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Potential use of seed lipases for fatty acid production from vegetable oil

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Abstract. Lipases are in high demand in food processing for various purposes, such as modifying food texture, aromatic development, and enhancing emulsifier properties. Plant-based lipases present an alternative to microbial lipases, offering advantages such as ease of preparation and purification and consequently lower production costs. This article describes the progress in research and potential applications of plant-based lipases. It highlights the biochemical properties of various plant lipases, including those from coconut flesh and ketapang seeds. Emerging research aims to better characterize lipases and match them to specific applications. Some seed lipases may prefer medium or long-chain fatty acids, often correlating with the dominant triacylglycerols from which the lipases are isolated. Exceptions to this rule exist, necessitating further studies to understand the general properties of seed lipase. Such information is crucial for the application of seed lipases.

1 Introduction

In the realm of sustainable industrial processes, the utilization of enzymes holds immense promise. Among these biocatalysts, lipases derived from seeds are emerging as key players in the conversion of vegetable oils into valuable fatty acids. This article explores the potential and current advancements in utilizing lipases for efficient fatty acid production, highlighting their significance in the context of renewable resource utilization and industrial applications. One special issue is the exploration of seed lipases as new sources of hydrolytic enzymes to replace traditional sources of enzymes.

Lipases are enzymes that catalyze the hydrolysis of fats and oils into glycerol and fatty acids. Seed lipases, in particular, are enzymes naturally found in seeds of various plants, where they play crucial roles in lipid metabolism. These enzymes exhibit high specificity towards different types of fatty acids, making them versatile tools in biotechnological processes.

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1.1 Characteristic of lipases

1.1.1 High Specificity and Efficiency

Lipases exhibit high substrate specificity [1,2]. They can act on specific positions of acyl ester. They also have a specific preference for fatty acids chain length. For example, coconut lipase has a preference for lauric acid [3]. Such properties enable the engineering of lipases for a specific purpose in the hydrolysis of triacylglycerol [4].

1.1.2 Environmentally Friendly

Enzymatic processes generally operate under mild conditions (e.g., moderate temperatures and neutral pH), reducing energy consumption and minimizing environmental impact compared to traditional chemical methods. Processing by lipase has been shown to be an environmentally friendly process [5].

1.1.3 Versatility

These enzymes can function in a variety of aqueous and non-aqueous environments, expanding their applicability across different industrial sectors [4,6]. Lipases hydrolyze triglycerides to produce free fatty acids, diglycerides and/or monoglycerides under aqueous conditions. However, in the presence of organic solvents, synthesis reactions such as esterification and transesterification are carried out [7].

1.2 Lipase sources

1.2.1 Microbes

Microbes are traditionally recognized as sources of lipases. They can be found in bacteria, mainly from the genera *Pseudomonas* and *Burkholderia*, which have high activity and a broad range of pH and temperature stability [4,8]. Other important microbial sources of lipases are fungi and yeast. It is estimated that more than half of commercial lipases are of filamentous fungi or yeast origin. Notable genera of these criteria are *Aspergillus, Rhizopus, Penicillium, Mucor, Geotrichum* and *Fusarium* [9,10].

1.2.2 Animals

Lipases are ubiquitous and are found in different parts of animals. Depending on the animal source, their abundance and properties vary [9,11]. Pancreatic, hepatic and gastric organs contain many lipases. They are required to process triglycerides prior to transport via aqueous environment. The main sources of animal lipases are insects, fishes and mammals [11].

1.2.3 Plants

Food reserve tissues are an important source of plant-based lipases. Seeds that are rich in triglycerides normally utilize lipases during gemination to break down lipid reserves as carbon sources for growing [3,11,12]. Plant lipases offer cheaper enzymes since they can be obtained through simpler methods. In most cases, it involves no genetic engineering process as frequently found in microbial enzyme production [13]. Seed lipases are described in more detail in section 3.

2 Biotechnological Application of Lipases

The majority of the biomass on Earth is made up of lipids, and lipolytic enzymes are crucial to the breakdown of these intractable substances. Lipases and other hydrolytic enzymes of microbial origins make up around 75% of commercial enzymes. Lipolytic enzymes have a role in both the breakdown and subsequent mobilization of lipids within individual species' cells, as well as the transfer of lipids across different organisms. The hydrolysis of the carboxyl ester linkages in triacylglycerols (TAGs) to create diacylglycerols, monoacylglycerols, fatty acids, and glycerol is catalyzed by lipase (triacylglycerol acylhydrolase, EC 3.1.1.3). Lipases also catalyze other esters' hydrolysis, acidolysis, aminolysis, and transesterification [11]. The application of lipases is described in the following.

2.1 Fatty Acid Hydrolysis

Lipases are utilized to break down triglycerides present in lipid substrates, such as vegetable oils, into free fatty acids. This process, known as hydrolysis, is essential for the production of fatty acids. The resulted in fatty acids and monoglycerides are used in numerous industries including food [14], pharmaceuticals [15], and cosmetics [16]. The fatty acids market accounted for $29.47 billion in 2022 and has steadily increased to reach $48.42 billion by 2032 [17].

2.2 Biodiesel Production

In biodiesel production, lipases are employed to catalyze the transesterification reaction [9,18]. This process converts vegetable oil into biodiesel by replacing glycerol with alcohol, yielding fatty acid methyl esters (FAMEs). In this case, lipases may be utilized as free or immobilized enzymes [9,19]. It has been recently described that further pyrolysis or decarboxylation reaction to the fatty acid may produce hydrocarbon that readily can be mixed with fossil fuel, as they have similar chemical structures [20].

2.3 Flavor Enhancement

In food processing, particularly in the production of speciality oils and fats, lipases can modify the fatty acid composition to enhance flavour profiles. This application is crucial in the culinary industry, where specific taste and aroma profiles are desired. An example is shown by controlling fatty acid release in low-fat cheese by immobilized lipase [21] and in the production of short-chain flavour ester [22].

2.4 Detergent Industry

Lipases are used in laundry detergents to break down lipid-based stains like oils and grease. This improves the cleaning efficiency of the detergent [23]. The need for lipases as complementary to proteases in the detergent industry is increasing in many developing countries. To serve the requirements of the detergent industry, research on alkaline and thermostable lipases, especially from microbes, is still an attractive field [24].

2.5 Textile Industry

Because they are environmentally friendly and may be applied to a variety of substrates depending on the application method, enzymes are frequently utilized in the textile industry. They are employed in the enzymatic finishing process, which enhances the material's quality and processing. Enzymes with certain characteristics are advantageous to the textile industry; they include substrate specificity, which lowers the activation energy and speeds up the reaction; mild conditions; ease of control over the enzymatic activity; and biodegradability. Lipases are used in the textile industry to remove fats and waxes from fabrics, thereby improving dye penetration and fabric quality [25,26].

2.6 Medical and Diagnostic Applications

The regioselectivity, enantio and chemoselective properties of lipases enable their utilization in vital reactions in drug synthesis within organic solvents [27]. For example, lipase derived from *C. antarctica* and *C. rugosa* catalyzes the enantioselective esterification of Profens (2-aryl propionic acids), an important class of non-steroidal anti-inflammatory medicines that are active only in a particular conformation [28]. Lipases are also used in diagnostic assays, for example, to determine acute pancreatitis [29].

2.7 Bioremediation

The development of agriculture in many developing countries is accompanied by an increase in waste products, which creates new environmental burdens. It may also contribute to gas emissions because they have the potential to serve as a source of nutrients for microbial crops; agro-industrial wastes like bran, cobs, and bagasse can be valued by turning them into microbial cultivations for the production of compounds. This makes the waste more valuable economically. In this regard, lipases are agents that help the bioremediation process. An example is shown by Kreling and co-workers, who utilized lipase in a solid-state fermentation to treat contaminated soil [30]. A broader application of lipolytic enzymes for bioremediation is also achieved in the cleaning of wastewater and plastic degradation, and it is even utilized as a biosensor to detect environmental pollution [31].

3 Seed Lipases

Plants are potential sources of lipase in terms of quantity and availability. However, more attention needs to be given to plant lipase in comparison to microbial and animal lipases. Although many protein sequences of plant lipases have been deposited in UniProtKB, there is no record of the crystal structure of plant lipases [32]. Seeds rich in lipids are enormous sources of lipase. The lipase's activity is mostly found in the germinating seeds, i.e., the phase where the lipid storage is mobilized as a carbon source and energy for growing.

Seed lipases offer an advantage over microbial lipases in terms of ease of purification. They are also abundant in oilseed, which makes them inexpensive [33]. However, seed lipases are less studied. Selectivity for the predominant fatty acids in the seed is demonstrated by seed lipases, and hence, within the last two, seed lipases became more interesting. Elm lipase is selective for tricaproin, Vermonia sp. lipase is selective for trivernolein, castor bean lipases are selective for triricinolein, and palm tree lipase is selective for either trilaurein or tricaproin. Canola and pinus seed lipases are two examples of other seed lipases that can rapidly hydrolyze a wide range of fatty acids. While canola seed lipase is generally active with most lipids, it is selective for fatty acids that have either cis-4 or cis-6 double bonds [34,35]

A simple purification step of seed lipase is shown by the fact that only a few 'contaminating' proteins are associated with the lipase. An example is shown by coconut and Ketapang lipase (Fig 1). The crude extract of coconut lipase consists of only a few protein bands in SDS-PAGE. Moreover, following fractionation by ammonium sulfate, native-PAGE of coconut lipase shows only single band (Fig 1). It suggests that coconut lipase might be a complex protein with several subunits.

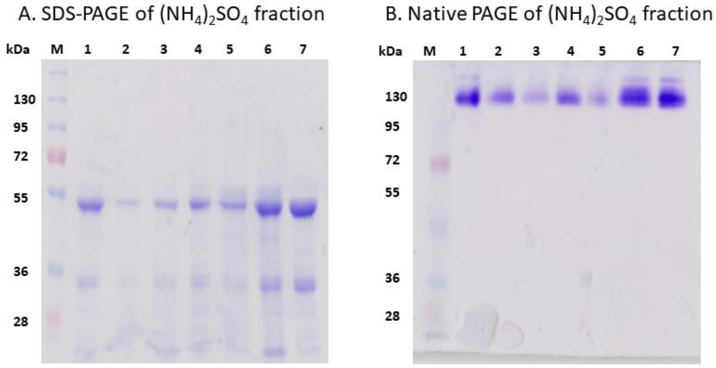

Fig 1. Coconut lipase is derived from coconut meat. Ammonium sulfate fractions of coconut lipase are separated by SDS-PAGE (A) and native PAGE (B). M: protein marker; 1: crude extract; 2: fraction 0-15%; 3: fraction 15-30%; 4: fraction 30-45%; 5: fraction 45-60%; 6: fraction 60-75%; and 7: fraction 75-90%.

As a general rule, it was proposed that seeds with >80% saturated fatty acids or unusual fatty acids may exhibit selectivity [35]. We have recently shown that coconut lipase has lauric acid as its preferred substrate [3] (Fig 2). Coconut lipid consists of more than 50% lauric acid. However, ketapang seed lipase prefers short chain (4 or 8 carbons chain) over long fatty acid (18 carbon chain) [12], despite the fact that more than 60% of ketapang seed lipids are 18 carbons fatty acid (stearic acid, oleic acid, and linoleic acid), Fig 2

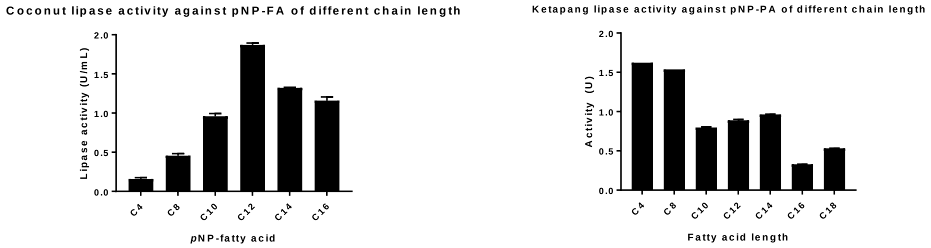

Fig 2. Substrate specificity of coconut lipase [3] and ketapang [12]

The above report underlines the potential of seed lipase. However, efforts are needed to exploit seed lipase for specific purposes. This includes identifying the specificity of seed lipase in hydrolyzing triglycerides of vegetable oil. Additionally, other biochemical properties such as optimum reaction conditions (pH, temperature, metal ion inhibition/activation, stability against detergent or organic solvents) need to be investigated for individual seed lipase.

4 Conclusion

Seed lipases represent a promising biocatalyst for the sustainable production of fatty acids from vegetable oils. Their ability to selectively hydrolyze triglycerides into valuable products under mild conditions makes them indispensable in various industrial applications. As advancements continue in enzyme engineering and process optimization, the integration of seed lipases into mainstream industrial processes is expected to grow, contributing to a more sustainable and environmentally friendly future.

While seed lipases offer significant advantages, several challenges remain. These include exploration of seed lipase specificity, optimizing enzyme stability and activity under industrial conditions, reducing production costs, and scaling up processes to meet commercial demands. Ongoing research focuses on engineering lipases for improved performance and exploring novel sources of these enzymes.

In conclusion, the harnessing of seed lipases for fatty acid production underscores a significant advancement in biotechnology, highlighting the potential of enzymes to revolutionize how we utilize renewable resources efficiently and sustainably.

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