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Synthesis and properties of fatty acid starch esters

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ABSTRACT

Being completely bio-based, fatty acid starch esters (FASEs) are attractive materials that represent an alternative to crude oil-based plastics. In this study, two synthesis methods were compared in terms of their efficiency, toxicity and, especially, product solubility with starch laurate (C_{12}) as model compound. Laurates (DS > 2) were obtained through transesterification of fatty acid vinylesters in DMSO or reaction with fatty acid chlorides in pyridine. The latter lead to higher DS-values in a shorter reaction time. But due to the much better solubility of the products compared to lauroyl chloride esterified ones, vinylester-transesterification was preferred to optimize reaction parameters, where reaction time could be shortened to 2 h. FASEs C_6–C_{18} were also successfully prepared via transesterification. To determine the DS of the resulting starch laurates, the efficient ATR-IR method was compared with common methods (elementary analysis, ^{1}H NMR). Molar masses (M_w) of the highly soluble starch laurates were analyzed using SEC-MALLS (THF). High recovery rates (>80%) attest to the outstanding solubility of products obtained through transesterification, caused by a slight disintegration during synthesis. Particle size distributions (DLS) demonstrated stable dissolutions in $CHCl_3$ of vinyl laurate esterified – contrary to lauroyl chloride esterified starch. For all highly soluble FASEs (C_6–C_{18}), formation of concentrated solutions (10 wt%) is feasible.

1. Introduction

Meeting the growing need for plastic products is a challenge that must be met now and in the future. At present, this demand is mainly satisfied by petroleum-based materials, however a steadily increasing oil price and oil shortages prompt the need for alternatives that are based on renewable resources. Starch has the capability of becoming a serious alternative polymer since it is easy to extract, abundant and renewable. Its drawbacks when processed and used as a material are that it requires low-molecular weight plasticizers because of its strong brittleness. This results in an unfavorable aging of the material during storage and application. Another drawback is its distinct hydrophilicity. These properties strongly limit the application fields of native starch. One industrial solution is to produce starch blends with natural or synthetic polyesters to achieve the properties needed for use as packaging materials. One interesting alternative is the development of hydrophobic starch which has thermoplastic properties. Early studies, whose aim was to find applications for modified starch, dealt with the esterification of carbohydrate polymers using acetic or propionic anhydride (Mark & Mehltretter, 1972; Schützenberger, 1865). Even if a significant hydrophobicity was achieved, plasticizers were still indispensable during material processing due to the brittleness of the product materials.

Starch esters with saturated medium and long chain fatty acids are a promising method for generating sustainable, bio-based polymeric materials. FASEs are highly hydrophobic, consist entirely of naturally based materials and exhibit an internal plasticizing effect caused by the long chain ester groups. In initial synthetic approaches to produce highly substituted FASEs, fatty acid chlorides were used with chinolin as a base, however the resulting DS was not determined (Karrer & Zega, 1923). The chloride-esterification approach was later modified by using pyridine (Aburto et al., 1999) or imidazole (Liebert et al., 2011) as an alternative reaction media. Initial systematic studies on the thermal and mechanical properties of a homologous series of fatty acid amylose esters dealt with films by casting chloroform-solutions of fatty acid amylose esters (Gros & Feuge, 1962) as well as molded starch ester samples (Wolff, Olds, & Hilbert, 1951) without applying plasticizers. The properties of native starch were completely altered to create hydrophobic, film-forming, thermoplastic materials. Though initial steps toward processing were made, its suitability for industry-oriented material manufacturing still had to be investigated. Further studies on the thermoplastic property

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of FASEs in later years revealed similar, but in some cases also contradictory, trends which are an indication of how strongly starch is impacted by its structure (Aburto et al., 1999; Funke & Lindhauer, 1994; Sagar & Merrill, 1995).

Due to the high sensitivity of the fatty acid chlorides to traces of water and impurities, alternative approaches for starch esterification have been examined. One promising method was the base-catalyzed transesterification of starch with carboxylic acid vinyl esters (Tanaka, 1998). This method was applied by Dicke and Klemm using vinylacetate and a low molecular weight salt in DMSO to obtain regioselectively acetylated starch at the C-2 position in a one-step reaction (Dicke, 2004; Dicke & Klemm, 2000). Using K_2CO_3 as a catalyst, the C-2 position was preferred even in the case of a total DS of 2.18. Although the detailed mechanism was not enlightened, the type of catalyst seemed to play an important role. This method was also applied by Mormann for acetylating starch in water or DMSO and further enabled a successful lauroylation of starch in DMSO (DS 2.3) using Na_2HPO_4 as a catalyst (Mormann & Al-Higari, 2004). In following studies, different catalysts for starch esterification using vinyl laurate and -stearate as reagents were made (Junistia et al., 2008, 2009). However, the system has not yet been applied toward a complete series of ester groups (C_6–C_{18}). The method was further applied to esterify cellulose, for example (Heinze et al., 2000). The use of the starch solvent DMSO enabled a homogeneous esterification process, but studies on the real solution state of amylose and amylopectin were lacking. Furthermore, the reaction time of 24 h for starch esterification (Junistia et al., 2008; Mormann & Al-Higari, 2004) is unfavorably long. Even if the change of the starch structure during synthesis seems to have a strong influence on the product properties, a comparison of both methods, chloride esterification and vinyl ester transesterification, has yet to be made. As a further strategy for a homogenous esterification of starch in DMSO, the in situ activation of pure fatty acids using carbonyldiimidazol (Grote & Heinze, 2005; Tessler, 1973) or carbodiimides was mentioned but resulted in DS values no higher than 2.

The current study first gives a comparison of the reaction parameters and product properties for the two main methods of FASE synthesis: chloride and vinyl ester esterification, with starch laurate being used as a model compound. The aim was to work out the differences in the structure of the starch as well as reaction parameters depending on the method of synthesis. Though the transesterification method was superior in sustainability as well as in the solubility of the resulting FASEs, it was further optimized (e.g. reaction time). SEC-MALLS and DLS measurements were obtained to give an insight into the structural properties of the soluble products. The study furthermore introduced a very quick and simple quantitative evaluation of ATR-IR spectra as an alternative to common DS-determination methods, enabling a high number of samples to be passed quickly. Although using IR-spectroscopy is a common method for determining the DS of carbohydrates, to our knowledge, quantitative studies for fatty acid starch esters have been fairly applied. Elomaa et al. found a cubic polynomial correlation between ester band integral (1120–1251 cm^{-1}) and absolute DS of starch acetates (Elomaa et al., 2004). Forrest and Cove (1992) revealed a linear calibration function in determining the DS of hydroxypropylated starch by using the integral of CH-bands (2974 cm^{-1}). In summary, important steps were taken toward optimized synthesis and use of a broad range of fatty acid starch esters as bio-based materials in the current study.

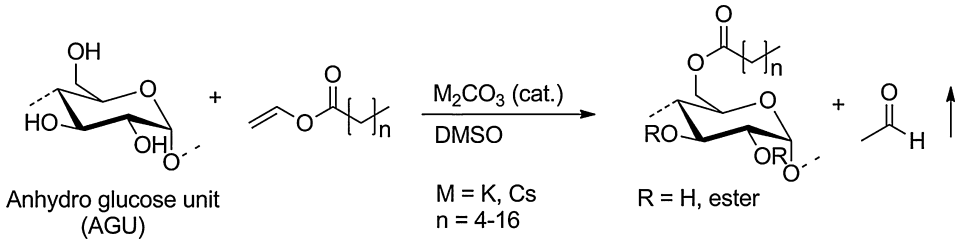
2. Materials and methods

2.1. Materials

Gelose 80, a maize starch with an amylose content of 80%, was supplied by Penford and dried under vacuum conditions before use in order to achieve a moisture content of <1.5%. All reagents were used as received: vinyl laurate (99%), vinyl stearate (95%), dimethyl sulfoxide (DMSO, 99.7%), K_2CO_3 (99%) and Na_2CO_3 (99.5%) were supplied by Sigma–Aldrich, Cs_2CO_3 by Alfa Aesar and pyridine (99.8%) by Prolabo. Lauric acid chloride (99%) was supplied by ABCR. Vinyl hexanoate, -octanonate, -decanoate (99%) and -palmitate (96%) were obtained from TCI Europe

2.2. Methods

2.2.1. Synthesizing FASEs through transesterification of the fatty acid vinyl esters

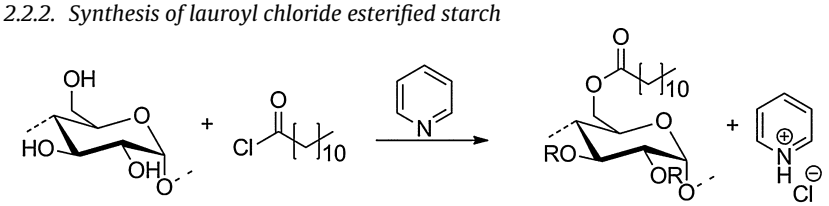


Anhydro glucose unit (AGU)

Initially, a clear and homogeneous dispersion of starch in DMSO (10%, w/w) was formed by stirring for 2 h at 95 °C. 40 g dispersion, containing 4.0 g starch (25 mmol AGU), were added with the desired amount of the fatty acid vinyl ester and heated to 110 °C. Then, the solid carbonate catalyst was added (3 mol% based on AGU) and the reaction mixture was stirred for 2 h at 110 °C. After cooling to room temperature, the mixture was added to 150 mL ethanol.[1] The brownish raw product was dissolved in chloroform or THF and precipitated by being added to ethanol one drop at a time.[1] After subsequent washing steps with ethanol,[1] the product was dried in vacuum (85 °C, 16 h) to obtain a moisture content of <1%, which was determined using a Sartorius moisture analyzer based on weight loss (15 min, 130 °C).

[1] In the case of starch hexanoate, an ethanol/water mixture (5:1, w/w) was applied instead of ethanol.

2.2.2. Synthesis of lauroyl chloride esterified starch



4.0 g of starch (25 mmol AGU) were suspended in 40 mL of pyridine at room temperature. Then, lauroyl chloride (75 mmol, 18 mL) was added a drop at a time. Subsequently, the reaction vessel was stirred for 1 h at 110 °C. After cooling, the processing was equivalent to that which was described for the transesterification of vinyl esters.

2.3. Characterization

2.3.1. Determining DS

2.3.1.1. Elementary analysis. Measurements were performed using the combustion technique on a FlashEA 1112 CHNS/O automatic elemental analyzer with 2 autosamplers MAS200R (Thermo Scientific). The software ChemBioDraw Ultra 12.0 (CambridgeSoft) was used in the evaluation. The best fit for C, H, O in DS-increments of 0.03 gave the desired DS.

2.3.1.2. NMR spectroscopy. The NMR spectra were measured on a Varian Unity Inova 500 spectrometer. The ^{1}H NMR spectra were recorded at a spectrometer frequency of 500 MHz at 25 °C using DMSO-d_6, $CDCl_3$ or THF-d_8 as solvents. The chemical shifts were related to the solvent signals, which in turn were related to TMS (δ = 0.0 ppm). In order to determine the DS, the distinguishable signals of the fatty acid (e.g. methyl group and methylene groups) were used to create an average value. This value was related to the single starch proton at 5.6 ppm or a group of 3 starch protons (4.5–6.0 ppm).

To obtain the substitution pattern, ^{13}C NMR spectra were measured at 60 °C in DMSO-d_6 at a spectrometer frequency of 125 MHz. To allow a quantitative determination of the degree of substitution, the measurements were performed with a relaxation delay of 5 s under using the inverse gated decoupling technique. That means, the Nuclear Overhauser Effect which could falsify the spectral intensities was suppressed. The overall DS was determined from the mean value of the fatty acid signals in relation to the starch C-1 signals. The splitting of the C-1 signal into a signal at 100 ppm (unsubstituted C-2) and a signal at 95 ppm (substitution at C-2), and the splitting of the C-6 signal (only the unsubstituted isolated) gave the individual DS for a determination of the substitution pattern.

2.3.1.3. ATR-FTIR spectroscopy. Measurements were performed on a Varian Scimitar 2000 FT-IR. The samples were measured in their original solid form at room temperature. 16 scans were taken with a resolution of 4 cm^{-1} in a wavenumber range of 400–4000 cm^{-1}.

2.3.2. SEC-MALL spectroscopy

Size exclusion chromatography (SEC) with multi angle laser light scattering (MALLS) was used to determine the molar mass weight average M_w and the molar mass distribution *MMD*. Polymer molecules were separated on columns with porous gel according to their molecular hydrodynamic volume.

The SEC-MALLS system that was used to investigate native starch in DMSO consisted of a Waters 515 HPLC pump, 717 autosampler, a Waters in-line degasser DG2 and a Waters 2414 refractive index detector (RI). The column series PSS Suprema S30000, S1000 and S100 was used for sample fractionation. Elution of the starch sample was carried out using dimethyl sulfoxide (DMSO) containing 0.09 m $NaNO_3$ at a flow rate of 0.5 mL/min and a temperature of 70 °C. A Wyatt Dawn HELEOS MALLS detector (λ = 658 nm) was used to detect the scattering light intensity.

In order to prepare the starch ester samples, about 8 mg were dissolved in 4 mL THF (2 mg/mL) and stirred for 24 h. Before the measurement, the solutions were filtrated using 1 μm PTFE membranes. Subsequently, the starch ester samples were measured at 25 °C using an SEC-MALLS system which consisted of a Waters 2695 separation module, a DAWN DSP-F laser photometer (Wyatt, 488 nm), a dual λ absorbance detector 2487 (Waters) and a refractive index detector 2414 (Waters, 35 °C). The column system PLgel 10 μm precolumn, 3× Plgel 20 μm Mixed-A; 2000–40,000,000 and 1× Plgel 20 μm Mixed-A LS; 2000–40,000,000 (supplied by Polymer Laboratories) was used. A flow-rate of 1 mL/min was applied.

The evaluation was conducted with Astra-software (Wyatt) using a refractive index increment d*n*/d*c* of 0.070 (THF) (Lang, 2000) and 0.068 (DMSO).

When discussing the degradation, the term "starch backbone" is used. It is described as followed:

$M_w - AGU_{(starch\ laurate)}/M_w - AGU_{(starch)} = X$
$M_{w(polymer)}/X = M_w$ of residual starch-chains with subtracted M_w of lauroyl groups.

$M_w - AGU_{(starch)}$ = 162 g/mol; $M_w - AGU_{(starch\ laurate)}$ = depending on DS; $M_{w(polymer)}$ = result of SEC-MALLS

2.3.3. Solubility studies (DLS)

As a qualitative preinvestigation, the solubility of the starch esters was examined as follows: About 50 mg of the sample were mixed with 1 mL of the solvent and heated for 2 h under constant stirring and reflux conditions. The resulting mixture/solution was visually categorized into soluble (homogeneous solution without visible particles), swellable (visible gel-particles) and insoluble (no interplay) solutions.

To obtain information about the particle size distributions of the products and with the information collected about structure changes and disintegration during synthesis, chloroform solutions were measured by means of the DLS technique using a Delsa Nano C from Beckmann Coulter. 100.0 mg of the sample were dissolved in 9.900 g of chloroform to obtain a 1% (w/w) solution. The mixture was stirred for 2 h under reflux conditions and was measured after cooling. The non-filtrated solutions were measured using quartz cuvettes at a wavelength of 658 nm and a detection angle of 165°. After standing overnight, the sample cuvettes were re-measured. All spectra were measured at 25 °C. For evaluation, the CONTIN-method was applied.

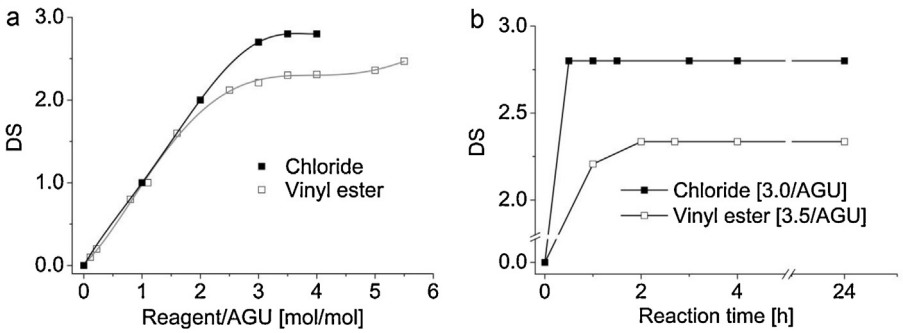


Fig. 1. Amount of reagent (a) and time (b) vs. product DS.

3. Results and discussion

3.1. Synthesis of starch laurate (C_{12})

3.1.1. Comparison of lauroyl chloride esterification and lauric acid vinyl ester transesterification

Both methods of synthesis were conducted with (commercially available) fatty acid derivatives due to the low reactivity of pure carboxylic acids. One important difference between chloride esterification and vinyl ester transesterification is that DMSO behaves as a starch solvent while pure pyridine does not. As a consequence, the conditions are initially homogeneous (DMSO) or heterogeneous which impacts the product's structure and properties. A further difference, caused by the choice of reagent and medium, was the resulting maximum DS, as shown in Fig. 1.

It was established that when vinyl laurate was used, a DS no higher than 2.5 could be achieved. Lauroyl chloride esterification led to an almost complete esterification of the starch. This can be explained by the fact that DMSO is a relatively polar solvent. As the DS increased during the reaction, the starch ester became more apolar, less soluble in DMSO, and finally precipitated. Methods using, for example, toluene as a co-solvent did not lead to any improvement. When lauroyl chloride/pyridine was used, the reaction started to become a heterogeneous suspension. With increasing DS, the solubility of the esterified product in pyridine was enhanced leading to an increased accessibility for further reagents and consequently a higher substitution level. In any case, organic solvent–soluble starch diesters (DS > 2) could be achieved using both methods with a moderate addition of reagent.

Compared to the vinyl esters, fatty acid chlorides have an even higher reactivity as shown in Fig. 1(b): The lauroyl chloride esterification reached its final DS after less than 1 h, while the vinyl laurate transesterification was fully completed after about 2 h. Nevertheless, 2 h are a moderate reaction time and a remarkable improvement over the time stated in the literature (24 h) (Junistia et al., 2008). One important drawback of the enhanced reactivity of chlorides is their higher toxicity and sensitivity to water and impurities.

In summary, for the synthesis of starch dilaurate (DS > 2), the chloride esterification was just slightly more efficient, whereas the vinyl laurate-transesterification was much more sustainable because the low-hazardous solvent DMSO, a carbonate catalyst and less toxic reagents were used. This demonstrates its high potential as a synthesis method for industrial scale applications. Further steps to optimize this method of choice are revealed below.

3.1.2. Reaction parameters for vinyl laurate transesterification

With a view of using the mildest conditions to synthesize starch dilaurate, the amount of the catalyst and the reaction temperature were varied as shown in Fig. 2. A 3 mol% based on AGU is needed to obtain starch laurate with a product DS > 2 at 110 °C. Beyond that, higher catalyst amounts did not lead to a further enhancement of the DS. A similar result was obtained by varying the reaction temperature: Decreasing temperature to under 110 °C led to a decrease in the product DS, while a further increase had no improvement. Hence a 3 mol% catalyst and a 110 °C reaction temperature were considered standard conditions for this type of synthesis.

Additionally, the type of basic alkali carbonate catalyst was varied by taking samples out of the reaction vessel. In the case of Na_2CO_3, there was an obvious deceleration of the reaction rate, while there was no marked difference in DS between K_2CO_3 and Cs_2CO_3. The latter, however, seemed to accelerate somewhat before reaching its final DS (Fig. 3). After 24 h, every carbonate catalyst achieved the same DS.

3.1.3. Solubility of the starch laurates studied using DLS

As a precondition for studying starch laurates using SEC-MALLS, the products should be completely soluble so that no product fraction is lost after the filtration procedure. The particle size distributions of the starch dilaurates obtained with both methods of synthesis are shown in Fig. 4.

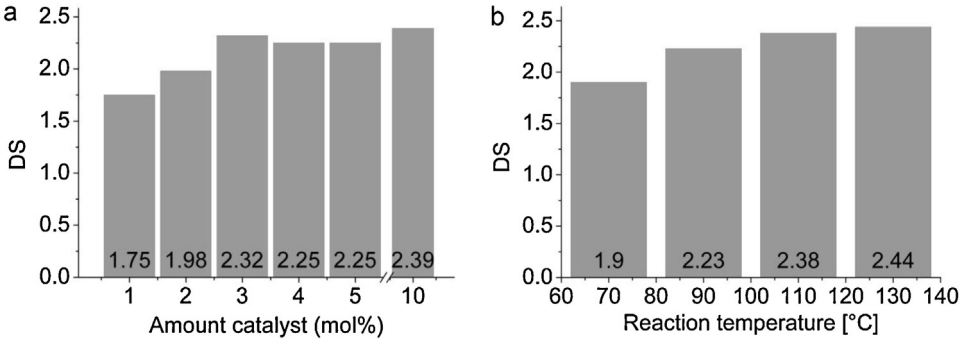


Fig. 2. Amount of catalyst (a) and reaction temperature (b) vs. DS [Cs_2CO_3].

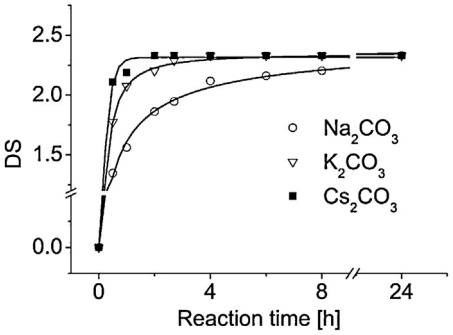


Fig. 3. Variation of the carbonate catalyst.

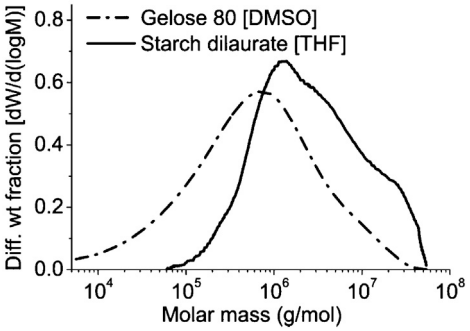


Fig. 5. Molar mass distributions.

The measurement immediately after stirring revealed a remarkable amount of insoluble material in the case of the lauroyl chloride esterified starch. An obvious peak in the area of a 2000–100,000 nm particle diameter was observed. Furthermore, the solution was optically inhomogeneous with visible gel particles. After standing for 16 h, the insoluble material rose to the surface of the cuvette out of the measuring range. So the D (90%) value for chloride esterified starch decreased dramatically from 14,484 to 534 nm after 16 h, revealing the absence of that insoluble material. The measurement of the soluble part of the mixture (Fig. 4b) showed a main particle size in the range of 100–1000 nm. In the case of the vinyl laurate esterified product, the differences between the measurements taken immediately and after 16 h were slight. The *D* (90%) value only decreased from 1706 to 1185 nm and both curves show the same maximum (Fig. 4b). In summary, the premise for taking SEC-MALLS measurements was substantiated in the case of the vinyl ester product, while measuring lauroyl chloride esterified products would produce insignificant results.

3.2. Molar mass distributions (SEC-MALLS)

Because it is a solvent for starch, DMSO enables the esterification procedure to be homogeneous. The starch granules were disintegrated in a pre-step and the resulting starch/DMSO solution was used for several synthetic approaches. Fig. 5 shows the molar mass distribution of gelose 80 after stirring for 2 h at 95 °C in DMSO. Since gelose 80 consists of 80% amylose and 20% amylopectin, there should be 2 fractions visible in the spectrum. Certainly, the distribution curve is mostly mono-modal and only a weak shoulder at the right edge is visible. This shoulder can be ascribed to the amylopectin fraction with its higher molar mass, but there is only a very low amount of it visible in the spectrum. The main part of the amylopectin fraction was lost during the filtration procedure prior to the measurement, which is underlined by a recovery rate of only 85%. In summary, a part of the amylopectin fraction did not completely disintegrate after stirring for 2 h at 95 °C.

Usually when the starch backbone does not completely disintegrate, the products exhibit a very weak solubility due to the existing starch-supermolecular structure. This is the reason for the partial insolubility of the products obtained by lauroyl chloride esterification in pyridine, where the starch structure did not disintegrate before or during synthesis. Thus it would be unfavorable for starch ester products to mix with other polymers e.g. to form blends with reduced interfacial tension and improved product properties. Furthermore, film formation through solvent-casting as well as solution analysis would be unfavorable as well.

Contrary to this, the vinyl laurate based-products were highly soluble as initially revealed in the DLS measurements. One important question was the development of the starch backbone during synthesis. To answer this question, a comparison was done of the M_w-starch-backbone-chain of the products (where the molecular weight of the fatty ester chains was subtracted) and the initial gelose 80 using varying reaction temperatures and amounts of catalyst (Table 1).

When the amount of the catalyst was increased slightly, the M_w of the product starch-backbone-chains decreased, as shown in Fig. 6a. The amount of the catalyst only played a minor role and lead to a further degradation only when high values (10%) are used. Fig. 5 compares the fitted molar mass distribution of starch laurate (DS 2.32, 3% catalyst, 110 °C) to the gelose 80 used initially. Both, the strong curve-decrease and the slight shoulder peak on the right edge of the product curve are the result of a very low amount of aggregated structures (as well as an influence of the fitting procedure). When the recovery rates of these two samples are compared, the rate of the starch ester product is remarkably higher, indicating an advanced disintegration of the amylopectin and/or the amylose fraction during the synthesis procedure. This indicates the superior solubility of the resulting starch ester products. However, the molar mass distribution curves all show one single peak when the amount of catalyst is varied and the reaction temperature is kept at 110 °C.

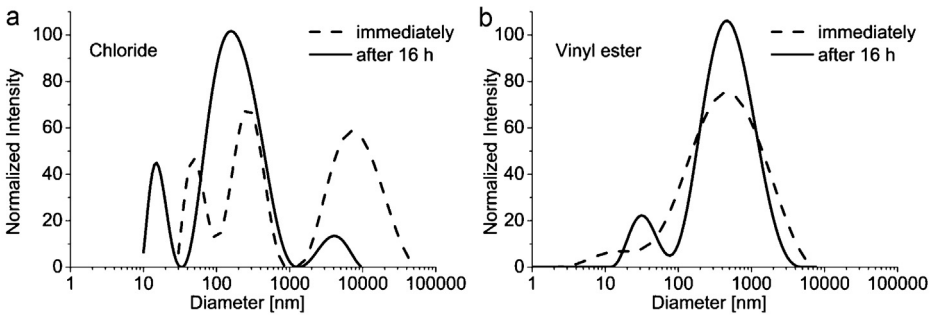


Fig. 4. Particle size distributions of starch laurates in $CHCl_3$, chloride esterification (a) and vinyl ester transesterification (b).

Table 1
Molecular weight distributions of starch laurates.

Amount Cs_2CO_3 [%]	Temperature [°C]	Recovery rate [%]	DS	M_w [10^6 g/mol]	M_w-starch [10^6 g/mol]	$M_{w\text{-Prod}}/M_{w\text{-G80}}$ [%]
Gelose 80 (DMSO)		85	–	1.90		–
1	110	92	1.75	4.92	1.67	88
3		92	2.32	5.93	1.64	86
5		84	2.25	5.79	1.65	87
10		86	2.39	5.52	1.49	78
3	70	82	1.90	6.07[a]	1.93	~102
3	90	85	2.23	6.23[a]	1.77	93
3	110	93	2.38	5.90	1.64	85
3	130	100	2.44	6.36	1.72	89

[a] Calibration over the complete curve (2 peaks).

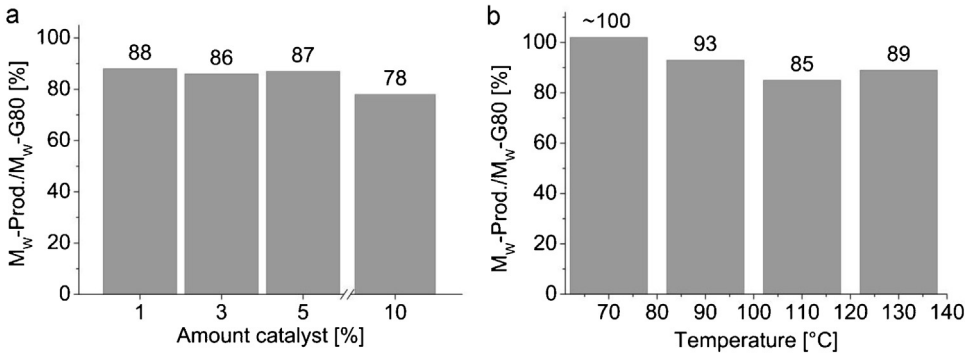


Fig. 6. Degradation of starch backbone vs. amount of catalyst (a) and temperature (b).

The reaction temperature played a significant additional role (Fig. 6b). When the temperature was 70–90 °C, the measured raw-curves (before calibration procedure) revealed two obviously separated peaks. The first peak at extremely high molar masses (>1 × 10^7 g/mol) was ascribed to aggregated structures which were not removed after filtration. It was only approximately <5% of the total measured sample. Presumably, the amylopectin fraction was not fully disintegrated during the reaction, revealing the role high temperature plays in producing products with high solubility. At temperatures above 100 °C, there was only one single peak visible, accompanied by an increased recovery rate. These conditions enabled the starch chains, especially the amylopectin fraction, to completely disintegrate during esterification procedure. In summary, about 110 °C are needed to obtain fully soluble fatty acid gelose 80 esters. However, aside from disintegrated and soluble products, polymers with very high molecular weights (M_w) are obtained which exceed many synthetic ones.

3.3. Determining DS

3.3.1. ^{1}H NMR-spectroscopy and elementary analysis

NMR spectroscopy is often referred to as one possible method for determining DS in FASEs; however, it is quite expensive and has some drawbacks in terms of reliability. Spectra of pure fatty acid starch esters can be measured in a suitable organic solvent and the integrals of the methyl group of the fatty acid and one or all starch signals are compared. Due to the remaining OH-groups, the starch protons (3–6 ppm) appear as broad and overlapping signals. For an improvement of the signal quality, a peracetylation procedure (Einfeldt et al., 2001) is typically applied and gives reliable results. However, it would be desirable to avoid this additional synthesis step. In the current study, the availability of the soluble FASEs for ^{1}H NMR-measurements is studied. Furthermore the substitution pattern is discussed using ^{13}C NMR spectra.

The quality of the ^{1}H NMR spectra of non-peracetylated samples generally depends on the solubility of the starch ester. The solubility of FASEs in DMSO decreased with increasing DS due to their apolarity. A DS of 0.5 turned out to be the limit (Fig. 7) for a reliable measurement in DMSO. The —CH_3-group of the fatty acid chain appears at 0.90 ppm and CH_2-fatty acid protons occur at 1.29, 1.57 and 2.40 ppm, respectively (Fig. 7a).

In the case of starch laurates with a DS of <0.5, the starch protons and the fatty acid chain signals are well resolved; the DS can be determined precisely and gives values comparable to elementary analysis (Table 3). At a DS of 0.8 (dotted gray curve), determining the DS in DMSO was no longer significant due to broad and poorly resolved signals accompanied by a strong swelling of the sample in the solution. THF-d_8 proved to be a suitable solvent for products with a DS > 1.2 and $CDCl_3$ was suitable for starch dilaurate (DS > 2.0).

Nevertheless, the determination of the DS for non-peracetylated samples in organic solvents was imprecise because fatty acid signals were over-dimensioned in the case of higher DS values. Here, ester-chain interactions might play a role as well as solvent-effects. The different proton signals inside the fatty acid chain also varied in their integrated intensity and so an average value was used. However, there still might be a divergence toward the real value.

In Table 2, substitution patterns of lauroylated Gelose 80 obtained by ^{13}C NMR are revealed. Fig. 7b shows one example of a ^{13}C NMR-spectrum of starch laurate with a DS of 0.56. As obtained by the splitting of C-1, it came out that for esterification with vinyl laurate in DMSO with Cs_2CO_3 as a catalyst, the C-2 was the preferred OH-group to be esterified. Although this fact is consistent with earlier studies of acetylation in DMSO by Dicke (Dicke, 2004), lauroylation concomitantly occurred at the C-6 position. At a DS of

Table 2
Substitution patterns of lauroylated Gelose 80.

Total DS (^{13}C NMR)	DS C-2	DS C-3	DS C-6
0.14	0.06	0	0.08
0.21	0.11	0	0.10
0.56	0.32	0.10	0.14
0.60	0.29	0.19	0.12
2.21	0.88	0.39	0.94

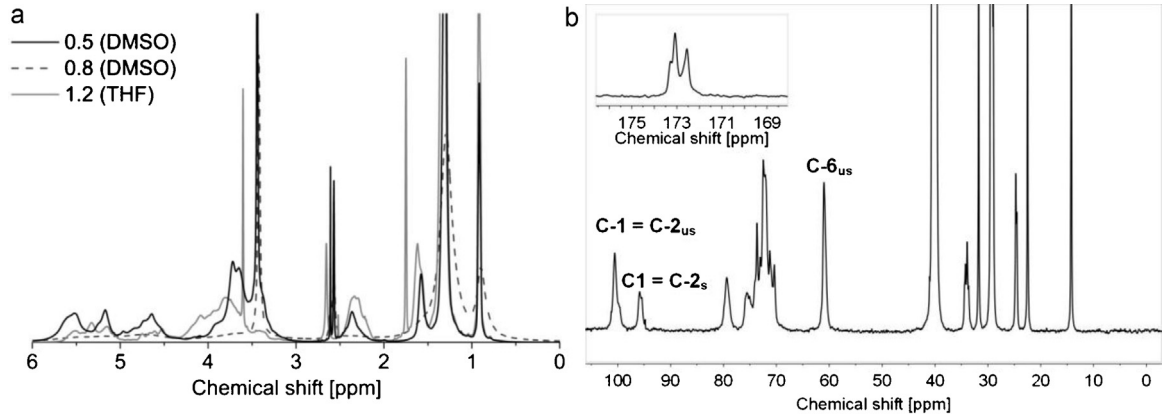


Fig. 7. (a) ^{1}H NMR spectra of starch laurate [DMSO-d_6, THF-d_8]; (b) ^{13}C NMR spectrum of starch laurate (DS 0.56) to obtain substitution pattern. s: substituted, us: unsubstituted.

>0.4, there is also a partial esterification at the C-3 position (determined indirectly by total DS, DS of C-2 and the difference of the integral of unsubstituted C-6 toward 1.0).

The reason for a more homogeneous substitution pattern in the current study might be on the one hand the improved solution state of the starch due to the higher temperature compared to studies made by Dicke. Furthermore, Cs_2CO_3 shows a better solubility in DMSO and with that, the detailed mechanism of the structure/esterification might be changed. However, at this point, further studies are necessary.

Elementary analysis is considered to be another possible and reliable method; In the case of low DS values, traces of impurities have a strong influence on the determination of the product DS and might lead to strong inaccuracies.

In summary, ^{1}H NMR and ^{13}C NMR-spectroscopy are possible methods for determining DS of FASEs without a further peracetylation step. However, there are difficulties dissolving starch fatty acid esters with a DS 0.8–1.2 and inaccuracies for FASEs measured in THF (medium DS). A combination of ^{1}H NMR and elementary analysis turned out to be useful.

3.3.2. ATR-FTIR

Fig. 8 shows infrared spectra of starch laurates with different DS-values (transmission view). The broad signal at 3300–3700 cm^{-1} is attributed to the vibration of the O—H groups. The bands at 2924 and 2852 cm^{-1} are ascribed to C-H vibrations, mainly of the fatty acid chain and the glucose unit. At 1740 cm^{-1} appears the C=O vibration of the ester group. Another ester signal is visible at 1157 cm^{-1}. The intensive signal at 1020 cm^{-1} is ascribed to the C—O—C ring vibration of the glucose units of the starch. It is visible, that the intensity of the C—H signals as well as the ester signals

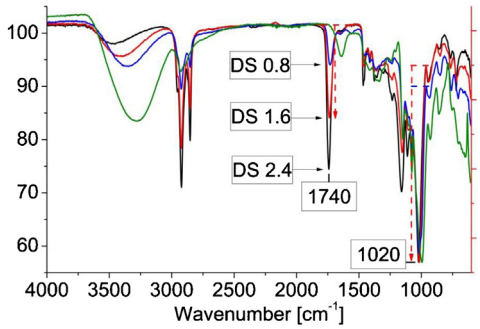


Fig. 8. ATR-FTIR spectrum of Gelose 80 (green) and starch laurates. (For interpretation of the references to color in text/this figure legend, the reader is referred to the web version of the article.)

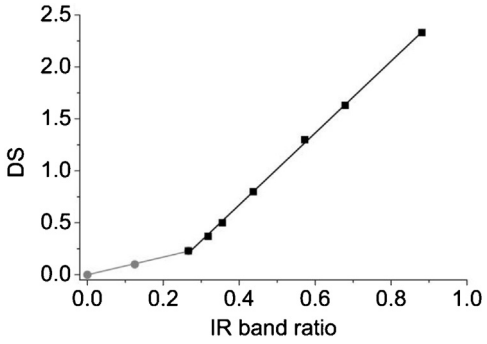


Fig. 9. IR band ratio vs. DS.

are increasing in their height with increasing degree of substitution. Simultaneously, the relative ratio of the height of the ester band (1742 cm^{-1}) and the height of the starch band (1020 cm^{-1}) is increasing (Fig. 8).

$$DS \sim \frac{height_{[C=O\,ester]}}{height_{[C-O-C\,starch]}} \quad \text{[red arrows in Fig. 8]}$$

For a quantitative comparability of different samples independent from the absolute y-scale, the intensities of the C—O—C starch bands (at 1020 cm^{-1}) were normalized from the baseline to a uniform height and the ratios with the ester bands height were taken as described (Fig. 8).

To be able to correlate the band ratio to absolute DS-values, a calibration curve of selected samples was previously done using the combination of ^{1}H NMR and elementary analysis (Table 3). It turned out that the ratio of the height of the bands correlated with the DS in form of two linear functions: One in the DS range of 0.0–0.2 and one in the DS range of 0.3–2.5 (Fig. 9).

Though all DS values of the synthesized samples were in DS range of >0.2, the following (second) linear function for DS calculations was applied (R^2 = 0.99925)

$$DS = -0.71072 + 3.45754 \times ratio\,(IR)$$

Table 3
DS obtained by ^{1}H NMR and elementary analysis.

DS [elem. anal.]	DS [^{1}H NMR]	IR band ratio
0.30	0.23 [DMSO]	0.27
0.43	0.37 [DMSO]	0.32
0.80	0.89 [DMSO]	0.44
1.33	1.45 [THF]	0.57
1.63	1.96 [THF]	0.68
2.33	2.41 [$CDCl_3$]	0.88

Table 4
DS values of a series of starch esters (o = possible, x = impossible).

Fatty acid ester chain	Appearance	DS value	10% solution (w/w)	Film-forming
Hexanonate (C_6)	Pale yellow, amorphous solid	2.6	o	o
Octanoate (C_8)		2.4	o	o
Decanonate (C_{10})		2.5	o	o
Laurate (C_{12})		2.3	o	o
Palmitate (C_{16})	Colorless, powder	2.2	o	o
Stearate (C_{18})		2.2	o	x

Table 5
Qualitative solubility of starch esters.

Ester chain	DS	Reagent of synthesis	Water	DMSO	THF	$CHCl_3$
Gelose 80 [pur]			Soluble	Soluble	Insoluble	Insoluble
C_6–C_{18}	0.5	Vinyl ester	Insoluble	Soluble	Insoluble	Swellable
C_6–C_{18}	1.5		Insoluble	Insoluble	Soluble	Swellable
C_6–C_{18}	>2		Insoluble	Insoluble	Soluble	Soluble
C_6–C_{18}	>2	Chloride	Insoluble	Insoluble	Swellable	Swellable

This function most likely varies with every IR spectrometer, type of ester group and can be influenced by a varied baseline detection.

In summary, ATR-IR turned out to be a quick and efficient method of determining DS after taking a calibration curve with the aid of ^{1}H NMR (DS < 0.5) and elementary analysis (DS > 0.5).

3.4. Applicability toward further fatty acid esters of starch

Homogeneous esterification of starch with vinyl laurate led to highly soluble starch derivatives. Starch laurate served as a model compound and esterification with other fatty acid vinyl esters under the same conditions is promising. In this regard, the synthesis method was tested with a series of fatty acid vinyl esters and the DS was determined (elementary analysis). For every type of fatty acid vinyl ester, a temperature of 110 °C, 3 mol% of Cs_2CO_3 and a reaction time of 2 h were applied. The resulting DS values of the products are revealed in Table 4.

It turned out that all esterified products fell within a comparable range of DS values (DS > 2). The use of short and medium fatty acid vinyl esters (C_6–C_{12}) led to slightly higher DS values than for higher (C_{16}–C_{18}) fatty acid ester groups. This can be explained by the increased hydrophobicity of the long palmitate und stearate groups which precipitated earlier out of the DMSO reaction medium, as well as a decreased availability of the free OH groups due to the high space requirement of the ester groups. The formation of concentrated solutions and subsequent film formation through casting was possible for all types of ester groups except starch stearate which turned out to be too brittle.

As mentioned above, the solubility of the organic solvent turned out to be an important product characteristic. As shown in Table 5, even at low DS, all of the starch esters became totally insoluble in water due to the strong hydrophobic character of the fatty ester chains. The solubility in DMSO decreased with increasing DS for all esters. In summary, all diesters of vinyl ester esterified starches (DS > 2) exhibited a remarkable solubility in THF, chloroform and even toluene.

4. Conclusion

Compared to the conventional chloride esterification method, sustainable vinyl ester transesterification turned out to be the preferred method for FASE synthesis with C_6-C_{18} ester chain lengths. DS values >2 were obtained in all ester groups in an optimized reaction time of only 2 h. During synthesis, a slight disintegration of the starch backbone was proven using SEC-MALLS. This resulted in products which were highly soluble in organic solvent, leading to high recovery rates after M_w determination. DLS was applied as a suitable method to reveal the differences in solubility for the two methods of synthesis.

Furthermore, after a calibration using ^{1}H NMR spectroscopy and elementary analysis, the efficient ATR-FTIR method could be applied to quickly and easily determine the DS of the products. The highly soluble FASEs were able to form concentrated solutions in organic solvents and have the potential of forming into a film through solvent casting.

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