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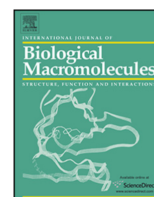

Morphological and physicochemical characterization of porous starches obtained from different botanical sources and amylolytic enzymes

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ABSTRACT

Porous starches might offer an attractive alternative as bio-adsorbents of a variety of compounds. However, morphology and physicochemical properties of starches must be understood before exploring their applications. Objective was to study the action of different amylolytic enzymes for producing porous starches. Wheat, rice, potato and cassava starches were treated with Amyloglucosidase (AMG), α-amylase (AM) and cyclodextrin-glycosyltransferase (CGTase). Morphological characteristics, chemical composition, adsorptive capacity and pasting/thermal properties were assessed. Scanning Electron Microscopy (SEM) showed porous structures with diverse pore size distribution, which was dependent on the enzyme type and starch source, but no differences were observed in the total granule surface occupied by pores. The adsorptive capacity analysis revealed that modified starches had high water absorptive capacity and showed different oil adsorptive capacity depending on the enzyme type. Amylose content analysis revealed different hydrolysis pattern of the amylases, suggesting that AMG mainly affected crystalline region meanwhile AM and CGTase attacked amorphous area. A heatmap illustrated the diverse pasting properties of the different porous starches, which also showed significant different thermal properties, with different behavior between cereal and tuber starches. Therefore, it is possible to modulate the properties of starches through the use of different enzymes.

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1. Introduction

Porous starch granules are becoming of great interest such as non-toxic absorbents, owing to their great absorption capacity derived from the major specific surface area [1]. Pores can protect sensitive elements as oils, minerals, vitamins, bioactive lipids, food pigments such as β-carotene and lycopene that are sensitive to light, oxidation or high temperature [2–4]. In fact, porous starches have been proposed as carriers or vehicles of colorants, spices, flavorings or sweeteners and pharmaceuticals [5]. Nevertheless, very scarce information exists regarding the characteristics of the pores and how to modulate them to extend the application of the porous starches [6].

Up to now, several enzymes, such as α-amylase (AM), β-amylase, amyloglucosidase (AMG), pullulanase, isoamylase and cyclodextrin-glycosyltransferase (CGTase) have been used for producing porous starches [7–10]. Pin-holes, sponge-like erosion, numerous medium-sized holes, distinct loci leading to single holes in individual granules and surface erosion are being observed after enzymatic action [11], but there is no clear understanding about the role of either the botanical origin of starch or the enzyme used. Aggarwal and Dollimore [12] observed an increase in the size of the pores on corn starch granules, when augmented the AMG concentrations, till the breakdown was so pronounced that walls around pinholes were broken, leading to large irregular holes and a disrupted structure. Recently, Benavent-Gil and Rosell [13] compared the effect of AMG, AM, CGTase and branching enzyme on corn starch properties, taking also into account the impact of enzyme level. Authors concluded that corn starches with varying number and size of pores could be obtained by controlling either the type of amylolytic enzyme or the level of enzyme.

In addition, it must be considered the intrinsic structural features of starches from different botanical origin, which might affect the amylolytic action. In fact, when corn, mung bean or sago starches were treated with a mixture of AM and AMG at 35 °C for 24 h, porous granules were obtained, whereas only enzymatic erosion occurred on the surface of cassava starch granule [14]. According to Rocha, Carneiro and Franco [15], AM degraded the

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external part of the granule surface of cassava, sweet potato, and potato starches after hydrolysis at 37 °C for 48 h; but Peruvian carrot starch showed only some granules with internal degradation.

In previous literature, substantial variation was found in terms of hydrolysis time and temperature, and enzyme type, which somewhat impedes the exploitation of porous starches; meanwhile there is no clear knowledge about the role of those factors on the pore development. Likewise, taking into account the variety of compounds to be adsorbed from foodstuffs, pharmaceutical, cosmetic and chemical products, the characterization of those starches would be needed from an industrial point of view.

Therefore, the main objective of this study was to identify the potential of starches from different botanical sources to obtain porous starches with different type of hydrolases. Particularly, to characterize and compare the effect of amyloglucosidase, fungal α-amylase and cyclodextrin-glycosyltransferase on the morphological and physicochemical properties of selected starches from cereals and tubers. In this study, morphological, chemical, thermal and pasting properties of different enzymatically modified starches were studied. Thereby, the granule characteristics as well as the enzyme attack on starch granules were visualized by scanning electron microscopy (SEM) and analyzed by a micrograph processing tool. In order to establish a possible correlation, these values were combined with chemical, pasting and thermal properties.

2. Materials and methods

2.1. Materials

Potato starch (Tereos Syral, Marckolsheim, France), wheat starch (NATILOR Chamtor company, Pomacle, France), intermediate amylose rice starch (Sigma-Aldrich, Spain) and cassava starch (local market) were used as substrates for enzymatic modification. Amyloglucosidase (EC 3.2.1.3), fungal α-amylase (EC 3.2.1.1) and cyclodextrin-glycosyltransferase (EC 2.4.1.19) activities were provided by commercial food grade preparations (Amyloglucosidase 1100 L declared activity 1100 AGU/g product, Fungamyl® 2500SG declared activity 2500 FAU/g product and Toruzyme® 3.0 L declared activity 3KNU/mL product) supplied by Novozymes (Bagsværd, Denmark). All other reagents were of analytical grade. The water used was deionized.

2.2. Preparation of porous starch

The preparation of porous starch was based on the method of Benavent-Gil and Rosell [13]. The selection of enzyme levels (16.5 AMG U/g, 11 AM U/g and 0.2 CGTase U/g) was based on preliminary experiments, which showed that under the experimental conditions used (50 °C, 2 h), maximum number of pores were obtained without distorting the granule. Native starches were included for comparison, and starches subjected to treatment conditions in the absence of enzymes were used as controls.

2.3. Scanning electron microscopy (SEM)

The granule morphology of native and modified starches was observed using a JSM 5200 scanning electron microscope (SEM) (JEOL, Tokyo, Japan). Samples were coated with gold in a vacuum evaporator (JEE 400, JEOL, Tokyo, Japan) prior to observation. The obtained samples were examined at an accelerating voltage of 10 kV and magnified 3500× times.

The microstructure analysis was carried out using the image analysis program (ImageJ, UTHSCSA Image Tool software). The SEM images were saved as 8-bit tiff format. Scale was initially set using the relationship between pixels and known distance. Threshold was assessed applying the default algorithm and then particle analysis was carried out. The following parameters were measured: granule and pore area. The area occupied by pores in a starch granule was calculated as the sum of the areas of all the pores of a starch granule divided by granule area. Values were the average of 20 independent measurements.

2.4. High performance anion exchange chromatography (HPAEC)

The hydrolysis compounds (oligosaccharides and cyclodextrins) lixiviated during enzymatic treatment were quantified using HPAEC (Dionex ICS3000, Thermo Fisher Scientific, Waltham, MA, USA) according to the methodology described by Dura and Rosell [16].

2.5. Analysis of chemical and physicochemical properties of modified starches

The amount of amylose/amylopectin in the starches was analyzed using a commercially available kit (Amylose/Amylopectin Assay Kit, Megazyme International Ireland Ltd., Bray, Co. Wicklow, Ireland) following supplier instructions. This enzymatic method is based on the concanavalin A method [17]. Water and sunflower oil adsorptive capacities of starches were determined following the method described by Yousif, Gadallah and Sorour [18], with slight modifications. Samples (0.100 g ± 0.005 g) were mixed with distilled water or oil (1 mL) and centrifuged at 3000× *g* for 10 min. Adsorptive capacities were expressed as percent weight of solvent retained by the sample. Each measurement was performed in duplicate.

2.6. Viscosity measurement

The pasting properties of native and enzymatically modified starches were measured using a Rapid Visco Analyzer (RVA-4500, Perten Instruments, Hägersten, Sweden). Starch (2.00 g ± 0.01 g based on 14% moisture content) was added to 20 mL of distilled water placed into the aluminum RVA canister. Slurries underwent a controlled heating and cooling cycle, from 50 to 95 °C in 282 s, holding at 95 °C for 150 s and then cooling to 50 °C. The initial speed for mixing was 960 rpm for 10 s, followed by a 160 rpm paddle speed that was maintained for the rest of assay. Peak viscosity, final viscosity, breakdown (peak viscosity-through), setback (final viscosity-through) and onset temperature for pasting formation were determined from the viscosity plot and recorded using Thermocline software for Windows (Perten Instruments, Hägersten, Sweden). The level of hydrolysis at 95 °C and 50 °C was defined as the%-change in paste viscosity recorded in the RVA at 50 °C and 95 °C.

2.7. DSC thermal analysis

Gelatinization properties of modified starches were measured using a differential scanning calorimeter (DSC) from Perkin–Elmer (DSC 7, Perkin–Elmer Instruments, Norwalk,CT). The slurry of starch and water (1:3) was placed into stainless steel capsules. The sealed capsules were equilibrated at room temperature for one hour before analysis. The samples were then heated from 30 to 120 °C at a heating rate of 10 °C/min under nitrogen atmosphere, using an empty stainless steel capsule as reference. The onset (To), peak (Tp) and conclusion (Tc) temperatures were determined from the thermogram. The enthalpy of gelatinization (ΔH) was estimated based on the area of the main endothermic peak, expressed as joule per gram sample (J/g).

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