Carbohydrate Polymers 93 (2013) 65–72

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Enzymatic synthesis of oligo- and polysaccharide fatty acid esters

Lambertus A.M. van den Broek*, Carmen G. Boeriu

Wageningen UR Food & Biobased Research, P.O. Box 17, 6700AA Wageningen, The Netherlands

ARTICLE INFO

Article history:
Received 29 November 2011
Received in revised form 10 May 2012
Accepted 16 May 2012
Available online 26 May 2012

Keywords:
Lipase
Protease
Organic solvents
Ionic liquids
Supercritical carbon dioxide

ABSTRACT

Amphiphilic oligo- and polysaccharides (e.g. polysaccharide alkyl or alkyl-aryl esters) form a new class of polymers with exceptional properties. They function as polymeric surfactants, whilst maintaining most of the properties of the starting polymeric material such as emulsifying, gelling, and film forming properties combined with partial water solubility or permeability. At present carbohydrate fatty acid esters are generally obtained by chemical methods using toxic solvents and organic and inorganic catalysts that leave residual traces in the final products. Enzymatic reactions offer an attractive alternative route for the synthesis of polysaccharide esters. In this review the state of the art of enzymatic synthesis of oligo- and polysaccharides fatty esters has been described.

1. Introduction

Nowadays, the interest for synthesis of modified oligo- and polysaccharides is increasing. Amphiphilic polysaccharides can function as polymeric non-ionic surfactants, whilst maintaining most of the properties of the starting polymeric materials such as emulsifying, gelling, and film forming properties combined with partial water solubility or permeability. Their functional properties can be fine-tuned by adjusting the ratio between the hydrophilic parts, the oligo- or polysaccharide, and the hydrophobic (lipophilic) parts, the amount and the chain length of the alkyl (fatty acid) residue. Derivatives with a low degree of substitution (DS) can exhibit insufficient amphiphilic character, whilst esters with a too high DS become water insoluble. Due to their unique properties, amphiphilic oligo- and polysaccharides can find multiple applications, such as structural components in food products due to their impact on texture of food and their flavour and/or bioactive release characteristics (Chang & Shaw, 2009). They can also be used in topical formulations, to increase the solubility of insoluble or poorly soluble drugs, where they replace synthetic polymers such as poloxamer (a copolymer of polyoxoethylene–polyoxopropylene), poly(vinyl ethers), or poly(metacrylic acids). Moreover, fatty acid esters of polysaccharides can be applied for delivery and controlled release of agrochemicals (fungicides and insecticides), since due to their lipophilic character they enhance the adhesion of the formulation to the hydrophobic surface of plant leaves and decrease the wash ability.

At present, esters of carbohydrates are commercially manufactured by chemical esterification with acid chlorides, in organic solvents (e.g. dimethyl sulfoxide) using organic and inorganic catalysts (pyridine, tionyl chloride, K_2CO_3, celite) that leave residual traces in the final products. Generally, esterification reactions require high temperatures which, in combination with the catalysts used, might induce partial degradation of the polysaccharide chains and discoloration. Currently solvent-free chemical processes for esterification of starch are under development, but they do not eliminate the problem of chain degradation and high volume of wastes produced in the downstream processing step. Chemical esterification is non-selective, and both primary and secondary hydroxyl groups are substituted, making this process suitable only for the production of carbohydrate esters with a high degree of substitution (DS). This is, however, a severe limitation when products with low DS are targeted, as in the case of amphiphilic polysaccharides. Since chemical esterification results in complex mixtures of mono-, di- and tri-esters with random distribution of the ester groups both on the carbohydrate monomer and the polymer backbone.

Enzymatic processes offer an attractive alternative route for the synthesis of oligo- and polysaccharide esters. Selective processes catalysed by enzymes may be performed under mild conditions of temperature and pressure, thereby avoiding polymer degradation. Application of enzymes for modification of polysaccharides will bring the advantage of the high specificity and

Abbreviations: CFAE, carbohydrate fatty acid esters; CLEAs, carrier-bound cross-linked enzyme aggregates; CMC, critical micelle concentration; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; DS, degree of substitution; ILs, ionic liquids; OAT, bis(2-ethylhexyl)sodium sulfosuccinate; $scCO_2$, supercritical carbon dioxide.

* Corresponding author. Tel.: +31 317 480104; fax: +31 317 483011.
E-mail address: Ben.vandenbroek@wur.nl (L.A.M. van den Broek).

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.05.051

regioselectivity of the reaction, which will generate products with controlled structure and functionality. The formation of ester linkages is thermodynamically favoured in low water content media and therefore alternative enzymatic procedures should employ solvent free, organic solvents, or organic solvent mixtures as reaction medium. Also media like ionic liquids (ILs) and supercritical fluids, in particularly supercritical carbon dioxide ($scCO_2$), can be used for enzyme catalysed reactions.

This minireview has been focused on the enzymatic synthesis of oligo- and polysaccharide fatty acid esters referred to in the text as carbohydrate fatty acid esters (CFAE). The esterification of mono- and disaccharides is not included since this topic is extensively addressed in literature (Allen & Tao, 1999; Chang & Shaw, 2009; Gumel, Annuar, Heidelberg, & Chisti, 2011; Kennedy et al., 2006; Polat & Linhardt, 2001).

2. Enzymes

Lipases (EC 3.1.1.3), esterases (EC 3.1.x.x), proteases (EC 3.4.21.x), and peptidases (EC 3.4.x.x) have been applied for the synthesis of CFAE. All these enzymes are highly stereo- and regioselective catalysts that usually operate under mild reaction conditions, are robust and easy to handle and are commercially available for industrial applications (Table 1). They are efficient catalysts for the hydrolysis of esters, but in the absence of water in neat organic solvents, they catalyse the reverse reaction, i.e. the syntheses of esters. Common to these hydrolases is the catalytic triad serine–histidine–aspartic acid in the active centre, although the residues occur in a different order in each protein sequence (e.g. Ser105-His224-Asp187 in *Candida antarctica* B lipase and Ser195-His57-Asp102 in trypsin). The coupling of fatty acids to carbohydrates can take place by esterification and/or transesterification reactions (Fig. 1). The esterification reaction (reaction A in Fig. 1) is an equilibrium, that is thermodynamically controlled using non-activated, free carboxylic acids as substrates and requires the continuous removal of the water produced in the reaction to shift the equilibrium and increase the ester yield. Strategies for water removal are discussed in Section 3. Transesterification (reaction B in Fig. 1) is preferred above esterification, due to (1) the higher reactivity of the ester group as compared to a non-activated carboxylic acid residue and (2) the easier removal of the alcohol by-products from the reaction mixture as compared to water, the secondary product in the esterification reaction. Methyl, ethyl, and vinyl esters are generally used as acyl reagents in the transesterification reaction. Vinyl esters are favoured acyl reagents in most studies, since the vinyl alcohol formed in the reaction tautomerises to the volatile acetaldehyde that is easily removed from the reaction mixture thus driving the reaction to completion. However, due to its high reactivity, acetaldehyde could react with the free amino groups of the lysine residues in the protein resulting in enzyme inactivation. It has been reported that some lipases (e.g. from *Candida rugosa*) loose most of their activity when exposed to acetaldehyde (Weber, Stecher, & Faber, 1995).

Proteases from *Bacillus licheniformis* and *Bacillus subtilis* have been used for the enzymatic synthesis of carbohydrate fatty acids esters (Table 2). However, esterases and in particularly lipases are preferred above proteases due to the high substrate specificity of the latter that narrows the range of carboxylic acids and alcohol substrates (Bordusa, 2002). Like esterases, proteases have a preference for short and medium chain fatty acids, whereas lipases are the most promising biocatalysts for long chain fatty acids (Plou et al., 2002).

Subtilisin, a serine protease from *B. subtilis*, appeared to be highly efficient for esterification of the primary OH-group of carbohydrates (Bruno, Dordick, Kaplan, & Akkara, 1998). Lipases from *C. antarctica* (lipase CA) and *Thermomyces lanuginosus* (lipase TL), and an alkaline protease from *B. licheniformis* have been used to synthesize CFAE using vinyl laurate as acyl reagent. Melezitose, raffinose, stachyose, and kestose were used as oligosaccharides. Although different reaction media were used, different regioselectivity for the acylation of the primary hydroxyl groups was observed for lipases as compared to subtilisin (Perez-Victoria & Morales, 2006a). Both lipase CA and lipase TL showed the highest regioselectivity for the 6-OH galactosyl of raffinose and stachyose and 6-OH glucosyl of melezitose, whilst subtilisin modified preferentially the primary hydroxyl of the fructose residues of the oligosaccharides, the 1′-OH fructosyl in raffinose and stachyose, 6-OH fructosyl in melezitose and 1‴-OH fructosyl in kestose. For the acylation of secondary hydroxyl groups thermolysin a metalloprotease from *Bacillus thermoproteolyticus* can be used (Perez-Victoria & Morales, 2006b). Thermolysin has been used also for the esterification of cyclodextrin with fatty acids in dimethyl sulfoxide (DMSO). The regioselectivity of thermolysin was mainly against the hydroxyl group in position 2 of the glucopyranose unit although the hydroxyl group at positions 3 and 6 were also partially esterified leading to multiple-substituted esterified cyclodextrins (Choisnard et al., 2011; Pedersen et al., 2005). Lipases have been used also to synthesize lipidyl-cyclodextrins by amidation. Mono-6-amino-permethylated β-cyclodextrin was used as substrate and ethyl caprylate or vinyl laurate as acyl donor. As all the OH-groups were methylated the lipases acylated the amine group to synthesize mono-substituted methylated cyclodextrin with only one fatty acid moiety (Favrelle, Bonnet, Sarazin, & Djedaini-Pilard, 2007; Favrelle et al., 2010). Lipase from *C. rugosa* was used for transesterification of dextran with vinyl decanoate in DMSO. Using a pH-imprinted enzyme, the extent of dextran modification (49%) was increased 16 fold as compared when native lipase was used (3%) (Kaewprapan, Tuchinda, Marie, Durand, & Inprakhon, 2007). It was also observed that the degree of modification was depending on the commercial enzyme preparation used. Three factors were mentioned as possible reasons (1) relative proportion of isoenzymes present, (2) amount of water in the lyophilized enzyme preparation, and (3) the amount of lipase protein present. In a follow up study it was noticed that addition of 18-crown-6-ether before lyophilisation of the pH-imprinted enzyme resulted in even a higher extent of dextran modification. Furthermore it was observed that the regioselectivity was equalled between the hydroxyl group at position 2 and 3 of the glucose residue when saturated fatty acids were used as acyl donor. In the case of unsaturated acyl donors a preference for the hydroxyl group at position 2 of the glucose residue was observed, whereas vinyl pivalate, a sterically hindered acyl donor, had a preference towards the hydroxyl group at position 3 (Kaewprapan et al., 2011). In Table 2 an overview is given of the different carbohydrates that have been esterified with fatty acids using enzymes. In general long incubation times (20–120 h) are needed to obtain CFAE. An exception is the esterification of starch with fatty acids from coconut oil using micro wave heating and lipase TL. In this case the reaction was performed in minutes instead of hours (Rajan, Prasad, & Abraham, 2006).

For the enzymatic reactions often immobilized enzymes have been used because immobilization enhances the stability of the enzyme in organic solvents. The immobilized enzymes can be generally classified into two groups. Namely the group of carrier supported immobilized enzymes (e.g. adsorption on polypropylene or macroporous acrylic resin, covalent attachment to eupergit C or magnetic particles, silica-granulation, and sol–gel encapsulation), and the other group is the carrier-free immobilized enzymes like bound cross-linked enzyme aggregates (CLEAs). For example lipase from *C. rugosa* was encapsulated into a polyacrylamide gel and showed improved stability in the transesterification reaction between dextran and vinyl decanoate in anhydrous DMSO (Ge, Lu,

Table 1
Commercial enzymes used for enzymatic synthesis of carbohydrate fatty acid esters.

Name	Micro-organism	Company	Remark
Novozyme 435	*Candida antarctica*	Novozymes	Immobilized
Novozyme SP525	*Candida antarctica*	Novozymes	Free enzyme
Subtilisin	*Bacillus subtilis*	Sigma Aldrich	Free enzyme
Protease N		Amano Enzyme Co.	
Proleather FG-F		Amano Enzyme Co.	
Subtilisin Carlsberg	*Bacillus licheniformis*	Novozymes; Sigma Aldrich	Free enzyme
Lipozyme IM 60	*Rhizomucor miehei*	Novozymes	Immobilized
Lipase A12	*Aspergillus niger*	Amano Enzyme Co.	Free enzyme
Lipase AY	*Candida rugosa*	Amano Enzyme Co.	Free enzyme
L-1754		Sigma Aldrich	
Lipolase TL IM	*Thermomyces lanuginosus* (*Humicola lanuginosa*)	Novozymes	Immobilized
Lipolase 100L		Novozymes	Free enzyme
Lipozyme TM 20	*Mucor miehei*	Novozymes	Immobilized
Thermolysin	*Bacillus thermoproteolyticus*	Sigma Aldrich Calbiochem	Free enzyme

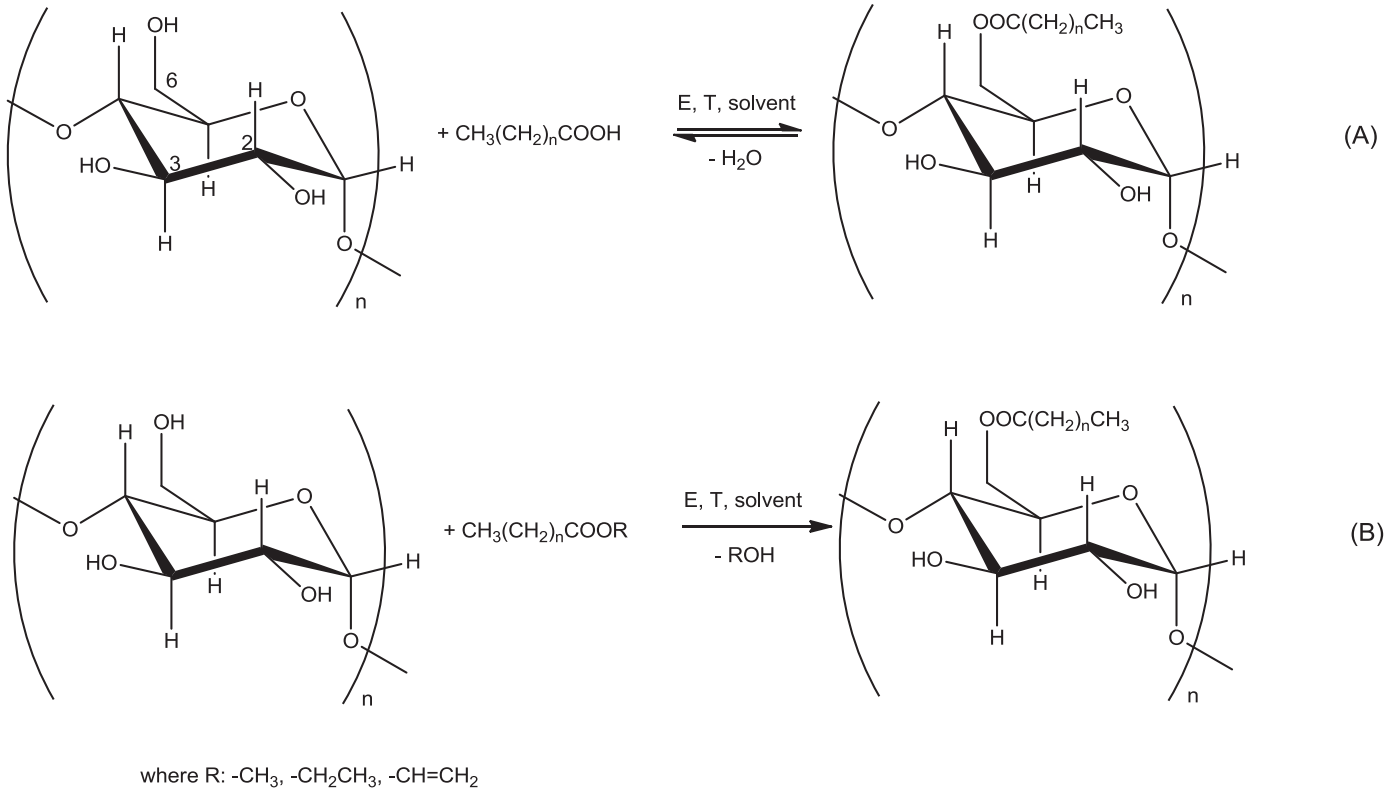

Fig. 1. Example of an esterification (A) and transesterification (B) reaction at the 6-OH glucosyl position of α-oligo and polyglucosides. E is enzyme, T is temperature.

Wang, & Liu, 2009). Another advantage of immobilisation is that it allows an easy separation and re-use of the enzyme (Cao, 2011; Plou et al., 2002). Furthermore it improves the transesterification reaction rate of enzymes (Ferrer, Cruces, Plou, Bernabe, & Ballesteros, 2000). A drawback is that immobilized enzymes are more expensive per 'activity unit' as their liquid counterparts, although new immobilization technology resulted already in a lower selling price for lipase (Nielsen, Brask, & Fjerbaek, 2008). In order to be cost effective re-use and improved stability of the enzymes are important factors. However, different types of immobilization may have different effects on the enzyme activity and/or stability, which is not always predictable at forehand (Arroyo, Sanchez-Montero, & Sinisterra, 1999; Cao, 2011). On the other hand it is known that any type of immobilization method has the potential to stabilize the enzymes relative to their native form (Cao, 2011). Thus for each enzyme and enzyme reaction the optimal immobilization parameters have to be determined.

Infrared spectroscopy (FT-IR) is generally used as a fast and reliable method to estimate the formation of esters during the enzymatic modification of carbohydrates. The spectra of carbohydrate fatty acid esters show the characteristic vibrations of the ester group between 1716–1751 cm^{-1} depending on the type of fatty acid coupled (Fig. 2). In the case of acetate a characteristic absorption at 1751 cm^{-1} becomes stronger (Chen, Zong, & Li, 2006), for laurate esters a signal is obtained at 1733 cm^{-1} (Pedersen et al., 2005) and 1716–1731 cm^{-1} (Sagis, Boeriu, Frissen, Schols, & Wierenga, 2008), the presence of a carbonyl C=O for esterified starch with fatty acids is detectable at 1746 cm^{-1} (Rajan et al., 2006), and the absorption peak at 1721 cm^{-1} was assigned to the decanoate ester attached to dextran (Kaewprapan et al., 2007). The intense peaks at 2700–3000 cm^{-1} are characteristic for the methyl and methylene groups of aliphatic fatty acid chains (Fig. 2). ^{1}H and ^{13}C NMR as well as 2D-NMR are generally used for the determination of the regioisomers formed. Malditof-MS was used

Table 2
Enzymatically synthesized carbohydrate fatty acid esters.

Carbohydrate	Acyl donor	Solvent	Time (h)	Yield (%)[a]	Enzyme	Reference
Amylose	Vinyl fatty acid ester	Isooctane	48	n.d.	*Bacillus subtilis* protease	Bruno et al. (1995)
	n-Capric acid vinyl ester	Isooctane	48	n.d.	*Bacillus licheniformis* protease	Bruno et al. (1998)
Starch	Fatty acids from coconut oil	DMSO	36–144	n.d.	*Thermomyces lanuginosus* lipase	Rajan et al. (2006)
		Hydrolysed coconut oil (microwave)	0.02	n.d.		
		Hydrolysed coconut oil with or without Triton X-100	0.33	n.d.		
	Vinyl stearate	Toluene	48	n.d.	*Candida antarctica* lipase	Chakraborty et al. (2005)
Maltotriose	Vinyl myristate	2-Methyl-2-butanol/DMSO	24	26	*Thermomyces lanuginosus* lipase	Ferrer et al. (2000)
	Vinyl stearate			27		
	Vinyl laurate			21–74		
	Vinyl palmitate	2-Methyl-2-butanol/DMSO	24	28		Ferrer et al. (2000, 2002, 2005)
Malto oligosaccharides	Trichloroethyl butyrate	DMF	96	n.d.	*Bacillus subtilis* protease	Riva, Chopineau, Kieboom, and Klibanov (1988)
Avicel cellulose	Vinyl propionate	Pre-treated with ionic liquid, hereafter solvent free incubation	20	n.d.	Hog liver esterase and *Fusarium solani* cutinase	Gremos et al. (2011)
	Vinyl laurate					
	Vinyl stearate					
Cellulose	Vinyl acetate	DMSO/para-formaldehyde	48	n.d.	*Aspergillus niger* lipase	Yang and Wang (2003) and Yang, Wang, and Kuo (2004)
	Vinyl propionate	Anhydrous pyridine	120	n.d.	*Bacillus licheniformis* protease	Xie and Hsieh (2001)
	Vinyl acrylate					
	Isopropenyl acetate	Sodium phosphate buffer	18	n.d.	*Arthrobacter viscosus* carboxylesterase	Cui, Winter, Tanenbaum, and Nakas (1999)
	Vinyl acetate	Sodium phosphate buffer	24	n.d.	*Aspergillus niger* lipase	Yang and Wang (2003)
Carboxymethyl cellulose	Vinyl acetate	Sodium phosphate buffer	48	n.d.	*Aspergillus niger* lipase	Yang and Wang (2003)
Cellulose acetate	Lauric acid	Acetonitrile	96	n.d.	*Candida antarctica* lipase	Sereti et al. (1998)
	Oleic acid					
Hydroxy ethyl cellulose	*n*-Capric acid vinyl ester	Isooctane	48	n.d.	*Bacillus licheniformis* protease	Bruno et al. (1998)
Hydroxypropyl cellulose	Lauric acid	*tert*-Butanol	192	n.d.	*Candida antarctica* lipase	Sereti et al. (2001)
Dextran	Vinyl acetate	DMSO	32	n.d.	*Candida rugosa* lipase	Kaewprapan et al. (2011)
	Vinyl propionate					
	Vinyl decanoate					
	Vinyl laurate					
	Vinyl acrylate					
	Vinyl methacrylate					
	Vinyl crotonate					
	Vinyl pivalate					
	Vinyl decanoate	DMSO	24–240	n.d.	*Candida rugosa* lipase	Ge et al. (2009) and Kaewprapan et al. (2007)
	Vinyl acrylate	DMSO	72	45	*Candida rugosa* lipase and *Bacillus subtilis* protease	Ferreira et al. (2005)
	Divinyladipate	DMSO	72	n.d.	*Candida rugosa* lipase, *Bacillus subtilis* protease, and *Pseudomonas cepacia* lipase	Ferreira et al. (2002)
β-Cyclodextrins	*n*-Capric acid vinyl ester	Isooctane	48	n.d.	*Bacillus licheniformis* protease	Bruno et al. (1998)
	Vinyl butyrate	DMSO	20–73	n.d.	*Bacillus thermoproteolyticus* protease	Pedersen et al. (2005)
	Vinyl decanoate					
	Vinyl laurate					

Table 2 (*Continued*)

Carbohydrate	Acyl donor	Solvent	Time (h)	Yield (%)[a]	Enzyme	Reference
Fructo oligosaccharides	Vinyl laurate	*tert*-Butanol/DMSO	24	n.d.	*Candida antarctica* lipase	Sagis et al. (2008)
	Vinyl laurate	*tert*-Butanol/DMSO	120	n.d.	*Candida antarctica* lipase	ter Haar et al. (2010)
	Lauric acid					
	Divinyladipate	DMF	72	n.d.	*Bacillus subtilis* protease	Ferreira et al. (2002)
	Vinyl acrylate	DMF	96	44	*Bacillus subtilis* protease	Ferreira et al. (2002)
Kestose	Vinyl laurate	*tert*-Butanol/pyridine	72, 72, 48	54, 57, 55	*Candida antarctica* lipase, *Thermomyces lanuginosus* lipase, and *Bacillus licheniformis* protease	Perez-Victoria and Morales (2006a)
		DMSO	72	32	*Bacillus thermoproteolyticus* protease	Perez-Victoria and Morales (2006b)
Melizitose	Vinyl laurate	DMF	24	50	*Bacillus subtilis* protease	Riva, Nonini, Ottolina, and Danieli (1998)
	Vinyl laurate	*t*-Butanol/pyridine	72, 72, 48	38, 54, 69	*Candida antarctica* lipase, *Thermomyces lanuginosus* lipase, and *Bacillus licheniformis* protease	Perez-Victoria and Morales (2006a)
	Divinyladipate		72, 72, 24	38, 50, 55	*Candida antarctica*, *Thermomyces lanuginosus* lipase, and *Bacillus licheniformis* protease	Perez-Victoria and Morales (2007)
	Vinyl laurate	DMSO	72	22	*Bacillus thermoproteolyticus* protease	Perez-Victoria and Morales (2006b)
Raffinose/stachyose	Vinyl laurate	DMF	24/24	49/49	*Bacillus licheniformus* protease	Riva et al. (1998)
	Vinyl laurate	*tert*-Butanol/pyridine	72/192, 24/72, 24/48	48/26, 79/68, 74/76	*Candida antarctica* lipase, *Thermomyces lanuginosus* lipase, and *Bacillus licheniformis* protease	Perez-Victoria & Morales (2006a)
	Divinyladipate	*tert*-Butanol/pyridine	72/–, 24/–, 24/–	42,–. 75/–, 65/–	*Candida antarctica* lipase, *Thermomyces lanuginosus* lipase, and *Bacillus licheniformis* protease	Perez-Victoria and Morales (2007)
	Vinyl laurate	DMSO	72/72	27/12	*Bacillus thermoproteolyticus* protease	Perez-Victoria and Morales (2006b)
Apple pectin	Vinyl laurate	$scCO_2$ *tert*-Butanol	96–116 72	n.d. 10	*Candida antarctica* lipase	This study
Beet pectin	Vinyl laurate	*tert*-Butanol	72	10	*Candida antarctica* lipase	This study
Konjac glucomannan	Vinyl acetate	*tert*-Butanol	24	n.d.	*Candida antarctica* lipase	Chen et al. (2006)
Xyloglucan oligosaccharides	Vinyl stearate	DMSO/2-methyl-2-butanol	20	n.d.	*Candida antarctica* lipase	Gustavsson et al. (2005)

[a] n.d. is not determined.

to determine the presence of mono-, and multiple esters in the product of the lipase catalysed esterification of inulin with vinyl laurate. Mono-esters were obtained using Novozyme 435 lipase, whereas molecular sieves resulted in multiple fructose oligosaccharide esters (ter Haar et al., 2010).

3. Reaction media

One of the main problems of the enzymatic synthesis of CFAE is the low solubility of the sugar substrates in organic solvents. In addition the enzyme should retain its activity in these solvents. Besides water, oligo- and polysaccharides are soluble in hydrophilic organic solvents like DMSO, 1-methyl-2-pyrrolidone (NMP), and dimethylformamide (DMF), which all have a strong inactivating effect on enzymes. To remain active in organic solvents the enzyme must maintain a small and essential amount of water which is tightly bound to the enzyme and is needed for stability and flexibility of the enzyme (Fernandez-Mayoralas, 1997). Several peptidases and proteases for example retain their activity in pyridine and DMF (Davis & Borer, 2001). In organic solvents the molar fraction of water is reduced by the addition of the organic solvent and this will shift the reaction equilibrium towards synthetic catalysis instead of hydrolysis. The activity of hydrolases such as lipases and proteases in organic solvent has been often correlated with the solvent hydrophobicity ($\log P$) (Laane, Boeren, Vos, & Veeger, 1987). A high $\log P$ is advantageous for these enzymes. Another way to correlate enzyme activity is the thermodynamic water activity (a_w). In this case a_w is correlated to the mole fraction of water and the water activity coefficient (Carrea & Riva, 2000; Halling, 1994). Apart from using only one solvent a mixture of two or more solvents can be used (Plou et al., 2002), although the hydrophobicity of solvent mixtures cannot be defined (Halling, 1994). Furthermore an advantage of organic solvents is that in some cases enzymatic properties such as specificity and selectivity can be influenced by these solvents (Carrea & Riva, 2000; Fernandez-Mayoralas, 1997). Another approach is using organic solvents with low concentrations of surfactants. In these experiments the enzyme, often proteases, is incorporated within reversed micelles using the anionic surfactant bis(2-ethylhexyl)sodium sulfosuccinate (OAT) in isooctane. By ion-paring between the surfactant and the enzyme it is possible to have (trans)esterification reactions (Bruno et al., 1995, 1998; Chakraborty, Sahoo, Teraoka, Miller, & Gross, 2005; Xie & Hsieh, 2001).

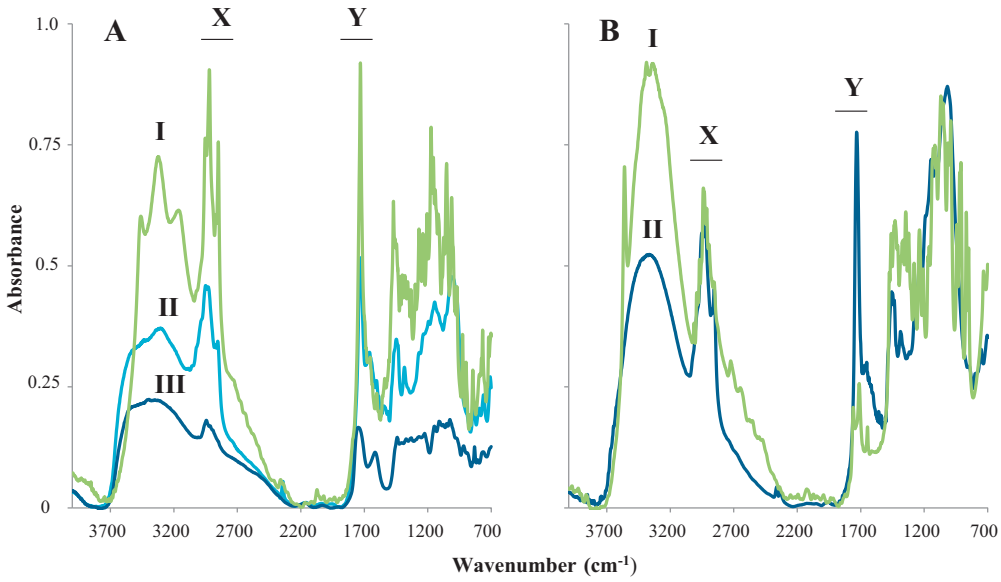

Fig. 2. FT-IR spectra of (A) laurate esterified apple pectin using *Candida antarctica* lipase (Novozyme 435) in *tert*-butanol (I) and $scCO_2$ (II), and as control native apple pectin (III). (B) Native beet pectin (I) and laurate esterified beet pectin using *C. antarctica* lipase in *tert*-butanol (II). Characteristic vibration for (*x*) the methyl and methylene groups of aliphatic fatty acid chains and (*y*) the carbonyl in the ester group.

To improve the solubility of carbohydrates in organic solvents hydrophobisation of the sugar moiety can be done. This is performed by complexation with phenyl boronic acids, formation of acetals, or chemical acetylation. The derivatisation is followed by solvent-free esterification (Plou et al., 2002). In the case of a solvent-free medium often the acyl donor is used as solvent.

Although the catalysis of ester synthesis in water is unfavourable, due to the ester hydrolysis reaction also catalysed by the enzyme, the presence of low water content in the reaction medium is desired for keeping the enzyme active as stated above (Carrea and Riva, 2000). Usually, a water content between 0.3% and 1% (v/v) is sufficient for the enzymes to maintain their catalytic active conformation. Water removal in solvent-free systems can be done by evacuation in vacuo, using open test tubes, pervapouration using membranes, and dry gas bubbling (Yahya, Anderson, & Moo-Young, 1998). When reactions are carried out in solution using solvents, the water from the media is removed under reduced pressure or by using molecular sieves. However, the presence of molecular sieves can lead to multiple-substituted products due to a chemical esterification catalysed by the molecular sieves. If no molecular sieves were used in the lipase catalysed esterification reaction of vinyl laurate and fructose oligosaccharides then only mono-substituted oligomers were synthesized. The presence of molecular sieves resulted in multiple-substituted oligomers. Therefore the application of molecular sieves should be carefully monitored as even pre-drying of solvents can release the catalysing components originating from the molecular sieves into the solvents (ter Haar et al., 2010). In addition chemical acylation of hydroxyl-containing compounds (e.g. oligo- and polysaccharides) can also occur with enol (e.g. vinyl) esters in polar solvents using immobilized enzymes due to the support (e.g. celite, eupergit C) and salts (Na_2HPO_4) employed during immobilization or lyophilisation (Plou et al., 1999).

Apart from organic solvent supercritical fluids can be used as reaction media. Supercritical fluids exist as a vapour and liquid in equilibrium above their critical temperature and critical pressure. $scCO_2$ has properties like a relatively low critical pressure (74 bar) and has a close to ambient critical temperature (31 °C). Furthermore it is non-toxic, non-flammable, and has a low cost and it can be considered as a green solvent under certain circumstances (Beckman, 2004; deCarvalho, deSampaio, & Barreiros, 1996; Sheldon, 2005). These characteristics make $scCO_2$ an attractive solvent for the esterification of carbohydrates with fatty acids. A drawback could be that the carbon dioxide reacts with free primary amino groups on the surface of the enzyme to form carbamate complexes that results for some enzymes in reduced enzyme activity (Beckman, 2004; deCarvalho et al., 1996). In our laboratory apple pectin (75% methylated) was esterified with vinyl laurate using Novozyme 435 lipase (*C. antarctica*) in $scCO_2$ (300 bar, 100 °C and 80 °C and an incubation time of 96 and 116 h, respectively). Molecular sieves were added to remove water from the reaction mixture. After release of CO_2 the final reaction mixture appeared to be a light grey powder. The powder was washed extensively with hexane to remove the residual vinyl laurate and was analysed using FT-IR. The spectrum (absorbance at 1720 cm^{-1}) clearly shows the presence of the new ester groups formed (Fig. 2). For comparison apple pectin and beet pectin were esterified (in duplicate) with vinyl laurate in *tert*-butanol using the immobilized Novozyme 435 lipase (60 °C, 72 h; Fig. 2). The DS estimated from the IR-calibration was higher for apple pectin and beet pectin (both DS ~0.29) incubated in *tert*-butanol as for apple pectin incubated in $scCO_2$ (DS ~0.14).

Ionic liquids (ILs), which can also be used as media for carbohydrate esterification reactions, are generally salts of organic cations and represent liquids that are composed of entirely ions. These liquids have essentially no vapour pressure. The polarity and hydrophilicity/hydrophobicity can be regulated by changing the combination of anion and cation (Sheldon, 2005). ILs have been found capable of dissolving cellulose and carbohydrates to a great extent and research is going on to develop ILs which are less hazardous for enzymes (Zhao et al., 2008). For example Avicel cellulose was dissolved in 1-butyl-3-methylimidazolium [BMIm][Cl] to make the structure more accessible. Hereafter the IL was removed and the remaining cellulose was incubated with immobilized esterase from hog liver or with cutinase from *Fusarium solani*. FT-IR analysis revealed the formation of ester linkages using vinyl propionate, vinyl laurate, or vinyl stearate as acyl donor. Lipase from *C. antarctica*, *Candida cylindracea*, and *Aspergillus niger* were not able to esterify the pre-treated cellulose (Gremos, Zarafeta, Kekos, & Kolisis, 2011). In literature no enzymes have been reported yet to be able to esterify carbohydrates with fatty acids directly in ILs.

Only the esterification of cellulose with methyl methacrylate in [Me(Oet)3-Et3N][OAc], [Me(OEt)$_2$-Et-Im][OAc], and [Me(OEt)$_7$-Et-Im][OAc] has been reported using lipase from *C. antarctica* (Zhao et al., 2008).

4. Products/application

As shown in Table 2 different oligo- and polysaccharides have been enzymatically esterified with fatty acids. The synthesized carbohydrate fatty esters can be applied in a whole range of (potential) products and applications. For example the CFAE can be used as biodegradable emulsifiers, compatibilisers, detergents and for surface modification of preformed polysaccharides-based materials. The advantage of oligo- and polysaccharides fatty acid esters as surfactants is that the sugars are more soluble in water as mono- and disaccharides due to their increased hydrophilicity of the sugar groups (Plou et al., 2002). The degree of substitution and the position of the fatty acid to the sugar moiety determine the critical micelle concentration (CMC) value. This value represents the concentration of surfactants in the bulk at which micelles start forming and can be used as a measure of surfactant efficiency. Esters of maltotriose with a low CMC value ranging from 2 to 52 μM displayed better surface-active properties and higher solubility compared with their monosaccharide esters. The longer the fatty acid chain the lower the CMC value (Ferrer et al., 2002).

CFAE with enhanced surface-active properties can be used also for the stabilisation of foams and emulsions. Raftiline LS, a mixture of linear-chains of β-(2-1) linked fructo-oligosaccharides containing a glucose moiety at the non-reducing end, has been enzymatically esterified with lauric acid. The carbohydrate fatty acid ester functionality has been tested for application in foams and it showed exceptional foam stability (Sagis et al., 2008).

Enzymatic synthesis using divinyl adipate as acyl donor can be used to link polymers with each other. Dextran-based hydrogels were prepared and showed superior mechanical properties (higher elastic modulus for a given swelling ratio) above the chemically prepared dextran-based hydrogels. These hydrogels are biodegradable and have potential in biomedical applications like tissue engineering and controlled drug delivery (Ferreira, Gil, Cabrita, & Dordick, 2005). Also inulin (fructo-oligosaccharides) could be intermolecular cross linked with divinyl adipate and longer chain polymers were obtained in comparison with a chemical approach (Ferreira et al., 2002).

Sugar esters are promising candidates as antitumor agents. Two fatty acids esters of maltotriose were synthesized (6′-O-dodecanoylmaltotriose and 6′-O-palmitoylmaltotriose) and showed inhibitory effects towards two different tumour cell lines (Hep-G2 and HeLa). 6′-O-palmitoylmaltotriose displayed the highest inhibitory effects and showed marginal cytotoxicity to rat hepatocytes. It was concluded that the trisaccharides were more promising inhibitors as their mono- and disaccharide counterparts (Ferrer, Perez, Plou, Castell, & Ballesteros, 2005).

Cellulose and cellulose esters can be used in the production of e.g. fibres, plastics, drugs, and films. In order to improve the thermoplastic properties of the acyl moieties of esterified cellulose, which can act as internal plasticizer, should contain six or more carbon atoms. Commercial synthesis is performed in heterogeneous reaction systems, however, this process is mainly limited to the incorporation of short chain fatty acids containing four or less carbon atoms. Direct esterification of the glucose units in cellulose with longer chain fatty acids can also be done chemically. Another way to obtain cellulose with longer chain fatty acids is described by Gremos et al. Avicel cellulose was made more amorphous by incubating it in ionic liquid and after isolation from the ionic liquid it could be esterified enzymatically with long chain fatty acids (12 or 18 carbon atoms). A drawback is the low esterification degree (1.3–0.9%) (Gremos et al., 2011). Another approach was the acylation of xyloglucan oligosaccharides with stearic acid moieties. Subsequently the acylated xyloglucan oligosaccharides were incorporated into cellulose molecules using xyloglucan endotransglycosylase (Gustavsson et al., 2005). Soluble derivatives of cellulose like cellulose acetate and hydroxypropyl cellulose have been used to obtain cellulose containing longer chain fatty acids residues. For cellulose acetate the yield was ~35% and mainly acetate groups were exchanged with lauric or oleic acid (Sereti, Stamatis, Koukios, & Kolisis, 1998). In the case of hydroxypropyl cellulose the secondary OH-group of the hydroxypropyl side chain was esterified with lauric acid (~11%) (Sereti, Stamatis, Pappas, Polissiou, & Kolisis, 2001).

5. Future trends

Nowadays, products made from renewable resources have received more and more attention and gained increasing importance in the frame work of a biobased economy. CFAE are made of natural raw materials like carbohydrates and fat/oil. Instead of using a chemical approach enzymes like lipases and proteases can be used for the synthesis of CFAE. This results in a 'green' product that is biodegradable and can be used for example as surfactant. However, there are no commercial processes yet for the enzymatic production of CFAE. The research over the past decade has, however, demonstrated the high potential of enzymes for the synthesis of a wide range of carbohydrate esters with large diversity of both the carbohydrate backbone and aliphatic residue. Future research should focus on the optimization of processes including the decrease in reaction time, the physico–chemical and functional characterization of the products and the development of applications. In addition a general method for determination and definition of the degree of modification is needed. At this moment it is difficult to compare results due to the different methods applied to calculate the degree of substitution, conversion, and yield.

For optimal enzymatic synthesis protein engineering and solvent engineering is one of the key areas for improvement. Protein engineering like site directed mutagenesis and directed evolution have made it possible to make tailored made enzymes with the desired and/or improved properties. Enzymes with higher stability at elevated temperatures have been obtained already. Further engineering of enzymes for carbohydrate modification should address the increase of stability in hydrophilic organic solvents and the modulation of the substrate specificity. Integration of in silico modelling with protein engineering and high through-put screening will allow the rational design of better enzymes for the esterification of carbohydrates. Immobilization is another approach for changing and improving the activity and selectivity of the enzymes and for the recovery and recycling of the enzymes. Development of robust and reliable enzyme preparation via immobilization would contribute to the implementation of technological viable and cost efficient enzymatic processes for the synthesis of carbohydrate esters. To make carbohydrate esterification reaction greener $scCO_2$ and ILs can be used as reaction media. However, the research for carbohydrate esterification in these media is still in its infancy. Furthermore ILs have still high prices and there is some discussion about their toxicity and biodegradability and their overall effect on the environment. On the other hand research for ILs is still on-going and ILs are now designed to overcome these problems.

In the last years more and more knowledge was obtained especially for the enzymatic synthesis of fatty acids esters of mono- and disaccharides. Part of this can be applied for the enzymatic synthesis of carbohydrate fatty esters. As already shown carbohydrate fatty esters can have superior properties above their monosaccharide counterparts. To make the enzymatic synthesis possible on industrial scale more research is needed in this field.

6. Conclusion

Mainly lipases but also proteases have been successfully used for the enzymatic synthesis of CFAE. The synthesized CFAE show new or improved properties for example for foaming, controlled drug delivery, and as plasticizer. For optimal CFAE production protein and solvent engineering are needed, next to improvement of the stability and re-use of the enzymes by immobilization, in order to obtain an economical feasible production process.

References

Allen, D. K., & Tao, B. Y. (1999). Carbohydrate-alkyl ester derivatives as biosurfactants. *Journal of Surfactants and Detergents*, *2*(3), 383–390.

Arroyo, M., Sanchez-Montero, J. M., & Sinisterra, J. V. (1999). Thermal stabilization of immobilized lipase B from Candida antarctica on different supports: Effect of water activity on enzymatic activity in organic media. *Enzyme and Microbial Technology*, *24*(1–2), 3–12.

Beckman, E. J. (2004). Supercritical and near-critical CO_2 in green chemical synthesis and processing. *Journal of Supercritical Fluids*, *28*(2–3), 121–191.

Bordusa, F. (2002). Proteases in organic synthesis. *Chemical Reviews*, *102*(12), 4817–4867.

Bruno, F. F., Akkara, J. A., Ayyagari, M., Kaplan, D. L., Gross, R., Swift, G., et al. (1995). Enzymatic modification of insoluble amylose in organic solvents. *Macromolecules*, *28*(26), 8881–8883.

Bruno, F. F., Dordick, J. S., Kaplan, D. L., & Akkara, J. A. (1998). Regioselective enzymatic transesterification of polysaccharides in organic solvents. In R. A. Gross, D. L. Kaplan, & G. Swift (Eds.), *Enzymes in polymer synthesis* (pp. 167–174). Washington: Amer Chemical Soc.

Cao, L. (2011). Immobilized enzymes. In M. Moo-Young (Ed.), *Bioreactions and bioreactor operation* (pp. 461–476). Elsevier.

Carrea, G., & Riva, S. (2000). Properties and synthetic applications of enzymes in organic solvents. *Angewandte Chemie-International Edition*, *39*(13), 2226–2254.

Chakraborty, S., Sahoo, B., Teraoka, I., Miller, L. M., & Gross, R. A. (2005). Enzyme-catalyzed regioselective modification of starch nanoparticles. *Macromolecules*, *38*(1), 61–68.

Chang, S. W., & Shaw, J. F. (2009). Biocatalysis for the production of carbohydrate esters. *New Biotechnology*, *26*(3–4), 109–116.

Chen, Z. G., Zong, M. H., & Li, G. J. (2006). Lipase-catalyzed acylation of konjac glucomannan in organic media. *Process Biochemistry*, *41*(7), 1514–1520.

Choisnard, L., Geze, A., Vanhaverbeke, C., Yameogo, J. B. G., Putaux, J. L., Brasme, B., et al. (2011). Physicochemical characterization of alpha-, beta-, and gamma-cyclodextrins bioesterified with decanoate chains used as building blocks of colloidal nanoparticles. *Biomacromolecules*, *12*(8), 3031–3038.

Cui, W., Winter, W. T., Tanenbaum, S. W., & Nakas, J. P. (1999). Purification and characterization of an intracellular carboxylesterase from Arthrobacter viscosus NRRL B-1973. *Enzyme and Microbial Technology*, *24*(3–4), 200–208.

Davis, B. G., & Borer, V. (2001). Biocatalysis and enzymes in organic synthesis. *Natural Product Reports*, *18*(6), 618–640.

deCarvalho, I. B., deSampaio, T. C., & Barreiros, S. (1996). Solvent effects on the catalytic activity of subtilisin suspended in compressed gases. *Biotechnology and Bioengineering*, *49*(4), 399–404.

Favrelle, A., Bonnet, V., Avondo, C., Aubry, F., Djedaini-Pilard, F., & Sarazin, C. (2010). Lipase-catalyzed synthesis and characterization of novel lipidyl-cyclodextrins in solvent free medium. *Journal of Molecular Catalysis B: Enzymatic*, *66*(1–2), 224–227.

Favrelle, A., Bonnet, V., Sarazin, C., & Djedaini-Pilard, F. (2007). Novel chemo-enzymatic access to amphiphilic cyclodextrins. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, *57*(1–4), 15–20.

Fernandez-Mayoralas, A. (1997). Synthesis and modification of carbohydrates using glycosidases and lipases. *Glycoscience: Synthesis of Oligosaccharides and Glycoconjugates*, *186*, 1–20.

Ferreira, L., Gil, M. H., Cabrita, A. M. S., & Dordick, J. S. (2005). Biocatalytic synthesis of highly ordered degradable dextran-based hydrogels. *Biomaterials*, *26*(23), 4707–4716.

Ferreira, L., Gil, M. H., Carvalho, R., Geraldes, C., Kim, D. Y., & Dordick, J. S. (2002). Biocatalytic polytransesterification of inulin with divinyladipate. *Chemistry of Materials*, *14*(10), 4009–4011.

Ferrer, M., Comelles, F., Plou, F. J., Cruces, M. A., Fuentes, G., Parra, J. L., et al. (2002). Comparative surface activities of di- and trisaccharide fatty acid esters. *Langmuir*, *18*(3), 667–673.

Ferrer, M., Cruces, M. A., Plou, F. J., Bernabe, M., & Ballesteros, A. (2000). A simple procedure for the regioselective synthesis of fatty acid esters of maltose, leucrose, maltotriose and n-dodecyl maltosides. *Tetrahedron*, *56*(24), 4053–4061.

Ferrer, M., Perez, G., Plou, F. J., Castell, J. V., & Ballesteros, A. (2005). Antitumour activity of fatty acid maltotriose esters obtained by enzymatic synthesis. *Biotechnology and Applied Biochemistry*, *42*, 35–39.

Ge, J., Lu, D. A., Wang, J., & Liu, Z. (2009). Lipase nanogel catalyzed transesterification in anhydrous dimethyl sulfoxide. *Biomacromolecules*, *10*(6), 1612–1618.

Gremos, S., Zarafeta, D., Kekos, D., & Kolisis, F. (2011). Direct enzymatic acylation of cellulose pretreated in BMIMCl ionic liquid. *Bioresource Technology*, *102*(2), 1378–1382.

Gumel, A. M., Annuar, M. S. M., Heidelberg, T., & Chisti, Y. (2011). Lipase mediated synthesis of sugar fatty acid esters. *Process Biochemistry*, *46*(11), 2079–2090.

Gustavsson, M. T., Persson, P. V., Iversen, T., Martinelle, M., Hult, K., Teeri, T. T., et al. (2005). Modification of cellulose fiber surfaces by use of a lipase and a xyloglucan endotransglycosylase. *Biomacromolecules*, *6*(1), 196–203.

Halling, P. J. (1994). Thermodynamic predictions for biocatalysis in nonconventional media—Theory, tests, and recommendations for experimental-desing and analysis. *Enzyme and Microbial Technology*, *16*(3), 178–206.

Kaewprapan, K., Tuchinda, P., Marie, E., Durand, A., & Inprakhon, P. (2007). PH-imprinted lipase catalyzed synthesis of dextran fatty acid ester. *Journal of Molecular Catalysis B: Enzymatic*, *47*(3–4), 135–142.

Kaewprapan, K., Wongkongkatep, J., Panbangred, W., Phinyocheep, P., Marie, E., Durand, A., et al. (2011). Lipase-catalyzed synthesis of hydrophobically modified dextrans: Activity and regioselectivity of lipase from Candida rugosa. *Journal of Bioscience and Bioengineering*, *112*(2), 124–129.

Kennedy, J. F., Kumar, H., Panesar, P. S., Marwaha, S. S., Goyal, R., Parmar, A., et al. (2006). Enzyme-catalyzed regioselective synthesis of sugar esters and related compounds. *Journal of Chemical Technology and Biotechnology*, *81*(6), 866–876.

Laane, C., Boeren, S., Vos, K., & Veeger, C. (1987). Rules for optimizationof biocatalysis in organic solvents. *Biotechnology and Bioengineering*, *30*(1), 81–87.

Nielsen, P. M., Brask, J., & Fjerbaek, L. (2008). Enzymatic biodiesel production: Technical and economical considerations. *European Journal of Lipid Science and Technology*, *110*(8), 692–700.

Pedersen, N. R., Kristensen, J. B., Bauw, G., Ravoo, B. J., Darcy, R., Larsen, K. L., et al. (2005). Thermolysin catalyses the synthesis of cyclodextrin esters in DMSO. *Tetrahedron: Asymmetry*, *16*(3), 615–622.

Perez-Victoria, I., & Morales, J. C. (2006a). Complementary regioselective esterification of non-reducing oligosaccharides catalyzed by different hydrolases. *Tetrahedron*, *62*(5), 878–886.

Perez-Victoria, I., & Morales, J. C. (2006b). Regioselectivity in acylation of oligosaccharides catalyzed by the metalloprotease thermolysin. *Tetrahedron*, *62*(10), 2361–2369.

Perez-Victoria, I., & Morales, J. C. (2007). One- and two-step enzymatic synthesis of polymerizable vinyladipoyl mono- and diesters of non-reducing trisaccharides. *Journal of Molecular Catalysis B: Enzymatic*, *48*(1–2), 8–15.

Plou, F. J., Cruces, M. A., Ferrer, M., Fuentes, G., Pastor, E., Bernabe, M., et al. (2002). Enzymatic acylation of di- and trisaccharides with fatty acids: Choosing the appropriate enzyme, support and solvent. *Journal of Biotechnology*, *96*(1), 55–66.

Plou, F. J., Cruces, M. A., Pastor, E., Ferrer, M., Bernabe, M., & Ballesteros, A. (1999). Acylation of sucrose with vinyl esters using immobilized hydrolases: Demonstration that chemical catalysis may interfere with enzymatic catalysis. *Biotechnology Letters*, *21*(7), 635–639.

Polat, T., & Linhardt, R. J. (2001). Syntheses and applications of sucrose-based esters. *Journal of Surfactants and Detergents*, *4*(4), 415–421.

Rajan, A., Prasad, V. S., & Abraham, T. E. (2006). Enzymatic esterification of starch using recovered coconut oil. *International Journal of Biological Macromolecules*, *39*(4–5), 265–272.

Riva, S., Chopineau, J., Kieboom, A. P. G., & Klibanov, A. M. (1988). Protease-catalyzed regioselective esterification of sugars and related compounds in anydrous dimethylformamide. *Journal of the American Chemical Society*, *110*(2), 584–589.

Riva, S., Nonini, M., Ottolina, G., & Danieli, B. (1998). Subtilisin-catalyzed esterification of di- and oligosaccharides containing a D-fructose moiety. *Carbohydrate Research*, *314*(3–4), 259–266.

Sagis, L. M. C., Boeriu, C. G., Frissen, G. E., Schols, H. A., & Wierenga, P. A. (2008). Highly stable foams from block oligomers synthesized by enzymatic reactions. *Langmuir*, *24*(2), 359–361.

Sereti, V., Stamatis, H., Koukios, E., & Kolisis, F. N. (1998). Enzymatic acylation of cellulose acetate in organic media. *Journal of Biotechnology*, *66*(2–3), 219–223.

Sereti, V., Stamatis, H., Pappas, C., Polissiou, M., & Kolisis, F. N. (2001). Enzymatic acylation of hydroxypropyl cellulose in organic media and determination of ester formation by diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy. *Biotechnology and Bioengineering*, *72*(4), 495–500.

Sheldon, R. A. (2005). Green solvents for sustainable organic synthesis: State of the art. *Green Chemistry*, *7*(5), 267–278.

ter Haar, R., Schols, H. A., van den Broek, L. A. M., Saglam, D., Frissen, A. E., Boeriu, C. G., et al. (2010). Molecular sieves provoke multiple substitutions in the enzymatic synthesis of fructose oligosaccharide-lauryl esters. *Journal of Molecular Catalysis B: Enzymatic*, *62*(2), 183–189.

Weber, H. K., Stecher, H., & Faber, K. (1995). Sensitivity of microbial lipases to acetaldehyde formed by acyl-transfer reactions from vinyl esters. *Biotechnology Letters*, *17*(8), 803–808.

Xie, J. B., & Hsieh, Y. L. (2001). Enzyme-catalyzed transesterification of vinyl esters on cellulose solids. *Journal of Polymer Science Part A: Polymer Chemistry*, *39*(11), 1931–1939.

Yahya, A. R. M., Anderson, W. A., & Moo-Young, M. (1998). Ester synthesis in lipase-catalyzed reactions. *Enzyme and Microbial Technology*, *23*(7–8), 438–450.

Yang, K., & Wang, Y. J. (2003). Lipase-catalyzed cellulose acetylation in aqueous and organic media. *Biotechnology Progress*, *19*(6), 1664–1671.

Yang, K., Wang, Y. J., & Kuo, M. I. (2004). Effects of substrate pretreatment and water activity on lipase-catalyzed cellulose acetylation in organic media. *Biotechnology Progress*, *20*(4), 1053–1061.

Zhao, H., Baker, G. A., Song, Z. Y., Olubajo, O., Crittle, T., & Peters, D. (2008). Designing enzyme-compatible ionic liquids that can dissolve carbohydrates. *Green Chemistry*, *10*(6), 696–705.