Nanostructure, morphology and functionality of cassava starch after pulsed electric fields assisted acetylation

Jing Hong [a], Xin-An Zeng [a, *], Roman Buckow [b], Zhong Han [a, **], Man-sheng Wang [a]

[a] College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510641, China
[b] CSIRO, Food and Nutrition Flagship, Werribee 3030, VIC, Australia

ARTICLE INFO

ABSTRACT

Pulsed electric fields (PEF) assisted acetylation of cassava starch with a higher degree of substitution (DS) of 0.131 was obtained in the presence of 10% (w/w) acetic anhydride after PEF treatment at 2 kV/cm for 51.0 ms. Fourier-transformed infrared spectroscopy showed a characteristic peak of the acetyl group in modified starch at 1730 cm^{-1}. The crystalline region of acetylated starch was destroyed and rearranged as confirmed by X-ray diffraction. Small-angle X-ray scattering indicated that the fractal structure was changed from a surface fractal structure to a mass fractal structure with increasing DS probed. Atomic force microscopy showed that PEF assisted acetylation of starch markedly increased the surface roughness. The prominent freeze-thaw stability of PEF-assisted, acetylated starch demonstrated its potential and superior application in frozen food products.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Native cassava starch has limited industrial applications due to its hydrophilic nature of hydroxyl groups, which limits its use in starch-based products. Thus, various physical, chemical, enzymatic and genetic techniques have been used to modify the starch molecules and improve its physiochemical properties to satisfy industry requirements (Kavlani Neelam and Singh, 2012). Chemical modification of starch is often carried out to improve functional and physicochemical parameters of starch including solubility, pasting properties, thickening power, shear stability or digestibility (Miao et al., 2014). The modification of starch with acetic anhydride at low degree of substitution (DS) of 0.01–0.2 is commonly prepared by esterification in aqueous medium in the presence of an alkaline catalyst (Wang & Wang, 2002). Acetylated (AC) starch at a low DS offers desirable properties such as low-temperature stability (reduced syneresis), high-thickening power, low-gelatinization temperature, clarity of cooked food, good film-forming properties and reduced tendency to retrogradation (Chi et al., 2008; Lawal, 2004; Zięba, Szumny, & Kapelko, 2011). This is mainly due to the introduction of acetyl groups, which interrupt the ordered structure of native starch and reduce the electrostatic interaction of amylose and amylopectin chains. In addition, AC starch is an effective emulsifier due to the addition of dual functional hydrophilic and hydrophobic groups (Bao, Xing, Phillips, & Corke, 2003).

The transformation of AC starch was actually attributed to the alternated nanostructure, fractal and lamellae revealed by combining molecular investigations in nanostructure (SAXS) and microscopic characterization (AFM). The semi-crystalline shells were characterized by alternating high- and low-density lamellae with a repeat distance of 9–10 nm (Vermeylen et al., 2006). Recently, AFM observations showed that starch esters exhibited an aggregative nature, with aggregates being comprised of small spherulites, lamellae of a few microns and some other ill-defined structures (Zabar, Lesmes, Katz, Shimoni, & Bianco-Peled, 2010; Zhu, Li, Chen, & Li, 2013).

In recent years, the method of preparation AC starch has changed from simply exposing starch granules to acetic anhydride at room temperature (Bhosale & Singhal, 2006) to methods applying micro-wave (Biswas et al., 2008; Jyothi, Rajasekharan, Moorthy, & Sreekumar, 2005), enzymes (Huang et al., 2010), high pressure (Shogren, 2003), high temperature (Kim, Sandhu, Lee, Lim, & Lim, 2010), ball milling pre-treatment (Zhang, Zhao, & Xiong, 2010) as well as chemical synthesis (Aini & Purwiyatno, 2010) before or during acetylation in order to achieve higher DS in

---

* Corresponding author.
** Corresponding author.
E-mail addresses: xazeng@scut.edu.cn (X.-A. Zeng), fezhonghan@scut.edu.cn (Z. Han).

starches and reduce the use of regents and processing time (Matsumoto, Tsubaki, Sakamoto, & Azuma, 2011). Pulsed electric fields (PEF), a non-thermal, low energy processing technique, has been used to modify properties of starches from different origins (Han et al., 2012; Han, Zeng, Zhang, & Yu, 2009; Han, Zeng, Yu, Zhang, & Chen, 2009). It has been shown that the PEF treatment can enhance starch esterification under appropriate conditions (Lin, Zeng, Yu, & Sun, 2012; Zeng, Han, & Zi, 2010). Therefore, acetylation of starch combined with PEF treatment could be a novel technique to improve the DS at the same acetic anhydride content.

The objective of the present study was to investigate the effect of different PEF conditions on acetylation of cassava starch. Furthermore, the crystalline structure and selected physicochemical properties of obtained AC starches will be investigated by spectroscopic, microscopic, thermal, and physical techniques and potential applications in industry discussed.

2. Materials and methods

2.1. Materials

Cassava starch was obtained from Guangzhou Mingyu Food Development Co., Ltd. (Guangzhou, China). Acetic anhydride and ethanol (100%) were purchased from Beihua Fine Chemicals Co., Ltd. (Beijing, China). All chemicals used in the study were of analytical grade.

2.2. PEF-assisted acetylation

AC starch was prepared using a method with modification (Sodhi & Singh, 2005). The cassava starch was dried at 45 °C for 24 h to a moisture content of approximately 8.0% (w/w). Cassava starch was dispersed in distilled water at a concentration of 35% (w/w). The starch slurry was agitated at 30 °C for 5 min in a 250 mL three-neck flask fitted with a mechanical stirrer at 300 rpm (IKA, RW 20 digital, Germany) to activate starch. Subsequently, diverse amounts of acetic anhydride was added drop-wise within 30 min while the pH was maintained at 8.0–8.5 using 0.75 M NaOH solution. The electrical conductivity of reaction mixtures were in 9.4–10.6 mS/cm according to the different addition of 6–10% acetic anhydride. The electrical conductivity of the suspensions was uniformly adjusted to 11 mS/cm by adding 2.0 mol/L sodium chloride solution. Starch samples were then pumped through the PEF system with a peristaltic pump (Yz1515x, Longer Precision Pump Co. Ltd., Baoding, China). Native and AC starch samples were treated in a bench-scale, continuous PEF system as illustrated in Fig. 1 (SCUT PEF Team, the South China University of Technology, Guangzhou, China). The PEF system features a co-linear treatment chamber with an inner diameter of the electrodes and insulator of 3 mm and an electrode gap of 3 mm. PEF treatment was performed at a flow rate of 60 mL/min applying bi-polar, rectangular pulses of 40 μs at 2000 Hz and the delay time between the positive and negative pulses was 460 μs. The input voltage of the system was 450–750 V corresponding to electric field strengths of 1.5–2.5 kV/cm. The samples were cooled to 20 °C before being pumped to the PEF chamber for next cycle. During the whole reaction, pH was sustained at 8.0–8.5. After the PEF treatment, samples were washed three times with distilled water and twice with 100% ethanol. The solid was oven dried at 45 °C for 12 h (LinpinIsotemp 202-00 A, Linpin Scientific Instrument Co., Shanghai, China). After drying, the samples were passed through a 120-mesh sieve for subsequent use. According to the conventional method of Ayucitra (2012), samples of 35% starch concentration, which were added 6%, 8%, 10% acetic anhydride content and reacted for 60 min, were defined as control samples.

2.3. Experimental design

To optimize the extraction conditions and verify the synergetic influence of variables, the Box–Behnken experimental design (BBD) of the response surface methodology (RSM) by the Design Expert Version 8.0.5 (Stat-Ease Inc., Minneapolis, USA) was used with three variables at three levels, respectively (Table 1a,b). The independent variables and their optimization ranges, such as strength of PEF (X_1, 1.5–2.5 kV/cm), total cycle times in PEF system (X_2, 27–51 ms), and amount of acetic anhydride (X_3, 6–10%) were selected as experimental factors. It is worth noting that the effective PEF treatment time were 27.2 ms (16 cycles passed through the PEF system), 39.1 ms (23 cycles passed through the PEF system) and 51.0 ms (30 cycles passed through the PEF system), respectively as calculated by Eq. (1-3). The specific PEF energy applied to the fluid during one cycle were 42 kJ/L (1.5 kV/cm), 72 kJ/L (2 kV/cm), 117 kJ/L (2.5 kV/cm), respectively. The samples were cooled to 20 °C before being pumped to the PEF chamber for next cycle. Thus, the temperature of starch slurry increased from 20 °C to 30 °C (1.5 kV/cm), 37 °C (2 kV/cm), 48 °C (2.5 kV/cm), respectively which can make use of the synergetic effects of mild heat on the PEF treatment efficiency (Buckow, Schroeder, Berres, Baumann, & Knoerzer, 2010). PEF treatment time (t) was calculated with the number of pulses (N_p) received in the chambers ($N_c = 1$), which is obtained from the residence time in a chamber (t_v) as Eq. (1).

$$t_v = V/\Phi \tag{1-1}$$

where V is the volume of PEF chamber (m^3) and Φ is the flow rate (m^3/s).

$$N_P = t_v \times f \tag{1-2}$$

where f is the pulse frequency (Hz).

$$t = N_p \times N_c \times \tau \tag{1-3}$$

where N_c is the number of treatment chambers and τ is the pulse width (s).

The energy input (Q) was calculated by the following equation used by Zhao et al. (2008):

$$Q\left(J/m^3\right) = E^2 \sigma t \tag{1-4}$$

where E is the electric field strength (V/m), s is the electrical conductivity of the product (S/m) calculated for each process temperature, and t is the PEF treatment time (s).

A second order polynomial equation model was used to fit the yield with interaction terms as given in Eq. (2). And each experiment was replicated at least three times and the DS (%, Y_i) was taken as the responses of the experiments. Sample 0 was the control of raw cassava starch which was washed and dried as AC starches.

$$Y_i = A_0 + A_i \sum X_i + A_{ij} \sum X_i X_j + A_{ii} \sum X_i^2 (i \neq j) \tag{2}$$

where Y_i are the predicted response values; A_0, A_i, A_{ij} and A_{ii} are the regression coefficients of the model for the intercept, linear cross product terms and quadratic, respectively; X_i are the variables.

2.4. DS determination

DS value was determined titrimetrically, following the method outlined by Singh, Chawla, and Singh (2004). AC starch (1.0 g, dry basis) was placed in a 250 mL flask and 50 mL of 75% (v/v) ethanol