

Enzyme-Assisted Extraction (EAE) of Bioactive Compounds: A Greener and More Effective Approach

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Introduction

Enzyme-assisted extraction (EAE) is an efficient, environmentally friendly technique for recovering bioactive compounds from plant materials. It uses hydrolytic enzymes, such as cellulases, pectinases, hemicellulases, and proteases, to break down plant cell wall components and facilitate the release of intracellular compounds. This method enhances solvent penetration and diffusion, leading to improved extraction yields of compounds like polyphenols, carotenoids, flavonoids, and proteins

(Łubek-Nguyen *et al.*, 2022; Puri *et al.*, 2012). Compared to traditional techniques like solvent-based or heat-assisted extraction, EAE operates under milder conditions, requires less energy, and better preserves sensitive compounds, making it a more sustainable alternative (Štambuk *et al.*, 2016; Mattusch *et al.*, 2006).

Enzymatic Mechanisms and Target Compounds

The success of EAE depends heavily on selecting enzymes that are specific to the cell wall components of the plant matrix. Cellulases target cellulose, breaking it down into simpler sugars, and are particularly effective for extracting flavonoids. For example, cellulase derived from *Penicillium decumbence* improved the solubility and extraction of flavonoids from *Ginkgo biloba* leaves due to its transglycosylation activity (Chen *et al.*, 2011). Pectinases, which act on pectic substances in the middle lamella, have been used to enhance the recovery of phenolics from grape seeds (Štambuk *et al.*, 2016) and carotenoids from sunflower waste (Ricarte *et al.*, 2020). Hemicellulases

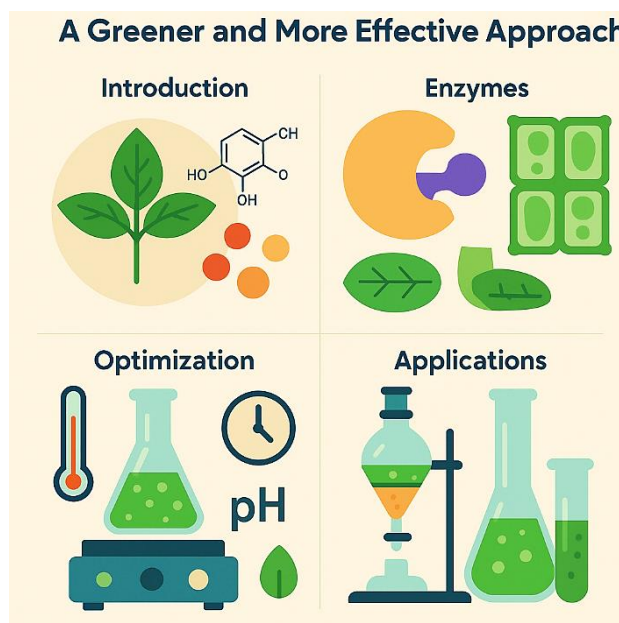


Figure 1: Enzyme-assisted extraction

complement cellulases by degrading hemicellulose-rich areas of plant walls, increasing the release of polyphenols, as seen in extractions from corn husks (Zuorro *et al.*, 2019). Proteases, on the other hand, catalyse the hydrolysis of protein matrices, improving the extraction of oils and soluble proteins. In soybean flake extraction, protease treatment increased oil recovery, with yields up to 15% higher compared to untreated samples (Wu *et al.*, 2009) whilst proteases were also effective for extracting soluble fibres and protein fractions from fruit by-products (Fuso *et al.*, 2022). Enzyme blends made up of combinations of cellulolytic, pectinolytic, and hemicellulolytic enzymes often achieve superior results compared to single-enzyme systems. One example is the 70% increase in shea butter yield when using a blend of cellulolytic, pectinolytic, and hemicellulolytic enzymes (Didia *et al.*, 2018). EAE has been successfully applied to various plant matrices, including grape skins (Tomaz *et al.*, 2016), guava peel (Amid *et al.*, 2016), and tomato waste (Zuorro *et al.*, 2011), for recovering high-value compounds.

Optimization Parameters in EAE

EAE is influenced by several key parameters that must be carefully optimized to achieve maximum yield and compound stability. One of the most critical factors is enzyme dosage, as the enzyme-to-substrate ratio significantly affects extraction performance. For instance, optimal doses of Lallzyme EX-V ranging from 10.52 to 20 mg/g were found to maximize flavonoid recovery from grape seeds and skins (Tomaz *et al.*, 2016; Štambuk *et al.*, 2016). The pH and temperature conditions also play a crucial role, as each enzyme exhibits peak activity within specific ranges. Pectinase showed maximum activity at pH 3.5–4 and 30–35°C for flavonoid extraction (Fu *et al.*, 2008), while cellulase performed best at pH 5.0 and 55°C for protein extraction from olive leaves (Vergara-Barberán *et al.*, 2015). Reaction time further influences outcomes, where extended incubation can enhance yields but may risk degrading sensitive compounds. For example, the optimized EAE of lycopene from tomato waste resulted in an 8–18 fold increase in recovery compared to untreated material (Zuorro *et al.*, 2011). Additionally, the use of enzyme combinations often yields better results than single-enzyme systems, as mixed enzymes can degrade various cell wall components simultaneously, as demonstrated in the phenolic extraction from rose

petals using both pectinase and cellulase (Kalcheva-Karadzhova *et al.*, 2014). To refine these variables, experimental design strategies such as response surface methodology (RSM) have been widely applied, enabling efficient process optimization and cost-effective production.

Combination with Ultrasound-Assisted Extraction (UAE)

Combining EAE with UAE has shown to significantly enhance the disruption of plant cell walls and improve enzyme-substrate interactions. This integrated approach, known as ultrasound-assisted enzymatic extraction (UAEE), leverages the cavitation effects generated by ultrasound waves to physically break down cellular structures, thereby increasing enzyme accessibility to target compounds. For instance, UAEE led to a notable increase in triterpenoid yields

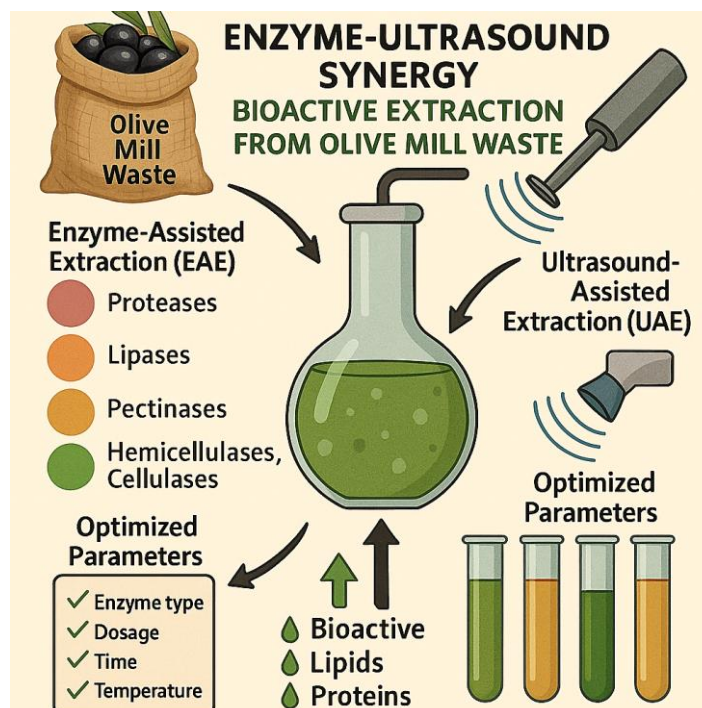


Figure 2: Enzyme-ultrasound synergy

from *Ganoderma lucidum* compared to conventional extraction methods (Dat *et al.*, 2022). UAE has also proven effective for enzyme recovery, as demonstrated in the extraction of pectinase from guava peel (Amid *et al.*, 2016) and β -carotene from orange processing waste (Shahram & Taghian Dinani, 2019). Under optimal conditions, such as 50% acoustic power and 10 minutes of sonication, UAE preserved enzyme activity while enhancing extraction efficiency (Amid *et al.*, 2016). However, excessive ultrasound exposure can compromise enzyme stability, necessitating careful regulation of sonication time and intensity (Fazlena *et al.*, 2013). When applied carefully, the combination of EAE and UAE offers synergistic benefits, resulting in higher yields and better preservation of bioactive compounds for nutraceutical and pharmaceutical use.

Applications and Benefits

EAE has demonstrated remarkable versatility and sustainability in the recovery of valuable bioactive compounds from a wide range of plant materials. Phenolic compounds extracted through EAE from winemaking by-products (de Camargo *et al.*, 2016) and rose petals (Kalcheva-Karadzhova *et al.*, 2014) have shown strong antioxidant activity, making them suitable for incorporation into functional foods. Similarly, the extraction of carotenoids from sunflower waste (Ricarte *et al.*, 2020) and flavonoids from corn husks (Zuorro *et al.*, 2019) has produced natural pigments applicable in both the food and cosmetic industries. Furthermore, EAE has enhanced the recovery of proteins from olive leaves (Vergara-Barberán *et al.*, 2015) and improved oil extraction from soybean flakes (Wu *et al.*, 2009), supporting the development of plant-based functional food products. Beyond its efficacy, EAE offers notable environmental benefits by reducing the reliance on chemical solvents, lowering energy consumption, and minimizing industrial waste, making it an attractive method for industries pursuing sustainable production practices.

Limitations of EAE

While enzyme-assisted extraction (EAE) offers numerous advantages, several limitations need to be addressed to enhance its industrial viability. One of the primary concerns is the stability and specificity of enzymes under varying extraction conditions. Enzymes such as cellulase and pectinase may denature when exposed to extreme pH, temperature, or ionic strength, resulting in reduced catalytic efficiency (Sowbhagya & Chitra, 2010). Moreover, regulatory requirements in food and pharmaceutical industries impose strict safety and quality standards, necessitating extensive testing and approval processes before enzymes can be used commercially (Štreimikytė *et al.*, 2022). Industrial scalability also poses a challenge, as optimizing enzyme concentrations and reaction parameters on a large scale can be technically complex and cost intensive. Additionally, enzyme mixtures may create emulsions that hinder downstream processing and purification (Wu *et al.*, 2009). The high production cost of commercial enzymes such as cellulase, pectinase, and protease further limits the economic feasibility of EAE for some applications (Didia *et al.*, 2018).

Conclusion

Despite its limitations, EAE remains a highly promising, sustainable, and efficient technique for recovering bioactive compounds from various plant-based materials. By enabling targeted breakdown of plant cell wall components, EAE enhances extraction yields while reducing reliance on harsh chemical solvents and excessive energy inputs. The integration of EAE with complementary technologies, such as UAE, further amplifies its efficiency and broadens its applicability across food, nutraceutical, pharmaceutical, and cosmetic industries. As advancements in enzyme engineering and process optimization continue, EAE is poised to play a pivotal role in developing environmentally responsible and economically viable extraction methods that support the transition toward greener industrial practices.

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References

1. Amid, B. T., Manap, M. Y. A., & Zohdi, N. (2016). Enzyme-assisted and ultrasound-assisted extraction of pectinase from guava peel: A comparative study. *Food and Bioproducts Processing*, 100, 245–253.
2. Chen, J., Li, W., & Wang, Y. (2011). Improved extraction of flavonoids from Ginkgo biloba leaves using transglycosylating cellulase from *Penicillium decumbens*. *Bioresource Technology*, 102(12), 6990–6996.
3. Dat, T. T. H., Pham, N. D., & Le, T. M. (2022). Enhanced triterpenoid extraction from *Ganoderma lucidum* using ultrasound-assisted enzymatic extraction. *Journal of Food Science and Technology*, 59, 2062–2070.
4. de Camargo, A. C., Regitano-d'Arce, M. A. B., & Da Silva, M. A. A. P. (2016). Phenolic profile and antioxidant activity of winemaking by-products. *Journal of Functional Foods*, 23, 94–102.
5. Didia, M. T., Ojokoh, A. O., & Akinlua, A. (2018). Enzyme-assisted extraction of shea butter: Yield and quality evaluation. *African Journal of Food Science*, 12(9), 220–226.

6. Fazlena, H., Yusoff, M., & Omar, A. (2013). Effect of ultrasound on enzyme activity and stability. *International Food Research Journal*, 20(5), 1983–1987.
7. Fu, X., Belwal, T., Cravotto, G., Luo, Z., & Peng, Z. (2008). Optimization of pectinase-assisted extraction of flavonoids from orange peel. *Food Chemistry*, 261, 241–248.
8. Fuso, A., Agcam, E., & Capanoglu, E. (2022). Emerging enzyme-assisted green technologies for valorizing fruit processing by-products. *Critical Reviews in Food Science and Nutrition*, 62(10), 2655–2672.
9. Kalcheva-Karadzhova, E., Bozhilov, D., & Baeva, M. (2014). Recovery of polyphenolic compounds from rose petals by enzyme-assisted extraction. *Journal of Essential Oil Bearing Plants*, 17(3), 427–436.
10. Łubek-Nguyen, P., Witrowa-Rajchert, D., & Nowacka, M. (2022). Enzyme-assisted extraction as a green and sustainable method for recovery of bioactive compounds. *Trends in Food Science & Technology*, 123, 92–105.
11. Mattusch, J., Wennrich, R., & Krüger, R. (2006). Bio-extraction and enzymatic extraction methods: A comparison with classical techniques. *Analytical and Bioanalytical Chemistry*, 384, 730–738.
12. Puri, M., Sharma, D., & Barrow, C. J. (2012). Enzyme-assisted extraction of bioactives from plants. *Trends in Biotechnology*, 30(1), 37–44.
13. Ricarte, D. F., Viganó, J., & Martínez, J. (2020). Carotenoid recovery from sunflower waste using enzyme-assisted extraction. *Food Research International*, 132, 109098.
14. Shahram, H., & Taghian Dinani, S. (2019). Ultrasound-assisted extraction of β -carotene from orange processing waste. *Journal of Cleaner Production*, 230, 1213–1221.
15. Štambuk, A., Šic Žlabur, J., Voća, S., & Brnčić, M. (2016). Enzyme-assisted extraction of polyphenols from grape skin and seeds. *Croatian Journal of Food Science and Technology*, 8(2), 20–26.
16. Štreimikytė, D., Tamošiūnas, A., & Mažeikienė, A. (2022). Regulatory challenges for enzyme use in food and pharmaceutical industries. *Regulatory Toxicology and Pharmacology*, 133, 105192.
17. Sowbhagya, H. B., & Chitra, V. N. (2010). Enzyme-assisted extraction of bioactives from plant materials: An overview. *Critical Reviews in Food Science and Nutrition*, 50(6), 584–596.
18. Tomaz, I., Štambuk, A., & Preiner, D. (2016). Optimization of enzyme dosage for polyphenol extraction from grape skins using Lallzyme EX-V. *Food Technology and Biotechnology*, 54(2), 169–177.

19. Vergara-Barberán, M., Lerma-García, M. J., & Simó-Alfonso, E. F. (2015). Enzyme-assisted extraction of protein from olive leaves. *Journal of Agricultural and Food Chemistry*, 63(29), 6627–6633.
20. Wu, H., Wang, Q., & Ma, T. (2009). Enhancing oil extraction from soybean flakes using protease treatment. *Journal of the American Oil Chemists' Society*, 86(7), 645–651.
21. Zuorro, A., Lavecchia, R., & Medici, F. (2011). Enzyme-assisted extraction of lycopene from tomato waste. *Chemical Engineering Transactions*, 24, 991–996.
22. Zuorro, A., Lavecchia, R., & Medici, F. (2019). Recovery of polyphenols and flavonoids from corn husk using enzyme mixtures. *Industrial Crops and Products*, 139, 111566.