chemistry proceedings

MDPI

Proceeding Paper

Critical Variables Influencing the Ultrasound-Assisted Extraction of Bioactive Compounds—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.)

[2] Centro de Investigaçao de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolonia 10, 5300-253 Bragança, Portugal

* 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, 1–15 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, such as short extraction time and low solvent consumption. The aim of this review is to critically present the different variables and parameters that can be modified in UAE, such as ultrasound power, time, temperature, solvent, and solid to solvent ratio that influence yield and extraction performance.

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

Citation: Carreira-Casais, A.; Carpena, M.; Pereira, A.G.; Chamorro, F.; Soria-Lopez, A.; Perez, P.G.; Otero, P.; Cao, H.; Xiao, J.; Simal-Gandara, J.; et al. Critical Variables Influencing the Ultrasound-Assisted Extraction of Bioactive Compounds—A Review. *Chem. Proc.* **2021**, *5*, 50. https://doi.org/10.3390/CSAC2021-10562

Academic Editor: Huangxian Ju

Published: 1 July 2021

1. Introduction

Ultrasound-assisted extraction (UAE) is a technique that belongs to the group of novel extraction methods, together with microwave assisted extraction (MAE), enzyme assisted extraction (EAE) or high-pressure assisted extraction (HPAE) [1,2]. UAE promotes the extraction of compounds of interest, lowering the consumption of resources, such as solvent and energy, whereas achieving remarkably higher extraction yields [3,4]. In addition, UAE is a multipurpose method that lends itself to be combined with other extraction methods, both conventional and novel [5]. UAE has been applied to obtain extracts rich in bioactive compounds, such as phenolic compounds, pigments, polysaccharides, and amino acids, among others from plant matrices [1,3,6,7].

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, ultrasound power and extraction time are closely linked to a fifth important factor, which is temperature. In practical terms, a correct optimization of these variables is essential to obtain a correct performance, resulting in a maximal extraction yield. In addition, temperature can affect the integrity of the bioactive compounds, since most of them are thermolabile. Considering that high ultrasound power linked to long extraction periods may lead to sample damage, 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, response surface methodology (RSM) can be a representative tool to generate meta-models. RSM allows to analyze and optimize several variables at the same time and minimize the loss of matrices [8,9].

2. Variables Influencing Ultrasound-Assisted Extraction

To obtain good extraction yields, an optimization of the variables that influence the process is required. Among the variables that affect UAE, there are three types of parameters, as follows: physical, medium-dependent, and matrix-dependent parameters. Regarding the first, physical parameters are related to the ultrasonic waves applied during UAE and the equipment used. In this sense, those attributed to ultrasonic waves are ultrasound power, frequency, and ultrasound intensity (UI), whereas those related with ultrasound equipment are extraction time, and shape and size of the ultrasonic reactor. Medium-dependent parameters are related with the space in which ultrasound waves are transmitted from the emitting source to the matrix. Solvent properties, temperature and the presence of gases are examples of medium-dependent parameters. Finally, matrix-dependent parameters are those that have a significant influence in the extraction of target compounds and considerably affect the effectiveness of the extraction. Type of matrix, structure, pre-treatment, particle size, or solid–liquid ratio are examples of those parameters [3,10]. Therefore, a correct design of the process, optimization of the variables, and appropriate equipment is needed to obtain extraction yields comparable to those obtained by the so-called traditional methods [11].

Regarding the ultrasound power, the use of high values usually improves extraction yields due to the generation of strong shear forces, so it is considered as one of the critical parameters to be optimized. Furthermore, higher ultrasound power reduces the time of extraction. For example, a study showed good results of the extraction of β-d glucans at a high extraction power (590 W), in only 58 min [12]. However, the use of high ultrasound power without control can overheat the reactor producing degradation of labile compounds and solvent evaporation [13]. In addition, the higher ultrasound power, the higher UI, so that when UI reaches the maximum value can produce liquid agitation and the consequent loss of ultrasound wave and the reduction of cavitation efficiency [14].

Regarding the extraction time, UAE allows to obtain good extraction yields with relatively short processing times (maximum 60 min) since longer times may cause undesirable changes in the extracted compounds. In this sense, optimized time commonly ranges between 20–60 min, minimizing the energy consumption and reducing the compounds' exposure to the process [15]. For example, one study shows that 7.25 min are enough to extract pigments from annatto seeds [16], while 37 min are needed to extract betacyanin and betaxanthin in bougainvillea flowers [17]. In the case of amino acid extraction, shorter extraction time was needed (6 min) [18,19], whereas other authors were able to extract polysaccharides from purple glutinous rice bran (*Oryza sativa*) with an extraction time of 20 min at 70 °C [20] (Table 1).

The type or polarity of the solvent used is closely linked to the nature of the compounds to be extracted. In addition, due to current concern for the environment, eco-friendly solvents are preferred. Predominantly, an aqueous medium is generally chosen for the extraction of polar compounds used in food matrices, while in the case of other organic compounds, ethanol, and methanol are usually employed. However, despite the use of methanol tends to obtain better extraction yields, ethanol is preferably chosen because of its lower toxicity [10]. For example, distilled water and ethanol are usually used for pigments extraction, while water is the most common solvent for the extraction of polysaccharides and amino acids [12,20,21] (Table 1). Furthermore, 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 [22]. In addition to the suitable solubility of the compounds of interest, it is also important to consider the vapor pressure, the surface tension, and the viscosity of the solvent, since those may affect cavitation and the extraction yield [3]. The

solid-to-solvent ratio used for each compound does not follow a certain pattern. It depends especially on the type of solvent and the matrix used. Table 1 shows that for the extraction of phenolic compounds, the most used solvent is ethanol and the solid-to-solvent ratio varies between 0.025 g/mL [23] and 0.1 g/mL [24], whereas ratios vary between from 0.058 g/mL for the extraction of betacyanin and betaxanthin in bougainvillea flowers [17] to 0.14 g/mL for the extraction of a natural pigment from annatto seeds [16].

Finally, high extraction temperatures not only affect the extraction yield but could also have negative effects due to the possible degradation of thermolabile compounds [14]. For this reason, the cooling system must allow the extraction of compounds avoiding the overheating of the medium by controlling the temperature of the system. For example, it is possible to extract phenolic compounds with temperatures up to 75 °C, β-d glucans at 81 °C or amino acids at 70 °C with optimal yields [12,18,25]. The increase in the temperature caused by the ultrasound probe itself is fundamentally produced when high ultrasound power is applied. The temperature increase produces a decrease in both viscosity and surface tension and induces an increase in the vapor pressure. Thus, too high temperatures can be harmful for the propagation of ultrasounds through the medium [13]. For these reasons, the optimization of the extraction temperature must be focused on both protecting the structure and function of the target components and improving the extractive properties of the solvent. Generally, the temperature does not exceed 80 °C, and it commonly works around 50 °C (Table 1).

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.
TFC	Blueberry pomace	EtOH/water 50%/50%; 0.05 g/mL	ET: 60 min; UPA: 64 W; T: 40 °C	[24]
TFC	*Zea mays* waste	EtOH/water 70%/30% 0.09 g/mL	ET: 40 min; UPA: 50 W; T: ND	[23]
Stilbenes	Grape canes	EtOH/water 60%/40% 0.025 g/mL	ET: 10 min; UPA: 200 W; T: 75 °C	[25]
Anthocyanins	Grape skins	EtOH 60% acidified pH = 3; 0.033 g/mL	ET: 28 min; UPA: 400 W; T: 50 °C	[26]
Natural pigment	Annatto seeds	Dw, previously treated with chloroform/ratio solid solvent 0.14 g/mL	ET: 7.25 min; UPA: 200 W; T: 72.7 °C. (Duty cycle of 0.8 s)	[16]
Betacyanin and betaxanthin	*Bougainvillea glabra* flowers	Distilled water 100% 0.058 g/mL	ET: 37 min; UPA: 88 W; T: 55 °C	[17]
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	[27]
B-d-glucan	*Ganoderma lucidum*	Distilled water 0.00004 g of fiber/mL	ET: 58 min; UPA: 590 W; T: 81 °C	[12]
Polysaccharides	*Oryza sativa* L.	Distilled water 0.05 g/mL	ET: 20 min; UPA: 150 W; T: 70 °C	[20]
PSMP	Perilla seed meal	Distilled water 0.038 g/mL	ET: 52 min; UPA: 229 W; T: 43 °C	[21]
Amino acids	Grapes	Distilled water 0.1 g/mL to 0.05 g/mL not significant differences	ET: 6 min; UPA: 140 W; T: 70 °C	[19]
Amino acids	*Apocynum venetum*	Distilled water 0.00047 g/mL	ET: 32 min; UPA: 187 W; T: NI	[18]

Abbreviations: TFC: total flavonoid content. UPA: ultrasonic power amplitude. T: temperature; ET: extraction time; PSMP: perilla seed meal polysaccharides; NI: not included; Dw: dry weight.

3. Conclusions

UAE is a useful method for obtaining different compounds of interest from plant matrices, since remarkably higher extraction yields are obtained with short extraction times. This extraction method belongs to "Green Chemistry" because it allows to decrease the consumption of resources, such as solvent and energy. However, it is still necessary to optimize the more relevant variables that influence the effectiveness of UAE, such as ultrasound power, extraction time and temperature, type of solvent, and solid-to-solvent ratio. Different studies have shown extraction yields of different bioactive compounds, such as phenolic compounds, polysaccharides, pigments, and amino acids, using UAE with short extraction times (maximum 60 min), medium ultrasound power (between 200–500 W), temperatures around 50 °C (maximum 80 °C), and environmentally friendly solvents (distilled water and ethanol). Therefore, UAE can be appropriately applied to obtain bioactive compounds through an efficient and eco-friendly process, considering and optimizing the different critical variables that affect the process.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/CSAC2021-10562/s1.

Author Contributions: Conceptualization, A.C.-C., A.G.P., M.C., P.O.; F.C., and A.S.-L.; methodology, P.O., and P.G.P.; validation, P.O.; formal analysis, P.O., and P.G.P.; writing—original draft preparation, A.C.-C., P.O., and M.C.; writing—review and editing, A.C.-C., A.S.-L., M.C., P.O., and M.A.P.; visualization, M.C., and A.G.P.; supervision, P.O., H.C., J.X., J.S.-G., and M.A.P.; project administration, M.A.P., and 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 (1) by MICINN supporting the Ramón y Cajal grant for M.A. Prieto (RYC-2017-22891) and the FPU grants for A. Carreira-Casais (FPU2016/06135) and A. Soria-Lopez (FPU2020/06140), (2) by Xunta de Galicia for supporting the predoctoral grants of A.G. Pereira (ED481A-2019/0228) and M. Carpena (ED481A 2021/313), and (3) by the program BENEFICIOS DO CONSUMO DAS ESPECIES TINTORERA-(CO-0019-2021) that supports the work of F. Chamorro. The authors are grateful to the Ibero-American Program on Science and Technology (CYTED—AQUA-CIBUS, P317RT0003), and 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.

References

1. Vinatoru, M.; Mason, T.J.; Calinescu, I. Ultrasonically Assisted Extraction (UAE) and Microwave Assisted Extraction (MAE) of Functional Compounds from Plant Materials. *TrAC Trends Anal. Chem.* **2017**, *97*, 159–178. [CrossRef]
2. Dias, A.L.B.; Arroio Sergio, C.S.; Santos, P.; Barbero, G.F.; Rezende, C.A.; Martínez, J. Ultrasound-Assisted Extraction of Bioactive Compounds from Dedo de Moça Pepper (*Capsicum Baccatum* L.): Effects on the Vegetable Matrix and Mathematical Modeling. *J. Food Eng.* **2017**, *198*, 36–44. [CrossRef]
3. Chemat, F.; Rombaut, N.; Sicaire, A.G.; Meullemiestre, A.; Fabiano-Tixier, A.S.; Abert-Vian, M. Ultrasound Assisted Extraction of Food and Natural Products. Mechanisms, Techniques, Combinations, Protocols and Applications. A Review. *Ultrason. Sonochem.* **2017**, *34*, 540–560. [CrossRef] [PubMed]
4. Chemat, F.; Zill-E-Huma; Khan, F.M. Applications of Ultrasound in Food Technology: Processing, Preservation and Extraction. *Ultrason. Sonochem.* **2011**, *18*, 813–835. [CrossRef]

5. Luque-García, J.L.; Luque De Castro, M.D. Ultrasound-Assisted Soxhlet Extraction: An Expeditive Approach for Solid Sample Treatment—Application to the Extraction of Total Fat from Oleaginous Seeds. *J. Chromatogr. A* **2004**, *1034*, 237–242. [CrossRef]
6. Saleh, I.A.; Vinatoru, M.; Mason, T.J.; Abdel-Azim, N.S.; Aboutabl, E.A.; Hammouda, F.M. A Possible General Mechanism for Ultrasound-Assisted Extraction (UAE) Suggested from the Results of UAE of Chlorogenic Acid from *Cynara scolymus* L. (Artichoke) Leaves. *Ultrason. Sonochem.* **2016**, *31*, 330–336. [CrossRef] [PubMed]
7. Wen, C.; Zhang, J.; Zhang, H.; Dzah, C.S.; Zandile, M.; Duan, Y.; Ma, H.; Luo, X. Advances in Ultrasound Assisted Extraction of Bioactive Compounds from Cash Crops—A Review. *Ultrason. Sonochem.* **2018**, *48*, 538–549. [CrossRef]
8. Chakraborty, S.; Uppaluri, R.; Das, C. Optimization of Ultrasound-Assisted Extraction (UAE) Process for the Recovery of Bioactive Compounds from Bitter Gourd Using Response Surface Methodology (RSM). *Food Bioprod. Process.* **2020**, *120*, 114–122. [CrossRef]
9. Jang, S.; Lee, A.Y.; Lee, A.R.; Choi, G.; Kim, H.K. Optimization of Ultrasound-Assisted Extraction of Glycyrrhizic Acid from Licorice Using Response Surface Methodology. *Integr. Med. Res.* **2017**, *6*, 388–394. [CrossRef]
10. Predescu, N.C.; Papuc, C.; Nicorescu, V.; Gajaila, I.; Goran, G.V.; Petcu, C.D.; Stefan, G. The Influence of Solid-to-Solvent Ratio and Extraction Method on Total Phenolic Content, Flavonoid Content and Antioxidant Properties of Some Ethanolic Plant Extracts. *Rev. Chim.* **2016**, *67*, 1922–1927.
11. Ghassempour, A.; Heydari, R.; Talebpour, Z.; Fakhari, A.R.; Rassouli, A.; Davies, N.; Aboul-Enein, H.Y. Study of New Extraction Methods for Separation of Anthocyanins from Red Grape Skins: Analysis by HPLC and LC-MS/MS. *J. Liq. Chromatogr. Relat. Technol.* **2008**, *31*, 2686–2703. [CrossRef]
12. Alzorqi, I.; Singh, A.; Manickam, S.; Al-Qrimli, H.F. Optimization of Ultrasound Assisted Extraction (UAE) of β- d -Glucan Polysaccharides from Ganoderma Lucidum for Prospective Scale-Up. *Resour. Technol.* **2017**, *3*, 46–54. [CrossRef]
13. Chuang, S.S.; Tamis-LeMonda, C.S. *Sonocrystallization of Fats*; Springer: New York, NY, USA, 2013; pp. 1–66. [CrossRef]
14. Carreira-Casais, A.; Otero, P.; Garcia-Perez, P.; Garcia-Oliveira, P.; Pereira, A.G.; Carpena, M.; Soria-Lopez, A.; Simal-Gandara, J.; Prieto, M.A. Benefits and Drawbacks of Ultrasound-Assisted Extraction for the Recovery of Bioactive Compounds from Marine Algae. *Int. J. Environ. Res. Public Health* **2021**, *18*, 9153. [CrossRef] [PubMed]
15. Aguilar-Hernández, G.; De Los Ángeles Vivar-Vera, M.; De Lourdes García-Magaña, M.; González-Silva, N.; Pérez-Larios, A.; Montalvo-González, E. Ultrasound-Assisted Extraction of Total Acetogenins from the Soursop Fruit by Response Surface Methodology. *Molecules* **2020**, *25*, 1139. [CrossRef]
16. Yolmeh, M.; Habibi Najafi, M.B.; Farhoosh, R. Optimisation of Ultrasound-Assisted Extraction of Natural Pigment from Annatto Seeds by Response Surface Methodology (RSM). *Food Chem.* **2014**, *155*, 319–324. [CrossRef] [PubMed]
17. Maran, J.P.; Priya, B.; Nivetha, C.V. Optimization of Ultrasound-Assisted Extraction of Natural Pigments from Bougainvillea Glabra Flowers. *Ind. Crops Prod.* **2015**, *63*, 182–189. [CrossRef]
18. Jin, Y.; Wang, C.Y.; Hu, W.; Huang, Y.; Xu, M.L.; Wang, H.; Kong, X.; Chen, Y.; Dong, T.T.; Qin, Q.; et al. An Optimization of Ultra-Sonication-Assisted Extraction from Flowers of Apocynum Venetum in Targeting to Amount of Free Amino Acids Determined by UPLC-MS/MS. *Food Qual. Saf.* **2019**, *3*, 52–60. [CrossRef]
19. Carrera, C.; Ruiz-Rodríguez, A.; Palma, M.; Barroso, C.G. Ultrasound-Assisted Extraction of Amino Acids from Grapes. *Ultrason. Sonochem.* **2015**, *22*, 499–505. [CrossRef]
20. Surin, S.; You, S.G.; Seesuriyachan, P.; Muangrat, R.; Wangtueai, S.; Jambrak, A.R.; Phongthai, S.; Jantanasakulwong, K.; Chaiyaso, T.; Phimolsiripol, Y. Optimization of Ultrasonic-Assisted Extraction of Polysaccharides from Purple Glutinous Rice Bran (*Oryza sativa* L.) and Their Antioxidant Activities. *Sci. Rep.* **2020**, *10*, 10410. [CrossRef]
21. Zhang, H.; Li, H.; Zhang, Z.; Hou, T. Optimization of Ultrasound-Assisted Extraction of Polysaccharides from Perilla Seed Meal by Response Surface Methodology: Characterization and in Vitro Antioxidant Activities. *J. Food Sci.* **2021**, *86*, 306–318. [CrossRef]
22. Ministerio de Sanidad BOE. Real Decreto 1101/2011, de 22 de Julio, Por El Que Se Aprueba La Lista Positiva de Los Disolventes de Extracción Que Se Pueden Utilizar En La Fabricación de Productos Alimenticios y de Sus Ingredientes. Real Decreto 1101/2011. 2011. Available online: https://www.boe.es/eli/es/rd/2011/07/22/1101 (accessed on 10 October 2021).
23. Aourabi, S.; Sfaira, M.; Mahjoubi, F. Optimization of Ultrasound-Assisted Extraction of Polyphenol Content from *Zea mays* Hairs (Waste). *Sci. World J.* **2020**, *2020*, 1–10. [CrossRef] [PubMed]
24. Bamba, B.S.B.; Shi, J.; Tranchant, C.C.; Xue, S.J.; Forney, C.F.; Lim, L.T. Influence of Extraction Conditions on Ultrasound-Assisted Recovery of Bioactive Phenolics from Blueberry Pomace and Their Antioxidant Activity. *Molecules* **2018**, *23*, 1685. [CrossRef] [PubMed]
25. Piñeiro, Z.; Marrufo-Curtido, A.; Serrano, M.J.; Palma, M. Ultrasound-Assisted Extraction of Stilbenes from Grape Canes. *Molecules* **2016**, *21*, 784. [CrossRef] [PubMed]
26. Tan, J.; Li, Q.; Xue, H.; Tang, J. Ultrasound-Assisted Enzymatic Extraction of Anthocyanins from Grape Skins: Optimization, Identification, and Antitumor Activity. *J. Food Sci.* **2020**, *85*, 3731–3744. [CrossRef] [PubMed]
27. Wang, H.; Shi, L.; Yang, X.; Hong, R.; Li, L. Ultrasonic-Assisted Extraction of Natural Yellow Pigment from *Physalis pubescens* L. and Its Antioxidant Activities. *J. Chem.* **2018**, *2018*, 1–6. [CrossRef]