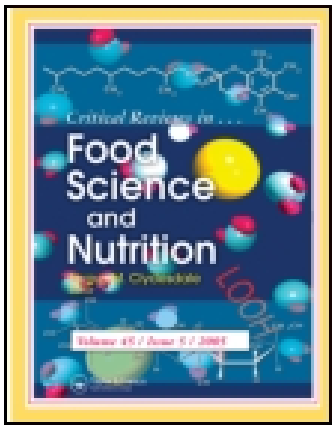

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Sugar Ester Surfactants: Enzymatic Synthesis and Applications in Food Industry

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Sugar Ester Surfactants: Enzymatic Synthesis and Applications in Food Industry

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Sugar esters are non-ionic surfactants that can be synthesized in a single enzymatic reaction step using lipases. The stability and efficiency of lipases under unusual conditions and using non-conventional media can be significantly improved through immobilization and protein engineering. Also, the development of de novo enzymes has seen a significant increase lately under the scope of the new field of synthetic biology. Depending on the esterification degree and the nature of fatty acid and/or sugar, a range of sugar esters can be synthesized. Due to their surface activity and emulsifying capacity, sugar esters are promising for applications in food industry.

Keywords Sugar ester surfactant, lipase, immobilization, organic reaction, food industry application

INTRODUCTION

Sugar-based surfactants are mainly characterized by having hydrophilic groups in their polar moiety. They are of great interest because they have good surface activity and can be produced from renewable resources (Soultani et al., 2003). Depending on carbon chain length and nature of the sugar head group, together with the many possibilities for linkage between the hydrophilic sugar head group and the hydrophobic alkyl chain, unique physicochemical properties can be attributed to these surfactants, some of them substantially different from the common non-ionic ethoxylated surfactants (El-Laithy et al., 2011). Also, these non-ionic surfactants cover a wide range of hydrophilic–lipophilic balance, thus enabling their use in a broad range of applications within fine chemistry, cosmetics, and food formulations (Coulon and Ghowl, 1998; Arcos et al., 2001; Ferrer et al., 2005; Queneau et al., 2008; Chang and Shaw, 2009; Gumel al., 2011).

These sugar-based surfactants are the final result of a product concept, which is based on the greatest possible use of renewable, inexpensive, and readily accessible feedstocks but also biodegradable, harmless to the environment and non-toxic resources (Tokiwa et al., 2000; Cruces et al., 2001; Castillo et al., 2003). While the derivatization of fats and oils to produce a variety of different surfactants for a wide range of applications has a long tradition and is well established, the production of surfactants based on fats, oils, and sugars on a bigger industrial scale is relatively new. Today, the most important sugar-based surfactants are alkyl polyglycosides, sorbitan esters, and sucrose esters (Hill and LeHen-Ferrenbach, 2009).

Considering the amphiphilic structure of a typical surfactant, it has always been a challenge to attach a sugar molecule as alternative to polyethylene glycol to a fat and oil derivative, such as a fatty acid. Although science has reported numerous ways of making such linkages and has also described a large number of different sugars used in such reactions, it is clear from an industrial perspective that only few sugars fulfill the criteria of price, quality, and availability to be an interesting raw material source. Those are mainly sucrose from sugar beet or sugarcane, glucose derived from starches, fructose from high fructose syrups, lactose from cheese whey, and sorbitol as the hydrogenated glucose derivative (Tokiwa et al., 2000).

The traditional chemical synthesis of sugar or polyol esters requires acidic and metal catalysts at high pressures and temperatures, resulting in most cases in complex mixtures of monoester and di- or tri-ester isomers and numerous by-products (Arcos et al., 2001; Kennedy et al., 2006). Until now, the sucrose esters

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synthesized by chemical way are the only sugar esters available on the market (Soultani et al., 2003). While most sugar fatty acid esters are chemically synthesized, the enzymatic synthesis of sugar esters has garnered considerable interest with regard to the creation of environmentally friendly processes (Liu et al., 2005; Chang and Shaw, 2009). As a result, in the latest years, several works have been done in this field mainly with sucrose, fructose, and glucose (Adachi and Kobayashi, 2005). These studies have shown that high conversion yields and productivities could be obtained. However, no or few data on the functional properties of these molecules are available, and no comparison has been made with commercial sucrose esters. For this reason, further studies on the surface properties of this novel class of surfactants are still required.

A comparison of the chemical and enzymatic routes for the production of sugar fatty acid esters is pertinent (Gumel et al., 2011). The industrial synthesis of sugar esters of glucose, fructose, and sucrose is achieved through the transesterification of the methyl esters of the corresponding fatty acid in the presence of a catalyst, at temperatures above 100°C and reduced pressures. Sucrose esters have been synthesized with decreasing yields, at 170–185°C and 133–400 Pa, using lithium oleate, sodium oleate, and potassium palmitate as catalysts, respectively (Liu et al., 1999). The use of high temperature in chemical synthesis implies a high-energy cost for the overall production process. In addition, the chemical process requires difficult and multistep separations. The esters synthesized by such high-temperature processes are contaminated with undesirable byproducts and commonly contain a heterogeneous mixture of products of different degrees of esterification and different positions of acylation (Gumel et al., 2011).

On the contrary, enzymatic sugar ester synthesis is a one-step process that does not typically involve protection/deprotection of the hydroxyl groups (Arcos et al., 1998). Enzymatic synthesis constitutes a low-energy (reaction temperature range between 40 and 60°C), environmentally friendly and popular alternative to chemical synthesis (Patil et al., 1991; Ward et al., 1997; Watanabe et al., 2001; Yan et al., 2001; Pedersen et al., 2003; Sabeder et al., 2006; Cauglia and Canepa, 2008; Queneau et al., 2008; Chang and Shawn, 2009). Enzymatic route offers a high degree of chemo-, regio-, enantio-, and diastereoselectivity (Chang and Shawn, 2009). The product is typically a monoester, although traces of diesters may occur. A relatively easy product mixture simplifies further downstream purification. The enzymatic route can achieve high yields. For example, Ferrer et al. (2005) reported a conversion yield of about 98% for 6-O-lauryolsucrose at 40°C using immobilized *Thermomyces lanuginosus* lipase.

Extensive literature exists on lipase-mediated synthesis of sugar fatty acid esters (Coulon and Ghowl, 1998; Adachi and Kobayashi, 2005; Divakar and Manohar, 2007; Karmee, 2008; Chang and Shaw, 2009; Hernandez-Fernandez et al., 2010) but important aspects of this reaction remain to be clarified. For example, why certain combinations of solvents favor synthesis, the effect of fatty acid chain length on the reaction and the maximum water activity within the reaction mixture at which the equilibrium shifts from synthesis to hydrolysis (Gumel et al., 2011). A major problem in synthesizing sugar esters by using enzymes in non-aqueous media is the selection of an appropriate solvent to dissolve sugars. To dissolve sugars, such as glucose, sucrose, fructose, or others, hydrophilic organic solvents are the preferred reaction media (Castillo et al., 2003; Lee et al., 2007). Also, most enzymes are quickly inactivated under hydrophilic organic solvents and there are some difficulties in controlling a specific degree of regioesterification (Plou et al., 2002). In order to cope with these limitations, strategies based on the modification of substrates to increase the solubility of the hydrophilic polyhydroxylated substrates in the hydrophobic media, have been reported (Castillo et al., 1994). Alternatively, other authors have selected reaction conditions that favor yield and selectivity (Khaled et al., 1991; Ducret et al., 1995; Gumel et al., 2011).

Sugar ester surfactants are increasingly used as important commodity chemicals in several industries. Furthermore, recently some ester derivatives have shown their therapeutic potential with antitumor activity, plant growth inhibition, and antibiotic activities. However, this potential has not been fully explored mainly due to low production yields and lack of specificity. This review will cover the major advantages and limitations of the enzymatic synthesis of sugar esters, as well as their potential applications in food industry.

LIPASES: BIOCATALYSTS FOR THE SYNTHESIS OF SUGAR ESTERS

The enzymatic synthesis of sugar esters has some advantages over the conventional chemical synthesis, such as one-step synthesis without the protection and deprotection of polyols (Arcos et al., 2001) and moderate reaction conditions (Adachi and Kobayashi, 2005). Lipases (EC 3.1.1.3) catalyze a condensation reaction (reverse hydrolysis) to produce various esters, such as aliphatic alcohol esters (Flores et al., 2000; Wu and Liu, 2000; Kobayashi et al., 2003), hydroxy fatty acid esters (Gargouri et al., 2002), polyesters (Sonwalkar et al., 2003), and polyol esters.

Lipases are also widely used in various reactions, such as transesterification and alcoholysis, for the production of optically active compounds (Ikeda and Kurokawa, 2002), as well as for the breakdown of fats and oil (Joseph et al., 2008). The customization of lipases for practical use has been extensively reviewed (Villeneuve et al., 2000; Hasan et al., 2006; Treichel et al., 2010).

Lipases

Several sources of lipases have been used in industrial processes, namely vegetal (*Asclepiadaceae*, *Euphorbiaceae* and *Caricaceae*), animal (pancreatic, hepatic, and gastric), and

Table 1 Biochemical properties of some lipases used for the synthesis of sugar esters

	Sources of lipases			
Properties	*C. rugosa*	*C. antarctica* (CALB)	Porcine pancreatic (PPL)	*Geotrichum candidum*
Molecular weight (kDa)	65	33	50	54
Optimum temperature (°C)	37	57	45	40
Optimum pH	7.0	7.0	8.0	6.3
Km value (mM)	0.17	0.19	0.30	0.71
Thermostability (°C)	37	70	40	55

microbial (bacteria and fungi). These enzymes have differences in their catalytic properties, and most lipases currently used for ester synthesis in non-aqueous media are produced by microorganisms (Cajal et al., 2000; Villeneuve et al., 2000; Tejo et al., 2004; Yu et al., 2008; Contesini et al., 2010; Guncheva and Zgiryakova, 2011). Table 1 presents a compilation of the biochemical properties of some lipases generally used for the synthesis of sugar esters.

Microbial lipases are mostly extracellular, thus easier to isolate/recover, are generally more stable, and far more diverse as compared to other sources of lipases (Hasan et al., 2006). The production of microbial lipases has been reviewed by several researchers (Sharma et al., 2001; Shu et al., 2010; Treichel et al., 2010). Microbial lipases can be produced by yeasts like *Candida* and *Torulopsis*; by filamentous fungi as *Rhizopus*, *Geotrichum*, and *Humicola*; and by bacteria, such as *Thermus thermophilus*, *Pseudomonas*, and *Staphylococcus*. The most common lipases from animal sources are the ones from porcine pancreas (PPL), while those from vegetal sources are extracted from soybean, barley and cotton (Gupta et al., 2004; Hasan et al., 2006; Fuciños et al., 2008).

The lipase obtained from *Candida antarctica* type B, also known as CALB, is the most frequently used lipase in organic reactions (McCabe and Taylor, 2004) and is commercially available in the free and immobilized forms (Ong et al., 2006). This enzyme can be used for industrial processes, such as the synthesis of triglycerides and esterification of terpene alcohols. Its use has also been reported for the synthesis of acyl hexose, and oleate esters of fructose or glucose.

Porcine pancreatic lipase (PPL) is the cheapest commercially available non-microbial enzyme. It presents a high thermostability and activity in anhydrous reaction media. Nevertheless, the enzyme preparations are often impure, containing various hydrolases, such as esterases, trypsin, and other proteases (Jaeger and Eggert, 2002) that can impair the success of a given reaction.

Finally, fungi also represent an important class of lipase producers. The most extensively studied are *Geotrichum candidum*, *Aspergillus niger*, *Aspergillus oryzae*, *Rhizopus delemar*, and *Penicillium cyclopium*. Fungi lipases constitute a source of new biocatalysts with special features and a high potential for organic reactions (Hasan et al., 2006).

In general, lipases consist of approximately 300 amino acid residues and present a molecular mass between 25 and 75 kDa, bearing the glycosylated hydrophilic part around the active site (Saxena et al., 2003; Treichel et al., 2010). Moreover, lipases are water-soluble enzymes that play an important role in the metabolism of fats in the digestion process (Reis et al., 2009). Lipases typically catalyze the hydrolysis of lipids in aqueous media, but this equilibrium reaction shifts towards synthesis, or esterification, in non-aqueous solvents in the presence of a sparing amount of water. A certain amount of water is essential for hydration of the enzyme even though water is not required for the synthesis of sugar fatty acid esters (Gumel et al., 2011). These enzymes are used in several applications in food, dairy, detergent, and pharmaceutical industries (Gupta et al., 2004). Novel biotechnological applications have been successfully implemented through the use of lipases, namely for the synthesis of biopolymers, the production of enantiopure pharmaceuticals, agro-chemicals, and flavor compounds (Jaeger and Eggert, 2002), among many other processes. Besides the advantages above mentioned, the use of lipases enables the minimization of the operational costs, such as reaction time, energy expenditure, and manpower (Saxena et al., 2003; Hasan et al., 2006). Lipases and their action have been extensively reviewed (Krishna and Karanth, 2002; Contesini et al., 2010; Rodrigues and Fernandez-Lafuente, 2010; Guncheva and Zgiryakova, 2011).

Furthermore, rational design of lipases has been shown to enhance their enantioselectivity (Magnusson et al., 2005), and the use of such engineered lipases in synthesis may have advantages. Specifically engineered lipases have been shown to have greatly enhanced specificity towards branched chain fatty acids (Juhl et al., 2010). Simple chemical modification of lipases can improve their activity, specificity and selectivity (Lee and Kim, 1995; Tsuzuki et al., 1999a, 1999b; Cabrera et al., 2010).

The Use of Lipases in Esterification

Synthesis of sugar esters and related compounds using a biocatalyst can provide high yields of specific isomers from cheap renewable material (Ducret et al., 1995; Arcos et al., 2001; Somashekar and Divakar, 2007) in comparison to the poor selectivity of the chemical synthesis method (Kennedy et al., 2006). Indeed sugar fatty acid esters can be obtained from less-expensive, renewable, and readily available agricultural materials viz. carbohydrate, fats, and oils (Hill, 2000; Johansson and Svensson, 2001; Patel, 2004). Further, as a result of the regioselectivity and mild reaction conditions of the enzymatic processes, there is growing interest in the application of lipases as biocatalyst for sugar fatty acid esters production (Chang and Shaw, 2009; Gumel et al., 2011). Lipases possess wide substrate specificity, have the ability to recognize chirality and do not require labile cofactors (Gandhi et al., 2000; Jaeger and Eggert,

2002). Since they are inexpensive, stable, and easy to recycle, these enzymes have been used for the synthesis of organic chemicals, mainly in aqueous media, and in some cases non-aqueous media (Villeneuve et al., 2000; Karmee, 2008). As mentioned above, in non-aqueous media, lipases are used in esterification, transesterification, amidation, hydrolysis, hydrazinolysis, and epoxidation reactions (Jaeger and Eggert 2002). Several studies reported that the lipases specificity is controlled by the molecular properties of the enzyme, substrate structure and factors affecting the enzyme-substrate binding (Hasan et al., 2006; Treichel et al., 2010).

A great amount of research has been conducted on the synthesis of various sugar ester surfactants by lipases in organic solvents, supercritical carbon dioxide, ionic liquids, or co-solvent systems to increase their production yield for industrial applications. Table 2 summarizes some of those examples for glucose, fructose, sucrose, and lactose esters.

Neta et al. (*data not published*) evaluated the synthesis of sugar esters by CALB and PPL using several combinations of sugars (fructose, sucrose, and lactose), fatty acids (oleic and linoleic acids), and solvents (ethanol and ethyl acetate). Generally, the use of CALB provided higher esterification yields and the best results were found for lactose, linoleic acid, and ethanol (83.5%). Using PPL, the highest esterification yields (47.6%) were found for sucrose esters. All the sugar esters obtained in this study were further characterized regarding their surface activity and emulsification capability.The monoacylation of various monosaccharides, using the trichloroethyl ester of acetic, butyric, caprylic, and lauric acids, was catalyzed by PPL in organic solvents such as anhydrous pyridine. These esterification reactions result in the acylation of the primary hydroxyl group of the monosaccharide. Disaccharides, such as sucrose, had very low reactivity towards this enzyme, probably the result of steric hindrance. The transesterification of glycosides of common disaccharides such as lactose or maltose, using vinyl esters and catalyzed by lipases also lead to the acylation of the primary hydroxyl group of the disaccharide non-reducing end (Lay et al., 1996). Lipases can also catalyze transesterification of the secondary hydroxyl groups of D-glucose, in which the C-6 hydroxyl group has been blocked by prior enzymatic acylation or chemical alkylation (Therisod and Klibanov, 1987). The transesterification of these secondary hydroxyl groups is also highly regioselective with positional specificity and is primarily dependent on the particular lipase selected as the catalyst.

Frequently, the problem during these lipase-mediated sugar fatty acid esters syntheses is the low solubility of sugars and fatty acids in non-polar organic solvents. To avoid this solubility problem many methods have been developed including the (i) use of a solvent mixture (Ferrer et al., 1999; Plou et al., 2002); (ii) use of highly polar organic solvents viz. pyridine or dimethyl formamide (Chopineau et al., 1988; Janssen et al., 1990; Riva et al., 1998); (iii) substrate immobilization (Sharma and Chattopadhyay, 1993); (iv) derivatization of sugars (Adelhorst et al., 1990; Fregapane et al., 1991); (v) complexation of sugar with organoboric acids (Ikeda and Klibanov, 1993); and use of activated acyl donors (Pulido et al., 1992).

Lipases Immobilization

Enzymes are naturally subjected to inactivation by chemical, physical, or biological factors. Also, they are unstable, have a rapid loss of catalytic activity and are not regenerated (Villeneuve et al., 2000). A great amount of work has been reported on the development of new immobilization techniques to provide stability to the enzymes, and improve their efficiency and recovery, especially under unusual conditions of temperature, pressure, and pH, or when using non-conventional media, such as in organic solvents. Lipases have been successfully immobilized on a variety of matrices to be used in organic reactions as summarized in Table 3.

Lipases can be immobilized by a number of methods, including physical adsorption (e.g., active charcoal), covalent binding (e.g., cellulose, silica), and entrapment (e.g., cellulose) (Hasan et al., 2006; Treichel et al., 2010). All methods present well-known advantages and disadvantages. Briefly, physical adsorption and entrapment are the simplest immobilization methods although desorption and leakage of the enzyme usually occur. These limitations can lead to a low stability and catalytic activity. Alternatively, covalent binding methods can be used, providing a stronger binding between the enzyme and the solid support. Nevertheless, the covalent binding methods result in a considerable decrease of the enzyme activity due to a possible damage of its active site and distortion of its nature structure (Villeneuve et al., 2000). To overcome this issue, methods of enzyme pre-treatment or supports types that prevent the loss of activity during the immobilization process are required.

Several materials have been reported as potential supports for lipases immobilization, including alginate gels, carrageenan and polyacrylamide, alumina, silica, acrylic resin and chitosan, among others (Moreno and Sinisterra, 1994; Makas et al., 2010; Orrego et al., 2010).

Silica gel has been widely used as a support due to its high mechanical strength, thermal and chemical stability, high resistance to microbial contamination and degradation, and high surface area. Silica gel was used to immobilize lipase from *Candida cylindracea* that catalyzes the hydrolysis of triglycerides into free fatty acids (Moreno and Sinisterra, 1994). Alumina has also been used to immobilize lipase from *C. antarctica* for the synthesis of butyl butyrate (Lozano et al., 2002). This support is highly resistant to high temperatures and pH values. Furthermore, Brígida and collaborators (2008) used waste green coconut fiber for the covalent immobilization of CALB.

Neta et al. (2012) studied the performance of CALB immobilized on chitosan to synthesize sugar ester surfactants. Similar lactose ester synthesis yield were obtained for the immobilization in chitosan (84.1%) or in acrylic resin (83.5%). Also, the synthesis of fructose ester was found to be higher for CALB immobilized on acrylic resin. These sugar esters were further

Table 2 Enzymatic synthesis of sugar esters by lipases in various organic solvents

Substrates		Enzymes	Solvents	Time (h)	Products	Yield	References
Acyl aceptor	*Acyl donor*						
D-Glucose	Vinyl acetate	PPL	Diisopropylether	72	6-*O*-acetylglucopyranoside	62%	Sharma and Chattopadhyay (1993)
D-fructose		1-*O*-acetylfructoside				70%	
Glucose	Lauric acid	Novozyme 435	Acetone	72	6-*O*-lauroylglucose	98%	Arcos et al. (1998)
D-glucose	Stearic acid	Lipozyme TM 20 (*Mucor miehei*)	Heptane	46	1 or 6-*O*-stearate glucose	9.3%	Oguntimein et al. (1993)
D-fructose	Stearic acid	Lipozyme SP 382	*tert*-Butanol	46	2 or 6-*O*-stearate fructose	8.6%	Chang and Shaw (2009)
D-fructose	Oleic acid	Lipozyme (*Rhizomucor miehei*)	2-Methyl-2-butanol	26	Fructose oleate	83%	Khaled et al. (1991)
D-fructose	Stearic acid	Lipozyme IM60 (*Rhizomucor miehei*)	*n*-Heptane	12	Fructose monostearate	40%	Schlotterbeck et al. (1993)
D-fructose Sucrose	Linoleic acid	NTG lipase (*Byssochlamys fulva*)	*tert*-Butanol	24	Mixture of esters Mixture of esters	71.3% 36.6%	Ku and Hang (1995)
Sucrose	Lauric acid Palmitic acid	Lipolase 100 L (*Humicola lanuginosa*)	*tert*-amyl alcohol:DMSO (4:1, v/v)	24 48	6-*O*-lauroylsucrose 6-*O*-palmitoylsucrose	70% 80%	Ferrer et al. (1999)
D-Fructose	Palm fatty acid distillates (PFAD)	Novozym SP435 (*C. antarctica*)	Acetone	72	6-*O*-palmitoyl-α-D-fructopyranose	38.8%	Chaiyaso et al. (2006)
D-Glucose	6-*O*-palmitoyl-α-D-glucopyranose					76%	
Glucose	Lauric acid	Lipase from *Thermomyces lanuginosus*	*tert*-amyl alcohol:DMSO (4:1, v/v)	20	6-*O*-lauroylglucose	98%	Ferrer et al. (2005)
D-Glucose (complexation phenylboronic acid)	Lauric acid Olive oil Soybean oil Corn oil Apricot seed oil Cotton seed oil	*Pseudomonas sp.* lipoprotein lipase	*tert*-butyl alcohol	24	Acylated glucose esters	97% 42% 32% 41% 54% 47%	Ikeda and Klibanov (1993)
D-Glucose	L-Alanine	Lipozyme IM20 (*Rhizomucor miehei, RML*)	CH2Cl2:DMF (90:10, v/v; 40°C)	72	Mixture of monoesters	2%	Somashekar and Divakar (2007)
D-Fructose	Mixture of diesters Mixture of monoesters					17% 6%	
Lactose	Mixture of diesters Mixture of monoesters					6% 14%	
Sucrose	Mixture of monoesters					8%	
Maltose	Ethyl butanoate	*C. antarctica* SP-435	*t*-butyl alcohol	24	6-*O*-monobutryl-D-glucopyranosyl-D-	93%	Oosterom et al. (1996)
D-glucose monohydrated	Tetradecanoic acid	*C. antarctica* SP-435	*tert*-butanol	24	6-*O*-glucose tetradecanoate	34 mg mL^{-1}	Degn et al. (1999)
Fructose	Oleic acid	Novozyme 435	Ethanol	37.8	Mixture of esters	88.4%	Neta et al. (2011)
Lactose	Linoleic acid	Novozyme 435	Ethanol	72	Mixture of esters	83.5%	Data not published
Sucrose	Linoleic acid	PPL	Ethanol	72	Mixture of esters	47.6%	Data not published

Table 3 Immobilization supports commonly used for lipases

Support	Microorganism	Method	Reference
Chitosan	*C. antarctica*	Activation agents: glycidol, glutaraldehyde and epichlorohydrin	Rodrigues et al. (2008a)
Eupergit C	*C. rugosa*	Covalent	Knezevic et al. (2006)
Alumina	*C. antarctica*	Gelatin and/or polyethy leneimine	Lozano et al. (2002)
Silica gel and alumina	*C. cylindracea*	Covalent	Moreno and Sinisterra (1994)
Green coconut fiber	*C. antarctica*	Adsorption	Brígida et al. (2008)
Activated carbon	*C. antarctica*	Adsorption	Rodrigues et al. (2008b)
Polystyrene	*C. rugosa*	Covalent	Ye et al. (2009)

studied regarding their ability to stabilize emulsions of fresh coconut milk.

ESTERIFICATION IN ORGANIC SOLVENTS

The most diverse organic reactions, such as hydrolysis, esterification, interesterification, alcoholysis, acidolysis, aminolysis, and lactonization can be conducted through synthesis routes using chemical or biochemical catalysts to achieve a given conversion under controlled conditions. The main advantages of these reactions are the enhanced solubility of non-polar substrates and the possibility of shifting the equilibrium of the reaction towards the synthesis (Persson et al., 2002). As previously mentioned, the synthesis of esters can be carried out through a chemical or enzymatic process. The chemical process occurs with a low selectivity and leads to a mixture of sugar esters with different degrees of esterification. It requires toxic organic solvents and is conducted at high temperatures, resulting in low quality of the final product (Kennedy et al., 2006). This limitation can be overcome using lipases. Additionally, the enzymatic method enables the reduction of the number of reaction steps, prevents the occurrence of racemization, and presents minimal protection due to regiospecificity (Chang and Shaw, 2009). The esterification reaction is both quantitative and regioselective towards the primary hydroxyl groups of the precursor. The selectivity of the process is based on the kinetic control of the reaction network. In particular, control of the selectivity is achieved via manipulation of the effects of temperature on the rates of acylation of primary and secondary hydroxyl groups, and selective in situ precipitation of the desired products (Arcos et al., 2001). A hydrolase-catalyzed reaction in a conventional aqueous system thermodynamically favors hydrolysis (Adachi and Kobayashi, 2005). Because a lipase-catalyzed reaction is also such a case, a condensation reaction to produce an ester is carried out in a non-aqueous medium, such as an organic solvent, a solvent-free system or an ionic liquid (Sheldon, 2001; Karmee, 2008). Some lipases have catalytic activity even in the presence of small amounts of water (Villeneuve et al., 2000). Reaction equilibrium constant is a crucial parameter in the prediction of the equilibrium yield of a desired product under any conditions. It is known that the constant is markedly affected by some factors, such as the hydration of a sugar substrate and the interaction of a reactant with a solvent (Chamouleau et al., 2001). The majority of the research reported on lipase-catalyzed reactions has been performed using a small-scale batch reactor, although a continuous reaction is preferred for the large-scale production of sugar esters (Adachi and Kobayashi, 2005; Gumel et al., 2011). As previously mentioned, many important aspects are involved in the lipase-mediated synthesis of sugar fatty acid esters, such as the reactants, the solvent system, the temperature, and the water activity.

Influence of the Reactants

The substrates used to synthesize sugar fatty acid esters, namely sugars and fatty acids, will greatly influence the efficiency of a lipase catalyzing a given esterification process. The catalytic efficiency of the lipases towards different substrates depends on their sources. As such, some lipases exhibit a high selectivity for long and medium chain fatty acids, while others are selective towards short and branched fatty acids (Alhir et al., 1990; Adachi and Kobayashi, 2005). Furthermore, lipase selectivity and activity are influenced by ionic and steric effects of the substrates, namely substitution, unsaturation, branching, and chain length (Adachi and Kobayashi, 2005; Divakar and Manohar, 2007). Indeed the effect of the fatty acid chain length on lipase activity cannot be generalized. For instance, the most commonly used lipase Novozyme 435 (*C. antarctica lipase* B immobilized on acrylic resin) is more specific to unbranched long fatty acids (Cao et al., 1999; Soultani et al., 2001; Juhl et al., 2010). Also, lipases from *Staphylococcus warneri* and *Staphylococcus xylosus* presented an increased activity with fatty acids increasing chain lengths (Kumar et al., 2005), while the lipase from *T. lanuginosus* showed reduced rates of esterification (Ferrer et al., 2005). Another important parameter that is well known to affect lipases selectivity is the nature of the material used for its immobilization (Cabrera et al., 2010; Severac et al., 2010). As reviewed by Gumel et al. (2011), the molar ratio of the two reacting substrates plays a significant role in the esterification reaction, since the solubility of a substrate in the medium is affected by the concentration of the other, thus affecting the polarity of the reaction medium. For example, for the reaction of glucose with stearic acid using CALB it was found that an excess of the fatty acid in the reaction medium leads to a significant increase of sugar ester synthesis (Yan et al., 2001). On the other hand, the sugar ester yield was reduced by decreasing the chain length of the fatty acid in a reaction involving glucose. Soultani and collaborators (2001) reported that the

esterification of fructose in *tert*-amyl alcohol was favored by an excess of short chain fatty acids (e.g., capric acid). On the other hand, an excess of long chain fatty acids (e.g., stearic acid) decreased the conversion rate, mainly due to the blockage of the catalytic site of CALB by the excessive amounts of fatty acid. Furthermore, the authors shown that for equimolar mixtures of fructose and fatty acids, increasing chain length of the fatty acid enhances the conversion, although the conversion rate decreases with an increasing molar ratio of the fatty acid to sugar. Other examples on the influence of the fatty acid chain length, molar ratio of fatty acid to sugar, concentration of the sugar and possible substrate inhibition, concentration and solubility of the product, have been reported by Tarahomjoo and Alemzadeh (2003); Salem et al. (2010); Cauglia and Canepa (2008); Tsuzuki et al. (1999a, 1999b) and Zhang et al. (2009). In general, the conversion rate of lipase-catalyzed transesterifications involving fatty acids with 10 or fewer carbons can be improved by supplying an excess of the fatty acid. On the contrary, for a reaction involving longer fatty acids, a lower molar ratio of fatty acid to sugar is preferable, as a high concentration of a non-polar fatty acid decreases the solubility of the sugar in the reaction medium. Sugars tend to be poorly soluble in non-polar organic reaction media (Gumel et al., 2011).

Influence of the Solvent System

Enzymatic reactions using organic solvents provide several advantages of industrial interest, such as increased solubility of non-polar substrates, reversibility of the thermodynamic equilibrium of the hydrolysis reactions, suppression of water-dependent side reactions, modification of substrate specificity and enantioselectivity, among others (Doukyu and Ogino, 2010). A non-aqueous solvent is essential for lipase-catalyzed synthesis of sugar fatty esters. A suitable solvent must be able to dissolve sufficient amounts of both substrates (sugar and fatty acid) (Gumel et al., 2011). The solubilities of sugars and fatty acids in organic solvents are generally markedly different, thus complicating the esterification reaction since a high concentration of both the reactants within a single phase is difficult to achieve. Additionally, the solvent must not adversely affect the stability and activity of the lipase. Furthermore, solvent selection is known to affect the enantioselectivity and specificity of lipase-catalyzed reactions (Sakurai et al., 1988; Klibanov, 1990; Wescott and Klibanov, 1994; Liu et al., 2009).

A condensation reaction is usually performed in a pure solvent with low water content. Even though the solvent provides only the field of the reaction, there are some cases in which the type of solvent affects equilibrium conversion (Adachi and Kobayashi, 2005). Both water-miscible and water-immiscible organic solvents have been used as reaction media for lipase-catalyzed condensation reactions. Depending on the miscibility of an organic solvent with water, and the relative proportion of solvent and water in the medium, there are three main types of organic solvent systems that can be used: water plus water-miscible organic solvent system (organic co-solvent system); water plus water-immiscible organic solvent system (two phase system or biphasic system); and nearly anhydrous organic solvent system (Khmelnitsky et al., 1988).

The use of water-miscible solvents (e.g., acetone, acetonitrile and tertiary alcohols) has the advantage that hydrophilic substrates (e.g., sugars) are solubilized to some extent without the addition of solubilizing reagents to facilitate the esterification of the substrates (Arcos et al., 2001; Castillo et al., 2003). Also, water-miscible organic solvent systems can reduce the mass-transfer limitations, leading to faster reaction rates for hydrophobic compounds. However, a water-miscible solvent removes water from the lipase molecule, which is essential for catalytic activity; as a consequence this water removal might promote the deactivation of the enzyme (Khmelnitsky et al., 1988).

The second system above mentioned consists of a two-phase system, namely an aqueous phase containing the enzyme, and another phase containing an immiscible organic solvent (Khmelnitsky et al., 1988). A hydrophobic substrate, such as steroids or fats, is mostly located in the organic solvent layer and is partitioned into the aqueous phase where the enzymatic reaction occurs. The substrate is converted by the enzyme, and then the product is extracted into the organic solvent phase. The advantage of this system is that it enables the reaction shift towards the synthesis of esters (Klibanov, 2001). In these systems, the solvent induced drying of the hydration layer of the enzyme reduces its catalytic power, activity and stability (Salem et al., 2010). The removal of hydration water is suggested to increase the enzyme rigidity (Hudson et al., 2005). As a consequence lipases that can better withstand a prolonged exposure to organic solvents is required and immobilization constitutes an alternative to improve their resistance. Further, the product solubility in the solvent should be low so it can be continuously removed and therefore the reaction equilibrium can be driven towards the product (Rubio et al., 1991).

Finally, in the last organic solvent system, involving lyophilization, immobilization or modifications with amphipathic compounds, the enzymes are required for solubilization purposes. Lyophilization causes a reversible damage in the enzyme structure (Lee and Dordick, 2002). Co-lyophilization with additives, such as carbohydrates, polymers, and salts prevents this damage and activates the lipases. The lipase lyophilized, or precipitated from an aqueous solution at its optimum pH, exhibits high activity. In this system, lyophilized lipases often exhibit high thermal stability, but show much lower catalytic activity than in water. The water content (water activity) in the system is essential to have sufficient lipase activity. It is generally known that hydrophobic solvents result in higher lipase activity comparing to the hydrophilic ones. Conformational mobility of lipases at such low water content is generally restricted. Therefore, the proteins are more rigid in this type of system than in water. This affects the catalytic activity, stereoselectivity, regioselectivity, and stability. This system has been demonstrated to be very useful in various enzymatic processes, such as in

the synthesis and transesterification of esters, peptide synthesis, and transformation of various hydrophobic compounds (Lee and Dordick, 2002).

Differences reported in the activity of an enzyme in different organic solvents have been attributed to the differences in the extent of enzyme hydration. In general, by affecting the hydration status of the enzyme, organic solvents significantly influence the reaction parameters such as the reaction rate, the maximum reaction velocity, the catalyst turnover rate, the substrate affinity for the catalyst and the specificity constant (Divakar and Manohar, 2007). The influence of organic solvents in enzymatic sugar ester syntheses has been widely studied (Chang and Shaw, 2009; Gumel et al., 2011). Parameters such as the solvent dielectric constant (Affleck et al., 1992), polarity and electron acceptance index (Valivety et al., 1994), as well as solubility (Brink and Tramper, 1985) and partition coefficient (Lu et al., 2008) have been used to explain the effect of different solvents on the rate of the lipase-mediated synthesis of sugar esters.

Influence of the Temperature

The temperature of the reaction affects the stability of the enzyme, the solubility of the reactants and product, the rate of the reaction and the position of the equilibrium. Most commonly used lipases, as Novozyme 435 that can be used in the range between 60 and 80°C without activity loss, are reasonably thermostable (Yan et al., 2001; Yoshida et al., 2006). For the lipase-mediated synthesis of glucose ester, it has been reported an increase in the enzyme activity as the reaction temperature was raised from 35 to 45°C (Yu et al., 2008). Also, the authors suggested that in addition to affecting the reaction rate directly, the use of a high reaction temperature improves the mass transfer rates.

Furthermore, it is important to evaluate the effect of the reaction temperature on the solubility of reactants and product. For instance the solubility of glucose in ethyl-methyl ketone, but also of the ester (glucose caprylate) was greatly enhanced when temperature was raised from 25 to 60°C (Yan et al., 2001). The increased solubility difficult the product removal by crystallization/precipitation and this in turn shifts the reaction equilibrium towards hydrolysis. Temperature dependence of dissolution kinetics of glucose and fructose in *tert*-amyl alcohol has been described (Engasser et al., 2008). One study reported a 78% conversion of fructose to its palmitate ester at 60°C after 72 h of reaction (Sabeder et al., 2006). The increase of temperature to 70°C significantly reduced the conversion due to thermal deactivation of the lipase and increased solubility of the sugar ester.

For lipase-mediated synthesis of glucose esters of short chain fatty acids, the best conversion was obtained between 35 and 45°C. At this temperature range the product could be removed by precipitation due to its low solubility. On the other hand, if long chain fatty acids are used the temperature can be raised up to 60°C (Yan et al., 2001). As such, the optimal temperature of reaction depends on the chain length of the fatty acids used (Soultani et al., 2001).

In summary, the optimal temperature for the enzyme is not necessarily the optimal temperature for the reaction, thus a case-by-case evaluation is required to establish the best operating temperature for a given combination of solvent, enzyme and reactants (Gumel et al., 2011).

Influence of the Water Activity

Water activity (a_w) has been described as an important parameter to optimize organic reactions in aqueous and non-aqueous systems and can be adjusted by a number of methods. In enzymatic reactions, a_w determines the equilibrium position of the hydrolase reaction in low water systems (Matsue and Miyawaki, 2000). Therefore, a minimal amount of water is necessary to ensure the enzyme optimal conformation and activity. On the other hand, high water contents reduce the reaction stability, since the particles of enzyme present in the medium could be covered by a layer of water, thus preventing the contact of a lipophilic substrate (i.e. fatty acid) with the enzyme (Chamouleau et al., 2001). According to Adachi and Kobayashi (2005), the water should be removed in order to shift the reaction towards the ester formation.

The reaction equilibrium constant significantly depends on the type of hexose, possibly due to the fact that the hydration of the hexose is considered to affect the equilibrium conversion. Adachi and Kobayashi (2005) showed that the hexoses that were more strongly hydrated further decreased the water activity in the system to shift the equilibrium towards the ester synthesis.

Numerous methods have been reported for the removal of water formed during the esterification reaction, such as evaporation under reduced pressure (Izák et al., 2005), azeotropic distillation (Yan et al., 2002), and the use of molecular sieves (Chamouleau et al., 2001) or silica gel (Sonwalkar et al., 2003). However, there is still no criterion regarding the amount of desiccant required to achieve the desired conversion (Adachi and Kobayashi, 2005). Furthermore, the molecular sieve acts as a catalyst, as well as an adsorbent. There are some cases in which undesirable reactions, such as the degradation of unstable substrates and diester formation, can occur (Chamouleau et al., 2001; Sonwalkar et al., 2003).

The influence of a_w is best predicted and analyzed in terms of its thermodynamic activity instead of water concentration. According to Gandhi and collaborators (2000), this is readily observed from the effect of the water content on the catalytic activity of lipase. In their study, the lipase presented a similar optimum at a thermodynamic a_w equal to 0.55 when used in solvents ranging from hexane to pentanone. In contrast, Awang et al. (2000) reported that a a_w ranging from 0.09 to 0.96 did not have a marked relevance on the yields of the ester synthesized from dihydroxy stearic acid with octanol by *Rhizomucor miehei* and Novozyme 435 lipases.

Studies conducted by Chowdary and Prapulla (2002), with the lipases from *Rhizopus oryzae*, *Mucor javanicus, A. niger*, and *Penicillium roqueforti*, demonstrated that at higher water activity levels (a_w 0.96), the enzymes promoted higher ester yields, probably due to protein aggregation. Therefore, the optimum a_w depends on the enzyme source, organic solvent used in the reaction, and type of immobilization support used (Gandhi et al., 2000).

SUGAR ESTER SURFACTANTS: PROPERTIES AND APPLICATIONS

Surfactants, biosurfactants, emulsifiers, bioemulsifiers, sugar esters, or fatty acid esters are compounds that possess surface activity. Additionally, some of them present a high emulsifying capacity. Among these, compounds obtained as microbial metabolic products or produced through microbial enzymes are named biosurfactants (Banat et al., 2000; Rodrigues and Teixeira, 2008; Kralova and Sjoblom, 2009).

A large number of synthetic surfactants have been reported in the literature (Hill and LeHen-Ferrenbach, 2009). However, these synthetic surfactants present some drawbacks, such as their costs, availability and environmental impact. Therefore, the lipase-catalyzed synthesis of sugar esters appears to be an interesting "green" alternative as compared to the chemical processes that are in place (Adachi and Kobayashi, 2005; Kennedy et al., 2006). Several natural and synthetic surfactants are currently available on the market. Examples of natural surfactants include biosurfactants, fatty acid amides, fatty acid amines, sucrose esters and sulfates of natural fatty alcohols, among others. On the other hand, the synthetic surfactants group includes alkyl and aryl ether carboxylates, alkyl aryl sulfates, co-polymers of ethyl oxide/propylene and ethoxylated fatty acids.

As previously mentioned, comparing to their chemical counterparts the microbial surfactants, such as the sugar fatty acid esters, are advantageous since they are biodegradable, present low toxicity and high selectivity, are ecological accepted and effective at extreme operational conditions (Banat et al., 2000; Rodrigues and Teixeira, 2008). Furthermore, these compounds are skin-compatible, odorless, tasteless, and can be produced from renewable and inexpensive sources, thus at lower costs (Cruces et al., 2001; Soultani et al., 2001; Tarahomjoo and Alemzadeh, 2003; Banat et al., 2010; Rodrigues and Teixeira, 2010).

Properties

Microbial surfactants possess several important physicochemical properties, such as foaming, emulsifying and stabilizing capacities, low critical micellar concentration, detergent solubility, and dispersion power, among others (Soultani et al., 2003; Banat et al., 2010). The fatty acid sugar esters are nonionic surfactants that exhibit high emulsifying, stabilizing, and detergency effect, thus finding applications in the food, cosmetic, detergent, and pharmaceutical industry (Yan et al., 1999; Sabeder et al., 2006; Szuts et al., 2007; El-Laithy et al., 2011).

The most important feature of any given surfactant is its ability to reduce the surface tension of a liquid medium. For example, sugar ester surfactants, such as sophorolipid esters can effectively reduce the surface tension of water to values below 38.7 mN m^{-1} (Zhang et al., 2004). Moreover, Neta et al. (2012) found that fructose esters, synthesized from oleic acid, fructose and ethanol by CALB, are able to reduce the surface tension to 35.8 mN m^{-1} and also to stabilize an emulsion (emulsification indexes (EI) between 54.4 and 58.4%).

Additionally, some microbial surfactants are considered good food emulsifiers. For instance, the fatty acid sugar esters find widespread use as water/oil emulsifiers in food products (Sabeder et al., 2006). The selection of a proper emulsifier is essential in the manufacture of food additives; the emulsifier must possess suitable functional properties to confer stability against droplet coalescence during the shelf life and they have to be non-toxic (Partal et al., 1999). Furthermore, in the food industry, the most useful property of a biosurfactant is its ability to form stable emulsions, which improves the texture and creaminess of dairy products (Banat et al., 2010). As an example, lactose esters (surface tension 38.0 mN m^{-1}; EI = 54.1%) were found to stabilize emulsions of fresh coconut milk (Neta et al., 2012).

Typically, the microbial surfactants are molecules bearing a hydrophilic and a hydrophobic part. In the case of the sugar fatty acid ester surfactants, the hydrophobic part consists of a fatty acid, whereas the hydrophilic part consists of a sugar molecule or one of its ester derivatives resulting from the reaction with organic acids, such as lactic, citric, acetic, or tartaric acid. The food industry is extremely interested in these additives since they can safely be consumed by humans in quantities up to 125 mg kg^{-1} body weight per day. Moreover, they have useful properties that improve the production of some food products, such as bakery commodities (Sawa et al., 2009). Nevertheless, although the potential of sugar fatty ester surfactants in food industry is high, it is important that they accomplish specific acceptance criteria, present certified performances and comply with the existing legal restrictions.

Applications

Many applications have been reported for biosurfactants including their use to retard staling, solubilize flavor oils, and improve organoleptic properties in bakery and ice cream formulations, and as fat stabilizers during cooking of fats (Banat et al., 2010). Sugar fatty acid esters used in ice cream, soup, and mayonnaise, are marked as E 473. In cosmetic industry they can be found in toothpaste, lotions, shampoos, and lip sticks (Sabeder et al., 2006).

Fructose esters can be used as antibacterial agents that suppress the cell growth of *Streptococcus mutans*, causative organism of dental caries. Among the different carbohydrate esters,

fructose laurate showed the highest growth inhibitory effect (Watanabe et al., 2000). Therefore, lipase-catalyzed synthesis of carbohydrate esters has potential for developing antibacterial agents applicable to food additives (Sabeder et al., 2006).

Sugar fatty acid esters have also been reported to inhibit biofilm formation by food-borne pathogenic bacteria (Furukawa et al., 2010). There are a few studies on chemicals with strong biofilm-specific inhibition (Chmielewski and Ftank, 2007) and in the food industry most of them are considered unsafe and cannot be used. Nevertheless, food additives are safe and some present biofilm inhibitory activity, as is the case of the sugar fatty acid esters. Furukawa et al. (2010) found that sucrose monomyristate and sucrose monopalmitate significantly inhibited biofilm formation by *Staphylococcus aureus* and *Escherichia coli* at a low concentration (0.001% w/w). The addition of sucrose monopalmitate at the early growth stage of *S. aureus* exhibited a strong inhibitory effect, suggesting that the ester inhibited the initial attachment of the bacterial cells to the abiotic surface. In a previous study, it was reported that the sugar fatty acid esters at 0.05% also inhibit the adhesion of *Salmonella enteriditis*. In contrast, biofilm formation by *Pseudomonas aeruginosa* was not inhibited with low concentrations of sugar fatty acid esters, probably due the presence of cell surface esterase that can decompose the added sugar fatty acid esters.

Sucrose esters are described as very mild with regard to their dermatological properties and are approved as food additives in many countries. As a consequence, these products seem to be perfect raw materials for food and cosmetic formulations, and their use in those applications as specialty emulsifier has long tradition (Hill and LeHen-Ferrenbach, 2009). In Asia, one can find sucrose esters with a low degree of esterification in special detergent products. The octaesters have been developed by Procter & Gamble and are used as non-caloric fat substitute in food industry. The glucose-derived surfactants, for example alkyl polyglycosides, depending on their size can be used as dishwashing agents, detergents, and personal care products, or as hard surface cleaners, agrochemicals, and products for industrial cleaning.

In the food industry, the modification of the structure and composition of oils and fats by enzymatic interesterification has become of great interest (e.g., coco butter). The lipolytic reaction in non-aqueous media has been suggested as an approach for the production of new nutrients, pharmaceuticals, and several food additives (Rajendran et al., 2009).

There is a variety of segments of the food industry in which sugar ester surfactants may be used, especially due to their good taste and aroma profile besides the other features already mentioned, such as in the production of aromas and maturation of cheeses, flavor synthesis, bakery products, cakes, biscuits, mayonnaise and sauces, instant products, sausages, wine and dairy products (Chang and Shaw, 2009; Rajendran et al., 2009).

The main emulsifiers used by the food industry are monoglycerides. These compounds are widely used as anti-staling agents and account for approximately one third of the emulsifiers used in the baking industry. These compounds can also act as mild dough conditioners, leading to improved handling properties of the dough, enhanced slicing performance and superior bread quality (Sawa et al., 2009).

In the last years, the bakery industry became more demanding regarding the increase of products shelf life and consistent quality through the use of food additives. These additives, including emulsifiers such as the sugar ester surfactants, enzymes, soy flour, oxidants, and reductants, are essential for improving dough handling, reducing resting time, and improving baked products shelf-life. Furthermore, these additives increase the volume and aeration thus reducing the stickiness; improve texture and shelf life of starch-containing products; improve crumb whiteness, aroma, and flavor (Moayedallaie et al., 2010). Surfactants are usually a broad spectrum of lipid chemicals that interact with the gluten network and starch that are represented in the dough system. Addition of surfactants in the dough results in the development of soft crust and crumb, finer cell development, and a more firm structure of the gluten network. So, these are also used to control and slow down the rate of the totally undesirable staling in the bakery applications (Asghar et al., 2011). The sugar ester surfactants are used to assist blending and emulsification of ingredients, to control the agglomeration of fat globules, stabilize aerated systems, modify rheological properties of wheat dough, improve consistency, and to interact with the components of the flour and other ingredients in the mix for softer crumb improving the palatability (Rajendran et al., 2009). According to their chemical structure, emulsifiers can interact and form complexes with starch, protein, shortening, and water. Interaction of an emulsifier with the protein can improve the strength and allow better retention of carbon dioxide (Nitschke and Costa, 2007).

Another segment of the food industry in which sugar ester surfactants may play a crucial role is in the manufacture of food-grade colloidal delivery systems, namely microemulsions and nanoemulsions. As reviewed by Rao and McClements (2011), there is a growing interest within the food and beverage industries in the use of colloidal delivery systems to encapsulate functional agents, such as flavors, colors, antimicrobials, micronutrients, and nutraceuticals (McClements et al., 2007; Velikov and Pelan, 2008; McClements, 2010; Sagalowicz and Leser, 2010). Microemulsions and nanoemulsions, consisting of oil, surfactant, and water, can easily be fabricated from food-grade ingredients using relatively simple processing operations (e.g., mixing, shearing, and homogenization), and also due to their specific physicochemical properties can be used in a number of particular applications (Flanagan and Singh, 2006). One of the most important applications of these type of emulsions is to incorporate lipophilic active ingredients into aqueous-based foods or beverages that need to remain transparent, such as some fortified waters, soft drinks, sauces and dips (Velikov and Pelan, 2008). Currently, the widespread use of microemulsions and nanoemulsions in many food products is limited by several technical and practical issues, including the limited number of food-grade surfactants available for preparing and stabilizing these systems (Kralova and Sjoblom, 2009); the scarce

knowledge on the influence of sample composition and environmental conditions on the formulation and stability of some colloidal delivery systems (Rao and McClements, 2011); and the difficulty to prepare microemulsions and nanoemulsions from many commonly used edible oils (e.g., fish oil, corn oil, or soybean oil) (Salager et al., 2005). Consequently, sugar ester surfactants seem to be a good alternative for the manufacture of such colloidal delivery systems. Several researchers studied the structure, properties and functionality of sucrose monoesters (Fanun, 2009a; Garti et al., 2000; Glatter et al., 2001; Yin et al., 2009). These studies have shown that sucrose monoesters can form a range of different colloidal structures depending on system composition and temperature; microemulsions, nanoemulsions, emulsions, and liquid crystals. Rao and McClements (2011) studied the factors that influence the formation and stability of microemulsions and nanoemulsions fabricated using sucrose monopalmitate as surfactant and lemon oil (generally used as flavoring agent in the food industry) as the oil phase. Moreover, Fanun (2009b) reported the properties of microemulsions manufactured from sugar surfactants, namely sucrose monolaurate and sucrose dilaurate, and peppermint oil (edible oil suitable for food, pharmaceutical and cosmetics applications).

FUTURE PERSPECTIVES

Commercial implementation of lipase-catalyzed processes is far from ideal since many issues remain unsolved and there is still room for process optimization. As pointed out by Karmee (2008) much more attention has to be drawn to topics such as solvent recycling; separation and reusability of lipases; removal of water from the reaction; and cost estimation of the overall process. Also, the reaction rate, conversion, regioselectivity, productivity and enzyme stability has to be evaluated case-by-case. Furthermore, methods should be developed for the isolation and purification of the sugar esters, such that its purity meets the demands to be used in the food industry.

Many mechanisms have been suggested to describe lipase-catalyzed reactions involving acylglycerols (Yamane et al., 1986; Chang and Shaw, 2009). However, the generally accepted mechanism for lipase-mediated transformations is the so-called ping-pong bi-bi mechanism (Lombardo and Guy, 1981). In this mechanism, a product is released between additions of two substrates. Either acylation or deacylation of the enzyme can then be the rate-controlling step in the reaction. The determination of rate expressions that describe the performance of the reactor in terms of the main factors that influence the reaction (e.g., temperature, enzyme loading, and substrate concentration) is required (Arcos et al., 2001). Also, the development of kinetic expressions to characterize the lipase-catalyzed reactions remains a challenge, due to the great variety of species that can be involved in these reactions, the number of positional isomers which can exist in glycerides, sugars, other potential substrates and the simultaneous occurrence of non-enzymatic side reactions. As a consequence, the corresponding network of equations is so complex that limits meaningful numerical solutions of these differential equations. To overcome this issue, most studies on the kinetics of lipase-mediated reactions have focused on model systems that contain only a few different chemical species and/or enzyme complexes, thus less representative of the whole process.

Current industrial synthesis of sucrose esters involves high temperature, reduced pressure, anhydrous conditions, and/or expensive catalysts. Furthermore, none of these processes is particularly selective (Cruces et al., 2001; Gumel et al., 2011). They all afford mixtures of compounds differing in their degree of esterification and/or the position of acylation. Although for many applications these mixtures of mono-, di-, and triesters are convenient, chemical reactions that preferentially produce sucrose monoesters in a single and economical procedure may represent an attractive approach to new uses. Derivatives containing three or more fatty acid residues are very hydrophobic and of limited application. For this reason, new methods oriented to achieve a higher selectivity in the reaction or to provide economical purification procedures are required (Cruces et al., 2001). The selective synthesis of monoesters may also be useful in studying the functional properties of single components of commercial derivatives (Banat et al., 2000).

Product design or process optimization can be performed using the response surface methodology (RSM), which is a useful methodology that combines mathematics and statistics tools. This methodology is useful for optimizing the reaction conditions that affect fatty acid sugar esters production to increase yield for possible industrial applications. It is valid to both chemical and enzymatic catalysis applications. Regarding the chemical process, Shieh and Lai (2000) reported the use of RSM to evaluate the effects of the synthesis parameters, such as reaction time, temperature and substrate molar ratio on the conversion to esters in a solvent-free system. For enzymatic process, there are limited reports in regard to evaluating the optimal lipase-catalyzed fatty acid sugar ester production by RSM. Neta et al. (2011) used RSM with a central composite rotatable design based on five levels to optimize three experimental operational conditions (temperature, agitation and reaction time), towards the maximization of fructose esters synthesis in oleic acid and ethanol using CALB. The optimum operational conditions for maximizing the synthesis of fructose esters were 57.1°C, 100 rpm and 37.8 h, and an experimental esterification yield of 88.4% was obtained.

Besides operational and process issues, the enzyme itself is also a key parameter in the lipase-catalyzed reactions. Protein engineering is useful in improving the catalytic efficiency, temperature and pH stability of biocatalysts for enzymatic synthesis of sugar esters (Chang and Shaw, 2009). The recombinant DNA technology (genetic engineering) can be used to produce large quantities of recombinant lipases thus lowering the enzyme cost, and therefore to make enzymatic production of sugar esters attractive for industrial applications. It can also be used for improving biochemical properties of biocatalysts such as regioselectivity, substrate specificity, solvent tolerance and

productivity for sugar esters production. As an example, Akoh and collaborators (2004) demonstrated the usefulness of site-directed mutagenesis in the production of pure lipase isoforms of *Candida rugosa*, as well as in engineering lipase catalysis and specificity. Furthermore, the catalytic efficiency of lipases can be significantly improved by gene shuffling (directed evolution) or rational design protein (Farinas et al., 2001; Jaeger and Eggert, 2002; Akoh et al., 2004). The development of *de novo* enzymes has seen a significant increase lately under the scope of the new field of synthetic biology (Golynskiy and Seelig, 2010). Synthetic biology is the use of engineering principles to create, in a rational and systematic way, functional systems based (or inspired) on the molecular machines and regulatory circuits of living organisms or to re-design and fabricate existing biological systems. The focus is often on ways of taking parts of natural biological systems, characterizing and simplifying them, and using them as a component of a highly unnatural, engineered, biological system (Endy, 2005). With the assistance of computational approaches, protein structures and functions have been designed, catalyzing functions not present in Nature. Directed evolution can complement this technique, by using mutagenesis and subsequent screening for improved synthetic properties.

Manipulating culture conditions and use of recombinant DNA technology are possible means of obtaining novel lipases with unique functions and different isoforms of lipases. Conventional expression systems such as *E. coli*, *Pichia pastoris* and *Saccharomyces cerevisiae* are frequently used to overexpress genes of interest (Jaeger and Eggert, 2002). Designing lipases/proteases/esterases that can tolerate hydrophilic solvent through protein engineering will undoubtedly lower the cost of sugar esters production at an industrial scale.

Finally, although a number of studies reported the successful immobilization of lipases on a range of supports to be used in organic reactions (Table 3), the enzymes stability, efficiency and recovery under unusual operational conditions and media can still be improved. Therefore, the development of novel supports and lipase immobilization methods will continue to drive research in this field.

CONCLUSION

Traditionally, the synthesis of sugar ester surfactants is conducted by chemical processes. Nevertheless, these processes present low selectivity and result in a mixture of esters with different degrees of esterification, besides requiring high temperatures and toxic organic solvents restricted in many industrial applications. Alternatively, using lipases, the sugar esters can be produced as monoesters through enzymatic synthesis from sugars and fatty acids. Lipases immobilization is beneficial, namely due to the possibility of reusing the biocatalyst in continuous processes; ease of handling; improvement of the enzyme thermal and chemical stability; regeneration and ease of recovery; and reduction of operational costs.

Sugar ester surfactants present many advantages as compared to their chemical counterparts, such as biodegradability, low toxicity, selectivity, biocompatibility, environmental acceptability, and effectiveness under extreme conditions. Therefore, these compounds potentially find applications in the pharmaceutical, detergency, cosmetic and food industries. In the food industry, sugar esters can be used in the production of aromas and maturation of cheeses, bakery products, cakes, cookies, mayonnaise and sauces, instant products, sausages, among others.

Furthermore, sugar ester surfactants are expected to gain a significant market share due to the increase of production yields and product diversity as a result of the advances in fields such as metabolic engineering, systems and synthetic biology. Additionally, efforts to increase lipases production involving protein engineering and recombinant DNA technology are being conducted. Moreover, many researchers are developing novel and improved downstream technologies that are expected to enhance product recovery.

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