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Impact of reagent infiltration time on reaction patterns and pasting properties of modified maize and wheat starches

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ABSTRACT

The impact of granular and molecular reaction patterns on modified starch properties was investigated as a function of the length of time allowed for reagent to infiltrate starch granules. A fluorescent reagent [5-(4,6-dichlorotriazinyl)aminofluorescein] was dispersed in aqueous normal maize or wheat starch slurries (35%, w/v) for 0, 5, 10, 30, or 60 min, after which reaction was initiated by increasing the pH to 11.5 and allowing reaction to proceed for 3 h. With increasing lengths of infiltration, the reaction became increasingly homogeneous within the granule interior (matrix) and the AM:AP reactivity ratio increased (wheat starch), as assessed by confocal laser scanning microscopy (CLSM) and size-exclusion chromatography (refractive index and fluorescence detection), respectively. A longer reagent infiltration time also led to a more inhibited (i.e., cross-linked) pasting viscosity, suggesting that both granular and/or molecular reaction patterns were altered by varied reagent infiltration times to ultimately impact modified starch properties.

1. Introduction

Native starch properties are improved by chemical modification, enhancing starch functionality for intended food and industrial applications. Though chemical modification of starch is industrially practiced, the impact of the granular and molecular reaction locale on the physical properties of modified starches has not yet been established.

At the granular level, microstructural features of starch granules such as pores and channels, which connect the central cavity region of granules (corn, sorghum, wheat, etc.) to the extra-granular environment, provide a direct path for reagent to access the interior regions of starch granules and facilitate reagent flow into granule matrix (Huber & BeMiller, 1997, 2000, 2001). These findings have cultivated the technical insight and capacity to directly detect reaction sites/patterns within starch granules. Reaction patterns within derivatized starch granules have been probed with anionic reagents and reagent analogs (Gray & BeMiller, 2004, 2005; Huber & BeMiller, 2001; Kim, 2009; Kim & Huber, 2013), as well as a fluorescent probe (Hong & Huber, 2015a; Kim & Huber, 2008), via confocal laser scanning microscopy (CLSM). Reaction conditions and parameters (pH, swelling inhibiting salt, and/or temperature) that promote a high degree of starch swelling generally enhance reagent penetration into granules and favor a uniform granular derivatization pattern (Gray & BeMiller, 2005). A high level of reagent (Huber & BeMiller, 2001; Kim & Huber, 2013) or an increasing hydration of granules prior to the derivatization (Hsieh & Huber, 2012) further increase reaction uniformity within starch granules. A model system approach, utilizing a fluorescent probe as a reagent, has the advantage of facilitating straightforward monitoring of not only granular, but also molecular, reaction patterns (Higley, 2005), allowing the effects of reaction conditions and parameters on starch reaction patterns to be directly assessed. On a molecular level, Hong and Huber (2015a) provided the first-ever report of reaction kinetics for amylose (AM) and amylopectin (AP) branch chains (long, medium, short) as a function of reaction time. As reaction time increased, reagent gradually diffused from external granular surfaces into the granule matrix, altering the relative extents of reaction among AM and AP branch chains. This result suggested that starch molecular, as well as granular, reaction patterns might be altered by manipulating reaction system conditions, and that specific combinations of granular and molecular reaction patterns

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might have potential to produce modified starches with different physical properties.

The objective of this study was to investigate both granular and molecular reaction patterns, and their collective impact on modified starch pasting behavior, as a function of varied reagent infiltration times (i.e., length of time for reagent to penetrate starch granules before formal reaction was initiated by addition of base). While the presence of surface pores and channel structures in cereal starches has been reported to provide reagent with direct access to the granule interior (Huber & BeMiller, 1997; Kim & Huber, 2008), a direct comparison of normal maize and wheat starches has not yet been conducted to contrast similarities and differences in their granular and molecular reaction patterns under similar reaction conditions. Starch derivatives, which were produced in a model reaction system utilizing a fluorescent probe as the reagent, were assessed at the granular (CLSM) and molecular (reactivity of AM and AP branch chains) levels via intermediate pressure size-exclusion chromatography (IPSEC) equipped with both refractive index (RI) and fluorescence (FL) detection. A Rapid Visco Analyzer (RVA) was used to evaluate the pasting properties of starch derivatives. This study seeks to contribute new insight into the interrelationship between granular/molecular reaction patterns and modified starch physical properties to improve technical practices for creating functional modified starch products.

2. Materials and methods

2.1. Starch sources and chemicals

Laboratory-isolated normal maize starch was prepared in the laboratory of J.N BeMiller (Purdue University, West Lafayette, IN) using the procedure described by Wongsagonsup, Varavinit, and BeMiller (2008). Commercial wheat starch (Midsol^{TM}50, MGP Ingredients, Inc., Atchison, KS) consisted of approximately 99.7% A-type granule starch (volume/weight basis), as determined by particle size analysis (Geera, Nelson, Souza, & Huber, 2006). The fluorescent probe, 5-(4,6-dichlorotriazinyl)aminofluorescein (DTAF), which was utilized as the starch modifying reagent, was purchased from Sigma-Aldrich Corporation (St. Louis, MO USA), while isoamylase (EC 3.2.1.68; 1000 U/ml) was acquired from Megazyme International Ireland Ltd (Co. Wicklow, Ireland) for debranching of starch chains. Other reagents and chemicals used were at minimum of analytical grade, and were obtained from Sigma-Aldrich Corporation unless indicated otherwise.

2.2. Starch derivatization with DTAF with varied reagent infiltration times

Starch (5.0 g, db) was dispersed in deionized water (13.8 and 14.0 mL for maize and wheat starch, respectively) with stirring to maintain granules in suspension, after which 300 μL (maize) or 50 μL (wheat) of DTAF solution (10%, w/v in dimethyl sulfoxide) was added to the reaction slurry. After DTAF addition, the starch reaction slurry was stirred for varied lengths of time (0, 5, 10, 30 or 60 min) prior to adding 2 M sodium hydroxide (230 μL and 275 μL for maize and wheat starches, respectively, to achieve a reaction pH of 11.5) to formally initiate the reaction. Following pH adjustment, derivatization was allowed to proceed for 3 h at ambient temperature. A few drops of 2 M NaOH (≤100 μL in total) were added as needed to maintain the pH at 11.5 during reaction. At the conclusion of the derivatization period, the reaction was terminated by neutralizing the reaction medium with 1 M hydrochloric acid. The entire reaction slurry was transferred to a conical tube (50 mL), and centrifuged (3000*g*, 10 min) to recover the modified starch pellet. The starch pellet was suspended (15 min) in 85% (v/v) aqueous ethanol (40 mL) on a wrist action shaker (Model 75, Burrell Co., Pittsburgh, PA), after which it was recovered by centrifugation (3000*g*, 10 min). This washing procedure was repeated until the resulting supernatant became colorless (i.e., unreacted DTAF was removed). The final starch pellet was dispersed in absolute ethanol, vacuum-filtered over a Büchner funnel, and air-dried.

A reaction control (Reaction-Control) was generated for each infiltration time/reaction condition described above without the addition of reagent. An additional set of controls (Infiltration-Controls) was subjected only to the reagent infiltration step (reagent included), after which each starch was recovered immediately thereafter without undergoing the formal 3 h reaction in the presence of sodium hydroxide. All reaction controls were subjected to the same washing and recovery procedures outlined previously.

2.3. Confocal laser scanning microscopy and light microscopy of starch granules

Granular reaction patterns for starch derivatives were visualized via confocal laser scanning microscopy (CLSM), with sample preparation conducted as described by Huber and BeMiller (2000). A CLSM system (Zeiss LSM 510, Thornwood, NY, USA) equipped with an inverted microscope was used to generate serial cross-sections (1 μm thickness) of modified starch granules as described by Kim and Huber (2008). Excitation was achieved with an Argon laser (488 nm) operated at 30% power, and emission was regulated through a LP 505 emission filter. During image acquisition, microscope parameters (confocal pinhole, detector gain, and amplification factor) were fixed for all starch derivatives regardless of starch type (maize or wheat) or reagent infiltration time, such that observed differences in fluorescent intensity and patterns reflected true differences in extent of reaction. A single cross-section representing the approximate geometric center of granules was utilized to assess granular reaction patterns. Acquired images were processed using Zeiss LSM Image Browser software.

Following pasting analysis (Section 2.5), pastes of native and DTAF-derivatized starches were visualized with a Nikon Eclipse E600 light microscope (Nikon Inc. Instrument Group, Melville, NY, USA) to compare the structural integrity of granule remnants. One drop each of starch paste and I_2/KI solution (0.2% I_2 in 2% KI, diluted 2:1 with distilled water) were placed on a glass slide, and overlaid with a glass cover slip in preparation for viewing.

2.4. Intermediate pressure size-exclusion chromatography of debranched starch derivatives

Starch was solubilized in DMSO assisted by microwave heating, debranched with isoamylase, and prepared for intermediate size-exclusion chromatography (IPSEC) as described by Hong and Huber (2015a), except that only 25 mg of starch (db) was utilized for solubilization, and the amount of isoamylase for debranching was reduced accordingly (6.25 μL of isoamylase with 1000 U/mL of activity).

Debranched starch solution was injected onto an IPSEC system (Waters Corp., Milford, MA, USA), equipped with a 1525 binary HPLC pump array, a Rheodyne 7723i manual sample injector with a 200 μL sample loop, and 2410 refractive index (RI, operated at 30 °C) and 2475 fluorescence (FL) detectors. Starch chains were eluted (0.4 mL/min) with deionized water containing 0.02% sodium azide (w/v) as a mobile phase through two successive Tricorn (10/300) columns packed with 30 cm of Superdex-G75 (M_r: 3×10^3–7×10^4) and Superdex-G30 (M_r: ~1×10^4) prep grade gels, respectively (Amersham Biosciences, Piscataway, NJ). DP_w for debranched starch chain fractions [AM and AP long (AP-LC), medium (AP-MC), and short (AP-SC)] were estimated using pullulan standards (Shodex Standard P-82, JM Science, Inc, NY)