



Lipases efficiently stearate and cutinases acetylate the surface of arabinoxylan films



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ABSTRACT

This is the first report on successful enzyme catalyzed surface esterification of hemicellulose films. Enzyme catalyzed surface acetylation with vinyl acetate and stearation with vinyl stearate were studied on rye arabinoxylan (AX) films. Different surface analytical techniques (FT-IR, TOF-SIMS, ESCA, CA) show that lipases from *Mucor javanicus*, *Rhizopus oryzae* and *Candida rugosa* successfully surface stearate AX films and that a cutinase from *Fusarium solani pisi* surface acetylates these films. The specificities of cutinase and lipases were also compared, and higher activity was observed for lipases utilizing long alkyl chain substrates while higher activity was observed for cutinase utilizing shorter alkyl chain substrates. The contact angle analysis showed films with increased initial hydrophobicity on the surfaces.

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1. Introduction

Current plastic production is chiefly based on non-renewable resources, and the carbon dioxide emissions resulting from this process and the accumulating non-biodegradable waste are a serious challenge to the ecosystem's balance. Hemicelluloses, being the second most abundant biopolymer on Earth, can make a major contribution toward a sustainable bio-based economy. Besides their potential conversion to biofuels and biochemicals, hemicelluloses and some of their derivatives can be utilized as novel value added bio-based and biodegradable materials such as gels, packaging material, matrices for composites and binders (Ebringerova and Heinze, 2000).

Hemicelluloses are a group of polysaccharides consisting of diverse monosaccharides building up a complex composition and structure (Timell, 1967). In contrast to cellulose, hemicelluloses are nonlinear heteropolymers with a lower molecular weight, which

gives them significantly different chemical and material properties compared to cellulose (Tombs and Harding, 1997). For example, arabinoxylan forms films with good oxygen barrier properties, which show a potential as novel packaging materials (Escalante et al., 2011; Grondahl et al., 2004; Hoije et al., 2008). Furthermore, the acetylated arabinoxylans have improved water resistance and attractive mechanical and thermal properties compared to other commercially available bio-based packaging materials (Stepan et al., 2012).

Due to the more complex structure of hemicellulose as compared with cellulose, targeted hemicellulose derivatization is still a challenge. Most of the hemicellulose conversions to date proceed through chemical reactions involving in most cases significant excess of several organic solvents for significant esterification levels (Sun et al., 1999, 2004; Timell, 1967). Surface-only modification, such as the surface octanoylation of starch films, has been developed to decrease the amount of chemicals needed for the conversion (Bengtsson et al., 2003). Enzymes offer an alternative option to carry out surface esterification. Such biocatalytic systems can decrease the amount of chemicals required, many of which can be toxic and dangerous, while enzymes, when immobilized, can be reused, reducing the cost of the enzyme production. Chemical surface modifications may also have high energy demands, with

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reactions taking place at high temperatures, such as 80 °C in the case of the octanoylation, while most enzymes used in this study have their temperature optimum at around 40 °C. Further advantage of enzymatic surface modifications is that it is less intrusive for the treated material, as they depend on its porosity to access the potential active groups. Enzymes can also provide high level of reaction specificity, which is important for the development of custom-made biomaterials and not always controllable in chemical synthesis systems. Nevertheless, the push toward environmental processes is a main trigger to turn toward enzyme applications for hemicellulose derivatization. The literature on enzyme catalyzed chemical modifications of carbohydrates is continuously expanding. Numerous studies have been done on the activity, stability and specificity of lipases and cutinases in hydrolyzing different ester bonds or transesterification reactions, which have inspired researchers to use lipases and cutinases in a reverse reaction for esterification of hemicelluloses (Kontkanen et al., 2004; Pio and Macedo, 2009; Poulsen et al., 2005; Sakai et al., 2008; Utsugi et al., 2009). It has been shown that some lipases, esterases and cutinases that show activity on esterified substances can also have monosaccharide, oligosaccharide and polysaccharide derivatives as a substrate (Guebitz and Cavaco-Paulo, 2009; Woudenberg-van et al., 1996). The work of (Sakai et al., 2008) and (Utsugi et al., 2009) provides a good overview of some lipases. Their research helped in choosing the lipases from *Mucor javanicus*, *Rhizopus oryzae* and *Candida rugosa* and a cutinase from *Fusarium solani pisi* in our study. Even though they were selected for their attractive properties, the enzymes used in the presented study have to the best of our knowledge not been applied for esterification of arabinoxylans. Klibanov and co-workers were pioneers in the field of using enzymes in non-aqueous media (Ikeda and Klibanov, 1993; Klibanov, 1989; Patel et al., 1996; Zaks and Klibanov, 1988). While the presence of water is essential in the hydrolysis reaction, esterification on various substrates has been demonstrated using esterases and lipases in non-aqueous media (Chen et al., 2006; Gremos et al., 2011; Kirk et al., 1995; Ljunger et al., 1994; Micaelo et al., 2005; Patel et al., 1996). Enzymatic acylation of polysaccharide derivatives in organic media was also an important landmark in the field, showing activity on not only monomer or oligomer units (Sereti et al., 1998, 2001). Native or unmodified polysaccharides remained beyond the scope of enzymatic functionalization until the last few years (Gremos et al., 2011; Matama et al., 2009). A significant breakthrough was achieved when Gremos et al., 2011 succeeded in performing acylation on cellulose with lipases and a cutinase with different esterifying reagents.

This study is the first report on enzymatic surface esterification of hemicellulose films. The work had two major goals. One of the aims of the project was to substitute the surface of rye arabinoxylan (AX) films in a heterogeneous reaction with a short acyl chain (acetyl) and a long acyl chain (stearate) and to characterize both with respect to chemical modification and possible changes in material properties. The second goal was to investigate and compare the activity of different lipases and a cutinase toward different alkyl chain lengths in esterification reactions of hemicelluloses. By modifying only the surface in a heterogeneous reaction, the reported good oxygen barrier properties of the bulk can be maintained while increasing the hydrophobicity of the film surface (Groendahl et al., 2006; Grondahl et al., 2003; Hoije et al., 2008; Stepan et al., 2012). Electron spectroscopy for chemical analysis (ESCA)/X-ray photoelectron spectroscopy (XPS) provided information about surface acetylation and steatation levels, after which samples (taken after three days of enzymatic surface treatment) were chosen for further analysis. To observe changes on a thinner outer layer of the surfaces, time of flight secondary ion spectroscopy (TOF-SIMS) and Fourier transform infrared (FT-IR) spectroscopy were used. Static contact angle measurements provided

information on the hydrophilic and hydrophobic properties of the film surfaces.

2. Materials and methods

2.1. Materials

Rye arabinoxylan (AX) was purchased from Megazyme (Ireland) (~95% purity, LOT 20601a). Deionized water was used to dissolve and cast the AX films to be treated. The reagents for the enzymatic surface modifications were purchased from Sigma-Aldrich: vinyl-stearate (95% purity, 43362-08) and vinyl-acetate (V1503). The lipases were also purchased from Sigma-Aldrich: lipase from *C. rugosa* (L1754), lipase from *R. oryzae* (86012), lipase from *Mucor miehei* (L9031) and lipase from *M. javanicus* (L8906). Cutinase from *F. solani pisi* was produced as described earlier (Araújo et al., 2007). Hexane (208752) and methanol (32213N) were used to wash the samples after the enzyme treatments.

2.2. Methods

2.2.1. Film preparation

1 g of rye AX was dissolved in deionized water (40 ml). The solution was used to cast films on polystyrene petri dishes. The films were dried in room temperature in a fume hood. The films were freed from the petri dish and cut into 1 cm-wide strips, of which a 2 cm-long portion was hung in the treatment solution. Thus each strip yielded a treated film surface of 4 cm² for each test specimen.

2.2.2. Enzymatic surface acylation

The enzyme loading was as follows: 10 mg of dry enzyme (cutinase or lipase) was suspended in 10 ml of reagent (vinyl acetate or vinyl stearate). A strip of (~80 μm thick) film with a total surface of 4 cm² (see above) was kept suspended in the reaction mixture under continuous stirring at 300 rpm and 40 °C in closed systems to avoid evaporation of the reagents. Separate batches were run for 3 h, 6 h, 12 h, 24 h, 72 h, 96 h and 168 h. The samples were then washed several times with methanol and hexane, left to dry in fume hood for 5 min and stored, covered until surface analysis. Blank samples were treated in pure vinyl acetate (VAc blank) or vinyl stearate (VSt blank) without enzymes for 3 and 7 days. Untreated AX film is also discussed as untreated blank (AX blank), to serve as double blank for both treated blank studies.

2.2.3. Time-of-flight secondary ion mass spectrometry (TOF-SIMS)

ToF.SIMS5 from ION-TOF was used and controlled by the Surfacelab 6.1 software for analysis of the outermost one to two monolayers of the modified films. The primary ions used were Bi³⁺ ions, and the distance to the sample surface was 2 mm with a primary beam angle of 45° with respect to the surface. The area analyzed was selected manually (~100 μm × 100 μm), and the spectra were recorded for 50 s in high current bunched mode. A flood gun was used for charge compensation. Measurements were made in both positive and negative modes.

2.2.4. Fourier transform infrared spectroscopy (FT-IR)

The surface of the AX films was scraped off with a scalpel, and this powder was mixed with KBr to press tablets. The samples were analyzed with a System 2000 FT-IR from Perkin Elmer controlled by Spectrum software version 5.3 in transmission mode using a triglycine sulphate (TGS) detector. Twenty scans were taken with a resolution of 4 cm⁻¹. Samples were also run in reflection absorption spectra (RAS) mode on a Spectratech FT-80 with a liquid nitrogen-cooled mercury cadmium telluride (MCT) detector. In this mode, 200 scans were taken with a resolution of 4 cm⁻¹. The

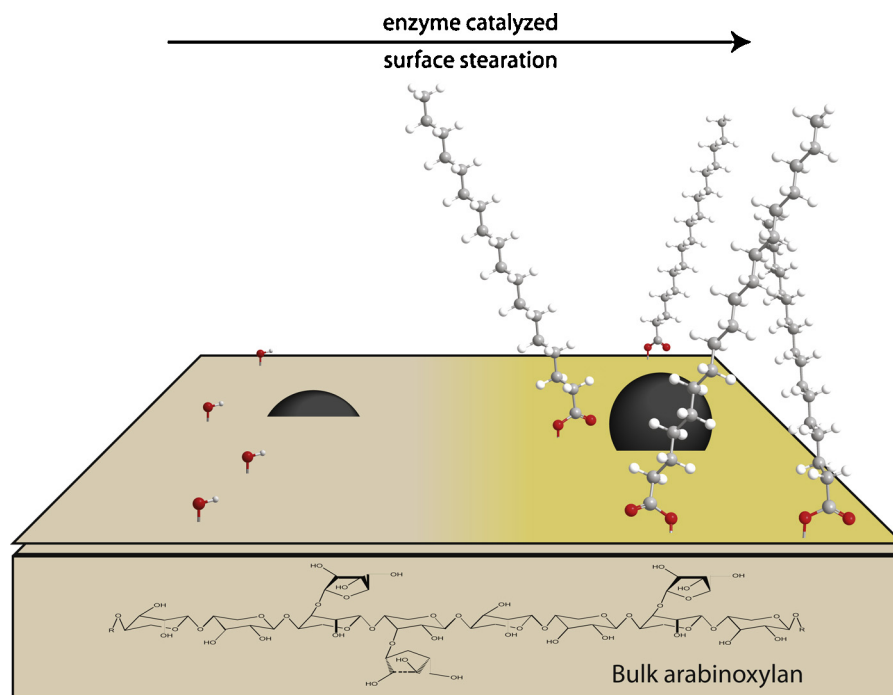


Fig. 1. Scheme of surface acetylation of AX film with vinyl acetate catalyzed by an enzyme. The hydroxyl groups on the surface are available for enzymatic esterification, while the hydroxyl groups in the bulk of the material are not available.

incident angle was near grazing angle (85°) with non polarized IR. The background was run on a quartz crystal microbalance (QCM) (from Qsense, QSX301) gold crystal. For the RAS mode, the samples were prepared as follows: AX films were drop-casted from 2.5% deionized water solution onto QCM gold crystals and, after drying, they were enzymatically surface acylated. After washing these samples in the same way as the films described above, the films were allowed to dry and were analyzed in RAS mode.

2.2.5. Electron spectroscopy for chemical analysis (ESCA)/X-ray photoelectron spectroscopy (XPS)

Surface composition analysis was done on a Quantum 2000 scanning ESCA microprobe from Physical Electronics controlled by Multipack software. An Al $K\alpha$ (1486.6 eV) X-ray source was used, and the beam size was $100\ \mu\text{m}$. The area analyzed was selected manually ($\sim 500\ \mu\text{m} \times 500\ \mu\text{m}$), and the take-off angle was 45° with respect to the sample surface. The information depth is approximately 4–5 nm.

2.2.6. Contact angle

Advancing contact angle against water was measured in a static contact angle measuring mode on an Attention device, model Θ . Pictures were taken with a camera once every second for 30 s for each measurement. The collected data was analyzed by Attention Θ software. The highest initial contact angle is reported.

3. Results and discussion

3.1. Enzymatic surface esterification

In the present study, the enzymatic surface esterification of arabinosylyan was performed in the absence of water in order to favor the esterification direction of the reaction. Since the activities of the enzymes for the esterification reactions were unknown, the enzyme load was uniformly set to 10 mg of freeze-dried enzyme in each experiment. AX was insoluble in vinyl acetate or in vinyl stearate; this resulted in heterogeneous reaction systems, which

are ideal for surface modifications. The enzymatic surface acylation of an AX film is schematically illustrated in Fig. 1.

In a heterogeneous reaction, it is reasonable to believe that the enzyme will access more easily hydroxyl groups that are available on the surface of the film. Various analytical methods (ESCA, FT-IR, TOF-SIMS, CA) with different sensitivities and information depths on the analyzed sample surfaces were applied to confirm the successful acetylation or steartion. In the first screening analysis of the modified films, an optimal treatment time for further analysis was selected based on ESCA results. It was found that three days of reaction provided a set of samples that allowed the comparison of the different reactions. TOF-SIMS, FT-IR and contact angle measurements were consequently performed on the samples that were enzymatically surface treated for 3 days. Results from the ESCA are shown for the 3 and 7 day samples to present consistency of the experiments.

3.2. TOF-SIMS

The TOF-SIMS is a highly surface sensitive analytical method. The information depth is 1 nm, which allows a more accurate composition analysis of the outermost molecular layer. The method is not quantitative, but provides supporting data on surface composition. Spectra of the VSt blank for 3 days are presented with the sample which was treated for 3 days in vinyl steartion in the presence of *M. javanicus* (Fig. 2). The spectral evaluation was straightforward for the steartion samples, since a characteristic signal group of the steartion molecule and its fragments were identified on the positive scan at 329 mass units (Fig. 2a) and on the negative scan at 283 mass units (Fig. 2b and c). As TOF-SIMS was not useful for identifying acetylation, other methods were applied for those samples.

3.3. FT-IR

Three different FT-IR techniques were used for characterization of the surface modified samples: two types of transmission modes

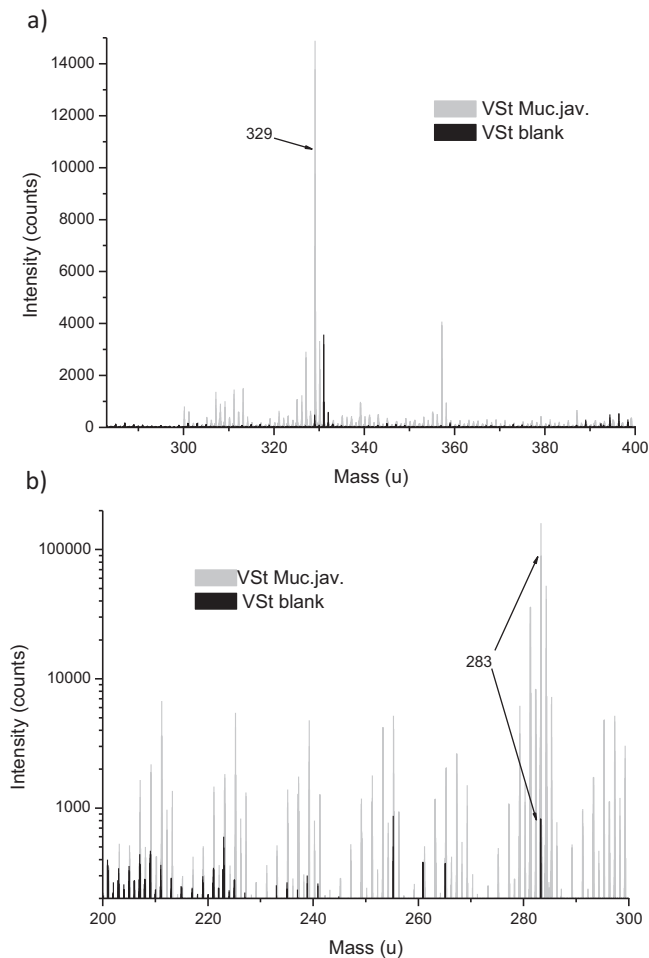


Fig. 2. (a) Positive TOF-SIMS scan of VSt blank film surface and enzymatically steared films (with *Mucor javanicus*) (300–400 mass units); (b) VSt blank film surface and enzymatically steared films (with *M. javanicus*) (200–300 mass units) shown on logarithmic y axis for better visualization of the VSt blank.

(one by preparing KBr tablets and one by using a free-standing thin film with a beam condenser) and a RAS mode. The KBr tablet contained the scratched off surface of the films, as well as some deeper unacylated molecular layers, resulting in a weak signal at the FT-IR (Fig. 3). Fig. 3a presents the untreated blank sample with the blank sample that was treated in vinyl acetate and the blank treated in vinyl stearate for 3 days. A signal at 1752 cm^{-1} indicates some carbonyl groups, but no relevant signal can be identified at 2918 cm^{-1} , which suggest no significant amount of acetyl groups on the surface. The VSt blank does not show characteristic signals of acylation compared to the AX blank. The spectra of the enzyme treated samples clearly show signals originating from the carbonyl group of the ester bonds appearing for all treated samples at 1752 cm^{-1} and from the alkyl chains at 2918 cm^{-1} also compared to their respective blanks (Fig. 3b and c). Using thin free films, very similar data were obtained for the method using KBr tablets (not shown). The reflection absorption spectra of the samples showed very clear and distinct peaks for the carbonyl (1752 cm^{-1}) and the alkyl groups (2918 cm^{-1}) (Fig. 3b). These were significantly more pronounced than for the technique with KBr tablets. The results of both FT-IR techniques demonstrate clear proof of surface acylation of the films.

3.4. ESCA/XPS

The ESCA spectra provide elemental surface composition of samples, and high resolution spectra also give information on the

chemical bonds between the elements. The peaks from right to left correspond to carbon bound only to carbon and hydrogen (C1), carbon with one bond to oxygen (C2), carbon with two bonds to oxygen(s) (C3) and carbon with three bonds to oxygens (C4) (Groendahl et al., 2006).

There are many signs of the presence of residual proteins in the pure starting material. The atomic concentration table and the survey spectrum showed nitrogen content of 4.1% in the sample. Annual plants, such as rye, have a significant amount of proteins in the cell walls, which means amino acids can probably be detected in hemicellulose extracts that are not 100% pure (Albersheim et al., 2011). That the oxygen to carbon ratio is 0.57 instead of 0.8 also indicates the presence of non-polysaccharide components in the sample. The spectrum of pure AX should not contain C1 and C4 peaks, but the traces of proteins and non polysaccharide residues together could give an explanation for the presence of both the C1 peak and the C4 peak. Without the presence of protein in the AX, the acylation of the samples could be determined by the appearance of C1 (the C–C bonds of the alkyl chains) and C4 (showing the presence of carboxylate groups) in the esterified samples. Full esterification would be indicated by the ratio of the areas of C4/C3 peaks being two for pure samples. Hence the existence of the C1 and C4 peaks in the blank AX sample, the relative increase of the C1 peak compared to the other peaks will indicate the new, non-protein originated, C–C bonds from the alkyl chains; and the increased ratio of the C4/C3 peaks will indicate new carbonyl groups from the ester bond on the surface.

Due to the presence of non-polysaccharide components, no quantitative conclusion could be made from C4/C3. Furthermore, the ESCA information depth is 4–5 nm, which is deeper than the thickness of the outermost molecular layer, and the information collected therefore also corresponds to molecular layers under the surface that were not accessible to the enzymes. Thus, in this case, the method is not surface sensitive enough to determine reaction yields or degrees of substitution, but changes on the surface can be followed.

The ESCA high resolution carbon peak of the AX blank can be seen in Fig. 4a as serving as a double blank for both of the treated blank samples. The treated blank samples were treated in pure vinyl acetate or vinyl stearate without enzymes for three days, Fig. 4b and d, respectively. These blanks were evaluated for adsorption of the reagent. The VAc blanks represented some acetate deposition on the surface based on the increase of the C4/C3 ratio (Table 1a). The VSt blanks showed a higher C1 peak as compared to the AX blank samples, but no difference was seen in the C4/C3 ratio. This means there may be some adsorbed stearate on the surface but that no ester bonds were formed (Table 1b). This can be caused by the fact that the vinyl stearate is solid in room temperature and, even though washing of the samples at the end of the treatment was done with warm methanol and hexane, there is a chance of some stearate solidifying on the surface of the films. The enzyme treated samples were therefore compared to their respective treated blanks.

The ESCA spectrum of an AX film after 3 days of acetylation with cutinase from *F. solani pisi* (Fig. 4d) and after 3 days of stearamation with lipase from *M. javanicus* (Fig. 4e) showed successful acylation with both an increased C1 signal and an increased ratio of C4/C3 compared to their respective treated blanks. The other two lipases (from *C. rugosa* and *R. oryzae*) showed activity similar to that of the lipase presented in Fig. 4e. The measurements from the samples treated for 7 days were in good agreement with the ones treated for 3 days, detecting similar or slightly increased levels of esterification (Table 1). The lower nitrogen content of the successfully enzyme treated samples confirms that no enzyme is adsorbed to the surface of the film. The amount of nitrogen detected by ESCA is lower than for the AX blank, since the composition of the detected top 4–5 nm on the surface should have increased oxygen and

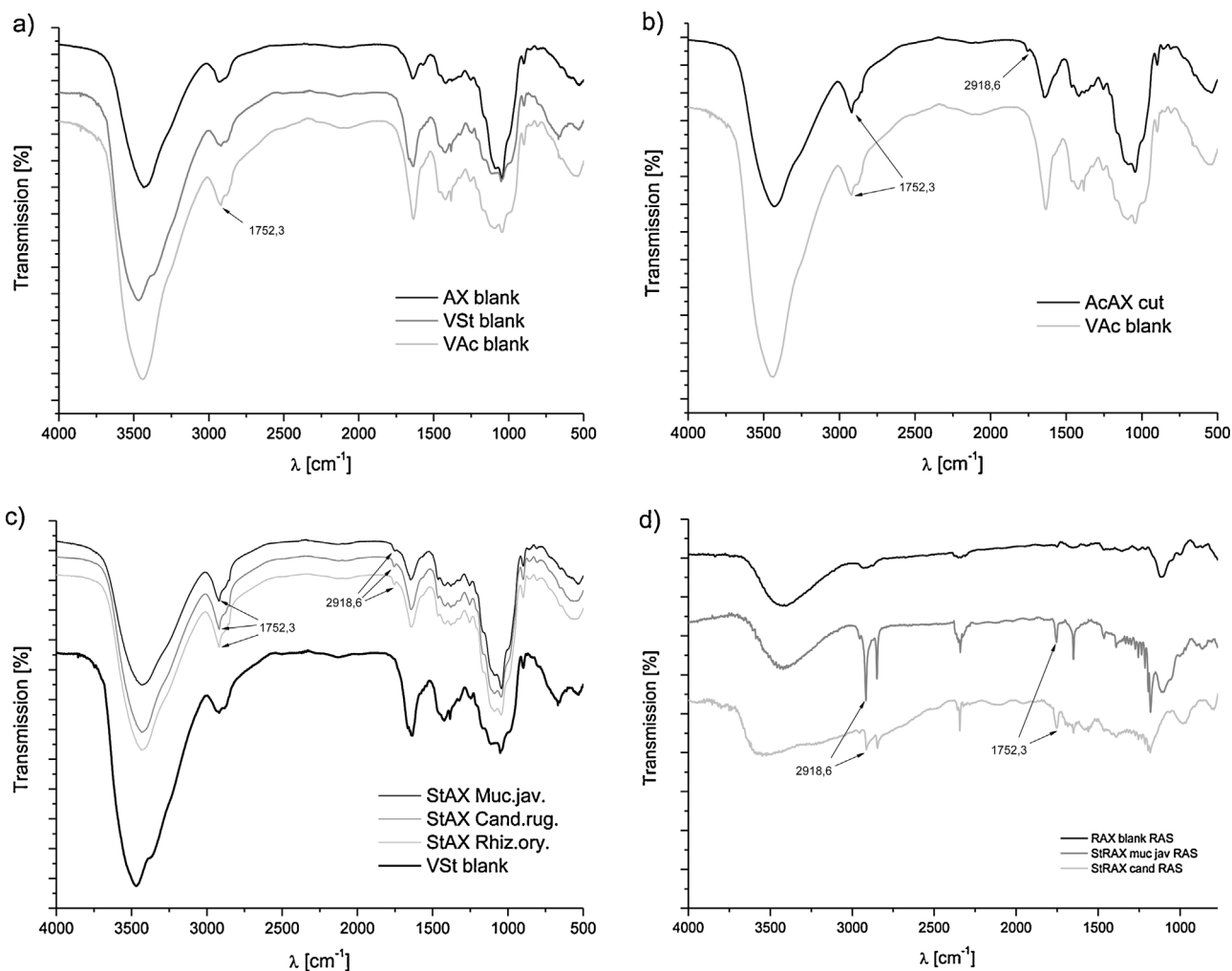


Fig. 3. FT-IR spectra of (a) AX blank with VAc blank and VSt blank. Transmission measured using KBr tablets. (b) VAc blank with enzymatically surface acetylated AX films with cutinase from *Fusarium solani pisi*. (c) VSt blank with enzymatically surface steared AX films with lipase (from *Mucor javanicus*, *Candida rugosa* and *Rhizopus oryzae*). (d) FT-IR spectra of AX blank with enzymatically steared AX films (lipase from *Mucor javanicus* and *Candida rugosa*) run in RAS mode.

carbon after acylation, covering some nitrogen, thus decreasing the detected proportion of it. This was confirmed in all successfully surface acylated samples compared to any blank sample. The detected ester bonds and C–C bonds are therefore from the acetates and stearates esterified on the surface of the film.

Often, esterases are distinguished from lipases by their higher activity for hydrolyzing short alkyl chain esters than for hydrolyzing long alkyl chain esters (in aqueous media). In this study it was expected to see similar activities in the reverse reaction in non aqueous media. We ran separate experiments with all the enzymes and with both short (vinyl acetate) and long chain (vinyl stearate)

Table 1
(a) Comparison of lipase and cutinase activity on vinyl acetate as substrate; (b) comparison of lipase and cutinase activity on vinyl stearate as substrate; based on the C4/C3 ratio from ESCA (where the C4/C3 for the untreated blank is 0.13).

(a)			
Enzyme	Enzyme origin	Acetylation 3 days	Acetylation 7 days
Lipase from <i>Mucor javanicus</i>	(Sigma) L8906	0.13	0.23
Lipase from <i>Candida rugosa</i>	(Sigma) L1754	0.12	0.23
Lipase from <i>Rhizopus oryzae</i>	(Sigma) 86012	0.22	0.22
Cutinase from <i>Fusarium solani pisi</i>	Research enzyme	0.80	0.80
Respective treated VAc blank	None	0.21	0.26
(b)			
Enzyme	Enzyme origin	Stearation 3 days	Stearation 7 days
Lipase from <i>Mucor javanicus</i>	(Sigma) L1754	0.22	0.34
Lipase from <i>Candida rugosa</i>	(Sigma) 86012	0.18	0.34
Lipase from <i>Rhizopus oryzae</i>	(Sigma) L9031	0.32	0.30
Cutinase from <i>Fusarium solani pisi</i>	Research enzyme	0.21	n.a.
Respective treated VSt blank	None	0.12	0.14

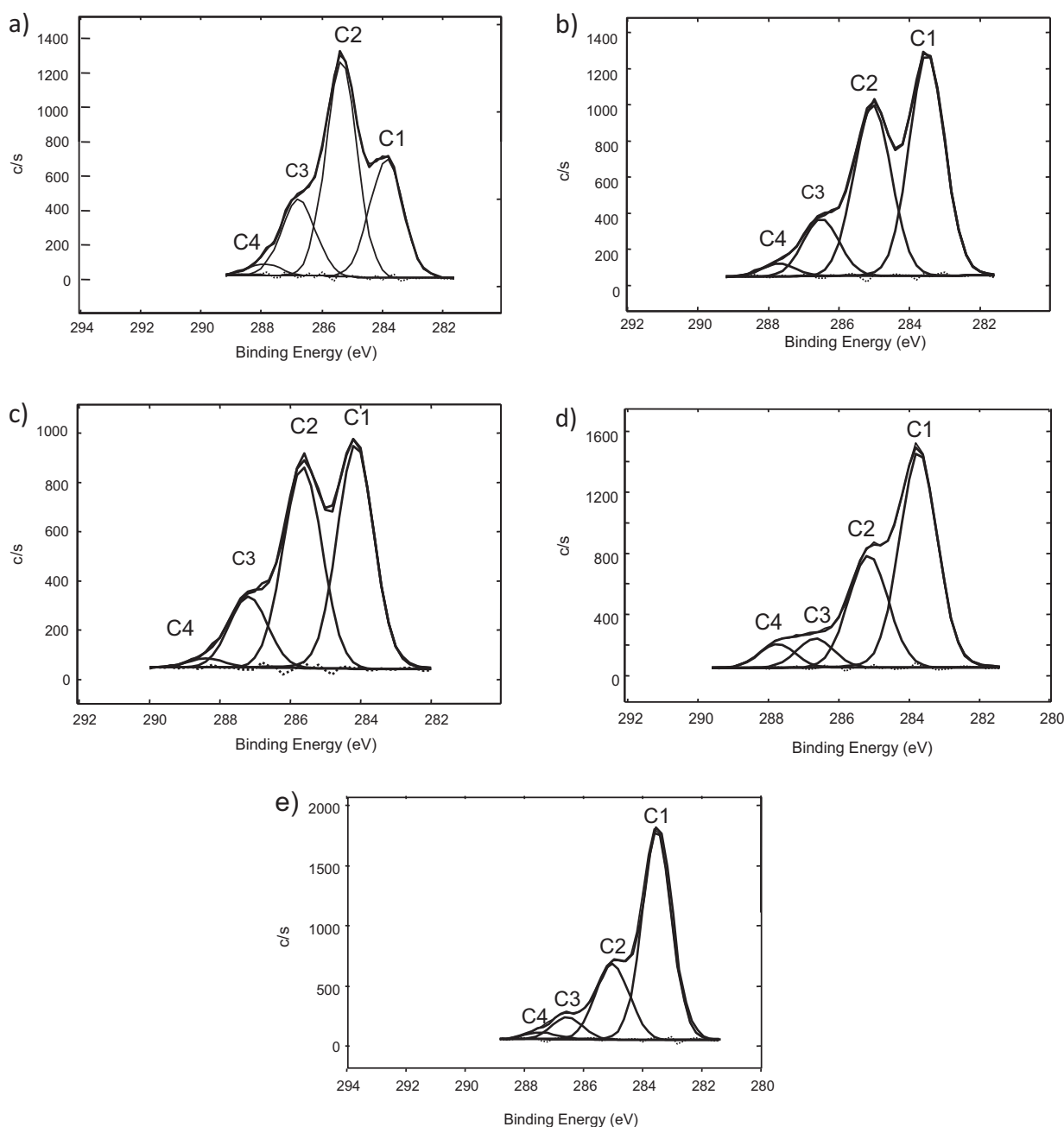


Fig. 4. ESCA spectra of rye AX films (a) untreated blank film (AX blank); (b) film treated with vinyl acetate for 3 days with no enzyme (VAc blank); (c) film treated with vinyl stearate for 3 days with no enzyme (VSt blank); (d) surface steared film with lipase from *Mucor javanicus* for 3 days; (e) surface acetylated film with cutinase from *Fusarium solani pisi* for 3 days.

substrates, and our findings on activity differences of lipases and cutinases toward different substrates are summarized in Table 1.

A general conclusion is that lipases work efficiently for stearament, whereas cutinase is more efficient for acetylation but also showed activity when vinyl stearate was used as reagent for stearament. None of the lipases from *M. javanicus* and *R. oryzae* showed activity on AX films when vinyl acetate was used for acetylation. A significant conclusion is that the cutinase resulted in mentionably higher substitution levels of acetylation than the lipases for stearament (based on the increase in C4/C3 ratios). The reason for this might be that, in most reports, cutinase has no hydrophobic lid, and the enzyme does not require a water–lipid interface for its activity in general, while the lipases used in the

experiments all had the hydrophobic lid structure (Martinez et al., 1992; Maruyama et al., 2003). It is important to note for the commercially available lipase from *C. rugosa* that it is a mix of isoenzymes with different specificities (Akoh et al., 2004). It has also been reported that the reproducibility of reactions is not satisfactory with *C. rugosa* lipase due to the variable proportion of isoforms, amount of water in the crude lyophilized lipase and the amount of lipolytic protein present in the powdered enzyme (Dominguez and Sinisterra, 1999). Interestingly, this enzyme had lower activity and a notable deviation between repeated batches compared to the other lipases. This limits the application of the crude lipase in the reproducible production of specific compounds with high purity.

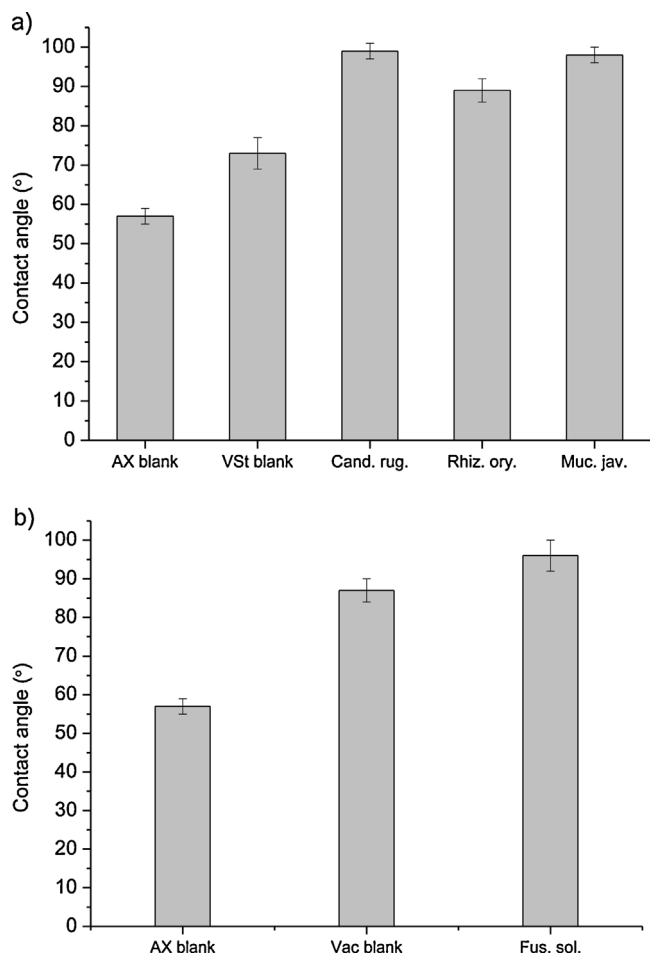


Fig. 5. Contact angle against water of (a) enzymatically surface steared and (b) enzymatically surface acetylated AX films.

3.5. Contact angle

Xylan films are hydrophilic, while their acetylated derivatives are less hydrophilic (Escalante et al., 2011; Grondahl et al., 2003; Stepan et al., 2012). The untreated blank AX films have an advancing contact angle of 57°, which increased to 87° for the VAc blank and to 73° for the VSt blank samples. The ESCA, ToF-SIMS and FT-IR did not detect stearate esters on the surface of the VSt blank. However it was also noted that traces of deposition on the surface are possible, which could also have a strong influence on the measured contact angle despite them not being covalently bound and at low quantities. For the VAc blank, the ESCA showed signs of ester bonds, thus the contact angle increase is not a surprise for the treated blank. The contact angle measurement resulted in an increased advancing contact angle for all enzymatically surface modified films compared to their treated respective blanks. The averages and standard deviations of the results are shown in Fig. 5. After about 20–30 s, the contact angle started to decrease significantly for all samples and the films dissolved with time under the droplet. A possible explanation is that a very thin monolayer of acyl substitution was achieved, and a water induced molecular rearrangement of the surface occurred (Andrade et al., 1983). The bulk of the film is still rich in hydroxyl groups, which are obviously more hydrophilic. Due to the molecular mobility of the surface, the more hydrophilic hydroxyl groups can turn toward the surface, come into contact with the aqueous layer and become hydrated, thus slowly allowing water to penetrate the whole film. Since the acylation of the surface of the films does not affect the bulk

material, the good oxygen barrier properties, which are characteristic for non-acetylated xylans, should be retained (Escalante et al., 2011; Hoije et al., 2008). The increased hydrophobicity of the surface could provide an opportunity to produce laminates with good adhesion to hydrophobic films (based on the chemical similarities of the surfaces).

4. Conclusions

Enzymatic surface esterification of arabinoxylan films was demonstrated for the first time. Different enzymes were evaluated for surface acetylation and steardation of rye arabinoxylan (AX) films. The reactants (vinyl acetate and vinyl stearate) also served as the reaction media, making the reaction a solvent free reaction. Surface analysis showed that lipases from *M. javanicus*, *R. oryzae* and *C. rugosa* successfully surface steardated, and a cutinase from *F. solani pisi* surface acetylated the AX films after 3 days of reaction time.

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References

- Akoh, C.C., Lee, G.-C., Shaw, J.-F., 2004. Protein engineering and applications of *Candida rugosa* lipase isoforms. *Lipids* 39, 513–526.
- Albersheim, P., Darvill, A., Roberts, K., Sederoff, R., Staehelin, A., 2011. *Plant Cell Walls*. Garland Science. Taylor & Francis Group, LLC, New York, USA/Abingdon, UK.
- Andrade, J.D., Gregonis, D.E., Smith, L.M., 1983. Polymer–water interface dynamics. In: Mittal, K.L. (Ed.), *Physico-Chemical Aspects of Polymer Surfaces*. Plenum, New York, pp. 911–922.
- Araújo, R., Silva, C., O'Neill, A., Micaelo, N., Guebitz, G., Soares, C.u.M., Casal, M., Cavaco-Paulo, A., 2007. Tailoring cutinase activity towards polyethylene terephthalate and polyamide 6,6 fibers. *Journal of Biotechnology* 128, 849–857.
- Bengtsson, M., Koch, K., Gatenholm, P., 2003. Surface octanoylation of high-amylose potato starch films. *Carbohydrate Polymers* 54, 1–11.
- Chen, Z.-G., Zong, M.-H., Li, G.-J., 2006. Lipase-catalyzed acylation of konjac glucomannan in ionic liquids. *Journal of Chemical Technology and Biotechnology* 81, 1225–1231.
- Dominguez, D.M.P., Sinisterra, G.J.V., 1999. Causes of unreproducibility of *C. rugosa* lipase-catalyzed reactions in slightly hydrated organic media. *Tetrahedron* 55, 8555–8566.
- Ebringerova, A., Heinze, T., 2000. Xylan and xylan derivatives – biopolymers with valuable properties. 1. Naturally occurring xylans structures, isolation procedures and properties. *Macromolecular Rapid Communications* 21, 542–556.
- Escalante, A., Goncalves, A., Bodin, A., Stepan, A., Sandstroem, C., Toriz, G., Gatenholm, P., 2011. Flexible oxygen barrier films from spruce xylan. *Carbohydrate Polymers* 87, 2381–2387.
- Gremos, S., Zarafeta, D., Kekos, D., Kolisis, F., 2011. Direct enzymatic acylation of cellulose pretreated in BMIMCl ionic liquid. *Bioresource Technology* 102, 1378–1382.
- Groendahl, M., Gustafsson, A., Gatenholm, P., 2006. Gas-phase surface fluorination of arabinoxylan films. *Macromolecules* 39, 2718–2721.
- Grondahl, M., Eriksson, L., Gatenholm, P., 2004. Material properties of plasticized hardwood xylans for potential application as oxygen barrier films. *Biomacromolecules* 5, 1528–1535.
- Grondahl, M., Teleman, A., Gatenholm, P., 2003. Effect of acetylation on the material properties of glucuronoxylan from aspen wood. *Carbohydrate Polymers* 52, 359–366.
- Guebitz, G.M., Cavaco-Paulo, A., 2009. Enzymes go big: surface hydrolysis and functionalization of synthetic polymers. *American Chemical Society, POLY-175*.
- Hoije, A., Sternemalm, E., Heikkinen, S., Tenkanen, M., Gatenholm, P., 2008. Material properties of films from enzymatically tailored arabinoxylans. *Biomacromolecules* 9, 2042–2047.
- Ikeda, I., Klibanov, A.M., 1993. Lipase-catalyzed acylation of sugars solubilized in hydrophobic solvents by complexation. *Biotechnology and Bioengineering* 42, 788–791.
- Kirk, O., Christensen, M.W., Beck, F., Damhus, T., 1995. Lipase-catalyzed regioselective acylation and deacylation of glucose derivatives. *Biocatalysis and Biotransformation* 12, 91–97.

- Klibanov, A.M., 1989. Enzymatic catalysis in anhydrous organic solvents. *Trends in Biochemical Sciences* 14, 141–144.
- Kontkanen, H., Tenkanen, M., Fagerstrom, R., Reinikainen, T., 2004. Characterisation of steryl esterase activities in commercial lipase preparations. *Journal of Biotechnology* 108, 51–59.
- Ljunger, G., Adlercreutz, P., Mattiasson, B., 1994. Lipase catalyzed acylation of glucose. *Biotechnology Letters* 16, 1167–1172.
- Martinez, C., De Geus, P., Lauwereys, M., Matthyssens, G., Cambillau, C., 1992. Fusarium solani cutinase is a lipolytic enzyme with a catalytic serine accessible to solvent. *Nature* 356, 615–618.
- Maruyama, T., Nakajima, M., Kondo, H., Kawasaki, K., Seki, M., Goto, M., 2003. Can lipases hydrolyze a peptide bond? *Enzymes and Microbial Technology* 32, 655–657.
- Matama, T., Araujo, R., Gubitz, G.M., Casal, M., Cavaco-Paulo, A., 2009. Functionalization of cellulose acetate fibers with engineered cutinases. *Biotechnology Progress* 26, 636–643.
- Micaelo, N.M., Teixeira, V.H., Baptista, A.M., Soares, C.M., 2005. Water dependent properties of cutinase in nonaqueous solvents: a computational study of enantioselectivity. *Biophysical Journal* 89, 999–1008.
- Patel, M.T., Nagarajan, R., Kilara, A., 1996. Lipase-catalyzed biochemical reactions in novel media. A review. *Chemical Engineering Communications* 152/153, 365–404.
- Pio, T.F., Macedo, G.A., 2009. Cutinases: properties and industrial applications. *Advances in Applied Microbiology* 66, 77–95.
- Poulsen, K.R., Snabe, T., Petersen, E.I., Fojan, P., Neves-Petersen, M.T., Wimmer, R., Petersen, S.B., 2005. Quantization of pH: evidence for acidic activity of triglyceride lipases. *Biochemistry* 44, 11574–11580.
- Sakai, S., Antoku, K., Yamaguchi, T., Kawakami, K., 2008. Transesterification by lipase entrapped in electrospun poly(vinyl alcohol) fibers and its application to a flow-through reactor. *Journal of Bioscience and Bioengineering* 105, 687–689.
- Sereti, V., Stamatis, H., Koukios, E., Kolisis, F.N., 1998. Enzymic acylation of cellulose acetate in organic media. *Journal of Biotechnology* 66, 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, 495–500.
- Stepan, A.M., Hoeije, A., Schols, H.A., de, W.P., Gatenholm, P., 2012. Arabinose content of arabinoxylans contributes to flexibility of acetylated arabinoxylan films. *Journal of Applied Polymer Science* (ahead of print).
- Sun, R., Fang, J.M., Tomkinson, J., Jones, G.L., 1999. Acetylation of wheat straw hemicelluloses in N,N-dimethylacetamide/LiCl solvent system. *Industrial Crops and Products* 10, 209–218.
- Sun, R., Sun, X.F., Tomkinson, J., 2004. Hemicelluloses and their derivatives. *ACS Symposium Series* 864, 2–22.
- Timell, T.E., 1967. Recent progress in the chemistry of wood hemicelluloses. *Wood Science and Technology* 1, 45–70.
- Tombs, M., Harding, S., 1997. *An Introduction to Polysaccharide Biotechnology*. Taylor & Francis, London, UK/Bristol PA, USA.
- Utsugi, A., Kanda, A., Hara, S., 2009. Lipase specificity in the transacylation of triacylglycerin. *Journal of Oleo Science* 58, 123–132.
- Woudenberg-van, O.M., van, R.F., Sheldon, R.A., 1996. Regioselective acylation of disaccharides in tert-butyl alcohol catalyzed by Candida antarctica lipase. *Biotechnology and Bioengineering* 49, 328–333.
- Zaks, A., Klibanov, A.M., 1988. Enzymatic catalysis in nonaqueous solvents. *Journal of Biological Chemistry* 263, 3194–3201.