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Application of Lipase in Food Biotechnology

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Abstract. Lipase is a biocatalyst capable of catalyzing the hydrolysis or synthesis of triglycerides. It is widely distributed in animals, plants, and microorganisms. Owing to its high efficiency, specificity, and mild reaction conditions, lipase has demonstrated significant value in food processing, nutritional enhancement, and waste treatment. This paper reviews the sources of lipase and its applications in food processing, flavor improvement, and nutritional enhancement, as well as discusses its future development trends. Lipase can be utilized in oil modification, dairy product processing, baked goods, meat product processing, and other areas to enhance food quality and nutritional value. Furthermore, lipase exhibits substantial potential in the utilization of food waste resources and the development of functional foods. With advancements in enzyme engineering technology and deeper insights into the mechanism of lipase, its application in food biology will become increasingly extensive and profound.

Keywords: Lipase, Food biology, Food processing

1. Introduction

Lipases (EC 3.1.1.3) are members of the carboxyl ester hydrolase family, which catalyze the hydrolysis of triglycerides into glycerol and fatty acids by acting on the ester bonds of triglycerides. The active site residues of lipases consist of serine, aspartate, and histidine, forming a catalytic triad characteristic of serine proteases. In homogeneous aqueous solutions, lipases typically adopt a closed conformation, with their active centers shielded by a hydrophobic domain, such as a "lid" formed by the folding of alpha-helices or beta-sheets, resulting in low enzymatic activity. Upon encountering an oil-water interface or hydrophobic substrate, the enzyme molecules adsorb to the interface via hydrophobic interactions, inducing a conformational change: the "lid" structure opens, exposing the hidden hydrophobic active sites and transitioning to an open conformation. This interfacial activation mechanism markedly enhances catalytic efficiency in heterogeneous systems. Consequently, the varying activities of lipases depend on the specific reaction systems, such as promoting ester hydrolysis at the oil-water interface and facilitating esterification or transesterification in organic phases. As a critical biocatalyst, lipases exhibit high catalytic efficiency, strong specificity, and mild reaction conditions, making them widely applicable in food, pharmaceuticals, and chemical industries [1]. In recent years, with the improvement of living standards and heightened health awareness, the demand for improved food quality and nutritional value has increased. As a green and safe biocatalyst, lipases have demonstrated significant

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application potential in the field of food biology [2]. In contrast, surface active agents and emulsifiers commonly used in processing such as sucrose fatty acid ester and glycerol monostearate need to be synthesized chemically, and are often accompanied by impurities, and their purity is difficult to control [3].

Through the catalytic action of lipases, the texture, flavor, and nutritional value of food can be improved, and more functional foods that meet consumer needs can be developed. However, there are still problems in the current application of lipases, such as the interference effect of food matrices on lipases, the environmental sensitivity of lipases that limits their application scenarios, and the high production cost of enzyme preparations.

This article aims to review the current application status of lipases in food biology from aspects such as flavor improvement, nutritional improvement, and waste processing, and explore their future development trends, to provide references for further research and application of lipases in the food field.

2. Biological characteristics of lipase

2.1. Sources of lipasee

Microorganisms are the main source of industrial lipases because of their rapid growth, genetic modifiability, adaptability, and efficient fermentation. Bacterial and fungal lipases are mostly extracellular, facilitating industrial extraction [4]. Yeast lipase, usually intracellular, requires cell wall disruption; however, some yeasts exhibit a high affinity for hydrophobic substrates [5]. Specific microbial lipases offer valuable characteristics for chemical synthesis: P. fluorescens [5] and P. cepacian [6] produce thermostable (50-70℃) and organic solvents-tolerant lipases suitable for lipid hydrolysis and transesterification (cocoa butter substitutes). Candida antarctica lipase B (CALB) has high stereoselectivity and is used to synthesize food-grade esters (such as flavors and fragrances) [7].

Animal and plant lipases, although available, are rarely used industrially due to cost, ethical considerations, and scale limitations. Animal-derived pancreatic lipases are mainly utilized in dairy products and digestive aids [8]. Castor seed lipase, while heat-resistant, requires strict purification due to the toxic ricin component [9]. Other examples include papaya lipase [10] and wheat germ lipase [11].

2.2. Catalytic properties of lipase

Lipase's catalytic versatility significantly impacts its application in food processing, dairy production, baking, and flavor enhancements [12]. Regiospecificity allows selective hydrolysis of a triglyceride (e.g., 1,3 or 2 positions) or for a specific fatty acid (e.g., short or long-chain fatty acid) [13,14]. Thermostability ensures activity at high temperatures (50-90℃), ideal for high-temperature processing [15]. pH Tolerance varies with enzyme source, influencing application conditions [16]. Stereoselectivity enables precise catalytic hydrolysis or ester bond formation involving chiral substrates [17]. Lipase activation occurs via interfacial activation, involving a structural shift exposing the active site upon hydrophobic interaction at oil-water interfaces [18]. Broad Substrate Specificity allows action on various substrates such as triglycerides, phospholipids, and sterols [19], while organic solvent tolerance facilitates non-aqueous catalytic reactions [20]. The texture, flavor, nutritional value, and safety of food can be accurately regulated through reasonable selection or modification of these characteristics.

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3. Application of lipase in the food industry

3.1. Modified food flavor

Hydrolysis of milk fat by lipase to generate short-chain fatty acids (such as butyric acid) can create unique flavors in cheese and butter. Lipase hydrolyzes the ester bonds of triglyceride, the main milk fat, into glycerol and free fatty acids [21]. Some lipases selectively hydrolyze the sn-1 and sn-3 positions of triglyceride, releasing short-chain fatty acids (C4-C12) [22]. The short-chain fatty acids generated by hydrolysis directly contribute to the unique flavor of dairy products. Butyric acid in butter, for example, has an iconic fermented bean curd and cheese-like odor, presenting a creamy aroma at low concentrations and a pungent taste at high concentrations, contributing about 50% of the volatile flavor compounds of fermented butter [23].

Similarly, in meat processing, lipases release free fatty acids (FFA) from animal fats, which subsequently form complex aroma compounds through reactions such as oxidation (e.g., hexanal in ham). A typical example is the formation of hexanal and 3-octen-1-ol from the oxidation of oleic acid, which gives Xuanwei ham its unique aroma [24]. Optimizing enzyme type, temperature, and reaction time allows precise control over product flavor profiles.

3.2. Improve food properties

In cheese production, lipases accelerate flavor formation and shorten ripening periods. Lipase addition promotes lactone formation in cheddar cheese and significantly improves cheese texture [25]. Besides shortening the ripening period, improving texture is also an important function of lipase. Reconstituted cheese with added lipase has significant differences in hardness, elasticity, adhesiveness, and cohesiveness [26].

In baking, lipase hydrolyzes flour triglycerides into monoglycerides (MAG), significantly improving the gluten network structure and enhancing the extensibility. Lipase hydrolyzes the ester bonds of triglycerides to generate MAG and FFA, among which MAG is the core pathway for enhancing the stability of the gluten network [27]. The polar head of MAG binds to the hydrophobic regions of gluten proteins, and the non-polar tail inserts into the lipid phase, forming a "protein-MAG-lipid" ternary complex. In this complex, MAG acts as a "molecular bridge", promoting the cross-linking of cysteine (-SH) in gliadin and the hydrophobic regions of glutenin, enhancing the compactness of the gluten network. Additionally, MAG can encapsulate free fatty acids, delay oxidation, and rancidity, and maintain the processing stability of the dough [28]. However, excessive lipase (beyond 0.02% flour weight) can lead to undesirable rancid flavors [29].

3.3. Produce functional lipids

Lipases are also used in the production of functional lipids such as medium and long-chain triglycerides (MLCT), which combine rapid energy supply and anti-obesity properties. Compared with traditional oils, the MCFA in MLCT is metabolized quickly, reducing fat storage, while the LCFA is metabolized slowly, prolonging satiety and stabilizing blood sugar. Moreover, the metabolic process of MLCT consumes more energy, making MLCT a promising representative of the next generation of healthy lipids [30]. Lipase-catalyzed transesterification efficiently synthesizes MLCT, which offers metabolic benefits.

Additionally, lipases create low-calorie fat replacers, reducing oil caloric content by approximately 30% without compromising texture or flavor [31]. Structured lipids synthesized

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enzymatically have been clinically shown to decrease body fat significantly. Clinical trials have shown that a daily intake of 20g of edible oil containing MLCT can reduce body fat by 3-5% [32].

3.4. Dispose of food processing waste

The lipase catalytic method can extract fish oil from fish processing by-products such as fish heads, organs, bones, and skin. Through enzymatic hydrolysis combined with physical adsorption and molecular distillation, fish oil can be produced with low energy consumption and minimal environmental impact. Fishy odors mainly caused by compounds like trimethylamine (TMA), aldehydes, ketones, and oxidation products are reduced using oxidase pre-treatment followed by lipase-assisted triglyceride hydrolysis [33]. Experiments showed that using immobilized Rhizopus oryzae lipase (ROL) increased tuna oil yield from 65% to 88%, with TMA reduced to below 5 ppm [34]. Compared to solvent extraction, the enzymatic method lowers overall production costs by 15-20% and reduces CO_2 emission by 30-40%. This approach also preserves nutrients such as omega-3 fatty acids, which meets the standards of food and pharmaceutical-grade fish oil [35]. The characteristics of lipases required for different food industry processing requirements are also different (see Table 1).

Table 1. Characteristic requirements and application cases of lipase in the food industry

Application field	Catalytic characteristic requirement	Specific case
dairy product	acidic pH tolerance location specificity	cheese maturation [36] yogurt flavor enhancement [37]
bake	thermal stability substrate broad spectrum	bread volume improvement [38]
oil modification	stereoselectivity	production of zero trans fatty acid fats [39]
flavored food	Stereoselectivity organic solvent tolerance	synthetic natural vanillin [40]
functional food	location specificity broad spectrum of substrate	production of OPO structure lipids [41]

4. Technological advancements in lipase utilization

4.1. Immobilized enzyme technology

Natural lipases often suffer from weak structure, poor reusability, and difficult separation, which limit their application in the industrial field. Immobilized enzyme technology blocks enzymes in solid materials or restricts them to the catalytic action area, which significantly improves the stability, reusability, and catalytic efficiency of the enzyme.

To improve recovery and reuse, magnetic nanoparticles (MNPs) such as Fe_3O_4@SiO_2 can be covalently bound to lipases through surface amino or epoxy groups, enabling rapid magnetic field recovery. These immobilized enzymes maintain over 90% activity even after 10 reuse cycles [42]. Similarly, 3D printing offers customized carriers with optimized pore structures. Chitosan-Ziziphi Spinosae protein-lipase composite particles have been shown to achieve a reaction conversion rate of 74.58±1.96%, 3.67 times that of the free enzyme system, retaining over 85% activity after 5 cycles [43].

Click chemistry, such as copper-catalyzed azide-alkyne cycloaddition, achieves high binding strength and an enzyme shedding rate of less than 5% [44]. To date, this approach has been

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successfully applied in the preparation of MLCTs and other domains. Carrier-free immobilization via crosslinked enzyme aggregates (CLEAs) reduces cost and enhances stability. CLEAs show improved thermal and pH tolerance. Following thermal preservation at 70℃ for 2 hours, residual enzyme activity remains at 54.88%. Moreover, after 10 cycles of repeated use, the residual enzyme activity of CLEAs is maintained at 50.26% [45].

Encapsulating lipase within phospholipid bilayers to mimic cellular membrane environments is another effective strategy for enhancing enzyme stability and application versatility. This technique's core advantages in the food industry include extended enzyme lifespan, precise catalytic control, and compatibility with clean-label requirements. It currently finds applications in areas such as milk powder processing [46], fat modification [47], and beyond.

4.2. Non-aqueous catalytic system

Traditional aqueous systems often face issues such as low substrate solubility and undesired side reactions. In contrast, non-aqueous catalytic systems can enhance the catalytic efficiency, selectivity, and sustainability of lipase [48]. These systems offer valuable applications in functional lipid synthesis, flavor production, and oil modification. Advances in materials, solvent systems, and reactor design have expanded their industrial relevance, aligning with green chemistry goals.

4.2.1. New non-aqueous solvent

Compared with organic solvents as extractants, ionic liquids (ILs) exhibit higher solubility, recyclability, low volatility, and structural designability. When temperature-sensitive ionic liquids are employed in lipase-catalyzed reactions, the product and solvent can be automatically stratified through cooling. In milk fat extraction, CALB lipase separates short-chain and long-chain triglycerides within temperature-sensitive ILs, achieving a recovery rate of over 90% [49]. Deep eutectic solvents (DESs) are environmentally friendly biocatalytic media due to their straightforward preparation, tunable properties, and biodegradability. The micro-water depth eutectic solvent enhances enzyme thermal stability by replacing CALB with hydrophobic DESs, whose carboxyl groups and terpene compounds DESs contribute positively to enzymatic activity enhancement [50]. Based on the correlation between DES properties and enzyme activity, DESs can be further tailored as optimal media for lipase-catalyzed reactions.

4.2.2. New non-aqueous carriers

A biodegradable membrane can serve as a carrier for lipase, enabling it to function at the oil-water interface. The catalytic efficiency of the nanofiber membrane-lipase reactor was 2.43 times higher than that of free enzymes [51]. Additionally, an optimized sandwich-type lipase-nanofiber membrane reactor achieved a catalytic efficiency 2.05 times higher than that of free enzymes, with the enzyme loading of the sandwich structure being controllable by adjusting the spinning time. Mesoporous silica foams (MCFs) have large pore sizes, high pore volume, extensive specific surface area, and ease of surface modification. Jin Wenbin modified MCFs using alkyl silanes of varying carbon chain lengths and utilized them as carriers for lipase immobilization [52]. The activity for synthesizing ethyl caprylate reached up to 20 times that of free enzymes. When aldehyde acacia was used as a coupling agent for enzyme immobilization, the resulting immobilized enzyme exhibited enhanced organic phase thermal stability and reusability.

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4.3. Precise development using artificial intelligence

Lipases currently face three major challenges in the food industry: cost control, enzyme stability, and regulatory compliance. To address these issues, researchers should focus on integrating advanced technologies such as metabolic engineering, structural biology, and artificial intelligence to overcome the bottlenecks in lipase application. Artificial intelligence can be employed to design and predict enzyme structures and functions, thereby accelerating the development of enzyme preparations [53]. De novo protein design offers the potential to transcend the limitations of natural evolutionary templates, expanding the functional sequence space of enzymes and providing innovative approaches for enzyme design. Ren Mengyuan [54] utilized a deep learning model based on Generative Adversarial Networks (GAN) to design malate dehydrogenase and fructose-1,6-diphosphate aldolase from scratch, thus offering new insights and technical references for the design of other enzymes. Constructing an integrated platform combining "engineered bacteria - intelligent fermentation - self-purification" represents a promising direction for achieving automated control throughout the process from gene editing to final product filling. Directed evolution combined with high-throughput screening enables the rapid evaluation of thousands of enzyme mutants per second through microdroplet encapsulation technology. For example, a three-region single-channel microfluidic paper chip (μPADs) was utilized as an analytical platform to assess the activity of acetylcholinesterase (AChE) inhibitors via colorimetry [55], showcasing advantages such as rapid and convenient evaluation, minimal sample consumption, and low analysis costs.

5. Conclusion

Due to their green and highly efficient catalytic properties, lipases have become indispensable tools in food biology. Despite challenges such as high industrial enzyme costs and insufficient adaptability to extreme processing conditions, their pivotal role in precision nutrition and clean-label food development continues to expand. With technological advancements, the application potential of lipases in emerging fields like precision nutrition and sustainable food manufacturing will be further realized. It is imperative to actively leverage cutting-edge electronic technologies to empower the food industry.

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