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Esterification of potato starch by a biocatalysed reaction in an ionic liquid

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ABSTRACT

In this study, potato starch was esterified with oleic acid, using 1-butyl-3-methylimidazolium chloride as a reaction medium and an immobilised lipase from *Thermomyces lanuginosus* as a catalyst. The degree of substitution (DS) of the products was determined by the volumetric method; and the best esterified product (with the highest DS) was determined by an elemental analysis. The effect of the reaction parameters on the DS, such as the time and the temperature, were also studied. The product with the highest DS (0.22) was found in the reaction carried out at 60 °C for 4 h. Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) analyses confirmed the esterification of the potato starch. Furthermore, the results of X-ray diffraction (XRD) and a scanning electron microscopy (SEM) revealed that the crystallinity and the morphology of the native potato starch was slightly changed during its partial gelatinisation in the ionic liquid, and was completely destroyed as a result of the formation of the esters. The thermal stability of the starch oleate decreased, when compared to the unmodified starch, as was indicated by a thermal gravimetric analysis (TGA).

1. Introduction

Natural polymers which are non-toxic, biodegradable and inexpensive, such as starch, are of great interest regarding to their possible use as materials in various industries, such as the food, paper, pharmaceutical and packaging industries (Fang & Fowler, 2003). Starch granules consist of two types of α-glucan: amylose and amylopectin (representing approximately 98–99% of the dry weight). These form linear polymer chains and hydrogen-bonded supramolecular structures (Jie, Wen-ren, Manurung, Ganzeveld, & Heeres, 2004). Native starch is characterised by high fragility and an incompatibility with hydrophobic polymers. It also has a low moisture resistance, meaning it has low processing qualities (due to high viscosity) and is highly hydrophilic. These properties can significantly limit the use of a starch to obtain a new type of material. Therefore, different types of modifications have been implemented to improve the mechanical properties of starch and its hydrophobisation (Fang, Fowler, Tomkinson, & Hill, 2002; Tomasik & Schilling, 2004).

Esterification is one of the oldest methods used for the modification of carbohydrate polymers (Mullen & Pacsu, 1942). The most important starch derivative – fatty acid starch esters – can be obtained through an acylation reaction of the free hydroxyl groups in the anhydroglucose unit (AGU). Usually, in order to enable an esterification reaction with the acids, a starch is first dissolved in dimethyl sulfoxide (DMSO) (Junistia et al., 2009), pyridine (Aburto et al., 1999) or another commonly used solvent, such as dimethylformamide (DMF) or tert-butanol (Boruczkowska et al., 2013; Lukasiewicz & Kowalski, 2012). However, the use of solvents also has some limitations and disadvantages, such as volatility, flammability and high levels of toxicity. They not only represent a significant hazard during the separation process, but can also be a source of environmental pollution. Therefore, it has become important to look for a "green", environmentally friendly solvent, which can be used for the esterification of polymers.

A few years ago, researchers discovered the some ionic liquids (ILs) have the ability to dissolve carbohydrate polymers (Wilpiszewska & Spychaj, 2011). One of the first of these ionic liquids was 1-butyl-3-methylimidazolium chloride, which was used during the chemically catalysed synthesis of a starch acetate using acetic anhydride (Biswas, Shogren, Stevenson, Willett, & Bhowmik, 2006). In 2007, a study was conducted on the influence of the same ionic liquid on the dissolution of starch from different botanical

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origins (corn, rice, wheat and potato) (Stevenson, Biswas, Jane, & Inglett, 2007). From this, it is known that the solubility of starch in an ionic liquid not only depends on its structure, but also on the temperature of the reaction. In order to reduce the time for starch depolymerisation, while maintaining a highly efficient reaction, the depolymerisation can be carried out in an oil bath, or even by heating using a microwave reactor in the presence of a chemical catalyst (Rajan, Prasad, & Abraham, 2006). Different esterifying factors have been used for the non-catalysed synthesis of starch esters in an ionic liquid, including: acetic and succinic anhydride (Luo & Zhou, 2012; Xie, Shao, & Liu, 2010); fatty acids such as stearic acid, lauric acid, and palmitic acid; or esters of these acids such as vinyl stearate (Biswas, Shogren, & Willett, 2009; Gao, Luo, & Luo, 2012). The chemically catalysed (using pyridine) esterification of starch in an ionic liquid has also been carried out, by reactions with acetic anhydride (Biswas et al., 2006); hexanoic, phthalic, and propionic anhydride (Lehmann & Volkert, 2009); and with fatty acid esters such as methyl laurate or stearate (Xie & Wang, 2011).

It is commonly known that the esterification of starch with fatty acids can be performed in the presence of biocatalysts (like lipases). The main sources of these enzymes are fungi, including: *Candida antarctica* (Xu et al., 2012); *Thermomyces lanuginosus* (Alissandratos et al., 2011); *Burkholderia cepacia* (Rajan & Abraham, 2006); and *Candida rugosa* (Rajan, Sudha, & Abraham, 2008). Sometimes, enzymes from bacteria are also used, such as: *Staphylococcus aureus* (Horchani, Chaabouni, Gargouri, & Sayari, 2010), and *Pseudomonas* sp. (Qiao, Gu, & Cheng, 2006). Studies have confirmed that some ionic liquids improved the stability of the enzymes, and in addition could be their activators (Van Rantwijk, Madeira Lau, & Sheldon, 2003). In many cases, the enzymatic esterification in an ionic liquid has been the selective and efficient method (Ganske & Bornscheuer, 2005; Sheldon, Madeira Lau, Sorgedrager, van Rantwijk, & Seddon, 2002), such as a successful attempt at the lipase-catalysed esterification of high-amylose corn starch in a mixture of imidazolium-based ionic liquids (Lu, Luo, Yu, & Fu, 2012). Fatty acid starch esters have also been prepared via a two-step method using two different types of imidazolium-based ionic liquids, where the pregelatinisation of the starch was performed using chloride, but the lipase catalysed synthesis was carried out in tetrafluoroborate (Lu, Luo, Fu, & Xiao, 2013).

The aim of present study is to optimise the conditions for the preparation of new functional materials based on starch, by a biocatalysed esterification with unsaturated fatty acids in an ionic liquid. Thus, potato starch was esterified with oleic acid, in the presence of an immobilised fungal lipase from *T. lanuginosus* as the catalyst, and with 1-butyl-3-methylimidazolium chloride as the reaction medium. The esterification of the starch with an unsaturated fatty acid may provide opportunities to further modify the obtained potato starch fatty acid esters, by an addition reaction to unsaturated double bond. Important and novel aspects of the study are the application of lipase immobilised on a polymer carrier, which can be used several times; and a simplification of the process by using the same ionic liquid for both the pregelatinisation of the starch and the preparation of the starch oleates. It should be emphasised that the proposed model esterification reaction may be applied to the esterification of potato starch with oils rich in oleic acid, such as high oleic sunflower oil and high oleic canola oil.

2. Materials and methods

2.1. Materials

The materials used in the study include: potato starch purchased from Roth (Karlsruhe, Germany); pure oleic acid purchased from Chempur (Piekary Slaskie, Poland); 99.8% pure anhydrous ethanol purchased from POCH (Gliwice, Poland); 95% ionic liquid 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) purchased from Sigma–Aldrich (Germany); and lipase from *T. lanuginosus* (Immozyme TLL-T1-350) adsorbed on a polymer carrier (with an activity of 10,000 TBU/g), purchased from Chiral Vision (The Netherlands).

2.2. Determination of lipase activity

The lipase activity was measured by the liberation of butyric acid during the hydrolysis of tributyrin (glycerol tributyrate) according to the assay method described by Chiral Vision for their Immozyme TLL-T1-350 lipase. Hydrolysis of tributyrin was monitored titrimetrically in a pH-stat titration system (Metrohm titrator). The butyric acid, which is formed, was titrated with 0.1 mol/l sodium hydroxide and the consumption of the latter recorded as a function of time. Used method is based on the speed at which the enzyme hydrolyses tributyrin at pH 7.5 and 40 °C. The activity of enzyme is expressed as tributyrin units per gram enzyme (TBU/g). 1 TBU (lipase unit) is the amount of enzyme which releases 1 μmol titratable butyric acid per minute under the given standard conditions.

2.3. Partial gelatinisation of the starch and preparation of the starch oleates

Initially, the potato starch was dried at 105 °C, to a water content below 5%. The dried starch was then added to the ionic liquid ([BMIm]Cl) at a concentration of 10% (w/w) in a two-necked round flask flushed with an inert gas (Ar). The suspension was heated in an oil bath at approx. 90 °C for 1 h. After the ionic liquid melted (and the suspension became viscous), the whole starch paste was stirred vigorously with a magnetic stirrer at 500 rpm. Then, the pregelatinised starch was cooled to the desired temperature for synthesis, and the oleic acid and the biocatalyst were added to initiate the esterification (Lu et al., 2013). A constant molar ratio of the starch (anhydroglucose unit, AGU) to the oleic acid (1:3) was maintained, as well as a constant weight ratio of the immobilised lipase (1.176 g per 1 g of the native starch). The synthesis of esters was carried out in an oil bath at different temperatures (60, 70 or 80 °C) for 4, 6 or 8 h, and then the mixture was again stirred using a magnetic stirrer at 500 rpm. After the completion of the reaction, the mixture was cooled to room temperature. In a further step, the products were precipitated using anhydrous ethanol. Then, the precipitates were centrifuged and washed with anhydrous alcohol to separate out the main product of the esterification – the starch oleates. The solid residues were rewashed (three times) to eliminate the byproducts, the ionic liquid and the untreated reagents. Finally, the residues from the filtration were dried at 50 °C for 36 h. The dried products were ground and then subjected to a physicochemical analysis.

2.4. Determination of the degree of substitution (DS)

The DS of the fatty acid starch esters was determined according to the titration method, with a slight modification (Varavinit, Chaokasem, & Shobsngob, 2001). A half gram of powdered starch ester was weighed accurately, and was then dispersed in 25 ml of deionised water containing 5 ml of a 0.5 mol/l NaOH solution. The mixture was stirred vigorously at room temperature for 1 h. After this time, a few drops of phenolphthalein were added as an indicator, and the mixture was titrated using a 0.5 mol/l HCl solution. A blank sample was simultaneously titrated using native potato starch instead of the starch ester. Each sample was measured in