

## Enzymatic aqueous extraction with ultrasound: a prospective overview on the state of the art

Extração aquosa enzimática com ultrassom: uma abordagem prospectiva sobre o estado da arte

Extracción enzimática acuosa con ultrasonido: una aproximación prospectiva al estado del arte

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### Abstract

The oil extracted from different oleaginous raw materials is usually obtained through mechanical pressing, associated or not with organic solvents. The objective of this prospectation was to evaluate if the aqueous enzymatic extraction (AEE) with ultrasound should be a cleaner and more effective technology for the production of lipids and other products. The state of the art of this issue was observed with a focus on food, and more precisely oleaginous fruits and seeds. The advanced search in the Science Direct, Scopus and Web of Science (WOS) databases was carried out in July 2021. A total of 1,099, 79, and 46 scientific papers were retrieved within the Science Direct, WOS and Scopus databases, respectively. Identifying the experiments made in these studies with EAE and ultrasound, there was a selection of 24, 13 and 4 original articles in the investigated bases. The articles described that ultrasound used as pre-treatment or during AEE increased the yield of food lipid extraction. This prospectation described that EAE technology and ultrasound are impactful techniques in obtaining lipids. The advanced research highlighted that there is a gap related to researches with oleaginous matrices and the replacement of conventional techniques with clean techniques can be economically feasible.

**Keywords:** Biocatalysis; Vegetable oil; Nuts and nut products; Green extraction.

### Resumo

O óleo extraído de diversas matérias-primas oleaginosas é comumente obtido através da prensagem mecânica, associada ou não a solventes orgânicos. O objetivo desta prospecção foi avaliar se a extração aquosa enzimática (EAE) com ultrassom pode ser uma tecnologia mais limpa e eficaz para a obtenção de lipídios e outros produtos. Observou-se o estado da arte sobre este assunto com foco em alimentos, e mais precisamente frutos e sementes oleaginosos. A pesquisa avançada nas bases de dados Science Direct, Scopus e Web of Science (WOS) foi realizada em julho de 2021. Houve a recuperação de um total de 1099, 79 e 46 trabalhos científicos dentro das bases Science Direct, WOS e Scopus, respectivamente. Identificando os experimentos feitos nesses estudos com EAE e ultrassom, houve a seleção de 24, 13 e 4 artigos originais nas bases averiguadas. Os artigos descreviam que o ultrassom utilizado como pré-tratamento, ou durante a EAE incrementou o rendimento da extração de lipídios alimentícios. Esta prospecção descreveu que a tecnologia EAE e o ultrassom são técnicas impactantes na obtenção de lipídios. A pesquisa avançada ressaltou que há uma lacuna relacionada à pesquisa com oleaginosas e que a substituição de técnicas convencionais por técnicas limpas pode ser viável economicamente.

**Palavras-chave:** Biotálise; Óleo vegetal; Nozes e derivados; Extração verde.

### Resumen

El aceite extraído de diferentes materias primas se obtiene comúnmente mediante prensado mecánico, asociado o no a disolventes orgánicos. El objetivo de esta prospección fue demostrar que la extracción enzimática acuosa (EEA) con

ultrasonido puede ser una tecnología más limpia y eficaz, en la obtención de lípidos y otros productos. Se observó el estado del arte de esta temática con foco en alimentos, y más precisamente frutos y semillas oleaginosas. La búsqueda avanzada en las bases de datos Science Direct, Scopus y Web of Science (WOS) se realizó en julio de 2021. Se recuperaron un total de 1099, 79 y 46 artículos científicos dentro de las bases de datos Science Direct, WOS y Scopus, respectivamente. Identificando los experimentos realizados en estos estudios con EEA y ultrasonido, se realizó una selección de 24, 13 y 4 artículos originales en las bases investigadas. Los artículos describieron que el ultrasonido utilizado como pretratamiento o durante la EEA aumentó el rendimiento de la extracción de lípidos alimentarios. Esta prospección describió que la tecnología EEA y el ultrasonido son técnicas impactantes en la obtención de lípidos. La investigación avanzada destacó que existe una brecha relacionada con la investigación con oleaginosas y que el reemplazo de técnicas convencionales por técnicas limpias puede ser económicamente viable.

**Palabras clave:** Biocatálisis; Aceite vegetal; Nueces y derivados; Extracción verde.

## 1. Introduction

Brazil stands out in terms of the world agricultural scenario. It has a huge cultivated area, remaining one of the largest countries in the production and exportation of its agricultural products, both as a supplier of raw materials and as an exporter of industrialized products (IBGE, 2021).

Tree nuts are raw materials that can be easily found worldwide. Almonds, Brazil nut, cashew nuts, macadamia nuts, pecan nuts and pistachios are the most researched tree nuts, due to the combination of nutrients they present (Demoliner, et al., 2018).

In most food industries, nuts kernels, when not exported, are used in food formulation or destined for oil extraction. The oil from this raw material is obtained, industrially, through mechanical cold pressing (Yang, 2009).

Currently, there is a growing interest in alternative methods of oil extraction, focused on increasing the oil yield and, simultaneously, ensuring the quality of the by-products generated in the process. If possible, these approaches should reduce environmental impacts. These factors may become important differentials in the industry, especially in times of global economic crisis, such as the one faced recently, due to the pandemic caused by the new coronavirus.

In this context, enzymes, natural catalysts, can be protagonists to increase the efficiency of vegetable oil extraction, guarantee the quality of both lipids and by-products obtained, and reduce the environmental impact of the process (Polmann, et al., 2019; 2021; Zhang, et al., 2011). This extraction involves the application of an aqueous system with a combination of hydrolases, mainly proteases and carbohydrases (Rosenthal, et al., 1996; Rovaris, et al., 2012; 2013), and occurs under mild reaction conditions (Sharma & Gupta, 2006; Yusoff, et al., 2016).

In a recent study, the enzyme-assisted extraction of pecan oil was found to lead an 8% increment in the oil yield, when compared to pressing, which could allow an important short-to-medium payback to the processing plant. In addition, the oil obtained had a quality comparable to that obtained by mechanical cold pressing (Polmann, et al., 2019).

The use of ultrasound for the pre-treatment of the raw material and its subsequent enzymatic hydrolysis, may result in improvements in the process yield of oil extraction and allow to obtain high-quality side fractions (aqueous and solid ones). The cavitation generated by the ultrasound allows changes in the matrix microstructure, exposing components originally compacted in it, thus facilitating the later access of the biocatalyst to the substrate in an aqueous medium (Li, et al., 2011; Sharma & Gupta, 2006). The use of ultrasound associated with enzymatic extraction happens in a similar way. During the extraction process, ultrasonic waves pass through the liquid medium, creating cavitation bubbles that collapse, increasing temperature and pressure in a microscopic level. In this way, the cellular structure of the raw material changes, increasing the release of substances in the solvent (Show, et al., 2007). This fact, associated with the use of enzymes, will favor the release of substances of interest, such as oil, for example (Goula, et al., 2018). Knowledge about existing studies in the literature, related to the use of enzymes and ultrasound for extracting oil from matrices such as pecan nut (Polmann, et al., 2019) or peanut (Jiang, et al., 2010), should guide future investigations on this topic and be applied to tree nuts of local interest.

Thus, the objective of this study was to carry out a scientific prospection, aiming to identify state of the art on the use of AEE with ultrasound for the extraction of oil of different food matrices, relating the data obtained and evaluating the possibility of applying enzymes as biocatalysts as an innovative and alternative technology for oil extraction. This study focused on oils from vegetable origin, mainly oilseed fruits and seeds.

## 2. Methodology

Data collection was made in July 2021, using the scientific databases Science Direct, Scopus and Web of Science. For this purpose, an advanced search was performed in each database for articles published between 2014 and July 2021, using suitable keywords for this study (Table 1). After retrieving the data, there was a selection of original articles using the following criteria: publications on the extraction of oil or ingredients from food matrices, with the use of ultrasound as a pre-treatment of the raw material or a simultaneous process for the AEE. The data that met these requirements were collected and exported to Microsoft Excel and Mendeley programs for data processing.

## 3. Results and Discussion

The results of the search are described in Table 1. The Science Direct database provided the most results of research articles with any truncations between the keywords used. The combined keywords with the most results for this database were “enzyme AND extraction”, however, this event was not observed in the other databases, which provided more results when the “ultrasound AND extraction” keywords were applied. This may be related to the journals that these three databases include, and Science Direct had more articles focused on enzymatic extraction than the other two databases.

Among the publications retrieved, it was possible to find a wide variety of areas where the ultrasound technology has been used (sciences of agriculture, environment, biology, food, chemistry, physics, engineering, and medicine). To achieve the objectives of this study, the results were refined (Table 2) showing that the investigations in which the ultrasound was applied, both as a pre-treatment and simultaneously with the enzymatic extraction, retrieved a total of 42 original articles, adding up all papers refined through the databases results. Comparing the Scopus and WOS databases with the Science Direct database results, it was possible to find 5 and 15 duplicate articles, respectively. Something opposite occurred when Scopus was compared with Science Direct. The Scopus database had 14 original articles and few duplicate articles.

**Table 1.** Keywords used and number of articles retrieved in the search for scientific articles on the aqueous enzymatic extraction, including the use of ultrasound, in the Science direct, Scopus and Web of Science databases.

Keywords	Science Direct	Scopus	Web of Science
Enzymatic AND ultrassound	7,923	917	975
Enzymatic AND extraction	65,224	4,582	4,254
Enzymatic AND oil	32,946	4,678	3,507
Ultrasound AND extraction	21,613	7,318	8,158
Ultrasound AND oil	12,690	3,259	2,782
"Ultrasound pre-treatment" AND oil	69	13	36

Source: Authors.

**Table 2.** Refined results referring to the scientific articles on aqueous enzymatic extraction using ultrasound, according to the selection criteria applied.

Articles and databases	Science Direct	Scopus	Web of Science	Total
Total	24	19	24	62
Repeated	0	5	15	20
Originals	24	14	4	42

Source: Authors.

To better assess the scientific quality of the papers, there was a definition of the impact that each journal has according to the Brazilian QUALIS CAPES platform (Table 3). Among the articles found, 40% (17) were published in journals qualified as A1, that is, with greater impact. The group of publications in journals classified as A contains more than 50% of the total results. Among the journals, the ones with the most publications related to ultrasound within the food science area, in descending order, were: Ultrasonics Sonochemistry (6 publications), Journal of Chromatography A (4), Food Chemistry (3), Industrial Crops & Products (3), Food Processing Engineering (2) and Molecules (2).

**Table 3.** Classification of the journals in the food science area in which the original articles on aqueous enzymatic extraction using ultrasound were published, according to the Qualis CAPES criteria (2013-2016)\*.

Classification according to Qualis CAPES*	Amount of articles
A1	17
A2	4
A3	1
B1	4
B2	1
B3	2
B4	2
B5	1
C	4
NB	6
Total	42

*NB: It does not belong to the food science field. \* Qualis CAPES – Brazilian journal evaluation system kept by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.* Source: Authors.

The journals considered to be in the B group contained 23% (10) of the refined results and the C journals, with the lowest impact on the QUALIS platform, had a share of 9.5%. Some journals were not part of the food science area and these, did not have a defined QUALIS. These results demonstrated that the use of ultrasound in the food science area generates publications with a high impact.

Among the selected articles, it was observed that 16 (38%) had the objective of extracting oil from the raw materials used. Among these publications, 87% used water as a solvent and 13% used *n*-hexane for the enzymatic extraction in

combination with ultrasound as a pre-treatment or assisted by this technology. Among the studies using water as solvent, it was also verified that 8 of them (57%) used a raw material that can be used for human consumption and 6 (43%) had dairy residues and medicinal plants as raw materials.

After a careful reading of the selected papers regarding the use of ultrasound, it was possible to observe that the power, time and frequency of pulses varied according to the type of raw material used, as well as the enzyme applied as a catalyst. The more relevant papers selected are shown in the Table 4. Other studies were not included in this table, as they were not directly related to the subject of interest, such as the use of ultrasound-assisted degumming of soybean oil with Phospholipase A (Lecitase Ultra) (More & Gogate, 2018) and Phospholipase C from *Bacillus cereus* (Jiang, et al., 2014).

**Table 4.** Enzymes used for oil extraction in ultrasound processes, reported as the optimal conditions, in the selected original articles.

Enzyme	Amount of articles	Authors and year	Provider	Enzyme origin
Cellulase	6	Hu, et al. (2020)	Shanghai Ruiyong Biological Technology Co., Ltd;	<i>Aspergillus niger</i>
		Amigh & Dinani (2020)	CN LAB Nutrition	Not described
		Goula, et al. (2018)	Sigma-Aldrich	<i>Trichoderma reesei</i>
		Han, et al. (2018)	Shanghai Huishi Biochemical Reagent	Not described
		Hu, et al. (2019)	Shanghai Yuanye Biological Technology	Not described
		Chen, et al. (2016)	Shanghai Yuanye Biological Technology	Not described
Pectinase	3	Hu, et al. (2020)	Shanghai Ruiyong Biological Technology Co., Ltd;	<i>Aspergillus niger</i>
		Hu, et al. (2019)	Shanghai Yuanye Biological Technology	Not described
		Chen, et al. (2016)	Shanghai Yuanye Biological Technology	Not described
Hemicellulase	3	Hu, et al. (2020)	Shanghai Ruiyong Biological Technology Co., Ltd;	<i>Aspergillus niger</i>
		Hu, et al. (2019)	Shanghai Yuanye Biological Technology	Not described
		Chen, et al. (2016)	Shanghai Yuanye Biological Technology	Not described
Peclyve V (Pectinase)	1	Goula, et al. (2018)	Lyven	Not described

Source: Authors.

Although the enzymes used to extract the oil vary according to the raw material used, it was observed that Cellulase@ alone or in consortium with another enzyme was applied in 66.7% of the experiments. This is consistent with the effect described by Ferreira (2016), who attributed the disruption of cellulose, present in the plant cell structure, caused by cellulase, for example, to greater efficiency in the oil extraction. Some reports from the selected original articles, where the ultrasound was used a pre-treatment or in combination with the enzymatic extraction, are shown in Table 5.

**Table 5.** Ultrasound and aqueous enzymatic extraction conditions reported in the selected original articles.

Authors and year	Sample	Experimental conditions	Result
Amigh & Dinani (2020)	Date seed ( <i>Phoenix dactylifera L. var Shahani</i> )	Baking pre-treatment in combination with AEE using Cellulase and ultrasound Ultrasound: Microwave ultrasonic synergistic extractor Power: 460W Frequency: 25 kHz Time: 30 min	Improved oil extraction
Hu, et al. (2020)	Roots of tiger nut ( <i>Cyperus esculentus L.</i> )	Ultrasound-assisted aqueous enzyme extraction with Cellulase, Pectinase and Hemicellulase Ultrasound: Microwave ultrasonic synergistic extractor Power: 460W Frequency: 25 kHz Time: 30min	Improved quality of extracted oil without using toxic or flammable solvents
Han, et al. (2018)	Corn germ ( <i>Zea mays</i> )	Ultrasound-assisted aqueous enzyme extraction Ultrasound: Ultrasound pretreatment Time: 20 min	It was observed that the use of ultrasound exerted a positive effect on the aqueous enzymatic extraction of the oil.
Hu, et al. (2019)	Seeds from <i>Prunus avium L.</i>	Ultrasound-assisted aqueous enzyme extraction Ultrasound: Ultrasonic cleaning bath Power: 130W Frequency: 20 kHz	The method used met the need for sustainable development, with faster extraction and without the use of organic solvents.
Goula, et al. (2018)	Pomegranate seeds ( <i>Prunus persica</i> )	Microwave ultrasound-assisted aqueous enzymatic extraction with Cellulase and Pectylve V Ultrasound: Ultrasonic cleaning bath Power: 130W Frequency: 20 kHz Ultrasound-assisted enzyme extraction (Phospholipase A)	It proved to be an economic and sustainable alternative, with an extraction yield equal to or higher than the conventional one.
Chen, et al. (2016)	<i>Pinus pumila</i>	Circulating ultrasound of homogenate in combination with aqueous enzyme extraction (Cellulase, pectinase and hemicellulase) Ultrasound: Microwave ultrasonic synergistic extractor Power: 600W Frequency: 20 kHz Time: 25.5 min	The process used provided an effective alternative method, extracting a high quality and environmentally friendly product for human consumption.

Source: Authors.

From Table 5, it is possible to observe several positive effects in the use of ultrasound in the aqueous enzymatic oil extraction. The use of this technology usually allows the recovery of a product with high quality. In general, the process with ultrasound application helps to reduce the extraction time and increases the oil yield. The absence of toxic organic solvents makes the process cleaner, contributing to its sustainability. Hu, et al. (2019) reported a lower oil yield in the ultrasound-assisted enzymatic aqueous extraction from seeds from *Prunus avium L.* Although these authors considered that the gains in less processing time and the absence of toxic solvents should justify the use of the ultrasound-assisted aqueous enzymatic extraction method, due to their greater efficiency and sustainability. Among the selected articles, it was observed that only

Han, et al. (2018) used ultrasound as a pre-treatment of a raw material intended for food use, demonstrating a knowledge gap in this field.

#### 4. Final Considerations

The results herein presented showed that the use of the aqueous enzymatic extraction with ultrasound greatly impacts the literature, which corroborates with one of the current needs in relation to sustainable development in the food processing chain. Enzymes can be advantageous for carrying out the oil extraction more efficiently and guaranteeing high quality products. A variety of hydrolases have been investigated in the literature and their combinations provided different results in the studies herein reported. The ultrasound was mainly used simultaneously with the application of enzymes. There are gaps in knowledge regarding the use of aqueous enzymatic extraction with ultrasound using oilseeds and nuts from the Brazilian biodiversity. Still, the use of ultrasound as a pre-treatment of raw materials is incipient, providing opportunities for studies in this area.

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