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Biodegradation of cross-linked and cationic starches

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ABSTRACT

The biodegradability and the influence of the degree of substitution of cationic groups or cross-linking level of starch were studied by using enzymatic hydrolysis and two aerobic degradation methods. Cationic starches with a degree of substitution varying from 0 to 0.54 were obtained by modifying native potato starch with 2,3-epoxypropyltrimethylammonium chloride, while cross-linked starches with a degree of cross-linking varying from 0 to 92.5% were obtained by reaction of native potato starch with epichlorohydrin. Enzymatic hydrolysis experiment was performed using α-amylase preparation, and aerobic degradation studies were carried out in liquid and solid media by using ISO 14855-2 and 14851 standards methods. The dextrose equivalent, molecular weight, viscosity and biodegradability parameters were used to assess biodegradation process. Biodegradability of modified starches decreased with increasing degree of modification. The addition of cationic groups to starch to the extent >0.1 mol/mol$_{AGU}$ reduced the biodegradability of starch derivatives, and CS became non-biodegradable when DS ≥ 0.54. The cross-linking of starch by building the alkyl chain cross-links between the polysaccharide macromolecules reduced ultimate biodegradability of starch derivatives, when the degree of cross-linking was higher than 92.5%.

1. Introduction

Synthetic polymeric materials are widely used in various fields of industry and technical areas, and most of them are made from petroleum products and are non-biodegradable under normal conditions [1]. However, resources of petroleum are limited and abundant use of non-biodegradable derivatives has been caused serious environmental problems already. Thus, in the recent years more attention has been given to polymeric materials which are easily biodegradable. Among the natural polymers, starch has been of great interest. It is generated from carbon dioxide and water by photosynthesis in plants [2], and it is completely biodegradable, renewable, easily modifiable and cheap [3]. Therefore, natural starch is a promising candidate for developing sustainable materials for various application areas [4–8]. By physical and/or chemical modification of starch new biocompatible and biodegradable materials can be developed which could be used in medicine, paper, packaging, cosmetic, textile industries, and environmental technologies etc. [9].

It is easy to chemically modify starch by introducing functional groups into starch backbone. By performing etherification of starch molecules cationic starch (CS) derivatives can be obtained. Cationic starch ethers can be easily prepared by using quaternary ammonium groups containing commercial reagents. Mainly, 2,3-epoxypropyltrimethylammonium chloride or 3-chloro-2-hydroxypropyltrimethylammonium chloride is used as cationization reagent for starch modification [10, 11]. The physicochemical properties of modified starches differ from those of native starch. For example, CS has higher affinity to water due to the cationic groups, therefore it is more attractive and has wider possibilities to be used in paper or textile industries, oilfield drilling and wastewater treatment technologies [10, 12, 13]. The important characteristic of CS is the degree of substitution (DS), and maximum theoretical DS value of CS could be 3. However, in praxis it is difficult to get CS with DS higher than 1.1 in one step aqueous cationization reaction [14]. There are some reports on synthesis of CS of higher DS but it is much more problematic, and the use of organic solvents or performing the reaction in two-steps is necessary [14, 15]. Cationic starch by the degree of substitution can be defined as CS of low DS (DS ≤ 0.1), medium DS (0.1 < DS < 0.5) and high DS, when DS ≥ 0.5. This classification is unsettled, for example, some authors indicate that modified starch is of low DS, when DS is lower than 0.07 or even as high as 0.20 [14, 16]. CS derivatives of low DS are widespread and used for commercial purposes for a long time [10, 17, 18]. However, only in the recent years more attention has been given to CS with higher DS by demonstrating some other properties and new application possibilities [11, 19–22]. Cross-linking is another important factor when imparting functional properties to starch – lower viscosity of paste, higher thermal stability, higher firmness of the granular structure etc. Commonly, native starch can be cross-linked with epichlorohydrin in presence of alkali, by obtaining cross-linked starch (CLS) [23, 24]. Cationized and/or cross-linked starches are of high interest in various fields of applications for their good technological properties, low price, natural origin and straightforward synthesis. However, their

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biodegradability and biotoxicity properties should be thoroughly evaluated before considering real applications.

Often biodegradability is indicated as an advantage of such modified starch derivatives. Many authors use the term "biodegradability" negligently or inaccurately in their papers, even in the abstracts or in the titles, despite the fact that biodegradation experiments are even not performed. It is very important to use the right definition because biobased materials are not necessarily biodegradable. The studies on biodegradation of modified starches are scarce. Several papers have been published on different biodegradation studies of modified starches and their composite films [25–28]. It was established that an increase of DS, from 0.02 to 0.13, did not seem to influence the primary enzymatic step and degradability level of molten hydroxypropyltrimethylammonium wheat starches [25]. However, by increasing the DS from 0.13 to 0.16 led to a 50% drop in the degradation level. Consequently, it can be seen that biodegradability of CS should depend on DS of CS and biodegradability testing results also should be of great interest when selecting the areas of application of CS derivatives. Meanwhile, by analysing the biodegradation of starch/polyvinyl alcohol films prepared with four different crosslinkers in composting medium, it was established that borax crosslinked films showed the highest rate of degradation by the fungi [28]. Meanwhile, samples crosslinked with epichlorohydrin were most degradation resistant.

The aim of the present work was to study the biodegradability of cationic starches of various degrees of substitution and cross-linked starches of different degrees of cross-linking by using enzymatic hydrolysis and aerobic degradation methods in solid and liquid media.

2. Materials and methods

2.1. Materials and chemicals

The native potato starch (intrinsic viscosity $[\eta] = 0.39$ l/g), containing 20–25 wt% of amylose and 75–80 wt% of amylopectin was received from Antanavas Starch Plant (Lithuania). 2,3-Epoxypropyltrimethylammonium chloride (EPTMAC, 70%), epichlorohydrin (EPI), lithium bromide, dimethyl sulfoxide Chromasolv® Plus (DMSO, HPLC grade, ≥99.7%) and *N*-allylthiourea (nitrification inhibitor, 98%) were supplied by Sigma–Aldrich. α-Amylase preparation from *B. licheniformis* (Liquozyme® Supra, 800 KNU/g) was obtained from Novozymes (Denmark). All other chemicals used were of analytical grade.

2.2. Preparation of modified starches

2.2.1. Synthesis of cationic starches

Cationic starches (CS) were obtained by the etherification of potato starch with EPTMAC in the presence of NaOH as a catalyst (the molar ratio AGU:EPTMAC:NaOH:H_2O was 1:(0.10–0.57):(0.038–0.040):(3–4.5) at 45 °C for 48 h. The molecular mass of the anhydroglucoside unit (AGU) was assumed as a mole of starch. After the reaction, CS derivatives were washed with a water–isopropanol mixture and dried at 105 °C. The obtained materials were purified by Soxhlet extraction with methanol for 16 h. The number of cationic groups in CS was expressed as the degree of substitution (DS), which was calculated from the nitrogen content estimated by the Kjeldahl method:

$$DS = \frac{162N}{1400 - 151.5N}, \tag{1}$$

where N is nitrogen content estimated by the Kjeldahl method (%), 162 is the molecular weight of the AGU, 1400 is the molecular weight of nitrogen multiplied by a hundred and 151.5 is the molecular weight of EPTMAC. The prepared cationic starches with a various DS were designated as CS_{DS}.

2.2.2. Synthesis of cross-linked starches

The starch was cross-linked with epichlorohydrin (EPI) in the presence of NaOH (the molar ratio AGU:EPI:NaOH:H_2O was 1:(0.1–0.005):0.04:(10–11) at 45 °C for 24 h. After reaction CLS was water washed and dried at 105 °C. The degree of cross-linking of CLS was determined by estimating viscosity parameters of aqueous slurries (8 g of starch derivative in 100 ml of distilled water) of modified starches according the procedure reported by Chatakanonda et al. [29] by using a Micro Visco-Amylo-Graph® (Brabender, Germany). Each slurry was added to the aluminium sample container and subjected to a controlled heating-and-cooling cycle under constant shear: heating from 30 to 95 °C at a heating rate of 6.5 °C/min and maintaining for 15 min and cooling to 50 °C at a cooling rate of 6.5 °C/min. The degree of crosslinking was estimated using the following equation:

$$Degree\ of\ crosslinking = \frac{A - B}{A} \times 100 \tag{2}$$

where A is the peak viscosity in Brabender units of the control sample (0% EPI) and B is that of the crosslinked starch sample.

2.3. Enzymatic hydrolysis and characterization of hydrolysis products

The enzymatic hydrolysis of native and modified starches was performed using α-amylase (Liquozyme® Supra) preparation. Water suspensions (3%) of native or cationic starches were mixed with various amounts of α-amylase preparation (0.2, 2.0 and 20 mg per g of polysaccharide), and their pasting properties were studied using Micro Visco-Amylo-Graph®. Each slurry was added to the aluminium sample container and subjected to a controlled heating-and-cooling cycle under constant shear: heating from 20 to 80 °C at a heating rate of 3 °C/min and maintaining for 10 min; heating from 80 to 95 °C at a heating rate of 6.5 °C/min and maintaining for 5 min and, finally, cooling to 50 °C at a cooling rate of 6.5 °C/min.

Starch derivatives hydrolysis was evaluated by the dextrose equivalent (DE), determined by the Schoorl method [30] and expressed as the reducing sugars content.

The intrinsic viscosity $[\eta]$ of 0.135% aqueous solutions of native and cationic starches after enzymatic hydrolysis was determined using Ostwald capillary viscometer (Ø = 0.34) at 25 °C.

The molecular weight parameters of native starch and cationic starch hydrolyzed samples were determined using size exclusion chromatography (SEC) system (Viscotek 270 Dual Detector, Malvern Instruments Ltd.) equipped with refractive index (RI) and light scattering (RALS and LALS) detectors, and two Viscotek-A columns (A2500 and A5000). Columns and detectors were operated at 60 °C and 50 °C, respectively. DMSO with 0.05 M LiBr was used as an eluent at a flow rate of 0.3 ml/min. The calibration was done with a pullulan standard (PUL-110 K) also provided by Malvern. Data were recorded, the obtained elution curves were analysed and the molecular weight ($M_{w)}$ was calculated using the Malvern Omnisec software (Version 4.7).

2.4. Aerobic biodegradation in solid medium

The aerobic biodegradation of non-modified and modified starch samples in solid medium was investigated by using MODA (Microbial Oxidative Degradation Analyser) apparatus (Saida Ironworks Co., Ltd) [31], comprised of six independent lines of columns in parallel. The samples and the solid medium were prepared by a standard ISO 14855-2:2007(E) procedure [32]. The temperature inside the columns was maintained at 58 ± 1 °C and the flow rate of CO_2-free but moisturized air was 10 ml/min. The microbial biodegradability of samples was evaluated by measuring the amount of released CO_2. The mass of produced CO_2 was calculated as a difference in the weight of two upper columns containing soda lime/soda talc and calcium chloride at the beginning and during the test. Sample weight was about 3 g (dry