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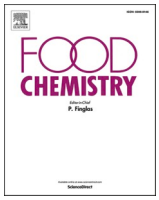

Review

Optimizing enzymatic oil extraction: critical roles of enzyme selection, process parameters, and synergistic effects on yield and quality

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ABSTRACT

Traditional plant oil extraction methods face challenges like low efficiency and pollution. In contrast, aqueous enzymatic extraction (AEE) offers advantages such as mild extraction conditions, no solvent residues, and sustainability, ensuring oil safety. The specificity of enzymes makes efficient oil extraction possible, requiring careful optimization of parameters such as enzyme selection, pH, temperature, solid-liquid ratio, enzyme concentration. This review explains how to enhance the yield and quality of oil through the synergistic effects between enzymes and these parameters. Conversely, inappropriate conditions reduce enzyme efficiency, cause oxidative reactions, and produce harmful substances, adversely affecting the flavor, color, and nutritional properties of the oil. Moreover, physical field auxiliary technology further improve enzyme permeability, boosting release rates, quality, and economic benefits. Future research should focus on cost reduction, energy efficiency, and by-product quality improvement. This review provides a theoretical framework for optimizing AEE processes and highlights its potential for sustainable oil extraction.

1. Introduction

Oil is a vital dietary component, supplying energy, essential fatty acids (FAs) such as linoleic and linolenic acids, and fat-soluble vitamins. However, the oil industry faces challenges including low oil quality, inefficient by-product utilization, and production inefficiencies. Improving oil quality, minimizing losses, and enhancing value are key developmental goals. Oil extraction predominantly employs pressing and leaching methods. Pressing, whether hot or cold, uses mechanical force to rupture oilseed cells but generally yields less oil and produces low-quality oil cakes, limiting by-product utilization (Jayaraman et al., 2016). Leaching, or solvent extraction, relies on the solubility of oil in solvents such as hexane, achieving a high oil yield of approximately 99 %. However, this method raises safety concerns due to the toxicity and flammability of the solvents involved (Dunford, 2022; Nde & Foncha, 2020). Therefore, developing green and flexible extraction technologies is crucial for advancing the industry.

Aqueous enzyme extraction (AEE), using water as a medium and leveraging enzymes' specificity, enhances oil yield while retaining or dissolving key nutrients. This technique offers the advantages of mild and sustainable reaction conditions (Cheng et al., 2019; Wang et al., 2023). AEE involves several stages, including oilseed pretreatment, enzymatic degradation of cellular structures such as the cell wall (CW) and oil body (OB) disruption, oil release, separation based on density differences, and emulsion breaking (Fig. 1). The enzymes primarily target and degrade macromolecular complexes (lipoproteins, lipopolysaccharides, etc.) present in the CW and OB, thereby facilitating oil release (Gao et al., 2024). The oil production rate is directly related to the degree of cell disruption, which is influenced by the type of enzyme used. Considering the specificity of various enzymes and the composition of the oil, selecting the appropriate enzyme for enzymatic digestion is fundamental to the success of AEE. Additionally, enzyme activity is impacted by factors such as reaction temperature and pH, while process parameters—including solid-liquid ratio, digestion time, and enzyme

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concentration—affect the degree of enzyme-substrate interaction, consequently influencing the yield (Mwaurah et al., 2020). Additionally, the stability of unsaturated fatty acids (UFAs) and other active compounds, including sterols and vitamins, is influenced by these factors, which in turn affects the quality, flavor, color, and nutritional value of the oil. To enhance the efficiency of AEE, auxiliary techniques such as ultrasound and microwave radiation have demonstrated a synergistic effect in promoting enzyme activity, facilitating the destruction of CW and OB, and increasing extraction rates (Hu et al., 2020; Li et al., 2016; Wang et al., 2023). However, it is crucial to carefully control the thermal effects of these techniques to maximize enzyme-substrate contact while preserving oil quality. As the oil is released and migrates into the aqueous phase, other macromolecules present in the oilseed, such as polysaccharides, proteins, and phospholipids, can function as surfactants. This results in a reduction of interfacial tension between the oil and water, leading to the formation of stable emulsions that can hinder oil separation. Therefore, efficient demulsification is essential for increasing oil production (Hoffmann & Reger, 2014). Enzymatic demulsification, which is environmentally friendly, has garnered significant attention, and the key to enhancing its effectiveness lies in the selection of appropriate enzymes.

Efficient extraction of high-quality oil relies on understanding oilseed cellular structure, enzyme mechanisms, and process parameters. However, studies on the combined effects of these factors on oil yield and quality remain limited. This review analyzed the composition, characteristics, and cellular microenvironment of various oilseeds, elucidating the oil release mechanism linked to enzymatic hydrolysis by different enzymes. It also explored how oilseed types influence AEE process parameters, including enzyme selection, concentration, hydrolysis time, pH, temperature, and solid-to-liquid ratio. Additionally, it examined how these parameters affect enzyme activity, impacting oil yield and quality. Furthermore, the review highlighted physical field auxiliary methods that enhance oil extraction by improving enzyme-substrate interactions. By systematically studying these factors, it provides a theoretical foundation for optimizing AEE and advancing sustainable oilseed processing, offering insights into improving oil yield and quality.

2. Oilseed cellular microenvironment

In the oilseed industry, seeds with oil content exceeding 10 % are classified as oilseeds. Based on their botanical characteristics, oilseeds are categorized into herbaceous oils (e.g., soybean, peanut, sunflower) and woody oils (e.g., palm, coconut). Plant oilseeds are further categorized by origin, including nut and regional oils (Fig. 2A). The cellular structures of these oilseeds are similar, with average diameters ranging from tens to hundreds of micrometers, primarily composed of protoplasm and CWs. To release oil, three barriers must be breached: the CW, the cell membrane (CM), and the OB surface barrier (OBSB). Understanding their structures aids in selecting appropriate enzymes.

2.1. Cell wall

The CW of oilseeds, acting as the main barrier for intracellular substance release, is composed of the intercellular layer, the primary wall, and the secondary wall (Fig. 2B). The intercellular layer mainly consists of pectin linked by divalent cations and hydrophobic bonds, facilitating cellular adhesion. The primary wall provides strength and flexibility via covalent and hydrogen bonds among hemicellulose, pectin, and cellulose. Upon cessation of cell growth, inner-region accumulation results in the formation of the secondary wall. Consequently, the CW forms a dynamic, complex network, which is characterized by cellulose microfilaments as the backbone, embedded in a matrix of pectin, hemicellulose, lignin, and structural proteins (Colosimo et al., 2021; Kubicek et al., 2014).

2.2. Protoplasm

The site closely linked to the CW is the protoplasm, which comprises the cell membrane, cytoplasm, nucleus, and organelles. Protoplasm is primarily composed of proteins and phospholipids, playing a crucial role in maintaining cellular stability. The CM exists between the CW and the protoplasm, primarily composed of proteins and phospholipids. The CM controls the exchange of substances between the intracellular and extracellular environments, serving as the second barrier that hinders

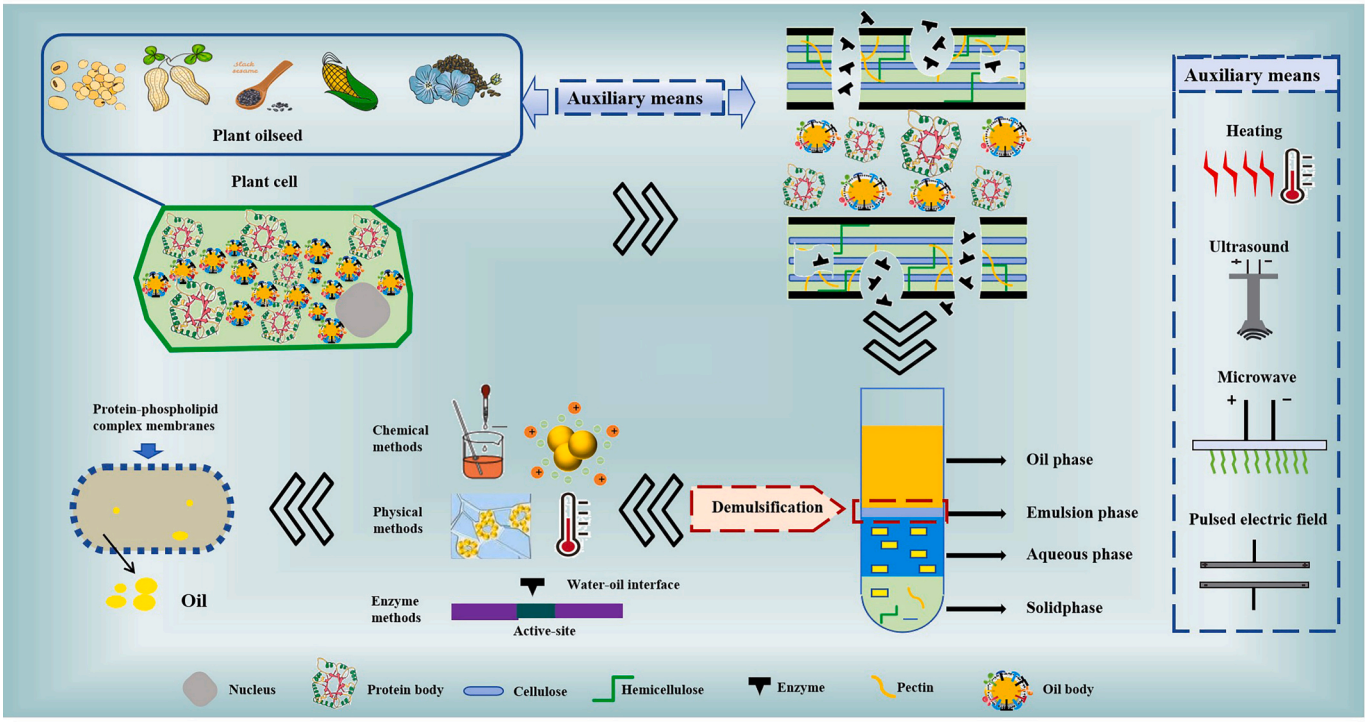

Fig. 1. Flow chart of oil extraction by Aqueous enzymatic extraction.

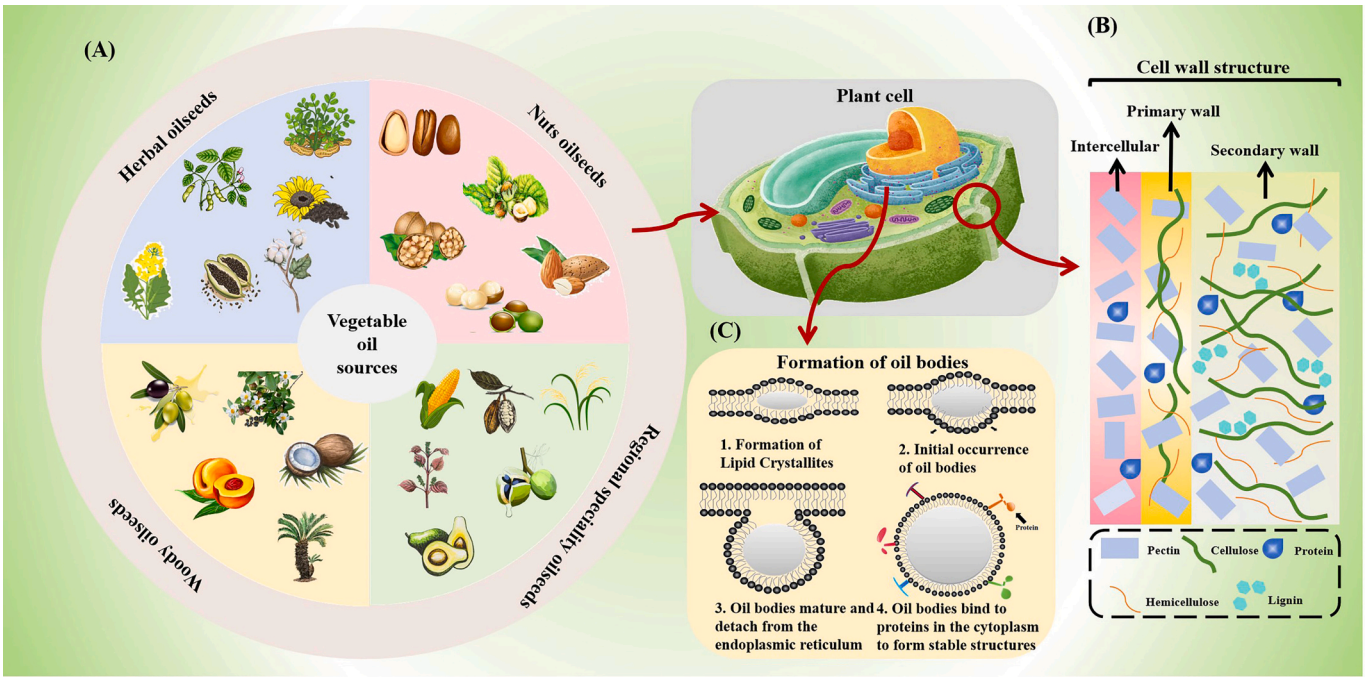

Fig. 2. Classification of plant lipids (A), Detailed structure of plant cell wall (B), Mechanism of oil body formation (C).

the release of oil. The cytoplasm is a fundamental component of protoplasm, containing various organelles such as plastids, mitochondria, vacuoles, and the endoplasmic reticulum. These organelles are involved in the synthesis of oil and significantly influence the properties of oil. Specifically, during the growth of oilseed, the enzymatic reaction of esterification of carbohydrates and glycerol-derived FAs catalyzed by lipases leads to the formation of oil droplets. These oil droplets are transported through the cytoplasm to the endoplasmic reticulum, where they aggregate and interact with phospholipids, forming a structure where a monolayer of phospholipid membranes surrounds the oil droplets. As the core of the oil droplets expands, they detach from the endoplasmic reticulum and enter the cytoplasm (Monson, Whelan, & Helbig, 2021). Numerous protein bodies (PBs) are present in the cytoplasm, and these oil droplets interact with the PBs to ultimately form homogeneous, discontinuous spherical droplets of varying sizes, referred to as OBs (Fig. 2C). Additionally, vacuoles contain polyphenols and pigments, which also influence the color of the oil.

2.3. Oil body

OBs serve as the primary organelles for triglyceride (TAG) storage. OBs form spherical aggregates (0.5–2.5 μm in diameter) near the PBs, occupying the entire cellular network. The OBSB is composed of phospholipids (0.60 %–2.00 %) and proteins (0.60 %–3.00 %), while their

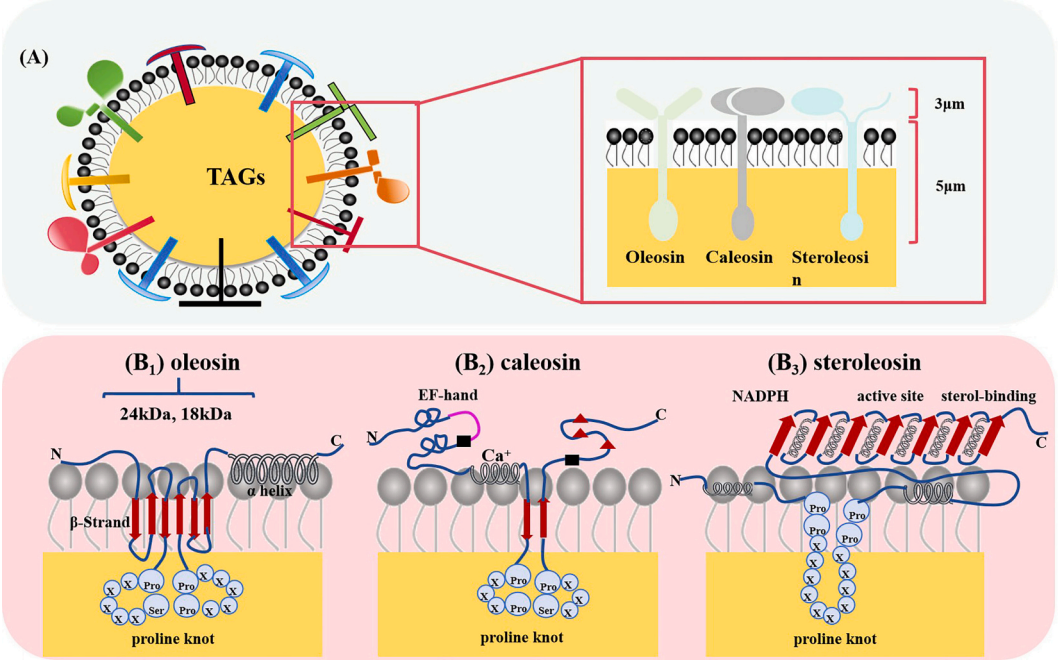

Fig. 3. Microstructure of the oil body. The structure of OB (A); The detailed structure of the three endogenous proteins (B).

interior stores TAG, which constitute 94.00 %–98.00 % of their total content (Yang et al., 2020). The OBSB exhibits stability, hindering TAG release and constituting the third barrier in AEE. Understanding its composition is critical for improving TAG release efficiency.

2.3.1. OBSB1 - endogenous proteins

The endogenous proteins of OBs are classified into three groups: oleosin, caleosin, and steroleosin. These proteins, the longest among OB proteins, contain hydrophobic domains (~5 nm) and outer hydrophilic domains (~3 nm) (Fig. 3A) (Liao et al., 2024).

Oleosin comprises an *N*-terminal domain, a hydrophobic central domain, and a *C*-terminal α-helical domain (Fig. $3B_1$). The *C*- and *N*-terminal domains flank the hydrophobic central domain embedded in the OB. This central domain contains an inverted parallel β-chain linked by a proline-encoding node, which regulates OB localization. Oleosin predominantly resides in the monomolecular layer of oleosomal phospholipids, maximizing surface area to facilitate its biological functions. Additionally, it promotes the binding of lipase, protease, and phospholipase enzymes on its surface, enabling lipolysis (Abell et al., 1997).

In contrast, caleosin has larger *N*-terminal domains containing Ca^{2+}-binding sequences and EF-hand chiral sequences, as well as potential phosphorylation sites in the *C*-terminal domains. These EF-hand chiral sequences suggest potential interactions between caleosin and other EF-hand chiral sequence-containing proteins in regulating lipid metabolism. Furthermore, the *N*- and *C*-terminal domains of caleosin contain heme-binding sites with conserved histidine sequences, allowing heme co-factor binding (Fig. $3B_2$) (Chen et al., 1999).

Steroleosin consists of only two structural domains. The first is a hydrophobic *N*-terminal domain containing a proline-encoding sequence, primarily anchoring to the OB surface. The second is a soluble sterol-binding dehydrogenase/reductase *C*-terminal domain, comprising an NADPH-binding domain, an NSYK-binding site (Asn-Ser-Tyr-Lys), and a sterol-binding subdomain (Fig. $3B_3$) (García-Llatas et al., 2008).

2.3.2. OBSB2 - phospholipids

The phospholipid composition in OBs is primarily composed of phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidic acid (PA). Four major phospholipids in OBs from oilseeds (e.g., soybean, rapeseed, maize, sesame) were identified: PC (41.2–64.1 %), PE (2.8–15.8 %), PI (6.9–20.9 %), and PS (18.3–33.1 %) (Bettini et al., 2014). Additionally, Simpson et al. reported trace amounts of PA in addition to PC, PE, PI, and PS in OBs. Elevated PC levels enhance hydrophobic interactions with interfacial proteins, thereby stabilizing the OB structure. Östbring et al. demonstrated oleosin protein, phospholipids and oil are mixed, creating emulsion droplets stabilized similarly to natural OBs (Östbring et al., 2020).

2.3.3. The stabilization mechanism of the OB

The OB membrane plays a key role in maintaining OB stability through several mechanisms: (1) strong hydrophobic interactions between endogenous proteins, phospholipids, and TAGs; (2) encapsulation of phospholipids by endogenous proteins, preventing their interaction with phospholipase and preserving OB stability; (3) electrostatic repulsion between positively charged amino acid residues in endogenous proteins and negatively charged phospholipids, which enhances OB stability; and (4) the spatial structure of endogenous proteins, which confers resistance to environmental stresses and chemical reagents, ensuring OB stability in oilseeds and isolated conditions (Liao et al., 2024). The enzymatic hydrolysis of surface endogenous proteins and phospholipids is critical in the AEE process to enhance oil yield.

3. Mechanism of enzyme action on oilseed cells

AEE primarily employs plant cell wall enzymes (PCWEs) to degrade CWs, followed by the enzymatic breakdown of CM and OBSB by proteases, and phospholipases, resulting in the release of TAGs. In the AEE process, selecting enzymes tailored to oilseed characteristics is essential. A thorough understanding of enzymatic mechanisms is vital for improving efficiency.

3.1. Enzymatic mechanism of PCWEs

The structural composition of the CW has been outlined in the section 2.1 (Fig. 2B). Therefore, Cellulases, hemicellulases, and pectinases are commonly used to enzymatically degrade CWs. These enzymes synergistically degrade the primary and secondary CWs of plant cells (Fig. 4A).

Cellulase, with a catalytic head and wedge-shaped tail, enzymatically degrades cellulose via synergistic action. Endoglucanase (EC 3.2.1.4) cleaves β-1,4-glycosidic bonds, forming oligosaccharides. Exoglucanase (EC 3.2.1.21) hydrolyzes these fragments into cellobiose, which β-glucosidase further breaks down into glucose, disrupting the cellulose network and enhancing CW porosity (Fig. 4B) (Ramachandran et al., 2022).

Hemicellulases, characterized by two glutamic acid and six cysteine residues forming three disulfide bonds, hydrolyze hemicellulose in primary and secondary CWs. Xylanase (EC 3.2.1.151) cleaves β-1,4-xylosidic bonds in xylan, yielding xylo-oligosaccharides and xylose. β-Mannanase (EC 3.2.1.78) and α-l-arabinofuranosidase (EC 3.2.1.55) degrade mannans and other components into smaller sugars, synergistically weakening the CW and enhancing enzyme accessibility to its components (Fig. 4C) (Chang et al., 2017).

Pectinases, active in the intercellular layer, degrade pectin, a right-handed cylinder composed of 7–9 parallel β-helices. They include protopectinases, pectin esterases (EC 3.1.1.11), polygalacturonases (EC 3.2.1.15), and pectin cleavage enzymes. These enzymes hydrolyze, deesterify, and cleave pectin into pectic acid and galacturonic acid, disrupting its structure, reducing intercellular adhesion, and facilitating cell fragmentation (Fig. 4D) (Satapathy et al., 2020).

3.2. Enzymatic mechanism of OBSB

Following CW disruption, the CM and OBSB emerges as the second and third obstacle to oil release. The disruption of phospholipids and proteins is pivotal for CM and OB destabilization during oil extraction.

Proteases disrupt the protein structure within the interfacial membrane, thereby damaging the network structure of the membrane and reducing its stability. Meng et al. observed a similar phenomenon, discovering that Alcalase 2.4L induces alterations in protein secondary structure during hydrolysis. The transition of protein conformation from ordered to disordered plays a pivotal role in facilitating oil release (Meng et al., 2018). Oleosin is particularly crucial for maintaining OB stability and integrity. There are two enzymatic pathways of Oleosin. The direct hydrolytic action of endogenous proteases on oleosin is one pathway for oleosin hydrolysis. This process is observed during seed germination or OB remodeling in plant physiology. The activity of endogenous proteases is influenced by factors such as pH and hormone levels, which in turn affect OB stability (Chen et al., 2021). Oleosin hydrolysis can also occur via the ubiquitin-proteasome system: oleosin first undergoes ubiquitinylation modification, after which the ubiquitinyl-modified oleosin dissociates from the OB and enters proteasome cavities, where it is enzymatically degraded by proteases (Fig. 5AII) (Vandana & Bhatla, 2006). On the other hand, exogenous proteases adhere to the surface of OB through hydrophobic domains or charge interactions, with the active sites of the proteases binding to specific peptide segments of oleosin. The *C*-terminal or *N*-terminal is preferentially cleaved, resulting in the disintegration of the OB membrane structure (Fig. 5AI). Based on the cleavage sites of the degraded proteins, proteases can be classified into two categories: endopeptidases and exopeptidases. Endopeptidases act on specific peptide bonds within

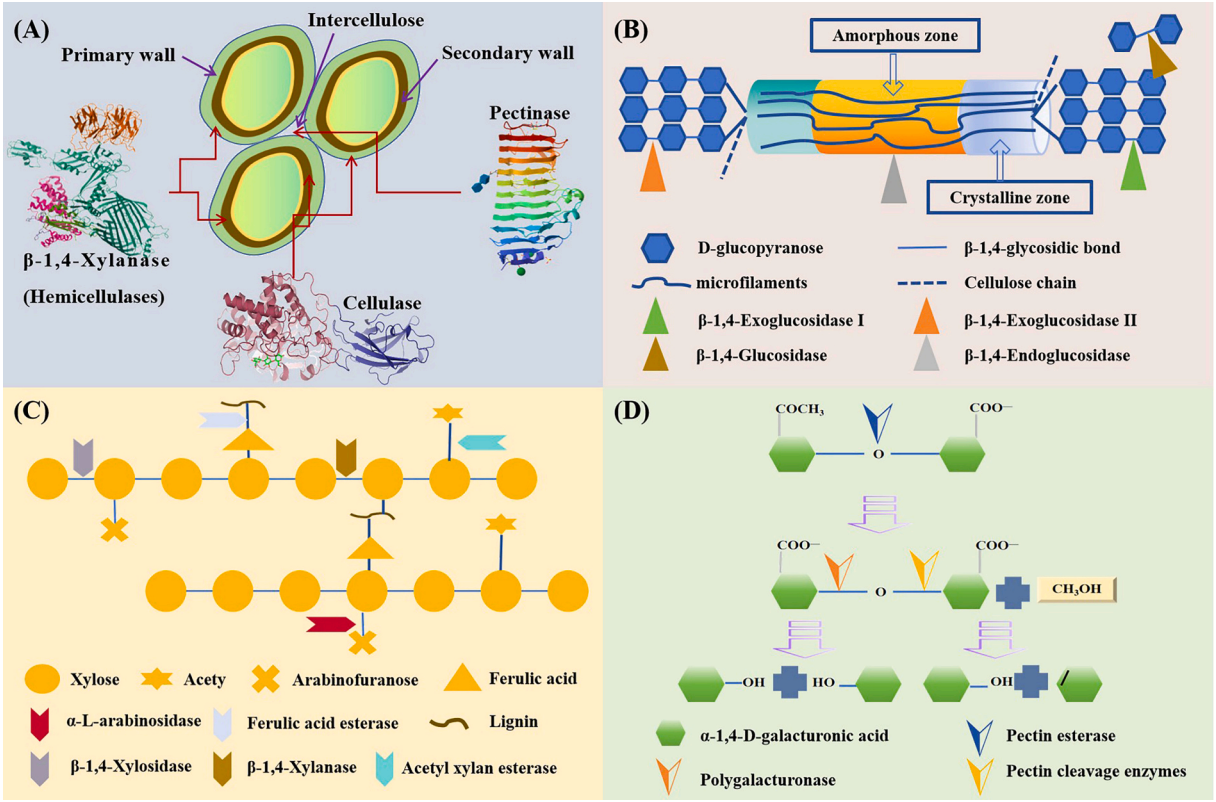

Fig. 4. Mechanism of cellulase, hemicellulase and pectinase enzymatic degradation of the cell wall. The schematic diagram of the main sites of action of the three enzymes on the cell wall (A); The mechanism of cellulase degradation (B); The mechanism of hemicellulase degradation (C); The mechanism of pectinase degradation (D).

the polypeptide chain. For example, serine proteases (such as trypsin, EC 3.4.21.4) cleave at the carboxyl terminal of arginine/lysine; cysteine proteases (such as papain, EC 3.4.22.2) cleave at hydrophobic residues; and metalloproteinases (such as collagenase, EC 3.4.24.27) rely on Zn^{2+} to cleave the Gly-Ile/Leu bond in collagen. In contrast, exopeptidases primarily remove amino acids progressively from the ends of the polypeptide chain. For instance, aminopeptidases cleave residues from the *N*-terminal (such as leucine aminopeptidase), while carboxypeptidases remove residues from the *C*-terminal (such as carboxypeptidase A, which preferentially targets hydrophobic ends). Furthermore, the phospholipid layer becomes loose after losing the anchoring of OB proteins (Müntz, 2007).

Phospholipases anchor to the phospholipid interface through amphipathic α-helices or circular domains. The active site of the enzyme then specifically cleaves the ester bonds or phosphodiester bonds in the phospholipid molecules on CM and OBSB through hydrophobic interactions, thereby disrupting the structural integrity of the lecithin layer interface. Based on their specific hydrolysis sites, phospholipases can be categorized into Phospholipase A (PLA1), Phospholipase B (PLA2), Phospholipase C (PLC), and Phospholipase D (PLD). As shown in Fig. 5B, PLA1 hydrolyzes the sn-1 ester bond, producing fatty acids and lysolipids. PLA2 cleaves the sn-2 ester bond, releasing signaling molecules such as arachidonic acid. PLC breaks the glycerophosphate bond, generating diacylglycerol and phosphoric head groups. PLD hydrolyzes the phosphodiester bond, resulting in phosphatidic acid and free head groups (such as choline) (He et al., 2016).

4. Effect of enzyme application on oil yield

As previously mentioned, the CWs of most plant seeds consist of cellulose, hemicellulose, and pectin; thus, studies often utilize PCWEs either individually or in combination. For instance, the combination of cellulases, hemicellulases, and pectinases has been shown to enhance oil yields in oat kernels, white pine kernels, and tiger nuts. These enzyme complexes significantly improve oil yields (Chen et al., 2016; Hu et al., 2020; Li et al., 2013). A specific enzyme can yield similar results. Viscozyme®L possesses xylanase, aconitase, β-glucanase, hemicellulase, and cellulase activities, which exhibit properties akin to an enzyme complex (Arroyo et al., 2019). Diaz et al. compared the effects of cellulase, hemicellulase, pectinase, and Viscozyme®L on the extraction rate of castor oil. Among the enzymes evaluated, Viscozyme®L produced the highest oil yield of 80 % (Díaz-Suárez et al., 2021). Liu et al. further demonstrated that Viscozyme®L acted on C—C stretching, C—O stretching, and CH_2 symmetric bending of cellulose, as well as C—O stretching and O—C—O asymmetric bending of cellulose, and C—C stretching and C—O stretching of pectin, resulting in CW degradation. Additionally, hydrolysis causes OBs to collide and merge, resulting in an increase in the size of some OBs and uneven distribution, thereby promoting the release of peanut oil (Liu, Hao, Chen, & Yang, 2020). However, the mechanism by which Viscozyme®L interacts with OBs remains unclear and warrants further investigation.

Proteases are commonly employed in oilseeds with high protein content, particularly in the extraction of protein-rich legume proteins. For instance, Jung et al. reported that the use of protease increased soybean oil extraction efficiency to 96.0 %, compared to 73.4 % achieved with phospholipase (Jung et al., 2009). Machida et al. utilized alkaline protease to hydrolyze chickpea CW structural proteins, such as stretch proteins and glycoproteins, as well as oleosins. This process disrupted the cell matrix porosity, elevating it to 65 %, while simultaneously reducing the interfacial tension of oil droplets from 28 mN/m to 12 mN/m, thereby promoting oil release to 95.80 % (Machida et al., 2022). In contrast, some oilseeds, such as sunflower seeds, contain lower protein content and exhibit smaller OB particle sizes (0.28 ± 0.03). In these cases, the addition of Alcalase 2.4L not only failed to effectively

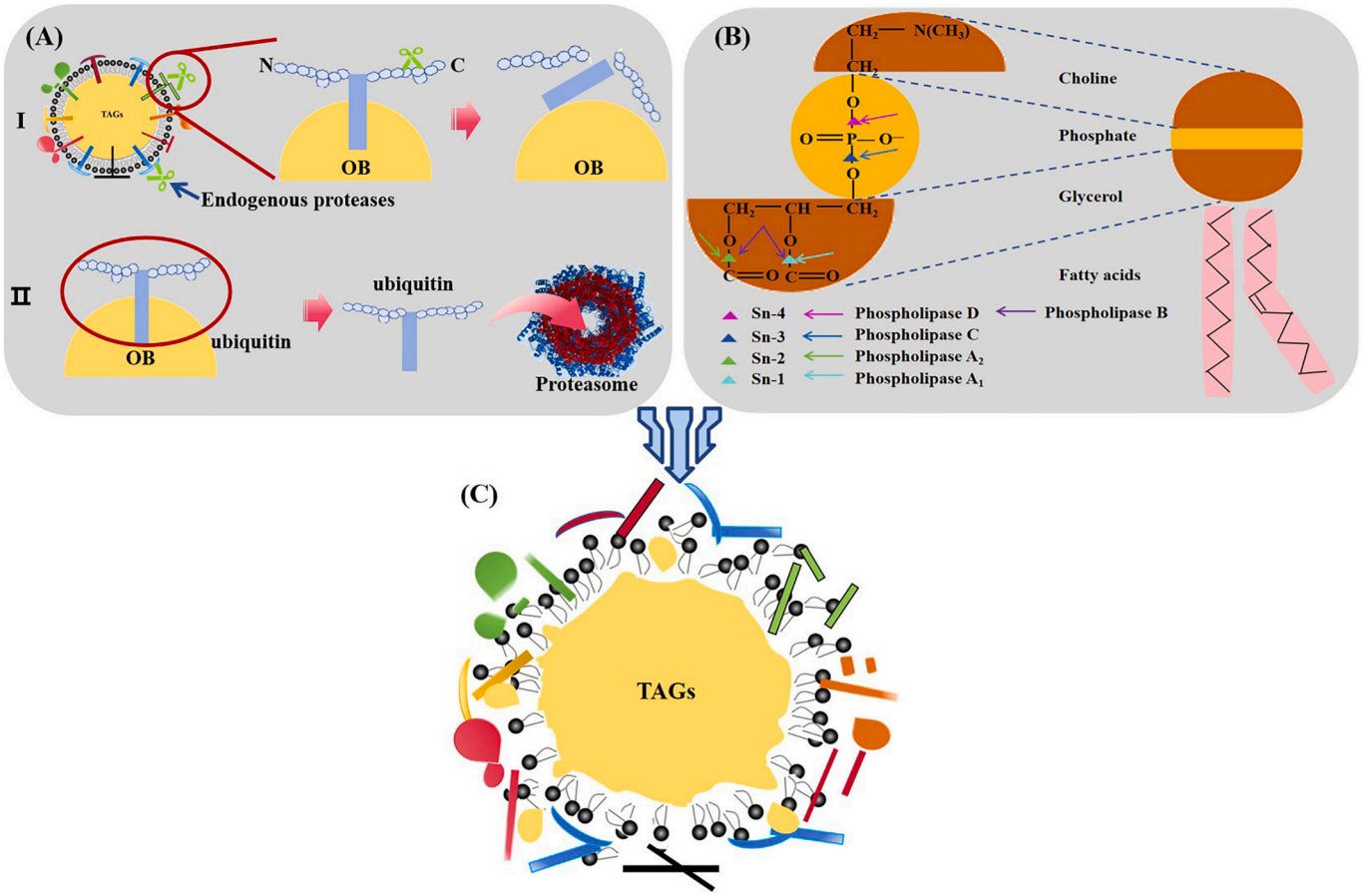

Fig. 5. OB destabilization mechanism in the presence of enzymes. The enzymatic mechanism of oleosin (A); The enzymatic mechanism of phospholipase (B); The schematic diagram of OB destabilization (C).

digest OBSB but also formed a stable emulsion with water, resulting in a reduced oil yield of 16.4 % (De Aquino et al., 2022). Furthermore, Nguyen et al. demonstrated that the selection of the appropriate enzyme based on the characteristics of the oilseed significantly influences oil yield. Their comparison of cellulase and papain in the extraction of Sacha inchi seed oil revealed that papain was more efficient, yielding 24.67 % compared to cellulase's 20.90 %, which was attributed to the high protein content of Sacha inchi seeds (Nguyen et al., 2020).

In most cases, combining PCWEs and proteases enhances oil yield. A 1.5 % (*w*/w) enzyme cocktail (cellulase/pectinase/protease = 1/1/1, w/w/w) for 90 min achieves an optimal oil yield of 21.62 % from forsythia seeds (Gai et al., 2013). Similarly, a 1 % (*v*/*w*) enzyme cocktail (Celluclast 1.5 L/Alcalase 2.4 L = 60/40, *v*/v) for 2.5 h achieves an almond oil yield of 47.93 % (Pawar et al., 2024).

However, not all enzyme cocktails are optimal, as this is also influenced by the composition of the oilseed. For instance, Nguyen et al. utilized a mixture of papain and cellulase to extract oil from sea buckthorn seeds. They observed a higher oil yield with papain alone, likely due to the low cellulose and hemicellulose content of sea buckthorn. The excessive addition of enzymes caused the reaction system to become sticky, resulting in a decrease in the extraction rate (Nguyen et al., 2020). Oilseed composition also influence the sequence of enzyme application. For instance, employing the cellulase-pentosanase method for extracting oil from *Acer truncatum* Bunge seeds resulted in a 3.5 % increase in the yield compared to the reverse order of enzyme addition. This enhancement can be attributed to the capacity of cellulase to initially hydrolyze the cellulose in the primary CW of the seeds, facilitating the degradation of the primary CW. The subsequent addition of pentosanase enables it to traverse the primary CW and hydrolyze the pentose-rich secondary CW, further compromising the cellular structure. Conversely, when the order of enzyme addition is reversed, pentosanase encounters challenges in enzymatically hydrolyzing the primary CW, leading to a decrease in oil yield (Hu et al., 2023).

In addition to cellulose, and protein, various oilseeds contain other components such as polysaccharides, starch, and other substances. The presence of these components tends to form stable emulsions with water, thereby influencing oil separation. For instance, camellia seeds have a high starch content, the starch forms a paste upon contact with water, which increases the viscosity of the system and impedes oil release. α-Amylase cleaves the α-1,4 glycosidic bonds in starch molecules, resulting in the production of short-chain dextrins and a small quantity of low molecular weight sugars, which reduces the viscosity of the reaction system, yielding an oil extraction rate of 39.6 ± 0.3 % (Zhang, Chen, Liu, et al., 2023). Pentosan, a major component of the secondary CW of *Acer truncatum* Bunge seeds, when combined with cellulase, enhances oil yield compared to cellulase alone (Hu et al., 2023). Certain wood oils, such as olive oil (Fig. 2), can be released more effectively due to the high lignin content in olives, which gives them a hard texture. Ligninase can be employed to disrupt the cellular structure, thereby facilitating the release of the oil (Liu et al., 2013).

From the above, it is evident that comprehending the primary components of oilseeds and strategically selecting suitable enzymes can significantly enhance the efficiency of AEE. Consequently, Table 1 provides a summary of the compositions of common oilseeds to assist in enzyme selection.

5. Effect of enzymatic conditions on oil yield

Enzymatic conditions encompass pH, temperature, solid-liquid ratio, enzyme concentration, and digestion time. Each enzyme must operate

Table 1
Information about main components in different oilseeds.

Oilseed	Lipid (%)	Protein (%)	Carbohydrates (%)	Major fatty acids (%)	Main fat concomitants	Reference
Soybean	14–21	32–42	~35	C16:0 (7–12) C18:0 (2–5.5) C18:1 (19–30) C18:2 (48–58) C18:3 (5–8.8)	Isoflavones (0.2–4.2 mg/g seed dry weight) Phytic acid (1.0–2.3 % on a dry seed basis)	(Medic et al., 2014)
Corn germ	30–50	15–20	15–20	C16:0 (8.6–16.5) C18:0 (< 3.3) C18:1 (20.0–42.2) C18:2 (34.0–65.6)	Ferulic acid (19 mg GAE/kg) Campesterols (1800–2700) Stigmasterol (300–1700) β-Sitosterol (3800–14,700) Sitostanol (350–460) △5-Oat sterenols (100–1800) Squalene (80–165) γ-Tocopherol (268–2468)	(Apetrei & Apetrei, 2015)
Peanut	~50	24–36	~21.51	C16:0 (9.2–10.9) C18:0 (1.3–2.3) C18:1 (40.8–56.3) C18:2 (24.1–40.6)	Tocopherol (223 ± 16 mg/100 g) Phospholipids (500–700 mg/100 g) Vitamin E (223 mg/100 g)	(Mingrou et al., 2022)
Coconut white kernel	~38.8	~6.2	Carbohydrates (~10.6) Crude fibre (~11.7)	C8:0 (~5.6) C12:0 (~52.8) C14:0 (~19.2) C16:0 (~7.4)	Total phytosterols (30.66 mg/100 g) Hydroxybenzoic acid (34.7 μg/100 g) Coumaric acid (6.9 μg/100 g) Gallic acid (15.9 μg/100 g) α-Tocopherol (2.5 mg/100 g)	(Appaiah et al., 2014)
Rapeseed	34–40	37–46	Fibre (~15)	Erucic acid (37.9–57) C18:1 (~13) C18:2 (~14) C18:3 (4.7–13.0)	Vitamin E (608.90 mg/kg) Flavonoids (164.1 mg/kg) Squalene (21.8 mg/kg) Carotenoid (29.4–358.7) β-Sitosterol (~3597 mg/kg) Tocotrienols (450–600)	(Nagaraj, 2009; Shen et al., 2023)
Palm kernel	49–55	~8	~19.59, which contains crude fibre (~11.38)	C16:0 (~44.0) C18:0 (~4.5) C18:1 (~39.2) C18:2 (~10.1)	Phytosterol (326–527) Squalene (250–800) Polyphenols (40–70) β-Sitosterol (25.0–67.0 mg/kg) Stigmasterol (6.0–40.0 mg/kg) Campesterols (11.0–35.0 mg/kg)	(Koushki et al., 2015; Sundram et al., 2003)
Rice bran	12–23	14–16	Dietary fibre (8–10)	C16:0 (14–23) C18:0 (0.9–4.0) C18:1 (51–70) C18:2 (15–30) C18:3 (5–14)	γ-Tocotrienic (142–790 mg/kg) α-Tocotrienic (49–583 mg/kg) γ-oryzanol (1.0–4.0 %)	(Modupalli et al., 2024)
Sesame	~41.20	~22.41	Crude fibre (~3.42)	C16:0 (0.09–0.14) C18:0 (5.41–6.42) C18:1 (35.9–47) C18:2 (35.6–47.6)	Sesamin (100.89 μg/g) Sesalinin (40.72 μg/g) Sesamol (240.96 μg/g)	(Abbas et al., 2022)
Flaxseed	~41	~20	Total dietary fibre (~28)	C16:0 (~5.0) C18:0 (~3.0) C18:1 (~19) C18:2 (~17) C18:3 (~53) C14:0 (5.0–7.0)	γ-tocopherol (552 mg/100 g) α-tocopherol (7 mg/100 g) Ferulic acid (10.9 mg/g) Chlorogenic acid (7.5 mg/g) Secoisolariciresinol (165 mg/100 g) Squalene (46–360 mg/kg)	(Shim et al., 2014)
Sunflower	48–53	14–19	Crude fibre (16–27)	C16:0 (3.0–5.0) C18:0 (0.3–0.8) C18:1 (22–50) C18:2 (40–67)	Phytosterol (150–1800 mg/kg) Canolasterol (140–1140 mg/kg) β-Sitosterol (1200–2900 mg/kg) α-Tocopherol (403–935 mg/kg)	(Anjum et al., 2012; Nagaraj, 2009)
Camellia	40–60	8–9	/	C16:0 (~8.12) C18:0 (~2.23) C18:1 (~77.81) C18:2 (~10.6)	The content of all the following substances is in mg/kg. Lanosterol (715.19–1202.80) β-Amyrin Lupeol (607.24–913.35) Cycloatonol (578.87–1093.67) Betulin (165.57–330.56) β-Sitosterol (106.96–240.12) α-Tocopherol (157–771) Squalene (122.02–248.24) Cinnamic acid (3.72–10.63) Protocatechuic acid (2.27–4.80)	(Zhang et al., 2022)
Walnut	52–70	15–20	10–15, Soluble Sugar (5–8)	C16:0 (5.82–13.50) C18:1 (21.0–79.30) C18:2 (2.31–57.46) C18:3 (0.06–11.58)	Tocopherols (382.28 mg/kg) Gallic acid (440.36 mg/kg) Total phenols (135.9–163.8 mg GAE·100/g) Total sterols (900–2830 mg/kg) β-sitosterol (772–2520 mg/kg)	(Song et al., 2022)

(*continued on next page*)

Table 1 (*continued*)

Oilseed	Lipid (%)	Protein (%)	Carbohydrates (%)	Major fatty acids (%)	Main fat concomitants	Reference
Cocoa bean	~50	10–15	Starch (3–7) Cellulose (~12)	C16:0 (23.7–25.5) C18:0 (32.9–37.1) C18:1 (33.2–37.4)	Caffeic acid (13.92–109.5 mg/kg) Chlorogenic acid (8.8–17.5 mg/kg) Caffeine (2–3 %) Cocoa polyphenols (unfermented: ~2; fermented: ~6)	(Bertazzo et al., 2013)
Olea europaea	~30	~17	Total fibre (~47)	C16:0 (7.5–20) C16:1 (0.3–3.5) C18:0 (0.5–5) C18:1 (55–83) C18:2 (3.5–21)	Tocopherols (460 mg/kg oil) Squalene (194 mg/kg) Phenolic (2.8 mg/g seed)	(Maestri et al., 2019)
Safflower seed	28–32	14–15	Crude fibre (32–34)	C16:0 (5.0–7.5) C18:0 (1.4–1.8) C18:1 (6.1–7.6) C18:2 (74.9–78.4) C20:0 (1.2–3.6)	Acacetin (98.82 mg/100 g) Phytosterols (430.06 mg/100 g) VE (47.29–69.74 mg/100 g)	(Matthaus et al., 2015; Nagaraj, 2009)
Castor	40–55	18–23	Soluble sugars (~5) Crude fibre (~25)	Ricinolic (87.7–90.4) C18:0 (0.7–1.2) C18:1 (2.0–3.3) C18:2 (3.4–4.7)	Ricinoleic acid (89–89.4 %)	(Nagaraj, 2009)

Note: / indicates not mentioned in the study.

within its optimal temperature and pH range to achieve maximum activity; deviations from this range can lead to diminished enzyme activity or inactivation, thereby reducing the efficiency of the enzymatic process. Furthermore, these factors influence the physical properties of the reaction, including fluidity, emulsification, and viscosity, which in turn affect the degree of enzyme-substrate contact and the rate of oil release. Additionally, the quality of the oil is also influenced by these parameters. Consequently, it is essential to consider these process parameters alongside the selection of an appropriate enzyme to optimize enzymatic efficiency.

5.1. Enzymatic concentration

Enzyme concentration is a key determinant in AEE, directly affecting the extraction rate. When the amount of enzyme is insufficient, the reaction sites are not fully covered, leading to a decrease in extraction efficiency. Conversely, an excess of enzyme may induce substrate inhibition or create steric hindrance between enzyme molecules, thereby reducing the reaction rate. In the extraction of sacha inchi seed oil, Nguyen et al. (2020) demonstrated that the oil extraction rate increased steadily with papain concentrations rising from 2 % to 4 %. When the enzyme concentration reached 4.46 %, the oil yield peaked at 48.45 %, after which it began to decline. This decline occurs because excessive enzyme aggregation reduces effective concentrations, restricting molecular flexibility and enzyme-substrate binding, thereby lowering enzymatic activity (Nguyen et al., 2020). Additionally, excess enzymes tend to form stable emulsions, hindering oil-water separation and reducing yield (Zhu et al., 2024). Therefore, controlling the enzyme dosage is crucial for achieving high yield and maintaining high oil quality.

5.2. Digestion time

Enzyme digestion time plays a critical role in determining the vegetable oil extraction rate. Within a specific range, prolonged enzyme digestion generally enhances oil yield. This is attributed to optimal enzyme-substrate contact during the initial digestion phase, enhancing enzymatic efficiency. However, when the digestion time exceeds a specific threshold, the degradation or transformation of reaction products, such as the excessive hydrolysis of proteins leading to small peptides and amino acids, increased the viscosity of the aqueous phase, impeding the oil-water separation and thus reducing the oil extraction rate (Nguyen & Dang, 2016). Additionally, extended hydrolysis can promote microbial growth in the reaction system. The metabolic activities of these microorganisms can consume oil or produce impurities, adversely impacting both extraction rate and oil quality (Guerrini et al., 2019). Optimizing digestion time requires evaluating factors such as oil type, enzyme characteristics, reaction temperature, and pH. Different oilseeds require tailored digestion times due to variations in their cellular structure and composition.

5.3. pH

pH affects the three-dimensional conformation of enzyme proteins by altering the distribution of surface charge, thereby influencing the binding capacity of the active site to the substrate. Each enzyme operates within an optimal pH range that maximizes catalytic efficiency, while deviations beyond this range can inhibit enzyme function, leading to incomplete hydrolysis and reduced oil extraction. For example, cellulase achieves peak efficiency at pH 4.8–5.5, while Alcalase 2.4L, a protease, performs best at pH 8.0–8.5 (Fang et al., 2016). pH effects on proteins also play a crucial role in determining oil yield. Near the isoelectric point, protein solubility reaches its minimum, facilitating protein separation from the solution. However, protein aggregation under these conditions can form dense structures that trap oil, reducing oil yield (Tirgar et al., 2017). pH also influences oil yield by altering OB stability. Rising pH dissolves interfacial OB proteins, disrupting membranes, enlarging particles, and creating porous surfaces. At pH 11, reduced hydrophobic amino acids weaken OB interfaces, promoting aggregation and lowering oil yield (Gao et al., 2021). Thus, selecting optimal pH conditions requires considering oil and enzyme characteristics.

5.4. Temperature

Temperature critically impacts enzyme activity, directly affecting oil yield efficiency. Enzymes typically exhibit peak activity within an optimal temperature range, facilitating oilseed cell degradation. Higher temperatures can reduce the viscosity of the reaction system, accelerate molecular motion, and increase the probability of enzyme-substrate binding. Rising temperatures also enhance the permeability and diffusion of oilseed cell membranes (Sun et al., 2020). However, elevated temperatures lead to protein denaturation, disrupting the active site of enzymes and decreasing their activity. For walnut oil extraction with Viscozyme®L, oil yield remains below 65 % at temperatures under 35 °C, peaks at 75 % at 41.8 °C, and declines above 50 °C (González-

Gómez et al., 2019). Temperature additionally affects the quality of oil and proteins. High temperatures can cause oil oxidation, increasing peroxide value and forming harmful oxidation products. These products not only affect the flavor and aroma of the oil but also diminish its nutritional value. Excessive temperatures during enzymatic reactions can oxidize polyphenolic antioxidants, diminishing the oil's antioxidant capacity. Furthermore, excessive heat can denature proteins, reducing their functional properties and lowering the quality of oil by-products (Mwaurah et al., 2020). Thus, maintaining lower temperatures is essential to optimize both oil yield and quality.

5.5. Liquid-solid ratio (L/S)

The L/S ratio plays a pivotal role in enzymatic hydrolysis by regulating enzyme-substrate interactions, mass transfer efficiency, and the physical state of the reaction system. An optimized L/S ratio ensures maximum oil yield. A low L/S ratio increases the proportion of solid oil materials, causing uneven enzyme distribution, which restricts hydrolysis and reduces oil yield (Zhang et al., 2007). In contrast, an excessively high L/S ratio improves mass transfer but increases reaction volume, escalating costs for separation, concentration, and processing. Additionally, excessive L/S ratios dilute enzyme and substrate concentrations, reducing collision frequency, slowing reaction rates, and hindering maximum oil yield (Tirgarian et al., 2019). Optimizing the L/S ratio is essential for efficient AEE.

5.6. Combined effect of oilseed properties on enzymatic conditions

Optimal enzymatic conditions must account for both the properties of the oilseed and the need to maximize enzyme activity. Table 2 summarizes the optimal conditions for several common enzymes, revealing variations in these conditions across different oilseeds. For example, the optimal digestion temperature of cellulase during walnut seed oil extraction is higher than that for rapeseed oil. This difference is attributed to lignin in walnut seed CWs, which requires higher temperatures to soften, degrade the structure, and improve enzyme accessibility (Ghasemi & Taghian Dinani, 2018). In castor oil extraction, Viscozyme®L showed a higher L/S ratio (1:5) compared to peanut and Mamey Sapote (1:3.5 or 1:4). This is due to castor oil's high viscosity, which hinders fluidity. Increasing liquid content improves intermolecular interactions and molecular collisions within the system (Liu, Hao, Chen, & Yang, 2020; Tacias-Pascacio et al., 2021; Díaz-Suárez et al., 2021). Similarly, sunflower oil extraction with Alcalase 2.4L required lower temperature, time, and enzyme dosage than sesame oil. This was because sunflower seeds have thinner CW, leading to weaker binding of oil droplets to proteins, allowing efficient extraction with reduced enzyme use and shorter times (De Aquino et al., 2022; Ribeiro et al., 2016). The site of extraction also affects enzyme requirements. For instance, pectinase concentration was higher (6.3 %) for *Acrocomia aculeata* pulp compared to oilseeds, as pulp contains more pectin (Sorita et al., 2024). These examples highlight how variations in oilseed composition and physical properties influence optimal enzymatic hydrolysis parameters. In conclusion, achieving maximum enzymatic efficiency requires tailoring conditions to specific enzymes, oilseed characteristics, and reaction system properties. However, research on the differences in enzymatic process parameters for various oils remains limited. This gap represents a promising area for future studies in AEE, enabling its broader application to diverse oils.

Table 2
Optimal process parameters of enzyme in AEE in different oilseeds.

Type of enzyme	Source of oil	Optimal enzymatic conditions	Yield (%)	Reference
Cellulase	Rapeseed	Enzyme concentration: 0.2–0.5 mL/100 g, digestion time: 2 h, temperature: 50 °C, pH: /, S/L: 1 mL/100 g	61.8 %	(Thomsen et al., 2024)
	Walnut seed	Enzyme concentration:2.0 % , digestion time:110.91 min , temperature:56 °C , pH: 5.0 , S/L: 1:4	28.85 %	(Ghasemi & Taghian Dinani, 2018)
Pectinase	*Acrocomia aculeata* Fruit pulp	Enzyme concentration: 6.3 %, digestion time: /, temperature: 50 °C, pH: 5.5, S/L: 1:1, stirring speed 350 rpm	88.6 %	(Sorita et al., 2024)
	peony seed	Enzyme concentration: 0.15 %, enzyme digestion time: 1 h, temperature: 50 °C, pH: 4.5, S/L: 1:5 (*w/v*)	92.06 %	(Song et al., 2019)
Viscozyme®L	*Arachis hypogaea*	Enzyme concentration: 1.25 %, Enzymatic digestion time: 80 min, Temperature: 50 °C, pH: 4–5, S/L: 1:4 (w/v)	38.86 %	(Liu, Hao, Chen, & Zhu, 2020)
	Mamey Sapote	Enzyme concentration: 3.5 %, digestion time: 5.5 h, temperature: 50 °C, pH: 4, S/L: 1:3.5 (w/v)	66 %	(Tacias-Pascacio et al., 2021)
	Ricinus communis seeds	Enzyme concentration: 2 %, digestion time: 4 h, temperature: 50 °C, pH: 4.0, S/L: 1:5 (w/v)	~80 %	(Díaz-Suárez et al., 2021)
Alcalase 2.4L	Sunflower seeds	Enzyme concentration: 9 %, digestion time: 3 h, temperature: 40 °C, pH: 8.0, S/L: 1:5 (w/v)	16.4	(De Aquino et al., 2022)
	Sesame	Enzyme concentration: 10 %, digestion time: 8 h, temperature: 55 °C, pH: 7.0, S/L: 1:6 (w/v)	36.65	(Ribeiro et al., 2016)
Papain	Sacha inchi	Enzyme concentration: 4.46 %, Enzymatic digestion time: 4.95 h, Temperature: 38.9 °C, pH: /, S/L: 4.5 mL/g	28.45 %	(Nguyen et al., 2020)
	Peanut	Enzyme concentration: 1400 U/g, digestion time: 3 h, temperature: 55 °C, pH: 7.5, S/L: 1:3 (w/v)	92.39 %	(Niu et al., 2020)
Alkaline protease	*Pinus koraiensis* nuts	Enzyme concentration: 3.23 mg/g, digestion time: 2.84 h, temperature: 44 °C, pH: 8.0, S/L: 1:5 (g/mL)	68.35 %	(Wang, Zhang, et al., 2023)
	Rice bran	Enzyme concentration: 1.6 mg/g, enzyme digestion time: 150 min, temperature: 55 °C, pH: /, C: 1:4 (g/mL)	85.6 %	(Yu et al., 2022)

Note: / indicates not mentioned in the study.

6. Application of enzymes in demulsification

In AEE proces, released oil occurs as free oil and OB. Some free oil fails to aggregate into large droplets during agitation, forming stable emulsions with proteins, polysaccharides, cellular debris, and water (Cui et al., 2023). Phospholipids on OBSB similarly form emulsions in water. These emulsions limit crude oil yield and pose a significant bottleneck for industrial AEE applications (Yang et al., 2019). Reducing emulsion formation and promoting their disruption have become key research focuses in AEE.

Enzymatic demulsification is a primary method. Protease serves as the primary degrading enzyme, depending on the emulsion composition. The mechanism involves enzymatic hydrolysis disrupting proteins' secondary and tertiary structures, producing short peptides with

diminished ability to stabilize spatial and interfacial membranes. Li et al. found that Alcalase 2.4L achieved a free oil recovery of (86.9 ± 3.3) %, compared to (69.0 ± 4.0) % with amylase and (56.7 ± 1.2) % with phospholipase, highlighting proteins as the primary stabilizing agents in the emulsion. Among proteases, Protex 50FP and papain demonstrated emulsion breaking rates exceeding 90 % (Li, Zhang, Han, et al., 2017). Zhang et al. reported a 94 % oil yield by digesting a water/peanut emulsion at a 1:1 volume ratio, 1600 IU/g enzyme concentration, 50 °C, and 70 min using alkaline endopeptidase (Zhang et al., 2013).

Another enzyme commonly used in demulsification is phospholipase. Lamsal et al. disrupted soybean emulsions using phospholipases, showing that specific phospholipases target distinct sites on phosphoglycerides, significantly destabilizing the emulsion(Lamsal & Johnson, 2007). However, the use of a single enzyme often neglects the roles of phospholipids and carbohydrates in stabilizing emulsions. Additionally, inadequate screening of specialized enzymes and optimal conditions hinders effective emulsion breaking, leading to suboptimal results. Thus, enzyme compounding warrants consideration. Chaberand et al. employed a two-step demulsification process: first using alkaline peptide chain endonuclease, followed by lysophospholipase A1 at pH 4.5, achieving a 95 % oil yield (Morales Chabrand, 2007).

7. Influence of enzymes on the quality of plant oils

High-quality plant oils require optimal flavor, color, nutritional content, and physicochemical properties. Both color and flavor significantly influence sensory perception. Nutritional content includes FAs and bioactive compounds like tocopherols, phytosterols, and polyphenols, known for their health benefits. Physicochemical properties include peroxide value (PV), iodine value (IV), and acid value (AV). In AEE, oil quality depends on enzyme type and reaction conditions. The following sections explore how enzymatic processes affect oil quality.

7.1. Effects on physicochemical properties of plant oils

AV is a crucial indicator of free fatty acid (FFA) levels in vegetable oils, with lower AV signifying higher oil quality. Oils treated with AEE demonstrate lower AV compared to those obtained through conventional methods, such as Soxhlet extraction (SE). For instance, Xu et al. (2021) reported that rice bran oil extracted using AEE exhibited an AV of 1.92 mg KOH/g, significantly lower than the AV of 4.56 mg KOH/g found in SE oil. This reduction is attributed to diminished hydrolysis and oxidation during the mild enzymatic process. However, this process must occur under optimal enzymatic conditions. Extended digestion activates endogenous lipases that hydrolyze TAG at sn-1 and sn-3 positions. This activity promotes the release of FFAs and results in an increased AV (Machado et al., 2021). Additionally, extended enzymatic digestion can lead to secondary oxidation of UFAs in plants oils, further exacerbating the increase in AV (Li, Zhang, He, et al., 2017).

PV is a crucial indicator for evaluating the oxidation of oils, representing the primary oxidation product of oil degradation. Similar to AV, the ability of AEE to effectively inhibit the increase of PV in oils is largely attributed to its mild reaction conditions (Xu et al., 2021). Furthermore, the selection of enzymes can also influence the extent of oil oxidation. For instance, targeting lipases to hydrolyze TAG ester bonds can indirectly lower the PV of the reaction solution by eliminating oxidized lipids, as seen in the operation of saponification during vegetable oil refining and valorization with enzymes (Casali et al., 2021). Alkaline proteases, such as Alcalase, can hydrolyze lipid oxidation precursors at pH 8, thereby inhibiting free radical chain reactions and reducing PV levels (Liu et al., 2021). Immobilized lipase (Lipozyme TL IM) can mitigate oil oxidation by shortening the reaction time to 2 h and decreasing the accumulation of oxidation intermediates (Belinska et al., 2022). Notably, it is essential to carefully regulate the L/S ratio, as excessive water can worsen the emulsification of the oil, resulting in an increase in PV (Liu et al., 2021).

In the process of AEE, lipases and proteases hydrolyze TAG and proteins, releasing fat-soluble pigments (e.g., carotenoids and chlorophyll derivatives) as well as flavor-active compounds (e.g., aldehydes and ketones). The flavor components of vegetable oils primarily consist of N-heterocyclic compounds, O-heterocyclic compounds, sulfur compounds, furans, aldehydes, alcohols, esters, and volatile phenolic compounds, all of which are volatile when heated and release pleasant aromas. For instance, Lee et al. reported that the enzymatic digest of perilla powder, when heated with coconut oil in a maillard reaction system, yielded volatile compounds such as hexanal and 2-pentylfuran. These compounds impart nutty and roasted flavors, enhancing the overall taste profile (Lee et al., 2024). Numerous studies have also indicated that high-temperature pretreatment of oilseeds prior to AEE, such as roasting, facilitates the Meladic reaction of proteins and polysaccharides, resulting in the formation of pyrazole compounds that subsequently yield volatile compounds, thereby enriching the flavor of the oil (Van Boekel, 2006). Nevertheless, attention must be given to the impact of various processing conditions on oil quality, which is closely linked to flavor quality. As previously mentioned, excessive enzymatic degradation, characterized by excessively high reaction temperatures or prolonged enzymatic degradation times, can lead to oxidative rancidity in the oil, resulting in off-flavors that negatively affect consumer sensory evaluations.

The color of oil serves as a significant indicator for assessing its quality. Generally, oil that is light or bright in color and free from turbidity is deemed to be of high quality. Shende et al. demonstrated that maintaining the light yellow color of corn oil could be achieved by optimizing the process parameters of the AEE, which minimizes both the oxidation of the oil and the leaching of impurities while efficiently extracting the oil (Shende & Sidhu, 2016). Furthermore, consumers possess varying psychological expectations regarding the color of oils derived from different sources. For instance, reddish chili oil is preferred by many, while some fruit-based oils, such as olive oil, exhibit a greenish hue to align with consumer preferences. Consequently, the release of natural pigments from oilseeds is one of the objectives of AEE, which can be facilitated through careful enzyme selection. In the AEE of castor oil, lipase hydrolyzes the chlorophyll-lipid complex, leading to the release of chlorophyll and a more pronounced green coloration in castor oil (Wang et al., 2025). However, Inappropriate enzymatic conditions (excessive enzymatic time or high enzymatic temperatures) accelerate oil oxidation, producing polycarbon compounds that form melanoidins, causing the oil to darken. Therefore, to maintain the desirable color of the oil, it is essential to consider appropriate enzymatic conditions.

7.2. Nutritional composition

The FA composition of plant oils critically determines their nutritional value and functionality. AEE minimally affects FA composition due to its mild reaction conditions. Xu et al. compared AEE with SE for plant oil extraction. Although no significant differences were found in FA fractions, a significant difference was observed in their content. The UFAs content of rice bran oil extracted via AEE (76.31 %) surpassed that obtained through SE (75.24 %). Linoleic and oleic acids were highest, at 38.84 % and 34.31 %, respectively (Xu et al., 2021). Similarly, Yan et al. used Alcalase 2.4L to extract lentil oil, yielding a slightly higher UFAs content (98 %) than cold pressing (97 %) (Yan et al., 2016). Zhang et al. showed no significant differences in the FA composition of high oleic acid rapeseed oil across methods like cold pressing, hexane extraction, AEE, and subcritical butane extraction (Zhang, Gao, Fang, et al., 2023).

The mild conditions of AEE effectively preserve and enhance thermally unstable trace active components, such as polyphenols, sterols, and tocopherols. The phenolic and tocopherol contents, as well as the antioxidant activities of lentil oil extracted using Alcalase 2.4L, were significantly higher than those obtained through SE and cold pressing. This increase is attributed to the organic solvents used in SE, which tend to solubilize the active constituents in the oil, while the de-oiling process

diminishes their activity. In contrast, cold pressing often restricts the release of active constituents due to the incomplete disruption of the oilseed cells (Yan et al., 2016). Sorita et al. also found that the oil extracted from the pulp of *Acrocomia aculeata* using AEE is rich in carotenoids. These compounds are associated with biological activities and antioxidant properties, which help reduce the risk of metabolic diseases such as cardiovascular diseases and obesity, as well as decrease the extent of lipid oxidation (Sorita et al., 2024). Abd Rashid et al. further demonstrated that, compared to SE, AEE not only increased the carotenoid and phenolic content in palm oil but also reduced the degumming step in palm oil refining (Abd Rashid et al., 2021). Furthermore, the incorporation of specific enzymes can effectively enhance the extraction of target components. For instance, squalene, which aligns with the FA tails in palm cell membranes, can be enriched in virgin palm oil through the addition of phospholipases (Abd Rashid et al., 2023).

8. Effects of physical field assisted technologies on oil seed

Despite the excellent performance of AEE in green oil production, the production process is hindered by high enzyme costs and time-consuming procedures. To enhance efficiency and reduce production costs, optimizing key steps and integrating supportive technologies is essential. Notably, physical field technology facilitates the loosening of densely arranged cells in the oilseed, effectively breaking down the polysaccharide barriers of CWs, disrupting the structure of cell membranes, and allowing for the easier release of oil that is tightly bound to other macromolecules within the oilseed cells. This also enables enzymes to penetrate the interior of the oilseed cells more effectively, thereby improving the oil yield. Concurrently, optimizing process conditions can enhance the characteristics of oilseeds and improve oil quality (Table 3).

8.1. Ultrasound-assisted aqueous enzymatic extraction (UAAEE)

Ultrasound primarily utilizes the cavitation effect to induce structural damage to oilseed cells, enhance matrix porosity, and accelerate the decomposition of the CW. The cyclic oscillation of the cavitation bubbles increases the intracellular space of the oilseed cells, improves cell membrane permeability. This process promotes the release of cell contents and enhances the permeation of enzymes, thereby increasing the contact area between enzymes and substrates, improving the reaction rate, shortening the reaction time, and ultimately enhancing the yield (Li et al., 2021). For instance, Kumar et al. (2022) utilized UAAEE to extract oil from sea buckthorn berries, achieving a higher UFA content compared to conventional methods (Kumar et al., 2022). Similarly, Liu et al. (2022) applied UAAEE in conjunction with pectinase to extract oil from hickory kernels, which not only improved the oil yield but also enhanced its antioxidant properties by increasing the levels of functional components such as total phenols, squalene, phytosterols, and oleic acid (Liu et al., 2022). Additionally, the mechanical effects generated by ultrasound directly impact the enzyme molecules, disrupting weak interactions such as hydrogen bonding and van der Waals forces, and altering their secondary and tertiary structures. This alteration results in improved enzyme activity and stability. Ma et al. demonstrated that ultrasound treatment significantly increased enzyme activity, with the highest pectinase activity observed at an ultrasound power density of 4.50 W/mL for 15 min. Furthermore, at lower enzyme concentrations, the degree of modification of pectinase by ultrasound was higher, resulting in a significant increase in pectinase activity. This made it possible to achieve relatively ideal enzymatic hydrolysis effects at low enzyme concentrations, which helped to reduce the amount of enzyme used (Ma et al., 2020).

However, optimal ultrasound power and treatment duration are crucial. Juhaimi et al. found that 30 min of ultrasound treatment maximized oil production in hazelnut and black cumin seeds. However, the physical and thermal effects produced by excessive sonication may lead to enzyme denaturation, thereby reducing enzymatic efficiency and resulting in lower oil yields. Additionally, thermal effects can diminish the oleic acid content in hazelnut oil and the linoleic acid content in peanut oil, adversely affecting oil quality (Al Juhaimi et al., 2018). Therefore, careful optimization of ultrasound power and treatment duration is essential.

8.2. Microwave-assisted aqueous enzymatic extraction (MAAEE)

Microwave irradiation increases the internal temperature of oilseed cells, leading to the formation of water vapor and the electroporation effect, which subsequently elevates intracellular pressure and disrupts the CW. Kaseke et al. demonstrated the deformation and rupture of oilseed cells following microwave treatment, as well as the rupture of lipoprotein membranes on the surface of the OBs (Kaseke et al., 2020). This indicates that the cell surface membrane produces pores that facilitate the entry of enzymes and the release of intracellular substances, thereby improving the efficiency of oil extraction. For instance, Li et al. showed that MAAEE significantly increased the oil yield of oat kernel oil by up to 55.8 %, while also enhancing the content of UFA and oxidative stability (Li et al., 2013). Similarly, Arroyo et al. reported that MAAEE achieved a 33.7 % higher yield of Jicaro seed oil compared to SE (Arroyo et al., 2019). Additionally, the extraction rate of Tephrosia chinensis seed oil reached 94.54 % after microwave pretreatment and alkaline protease hydrolysis before AEE (Chemat et al., 2019). The efficient mass transfer technology of microwave treatment allows for uniform heating of the oil, which can stimulate flavor compounds and enhance the release of nutritional factors, thereby improving the overall quality of the oil. Abd Rashids et al. utilized palm fruit peel as a raw material, applying microwave treatment at 850 W for 1 min followed by enzymatic digestion, ultimately producing squalene-rich palm oil with a concentration of (961.77 ± 53.16) mg/100 g (Abd Rashid et al., 2023). While microwave treatment offers numerous advantages, it also presents the drawback of uneven heating. This issue arises from the accumulation of electromagnetic wave energy and the uneven temperature distribution during microwave treatment, leading to localized overheating and underheating of the oil. Therefore, it remains essential to investigate and control the conditions of MAAEE to achieve efficient oil extraction.

8.3. Pulsed electric field-assisted aqueous enzymatic extraction (PAAEE)

Pulsed electric field (PEF) technology utilizes short bursts of high-voltage electric fields to treat biomaterials, inducing electroporation that disrupts cell membranes and increases permeability. This process facilitates the release of intracellular components, such as lipids, and enhances enzyme-substrate interactions. For instance, Ferraz et al. demonstrated that PEF significantly increased oil production by breaking down CWs and allowing deeper enzyme penetration into plant tissues (Ferraz & Silva, 2025). Similarly, Moradi et al. highlighted that in sunflower oil extraction, PAEE with an electrolysis index of 0.70 significantly improved mass transfer efficiency, enhanced cell disruption and enzyme penetration, and achieved higher oil yields with lower energy consumption (Moradi & Rahimi, 2019). Ranjha et al. emphasized the role of PEF in promoting enzyme-induced autolysis, which is essential for breaking down cellular structures during oil extraction (Ranjha et al., 2021). Therefore, PAAEE offers a sustainable and efficient alternative to conventional oil extraction methods.

8.4. Other auxiliary methods

Ultrasound and microwave technologies hold significant potential in enhancing AEE. However, limited thermal effects of ultrasound and uneven heating of microwaves constrain further advancements in AEE. Research has shown that combining these technologies effectively disrupts CW and improves enzymatic efficiency. Hu et al. employed a

Table 3
Extraction of oil and related parameters from oilseeds by intensification extraction technologies of AEE.

Oilseed	Enzyme	Enzymatic conditions						Oil quality	Oil yield (%)	Ref.
		Enzyme addition (wt%)	Temperature (°C)	Time (h)	S/L	pH	Auxiliary means			
Hemp seed	Cellulase/ Hemicellulase/ Pectinase = 1/1/ 1, *w*/*w*/w	37,800 U/ g	32	189 min	10.4 mL/g	5	Ultrasonic-ethanol pre-treatment: ultrasound 200 W, 60 % (*v*/v) ethanol, combined treatment 30 min	AV: 1.76 mg KOH/g; PV: 0.93 meq O_2/kg; Iodine value: 167.80 g I_2/ 100 g; Saponification value: 123.41 mg KOH/g; C18:2 (linoleic acid, 54.82 %); C18:3 (α-linolenic acid, 19.25 %); ω-6/ω-3 ratio: 3.59; Δ9-THC: 9.58 mg/kg	23.32	(Zhang et al., 2024)
Sunflower Seed	Cellulase /Pectinase = 2/1, w/w	2	40	2	1:6	4.5	Ultrasonic 250 W; Pulsed electric field strength 1.2 kV/cm	PV of PAAEE: 6.13 meq O_2/ kg; PV of UAAEE: 17.11 meq O_2/kg; Tocopherols for UAEE: 543.5 mg/kg; Tocopherols of PEF-AEE: 561.01 mg/kg	UAAEE: 45.13; PAAEE: 28.13	(Moradi & Rahimi, 2019)
Moringa oleifera seed	Neutrase 0.8 L/ Celluclast 1.5 L = 3/1, *w*/w	2	50	12.5	1:4 g/g	/	High Pressure Processing, 50 MPa	High oxidative stability	73.02	(Yusoff et al., 2017)
Gardenia fruits	Cellic CTec3, Alcalase 2.4 L	1.7, 1.3	45, 51	2	1:6 g/ mL	/	Ultrasonic power: 480 W; Processing time: 30 min	Oleic acid (18:1): 26.26 %; Linoleic acid (18:2): 49.75 %; VE: 1.01 μg/mg; Squalene: 333.36 μg/g; α-Tocopherol: 0.86 μg/mg	18.65	(Wang et al., 2023)
Cherry seed	Cellulase/ Hemicellulase/ Pectinase = 1/1/ 1, w/w/w	2.7	40	4	12 mL/ g	3.5	Ultrasonic power: 560 W; Microwave power: 323 W; Processing time: 38 min	AV (mg KOH/g): 2.42 ± 0.01 PV (meq O_2/kg): 2.56 ± 0.05 Oleic acid (18:1): 53.82 %; Linoleic acid (18:2): 38.25 %; Total phenolics: 78.85 ± 2.14 mg GAE/kg oil; VE: 155.39 ± 5.24 mg/kg oil; β-carotene: 26.03 ± 0.62 mg/kg oil; Phospholipids: 0.40 %; Total phytosterols 462.35 ± 18.77 mg/100 g oil; β-sitosterol 225.64 ± 15.16 mg/100 g oil; AV (mg KOH/g): 3.64 ± 0.21; PV (meq O_2/kg): 12.17 ± 0.41	83.85	(Hu et al., 2019)
Sunfower seeds	Alkaline serine endopeptidase	2.835	45.87	2	1:7.375	8	Automatic continuous stirred tank reactor with 120 s^{-1} stirring rate	High oleic acid content: 75–91 %	82.18	(Munder et al., 2020)
Peanut	Pectinase	1.5	50	1	1:4 (w/ v)	4.2	Microwave, 700 W, processing 4 min	Increased protein and oil body production	48.4	(Guo et al., 2024)
Camellia	Cellulase/ Alcalase = 1/1, w/ w	800 mg/ 50 g Camellia powder	50	7	1:8 (w/ v)	9.0	Ultrasonication power: 100 W; processing time: 30 min	Oleic acid (C18:1): 80–83 %; Linoleic acid (C18:2): 7–13 %; Significant reduction of tea saponin content in the oil (from 2.89 % to 2.31 %)	78.03	(Lu et al., 2023)
Peanut	Viscozyme®L	/	50	2	1:4(w/ v)	/	Baking 90 °C, 30 min	Oleic acid (C18:1): 37.37–39.02 %; Linoleic acid (C18:2): 37.28–41.05 %; AV: 0.18–0.35 mg KOH/g; PV: 0.03–0.12 g/100 g;	92.21	(Zhang, Chen, Liu, et al., 2023)

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Table 3 (*continued*)

Oilseed	Enzyme	Enzymatic conditions						Oil quality	Oil yield (%)	Ref.
		Enzyme addition (wt%)	Temperature (°C)	Time (h)	S/L	pH	Auxiliary means			
Sapium sebiferum seed	Neutral proteinase	2.95	50	3	8 mL/g	/	Ultrasound 485 W, 39 min	Tocopherol: 24.56–25.24 mg/100 g; α-Tocopherol: 10.51–11.49 mg/100 g; γ-Tocopherol: 12.46–13.22 mg/100 g; δ-Tocopherol: 0.98–1.06 mg/100 g Linolenic acid (C18:3): 64.33 %; Linoleic acid (C18:2): 23.66 %; AV: 4.61 mg KOH/g; IV: 186.85 g I_2/100g	40.60 %	(Liu et al., 2023)
Cucurbita pepo L.	Cellulase/ Pectinase/Neutral proteinase = 1/1/ 1, w/w/w	1.05	45	69 min	/	4–6	Negative pressure cavitation system: Vacuum: −0.07 MPa Extraction time: 69 min	Linoleic acid (C18:2): 47.67 %; Oleic acid (C18:1): 37.35 %; VE: 843.92 mg/kg oil; Polyphenol: 117.74 mg GAE/kg oil; PV: Lower than international standard, 2.08 meq O_2/kg; AV: 1.40 mg KOH/g; α-Amylase inhibitory activity: IC_{50} is 40.68 μg/mL	58.06	(Li et al., 2016)
Peanut	Alcalase 2.4L	1.5	60	3	1:5 w/v	8.5	Short-wave infrared radiation: 150 °C, 55 min	Polyphenols: 2.79 ± 0.05 mg GAE/kg Total Sterols: 1182.62 ± 49.51 mg/kg β-sitosterol: 830.98 mg/kg; Campesterol: 203.15 mg/kg; Soy sterols: 148.49 mg/kg; Tocopherols: 420.09 ± 8.04 mg/kg; α-Tocopherols: 223.98 mg/kg; γ-Tocopherols: 158.87 mg/kg Oleic acid (C18:1): 63.13 %; Linalool: 48.63 %; Methylcyclopentane: 21.69 %;	83.75	(Deng et al., 2018)
Macadamia	Cellulase/ Pectinase/ Proteinase = 1/ 1:1, w/w/w	1.60 %	45	0.5	/	6.0	Microwave 450 W	DPPH free radical scavenging capacity: IC50 value of 94.42 mg/mL; β-carotene bleaching inhibitory ability: IC50 value of 131.68 mg/mL Oleic acid (C18:1): 22.09 %; Linoleic acid (C18:2): 51.56 %; Linolenic acid (C18:3): 0.50 %;	/	(Ma et al., 2022)
Corn Germ	Cellulase and α-amylase	/	50	2	/	4.8	Ultrasonic 20 min	AV: 3.36 mg KOH/g; POV: 0.86 meq/kg; IV: 102.72 g/100 g; DPPH radical scavenging: IC50 value of 27.38 mg/mL; Hydroxyl radical scavenging: IC50 value of 1.574 mg/mL	67.54	(Han et al., 2018)
Pecan Nut Kernel	Cellulase/ Hemicellulase/ pectinase/ Neutrase	2.9	53	2.0	4 mL/g	4.0	Ultrasonic power 432 W, 20 min	Oleic acid (C18:1): 70.4 %; Linoleic acid (C18:2): 19.11 %; Linolenic acid (C18:3):	78.83	(Liu et al., 2022)

(*continued on next page*)

Table 3 (*continued*)

Oilseed	Enzyme	Enzymatic conditions						Oil quality	Oil yield (%)	Ref.
		Enzyme addition (wt%)	Temperature (°C)	Time (h)	S/L	pH	Auxiliary means			
	=1:1:1:1, w/w/w/w							4.17 %; Polyphenols:14.17 mg GAE/kg; Squalene: 1963.42 mg/kg; γ-Tocopherol: 26.26 mg/100 g; α-Tocopherol: 0.04 mg/100 g; Phytosterols: 1068.1 mg/100 g; β-Sitosterol: 892.06 mg/100 g; Stigmasterol: 123.21 mg/100 g; Campesterol: 52.83 mg/100 g; AV: 2.48 mg KOH/g; POV: 1.43 mEq/kg		
Litsea cubeba	Hemicellulase/Pectinase/Protease = 1/1/1, w/w/w	3 (w/v)	52.78	2.53	9.31 mL/g	5.0	Ultrasonic power 400 W, Microwave 540 W	D-Limonene:17.94–18.81 %; *trans*-Citral: 14.56–16.92 %; *cis*-Citral: 11.88–13.35 %; Citronellal: 10.25–11.67 %	240.56 (mL/kg Dry weight)	(Yang et al., 2024)
Tiger nut	Cellulase/Hemicellulase/Pectinase = 1/1/1, w/w/w	2	45	4.9	10 mL/g	3	Ultrasonic power 460 W, Microwave power 300 W	Polyphenol: 83.57 mg GAE/kgoil; α-Tocopherol: 329.76 mg/kg; Sterols: 673.92 mg/100 g, β-Sterols: 502.75 mg/100 g AV: 2.35 mg KOH/g; PV: 7.63 meq O_2/kg; Oil light in color; L*: 72.76	83.85 %	(Hu et al., 2020)
Peanut	Cellulase	1.47	56	2.0	1:4 g/mL	4.61	Ultrasound power 250 W, 33.23 min	Polyphenol: 411.40 mg GAE/kg; IV: 3.50 meq O_2/kg; L*: 4.44 (Lighter oil color); b: 10.63 (Oil color more yellow)	27.35 %	(Heidari & Dinani, 2018)

Note: / indicates not mentioned in the study.

combined ultrasound-microwave treatment to enhance enzymatic hydrolysis during oil extraction from *Cyperus esculentus*. Optimization of ultrasound-microwave treatment, followed by enzymatic hydrolysis, increased the oil yield from *Cyperus esculentus* to 85.23 %. The cavitation effect of ultrasound, microwave radiation, and enzyme preparation synergistically disrupt cell structures, facilitating oil extraction (Hu et al., 2020).

In addition to other physical field processing techniques, such as high pressure (HP) and infrared radiation (IR), the combination of these methods with AEE has been shown to enhance the efficiency of oils. HP typically utilize water as a medium to pressurize the system, which disrupts the cellular structure of the oilseed. This disruption leads to increased cell permeability, facilitating the exchange of environmental substances both inside and outside the cell. Yusoff et al. (2017) extracted moringa seed oil using ultra-high pressure (UHP) in conjunction with AEE, finding that UHP not only increased the yield of clear oil but also reduced the emulsion layer. This reduction was attributed to the physical modification of moringa seed proteins by UHP, which caused structural changes that diminished their emulsifying properties. Consequently, this streamlined the process of demulsification (Yusoff et al., 2017). IR involves the transfer of energy through radiation from an infrared generator, where the oil absorbs this energy and converts it into thermal energy, resulting in uniform heating. This heat treatment is conducive to improving the flavor quality of the oil. For instance, Deng et al. treated peanuts with short-wave infrared radiation (SIR) at 150 °C for 55 min prior to enzymatic digestion. Compared to the control group, the peanut oil extraction rate increased by 8.74 %, and the polyphenol content rose by 62.21 %, thereby enhancing the nutritive properties of the peanut oil. The heat energy provided by SIR facilitated a Maillard reaction, which increased the volatilization of pyrazines and pyridines, culminating in peanut oil with a strong nutty aroma (Deng et al., 2018). The combination of enzymatic extraction methods with other auxiliary techniques can increase the oil yield from plant sources; however, it complicates the operation and requires high-energy-consuming equipment. Therefore, the process needs further optimization to suit industrial production. Additionally, the heat generated by these auxiliary methods may alter the quality of the oil. Some thermosensitive active substances can easily degrade when exposed to heat. Therefore, plant oils containing such substances, such as olive oil and perilla seed oil, should be comprehensively evaluated for the impact of auxiliary methods on both yield and quality.

9. Future trends and challenging opportunities

The utilization of complex enzymes, in conjunction with various physical field technologies, enhances enzyme efficiency and consequently increases both oil yield and quality. However, the intricacy of this process necessitates precise control over enzymatic parameters, such as pH and temperature, to sustain the activity of each enzyme involved. Furthermore, employing multiple enzymes escalates costs and generates substantial wastewater post-enzymatic hydrolysis, which further inflates expenses associated with aqueous enzymatic oil

extraction, thereby diminishing its economic viability and limiting the industrial application of AEE. Thus, there is an urgent need to develop cost-effective enzyme engineering solutions. For instance, microbial fermentation can be harnessed to produce heat-resistant and substrate-specific enzymes, thereby reducing the diversity and quantity of enzymes (Patel et al., 2023). The application of specific enzymes can effectively target and regulate various nutritional factors. For instance, the use of phytase during oilseed processing significantly reduces the phytic acid content in canola meal, thereby enhancing the quality of oilseed by-products (Darambazar et al., 2019). Immobilized enzyme technology facilitates the separation of enzymes from products, enables reuse, and enhances enzyme stability and longevity. The immobilization of cellulase in Fe_3O_4/SiOx-g-P (glycidylmethacrylate) within a magnetic fluidized bed not only improves enzyme utilization but also, when combined with magnetically immobilized protease, allows for the efficient extraction of rice bran oil (Yu et al., 2022). Furthermore, in addition to the aforementioned physical field-assisted technologies, the integration of AEE with other emerging technologies, such as subcritical water extraction or deep eutectic solvents, can minimize wastewater generation and reduce costs while being more environmentally friendly (Lampakis et al., 2021). In summary, the future of AEE will focus on low cost, high extraction rates, and high quality as the primary development directions.

10. Conclusion

AEE is a safe and sustainable oil extraction technology that aligns with modern trends of low carbon emissions and safety. It produces high-quality oil without altering FA composition and meets quality standards by eliminating the refining step. Additionally, it retains more beneficial components, catering to contemporary nutritional and health demands. To enhance AEE efficiency, it is crucial to understand the components, structures, and cellular microenvironments of different oilseeds and their extraction sites. Based on this, enzymes must be precisely selected, and process parameters (enzyme concentration, temperature, time, pH, and L/S ratio) optimized to improve hydrolysis efficiency. The relationship between process parameters and enzyme activity should also be considered, as vigorous molecular activity increases enzyme-substrate interactions, boosting hydrolysis and oil yield. Oil quality is also key, and consumer demands must be considered. For instance, for high-nutritional plant oils, enzyme activity should be maintained while minimizing extraction temperatures to prevent degradation of active substances. For flavor-type oils, moderate heating is needed to enhance aromatic compound release. These outcomes can be achieved by fine-tuning enzymatic hydrolysis parameters. When integrating physical field-assisted technologies with AEE, it is necessary to account for the thermal energy introduced, its effects on enzyme and protein structures, and economic costs. Careful adjustments to power and duration are required to balance efficiency and cost. Future advancements in enzyme engineering will aim to reduce reaction costs while improving oil and by-product quality. Considering the specific characteristics of various oilseeds and their market niches, achieving significant reductions in enzyme costs and enhancing digestion efficiency will be critical for the broader application of AEE.

CRediT authorship contribution statement

Xiujun Lin: Writing – original draft, Methodology, Data curation, Conceptualization. **Zihan Ma:** Visualization, Software, Investigation. **Fangfang Liu:** Software, Data curation. **Yang Li:** Supervision, Resources, Project administration. **Huanyu Zheng:** Writing – review & editing, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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