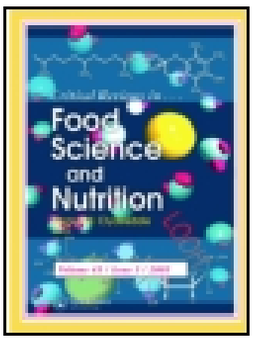

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REVIEW

Immobilized microbial lipases in the food industry: a systematic literature review

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ABSTRACT

Several studies describe the immobilization of microbial lipases aiming to evaluate the mechanical/thermal stability of the support/enzyme system, the appropriate method for immobilization, acid and alkaline stability, tolerance to organic solvents and specificity of fatty acids. However, literature reviews focus on application of enzyme/support system in food technology remains scarce. This current systematic literature review aimed to identify, evaluate and interpret available and relevant researches addressing the type of support and immobilization techniques employed over lipases, in order to obtain products for food industry. Fourteen selected articles were used to structure the systematic review, in which the discussion was based on six main groups: (i) synthesis/enrichment of polyunsaturated fatty acids; (ii) synthesis of structured lipids; (iii) flavors and food coloring; (iv) additives, antioxidants and antimicrobials; (v) synthesis of phytosterol esters and (vi) synthesis of sugar esters. In general, the studies discussed the synthesis of the enzyme/support system and the characteristics: surface area, mass transfer resistance, activity, stability (pH and temperature), and recyclability. Each immobilization technique is applicable for a specific production, depending mainly on the sensitivity and cost of the process.

KEYWORDS
Food raw materials; food technology; functional foods; health; nutrition

SUBJECT CLASSIFICATION CODES
food processing

Introduction

Enzymes are biological catalysts that accelerate biochemical reactions in living organisms (Okino-Delgado et al. 2018) and provide chemical reactions under mild conditions, with better efficiency/specificity and low costs compared to their chemical counterparts (Penha et al. 2015). These remarkable features enable their application in almost every field of biotechnological processes, including fine chemistry, food/feed production, biofuels, cosmetics, pharmaceuticals and papermaking (Okino-Delgado et al. 2018; Peil et al. 2016).

Lipases (triacylglycerol acylhydrolases, EC 3.1. 1.3) catalyze the hydrolysis of triacylglycerols in diglycerides, monoglycerides, free fatty acids and glycerol. Besides, their catalytic properties include esterification, interesterification (acidolysis, alcoholemia and transesterification) and aminolysis reactions, in both aqueous and organic media—in which the water content is restricted (Facin et al. 2019; Peil et al. 2016). Literature data highlight genera *Bacillus*, *Burkholderia*, *Pseudomonas*, *Staphylococcus* (bacteria), *Aspergillus*, *Geotrichum*, *Mucor*, *Penicillium*, *Rhizopus* (filamentous fungi) as the best lipase-producing sources; in addition, *Candida rugosa*, *Candida utilis*, *Pichia* sp., *Rhodotorula* sp. and *Yerrowia* sp. and are remarkable lipase-producing yeasts (Bharathi and Rajalakshmi 2019).

It is worth to notice that before commercialization or direct application in food sector, enzymes (free or immobilized) must undergo a series of protocols to certify their safety to human health.

Currently, in European Union (EU), EFSA (European Food Safety Authority) is responsible for issuing the authorization for marketing of enzymes and food process involved enzymes, following regulation EC 1331/2008, EC 1332/2008 and directive 2000/13/EC (EFSA 2020; www.efsa.europa.eu/en/topics/topic/food-enzymes). Some aspects required by legislation include certification of the enzyme production process (EC 852/2004) and purity, microorganism gene mutation assays, *in vitro* mammalian chromosomal aberration test, dose 90-day oral toxicity study and allergenicity assessment (EFSA 2020; www.efsa.europa.eu/en/topics/topic/food-enzymes).

Compared to free enzymes in solution, the immobilization of enzymes represents a more advantageous configuration since it improves biocatalysts stability, especially in relation to pH and temperature variations. Besides, supported enzymes also facilitate the separation of biocatalysts from reaction medium, allowing the recyclability of material (Bilal et al. 2019; Nicoletti et al. 2015; Oliveira et al. 2014; de Souza et al. 2018).

Some factors—such as immobilization protocols, lipase type and support employed—affect the immobilized lipases activity, because the intensity of the enzyme-support interaction and the orientation of the enzyme on the surface

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support results in systems with different stability and specificity. Thus, the immobilization procedure becomes a crucial point that can alter the enzyme functionality due to: (i) enzyme distortion, especially when there are multiple interactions between the enzyme and support; (ii) active site block due to the immobilization process itself and (iii) substrate diffusion problems (Bayramoglu et al. 2011; de Souza et al. 2018).

Although metagenomic tools have paved the way for production of new biocatalyst from noncultivable microorganisms, this technique has not circumvented the fact that biocatalysts still need to be purified, and their recovery after such procedure can be even more complex. In this sense, enzyme immobilization remains as the most attractive method to increase enzyme activity (Bilal et al. 2019).

In general, lipases are part of the industrial production of several food products consumed daily by humans. These enzymes are applied to improve the quality of food and processes, not only in relation to yield but also as regards environmental concerns. The literature presents several studies that address lipase application in food industry in dairy processing—baking, oil, meat and fish and beverages (Negi 2019)—however, studies with similar niche focusing on immobilized lipases remain poorly explored.

In this way, in this article we performed a systematic literature review focusing on immobilized microbial lipases applied to food technology, highlighting the influence of supports type, immobilization protocol, and the experimental reaction conditions.

Material and methods

The protocol of this systematic review was based on PRISMA method (Systematic Reviews and Meta-Analyses), proposed by Moher et al. (2009). The systematic literature review aims to identify, evaluate and interpret relevant researches on a particular issue, thematic area or phenomenon of interest (Kitchenham 2004).

Then, the present study focuses on the analysis of the application of immobilized lipases on the food industry, emphasizing the benefits/limitations of such method, aiming to answer the question: What is the influence of different types of support/immobilization technique on the activity of lipases directed for the synthesis of specific products for the food sector?

Bibliographic research was performed in electronic database Google Scholar, Scientific Electronic Library Online (SciELO), Web of Science and Scopus, until August 15, 2019.

In order to answer aforementioned question, the following aspects were adopted:

1. date range (2014–2019);
2. two keywords groups:
   A. lipases AND food AND support
   B. lipases AND food AND immobilization
   with words inserted in the title, keywords and abstract of documents; and
3. search in any language.

Then, exclusion/inclusion criteria were established. The initial bibliographic review (Figure 1) resulted in 167 and 314 potentially eligible scientific articles for a set of keywords A and B, respectively. Then, the works that did not have as its main focus the relevance of immobilization process on the performance of enzyme/support systems were excluded. A second analysis was carried out, and articles not directly related to the areas of food technology, food chemistry/microbiology, nutrition, packaging/storage and waste/effluent treatment from the food industry, were not considered for data extraction.

Finally, data abstraction and quality analysis were performed, and we sought emphasize the advantages and disadvantages of different immobilization techniques and supports and how these systems improve the productivity of production process. Besides, the performance of immobilized biocatalysts was also compared to the free systems.

Results and discussion

The 14 selected articles were published according to the following distribution: 2014 (4 articles, 28.6%), 2015 (1 article, 7.1%), 2016 (1 article, 7.1%), 2017 (2 articles, 14.3%), 2018 (5 articles, 35.7%) and 2019 (1 article, 7.1%). As for scientific journals, 50% are in the area of Food Science (Journal of Food Biochemistry, European Journal of Lipid Science and Technology, Food and Bioproducts Processing, Journal of Food Science and Agriculture, LWT-Food Science and Technology and Journal of Agricultural and Food Chemistry), and other articles belong to the areas of Biological Sciences, Chemical Engineering and Pharmacy.

Table 1 details the type of support, the methods of enzyme immobilization, the main objective and the most relevant results of the selected studies. For discussion, articles were grouped according to relevance for food sector: (i) synthesis/enrichment of polyunsaturated fatty acids; (ii) structured lipids synthesis; (iii) flavors and food coloring; (iv) additives, antioxidants and antimicrobials; (v) synthesis of phytosterol esters; and (vi) synthesis of sugar esters.

Synthesis/enrichment of polyunsaturated fatty acids (PUFAs)

In the last decades, the world population are experiencing a nutritional transition. This can be due to the continuous increase of overweight and obesity and their related diseases, such as type II diabetes, cardiovascular diseases and some carcinomas (Chilton et al. 2017; Elagizi et al. 2018). In order to reverse this scenario, the current medicine advocates for healthier eating habits, including for example, the replacement of saturated fats by PUFAs (Matuoog, Li, and Yan 2018; Elagizi et al. 2018), which are divided into three main categories: docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) (Rasti, Erfanian, and Selamat 2017; Walker, Decker, and McClements 2015).

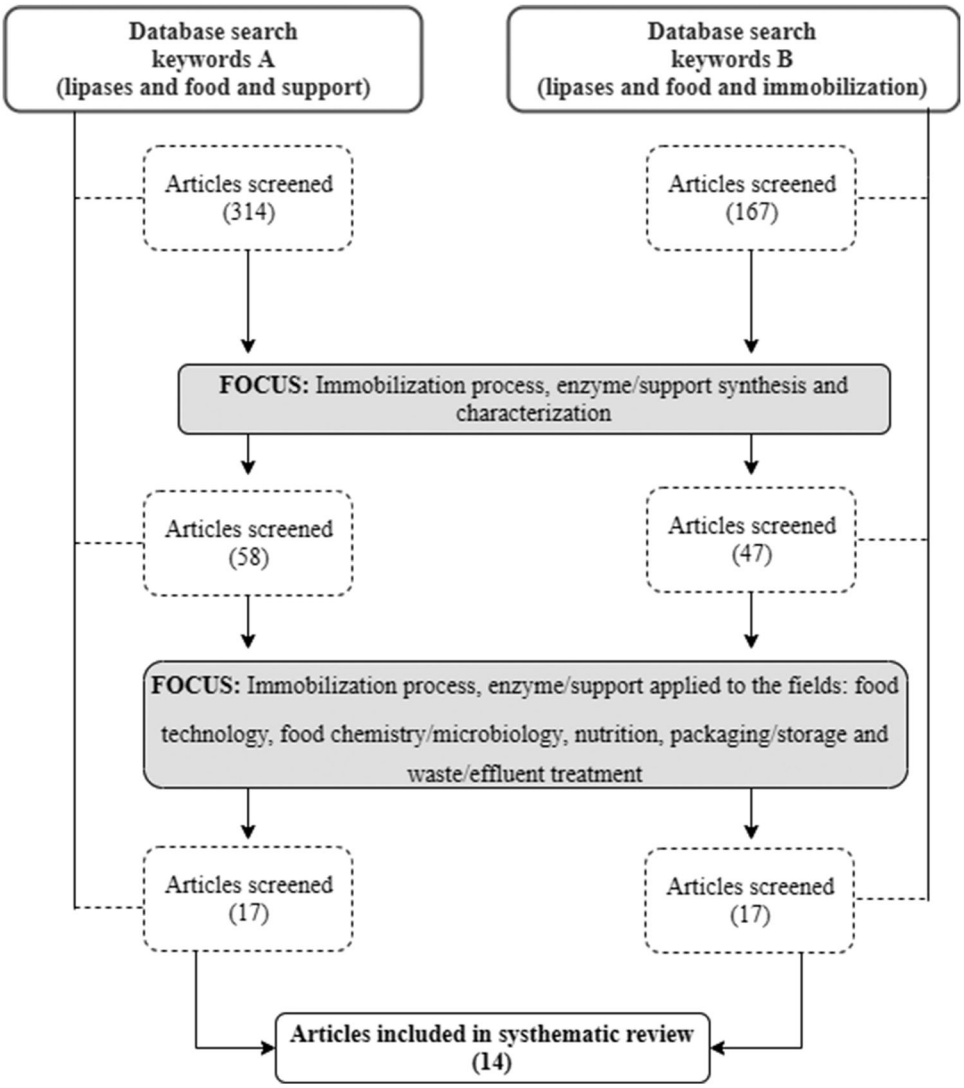

Figure 1. Flowchart of the study design of systematic review.

In this context, the health-beneficial properties of food products enriched with omega (ω-3, ω-6 and ω-9) fatty acids attract particular interest (Matuoog, Li, and Yan 2017; Rasti, Erfanian, and Selamat 2017; Walker, Decker, and McClements 2015) due to singular characteristics, such as: increase of high-density lipoprotein (HDL) and reduction of low-density lipoprotein (LDL) levels in the blood, minimizing the risk of cardiovascular diseases and strokes; better physical performance; greater energy availability and good mood; prevention/treatment of hypertension, diabetes and arthritis; induction of anti-inflammatory hormones secretion, and improvement in the immune system (Pérez et al. 2018; Morais Júnior et al. 2017).

According to Pérez et al. (2018), the use of enzymes to synthesize mono- and polyunsaturated fatty acids have many advantages over traditional chemical methods—fractional distillation and concentration with urea—which can promote the oxidation of PUFAs (Moharana et al. 2016). Besides, enzymatic reactions are conducted under milder conditions, have lower costs and do not generate undesirable compounds in the final products (Pérez et al. 2018). Nevertheless, there are still several bottlenecks associated with the use of free-form lipases (e.g., inactivation, difficult separation from reaction medium, sensitivity to pH and temperature variations, low operational stability) which paves the way for studies of immobilized biocatalysts in the synthesis and/or enrichment of PUFAs (Matuoog, Li, and Yan 2018).

Omega-3 (ω-3) fatty acids are partially susceptible to lipase hydrolysis, and therefore can be partially separated from monounsaturated fatty acids present in fish oil. For this purpose, Matuoog, Li, and Yan (2018) immobilized the lipase from *Thermomyces lanuginosus* (TLL) on magnetic nanoparticles by physical adsorption. Nanoscale magnetic materials offer several advantages, including high surface area, surface modification with various active groups, low mass transfer resistance, easy removal from reaction mixture and recyclability due to selective segregation under a magnetic field. Thus, the choice of support was based on two main aspects: the first one (resistance to mass transfer) is related to the fact that nano-immobilized enzymes are dispersed homogeneously in the reaction system, which prevents the formation of different mass resistance zones. Regarding the second aspect (separation of biocatalysts from the reaction medium), one of the limitations of nanoscale immobilized

Table 1. Lipases immobilized on different supports for food industry processes

Support	Immobilization technique	Objective	Main results	References
LRM-IM (Lipozyme from *Rhizomucor miehei*, immobilized on ion exchange resin, sn1,3-regiospecific); LTL-IM (Lipozyme from *Thermomyces lanuginosa*, immobilized on acrylic resin)	Not reported	Application of LRM-IM and LTL-IM on the production of structured lipids by interestification of avocado oil with caprylic acid (CA), in solvent free medium. Investigation of the effect of temperature and enzyme concentration on the incorporation of CA in the avocado oil structure	The temperature of 30 °C was ideal for the incorporation of CA in avocado oil. At all enzyme concentrations evaluated, the total activity values were 3.24-fold higher for LTL-IM, which is due to the differences in the surface nature of the supports used, providing different features in the lipase stability. LTL-IM has protein content was 3-fold higher than that of LRM-IM, which could explain the difference observed between the two immobilized lipases	Caballero et al. (2014)
Candida antarctica lipase B (CALB), Novozym 435. Support: macroporous acrylic resin	Adsorption	Synthesis of n-butyl lactate by Novozym 435, using lactic acid as substrate and supercritical fluoromethane as solvent	Lactic acid conversion to n-butyl lactate was dependent of pressure and temperature. The highest conversion (88.2%) was obtained after 26 h at 55 °C and 30 MPa. Conversion and reaction yield increased when pressure was changed from 7.5 MPa to 10 MPa and from 20 MPa to 30 MPa	Kavčič, Knez and Leitgeb (2014)
Candida rugosa lipase (CRL) free (AYS) and immobilized on porous cross-linked polystyrene resin beads (NKA)	Adsorption	Development of a new functional oil rich in phytosterol and diglyceride esters by enzymatic esterification using One Pot system. Evaluation of the catalytic activity and thermal stability of AYS and AYS@NKA systems during the transesterification reaction	At 50 °C, the conversion of phytosterol esters (PE) increased rapidly during the first 12 h and achieved a relatively high conversion (92.4%) with the AYS@NKA system. Free-AYS showed the conversion of 68.6 % after 24 h. AYS performance declined sharply at 60 °C, while AYS@ NKA retained most of its initial activity, with 87.2% of conversion	Zheng et al. (2014)
CRL immobilized on sol-gel matrix	Encapsulation	Study the sucrose acetylation process by immobilized CRLs on sol-gel support. Evaluation of the efficiency of immobilization process with different surfactant employed as coating. Comparison of the performance of immobilized and free enzymes	Nonionic surfactants (Tween 80, Tween 20, Span 80) enhanced the lipases activity. An increase in the yield of the sucrose-6-acetate synthesis reaction was obtained when the lipase:surfactant ratio was below 4:1, and the glycerol content was 0.1 mL. The optimal PEG concentration was 20 $mg.mL^{-1}$. The immobilized biocatalyst achieved maximum conversion at 45 °C and remained stable above 60 °C. The free enzyme lost its initial activity within about 2 h, while the immobilized/coated lipase maintained its initial activity for 16 h. The recyclability of the immobilized system was six cycles, yielding at the end 57.81%	Zhong et al. (2014)
Novozyme 425	Not reported	Synthesis of soybean oil PE by transesterification reaction, catalyzed by Novozyme 425. Investigation of the influence of temperature, reaction time, phytosterol concentration, reaction pressure, lipase dosage and velocity of agitation over conversion rate	Conversion rate increased rapidly when the amount of enzyme was increased to 1% (optimal concentration). Temperature had a significant effect on the conversion rate, raising it at range of 55-85 °C. Above 85 °C partial lipase denaturation occurred. Optimal values of phytosterol concentration, reaction time and pressure were 5%, 0.5–1 h, 6–8 MPa, respectively	Hu et al. (2015)
Hydroxyapatite (HAP)	Adsorption	Synthesis of methyl acetate (short chain ester) by free-CRL and immobilized-CRL on the HAP, using methanol as solvent	HAP-CRL showed remarkable thermal stability compared to free enzyme. Among polar organic solvents, the most striking effect was obtained for ethanol: after 3 h of incubation, CRL was completely inactivated, while HAP-CRL preserved residual activity of approximately 50%. Free CRL produced only 2.6% methyl	Trbojević Ivić et al. (2016)

(*continued*)

Table 1. Continued.

Support	Immobilization technique	Objective	Main results	References
			acetate, while 52.5% was obtained for the reaction with HAP-CRL	
Magnetic nanoparticles	Adsorption	Immobilization/stabilization of *Thermomyces lanuginosus* lipase (TLL) on magnetic nanoparticles, for application in docosahexanoic acid (DHA) enrichment	Maximum activity was reached at concentration of 4.4 mg TLL protein/g support. Optimum temperature and immobilization time values were 40 °C and 4 h. At pH 7.5, the best adsorptive capacity of the biocatalysts on the support was obtained. Under such conditions, enzymatic activity was increased to 82% compared to the free system. Fish oil hydrolysis was satisfactory after six cycles	Matuoog, Li and Yan (2017)
Chitosan coated Fe3O4-nanoparticles (CS-MNPs)	Covalent bond	Investigation of the efficiency of extremophilic lipases from *Rhodothermus marinus* (RD) in free and immobilizated form to synthesize methyl acetate. Evaluation of the support influence over stability at different pH and temperature conditions; storage and recycling stability; synthesis of aromatic methyl acetate ester	Residual activity of free-enzyme reduced to 83.2%, 72% and 35% after 60 min incubation at 60, 70 and 80 °C, respectively. Residual activity of the immobilized enzyme remained unchanged at 60 °C, decreasing to 98% (at 70 °C) and 92% (at 80 °C) after 60 min incubation. The free enzyme remained stable at pH 8, 9 and 10, retaining 90% of its initial activity. RD-MNPs was stable at different pH values and at high temperatures. RD-MNPs retained approximately 80% of their initial activity after 20 cycles. The reduction in enzymatic activity was attributed to biocatalyst denaturation and/or permeation of enzyme on the supports	Memarpoor-Yazdi, Karbalaei-Heidari and Khajeh (2017)
Rhizopus oryzae lipase immobilized on amberlite (rROL)	Not reported	Production of dietary triacylglycerides (MLM) rich in linoleic acid, by solvent-free acidolysis using grape oil as a source of polyunsaturated fatty acids and recombinant rROL and *Carica papaya* lipase (CPL) autoimobilized in papaya latex as catalytic system	MLM TAG production was quantified by TAG yields and consumption of capric or caprylic acids. The highest TAG consumed was obtained after 48 h of reaction for systems containing caprylic acid, and catalyzed by rROL (81.5%) and CPL (81.7%). The yields corresponding to the formation of new TAGs was 66.8 and 40.8% for rROL and CPL, respectively. This can be justified based on high hydrolytic activity CPL, which was 1.4 times higher than rROL	Costa et al. (2018)
CALB, LRM-IM; LTL-IM, CRL, *Rhizopus arrhizus* lipase and *Pseudomonas cepacia* lipase	Immobilization on acrylic resin (CALB) or microporous polypropylene by adsorption (other enzymes used)	Alter hydrophilicity of bixin by introduction of sorbitol in their chemical structure using different lipases	All enzymes presented similar performance, with no influence of the support and solvent types	Jahangiri et al. (2018)
Heterofunctional support (octyl sepharose)	Covalent bond and adsorption	Production and characterization of omega-6 and omega-9 obtained by hydrolysis of açai (*Euterpe oleracea*) and buriti (*Mauritia flexuosa*), using lipases isolated from *Beauveria bassiana* (BBL) and *Fusarium oxysporum* (FOL), immobilized on heterofunctional support	The immobilization process improved the activity of BBL and FOL in approximately 23 and 11 times, respectively. Immobilization provided an increase in thermostability of both enzymes, but only FOL remained with higher relative activity over a broader temperature range, with minimal loss of activity at 30–50 °C range	Pérez et al. (2018)
Celite, chitosan, amberlite and kaolin	Adsorption	Application of psychrophilic lipase from *Pseudomonas* sp. AMS8 in the synthesis of ethyl hexanoate by direct esterification, with or without the presence of toluene as a solvent. Study the influence of different immobilization materials over esterification reaction	Chitosan and kaolin showed the highest (76.2%) and lowest (31.2%) percentage of immobilization, respectively. The activity of native AMS8 lipase was ≈30.5 $U.mL^{-1}$, while immobilized enzyme activities were 37.2 (in chitosan), 33.5 (celite), 28.1 (amberlite XAD7) and 15 (kaolin) $U.mL^{-1}$. Chitosan immobilized lipase showed higher	Musa et al. (2018)

(*continued*)

Table 1. Continued.

Support	Immobilization technique	Objective	Main results	References
			conversion compared to native enzyme, with ethyl hexanoate production of 125.7% and 115.6%, in the absence and presence of toluene, respectively	
Lipozyme 435 (hydrophobic support), LTL-IM (silica gel), LRM-IM (weak-base anion exchange resin)	Not reported	Evaluate the performance of three commercial lipases immobilized on different supports in the delay/control of sardine oil oxidation	In the presence of immobilized lipases, the primary oxidation products were formed in much lower amounts than in control sample. Regarding secondary oxidation products, all immobilized systems presented similar behavior. As an exception, LTL-IM (at 40 °C) showed similar or higher values of secondary oxidation products compared to sardine oil in the absence of immobilized lipases	Solaesa et al. (2018)
Sepabeads C-18 resins coated with polyethylene glycol	Adsorption	Synthesis of omega-3 from chia oil using immobilized TLL lipase.	The performances of synthesized biocatalyst and commercial lipases (LTL-IM and TLL immobilized in Immobead 150) were compared. The system developed by the authors demonstrated better regioselectivity, obtaining 100% conversion of acylglycerols in omega-3. The recycled condition minimally reduced the activity of TLL immobilized on C-18, which demonstrated better performance compared to commercial enzymes	Castejón et al. (2019)

mes applications is that they become too small, making their recovery more difficult. Thus, the use of magnetic nanoparticles helps to solve this limitation, being widely used in the field of homogeneous catalysis (Matuoog, Li, and Yan 2018).

Matuoog, Li, and Yan (2018) reported the optimal conditions for immobilization of *T. lanuginous* onto magnetic nanoparticles, which were shown to occur at 40 °C, pH 7.5, 4 h and 4.4 mg TLL protein (g of support of lipase)$^{-1}$. Thermal (20–50 °C) and pH (0.1 M phosphate buffer pH 6–7.5 and 0.1 M Tris–HCl buffer pH 7.5–8.5) stability measurements were also performed for immobilized lipase. The experiments showed that catalytic system was stable at 40 °C/1 h, while 20% of the enzyme activity was lost after incubation at 50 °C for 1 h. Under pH conditions close to neutrality (7.5) the relative activity of immobilized TLL was 90.32%, for the two buffers used.

Moharana et al. (2016) reported that the selectivity of lipases is major challenge in the hydrolysis of ω-3 fatty acids. This is because fish oil triglycerides are complex in their composition, and encompass several fatty acids types (mainly saturated, monounsaturated and polyunsaturated) that are unevenly distributed in the glycerol skeleton. For anchovy oil, for example, DHA is more abundant at sn-2 than at sn-1 and sn-3, while EPA distribution is more abundant at sn-1 and sn-3 compared to sn-2 position (Akanbi, Adcock, and Barrow 2013; Moharana et al. 2016).

The current demand for ω-3 has caused the overfishing of many marine species, since they constitute the main source of these products. In order to overcome this problem, nonanimal sources have been proposed to meet the worldwide demand (Röhrl et al. 2020; Castejón et al. 2019). In this context, algae appear as an alternative source of ω-3, EPA and DHA, products that are sensorially more pleasant to the consumer compared to those of fish origin (Röhrl et al. 2020; Harwood 2019) However, limited information is available in scientific literature concerning the use of this raw material in the scope of this review.

Regarding plants of terrestrial origin, chia (*Salvia hispanica* L.) is a rich source of α-linolenic acid (ALA, 18:3), that can be used as a precursor for DHA and EPA synthesis (Karbowska and Kochan 2018). Indeed, the highest known natural percentage (about 65%) of ω-3 fatty acids are present in chia oil, while a smaller amount (30%) is found in fish oil (Moharana et al. 2016).

Therefore, the nutritional benefits of chia oil (such as relevant antioxidant content) added to the permission for their application in the food industry by European Commission authorization (Regulation (EC) n° 258/97) have given rise to an emerging interest of food industry in this oil (Castejón et al. 2019).

In a recent study (Castejón et al. 2019), TLL lipase was immobilized for synthesis of ω-3 from chia oil. The catalytic system was constructed by hydrophobic adsorption on Sepabeads C-18, and coated with polyethylene glycol (PEG), and prove to be an efficient, stable and active material for ω-3 production. Moreover, immobilized lipases were successfully operated in a recycle mode, which is considered a good way to balance the cost of production. The use of PEG is an emerging technology for biological catalysis that improves the stability of covalently immobilized enzymes on hydrophobic support. PEG forms a viscous layer around bioactive substances, which avoids changes of enzyme active sites. In addition, it is a nontoxic polymer considered GRAS (generally recognized as safe, status determined by FDA,

Food and Drug Administration) and applied in the manufacture of pharmaceuticals (Castejón et al. 2019). Table 2 shows other studies on production of PUFAs using immobilized lipases produced by *T. lanuginosus*, as well as lipases from other microbial sources.

It is worth to notice that precautions must be taken regarding lipases immobilization supports and methods, which results in different open/closed forms of the enzyme, that in turns provide different catalytic properties. When lipases are adsorbed on hydrophobic supports, for example, the open active site is fixed on the support; however, interaction with solvents can promote slight changes in their conformation, even if it already been fixed (Moreno-Perez et al. 2017).

According to Eş, Vieira, and Amaral (2015), the frequent choice of physical adsorption for PUFAs synthesis and enrichment results from the simplicity and low cost of processes allied to the lipase maintenance at high rates. However, this technique does not offer high enzymatic stability in the support and can lead to biomolecules leaching during food processing and/or washing of the enzyme carrier.

A simple and useful tool for improving/adapting enzyme properties to industrial processes (enzyme stabilization and reuse, long-term biocatalyst use) is the immobilization on hydrophobic supports. This strategy involves the surrounding of lipases active site, keeping it in an open conformation, which causes its hyperactivation and stabilization. Furthermore, enzymatic immobilization on hydrophobic surfaces is a reversible process (Pérez et al. 2018). After inactivation of the biocatalysts, lipases can be desorbed from the support, which can be reuse in a new immobilization and reaction cycles (Palomo et al. 2002; Pérez et al. 2018).

The application of octyl-sepharose, a hydrofobic support, has been found to be an interesting strategy to increase the activity of lipases produced from *B. bassiana* (23.5-fold) and *F. oxysporum* (11-fold), in the hydrolysis of amazon oils for production of omega 6 and 9. Moreover, the immobilization procedure doubled the thermostability of *B. bassiana* lipase at 40, 50 and 60 °C during 24 and 48 h. Besides, the supported biocatalysts remained stable at pH 5 and 6 up to 48 h under 40 °C. Regarding *F. oxysporum* lipases, they were stable in the range of 30–70 °C. The hydrofobic support promoted an increase in the stability of lipases at pH 5 and 6 (40 °C), at more acid condition, however, the stability of enzymes was halving (Pérez et al. 2018).

Notwithstanding, hydrophobic supports have some disadvantages, including the release of free lipases into reaction medium, due to exposure to high temperatures and solvent concentrations. In addition, many substrates and lipase products possess typical detergent properties that favor enzyme desorption (Pérez et al. 2018).

The use of heterofunctional supports can be employed to overcome the aforementioned limitations (Albuquerque et al. 2016; Guajardo et al. 2015; Pérez et al. 2018). A characteristic of this type of material is the several functional group on its surface, that combine the effects of physical adsorption and covalent bonding, thereby altering the three-dimensional structure, activity, stability and selectivity of the enzymes. Although heterofunctional supports encompasses the benefits previously described, the binding nature of this immobilization strategy becomes an irreversible process, preventing the reuse of the support. Additionally, unsuccessful enzyme-support linkages can lead to decreased in the lipase activity and even enzyme leakage (Pérez et al. 2018).

Synthesis of structured lipids (SLs)

SLs are defined as restructured triglycerides obtained by chemical or enzymatic interesterification of triacylglycerols (TAGs) containing short, medium and/or long chain fatty acids, from vegetable oils or animal fats (Moreira et al. 2017). SLs are rapidly metabolized by human body, basically the fatty acids at sn1,3 positions are hydrolyzed by pancreatic lipase, absorbed by portal vein and transported to the liver, preventing their accumulate as body fat (Costa et al. 2018; Caballero et al. 2014).

Ingestion of SLs increase the availability of essential fatty acids in human body, which have a positive effect on the health, including enhancement of healthy fats absorption, reduction of metabolic disorders as well as abdominal fat deposition (Caballero et al. 2014; Moreira et al. 2017; Verdasco-Martín et al. 2018). Besides, SLs containing ω-3 fatty acids possess interesting features, such as improvement of immune response, increase the synthesis of eicosanoid and anti-inflammatory effect (Kleiner and Akoh 2018; Akanbi and Barrow 2015). In this concern, SLs can be inserted in the formulation of several food industry products, as margarines, modified butters, shortenings, infant formulas, enteral supplements and chocolate (Osborn and Akoh 2002; Kim and Akoh 2015; Kleiner and Akoh 2018).

Regarding food technology field, SLs can modify the physical (melting point), chemical (reduction of caloric value) and rheological properties of edible oils and fats (Caballero et al. 2014; Moreira et al. 2017; Verdasco-Martín et al. 2018).

According to Osborn and Akoh (2002) several SLs such as Caprenin (Procter & Gamble's, USA), Salatrim® (Nabisco Foods Group, USA) and Benefat (Cultor Food Science, USA) have been produced industrially by chemical route since the 1990s. Nevertheless, the current demand for natural products and green processing open a new scenario for the synthesis of SLs by biocatalysis, since they not only allow environment-friendly production but also increase in the consumption/marketing of products considered "natural" (Şahin-Yeşilçubuk and Akoh 2017). Moreover, high regiospecificity, reaction conducted at low temperatures, and the possibility of non-solvent addition in reaction medium, makes lipases a more efficient system in the interesterification process, compared to chemical ones (Caballero et al. 2014).

Loders Croklaan (USA) was a pioneer in the production and commercialization of SLs synthetized by enzymatic means. The final product obtained and named Betapol®, was designed to imitate the profile of human milk in infant formulas. InFAT®, a product with the same purpose, was developed by Advenced Lipids in conjunction with Aarhus Karlshamn and Enzymotec (Osborn and Akoh 2002; Şahin-Yeşilçubuk and Akoh 2017).

Table 2. Literature compilation of lipase/support systems for synthesis/enrichment of polyunsaturated fatty acids

Support	Immobilization technique	Objective	Main results	References
Polyurethane (PU)-polyethylene glycol (PEG), 10% PEG (400, 4000 or 6000 Da)	Adsorption	Application of PU-PEG particles for TLL (*Thermomyces lanuginosus* lipase) immobilization. Evaluation of the effect of PEG molar mass on the enzyme-support interaction for ethanolysis of sardine oil	TLL was efficiently immobilizated on the PU-PEG particles. Different PEG fractions on the enzyme/support systems provided different functional properties for ethanolysis reaction. TTL-PU-PEG 6000 provided higher production of ethyl esters, with greater selectivity under tested temperature ranges	Cipolatti et al. (2015)
PU-PEG coated with trehalose, PU-PEG coated with polyethyleneimine (PEI)	Adsorption	Application of immobilized TLL lipases on trehalose or PEI coated PU-PEG supports in fish oil transesterification process for AGP production	PU-PEG-PEI and PU-PEG-trehalose improved the stability and catalytic activity of TLL. Both system were effective in the production of ethyl esters, with production 4-fold higher than uncoated PU particles. High percentages of immobilization and enzyme recovered activity were reached	Cipolatti et al. (2016)
Candida antarctica lipase B (CALB) immobilized on ECR 1030 resin (acrylate/divinyl benzene polymer)	Adsorption	Evaluation of ECR1030-CALB system with respect to temperature, acidity, alkalinity, tolerance to organic solvents stability, and fatty acid specificity. Comparison of the yields of ECR1030-CALB for EPA and DHA synthesis with performance of Novozym 435	ECR1030-CALB stability was similar to that obtained by commercial enzyme Novozym 435. ECR1030-CALB demonstrated high specificity for EPA and DHA, present excellent esterification activity, properties and promising catalytic performance	Li et al. (2017)
Carbon nanotubes (MWCNTs)	Adsorption	Evaluation of the effect of enzyme concentration, pH, temperature, time and specific activity over TLL immobilization on the MWCNTs by physical adsorption. Study on the performance of synthesized biocatalysts in docosahexaenoic acid (DHA), enrichment using fish oil as substrate	The highest enzymatic activity and immobilization efficiency were obtained at pH 8, at 45 °C, 30–60 min. DHA content of fish oil increased when the lipase amount added was 9 $mg.g^{-1}$. DHA concentration was 4.2 and 2.5 fold higher than the initial content for TLL-MWCNTs and free TLL, respectively. After 6 cycles of successive use, the hydrolysis yield was 80% for TLL-MWCNTs and 62% for free TLL	Matuoog, Li, and Yan (2017)
Candida rugosa lipase (CRL) immobilized on octyl sepharose, diethylaminoethyl (DEAE), bromocyanogen (CNBr), monoaminoethyl n-ethyl (MANAE), carboxymethyl and sulfopropyl, heterogeneous support aminoglyoxyl (AMG)	Adsorption (CRL), ionic bond (DEAE), one- (CNBr) and multipoint covalent bond (other supports used)	Use free and immobilized CRL (different supports) on the production of omega-3-PUFAs (polyunsaturated fatty acids) from fish oil in a two-phase system (water/solvent), supplemented with soybean molasses	DEAE support showed better immobilization yield (≈98%) and specific activity (48.9). CRL carboxymethyl and CRL/sulfopropyl systems were more stable in all solvents tested (propanol, methanol, cyclohexane). During hydrolysis of fish oil, immobilized lipase showed high specificity than free enzyme. Ionic supports were more active and specific for PUFAs production	Morais Júnior et al. (2017)
TLL-sepabeads, TLL-duolite, Lipozyme TL-IM (prepared by Novozymes), lecithase-sepabeads, and lecitase-duolite	Adsorption	Evaluate the influence of different supports (different orientation of enzyme on the support) over activity of lipases used in sardine oil ethanolysis, in a solvent free system	Under solvent free system, TLL-sepabeads was the most active (synthesis of EE-EPA and EE-DHA) and selective biocatalyst, being more stable than the commercial derivative, keeping 90% of activity after 48 h. Lecithase-sepabeads and Lecitase-duolite showed very low catalytic activity. Lecithase-	Moreno-Perez et al. (2017)

(*continued*)

Table 2. Continued.

Support	Immobilization technique	Objective	Main results	References
			sepabeads selectivity was 43-fold higher for EPA than DHA	
Butyl Sepharose, phenyl sepharose, octyl sepharose, octyl + PEI (Polyethyleneimine), octyl + trealose (diethylaminoethyl) DEAE agarose, CNBr-activated sepharose	Adsorption	Immobilization of *Moniliella spathulata* R25L270 lipase on hydrophobic, covalent and ionic supports, for PUFA production from sardine oil hydrolysis	Adsorption on hydrophobic supports demonstrated greater temperature stability than free lipase. No changes in pH and temperature stability occurred when ionic supports were employed. Immobilized lipases on the octil-sepharose had better activity and selectivity for sardine oil hydrolysis. The maximum selectivity (7.53) was obtained at pH 5.0	Souza et al. (2017)

The Archer Daniels Midland Company has in its portfolio several SLs (oils and shortenings) produced by lipases. Bunge Oils offer products in the same category, while medical nutrition products containing SLs are produced and sold by Stepan Specialty Products LLC (Kleiner and Akoh 2018).

The immobilized lipases Lipozyme RM IM, Lipozyme TL IM and Novozym 435 designed and commercialized by Novozymes (Denmark, Bagsvaerd, Denmark), are examples of biological catalytic systems used in industrial scale to produce SLs (Kim and Akoh 2015).

One of the most relevant parameters in the synthesis of SLs by lipases is the choice of TAG used as a substrate. In this context, avocado oil appears as a potential raw material for SLs production, because it is cold extracted, which leads to retention of significant amounts of natural bioactive phytochemicals.. In addition, this oil still has high amounts of campesterol, sitosterol and phytosterols, compounds that remain in the final product, increasing the nutritional quality of SLs (Caballero et al. 2014).

Medium-long-medium triacylglycerols (MLMs) are another type of SLs, synthesized by modifying fatty acid composition. MLMs are composed of medium chain fatty acids (M) in the external positions (sn-1 and sn-3) and long chain fatty acids (L) in the internal position (sn-2) of glycerol skeleton (Costa et al. 2018; Vieira 2018). These compounds are of dietary interest due to their potentially in control of obesity and metabolic disturbs, are also indicates for patients with fat malabsorption, and present low caloric value (about 5 $kcal.g^{-1}$) compared to conventional oils and fats (9 $kcal.g^{-1}$) (Costa et al. 2018; Vieira 2018).

According to literature data, a noncommercial catalyst, *Rhizopus oryzae* lipase (rROL) expressed in *Pichia pastoris*, clearly demonstrated a promising performance in the MLM-type TAG synthesis (Costa et al. 2018). In this concern, Costa et al. (2018) observed that immobilized rROL featured high stability under different operational conditions, which was attributed to high hydration degree of supports, since loss of activity may occur during the course of the reaction.

Flavors and food coloring

Currently, the drive among consumers is the pursuit of more natural and healthy food products. Different flavors for food industry are extracted from natural sources (as plants, fruits and flowers) or produced through chemical synthesis. However, the commercial use of natural extracts is hampered by limited supply and high production costs. Similarly, chemical route for flavors synthesis results in compounds associated with side effects to human health, due to the use of hazardous chemicals. On the other hand, enzymatic production of flavors has received increasing attention due to the inherent catalytic selectivity of enzymes, higher product purity and moderate reaction conditions (Musa et al. 2018).

In the dairy industry, for example, lipases play an important role in the hydrolysis of milk fat, enhancing cheese flavor and speeding up the cheese ripening, and are also involved in butterfat lipolysis (Memarpoor-Yazdi, Karbalaei-Heidari, and Khajeh 2017). These enzymes are excellent catalysts for production of food additives and ingredients, with potential application on the synthesis of short chain esters used as taste modifiers or aroma compounds (Memarpoor-Yazdi, Karbalaei-Heidari, and Khajeh 2017).

It is important to highlight that several health and safety committees, among which FDA and JECFA (Expert Committee on Food Additives), have no reservations about using of flavors sintetized by enzymes in the food sector (Sá et al. 2017).

Annatto (*Bixa orellana*) seed extract (containing bixin and norbixin as major components) is one of the most commonly natural colorants used in the food industry (Jahangiri et al. 2018). Besides, annatto derivate colorants are considered a safety additive for application in food processing. In this concern, FAO, WHO (World Health Organization) and JECFA consider the daily consumption of bixin up to 0.065 mg (kg body weight)$^{-1}$ as safe. In turn, for norbixin this value is 0.051 mg. (kg body weight)$^{-1}$ under United States regulations (Raddatz-Mota et al. 2017).

In vitro studies conducted with animals have shown that colorants extracted from Annatto can cause allergic reactions, due to the sensitivity of certain proteins of the achiote seed. It should be noted, however, that such products have not demonstrated any effect of genotoxicity and chronic oral toxicity (EFSA, 2017; Raddatz-Mota et al. 2017). Interesting, Beni et al. (2020) demonstrated that bixin and norbixin possess antihemolytic properties, that is, they act by protecting

erythrocytes from lipid peroxidation, and also improved the cellular redox environment.

The polyene chain of bixin makes it highly hydrophobic and unsuitable for application in water-based food formulations, while norbixin is a water-soluble colorant only in alkaline solutions, precipitating under acidic and neutral conditions (Jahangiri et al. 2018). In this regard, the food industry does not possess a stable and natural hydrophilic colorant in the yellow-red range that can be used in low pH water-based food products such as beverages. As an alternative, the water solubility of bixin could be increased by attaching a hydrophilic molecule to it, as proposed by Jahangiri et al. (2018). These authors employed five lipases to catalyze the transesterification reaction between bixin and sorbitol: two immobilized commercial lipases, lipozyme RM-IM (LRM-IM; lipase isolated from *Rhizomucor miehei*) and lipozyme TL-IM (LTL-IM; lipase from *T. lanuginosus*); three lipases (from *Rhizopus arrhizus*, *C. rugosa* and *Pseudomonas cepacia*) acquired in free form and immobilized on microporous polypropylene. Although immobilization can improve the activity of biocatalysts, Jahangiri et al. (2018) did not observe the influence of the type of support, and all enzymatic systems demonstrated similar performance.

Lipases are also used to produce food flavorings that successfully mimic the most complex natural ones (Trbojević Ivić et al. 2016), as observed in Table 3. The production of flavoring esters by lipases is well known, however, almost all literature data focus on the use of mesophilic lipases—*C. rugosa*, *Candida antarctica* and *Mucor miehei*—and the synthesis of esters by psychrophilic lipases are underexploited. In this context, Musa et al. (2018) demonstrated that lipase AMS8, isolated from psychrophilic *Pseudomonas* sp. AMS8 and expressed in cold-active *Escherichia coli*, exhibits high specificity and activity for flavoring production in the range of 0 and 20 °C. In addition, AMS8 biocatalysts showed greater flexibility and stability compared to mesophilic and thermophilic enzymes.

Additives, antioxidants and antimicrobials

Chlorine, chlorine dioxide, paracetic acid, ozone, hydrogen peroxide, UV radiation are among the most commonly antimicrobial used by the food industry. Nevertheless, these compounds have a drawback as they may induce food modifications (Kavčič, Knez, and Leitgeb 2014).

Commercial biocatalyst Novozym 435 (immobilized *C. antarctica* lipase B) was applied for synthesis of *n*-butyl lactate, using lactic acid as substrate and supercritical fluoromethane as solvent. *N*-butyl lactate is considered GRAS by the FDA, has broad-spectrum microbicidal activity; it is biodegradable and environmentally friendly (Kavčič, Knez, and Leitgeb 2014). The enzymatically synthesized *n*-butyl lactate exhibited 100% inhibition over *Saccharomyces cerevisiae, Aspergillus niger*, *Penicillium cyclopium* and *Trichoderma viride*. Therefore, the combined use of a sustainable and clean technology (such as biocatalysis) and green solvent is very attractive for the development of "green" processes for the synthesis of "natural" food additives (Kavčič, Knez, and Leitgeb 2014).

Sardine oil has a high amount of omega-3 polyunsaturated fatty acids (n-3 PUFA), mainly EPA (20:5n-3) and DHA (22:6n-3). Despite the noble nutritional value, the high degree of unsaturation in the chemical structure makes this oil prone to autoxidation. Oxidation compounds cause off-flavors and rancidity, loss of nutritional value and consumer rejection. The level of lipid oxidation is influenced by fatty acid unsaturation, oxygen concentration, temperature, water activity and presence of anti- and pro-oxidants (Solaesa et al. 2018).

Solaesa et al. (2018) evaluated the role of commercial lipases in the control of autoxidation kinetics of sardine oil. For this purpose, control experiments were performed at 40, 65 and 90 °C, and then immobilized enzymes were added to the reaction medium to obtain the net formation rate of primary and secondary oxidation products.

The data revealed that at 40 °C the values of primary oxidation products of sardine oil was poorly modified by the presence of catalytic systems. On the other hand, at 65 and 90 °C enzymatic reactions promote a strong decrease in the peroxide content, which was even lower than that of the control samples. Similar results were obtained for secondary oxidation products—anisidine and thiobarbituric acid reactive substances.

In general, lipozyme RM showed better performance on the control of sardine oil autooxidation, followed by Lipozyme 435 and Lipozyme TL. Nonetheless, Solaesa et al. (2018) did not carry out experimental tests to propose a plausive mechanism to justify the aforementioned results.

In addition to the direct action of lipases, the authors also attributed the results mentioned above to the nature of the catalytic supports, based on previous studies (Solaesa et al. 2017) of their research group. Lipozyme RM, for example, is immobilized on a weak base anion exchange resin, so the hydrophilic nature of this support may result in greater affinity for hydrophilic oxidation products. Consideration should also be given to contact area between support and enzyme: Lipozyme TL poses a smaller contact area with support (silica), which reduces the frequency of collisions between adsorbent and adsorbate, promoting a low system activity. On the other hand, enzymes immobilized on hydrophobic support (e.g., Lipozyme 435) tend not to perform well in relation to secondary oxidation products, since they are polar molecules.

Synthesis of phytosterol esters (PE)

Phytosterols had attracted a great attention in the food field, especially due to their hypocholesterolemic properties, thereby reducing plasma levels of total and LDL cholesterol, without affecting plasma HDL concentration (Yu et al. 2018; Zheng et al. 2014). Notwithstanding, these compounds are not synthesized in the human body, so it must be supplied by diet. In addition, they are generally insoluble in water and their solubility in edible oil is approximately 1%. This feature limits the application in the food matrix and makes their disposition in the human body difficult, namely

Table 3. Flavors for the food industry produced by immobilized lipases

Flavors	Support/enzyme	Produtictivity	References
Citronellyl laureate (lemon flavor)	*Burkholderia cepacia* lipase, immobilized on biodegradable chitosan copolymer and polyvinyl alcohol	Optimal catalytic performance: 94 ± 1.52% yield (8.5 mmol:19.87 mmol citronellol: vinyl laurate molar ratio; mass of biocatalyst: 175.6 mg; temperature: 46.02 °C; pressure: 8.81 MPa)	Badgujar and Bhanage (2015)
Banana flavor	*Thermomyces lanuginosus* lipase (TLL), immobilized on Fe_3O_4 nanoparticles.	Optimal flavor production: temperature: 40 °C; conversion of 88 % and selectivity of 100%.	Sarno et al. (2017)
Ethyl caprylate (fruit flavor)	Novozym 435 (*Candida antarctica* lipase, immobilized on acrylic resin)	Maximum conversion: 92%/5 h/ stirred tank reactor. Conditions: 60 °C, 1:2 caprylic acid /butanol molar ratio, 2% lipase concentration, 250 rpm, 4g molecular sieves).	Sose, Bansode, and Rathod (2017)
Geranyl butanoate (cherry flavor)	Novozyme 435, NZL102-LYO-HQ (*C. antarctica* B lipase) immobilized on polyurethane foam	Novozyme 435: conversion of 97% (3: 1 substrate molar ratio, 150 rpm, 5% enzyme weight, 1 h of reaction). Novozyme NZL 102-LYO-HQ: conversion of 95% (substrate molar ratio 5:1, 70 °C, 150 rpm, 5% enzyme weight and 1 h of reaction).	Sbardelotto et al. (2018)
Butyl butyrate (pineapple flavor)	*Aspergillus niger* lipase, in free-form and encapsulated in sol-gel matrix	Conditions for maximum yield: 3:1 substrate (butanol:butyric acid) molar ratio, temperature 60 °C, 0.5 g free or encapsulated lipase.	Travalia et al. (2018)
Benzyl butyrate (similar to the plum and melon aroma)	Novozym 435	Conversion of 80% in the synthesis of benzyl butyrate.	Meneses et al. (2019)
Methyl and ethyl butyrate (apple flavor)	*Rhizomucor miehei* lipase immobilized on chitosan	Under optimized conditions, the maximum esterification yield was 92% for ethyl butyrate and 89% for methyl butyrate.	Oliveira et al. (2019)

absorption and metabolization (Yang, Oyeyinka, and Ma 2016).

In order to overcome the above-said shortcomings, the synthesis of PE via chemical or enzymatic routes has been exploited. A traditional chemical method consists on esterification between phytosterol and organic acids such as fatty acids. However, this procedure occurs at high temperatures, had low yield, selectivity and quality of the final product. Additionally, an efficient removal of the catalysts (MgO, La_2O_3, ZnO, Al_2O_3, and AlI_3) employed in the process must be carried out (Yu et al. 2018; Yang, Oyeyinka, and Ma 2016; Zheng et al. 2014). Enzymatic catalysis, in turn, has proved to be environmentally friendly, energy and cost-efficient technology. The reactions are proceeded under mild conditions, producing fewer byproducts, and has high specificity, making it an attractive methodology for PE synthesis (Zheng et al. 2014).

PE and their precursors (phytosterols) have similar biological properties. However, physical-chemical features of both compounds are different, and the presence of esters provides greater solubility in comparison with phytosterols. Such aspects facilitate the incorporation of PE in a wide variety of diets and fat-based food products. Furthermore, PE can be conjugated with dietary diglycerides, a new type of functional lipids that suppresses body fat accumulation, inducing body weight loss and low levels of postprandial triglyceride. These compounds serve as functional food ingredients for people with metabolic syndrome or diabetes (Zheng et al. 2014).

Thus, the study of Zheng et al. (2014) was premised on the development of a new functional oil rich in phytosterol and diglyceride esters by enzymatic esterification in One Pot system. In this process, the reagent is subjected to successive chemical reactions in just one reactor, aiming to avoid a lengthy separation process, and purification of the intermediate chemical compounds, saving time and resources. For this purpose, free form of lipases (AYS) produced by *C. rugosa* and immobilized enzymes on porous cross-linked polystyrene resin beads (NKA) were used. The resulting immobilized lipase (designated AYS@NKA) resulted in higher catalytic activity, which can be justified by dispersion of enzymes on the support, and as consequence, higher mass transfer rate. On the contrary, AYS system easily aggregate within the reaction system. Besides, NKA surface has a stabilizing effect, which prevents extensive structural changes typical of thermal denaturation. The ability to retain enzyme activity at high temperatures provides several processing advantages, such as better substrate solubility (Zheng et al. 2014).

Hu et al. (2015) highlighted the health-beneficial attributes of phytosterol ingestion, suggesting its application as a dietary supplement in various industrialized foods, such as margarine, salad dressing, edible oil and milk. The authors proposed a green synthesis method to produce PE using enzymatic catalysis and supercritical carbon dioxide (SC-CO_2). The combined use of lipases/SC-CO_2 can be applied in several reactions performed in food industry (including hydrolysis, esterification, transesterification, acylation, glycerolysis and fat randomization), increasing the solubility of hydrophobic lipid substrates, and providing better separation/recovery of enzymatic catalysts from the reaction medium, since they are not soluble in non-polar supercritical fluid (Hu et al. 2015).

Novozym 435/SC-CO_2 was employed in transesterification of soybean oil for PE production. The high conversion rate of 92% was achieved at optimal conditions of 85 °C, 8 MPa, phytosterol concentration of 5%, enzyme loading of 1% and reaction time of 1 h (Hu et al. 2015).

Finland was the first country to insert and commercialize phytosterols in food, specifically in margarines. Later, in 1999, the American food industry followed the same trend. Currently, they produce functional food that contain phytosterols not only in margarines, but also in dairy beverages, fat spreads, juices, chocolates and rye bread (Corrêa et al. 2017). Besides, phytosterols and their esters received GRAS certification by FDA (Food and Drug Administration), and they are also recognized as a safe compound by European legislations (He et al. 2018).

Synthesis of sugar esters (SE)

SE are nonionic surfactants produced from fatty acid, which possess several important physicochemical properties, such as foaming, emulsifying, stabilizing and high dispersing power. SE are widely used as water/oil emulsifiers in food products; as example, lactose esters stabilize fresh coconut milk emulsions; sucrose mono-myristate and sucrose monopalmitate inhibit biofilm formation by foodborne pathogenic bacteria. This finding is of great relevance, since chemicals with similar properties are unsafe and cannot be used in the food industry (Neta, Teixeira, and Rodrigues 2015). Other applications of sugar ester surfactants in the food industry include the flavors production, maturation of cheeses, bakery products, cakes, biscuits, mayonnaise and sauces, instant products, sausages, wine and dairy products (Neta, Teixeira, and Rodrigues 2015; Banat et al. 2010).

The insertion of SE in food processing is allowed by FDA (21CFR 172.859) authorities (Pérez et al. 2017). Thus, several scientific articles contemplate a foray about the technological aspects of SE. For instance, Farooq and Haque (1992) verified that SE (stearates) improved texture and mouthfeel of yogurt. Neta et al (2012), demonstrated that fructose, sucrose and lactose esters act by stabilizing the physical properties of fresh coconut milk, with a decrease in surface tension and stabilization of the emulsion being observed. In addition, Chen, Nummer and Walsh (2014) have presented that it is possible to inhibited the growth of a five-strain cocktail of *Listeria monocytogenes* in milk, yogurt and cottage cheese, due to the presence of lactose monolaurate, in the range of 3–5 $mg.mL^{-1}$.

Chemical acetylation of sucrose is generally performed under high temperature conditions and in the presence of an alkaline catalyst, which results in low specificity, high-energy consumption, darkening of products and even the production of toxic substances (Zhong et al. 2014). In this context, Zhong et al. (2014) studied the sucrose acetylation process by *C. rugosa* lipases immobilized in sol-gel supports using encapsulation procedure. In this case, the use of nonionic surfactants favored the enzyme activity. The reason for this could be the soft interaction between nonionic surfactant and enzymes via van der Waals forces, which maintain the three-dimensional lipase structure under optimal conditions, and promotes better catalytic activity compared to cationic surfactants. Other relevant aspect emphasized by Zhong et al. (2014) was the lipase:surfactant ratio employed during immobilization. Incomplete coating may occur for conditions in which enzymes are in greater proportion; on the other hand, a thicker layer may be formed when biocatalysts are at lower concentrations. Both conditions compromise the activity of the enzyme system.

Thus, Zhong et al. (2014) point out that the yield of sucrose-6-acetate as well as the maintenance of the active state of biocatalysts in sol-gel matrix further depends on the glycerylsilane precursor composition, and specifically in this case, the glycerol ($mL.(mL\ sol)^{-1}$) and PEG 600 ($mg.(mL\ sol)^{-1}$) loading. Glycerol, for example, acts by protecting the active site conformation of encapsulated enzymes; however, their excessive contents affect the pore volume and surface area of the sol-gel, which could block the lipase interface. PEG mainly affects the pore morphology of the matrix and reduce enzymatic stress during the lyophilization process. At high concentrations ($>20\ mg.mL^{-1}$), PEG can increase internal mass transfer resistance due to its viscosity and minimize the matrix pore size, which possibly reduces the accessibility of enzymes to the substrate. The association of *C. rugosa* lipase with sol-gel support expanded the thermal stability of the enzymes under 60 °C, also promoting a greater tolerance to contact with organic solvents (Zhong et al. 2014).

Conclusions

The functional groups present on the surface of the immobilization material alter the three-dimensional structure of the enzyme, affecting its activity, stability and selectivity, during synthesis/enrichment of PUFAs and SLs. The nature of the support—hydrophobic or hydrophilic—is considered an important aspect as it results in different forms of opening of the enzyme active site, providing alteration in the catalytic properties. The proportion of the immobilization protocol components and enzyme concentration is also a preponderant factor, acting in the increase/reduction of the enzymatic stress.

Regarding food coloring, lipases can be employed to modify chemical structure for increase/decrease their solubility. With respect to flavors, enzymatic production is related to the inherent catalytic selectivity of the lipases, not mention the higher purity of the final product. Furthermore, lipases are efficient in the synthesis of antimicrobial compounds, such as *n*-butyl lactate, a very attractive technology in view of the current search for "natural" food preservatives. Furthermore, it has been shown that immobilized lipases may act to control the autoxidation of fish oil, a well-known source of PUFAs.

Due to their high specificity, immobilized lipases can be successfully employed in the synthesis of PE, since mild process conditions minimize the production of byproducts. As an attractive technology, this technique allows the solubilization of PE in a wide variety of diets and fat-based food

products. Finally, in the case of ester surfactants synthesis, relevant factors for better performance of enzymatic system is lipase: surfactant ratio and support gel composition, which act on the active state of biocatalysts.

Disclosure statement

Authors declare no conflict of interest.

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