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Influence of glucan structure on the swelling and leaching properties of starch microparticles

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ABSTRACT

Microparticles were made by a water-in-oil emulsion technique from acid-hydrolyzed and debranched normal, waxy and high-amylose corn starches. The starches prepared had a weight-average molecular weight (M_w) ranging 3.6×10^7–2.5×10^4, a polydispersity ranging 1.16–9.16, an apparent amylose content ranging 2.84–100%. These microparticles exhibited crystallinity ranging 4.41–22.84%, swelling power ranging 2.45–7.84 and percentage of leaching ranging 1.72–74.91%. Swelling power in water ($R^2 = 0.86$) and percentage of leaching in water ($R^2 = 0.89$) were modeled by a response surface method, using the following parameters: M_w, polydispersity, apparent amylose content and crystallinity of starch in microparticles. Overall, this study showed the key parameters for controlling the behavior of starch microparticles were related to the cohesiveness of the three-dimensional network, particularly through the retrogradation of starch polymers, the formation of crystallites and junctions zones. Such microparticles could be used for designing economical and biocompatible delivery systems of compounds for food, drug, or other applications.

1. Introduction

Over the last decades, polymer-based delivery systems have gained considerable interest. These systems, commonly based on the formation of hydrogels from synthetic or natural polymer, have been extensively studied and developed (Hamidi, Azadi, & Rafiei, 2008). On the one hand, the preparation of synthetic polymers is often well understood and controlled, leading to "smart" materials with finely tuned characteristics. However, these polymers cannot be guaranteed for innocuousness, because of potential reactants remaining from their synthesis or products resulting from the polymer enteric degradation (Goddard & Hotchkiss, 2007). Moreover, these systems rely on the use of non-renewable resources. Biopolymers also exhibit disadvantages that must be overcome for delivery applications (e.g. variability of their physicochemical characteristics depending on their source). However, biopolymers that can readily make hydrogels (e.g. chitosan, carrageenan and starch) are well known for their biocompatibility and non-toxicity (Chandra & Rustgi, 1998; Reis & Cunha, 2001; Varshosaz, 2007). In this perspective, starch has been widely studied, after modifications or in combinations with other polymers (Calinescu, Mulhbacher, Nadeau, Fairbrother, & Mateescu, 2005; Elvira, Mano, San Román, & Reis, 2002; Fransén, Björk, & Edsman, 2008; Oechslein, Fricker, & Kissel, 1996; Onofre, Wang, & Mauromoustakos, 2009; Rahmouni et al., 2003; Tuovinen et al., 2004).

In the case of in vivo delivery of bioactive compounds, whether the carriers are pre-hydrated hydrogels or hydrogels formed in situ from dry particles, these systems rely on the capability of the polymer network to swell upon water absorption and eventually to disintegrate, in order to let active compounds be released and diffuse (Peppas & Brannon-Peppas, 2001). This disintegration can also be achieved via digestion of starch in the gastrointestinal tract: swelling and leaching of the loaded particles are here crucial parameters to tune the kinetics of degradation, because they will affect the accessibility of glucan chains to the digestive enzyme α-amylase (Cristina Freire, Fertig, Podczeck, Veiga, & Sousa, 2009). Achieving a wide range of swelling and leaching characteristics can then lead to controlled delivery profiles of target compounds. Thus, this study aims to show that microparticles having potential for simple delivery systems can be prepared from starch only, by taking advantage of starch molecular properties and characteristics.

Starch is composed of amylopectin and amylose, two high molecular-weight, polydisperse (1,4)-α-D-glucans. Amylopectin is highly branched through (1,6)-α linkages (M_w ~5×10^7–5×10^8, average chain length ~20–30 units) and soluble in water as an isolated polymer (as opposed to native state in granular form), and amylose is linear or slightly branched (M_w ~10^5–10^6, average chain length ~10^2–10^3 units), nearly insoluble in water as an isolated

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polymer (as opposed to native state in granular form) (Calvert, 1997; Smith & Denyer, 2003). The structure–function relationships of amylose and amylopectin and their interactions in starch networks are well understood (BeMiller, 2007, chap. 6). Among other features, amylopectin and amylose chains are able to form double helices which may in turn associate to form crystallites. In general, native starch is isolated under the form of semi-crystalline granules where amylopectin is mainly responsible for crystallinity (Calvert, 1997). When heated in presence of water, starch granules disrupt and polymers leach out to form a three-dimensional network that turns into a gel upon cooling. Cohesiveness of this gel is provided by the double helices formed by associated glucan chains (junction zones) and amylopectin is mainly responsible for long-range interactions. These associations can reach a further extent until partially recreating crystallinity (Mitchell, 2005; Smith & Denyer, 2003). This process, called retrogradation, involves amylose in the first stages and amylopectin over a longer time-scale and in a lesser extent. From the sole standpoint of starch characteristics, crystallinity of retrograded starch depends on factors such as molecular weight and chain lengths of both amylose and amylopectin and branching patterns of amylopectin (Gidley, 1989; Gidley & Bulpin, 1989; Mua and Jackson, 1997a, 1997b). However, water content (interfering with hydrogen bonding of glucan chains) and temperature profile (modifying kinetics of associations) also play major roles in network formation and retrogradation (Gidley & Bulpin, 1989; Kim, Kim, & Shin, 1997; Robin, Mérinat, Simon, & Lehmann, 2008).

In this study, molecular weight, branching degree, amylose/amylopectin ratio and crystallinity of starch polymer were considered as essential parameters to characterize starch microparticles designed as potential delivery systems, all of which affect the swelling and leaching behaviors of starch-based retrograded particules.

Indeed, weight average molecular weight (M_w) and polydispersity are two obvious parameters characterizing starch at the molecular level. Considering polydispersity allows taking into account the distribution of molecular masses in two given starches that would have the same weight average molecular weight (Gidley et al., 2010). It gives a realistic idea of what the starch network is composed of (a blend of extremely long and extremely short chains vs. a homogeneous blend of medium length chains, for example).

Apparent amylose content and crystallinity were chosen as potential parameter controlling swelling and leaching properties of the particles. Indeed, amylose chains are known to interact rapidly by forming double helices referred as junction zones in these networks. Amylopectin chains are also known to exhibit similar interactions, but these latter need a much longer period of time to occur, which may be over a week-long storage period (Gidley, 1989; Gidley & Bulpin, 1989; Mua and Jackson, 1997a, 1997b). These double helices can be packed and arranged in crystalline structures, which represent a higher degree of order compared to junction zones and are quantified by the crystallinity parameter. Apparent amylose content and crystallinity could a priori be considered respectively as indirect and direct markers of the strength of the three-dimensional network created by amylose and amylopectin (Gidley et al., 2010; Rindlav, Hulleman, & Gatenholm, 1997; Rindlav-Westling, Stading, Hermansson, & Gatenholm, 1998; Rindlav-Westling, Stading, & Gatenholm, 2002).

In this study, it was decided not to choose the branching degree as a pertinent parameter. The branching degree would have allowed differentiating the ordered structure occurring from amylose or amylopectin (Reis & Cunha, 2001). However, this differentiation appeared to be already considered in our study, since debranching (i.e. the reduction of branching degree) directly leads to increased apparent amylose content. In addition, lightly branched amylopectin molecules (after debranching treatment) could also be involved in junction zones or crystalline patterns and could participate to the strengthening of the starch network, affecting the swelling and leaching properties of the particles. These effects are taken into account by the measurement of apparent amylose content and crystallinity, as well as M_w and polydispersity, and the characterization of branching degree could be considered redundant in this perspective.

Subsequently, this study aims to: (1) provide a practical platform for manipulating the swelling and leaching properties of "starch-only" microparticles, and (2) reveal the impact of amylose and amylopectin on the specific properties of starch particulates as potential delivery agents. To achieve this aim, microparticles were made by water-in-oil emulsion from starches that were specifically degraded and debranched. Their swelling and leaching properties in water were characterized, and these two parameters were modeled according to molecular parameters of starch, including molecular weight and polydispersity, amylose content, and crystallinity.

2. Experimental

2.1. Materials

Normal corn starch (NCS, 24.8% amylose), high amylose corn starch (HACS, 74.1% amylose) and waxy corn starch (WCS, 0% amylose) were provided by Cargill Inc. (Minneapolis, MN). All chemicals were purchased from VWR International (Radnor, PA) except for those indicated otherwise.

2.2. Starch modification

2.2.1. Acid hydrolysis

A 30% (w/w on dry starch basis) slurry was made by dispersing starch in 0.6 M hydrochloric acid. The slurry was placed in an oscillating water bath at 50 °C and agitated at 90 rpm over 4, 8 or 16 h. After acid treatment, the slurry was neutralized with 1.0 M sodium hydroxide and centrifuged at 3200 × g and 4 °C for 10 min (Allegra 6KR centrifuge, Beckman Coulter, Brea, CA). The starch recovered was then washed three times with de-ionized water and recovered each time by centrifugation (3200 × g, 4 °C, 10 min). The recovered starch was then dried overnight in a ventilated oven (FED53 with R3.1 controller, Binder, Bohemia, NY) at 55 °C. In further description of the materials, a 0-h acid treatment means that starch did not undergo the acidic treatment but was dried overnight along the samples with acid treatment.

2.2.2. Debranching

A 5% (w/w on dry starch basis) slurry was made by dispersing starch in a 200 mM sodium acetate aqueous solution (pH 5.0), and autoclaved at 121 °C for 10 min. The gel obtained was cooled to 40 °C in an agitated water bath (90 rpm, 40 °C) and pullulanase (P5420 from *Klebsiella pneumoniae*, Sigma–Aldrich, St. Louis, MO) was added (2.0%, w/w on dry starch basis). Debranching was performed for 9, 18 or 36 h in a water bath. At the end of the treatment, the starch dispersion was placed for 10 min in a boiling water bath to inactivate the enzyme. Starch was then precipitated with ethanol, recovered by centrifugation (3200 × g, 4 °C, 10 min), washed three times with ethanol and recovered each time by centrifugation (3200 × g, 4 °C, 10 min). The recovered starch was then dried overnight in an oven with air-circulation at 55 °C. In further description of the materials, a 0-h debranching refers to starch that did not undergo the pullulanase treatment but was autoclaved, placed in a boiling water bath, precipitated with ethanol, washed with ethanol and dried overnight like debranched starches.

Materials are subsequently coded as follows: "type of starch-acid treatment time-debranching time". For example, high amylose