéticas de degradación del orujo restante en un proceso de pirólisis.

2.8. MEDIDA DE LAS PROPIEDADES DIELÉCTRICAS

Las propiedades dieléctricas del orujo de uva y del alperujo fueron medidas con dos equipos diferentes, ambos basados en los cambios en una cavidad resonante y desarrollados por el Instituto ITACA de la Universidad Politècnica de València (España). Las variables analizadas en los dos casos fueron la temperatura y la composición.

Para el orujo de uva se utilizó un "*Dielectric Kit for vials*" en el que se insertó la mezcla multifásica después de haber sido calentada por medio de radiación electromagnética. Se midió la evolución de la permitividad con la temperatura.

En el caso del alperujo se utilizó un equipo innovador que permitió medir la permitividad mientras el medio era calentado con microondas. Al igual que en el caso anterior, se midió la variación de las propiedades dieléctricas con la temperatura.

Los datos así obtenidos fueron utilizados para desarrollar un modelo capaz de estimar la permitividad de estas mezclas multifásicas.

2.9. MEDIDA DE LA SOLUBILIDAD BAJO RADIACIÓN MICROONDA

La solubilidad de sistemas compuestos por ácido gálico o sulfato de sodio en agua, etanol, acetato de etilo y alcohol isopropílico fueron medidos bajo la influencia de microondas. El objetivo fue comprobar si las microondas provocaban un cambio en el equilibrio sólido-líquido capaz de explicar la mejora encontrada en el rendimiento en los procesos de extracción asistidos por microondas. Para ello, se comparó la solubilidad obtenida por medio de calentamiento convencional con la de calentamiento por microondas.

Cabe destacar la dificultad para mantener una temperatura constante en el sistema con microondas. Dado que las microondas generan calor al interaccionar con el medio, fue necesario retirar el exceso de energía mediante una corriente de aire. Por tanto, el sistema utilizado se puede clasificar como dinámico, ya que empleó dos flujos continuos de energía. El equilibrio entre estos dos flujos determinó la temperatura del sistema. Las pequeñas variaciones que se produjeron en dichos flujos conllevaron fluctuaciones en la temperatura, que se tradujeron en incertidumbres en la determinación de la solubilidad (calculadas considerando la propagación de errores).

2.10. ANÁLISIS ESTADÍSTICO

En los casos en los que se empleó un diseño estadístico de experimentos, se optó por un diseño compuesto centrado en las caras (α=1). El análisis de los efectos significativos se realizó a partir de la tabla ANOVA obtenida para cada diseño con un nivel de confianza del 95% (p-valor=0.05). Los efectos significativos fueron discutidos de acuerdo a las gráficas de efectos principales y de interacción generados por el software Design Expert, al igual que la optimización del proceso por medio de una función de deseabilidad.

3. RESULTADOS Y DISCUSIÓN

3.1. PRETRATAMIENTO CON MICROONDAS PARA MEJORAR LA EFICIENCIA DE EXTRACCIÓN Y LA RIQUEZA DEL PRODUCTO EN UN PROCESO DE EXTRACCIÓN DE POLIFENOLES A PARTIR DEL ORUJO DE UVA. EFECTO SOBRE LA BIOACTIVIDAD ANTIOXIDANTE.

Durante el proceso convencional de extracción de polifenoles del orujo de uva, es imposible obtener simultáneamente un rendimiento alto y un producto rico, tal y como demuestra la Figura 1(A). O bien la extracción tiene lugar a tiempos cortos en los que se obtiene un producto diluido pero rico en polifenoles, o bien se emplean tiempos largos en los que se agota la materia prima a cambio de obtener un extracto con una menor riqueza. No obstante, la adición de un pretratamiento con microondas a la extracción convencional sólido-líquido permitió acelerar la extracción de polifenoles, de manera que se hizo posible encontrar un tiempo óptimo de extracción en el que tanto el rendimiento como la riqueza eran máximos. Esto fue debido a que el pretratamiento con microondas aceleró sustancialmente la extracción de polifenoles (entre un 42%-66% más de polifenoles se obtuvieron por gramo de materia prima), y de una manera más tenue los compuestos no-polifenólicos (azúcares, fibras…). Esta diferencia de velocidad de extracción entre los polifenoles y otras sustancias permitió encontrar un tiempo óptimo de extracción en el que los polifenoles habían sido completamente extraídos (alto rendimiento) mientras que las sustancias no deseadas que contribuían a reducir la riqueza no habían sido liberados en una gran proporción (alta riqueza).

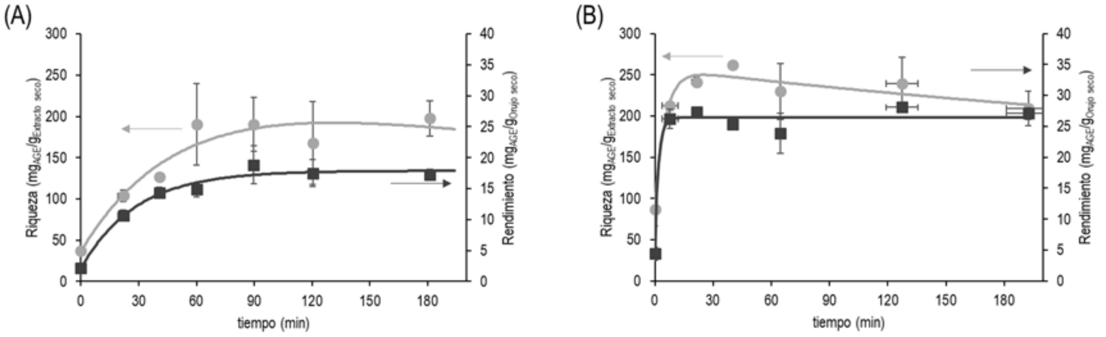

Figura 1. Rendimiento y riqueza en la extracción de polifenoles para la extracción convencional (A) y con un pretratamiento con microondas hasta 100ºC (● riqueza, ■ rendimiento).

En cuanto a la intensidad del pretratamiento, la extracción de polifenoles se vio favorecida por la combinación de microondas y presión, con un pretratamiento en el que la temperatura se aumentó hasta 100ºC (energía especifica absorbida de 0.33 kJ/mL). Como puede observarse en la Figura 1, este pretratamiento permitió obtener un rendimiento un 48% más alto respecto de la extracción convencional, así como reducir en 7 veces el tiempo de extracción necesario. Además, deteniendo la extracción a los 40 minutos, fue posible aumentar la riqueza del extracto un 33% más. En el caso de las antocianinas, los mejores resultados se obtuvieron con un pretratamiento con microondas hasta 80ºC (0.14 kJ/mL). Este permitió obtener un extracto un 85% más concentrado en antocianinas a los 30 minutos de extracción. Estos resultados pueden encontrarse en la Figura 2.

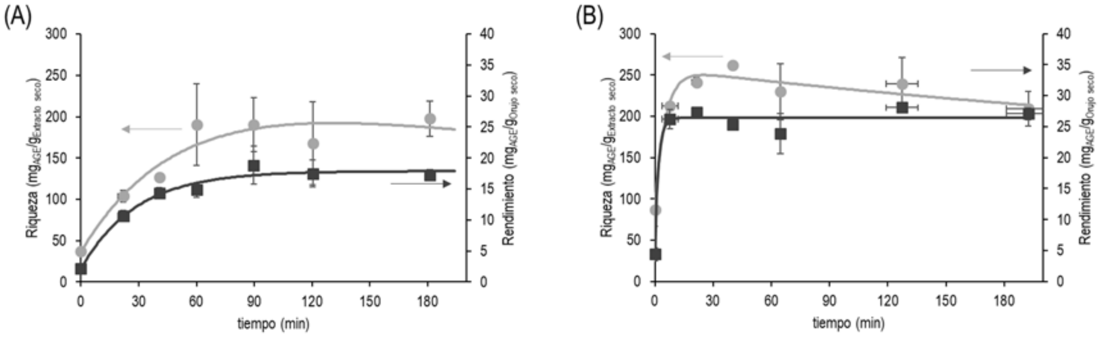

Figura 2. Rendimiento y riqueza en la extracción de antocianinas para la extracción convencional (A) y con un pretratamiento con microondas hasta 80ºC (● riqueza, ■ rendimiento).

Dado el sustancial aumento en la riqueza del extracto, las características del producto final fueron analizadas con mayor detalle. Lo cual conllevó la cuantificación de compuestos de interés y la medición de su capacidad antioxidante.

Las concentraciones de ácido gálico, catequina, procianidina B2, epicatequina, mirecitina, quercitina y malvidina fueron medidas, encontrándose que no hubo diferencia en los perfiles de concentración para los diferentes pretratamientos estudiados.

Por el contrario, si se encontró una diferencia significativa entre los pretratamientos al considerar la actividad antioxidante. Los pretratamientos que proporcionaron una alta riqueza en polifenoles y en antocianinas también presentaron una alta actividad antioxidante, tanto química como celular. Siendo esta mejora de entre el 83% y el 133% respecto a la capacidad antioxidante del extracto convencional.

En consiguiente, puede afirmarse que el pretratamiento con microondas consiguió mejorar sustancialmente el proceso de extracción de polifenoles, y en especial de antocianinas, del orujo de uva.

3.2. MEDICIÓN Y CORRELACIÓN DE LAS PROPIEDADES DIELÉCTRICAS DE UN MEDIO DE EXTRACCIÓN DISOLVENTE-ORUJO DE UVA. EFECTO DE LA TEMPERATURA DE Y DE LA COMPOSICIÓN.

En este apartado, la permitividad de una mezcla multifásica de orujo y disolvente fue medida para diferentes valores de temperatura (293-343 K), composición del disolvente (25-75% etanol:agua) y ratio sólido-líquido (0.50-1.00 g/mL). Estas medidas fueron utilizadas para desarrollar un modelo capaz de estimar las propiedades dieléctricas de la mezcla a partir de los compuestos puros (agua, etanol y orujo de uva).

Primeramente se estudiaron los disolventes puros etanol y agua. Se utilizaron regresiones con la temperatura para estimar los parámetros de la ecuación de Debye. La permitividad a baja y alta frecuencia se ajustaron a una regresión lineal, mientras que el tiempo de relajación se ajustó a una curva exponencial. La bondad de estas regresiones se cuantificó comparando los resultados con valores experimentales. Cabe destacar la gran discrepancia que se encontró entre los datos bibliográficos del etanol y que puede observarse en la Figura 3. Se concluyó que estas discrepancias eran consecuencia de los diferentes métodos de medición utilizados. Los valores medidos con una cavidad resonante y reflectometría se consideraron más fiables que los obtenidos con sondas coaxiales y celdas de admitancia, por lo que el primer grupo fue seleccionado para comparar los resultados de este estudio. El modelo propuesto fue capaz de estimar la permitividad del etanol con una precisión del 8.3% para la constante dieléctrica y del 4.6% para el factor de pérdidas. En el caso de la permitividad del agua, se obtuvieron errores del 1.6% y del 17.0% para la constante dieléctrica y el factor de pérdidas, respectivamente.

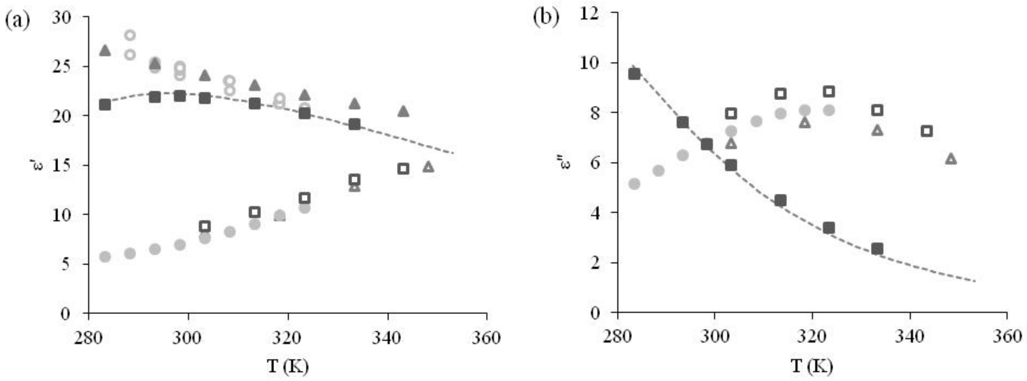

Figura 3. Constante dieléctrica (a) y factor de pérdidas (b) del etanol en función de la temperatura. Modelo propuesto (línea) y datos bibliográficos (símbolos).

Una vez establecido el modelo para calcular la permitividad de los disolventes puros, se procedió a combinarlos mediante una regla de mezcla de acuerdo con su composición. Se probaron diferentes ecuaciones, encontrándose que la recogida en la expresión [5] proporcionaba los mejores resultados para describir el comportamiento del disolvente a diferentes temperaturas y composiciones. La desviación relativa obtenida fue de 9.4% y 9.6% para cada componente de la permitividad.

$$\varepsilon^{*1/2} = X_1 \varepsilon_1^{*1/2} + X_2 \varepsilon_2^{*1/2} \tag{5}$$

Finalmente, se estudió cómo combinar la permitividad del disolvente y la del orujo de uva (obtenida a partir de una regresión bibliográfica). Fue necesario incluir las pérdidas iónicas al factor de pérdidas, dado que los electrolitos que inicialmente se encontraban en la materia prima fueron liberados en contacto con el disolvente. Este nuevo término supuso una importante contribución, de entre el 9% y el 83%, al factor de pérdidas. Las pérdidas iónicas se agregaron como un término independiente a la regla multifásica, dado que no se pudo atribuir a ninguno de los componentes de la mezcla. La ecuación de mezcla seleccionada finalmente para estimar la permitividad del medio de extracción es la indicada en la expresión [6].

$$\varepsilon_{Mix}^{*} = \left[\left(X_{Pomace} \cdot \varepsilon_{Pomace}^{*}\right)^{1.425} + \left(X_{Solvent} \cdot \varepsilon_{Solvent}^{*}\right)^{1.425}\right]^{1/1.425} + \frac{\sigma}{j\omega\varepsilon_0} \tag{6}$$

Esta ecuación corresponde a una modificación de la regla de mezcla potencial. Debido a que la gran influencia de la temperatura ocultaba la dependencia de la permitividad con la composición, esta fue incluida dentro de la potencia para darle una mayor importancia. Se consiguió así obtener un modelo capaz de predecir la permitividad de la mezcla con una precisión del 8.1% para la constante dieléctrica y el 24.8% para el factor de pérdidas.

En definitiva, cabe destacar que, a pesar de los numerosos parámetros utilizados para caracterizar los constituyentes puros de la mezcla, solo se ha utilizado un parámetro ajustable (exponente de la regla multifásica) para correlacionar los componentes de la mezcla. A pesar de que el error final es significativo, puede tomarse como aceptable dada la naturaleza heterogénea del orujo de uva y la gran variación de la permitividad en el intervalo estudiado (la constante dieléctrica casi se duplica y el factor de pérdidas se triplica). Asimismo, el procedimiento de cálculo aquí establecido puede ser empleado para caracterizar otras mezclas multifásicas.

3.3. DESARROLLO DE UNA PLANTA PILOTO EN CONTINUO CON UN PRETRATAMIENTO DE MICROONDAS PARA LA EXTRACCIÓN DE COMPUESTOS ACTIVOS.

Una vez conocidas las condiciones óptimas de extracción para el pretratamiento con microondas y las propiedades dieléctricas del medio de extracción, se procedió al diseño y construcción de un horno microondas capaz de realizar este proceso a mayor escala. Concretamente, las condiciones de operación que se fijaron fueron:

i. Una composición de disolvente de aproximadamente 50% de etanol-agua.
ii. Un ratio sólido-líquido de 0.75 g/mL.
iii. Una densidad de energía absorbida de en torno a 0.40 kJ/mL para llegar a una temperatura máxima de 120ºC.
iv. Un tiempo de residencia de 90 segundos.
v. Una capacidad de tratamiento de 20 kg/h de orujo y disolvente.

A partir de estas especificaciones, se fijó un intervalo de estudio para las variables del proceso potencia (2-3 kW), tiempo de residencia (45-120 segundos), temperatura final (100-120ºC) y diámetro de la conducción (0.020-0.40 m). Se analizaron las diferentes combinaciones de estas cuatro variables, y mediante balances de energía, se estimó el flujo másico que se podría tratar, la densidad de potencia en el medio de extracción, la densidad de energía, la longitud del horno y la velocidad con la que el medio de extracción avanzaría por este. La Tabla 1 resume las variables seleccionadas a partir de este análisis para llevar a cabo el diseño del horno.

Tabla 1. Variables seleccionadas para el diseño de un horno microondas para el pretratamiento en continuo en procesos de extracción.

P (W)	D (m)	t_R (s)	T_{salida} (ºC)	Flujo másico (kg/h)	Densidad de potencia (kW/L)	Densidad de energía (J/mL)	Longitud (m)	Velocidad (cm/s)
3.0	0.035	90	120	18	3.9	351	0.9	0.58

Una vez fijadas las especificaciones que el horno microondas debía cumplir, se procedió a su diseño. Como novedad se escogió que el horno tuviese una sección cuasi cilíndrica (rectangular con los bordes redondeados), a fin de facilitar la incorporación de sistemas de homogenización del campo electromagnético, como por ejemplo ranuras para la mejor distribución de las ondas. Las dimensiones del diseño finalmente escogido pueden encontrarse en la Tabla 2. Entre las ventajas de este diseño destaca su versatilidad: la cavidad del horno puede ser fácilmente modificada por medio de unos sintonizadores que modifican el campo para adaptarlo a otros materiales de extracción. Además, se caracteriza por su alta eficiencia energética y su volumen compacto que proporciona una homogénea irradiación del medio de extracción.

Tabla 2. Dimensiones del horno microondas para las secciones detalladas en la Figura 4.

t_x	t_{x2}	t_{x3}	t_{x4}	$desplaza_x$	$desplaza_y$	$R_{\_PTFE}$	r_{iris}
45	44	45	45	5	10	32	33

Guide width	d_1	d_{1_cav2}	d_{1_cav3}	d_{1_cav4}
32	216	247	155	212

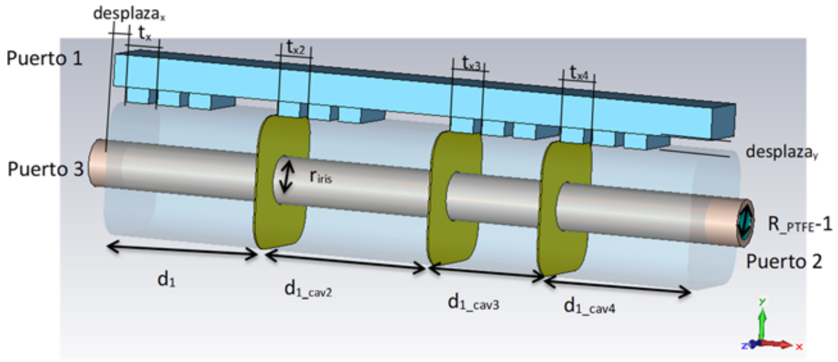

Figura 4. Dimensiones del horno microondas.

La construcción del horno fue llevada a cabo por la empresa Sistemas RBT, que además dotó al equipo de un sistema de control para medir la potencia absorbida y de un circuito de agua con refrigeración para disipar la energía reflejada, y así evitar dañar el magnetrón.

La operación en continuo con este equipo no fue sencilla, dadas las dificultades que se encontraron para bombear un flujo de 18 kg/h con una alta proporción de sólidos de gran tamaño. La alimentación por medio de un tornillo sin-fin fue la alternativa probada con mejores resultados, por lo que se construyó un prototipo para las dimensiones del horno. Este sistema permitió operar en continuo durante 40 minutos con un flujo aproximado de 20 kg/h. Durante este tiempo, la eficiencia de absorción de la radiación microondas estuvo siempre por encima del 97%. En cuanto a los resultados de extracción obtenidos, estos fueron similares a los esperados: un rendimiento de 14.00±0.30 $mg_{AGE}/g_{Orujo\ seco}$ y una riqueza del 166±5 $mg_{AGE}/g_{Extracto\ seco}$.

Por lo tanto, quedó probada la eficacia del diseño presentado, a la vez que la viabilidad de la configuración propuesta para el escalado de procesos de extracción de productos naturales con un pretratamiento con microondas.

3.4. INTENSIFICACIÓN DE LA HIDRÓLISIS DEL ORUJO DE UVA POR MEDIO DE RADIACIÓN MICROONDA

La influencia de la temperatura, el tiempo y el ratio sólido-líquido se ha estudiado en un proceso de hidrolisis hidrotermal y asistido por microondas. El efecto de estas variables puede encontrarse en la Figura 5.

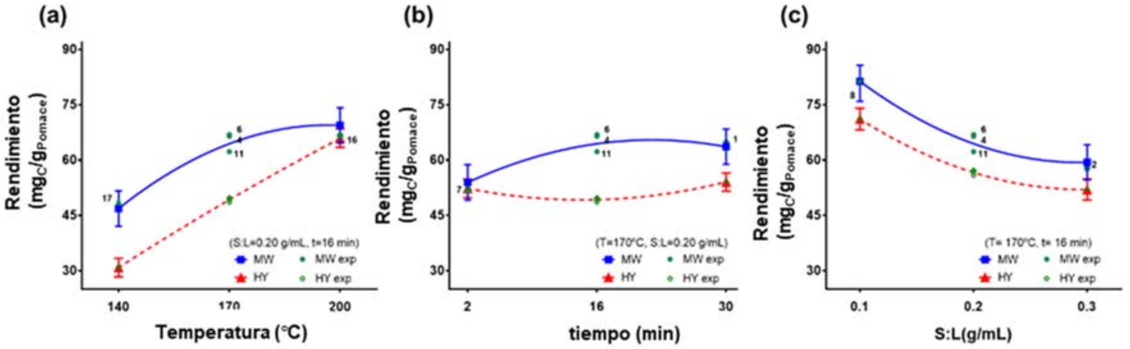

Figura 5. Comparación de la influencia de la temperatura, el tiempo y el ratio sólido-liquido en la hidrolisis hidrotermal (▲) y en la asistida por microondas (■).

Como puede observarse, la temperatura y el ratio son las variables que presentan una mayor influencia. Destaca la reducción de la temperatura en el proceso con microondas respecto al hidrotermal. Se concluyó que pudo ser debido a un efecto atermal de la radiación en la cristalinidad de la celulosa (interacción previamente referida en la bibliografía). Sin embargo, si hubiese tal efecto atermal en el sólido, este debería haber sido más notable con medios más concentrados, es decir, con ratios sólido-líquido más altos. Por el contrario, se encontraron mejores resultados con ratios bajos (mayor proporción de disolvente). Por tanto, puede concluirse que dicho efecto no fue observado, pues debería haber sido más evidente en medios concentrados.

En cuanto a la optimización del proceso de hidrólisis con microondas, esta se realizó de manera que se maximizase el rendimiento global así como la selectividad de azúcares, y que la formación de productos de degradación fuese mínima. Se llegó a la conclusión de que una temperatura de 155ºC, un tiempo de 7.5 minutos y un ratio de 0.10 g/mL proporcionaban los mejores resultados, obteniéndose un rendimiento global de 54.25 mg_C/g_{Orujo}. Si estos resultados se comparan con los presentados en la bibliografía (proceso optimizado a 180ºC y 45 minutos para obtener un rendimiento de 48.40 mg_C/g_{Orujo}), puede observarse una considerable reducción de las condiciones de operación. Por otra parte, se encontró una importante diferencia en las selectividades: mientras que el proceso hidrotermal presentó una mayor selectividad de azúcares, la hidrólisis asistida por microondas conllevó una mayor formación de productos de degradación. No obstante, esta diferencia puede ser debida a la diferente configuración utilizada: proceso en continuo frente a batch. Queda pues por determinar si disminuyendo el tiempo de exposición del líquido a la radiación y manteniendo el del sólido se consigue aumentar la selectividad en azúcares del hidrolizado.

Respecto a la valorización de la fase sólida resultante de la hidrólisis mediante un proceso de pirólisis, el análisis de las cinéticas de degradación llevó a la conclusión de que la hemicelulosa fue la fracción más afectada por el tratamiento. La celulosa y la lignina también mostraron modificaciones, pero en menor medida dado que requieren condiciones más severas que las usadas en este trabajo para ser extraídas. Por tanto, durante la hidrólisis solo las fracciones más accesibles del orujo se vieron afectadas.

En conclusión, con este estudio se consiguió completar la valorización fraccionada del orujo de uva hasta su completo aprovechamiento.

3.5. ESTUDIO DEL POSIBLE EFECTO DE LAS MICROONDAS EN EL EQUILIBRIO SÓLIDO-LÍQUIDO.

Con el objetivo de intentar encontrar una explicación para la sustancial mejora del rendimiento en los procesos de extracción asistidos por microondas, se procedió a analizar los posibles efectos de la radicación en el equilibrio sólido-líquido. Los sistemas que se escogieron para este estudio fueron: ácido gálico y sulfato de sodio como solutos, y agua, etanol, acetato de etilo y alcohol isopropílico como disolventes. El ácido gálico se escogió por ser uno de los polifenoles más abundantes. Su solubilidad se midió en los cuatro disolventes, que fueron seleccionados por sus propiedades dieléctricas (variando desde casi opacos a más transparentes a la radiación). El estudio de la solubilidad del sulfato de sodio en agua se incluyó a fin de comprobar los efectos de aumentar considerablemente el factor de pérdidas (debido a la presencia de electrolitos libres) y analizar su posible influencia en el equilibrio.

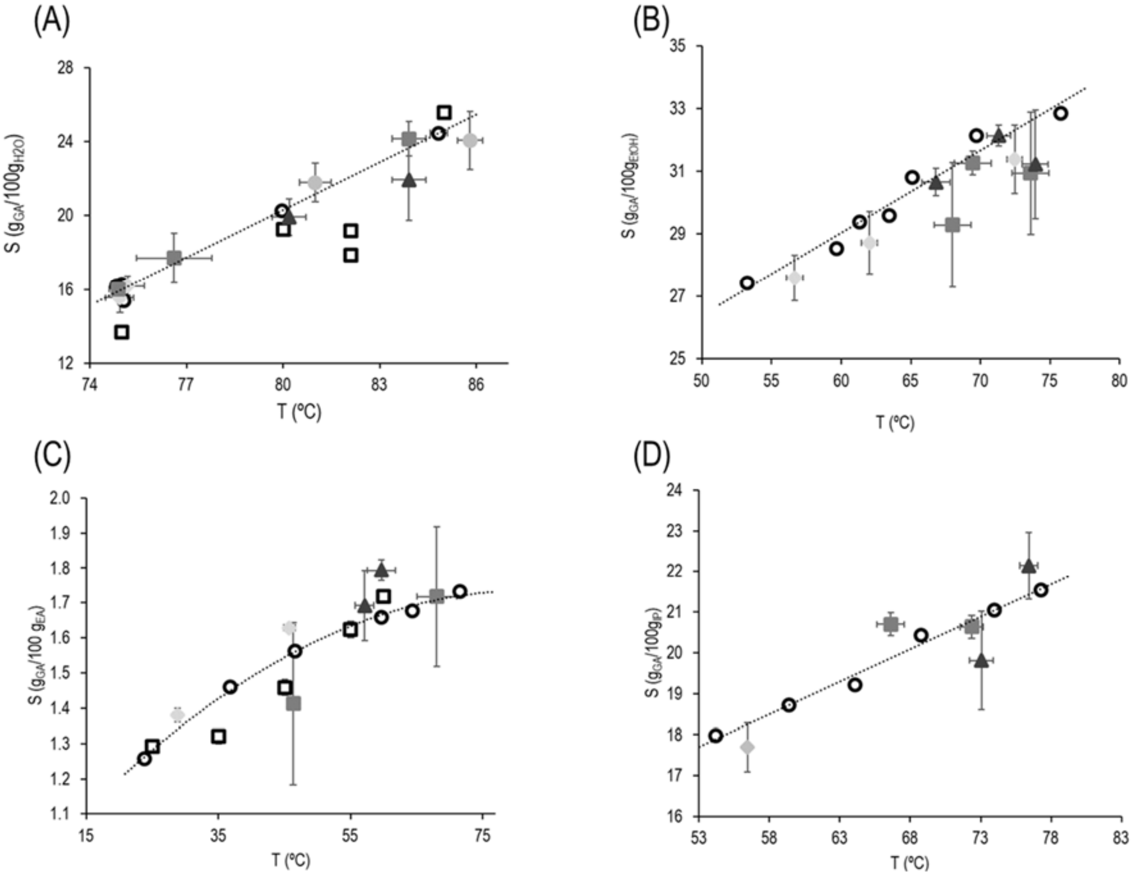

Figura 6. Solubilidad del ácido gálico en agua (A), etanol (B), acetato de etilo (C) y alcohol isopropílico (C) en sistemas de calentamiento convencional (□ datos bibliográficos, ○ medidas experimentales, ⋯⋯ regresión) y con adición de microondas (con potencias de ♦30 W, ■50 W, ▲ 70 W, ● 90 W)

La Figura 6 recoge la solubilidad del ácido gálico en los cuatro disolventes utilizados. De estas gráficas destacan los grandes errores para los sistemas con microondas. Estos fueron debidos a las fluctuaciones de temperatura causadas por el comportamiento dinámico en la celda de equilibrio con microondas. Dado que la temperatura del medio dependió del equilibrio entre dos flujos de energía (radiación y refrigeración), un pequeño cambio en uno de estos conllevaba una desviación en la temperatura. Por otro lado, los errores asociados a la solubilidad causados por dichas variaciones en la temperatura no fueron tan altos como para impedir la identificación de un posible efecto atermal de las microondas. Como puede observarse en la Figura 6, las solubilidades medidas en un sistema con microondas están en el mismo rango que las medidas realizas en sistemas de calentamiento convencional. Por tanto, puede afirmarse que ninguno de los disolventes proporcionó una mejora apreciable de la solubilidad del ácido gálico bajo radiación electromagnética. Luego no se encontraron evidencias de que las microondas puedan influir en el equilibrio sólido-líquido. En consecuencia, tampoco las propiedades dieléctricas del disolvente tuvieron ningún efecto sobre el equilibrio.

Por último, los experimentos con sulfato de sodio no mostraron mejores resultados que los anteriormente presentados. A pesar de haber aumentado el factor de pérdidas un 54% con la incorporación de iones libres al sistema, los experimentos de solubilidad con microondas presentaron resultados similares a los obtenidos por calentamiento convencional.

Por lo tanto, se concluyó que las microondas no ejercieron ningún efecto sobre los equilibrios sólido-líquido analizados y por consiguiente, no se encontró ninguna explicación para el aumento del rendimiento en los procesos de extracción asistidos por microondas.

3.6. OPTIMIZACIÓN DEL PRETRATAMIENTO CON MICROONDAS PARA LA EXTRACCIÓN DE POLIFENOLES DEL ALPERUJO

Una vez que se hubo concluido el estudio de la valorización fraccionada del orujo de uva, se procedió al análisis de una nueva materia prima: el alperujo.

A fin de tener una referencia para estudiar la influencia del pretratamiento con microondas en la extracción de polifenoles del alperujo, se realizó un estudio previo de la extracción convencional sólido-líquido. Las condiciones que se seleccionaron como referencia fueron un ratio de 0.50 g/mL, una temperatura de 70ºC y una mezcla al 50% (v/v) de etanol:agua como disolvente.

En cuanto al pretratamiento con microondas, se probaron diferentes condiciones: la aplicación solo de microondas y su combinación con presión, tanto en presencia de disolvente (etanol:agua) como sin disolvente. El efecto del pretratamiento se estudió en el rendimiento, la velocidad inicial y la riqueza del extracto.

Con la adición del pretratamiento, el rendimiento no se vio sustancialmente mejorado. Tan solo el pretratamiento sin disolvente consiguió aumentar levemente el rendimiento final de la extracción. No obstante, la mejora en la velocidad de extracción y en la riqueza del extracto con este último pretratamiento fue notable. La velocidad inicial se aceleró entre 1.4 y 6.3 veces el valor de la convencional. Estos resultados se encuentran recogidos en la Figura 7. Esta aceleración permitió alcanzar el rendimiento final de la extracción en menos tiempo. En particular, el tiempo de extracción se redujo desde los 39 minutos con el proceso convencional a los 6 con el pretratamiento.

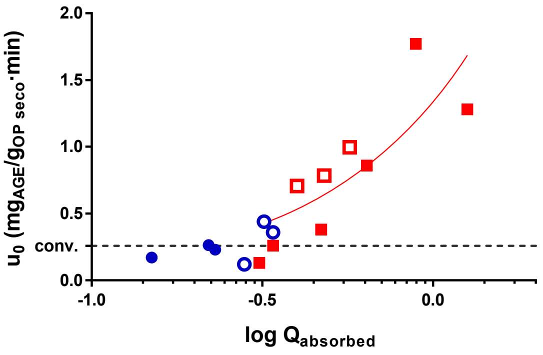

Figura 7. Velocidad inicial de extracción para los pretratamientos estudiados (● MW etanol:agua, MW&P etanol:agua, ■ MW sin disolvente, □ MW&P sin disolvente) en comparación con la extracción sólido-líquido convencional (0.26±0.02 $mg_{AGE}/g_{OP\ seco}$·min; - - - -).

Sin embargo, la mejora más significativa tuvo lugar con la riqueza del producto final, tanto en polifenoles totales como en compuestos individuales. La riqueza polifenólica aumentó entre un 25% y un 40% respecto al proceso convencional, alcanzándose un valor máximo de riqueza de 74.46±3.56 $mg_{AGE}/g_{Extracto\ seco}$ tras un pretratamiento sin disolvente, en el que se absorbió una energía específica de 0.55 kJ/g. Dado que el rendimiento permaneció constante independientemente del tiempo, se descartó la degradación térmica de los polifenoles. Un hecho importante a tener en cuenta con la utilización de pretratamientos más energéticos es la formación de compuestos de degradación de Maillard, como el hidroximetil furfural. La aparición de este compuesto fue significativa en pretratamientos sin disolvente, dado que la radiación tendía a concentrarse en puntos calientes, causando que la materia prima se quemase, generándose así este compuesto. Aunque se encontró en una baja proporción (una concentración máxima de 1.5 $mg_{HMF}/g_{Extracto\ seco}$), se trata de un compuesto no deseado debido a sus propiedades adversas para la salud, y que por tanto va en contra de las aplicaciones de un extracto polifenólico.

Como se ha apuntado anteriormente, el incremento en la riqueza también se encontró para dos compuestos de interés, específicamente el hidroxitirosol y la oleuropeína, como muestra la Figura 8. El tirosol también fue analizado, pero la adición del pretratamiento no fue capaz de mejorar su extracción. En el caso del hidroxitirosol, los pretratamientos que combinaron microondas y presión, así como el pretratamiento sin disolvente proporcionaron un aumento en la riqueza de este compuesto de entre el 25% y el 108%. La mejora en la oleuropeína fue considerablemente mayor, obteniéndose un extracto entre 2.7 y 5.0 veces más rico que con la extracción convencional. Por tanto, puede afirmarse que la implementación del pretratamiento en el proceso de extracción mostró una alta especificidad hacia estos dos compuestos.

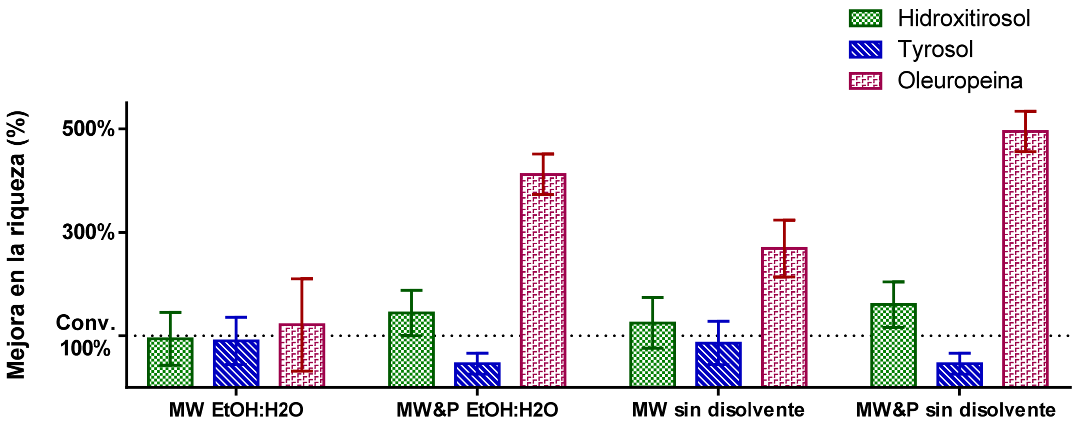

Figura 8. Mejora en la riqueza de hidroxitirosol, tirosol y oleuropeína con el pretratamiento con microondas en comparación con la extracción convencional.

En definitiva, se ha llevado a cabo un análisis exhaustivo de las ventajas y desventajas de la adición de un pretratamiento con microondas a la extracción convencional sólido-líquido de polifenoles del alperujo. Se concluyó que un pretratamiento de microondas sin solvente proporcionó los mejores resultados en términos de eficiencia del proceso y calidad del producto, con una gran riqueza en hidroxitirosol y especialmente en oleuropeína. Aunque también presentó el inconveniente de la formación de compuestos no deseados, como el hidroximetil furfural.

3.7. ESCALADO DEL PRETRATAMIENTO CON MICROONDAS PARA LA EXTRACCIÓN DE POLIFENOLES DEL ALPERUJO.

En este apartado se ha estudiado el escalado de los resultados obtenidos en la sección anterior. Al igual que en el estudio del orujo de uva, en este caso se han medido las propiedades dieléctricas del alperujo para optimizar la eficiencia energética del horno. La novedad en este trabajo ha sido la utilización de una cavidad resonante para la determinación de la permitividad que permitió medir las propiedades dieléctricas a la vez que el medio era calentado con microondas. Por tanto, estos resultados proporcionan el comportamiento real del alperujo durante su irradiación.

Las ecuaciones [7] y [8] recogen el modelo propuesto para estimar, respectivamente, la constante dieléctrica y el factor de pérdidas del alperujo, en función de la temperatura (25-99ºC) y la humedad (4%-69% w/w) con una precisión del 9% y del 29%, respectivamente.

$$\varepsilon' = 2.16 + 0.61H + 86.39H^2 + 0.19HT + 3.13 \cdot 10^{-3}TH^2 \quad [7]$$

$$\varepsilon'' = 7.12H + 2.53H^2 + 0.50TH^2 + 2.66T^2H^2 \quad [8]$$

En cuanto al análisis de la influencia de las variables, como puede observarse en la Figura 9, la humedad fue el factor más significativo, causando un substancial aumento en la permitividad, y sobre todo en el factor de pérdidas (llegando a multiplicarse hasta por 98). La temperatura no tuvo una influencia notable en la constante dieléctrica, aunque si presentó un efecto significativo en el factor de pérdidas dentro de la interacción temperatura-humedad, llegando a variar desde 0.30 hasta 49.34.

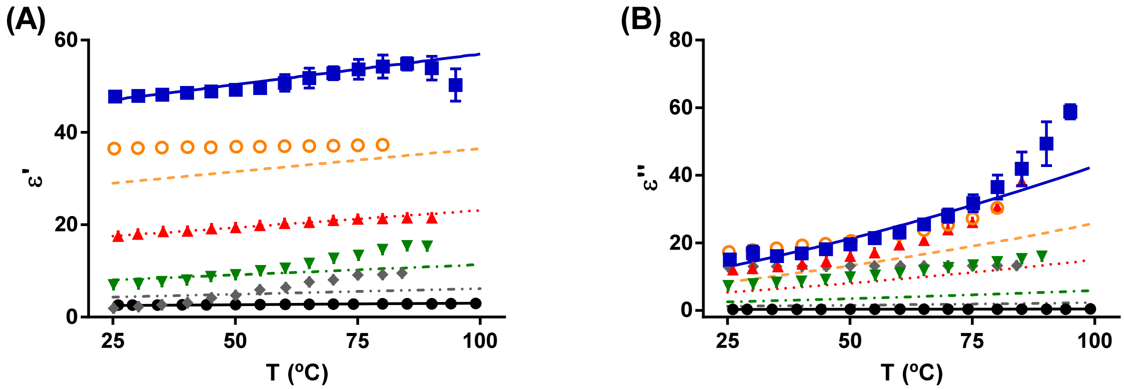

Figura 9. Constante dieléctrica (A) y factor de pérdidas (B) del alperujo en función de la temperatura y la humedad (■ H=69%, ○ H=53%, ▲ H=39%, ▼ H=23%, ♦ H=13%, ● H=4% w/w).

Estos resultados se utilizaron para ajustar los sintonizadores del horno continuo y así asegurar una gran eficiencia energética, que finalmente resultó ser del 97%. No obstante, debido a dificultades para asegurar un flujo de alperujo constante a través de horno, se descartó operar con flujo en continuo. En su lugar, se utilizaron cargas de 500 gramos. El procedimiento experimental seguido reprodujo condiciones similares a las óptimas fijadas anteriormente. Este consistió en un pretratamiento de dos minutos del alperujo en ausencia de disolvente, tiempo durante el cual se aumentó la temperatura del material desde 23ºC a 100ºC, produciéndose una reducción de la humedad del 17% (energía específica absorbida de 0.40±0.07 kJ/g). En cuando a los resultados de la extracción, se encontró un rendimiento similar al observado en estudios previos (24.73±2.05 $mg_{AGE}/g_{OP\ seco}$). Por otro lado, se consiguió un significativo aumento del 56% en la riqueza del producto final (105.71±7.85 $mg_{AGE}/g_{Extracto\ seco}$) respecto al observado con el pretratamiento a escala de laboratorio. Esta diferencia se achacó a la operación con cargas del horno en lugar de en continuo. La distribución de energía dentro del horno presenta zonas con una mayor intensidad que otras, pero esta diferencia es mitigada por el flujo en continuo. Por lo cual, se cree que el cambio en la configuración del horno es lo que conllevó el considerable aumento de la riqueza del extracto final.

En conclusión, con este trabajo queda probada la viabilidad del horno desarrollado como una unidad multi-propósito. No obstante, el trabajo futuro debe centrarse en la mejora del sistema de impulsión.

3.8. PRETRATAMIENTO CON MICROONDAS PARA LA EXTRACCIÓN DE ANTOCIANINAS A PARTIR DE FLORES DE AZAFRÁN. EVALUACIÓN DE LA CALIDAD DEL PRODUCTO

Finalmente, se ha estudiado la valorización de la flor de azafrán con el objetivo de obtener un extracto polifenólico, especialmente rico en antocianinas, que pueda ser utilizado como colorante ciánico. Para ello se ha empleado el pretratamiento con microondas anteriormente presentado y desarrollado. Las variables analizadas han sido la temperatura alcanzada durante el pretratamiento y el ratio sólido-líquido. El efecto de la extracción con el pretratamiento se comparó con el proceso convencional (sin pretratamiento), cuyas condiciones también han sido optimizadas en este trabajo.

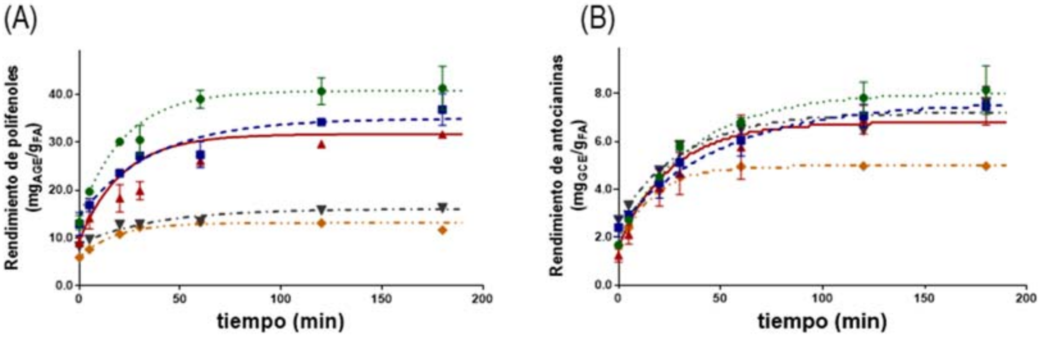

Figura 10. Influencia del ratio sólido-líquido en la extracción convencional de (A) polifenoles y (B) antocianinas. ●S:L=0.10 g/mL, ■S:L=0.20 g/mL, ▲ S:L=0.30 g/mL, ▼ S:L=0.50 g/mL, ♦ S:L=0.75 g/mL.

Respecto a la extracción convencional sólido-líquido, la proporción flores-disolvente fue de gran importancia. Su influencia puede observarse en la Figura 10. Se encontró que el rendimiento final disminuía a medida que aumentaba el ratio. Este efecto fue atribuido a diferencias en la transferencia de materia, aunque la posible hidrólisis ácida causada por el disolvente también fue considerada.

En cuanto a la selección de las condiciones de extracción de referencia, se seleccionó un ratio de 0.30 g/mL, ya que este valor implicaba un equilibrio entre la eficiencia de la extracción y el consumo de disolvente. Además, este ratio aseguraba un alto rendimiento en antocianinas, similar al presentado por ratios más bajos. Adicionalmente, se escogió una temperatura de referencia de 30ºC, frente a temperaturas más altas (60ºC), con el objetivo de minimizar la degradación térmica de las antocianinas.

Una vez optimizada la extracción convencional, se procedió a analizar la influencia de la adición del pretratamiento con microondas. Las principales ventajas logradas fueron la reducción del tiempo de extracción y la mejora de la calidad del producto final. La velocidad de extracción inicial fue el parámetro más afectado. Se aceleró enormemente cuando se emplearon temperaturas moderadamente altas y relaciones sólido-líquido bajas. Tal mejora permitió obtener un alto rendimiento en tan solo 5 minutos, en lugar de casi una hora como requería el proceso convencional. Por otro lado, la calidad del producto final aumentó en términos de riqueza, composición, actividad antioxidante y color. Por otra parte, se encontró que la riqueza en polifenoles y antocianinas presentaba tendencias contrarias. La riqueza polifenólica se vio favorecida por altas temperaturas en el pretratamiento (aumentando un 25% a 100ºC), mientras que la riqueza en antocianinas disminuyó un 9% con la temperatura debido a la degradación térmica.

Finalmente, se establecieron las condiciones óptimas de extracción para la obtención de un extracto rico en antocianinas. Por lo tanto, se fijó una temperatura de pretratamiento de 65ºC y un ratio de 0.30 g/mL. Además, estas condiciones permitieron maximizar la capacidad antioxidante del extracto (un 130% mayor que el extracto convencional) y obtener un tono más azulado en el extracto. Respecto a esta último parámetro, cabe destacar que condiciones de extracción leves (baja temperatura y ratio) incrementaron el tono violáceo del extracto, mientras que condiciones más severas (alta temperatura y ratio) conllevaron la obtención de un extracto más amarillo, probablemente debido a la degradación térmica. En relación a la composición del extracto, se encontró que la delfinidina fue el compuesto mayoritario, llegando a representar hasta el 80% de las antocianinas (15% respecto a los polifenoles totales). Tal especificidad incrementó sustancialmente el valor del extracto, ya que mediante una etapa de purificación puede obtenerse un producto ya no solo rico en antocianinas o polifenoles, sino en delfinidina, un compuesto con alto valor añadido.

En resumen, se concluyó que la adición del pretratamiento con microondas a la extracción convencional fue significativamente beneficiosa, ya que permitía obtener un producto de alta calidad en un proceso de extracción eficiente.

4. CONCLUSIONES

Respecto a los objetivos inicialmente fijados, puede afirmarse que la viabilidad del pretratamiento con microondas ha quedado demostrada para la extracción de polifenoles (y especialmente de antocianinas) a partir de orujo de uva, alperujo y flores de azafrán. Con la adición del pretratamiento a la extracción convencional sólido-líquido se han encontrado tanto mejoras en el proceso (significativa reducción del tiempo de extracción y aumento del rendimiento) como en la calidad del producto. De este último punto destaca el aumento en la riqueza polifenólica (proporción de compuestos activos respecto a todas las sustancias obtenidas en el extracto seco), dado que es un parámetro crucial en la comercialización del producto final. Otros parámetros considerados para la evaluación de la calidad del extracto han sido la capacidad antioxidante y las variaciones en el color.

El pretratamiento con microondas aquí presentado ha sido ideado para superar las limitaciones que esta tecnología presenta en cuanto a su implementación a escala industrial. Mediante el uso de un tiempo de irradiación muy corto (de hasta 2 minutos), ha sido posible diseñar un horno compacto para el tratamiento homogéneo del medio de extracción. Para ello, primeramente fue necesario contar con un modelo capaz de estimar las propiedades dieléctricas de las mezclas biomasa-disolvente utilizadas. Con este fin se desarrollaron para el orujo de uva y el alperujo modelos capaces de predecir la constante dieléctrica y el factor de pérdidas con una precisión del 8% y 25%, y del 9% y 29%, respectivamente. Estos modelo permitieron alcanzar un diseño final del horno con una alta eficiencia energética, que resultó ser del 97%. Su efectividad quedó probada con la reproducción de los resultados de extracción obtenidos a escala de laboratorio.

En cuanto a las diferencias observadas entre los diferentes residuos estudiados, se puede concluir que el pretratamiento con microondas es altamente recomendable para la extracción de compuestos termosensibles, como fue el caso de la obtención de antocianinas a partir de orujo y de flores de azafrán. En el caso de los polifenoles del alperujo, no se encontró una sustancial mejora, debido a que estos se encuentran altamente ligados en la estructura de la oliva, por lo que requieren tratamientos más agresivos para ser liberados. Aun así, en este último caso destaca la conveniencia del pretratamiento por permitir una alta especificidad en la extracción de los compuestos de alto valor añadido hidroxitirosol y oleuropeina.

Adicionalmente se estudió la completa valorización fraccionada del orujo de uva en una biorrefinería intensificada por microondas, por medio de una consecutiva hidrólisis y pirolisis. La implementación de la radiación al proceso de hidrolisis permitió reducir considerablemente la temperatura y el tiempo de operación, obteniéndose una eficiencia global similar a la del proceso hidrotermal. No obstante, el estudio de las cinéticas de degradación durante la posterior pirólisis determinó que tan solo la fracción más débil (hemicelulosa) fue modificada durante la hidrolisis, proporcionando así un subproducto más difícil de volatilizar.

Estas significativas mejoras observadas con la adición de las microondas llevaron al estudio del efecto de la radiación en el equilibrio sólido-líquido, a fin de intentar comprender qué sucede durante la extracción asistida por microondas. Para ello se procedió a medir la solubilidad de una especie molecular (ácido gálico) y una iónica (sulfato de sodio) en diferentes disolventes (agua, acetato de etilo, alcohol isopropílico y etanol), caracterizados por sus diferentes propiedades dieléctricas. A pesar de que los resultados conllevaron grandes incertidumbres debido a inestabilidades en la temperatura (sistema de medición dinámico), la precisión fue suficiente como para rechazar la hipótesis de un posible efecto atermal de la radiación, capaz de explicar la mejora en los procesos de extracción asistidos por microondas.

En conclusión, puede afirmarse que la innovadora configuración propuesta para la intensificación de los procesos de extracción, un pretratamiento con microondas, supone una prometedora alternativa para la implantación a escala industrial de las ventajas de esta tecnología. La utilidad del pretratamiento ha quedo además ampliamente probada por el exhaustico análisis de sus beneficios tanto en la eficiencia del proceso, como en la calidad del producto final. Es por ello que, el conocimiento desarrollado en este trabajo puede resultar de gran utilidad, no solo para la comunidad científica, sino también para las industrias que pueden contar con una herramienta factible para la intensificación de sus procesos.

AGRADECIMEINTOS

La investigación aquí presentada ha podido ser realizada gracias a la financiación recibida por parte del proyecto europeo WineSense (FP7-386 MC-IAPP) y de los proyectos VA330U13 y VA04U16 de la Junta de Castilla y León. A.Á. agradece al Ministerio de Educación las becas de doctorado FPU13/04678 y EST16/00177.

ACKNOWLEDGMENTS

AGRADECIMIENTOS

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Tengo tanto que agradecer a tanta gente que me ha ayudado durante esta etapa que necesitaría infinitas líneas para poder ser justa. Dado que no es posible, aquí está mi pequeña dedicatoria en comparación con el inmenso apoyo con el que he contado.

En primer lugar, gracias a María José, quien confió en mi para realizar un doctorado. Solo puedo añadir que me he esforzado mucho para estar a la altura de un grupo de investigación como HPP. Gracias a Rafa, que con tanta paciencia me ha ayudado. Siempre dispuesto y con buen humor me ha guiado como solo un gran profesor sabe. Ha sido un inmenso placer poder trabajar con vosotros, y me llevo un ejemplo de dedicación y ética en el trabajo, que tengo intenciones de seguir fielmente.

Regarding my secondment in York, I have to acknowledge Dr. Vitaly Budarin for accepting me in his research group, giving me the opportunity of an outstanding collaboration in York. Thanks Javier for your guidance, I don’t know what I would have done without you. To my lab mates there, Alexandre, Andrew, Anna, Coraline, Edu, Emma, Gavin, Hanna, Ian, Jenny, Jonny, Maxime, Roxanna, Thomas, Yann and all the Green Chemistry members, it was awesome sharing the lab with you and getting to know you. How I cannot be more than grateful to my dearest housemates, Abi, Lucy, Meg, and Rebekah, who accepted me as one of their close friends and gave me so many wonderful experiences that I will never forget.

Echo la vista atrás y me doy cuenta de que hace ya diez años tomé una de las mejores decisiones de mi vida: matricularme en Ingeniería Química. Qué grandes profesores me he encontrado en este departamento. Y los compañeros con los que he tenido la suerte de trabajar día a día no se han quedado atrás. Solo puedo agradecerles todos los buenos momentos que hemos compartido juntos, dentro y fuera de la universidad. En especial, gracias a mis amigos (mucho más ya que compañeros) con los que empecé este camino: Celia, Deodato, Gerardo, Gianluca, Gyuri, Nerea, Nuria, Reini, Vanessa y Yoana. Gracias a los divertidísimos marichochis de Alberto, Álvaro, Marta y Víctor, por enseñarme a relativizar qué es importante. Gracias a los magníficos técnicos con los que nada es impensable y todo es posible. Alvar, Dani, Isa, y Sergio no entiendo como no os ha reclutado ya la NASA. Gracias a mis compañeros de comidas, Araceli, Celia, Jonny, Lauri, Patri y Roxi, pero sobre todo por las sobremesas. Gracias a todos los visitantes internacionales por todos los momentos que hemos compartido. Me gustaría hacer un inciso especial para agradecer sobre todo a Rut y Álvaro su apoyo. Rut, mi Corks, mi gran compañera que tantas veces me ha aguantado desahogándome y con la que siempre he podido contar. Y como no, Álvarito, de las mejores personas que encontraré en la vida: noble, generoso y tan buena persona. Que suerte de haberme cruzado con vosotros.

A Patricia, Adrián, Héctor, Cristina, Jaime y Sara, gracias por vuestra dedicación a la investigación que tanto ha enriquecido esta tesis.

Gracias a mis amigos de siempre. Pienso en todas las noches que hemos compartido juntos y no puedo evitar sonreír y sentir ese calor en las mejillas por todas las risas y buenos momentos. Alfredo, Andrea, Blanca, Cris, Fran, Inés, Javi, Jose, Lorena, Marta, Martín, Pelu, Polle, Sansy, Sara, sois los mejores amigos que podría encontrar, fáciles de querer y difíciles de olvidar. Espero teneros siempre conmigo. Y en especial, gracias Jimmy por tu cariño, por ser mi refugio, por apoyarme siempre, por confiar en mi más que yo misma, por aguantarme (incluidos todos mis cambios de opinión), por no enfadarte nunca. Y con todo esto, cómo no te voy a querer.

Y por último, porque quien me conoce bien sabe que siempre dejo lo mejor para el final, mis padres. Referente de esfuerzo y sacrificio. Todo lo que tengo, lo que he conseguido y seré es gracias a estas dos excepcionales personas, a las que les digo menos de los que debería lo mucho que significan para mí. Su incondicional abnegación y dedicación, no solo a mi formación sino a todas las ideas (algunas más que disparatadas) que he tenido, ha sido inmensurable. Tanto que, en este caso, el mérito de realizar esta tesis lo tenemos que repartir a partes iguales.

ABOUT THE AUTHOR

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Ana Álvarez (1990) started her University studies in 2008 in the Chemical Engineering degree. In 2012, she had her first approach to research by a collaboration internship to analyse glucose degradation path under sub/supercritical water conditions. An interest in research that continued during her abroad year (2012/2013) in the University of Birmingham (UK). In this period, she had the chance to work in an international environment, where she developed the ability to adapt to new situations. In 2013, she was graduated as a Chemical Engineering, but she decided to continue her training by enrolling in Research in Fluid Thermodynamics Engineering Master. Afterwards, in 2014 she joined the High Pressure Process Group as a PhD student. Her thesis topic dealt with process intensification by means of microwave technology. Under the supervision of professors R.B. Mato and M.J. Cocero and in the frame of the European Project WineSene, she became aware of the importance of the transfer of knowledge between industry and academia. The development of the thesis led her to participate in international congresses and stablish international collaborations with other institutions. In particular, during 2017 she had the chance to do a three-month secondment in the University of York (UK).

From her expertise she highlights the autonomy and the ability to face multidisciplinary issues gained during her university career.

In a near future, she hopes to make a good use of the skills and knowledge attained during her training

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PERSONAL DETAILS

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RELEVANT SKILLS

Hard worker	Resourceful	Team building	Shared leadership
Self-driven	Multitasking	Results orientated	Project management

SUMMARY

- 5-year degree in Chemical Engineering by University of Valladolid.
- Master in Fluid Thermodynamic Engineering.
- PhD in Chemical Engineering. Thesis topic: process intensification.
- International experience.

PUBLICATIONS

- Cantero D. A., Álvarez A., Bermejo M. D., Cocero M. J. (2015) Transformation of glucose into added value compounds in a hydrothermal reaction media. The Journal of Supercritical Fluids, 98, 204-210. DOI: http://dx.doi.org/10.1016/j.supflu.2014.12.015.

- Álvarez A., Fayos-Fernández J., Monzó-Cabrera J., Cocero M. J., Mato R. B. (2017) Measurement and correlation of the dielectric properties of a grape pomace extraction media. Effect of temperature and composition. Journal of Food Engineering, 197, 98-106. DOI: http://dx.doi.org/10.1016/j.jfoodeng.2016.11.009.

- Álvarez A., Poejo J., Matias A.A., Duarte C.M.M., Cocero M.J., Mato R.B. Microwave pretreatment to improve extraction efficiency and polyphenol extract richness. Effect on antioxidant bioactivity. Food and Bioproducts Processing, 106, 162-170. DOI: https://doi.org/10.1016/j.fbp.2017.09.007

INTERNATIONAL CONGRESS

- Álvarez A., Llorens-Vallés G., García-Baños B., Cocero M.J., Catalá J.M., Mato R. Microwave pretreatment for the extraction of active compounds from olive pomace. Extraction optimization and dielectric properties measurement. 11th European Symposium on Biochemical Engineering Sciences (ESBES2018). Lisbon (Portugal), 9-12 September 2018. Poster.

- Mato R., Álvarez A., Llorens-Vallés G., Fayos-Fernandez J., Catalá J.M., Monzó-Cabrera J., Cocero M.J. Continuous microwave processing of olive-pomace for active compounds recovery. XXXV Jornadas Nacionales de Ingenieria Química. Salamanca (Spain), 4-6 July 2018. Oral communication.

- Álvarez A., H. Trigueros, M.J. Cocero, R.B. Mato. Microwave influence on solid-liquid equilibrium. 10th World Congress of Chemical Engineering. Process Intensification. Barcelona (Spain), 1-5 October 2017. Poster
- Álvarez A., Matias A.A., Cocero M.J., Mato R.B. A microwave pretreatment to enhance grape polyphenol extraction yield, extract richness and bioactivity. 8th World Bulk Wine Exhibition. Amsterdam (The Netherlands), 21-22 November 2016. Poster
- Álvarez A., Matias A.A., Mato R.B., Duarte C.M.M., Cocero M.J. Yield vs selectivity in grape pomace polyphenol microwave extraction. 3rd Global Congress on Microwave Energy Applications (3GCMEA). Cartagena (Spain), 25-29 July 2016. Oral communication.
- Álvarez A., García-Serna J., Mato R.B., Cocero M.J. Tuned biofunctional polyphenol extracts obtained by microwave and pressure pretreatment. 15th European Process Intensification Conference (EPIC 5). Nice (France), 27 September-1 de October 2015. Poster.
- Moreno T., Matias A., Álvarez A., De Paz E., Gonçalves V.S.S., Deodato D., Gil Y., Romero R., Benito O., Rodriguez-Rojo S., Martín A., Mato R.B., Duarte C.M.M., Guadarrama A., Every H., Cocero M.J. Extraction and formulation intensification processes for natural actives of wine. 1st International symposium on the valorization of wine-making by-products for the food sector. Pavia (Italy) 2 October 2015. Oral communication.
- Álvarez A., Fayos-Fernández J., Mato R.B., Monzó-Cabrera J., Cocero M.J. Dielectric properties of a multiphase mixture. Effect of temperature and composition. 15th International Conference on Microwave and High Frequency Heating. Cracow (Poland), 14-17 September 2015. Oral communication.
- Álvarez A., Mato R.B., Cocero M.J. Microwave and pressure pretreatment fot the extraction of polyphenols from grape pomace. WineSense First Summer School. Lisbon (Portugal), 22-26 June 2015. Poster
- Moreno T., Álvarez A., de Paz E., Gonçalves V., Rodríguez-Rojo S., Martín A., Mato R.B., Cocero M.J., Extraction and formulation intensification for natural actives of wine. Hungarian National Conference on Supercritical Fluids. Budapest (Hungary) 21 de May de 2015. Poster.
- Solyom K, Álvarez A., Cocero M.J., Mato R.B. Microwave assisted extraction of grape marc: comparison to conventional process and economic evaluation. 10th International Conference on Renewable Resources & Biorefineries (RRB10). Valladolid (Spain), 4-6 June 2014. Oral communication.

INTERNATIONAL SECONDEMENTS

University of York (UK)
From 04/04/2017 to 03/07/2017
Funded by Spanish Ministry of Education.
Objective: valorisation of biomass and structural characterization

Matarromera and Abrobiotec S.L. Winery (Spain)
From 04/11/2014 to 03/01/2015.
Funded by the European Project Winesense
Objective: academia-industry transfer of knowledge

University of Birmingham
From 20/09/2012 to 07/07/2013
Funded by Erasmus program
Objective: chemical engineering modules and research project.

GRANTS

- Collaboration grant for the development of a PhD thesis in an international institution, Spanish Ministry of Education, 2016, 3 months.
- Attendance to conferences and congress for the development of a PhD thesis, University of Valladolid, 2015, 2017 and 2018.
- Grant University Lecturers Training for the development of a PhD thesis. Spanish Ministry of Education, 2013, 4 years.
- Erasmus grant at University of Birmingham, 2012/2013, 9 months.
- Collaboration grant in research tasks, University of Valladolid, 2011/2012.

CONTRACTS

- European project WineSense. Active collaboration from 2014 to 2017.
- European project “Sustainable Hydrothermal manufacturing of nanomaterials”. From 01/01/2014 to 30/09/2014. University of Valladolid
- Project: “Decomposition and valorisation of biomass to obtain high added value compounds. Study of the dissolution and hydrolysis of biomass in supercritical water”. From 15/07/2013 to 31/12/2013. University of Valladolid.

LECTURING EXPERIENCE

Academic year 2017/2018.

- Projects in Chemistry Industry, Chemistry degree. 10 hours
- Experimentation in Chemical Engineering, Chemical Engineering degree. 15 hours
- Supervision of research project students.

Academic year 2016/2017.

- Introduction to research. Master in Thermodynamic Fluid Engineering. 15 hours.
- Experimentation in Chemical Engineering, Chemical Engineering degree. 10 hours
- Supervision of research project students.

Academic year 2015/2016

- Introduction to research. Master in Thermodynamic Fluid Engineering. 15 hours.
- Process engineering with supercritical fluids: separation. Master's Degree in Thermodynamic Fluid Engineering. 10 hours.

OTHERS

Prizes:

- Third prize at the International Microwave Congress of Cartagena (3GCMEA July 2016) for academic achievements and innovation in microwave research. Endowed with 250 USD.
- "Kaiser West Design Project Award for the design design of a silica sand processing and rare earth extraction plant" in July 2013 by the Department of Chemical Engineering, University of Birmingham. Endowed with 100 GBP.

Active collaboration with other research centers:

- iBET Institute (Portugal) for the extraction of active compounds from lees (wine residue)
- Callagham Innovation (New Zeland) for the study of microwave radiation on the extraction of active compounds from avocado seed and skin.
- Kumamoto University (Japan) for the extraction of polyphenols from *citrus depressa*.

The valorization of natural by-products from the region of Castilla y León has been studied through the development of a microwave pretreatment. This novel configuration has been proposed due to its industrial implementation feasibility. The comprehensive analysis performed about the pretreatment impact on the extraction of polyphenols has proved its advantages in terms of process efficiency and final product quality. The outstanding results obtained supports the viability of the microwave pretreatment as a convenient tool for the intensification of industrial processes.