chemistry proceedings

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Critical Variables Influencing the Ultrasound-assisted Extraction of Bioactive Compounds and a Review†

Anxo Carreira-Casais[1], Maria Carpena[1], Antia G. Pereira[1,2], Franklin Chamorro[1], Anton Soria-Lopez[1], Pascual Garcia –Perez[1], Paz Otero[1], Hui Cao[1], Jianbo Xiao[1], Jesus Simal-Gandara[1]* and Miguel A. Prieto[1,2]*.

[1] Nutrition and Bromatology Group, Department of Analytical and Food Chemistry, Faculty of Science, University of Vigo, Ourense Campus, E-32004 Ourense, Spain; anxocc@uvigo.es (A.C.-C.); mcarpena@uvigo.es (M.C.); antia.gonzalez.pereira@uvigo.es (A.G.P); franklin.noel.chamorro@uvigo.es (F.C.); anton.soria@uvigo.es (A.S-L.); pasgarcia@uvigo.es (P.G.-P.) paz.otero@uvigo.es (P.O.); hui.cao@uvigo.es (H.C.); jianbo.xiao@uvigo.es (J.X.); jsimal@uvigo.es (J.S.-G.); mprieto@uvigo.es (M.A.P.)

[2] Centro de Investigaçao de Montanha (CIMO) Instituto Politécnico de Bragança, Campus de Santa Apolonia 10, 5300-253 Bragança, Portugal; antia.gonzalez.pereira@uvigo.es (A.G.P); mprieto@uvigo.es (M.A.P)

* Correspondence: jsimal@uvigo.es (J.S.-G.); mprieto@uvigo.es (M.A.P.)

† Presented at the 1st International Electronic Conference on Chemical Sensors and Analytical Chemistry, 01—15 July 2021 ; Available online: https://csac2021.sciforum.net/.

Abstract: Ultrasound-assisted extraction (UAE) is a novel methodology, belonging to the so-called green chemistry which has gained interest in recent years due to the potential to recover bioactive compounds, especially those from plant matrices. It is widely recognized that the extraction of molecules by UAE gives rise to higher or similar yields than those obtained by traditional extraction methods. UAE has certain advantages inherent to green chemistry extraction methods like short extraction time and solvent consumption. The aim of this review is to critically present the different variables and parameters that can be modified in UAE, such as power, time, temperature, solvent, and solid solvent ratio and thus, has influence in yield and extraction performance.

Keywords: ultrasound-assisted extraction; critical variables; power; temperature; time; solvent

Citation: Carreira, A.; Carpena, M.; Pereira, A. G; Chamorro, F.; Soria-Lopez, A.; Garcia-Perez, P.; Otero, P.: Cao, Hui.; Xiao, Jianbo.; J. Simal-Gándara, M. A. Prieto. Critical variables influencing the ultrasound-assisted extraction of bioactive compounds, a review. *Proceedings* **2021**, *68*, x. Lastname, F.; Lastname, F.; Lastname, F. Title. *Chem. Proc.* **2021**, *3*, x. https://doi.org/10.3390/xxxxx

Published: 1 July 2021

1. Introduction

Ultrasound-assisted extraction (UAE) is a technique that belongs to the group of novel extraction methods belonging to green chemistry, like microwave assisted extraction (MAE), enzyme assisted extraction (EAE) or high-pressure assisted extraction (HPAE) [1], because it promotes the extraction of compounds of interest, with a decrease in the consumption of resources, such as solvent and energy, and achieving remarkably higher extraction yields [2,3]. UAE has been mainly used to plant matrices to obtain extracts rich in bioactive compounds, such as phenolic compounds, pigments, polysaccharides, amino acids, among others [1,2,4,5]. This methodology is based on the principle of cavitation, which leads to cell collapse of the matrix and allows the release of their inner substances. Several variables are relevant for the performance of UAE, including the solid-liquid ratio, the type of solvents used, the extraction time and the ultrasound power applied. Besides, power and extraction time are closely linked to a fifth important factor: temperature. In practical terms, a correct optimization of these variables is essential to obtain an excellent performance, resulting in a maximal extraction yield. In addition, temperature is very important, as it can affect the integrity of bioactive compounds since most of them are thermolabile. Thus, large amounts of power linked to long extraction periods may lead to sample damage, so temperature control is essential for a correct design of the cooling reactor and the optimization of UAE extraction protocols. Keeping all this in mind, this critical review is focused on the influence of all the variables that affect UAE, to analyze the critical factors involved in the optimization of this technique. In addition, the so-called

response surface models (RSM) are usually used to carry out the optimization of the variables that is a representative method to generate meta-models. RSM has the advantage over other methods in that it allows the effect of several variables to be analyzed at the same time [6].

2. Variables Influencing in Ultrasound-assisted Extraction

To obtain a good extraction yield, an optimization of the variables that influence the method is required. Among the variables that affect UAE, there are 3 types of parameters: physical parameters like the power, extraction time, medium-dependent parameters (solvent type and ratio, viscosity, surface tension and vapor pressure, temperature) and matrix-dependent parameters [2,7]. Here below we mention some issues regarding these parameters for method optimization of bioactive compounds from plants.

Power represents the unit of power emitted per unit of time. The use of high power usually improves extraction yields, for this reason is one of the main parameters to be optimized. However, the use of high power can overheat the reactor producing degradation of labile compounds and solvent evaporation [8]. Another variable that is commonly present in UAE is the extraction time, generally one of the advantages of UAE is that it allows obtaining good extraction yields with relatively short process times. It is common to achieve method optimizations in 30-60 min, minimizing the energy consumption and reducing the compounds exposure to the process [9]. The type of solvent used is closely linked to the nature of the compounds to be extracted; in the case of polar compounds, in food matrices, a predominantly aqueous extraction medium is generally chosen; in the case of matrices with organic bioactive compounds, ethanol is usually chosen while methanol tends to obtain better extraction yields. Some authors prefer to the use of ethanol because of its lower toxicity [7]. European Directive 2010/59/EU lists the solvents that can be used for the extraction of compounds from foodstuffs, as well as their uses and limitations [10]. In addition to the suitable solubility of the compounds of interest in the solvent, it is also important to consider the vapor pressure, surface tension and viscosity of the solvent [2]. The solid/solvent ratio used per each compound does not follow pattern, however there is a minimum of solvent per matrix used which is generally in the range from 0.1 g/mL to 0.025 g/mL solid/solvent. This depends especially on the type of solvent and the matrix used. Finally, the extraction temperature is a double-edged sword. Besides affecting the extraction, it could also have negative effects due to the possible degradation of thermolabile compounds. For this reason, the cooling system can allow the extraction of compounds by ultrasound avoiding the overheating of the medium and therefore the degradation. In this sense, one of the advantages of UAE is the temperature control to avoid damage certain compounds. The increase in temperature caused by the ultrasound probe itself is fundamentally produced when high powers are applied. A temperature increase produces a decrease in both viscosity and surface tension and induces an increase in the vapor pressure. Thus, very high temperatures can be harmful for the propagation of ultrasounds through the medium [8].

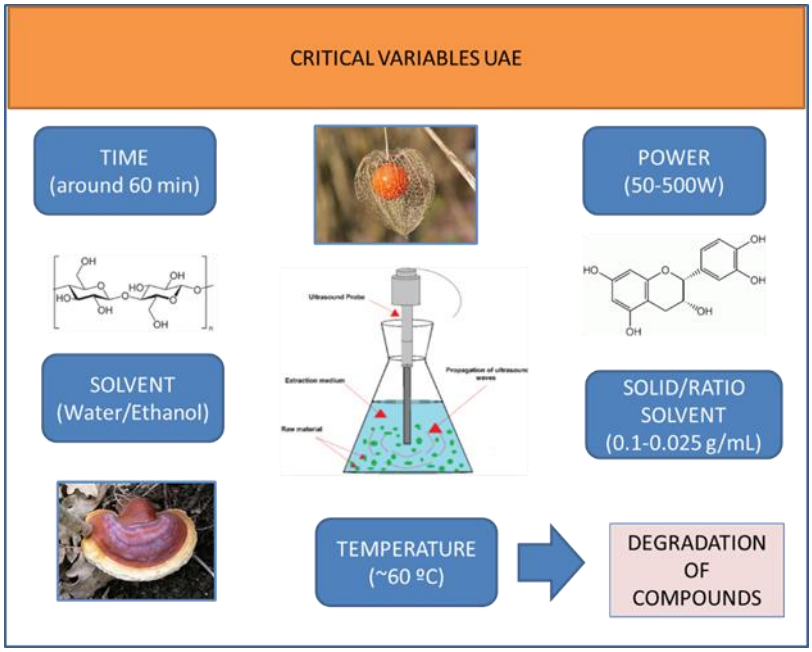

Figure 1. Variables in UAE studied in this review.

3. Results

Table 1 shows the optimized parameters for the extraction of phenolic compounds, pigments, polysaccharides, and amino acids from different plant matrices and all of them are described below.

For the extraction of phenolic compounds, it seems that power is a dependent parameter of the extraction time. If the power is increased, the extraction time is decreased (table 1). The most used solvent is ethanol and the solid solvent ratio varies between 0.1 g/mL [11] and 0.025 g/mL [12]. For these molecules, temperature must be controlled to avoid the degradation of the thermolabile compounds. In this case, it is possible to extract phenolics with temperature up to 75ºC [13].

In parallel, bibliography shows a variety of solvent treatments for pigment extraction depending on their characteristics. Among them, distilled water and ethanol are suitable. Extraction times employed are different depending on the compound. For example, one study shows the extraction of anatto seeds in 7.25 min [14] while 37 min are needed to extract betacyanin and betaxhantin in bougainvillea flowers [15]. Comparing to phenolic compounds, the potencies used are lower and solvent to solid ratios are higher, varying between 0.14 g/mL for the extraction of the natural pigment [14] from seeds to 0.058 g/mL for the extraction of betacyanin and betaxhantin in bougainvillea flowers [15].

For the extraction of polysaccharide by UAE, water is a common solvent used [16–18]. It is relevant to highlight that the extraction times are high, except for some studies (for example, pigments recovered from *Oriza sativa*, which is relatively performed in 20 min). Extraction temperatures are also medium-high [16,18]. For example, a study shows that the extraction of β-d glucans is performed at high extraction power (590 W) and temperature (81ºC) [16].

Regarding amino acid extraction, distilled water is a commonly solvent used, with an extraction power around 200 W. Some examples found in the bibliography described the variability in the solid-solvent ratio [7]. It is also noteworthy that in the case of grape amino acid extraction, the extraction time is really short (6 min) [19,20] and the extraction temperature is around 70ºC [19].

Table 1. Optimized extraction conditions for phenolic acids, pigments, polysaccharides, and amino acids from different vegetal matrices by UAE.

Compounds	Matrix	Solvent type and ratio	Optimized parameters	Ref.
Phenolic compounds				
Phenolics (TFC)	Blueberry pomace	EtOH/W 50%/50%; 0.05 g/mL	ET :60 min; UPA: 64 W; T: 40ºC	[11]
Phenolics (TFC)	*Zea mays* waste	EtOH/W 70%/30% 0.09 g/mL	ET: 40 min; UPA: 50W; T:ND	[12]
Stilbenes	Grape canes	EtOH/W 60%/40% 0.025 g/mL	ET: 10 min; UPA: 200W; T :75ºC	[13]
Anthocyanins	Grape skins	EtOH 60 % acidified pH= 3; 0.033 g/mL	ET:28 min; UPA: 400W; T: 50ºC	[21]
Pigments				
Natural pigment	Annato seeds	Dw, previously trated with chloroform/ratio solid solvent 0.14 g/mL	ET :7.25 min; UPA:200W; T: 72.7ºC. (Duty cycle of 0.8 s)	[14]
Betacyanin and betaxhantin	*Bougainvillea glabra* flowers	Distilled water 100% 0.058 g/mL	ET: 37 min; UPA: 88 W; T: 55ºC	[15]
Natural yellow pigment	*Physalis pubescens L.*	EtOH 75% 0.083 g/mL	ET: 14 min; UPA: 180 W; T: ND ultrasonic interval time of 10.55 s	[22]
Polysaccharides				
B-d-glucan	*Ganoderma lucidum*	Distilled Water 0.00004 g of fibre/mL	ET:58 min; UPA: 590 W; T: 81ºC	[16]
Polysaccharides	*Oryza sativa L.*	Distilled water 0.05 g/mL	ET: 20 min; UPA: 150 W; T: 70ºC	[17]
Perilla seed meal polysaccharides (PSMP)	*perilla seed meal*	Distilled water 0.038 g/mL	ET:52 min; UPA: 229 W; T: 43ºC	[18]
Aminoacids				
Amino acids	Grapes	Distilled water 0.1 g/mL to 0.05 g/mL not significant differences	ET:6 min; UPA: 140 W; T: 70ºC	[20]
Aminoacids	*Apocynum venetum*	Distilled water 0.00047 g/mL	ET:32 min; UPA:187 W; T: NI	[19]

Definitions:, UPA: ultrasonic power amplitude. T: temperature; ET: extraction time; NI: no included

4. Discussion and Conclusions

From these results, we can conclude that UAE is a useful method for obtaining different compounds from plant matrices [23]. Regarding solvent extraction, most studies use distilled water for the four group compounds, the second most solvent used is ethanol/water mixtures. In both cases solvents are considered environmentally friendly solvents [25]. Another relevant fact is that the extraction time is small comprised from 60 min to 7 min, this makes the UAE a fast method. Regarding temperatures, the maximum temperatures used are around 80 ºC, however the most common are those around 50 ºC. A fact that plays an important role is the nature of the compounds to be extracted, since an excessive increase in temperature is also undesirable because it produces unnecessary modifications in the physical-chemical properties of the solvent such as vapor pressure, which can modify the transmission of the ultrasounds. In addition to this, it is essential to pay attention to the nature of the ultrasound equipment used, since in addition to the power, the frequency used, the intensity and the design of the reactor , as well as the volume of solvent used have an influence on the extraction [1]. However, in those cases in which a correct design and a correct optimization of the variables and equipment have been carried out, it can be concluded that UAE is an adequate method for obtaining compounds such as: polyphenols, pigments, amino acids and polysaccharides, among other compounds, from different plant matrices, obtaining good extraction yields, comparable to those obtained by so-called traditional methods [26]. In addition to all this, UAE is a multipurpose method that lends itself to be combined with other extraction methods, both conventional and novel [27]. Finally, studies point out the importance of using mathematic models like response surface matrix (RSM) for the optimization of extraction performance before performing large-scale extractions, since it allows the proper management of resources and avoids the loss of matrices [24].

Author Contributions: Conceptualization, A.C-C., A.G.P., M.C., P.O.; F.C., A S-L.; methodology, P.O, P.G.-P; validation, P.O, formal analysis, P.O, P.G-P writing—original draft preparation, A.C.C, P.O, M.C; writing—review and editing, A.C.C, P.O, M.A.P.; visualization, M.C, A.G.P.; supervision, P.O, H.C., J.X., J.S-G., M.A.P.; project administration, M.A.P, J.S-G. All authors have read and agreed to the published version of the manuscript.

Funding: The JU receives support from the European Union's Horizon 2020 research and innovation program and the Bio Based Industries Consortium. The project SYSTEMIC Knowledge hub on Nutrition and Food Security, has received funding from national research funding parties in Belgium (FWO), France (INRA), Germany (BLE), Italy (MIPAAF), Latvia (IZM), Norway (RCN), Portugal (FCT), and Spain (AEI) in a joint action of JPI HDHL, JPI-OCEANS and FACCE-JPI launched in 2019 under the ERA-NET ERA-HDHL (n° 696295).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data sharing not applicable.

Acknowledgments: The research leading to these results was supported by MICINN supporting the Ramón y Cajal grant for M.A. Prieto (RYC-2017-22891) and the FPU grant for A. Carreira-Casais (FPU2016/06135); by Xunta de Galicia for supporting the predoctoral grant of A.G. Pereira (ED481A-2019/0228); by the program BENEFICIOS DO CONSUMO DAS ESPECIES TINTORERA-(CO-0019-2021) that supports the work of F. Chamorro ; by University of Vigo for supporting the predoctoral grant of M. Carpena (Uvigo-00VI 131H 6410211) and by Becas de Fundación ONCE Programme "Oportunidad al Talento" that supports the work of A. Soria-Lopez. Authors are grateful to Ibero-American Program on Science and Technology (CYTED—AQUA-CIBUS, P317RT0003), to the Bio Based Industries Joint Undertaking (JU) under grant agreement No 888003 UP4HEALTH Project (H2020-BBI-JTI-2019) that supports the work of P. Otero and P. Garcia-Perez.

Conflicts of Interest: "The authors declare no conflict of interest".

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