Food Bioscience 45 (2022) 101509

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Microbial lipases: Propitious biocatalysts for the food industry

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ARTICLE INFO

Keywords:
Microbial lipases
Food industry
Agro-industrial residue

ABSTRACT

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are one of the largest groups of enzymes and are used in various industrial processes. Lipases of microbial origin are currently receiving increased attention for industrial application as microorganisms grow quickly and are easily genetically manipulated. Furthermore, they offer several advantages, such as catalysis of diverse reactions, high specificity, high yields, low energy consumption and reduced processing time and production costs. There is a relentless ongoing effort to optimise the production of microbial lipases for potential application in the food industry. In this context, this review highlights the most promising techniques for producing microbial lipases and the recent applications of these lipases in dairy, oils and fats, bakery and confectionery, meat, flavours and aromas and other food industries. Microbial lipases are normally obtained by fermentation, but the high costs of carbon and nitrogen sources limit the process. To overcome this problem, low-cost agro-industrial residues in the lipase production process are explored. To obtain lipases with high yields and improved characteristics, the technique of protein engineering is described as promising, and the immobilization method that allows the recycling of lipases to improve their catalytic performance is focused. Due to their catalytic properties and versatility, lipases of microbial origin are considered extremely important catalysts in the food industry, meeting the demand for tastier foods with pleasant aromas and textures. Therefore, microbial lipases are considered safe and sustainable biocatalysts.

1. Introduction

1.1. Lipases as biocatalysts

Lipases are recognized as lipolytic enzymes belonging to the serine hydrolase group, responsible for catalysing the hydrolysis of ester-carboxylic bonds of triacylglycerols (TAG) with the release of free fatty acids, diglycerides (DAG), monoglycerides (MAG) and glycerol (Chen et al., 2003; Jaeger et al., 1994; Nagarajan, 2012). Lipases preferentially hydrolyse substrates with long-chain fatty acids greater than 10 carbons. However, lipases can also hydrolyse substrates with short- and intermediate-chain fatty acids (Anthonsen et al., 1995; Chen et al., 2003; Jaeger et al., 1999).

In addition to hydrolysis, in which the nucleophilic agent is a water molecule, lipases can catalyse the reverse reaction, esterification (Borrelli & Trono, 2015). Depending on the transformation of the ester group, other reactions can also be catalysed, such as (1) transesterification, which encompasses reactions with alcohols acting as nucleophiles, for example, glycerolysis; (2) interesterification, which is characterized by exchange between the substituents of two different esters and (3) acidolysis and (4) aminolysis, which, as the names suggest, refer to breakdown by carboxylates and amines as nucleophilic agents, respectively (Alfonso & Gotor, 2004; Farfán et al., 2013; Otera, 1993; Parker & Baker, 1968) (Fig. 1). Due to the versatility of reactions catalysed by lipases, they are considered biotechnologically important biocatalysts, becoming green alternatives to chemical methods, providing safe and invaluable tools for industrial transformations to synthesize natural or synthetic materials with lower energy consumption under moderate reaction conditions (Memarpoor-Yazdi et al., 2017).

Based on specificity, lipases can be grouped into four major types: i) enantioselective: can distinguish enantiomers in a racemic mixture; ii) substrate-specific: selectively act on a specific substrate in a mixture of different materials, facilitating the synthesis of the desired product, iii) regioselective (which are divided into 1,3 regiospecific and 2-regiospecific lipases) and iv) non-specific (Borrelli & Trono, 2015; Verma et al., 2021). The 1,3 regiospecific lipases release fatty acids from positions 1 and 3 of TAG and cannot hydrolyse ester bonds at secondary positions.

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https://doi.org/10.1016/j.fbio.2021.101509
Received 23 September 2021; Received in revised form 18 November 2021; Accepted 12 December 2021
Available online 14 December 2021

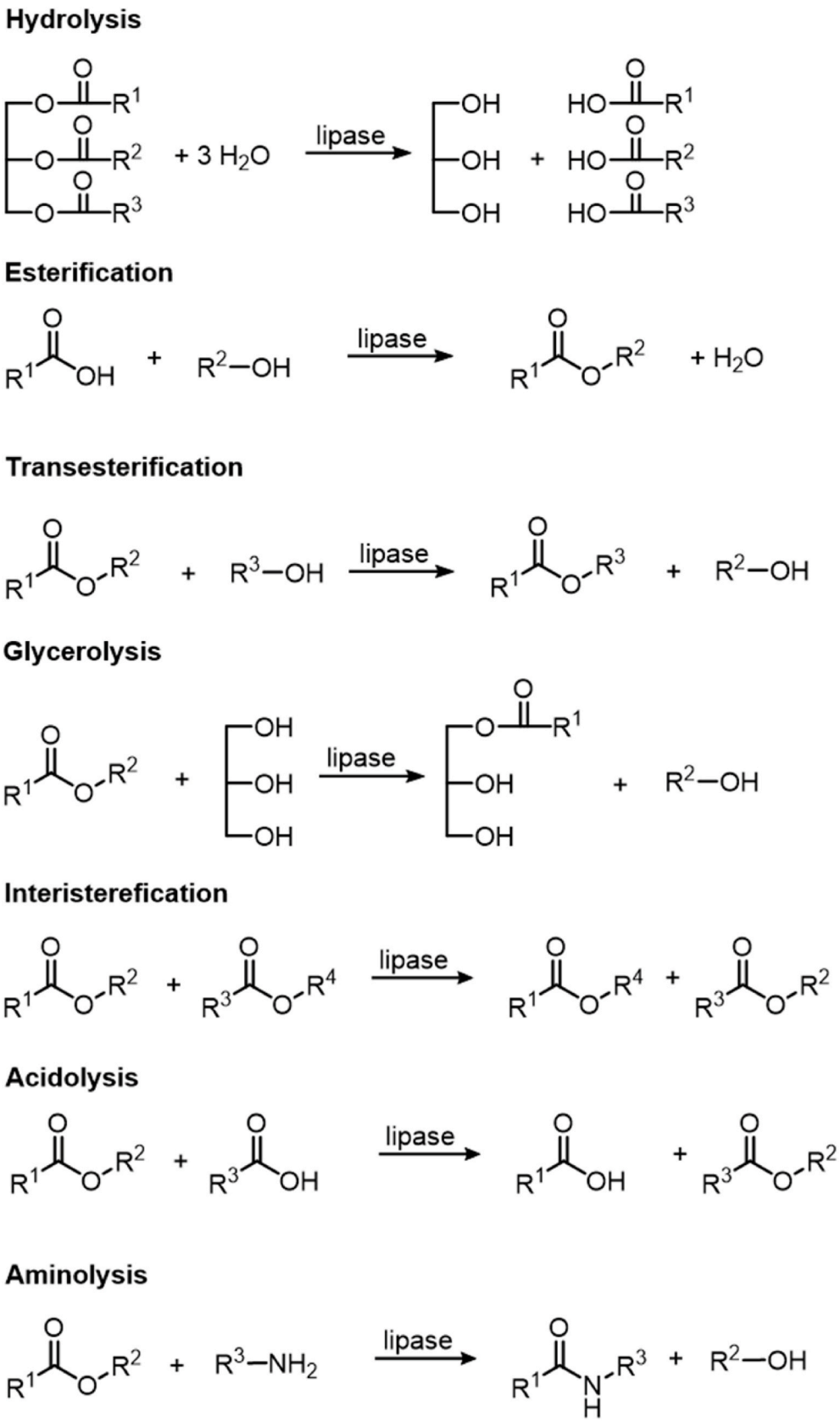

Fig. 1. Reactions catalysed by lipases.

The 2-regiospecific lipases release fatty acids at the secondary position of TAG, specifically producing 1,3-diacylglycerol. Non-specific lipases catalyse the hydrolysis of TAG into fatty acids and glycerol, with MAG and DAG intermediates, and can hydrolyse the ester group at any position on the substrate (Hari Krishna & Karanth, 2002; Jensen, 1974; Kapoor & Gupta, 2012).

Considering the biotechnological importance of lipases as biocatalysts, this review highlights the production and applications of microbial lipases in the dairy, oil and fat, bakery and confectionery, meat, flavour and aroma and other food industries.

1.2. Production of microbial lipases

Lipases are ubiquitous in plants, animals and microorganisms and vary considerably in their characteristics. Lipases of microbial origin have attracted much attention from industry, due to their catalytic versatility, specificity, high yields, superior stability and greater availability than lipases of animal and vegetable origin (Chandra et al., 2020; Geoffry & Achur, 2018). Microorganisms represent an excellent source of lipases, as they have a high growth rate and low production cost, are more easily genetically manipulated and can regularly supply lipases due to the absence of seasonal fluctuations (Borrelli & Trono, 2015; Hasan et al., 2006). Because of these properties, microbial lipases have wide application in the food, detergent, chemical, pharmaceutical, pulp and paper, bioremediation, cosmetic and other industries (Hasan et al., 2006; Uppada et al., 2017). Lipase-producing microorganisms are generally isolated from environments where lipids and conditions suitable for the action of lipases are present. The isolated microorganisms are evaluated in a solid or liquid media to produce lipases (Geoffry & Achur, 2018), and then the enzyme is characterized and purified. Several factors influence enzyme activity, such as pH; temperature and the presence or absence of solvents, inhibitors and prosthetic groups. Table 1 shows the main characteristics of some of the microbial lipases used in the food industry.

The market value of microbial lipases was estimated at US$425.0 million in 2018 and is projected to reach US$590.2 million in 2023 (Chandra et al., 2020). For industrial applications, lipases must be produced on a large scale by fermentation. Fermentation technology is the process by which microorganisms grow and metabolize complex substrates by breaking them down into simple compounds and in due course, yield many enzymes and by-products (Fasim et al., 2021). As microbial lipases are mainly extracellular, they can be produced by solid-state fermentation (SSF) or submerged fermentation (SMF). The type of fermentation, SSF or SMF, influences microbial growth and large-scale enzyme production. The main difference between them is the amount of water. In SSF, microorganisms grow on natural or inert solid support materials with a low moisture. SSF is widely used to produce lipases from fungi and yeasts using various simple, inexpensive and abundant agricultural and industrial residues as substrates. Therefore, SSF is an economical alternative for the large-scale production of enzymes that are produced by fungi (Bharathi & Rajalakshmi, 2019; Geoffry & Achur, 2018).

In SMF, microorganisms grow in a liquid medium with high moisture. It has some advantages, such as greater homogeneity of the culture medium and greater ease of controlling variable parameters such as temperature and pH. In addition, recovery and purification of extracellular lipases by SMF are simple, and agro-industrial residue substrates can also be used as carbon and nitrogen sources (Bharathi & Rajalakshmi, 2019; Geoffry & Achur, 2018; Wu et al., 2020).

A general disadvantage of industrial enzyme production, including that of lipases, is the production cost due to expensive sources of carbon and nitrogen, which account for 50% of the total cost of enzyme production. The exploitation of low-cost raw material residues becomes essential when scaling up the production of enzymes from microbial sources (Gaonkar & Furtado, 2021; Singh & Bajaj, 2017). The current emphasis is on the development of new and sustainable methods for industrially important enzyme production to make the whole process commercially cost effective (Sodhi et al., 2022). Residues such as bran, sugarcane bagasse, oil cakes, peels, feathers, whey, crustacean and fish residues, chicken feathers, sweet potato flour and bagasse and others are generated worldwide (El Sheikha & Ray, 2017; Gaonkar & Furtado, 2021). The exploration and valorisation of these residues are considered a widely sustainable approach once contribute to the production of enzymes of microbial origin and reduce production costs and problems associated with their elimination (Gaonkar & Furtado, 2021).

Recent reports exemplify the use of agro-industrial residues as substrates for the production of microbial lipases. Wu et al. (2020) showed the viability of industrial production of lipase A by *Bacillus subtilis* using glycerol and chicken feather hydrolysate as an inexpensive source of carbon and nitrogen. Knob et al. (2020) isolated lipolytic yeasts from slaughterhouse refrigerator effluent and oil mill effluent. The species identified as *Meyerozyma guilliermondii* was grown in 2% cheese whey at pH 4.0 for 24 h, with a 6.7-fold increase in lipase production. Cheese whey was also used for lipase production by *Geotrichum candidum* and productivity was increased with the enrichment of the medium with corn steep liquor (Ramos et al., 2021). Gaonkar and Furtado (2021) used different agro-industrial residues for the growth of the archaeon *Haloferax lucentensis* and verified that the use of coconut oil cake under optimized conditions increased lipase production.

The use of free-form lipases obtained from the fermentation process has technical limitations due to difficulty of their recovery for reuse, sensitivity to pH and temperature variations and low operational stability which increases cost. An alternative to circumvent this is to follow protocols of immobilization or encapsulation of the enzyme.

Table 1
Microbial lipases used by the food industry and their main characteristics.

Microbial source of lipase	Main characteristics of lipase	Reference
Penicillium cyclopium	Stability at 4 °C at pH values ranging from 5.0 to 10.0	(Huang et al., 2013)
Lactobacillus plantarum	Active on tributyrin and also on other long-chain substrates Optimum temperature: 40 °C Optimum pH: 7.0 Active under NaCl concentrations higher than 20% 40% of activity at refrigeration temperature (5 °C)	Esteban-Torres et al. (2015)
Lactobacillus plantarum	Lipase immobilized: Optimum temperature: 45 °C Optimum pH: 6.5 Synthesizing different short-chain fatty acid esters	Uppada et al. (2017)
Rhodothermus marinus	Preferential hydrolysis by esters with short chain fatty acids (<C8). Optimum temperature: 70 °C Optimum pH: 8.5 Optimum concentration of NaCl: 0.2 M Organic solvent tolerant	Memarpoor-Yazdi et al. (2017)
Malbranchea cinnamomea	Optimum temperature: 40 °C Optimum pH: 7.5 Specificity towards triglycerides with short and medium-chain fatty acids	Duan et al. (2019)
Streptomyces violascens	Better regioselectivity at the *sn*-1 and *sn*-3 positions Optimum temperature: 30 °C Optimum pH: 9.0 Organic solvent tolerant	Gao et al. (2020)
Cersospora kikuchii	Lipase immobilized: Optimum temperature: 40 °C Optimum pH: 6.5 Residual activity was high 88.7% after storage at 5 °C for six months.	Costa-Silva et al. (2021)

1.3. Lipase immobilization

Immobilization methods have been considered as a promising approach to boost enzyme productivity and operational stability and to facilitate the recycling of enzyme, which decreases cost. The immobilization of enzymes represents a more advantageous configuration since it also improves the separation from reaction mixtures without contaminating the products.

Enzymes can be immobilized by various methods, which can be broadly classified as physical, where there are weak interactions between the support and the enzyme, and chemical, where covalent bonds are formed between the support and the enzyme (Homaei et al., 2013). The physical method involves the adsorption of the enzyme on a support or its imprisonment inside a porous matrix, and interactions between the enzyme and the support are via weaker bonds such as van der Waals forces and hydrogen-type bonds (Chandra et al., 2020; Girelli et al., 2020). In the chemical method, covalent bonds are usually formed between the support with functional groups present on the protein's surface (Homaei et al., 2013). The only rule is that the covalent bond must not be formed with the enzyme's catalytic activity sites and that the binding reactions must be carried out under mild conditions (Girelli et al., 2020).

Different agro-industrial residues, called lignocellulosic (coconut fibre, corn cob, spent grain, spent coffee, husk, husk ash, straw rice, soybean and wheat bran) and not lignocellulosic by-products (eggshell and eggshell membranes) are materials with great potential for enzymatic immobilization. These highly available residues are underutilized and cause significant environmental problems due to inadequate storage but are of great interest due to their low cost (Girelli et al., 2020). Indeed, these natural polymers display a set of desired attributes and advantages such as nontoxicity, biodegradability, biocompatibility, hydrophilicity, antibacterial properties, physiological inertness, gel formation, heavy metals chelation, and high proteins affinity (Bilal et al., 2021).

Recently, nanostructure supports appear as strong alternatives in the field of enzyme immobilization for the application of industrial nanobiocatalysis, since they provide additional advantageous properties as higher surface areas and ease of recovery and reuse (de Oliveira et al., 2021). Different nanomaterials as metal-organic frameworks, carbon nanotubes, silica-based nanoparticles, nanoflowers, nanofibrous membranes, graphene oxide and hybrid nanomaterials have been considered as promising structures for immobilizing lipases. Recent reviews take a broad approach to lipase immobilization in nanomaterials (Bilal et al., 2021; de Oliveira et al., 2021; Ismail & Baek, 2020; Liu et al., 2020; Zhong et al., 2020).

Lipase immobilization technology has been used in the food industry for the synthesis of aromatic esters (Costa-Silva et al., 2021; Memarpoor-Yazdi et al., 2017), flavour esters (Narwal et al., 2016; Uppada et al., 2017) and emulsifiers (Bavaro et al., 2020); interesterification of oils and fats (Samoylova et al., 2017; Xie & Zang, 2017); production of margarine (Sellami et al., 2012); addition of value to oils (Binhayeeding et al., 2020) and others. However, according to Coelho and Orlandelli (2021), niche focusing on immobilized lipases remain poorly explored.

1.4. Protein engineering

To produce microbial lipases on an industrial scale, the protein engineering technique is used to obtain enzymes designed with better kinetic properties, greater substrate specificity, greater thermostability, altered pH optima and increased/decreased optimal temperatures, depending on the industrial applications (Verma et al., 2021). Proteins can be engineered by employing various methods; however, the basis remains recombinant DNA technology: altering the parent amino acid sequence to produce the required pattern. It is generally performed by manipulating the genes from original protein molecules and constructing unique proteins with varied structures, resulting in enhanced performance (Kataria et al., 2021).

Protein engineering methods, when used individually or in combination, mainly with directed evolution and rational design, exhibit a high potential to increase the functionality of existing proteins. This technique has proven an effective tool mainly for the amelioration of enzymes utilized in the food industry (Kataria et al., 2021). Directed evolution involves generating a series of random mutations in the gene that encodes the target protein. Rational design targets specific sites in the gene to make specific changes in the enzyme based on information gained from the protein's sequence, structure and function (Kapoor et al., 2017; Verma et al., 2021). Protein engineering, together with the possibility of immobilizing enzymes on suitable supports, can overcome the limitation of enzyme instability under different conditions, where parameters such as temperature and the presence of solvents and/or reagents and products can cause denaturation of the biocatalyst (Girelli et al., 2020).

Escherichia coli is by far the most used prokaryotic microorganism for recombinant production of lipases, and suitable host organism for bacterial lipases, such as from the genera *Pseudomonas*, *Bacillus*, *Staphylococcus*, *Serratia*, *Burkholderia*, and from metagenomic libraries (Contesini et al., 2020). The first recombinant enzyme approved by the Food and Drug Administration (FDA) of the United States for use in food was bovine chymosin expressed in *E. coli* K-12, for cheese production (Flamm, 1991), and from there, the study and improvement of recombinant enzymes in food manufacturing have been intensified. Protein engineering is a promising technique, and many lipases currently used in food processing are derived from recombinant microorganisms. Table 2 highlights some recombinant lipases approved by the FDA with GRAS (Substances Generally Recognized as Safe) status for use in the food industry.

Gonçalves et al. (2020) used several techniques simultaneously to produce and use lipase, such as protein engineering, the use of agro-industrial residues and enzyme immobilization. The lipase 1 from

Table 2
Lipases from recombinant microorganisms based on FDA regulations, GRAS affirmation petitions and intended use.

Microbial source of lipase	GRAS Notice No. /year of closure	Intended Use
Thermomyces lanuginosus	GRN 43/ 2000	Use in dough, baked goods, and the fats and oil industry as a processing aid for the interesterification or hydrolysis of glycerides in brewing beer
Fusarium oxysporum	GRN 75/ 2001	Use in the fats and oils industry, the baking industry, the hydrolysis of lecithin, and the modification of egg yolks
Thermomyces lanuginosus and ***Fusarium oxysporum***	GRN 103/ 2002	Use in bakery products, egg yolks, whole eggs, and fats and oils
Candida antarctica	GRN 158/ 2005	Use in the production of triglyceride products
Aspergillus niger	GRN 296/ 2009	Use in baked goods
Pseudomonas fluorescens **Biovar I**	GRN 462/ 2013	Use in the production of refined triacylglycerol-based oils used in food
Fusarium oxysporum	GRN 631/ 2016	For use in the production of baked goods
Rhizopus oryzae	GRN 783/ 2018 GRN 708/ 2017	For use in the production of cocoa butter substitutes and human milk fat substitutes
Aspergillus tubingensis	GRN 808/ 2019	For use as a processing aid in baking, in brewing processes and cereal manufacturing, in paste production, and potable alcohol production

GRN: GRAS affirmation petitions that have been converted to GRAS notices.

Beauveria bassiana was efficiently expressed in *Aspergillus nidulans* A773. The mutant strain proved to be viable for enzyme secretion with different carbon sources derived from the agro-industrial process, such as cassava husk, cornmeal, corn syrup, sorghum seed and wheat bran. Finally, the enzyme was immobilized on different hydrophobic supports for the transesterification reaction. An acidic lipase gene from the lipolytic bacteria *Micrococcus luteus* was successfully cloned to the yeast *Pichia pastoris* and utilizing combined codon optimization and optimization of signal sequence for extracellular expression resulted in increased gene expression compared to the previous expression in *E. coli* (Adina et al., 2021).

2. Application of microbial lipases in food industry

Microbial lipases are widely used during food processing, resulting in desirable changes in sensory quality, such as texture, flavour and aroma. Moreover, the food products obtained may have increased nutritional value. Due to the characteristics of microbial lipases, there is currently interest in a decisive search for them to improve the processing of food products. In this context, we will highlight the most recent studies on microbial lipases for application in the food industry. Fig. 2 presents a summary of the applications of microbial lipases in the food industry, in which the products are obtained from the versatility of reactions that lipases catalyse.

2.1. Dairy industry

Microbial lipases are used in the dairy industry to hydrolyse milk fat, improve yogurt flavour, enhance cheese flavour and lipolysis of butter and cream according to processing needs and accelerate cheese ripening (Jooyandeh et al., 2009; Memarpoor-Yazdi et al., 2017). Milk fat is the most complex fat found in nature, and more than 400 different fatty acids have already been identified in the TAG present inside milk fat globules (Lopez et al., 2011). According to the fatty acids released, the hydrolysis of TAG can contribute to desirable and undesirable flavours for dairy products. Short-chain fatty acids impart a strong, spicy taste; medium-chain fatty acids play a greater role in soapy flavour formation and long-chain fatty acids contribute little to flavour (Chen et al., 2003; Deeth, 2022; Fox & Stepaniak, 1983; Gupta et al., 2003).

To obtain the desired product, it is essential to choose the correct lipase, as each enzyme has a unique fatty acid release profile. Peng et al. (2014) expressed the lipase Est_p6 from a metagenomic library in *E. coli*, and the enzyme showed high hydrolytic specificity for myristate (C14) and palmitate (C16), which are long-chain fatty acids, concluding that Est_p6 is safe for imparting a distinctive and desirable flavour and odour in milk fat flavour production. Memarpoor-Yazdi et al. (2017) showed that GDSL lipase from *Rhodothermus marinus* cloned and expressed in *E. coli* and recovered by covalent immobilization on chitosan-coated Fe_3O_4 nanoparticles has the potential for hydrolysis of short-chain esters, suggesting its potential to improve dairy products. A lipase gene *McLipB* was cloned from a thermophilic fungus *Malbranchea cinnamomea* and expressed in *Pichia pastoris* (Duan et al., 2019). The produced lipase had high specificity for triglycerides with short and medium-chain fatty acids and had no positional specificity, exhibiting high stability under acidic conditions. McLipB protein may also be a potential candidate for producing milk fat flavour.

Lipases can also play an essential role in the flavour of yogurt. Huang, Yu, et al. (2020) used 1,3 regiospecific lipase from *Aspergillus oryzae* (AY30) to exclusively hydrolyse milk fat at positions 1 and 3 of TAG to release a large amount of free fatty acids. The addition of lipase

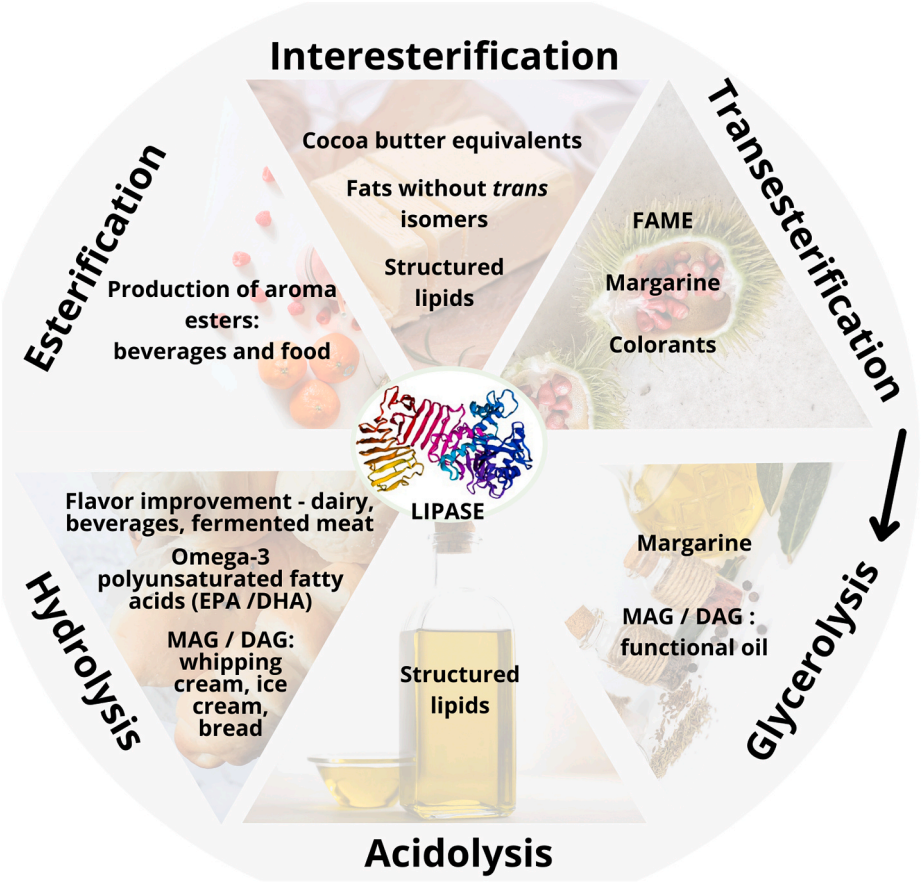

Fig. 2. Mainly important reactions catalysed by lipases and applied in the food industry. FAME = fatty acid methyl esters; DAG = diglycerides; MAG = monoglycerides; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

AY30 to the mixed fermentation conducted by *Streptococcus lactis* ACCC 11093 with *Lactobacillus casei* subsp. *rhamnosus* 6013, *Lactobacillus acidophilus* 1.1878 and *Lactobacillus plantarum* DMDL 9010 significantly improved the physicochemical properties of yogurt, especially the total volatile organic acid content, and efficiently improved the quality of yogurt-flavoured bases.

In cheese processing, lipolysis is essential for the development of a characteristic flavour. In ripened cheeses such as Brie, Camembert and Roquefort, *Penicillium* spp. are the essential lipolytic microorganisms. The white mould *Penicillium camemberti* is used for the maturation of soft cheeses, such as Camembert, Brie and Neufchatel (Ropars et al., 2020). Fatty acids produced by lipases of the blue *Penicillium roqueforti* mould make a major contribution to the flavour of blue-vein cheeses (Deeth, 2011). Caron et al. (2021) showed that the *P. roqueforti* population strongly impacts cheese quality, appearance and aroma. The populations used for cheesemaking led to bluer cheeses, with a better aroma, probably due to domestication involving the selection of multiple fungal traits by humans seeking to produce the best possible cheeses.

Cheese maturation is an expensive process, and its duration varies according to the type of cheese but contributes to the textural, functional and sensory attributes of the finished product. Karaca and Güven (2018) investigated the supplementation of milk for cheese making with commercial lipase Piccantase A from *Mucor miehei* for 90 days and reported increased lipolysis and acceleration of cheese ripening. Rani and Jagtap (2019) showed that lipase from *Bacillus tequilensis* PR13 reduced the period of maturation of Swiss cheese from 3 to 2 months. This was achieved without compromising the desirable physicochemical properties of the final product. Thus, lipase would result in decreased production costs by reducing the ripening period without affecting the quality characteristics of Swiss cheese. Therefore, the enzyme could be used for the cost-effective production of Swiss cheese. Kendirci et al. (2020) verified that cheeses modified by lipases obtained from *Rhizomucor miehei* and *Candida rugosa* presented a similar flavour to cured white cheeses. It was also observed a 12.5- to 81.9-fold increase in the content of total free fatty acids and an increase in volatile compounds compared with that of cured white cheeses.

2.2. Oil and fat industry

Fat and oil modification is one of the main areas of the food processing industry that requires new economic and green technologies (Hasan et al., 2006). Interesterification can be carried out chemically (using chemical catalysts) or enzymatically (using enzymatic catalysts). Interesterification catalysed either by chemicals or by enzymes leads to the exchange of fatty acids on the glycerol backbone or to a change in the position of fatty acids on the glycerides, resulting in structured lipids with modified physicochemical and nutritional properties. Despite the high cost, enzymatic interesterification offers additional advantages, such as milder processing conditions, fewer by-products and easier product recovery than chemical interesterification (Adhikari et al., 2010; Sivakanthan & Madhujith, 2020). In addition, it is possible to obtain modified oils and fats with a low content of *trans* isomers (Samoylova et al., 2016).

Unfortunately, inexpensive natural oils with a high melting point cannot be used directly for edible purposes as they cause the food product to have low plasticity and incomplete melting at body temperature (Samoylova et al., 2017; Xie & Zang, 2017). Interesterification is generally used to customize fat with a range of melting points for different food products and modify crystallization (Adhikari et al., 2010). Samoylova et al. (2017), through enzymatic interesterification catalysed by a lipase immobilized on recombinant thermostable silica of *Geobacillus stearothermophilus* G3, using sunflower oil and hydrogenated soybean oil, verified that this biocatalyst has high stability in the interesterification process. Thus, this biocatalyst is very effective for application in the interesterification of oils with high melting points, such as palm stearin, cacao butter, hydrogenated oils and other oils, which is an advantage to the production process of modified oils. Xie and Zang (2017) also found that *C. rugosa* lipase immobilized via covalent bonds in HAp-γ-Fe_2O_3 nanoparticles showed excellent catalytic performance for the interesterification reaction of soybean oil.

An ecologically sound approach to using relatively low-value biological resources was described by Sellami et al. (2012). Palm stearin and palm olein blends in different ratios were enzymatically transesterified in a solvent-free system using a *Rhizopus oryzae* lipase immobilized onto $CaCO_3$ to produce a suitable fat for margarine formulation. Results indicated that all transesterified blends had lower slip melting points than their non-transesterified counterparts. The rheological analysis showed that margarine prepared with the transesterified blend showed better spreadability than that of control margarine prepared with non-transesterified fat. Another example of the use of transesterification to add value to oils is the conversion of waste frying oil (WCO) into fatty acid methyl esters (FAME). Elhussiny et al. (2020) selected two *Aspergillus* isolates capable of producing lipolytic enzymes and transesterifying WCO into relatively high FAME yields. Binhayeeding et al. (2020) showed that a mixture of lipases from *C. rugosa* and *R. miehei* immobilized on polyhydroxybutyrate was also able to transesterify WCO, producing FAME. In the work by Da Silva et al. (2018), a lipase NS 40116, obtained from genetically modified *Thermomyces lanuginosus* was used to synthesize FAME from chicken abdominal fat, demonstrating high potential in transesterification reactions using this fat as a substrate.

Omega-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have shown important human-health-promoting biological functions, especially in the development and maintenance of brain function and vision (Castejón & Señoráns, 2020), and has aroused interest in the enrichment of oils using microbial lipases. For example, lipase from *Pseudomonas fluorescens* MTCC 2421 was used to enrich PUFAs from sardine oil, improving the EPA content from 17.8% to 42.5% (Chakraborty & Paul Raj, 2009). Gao et al. (2020) expressed *Streptomyces violascens* OUC-Lipase 6 in *B. subtilis* WB800 under ideal lipase conditions and found that OUC-Lipase 6 could selectively hydrolyse fatty acids on the glyceride backbone, thus improving the contents of DHA and EPA in codfish oil. In a study by Cao et al. (2020), lipase from *Trichosporon* sp. F1-2 also showed potential as a catalyst in enriching EPA and DHA in fish oil. Yang et al. (2021) used the lipase from *Candida cylindracea* to produce omega-3 PUFA from tuna oil. Under ideal conditions, the omega-3 PUFA content in the glyceride fraction of hydrolysed tuna oil increased significantly from 34.30% to 57.7%.

In addition to traditional sources of omega-3, there has been a recent effort to enzymatically enrich oils from vegetable sources, such as chia oil, coconut oil, linseed oil and others. Imanparast et al. (2018) showed that the immobilized lipase from *Actinomadura sediminis* represents a promising biocatalyst for producing PUFA-rich acylglycerols by direct esterification from free fatty acids in linseed oil. Castejón et al. (2019) synthesized omega-3 ethyl esters from chia oil catalysed by a lipase from *T. lanuginosus*. Consequently, omega-3 ethyl esters from chia oil with up to 65% α-linolenic acid were produced. Cipolatti et al. (2021) produced PUFA-enriched edible oils, obtaining lipids containing 181.6 mg of EPA and DHA in coconut oil, with the recombinant *Candida antarctica* lipase, obtained by fermentation in an alternative medium of low cost and immobilized on various particles of mesoporous silica.

Due to the recognition of the benefits of PUFA, PUFA-enriched MAG and DAG are used as emulsifiers and are widely applied in the food industry. Bavaro et al. (2020) established an enzymatic protocol for the continuous production of PUFA-enriched MAG and DAG from hemp seed in a continuous-flow fixed-bed reactor containing lipase from *Pseudomonas cepacia* covalently immobilized on Sepabeads EC-EP. A mixture of MAG and DAG (40% yield, 620 mg) composed of α-linolenic acid (82%) and oleic acid (16%) was obtained in less than 2 h. This bioprocess facilitates recovery of the different oil components (free fatty acids, MAG and DAG) deriving from oil hydrolysis and the use of lipase

in several cycles without any loss of activity.

Currently, the increased interest in improving the structural and functional quality of edible oils is remarkable. Yao, Wang, Yang, et al. (2020) developed a method to synthesize a functional oil rich in DAG, phytosterol esters and α-linolenic acid, from commercial soybean oil, peanut oil and rapeseed oil, using co-immobilized bi-lipases from *C. rugosa* and *T. lanuginosus*, on polydopamine-modified Fe_3O_4 surfaces. Three kinds of functional oils have been produced with high DAG, phytosterol esters and α-linolenic acid content (≥30.09%, ≥15.61% and ≥26.45%, respectively). Mota et al. (2020) extracted crude oils from coffee grounds and olive pomace that were used as raw material to produce low-calorie structured lipids by acidolysis with capric acid or interesterification with ethyl caprate, catalysed by 1,3 regiospecific lipase from *R. oryzae* immobilized on magnetic nanoparticles. Nicholson and Marangoni (2021) demonstrated a viable technique for structuring cottonseed and peanut oils into structural fats used in food applications. The non-regiospecific *C. antarctica* lipase B was able to perform glycerolysis, converting liquid oils into structural fats. The product of glycerolysis was used to make margarine with similar plasticity to that of commercial margarine and butter. Gunathilake et al. (2021) used pure concentrates of EPA and DHA to produce MAG and DAG oils through glycerolysis catalysed by the immobilized lipase of *C. antarctica* B. The omega-3 acylglycerols produced by the lipase were used for the fortification and stabilization of extra virgin olive oil using the antioxidant hydroxytyrosyl palmitate, and this blend of oils can be referred to as 'functional olive oil' or 'omega-3 enriched olive oil'.

2.3. Bakery and confectionery industry

In the manufacture of bread, it is necessary to use emulsifiers to improve the dough's volume, texture and stability. These emulsifiers are detected after bread processing and, therefore, must be described on the bread label. Due to the emergence of the clean label trend in recent years, a part of the strategy of some manufacturers has been to use the enzymatic approach, using lipases that are denatured after processing and therefore do not need to appear on the label (Monié et al., 2021; Sangeetha et al., 2011). Moayedallaie et al. (2010) made comparisons between three categories of commercial lipases and an emulsifier. Both the lipases and the emulsifier caused a significant increase in bread oven rise and specific volume. Lipases, as catalysts of the hydrolysis reactions of TAG, release MAGs and DAGs, which are considered emulsifiers and are capable of forming inclusion complexes with the amylose contained in the flour, improving the specific volume and firmness of the bread (Monié et al., 2021; Purhagen et al., 2011).

Huang et al. (2013) cloned the mono- and diacylglycerol lipase genes from *Penicillium cyclopium* and expressed them in *P. pastoris* strain GS115. The recombinant enzyme was named Lipase GH1 and was more efficient in the synthesis of MAGs and DAGs than was Lipase G50, a similar, commercially available lipase derived from *P. camemberti*, when oleic acid was used as an acyl donor. According to the authors, lipase GH1 has the potential for food emulsifier preparation.

Milk fat has been used as a functional ingredient in bread making, and Huang, Brennan, et al. (2020) evaluated the effects of the incorporation of the fungal lipase Lipopan F extracted from the *Fusarium oxysporum* strain in milk fat. As a result of the lipase treatment, the dough structure was strengthened, and the bread volume improved. Furthermore, the lipase treatment reduced the residual water activity, attenuating the hardening process of the bread crumb. Thus, this study demonstrates the potential application of exogenous milk fat treated with microbial lipase to replace synthetic surfactants, allowing for improved product properties and functionality.

The lipases available for bakery applications hydrolyse several lipid structures in flour, resulting in an increase in bread volume. Schaffarczyk et al. (2014) found that digalactosyl diglycerides, monogalactosyl diglycerides and *N*-acyl-phosphatidyl ethanolamine were hydrolysed with concomitant formation of digalactosyl monoglycerides, monogalactosyl monoglycerides and *N*-acyl-lysophosphatidyl ethanolamine by the action of lipase. Changes induced by the lipid fraction of wheat caused increases in bread volume of 56%–58%, depending on the type and concentration of added lipase.

Cocoa butter is the base ingredient for chocolate and other confectionery products and is one of the most expensive fats in the world. The current trends in the market suggest further rises in cocoa butter prices, shortages, strong demand in emerging countries, and profit squeezes for companies in the future. This means that finding a practical alternative is necessary (Ghazani & Marangoni, 2018; Verstringe et al., 2012). Cocoa butter can be replaced by other vegetable fats, and the most important requirement is that they be comparable with respect to melting behaviour and polymorphism, fatty acid and TAG composition and processing properties (Rohm et al., 2018).

Cocoa butter equivalents was synthesized from a 60:40 (w/w) mixture of a commercial enzymatically synthesized shea stearin and palm mid-fraction, catalysed by a commercially available immobilized fungal lipase, Lipozyme RM IM (Ghazani & Marangoni, 2018). The result showed that it is possible to synthesize cocoa butter equivalents with molecular structure and physical properties that resemble cocoa butter very closely. This was achieved in a two-step solvent-free reaction, with minimal downstream processing. Huang et al. (2021) prepared cocoa butter equivalents by enzymatic interesterification from the palm mid-fraction with stearic acid, using 1,3 regiospecific lipase from *R. miehei*, immobilized on a macroporous anion-exchange resin. They investigated the reaction process parameters and compared the physicochemical properties of the product with those of cocoa butter, concluding that the product obtained can be used as an excellent alternative to cocoa butter. Additionally, lipases are widely used in the chocolate industry to enhance the flavour of toffees, milk chocolate, caramels and buttercreams; decrease excess sweetness and improve the buttery character of caramels and toffees (Negi, 2019).

2.4. Meat industry

Lipases are also explored in the meat industry. In fermented meat products, which are appreciated by consumers for their sensory traits, lipid hydrolysis and oxidation in muscle and fat tissues are important processes that contribute to the development of the fermented flavour (Chen et al., 2017). In fermented meat products, lipase activity originates from a starter culture or from endogenous enzymes of fat cells and muscle fibres (Gandemer, 2002).

Lipases are active during the fermentation and maturation steps of sausage making. Chen et al. (2017) evaluated the role of several single strains or mixed strains (*Pediococcus pentosaceus*, *Lactobacillus curvatus*, *Lactobacillus sakei* and *Staphylococcus xylosus*) in lipolysis and lipid oxidation in Harbin sausages. The free-fatty-acid contents of both muscle and fat tissues were higher in the inoculated sausages than in the non-inoculated control, especially with mixed strains. The results demonstrate that Harbin dry sausage can be inoculated with a starter culture mixture of *P. pentosaceus*, *L. curvatus* and *S. xylosus* to promote lipid hydrolysis, inhibit lipid autoxidation and improve fermented flavour development.

Xiao et al. (2020) evaluated the effects of *L. plantarum* R2 and *S. xylosus* A2 inoculation on the microbial community, lipolysis, proteolysis and volatile compounds in Chinese dry fermented sausages. The results showed that the total contents of free fatty acids and free amino acids were increased by inoculation of starter cultures, especially a mixed culture. Thus, flavour development in the inoculated dry fermented sausages was attributed to improvements in microbiological quality.

Lipases are used to remove excess fat in the meat and fish processing industries to produce leaner meat. Developing techniques for deep processing of by-products of animal slaughter, such as bones, will have important social and economic benefits while promoting the production of a coordinated and sustainable source, which is desirable for eco-

friendly development of the slaughter industry (Yao, Wang, Liu, et al., 2020). Lipase pre-treatment of bone improved the enzymolytic efficiency of alkaline protease by significantly decreasing the lipid content and changing the surface structure and surface element content, promoting attachment to the sample (Yao, Wang, Liu, et al., 2020).

2.5. Flavour and aroma industry

Flavour esters that possess an aromatic ring in their molecular structure are also known as aromatic esters. These esters are widely found in nature (fruits and plants), and both synthetic (i.e. via chemical) and natural routes (i.e. via direct extraction from nature or via biotechnology) are suitable for their biocatalysis. From the industrial point of view, they are the most economical approach to reaching final green products with no toxicity and no harm to human health (Sá et al., 2017). The synthesis of different short-chain fatty acid esters for use as flavoring agents in the food industry is promissing. Xiao et al. (2015) demonstrated that lipase B from immobilized *C. antarctica* could carry out the esterification reaction of acetoin and fatty acids to synthesize fatty acid esters of acetoin, which are used in food and beverages to enhance the flavors of products. Narwal et al. (2016) immobilized lipase from *Bacillus aerius* and verified the esterification capacity of acetic acid and isoamyl alcohol, and the lipase catalysed the esterification with a yield of about 68% under optimized reaction conditions. The product was identified as isoamyl acetate, which has a pear or banana flavor and is widely used in the food, beverage, cosmetic and pharmaceutical industries. Uppada et al. (2017) explored esterification efficacy of the immobilized lipase from *L. plantarum* in synthesizing different short chain fatty acid esters which find use as flavoring agents in food industry.

Costa-Silva et al. (2021) combined spouted bed drying with conventional immobilization to increase the activity and stability of the lipase from *Cercospora kikuchii*. The lipase was immobilized in agricultural by-products known as 'green' supports such as rice husks, corn straw, green coconut fibre or sugarcane bagasse through covalent bonding. The rice husk system was used as a biocatalyst for aroma production by esterification of butyric acid to butyl butyrate, with a yield of 95%, reaching a concentration of 15.2 g/L of butyl butyrate, a fruity aroma widely used in the food industry. Oliveira et al. (2017) optimized the lipase yield of *Aspergillus ibericus* in agro-industrial residues and presented an environmental friendly strategy to naturally produce an aroma ester (butyl decanoate) in a solvent-free system, with application in the food industry, while adding value to the agro-industrial residues. Memarpoor-Yazdi et al. (2017) verified that the GDSL lipase from *R. marinus* exhibited a high potential for synthesizing short-chain esters and methyl acetate, suggesting its potential for aromatic compound synthesis.

Chen et al. (2021) proposed a two-step enzymatic catalysis method to modify goat milk fat, with the objective of generating a pleasant aroma to improve the flavour of goat milk products. First, the milk fat was hydrolysed to release free short-chain fatty acids as substrates, and then esterification was carried out to synthesize esters with pleasant notes. Lipase produced from *T. lanuginosus*, proved to be efficient in catalysing the hydrolysis of goat milk fat.

2.6. Other food industries

Recently, other sectors of the food industry have used microbial lipases as essential components for industrial application. Sofian-Seng et al. (2017) reported that direct incorporation of a lipase derived from *R. miehei* into palm oil-in-water emulsion formulations produced a combination of predominantly oleic and palmitic fatty acid and MAG fractions. When applied as part of the processing pathway for whipping cream and ice cream, controlled emulsion lipolysis has been shown to allow structure-function properties comparable to the use of commercial MAG.

The annatto is one of the most used natural colorants in the food industry, and bixin is one of the components responsible for the yellow-orange colour of the annatto extract. The structure of bixin is hydrophobic, which limits its application in water-based food products. To increase the solubility of bixin in water, it could be by attaching a hydrophilic molecule to this pigment. For this, Jahangiri et al. (2018) immobilized the lipase from *C. antarctica* to catalyse the transesterification reaction between bixin and sorbitol, forming a product that will have superior hydrophilic properties to the alcohol group of sorbitol.

Esteban-Torres et al. (2015) cloned the *lp_3562* gene from *L. plantarum* encoding a putative esterase/lipase and expressed it in *E. coli* BL21 (DE3), and the overproduced enzyme Lp_3562 showed biochemical properties that make it attractive for use in food fermentations. Park et al. (2020) produced ricinoleic acid vanillic ester through transesterification of vanillic acid and castor oil, mediated by recombinant *Proteus vulgaris* K80 lipase expressed in *E. coli* BL21 (DE3) cell and lipase acrylic resin from *C. antarctica*. This multifunctional compound showed potent antioxidant and antibacterial activity against Gram-positive bacteria and food spoilage microorganisms and can be used as an important biomaterial in the food and cosmetics industry. The production of a new maltoheptaose-based sugar ester with an excellent emulsifying property was proposed by Nguyen et al. (2021), the ester synthesis reaction was better when catalysed by lipase B from *C. antarctica*.

In this review, several studies of lipases produced by microorganisms were reported, and with this, many lipases with specific characteristics and improved properties were highlighted. Most of the studies are recent and have shown that microbial lipases are responsible for a range of improvements in foods, including flavour and aroma improvements in dairy products; cheese ripening; structured lipid synthesis; PUFA-enriched and functional oils; cocoa butter equivalents; improvements in the texture and volume of bread; synthesis of emulsifiers, aromatics and flavour esters and others.

3. Future directions

Microbial lipases have great potential in the food industry due to their properties, such as specificity, versatility of the reactions they catalyse and varied stability in relation to the presence of solvents, temperatures and pH, making them excellent biocatalysts. Due to its catalytic properties, it is possible to find or target a suitable lipase for each industrial application, allowing reproducible, efficient and devoid of undesirable by-products catalysis conditions, with lower environmental impacts and greener results.

Despite the numerous studies that focused on the use of lipases for application in food, few of them reached a final product with commercial importance. Therefore, the application of these enzymes in the food industry requires scientists to make a greater effort to obtain enzymes that are commercial and recognized as safe. Advances in more sustainable, economical and advantageous methods such as the use of agro-industrial residues for the production and immobilization of lipases, combined with protein engineering, provide the creation of biocatalysts with improved properties, bringing benefits to industrial processes, which include: environmental impact reduction, improved catalytic resources, reusability and more sustainable processes. A promising field for future research is the integration of different techniques to develop desired characteristics in lipases, with low cost production, making them excellent biocatalysts in industrial processes. Recombinant lipases are highly promising and some have been recognized with GRAS status and introduced in several food sectors, showing that application of lipases directly to food tends to increase and will be expanded in the coming years. The large-scale production of lipases for application in food industries is hampered by their high cost, and for this reason, extensive research using low-cost agro-industrial residues is aimed at reducing this expense. Furthermore, lipase immobilization strategies are growing

rapidly and these by-products can also be used for this purpose, allowing for cheaper, more efficient and more reusable reaction conditions. It is foreseeable that in the coming years, the focus of production and immobilization of enzymes will be on the reuse of agro-industrial residues, since they are considered sustainable and safe. In the near future, the large-scale application of enzymes, such as lipases, in foods will certainly make food products with better sensory characteristics and, consequently, more acceptable to consumers and with greater added value for the products, leading to an increase in financial return for manufacturers.

4. Conclusions

Microbial lipases are more advantageous over other natural sources of lipases, so their importance for application in food industries tends to increase and, for this, scientists are looking for simple and low-cost methods. Throughout this review, the main forms of production and immobilization of lipases were shown and the recent uses of microbial lipases in some food industries were highlighted. The use of lipases for food improvement is considered a more sustainable approach as it replaces traditional chemical methods and, therefore, it can be considered a green and promising tool to perform various reactions in food production. Finally, it is expected that in the coming years, the exploration of this biotechnological resource will be expanded due to the several advantages that microbial lipases have.

Declaration of competing interests

No conflict of interest exists.

Author contributions

Salgado, C. A.: Conceptualization, Visualization and Writing - Original Draft. Dos Santos, C. I. A.: Conceptualization, and Writing - Original Draft. Vanetti, M. C.D.: Conceptualization, Supervision and Writing - Review & Editing.

Acknowledgments

The authors thank the financial support from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and Universidade Federal de Viçosa (UFV).

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