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Effects of citric acid esterification on digestibility, structural and physicochemical properties of cassava starch

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ABSTRACT

In this study, citric acid was used to react with cassava starch in order to compare the digestibility, structural and physicochemical properties of citrate starch samples. The results indicated that citric acid esterification treatment significantly increased the content of resistant starch (RS) in starch samples. The swelling power and solubility of citrate starch samples were lower than those of native starch. Compared with native starch, a new peak at 1724 cm^{-1} was appeared in all citrate starch samples, and crystalline peaks of all starch citrates became much smaller or even disappeared. Differential scanning calorimetry results indicated that the endothermic peak of citrate starches gradually shrank or even disappeared. Moreover, the citrate starch gels exhibited better freeze–thaw stability. These results suggested that citric acid esterification induced structural changes in cassava starch significantly affected its digestibility and it could be a potential method for the preparation of RS with thermal stability.

1. Introduction

Starch is the main component of cereal grains and the most important dietary source of energy for humans. It is composed of essentially linear amylose and highly branched amylopectin with α-D-glucopyranose as the structural unit (Zhang, Sofyan, & Hamaker, 2008).

For nutritional purposes, starch is generally classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) in terms of the rate and extent of its digestion (Englyst, Kingman, & Cummings, 1992). RDS induces a rapid increase in blood glucose and insulin levels after ingestion. SDS prolongs the release of glucose, thus preventing hyperglycaemia-related diseases. RS reduces starch availability for digestion and produces short-chain fatty acids in the large bowel through fermentation, which is beneficial to colon health and protection against colorectal cancer (Lehmann & Robin, 2007). Furthermore, resistant starch has been categorized into four classes: physically inaccessible starch (RS1), granular starch (RS2), retrograded amylose or high amylose starches (RS3), and chemically modified starches (RS4) (Eerlingen & Delcour, 1995). Consequently, starch ingredients containing high levels of SDS and RS can improve the nutritional function of foods.

In recent years, many types of chemically modified starches have been prepared by acid hydrolysis, oxidation, etherification and cross-linking (Santacruz, Koch, Svensson, Ruales, & Elisson, 2002). Esterification is one of the most important chemical modification methods for starch. Acetic, citric and formic acids have been often used for chemical modification of starch (Klaushofer, Berghofer, & Steyner, 1978). Compared with other substances, citric acid is nutritionally harmless, and the rate of digestion of esterified starch by pancreatin is decreased with increasing degree of substitution (DS) by citrate (Klaushofer et al., 1978). Citric acid, when heated, will dehydrate to form an anhydride, which will react with starch to form a starch-citrate adduct. Further heating can result in additional dehydration of citric acid and give rise to cross-linking (Wing, 1996). Xie and Liu (2004) reported that 78.8% resistant starch was obtained when citric acid (40% of starch dry weight) was reacted with normal corn starch at 140 °C for 7 h. Shin et al. (2007) showed that the SDS fraction of citric acid-treated rice starch increased to 54.1% after heat treatment.

Cassava starch contains less amylose as compared to wheat, potato and maize starches, and it has some superior qualities like bland taste and flavor, high paste clarity and less tendency to retrograde (Raja, 1995). Esterification of cassava starch for preparing low DS acetates and citrates was investigated by Agboola, Akingbala, and Oguntmein (1991). However, the effect of citric acid esterification on the structural and physicochemical properties of cassava starch has not been reported.

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The aims of the present study were to investigate the influence of different citric acid concentrations on the esterification reaction of cassava starch, and the digestibility, structural and physicochemical properties of citrate starch samples with different DS were compared.

2. Materials and methods

2.1. Materials

Cassava starch was obtained from the Guangxi Hongfeng Starch Company (Guangxi, China). α-amylase type VI-B from porcine pancreas (EC 3.2.1.1, A3176) and amyloglucosidase (EC 3.2.1.3) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA) and Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China), respectively. All other chemicals were of analytical grade (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China).

2.2. Preparation of citrate starch samples

Citrate starches were prepared based on the method described by Klaushofer et al. (1978) with some modifications. Citric acid (10%, 20%, 30%, 40% of starch dry weight) was dissolved in 12 mL distilled water, then the pH of the solution was adjusted to 3.0 with 10 M NaOH, and finally diluted to the final volume of 24 mL. The citric acid solution (24 mL) was mixed with 20 g cassava starch in a stainless steel tray and conditioned at room temperature for 8 h. The tray was then placed in a forced-air oven and dried at 50 °C for 24 h to a moisture level of 5–10% (w/w). The mixture was ground and dried at 130 °C for 5 h in a forced-air oven. The dry mixture was washed three times with distilled water and centrifuged to remove unreacted citric acid. Finally, the washed starch was air-dried at 45 °C and ground again.

2.3. Determination of in vitro digestibility of starch samples

The *in vitro* digestibility of starch samples was determined according to the method of Englyst et al. (1992) with a slight modification. Starch sample (200 mg) was dispersed in distilled water (5 mL) in 50-mL screw-capped polypropylene centrifuge tube and mixed well. After mixing, the tube was placed in a boiling water bath for 20 min. The sample was stirred with magnetic stirring bars during heating. After cooking, the tube was placed in a 37 °C water bath until it is cooled. Then phosphate buffer (10 mL, 0.2 mol/L, pH 5.2) and five glass balls (10 mm in diameter) were added to centrifuge tube. After equilibration at 37 °C for 5 min, the enzyme solution (5 mL) was added to the sample tube, followed by incubation in a water bath at 37 °C with shaking (170 rpm).

Aliquots (0.5 mL) were taken at intervals of 20 and 120 min and mixed with 4 mL of 80% ethanol to deactivate the enzymes. The mixed solution was centrifuged at 2000 rpm for 10 min, and the glucose content in the supernatant was measured using the 3, 5-dinitrosalicylic acid (DNS) method (Miller, 1959). The percentage of hydrolyzed starch was calculated by multiplying a factor of 0.9 with the glucose content (Zhang et al., 2011). Each sample was analyzed in triplicate. The percentages of RDS, SDS, and RS in the samples were calculated by the following equations:

$$\text{RDS}\ (\%) = [(G_{20} - \text{FG})/\text{TS}] \times 0.9 \times 100$$

$$\text{SDS}\ (\%) = [(G_{120} - G_{20})/\text{TS}] \times 0.9 \times 100$$

$$\text{RS}\ (\%) = [(\text{TS} - \text{RDS} - \text{SDS})/\text{TS}] \times 100$$

where, G_{20} and G_{120} are the amounts of glucose released within 20 and 120 min of hydrolysis, respectively, and FG is the amount of free glucose in starch and TS is total starch weight.

2.4. Determination of degree of substitution (DS)

The amount of citric acid esterified to the starch was analyzed by the method of Klaushofer, Berghofer, & Pieber (1979), which was based on the reaction of citric acid and Cu^{2+} that formed a stable complex during titration with a solution of copper sulfate. DS was calculated based on the average number of substituent groups per anhydroglucose unit. Briefly, the starch sample (450 mg) was dispersed in 2 mL deionized water and dissolved in 50 mL of 1 M KOH. The solution was then boiled in a water bath for 10 min. After the solution was cooled to 25 °C, the pH was adjusted to 8.5 with 5 M acetic acid. The solution was added to sodium borate buffer (25 mL, pH 8.5H) containing indicator (0.3 g; Murexide:sodium sulfate = 1:500, w/w) and diluted to 300 mL with deionized water. The solution was titrated with 0.05 M copper sulfate solution until the color of the indicator (red-violet) disappeared. DS was calculated by the following equation:

$$\text{DS} = (162 \times W)/(100 \times M) - (M - 1) \times W$$

where W (% by weight of substituent) = [bound citrate (g)/sample (g) – bound citrate (g)] × 100, and M = molecular weight of the citric acid substituent which was 175.1. Each sample was analyzed in triplicate.

2.5. Swelling power and solubility

Swelling power (SP) and solubility (S) were measured according to a modified method of Schoch (1964). A starch sample (0.5 g) was suspended in the centrifuge tube containing 50 mL distilled water and kept in a shaking water bath at 55 °C, 65 °C, 75 °C, and 85 °C for 30 min, respectively. The centrifuge tube was then cooled rapidly to 25 °C. After centrifugation at 2000 rpm for 15 min, the supernatant was collected and dried at 105 °C for 2 h and the remnant was weighed, and the precipitate was weighed. The solubility (%) was determined as the weight ratio of the dried supernatant to the dry starch. Swelling power (g/g) was determined as the weight ratio of the precipitate in the tube to the dry starch.

2.6. Fourier-transform infrared spectroscopy (FT-IR)

FT-IR was measured according to the method of Li, Ward, and Gao (2011) and Xie, Hu, Jin, Xu, and Chen (2014). All infrared spectra were obtained on a Nicolet 6700 spectrometer (Thermo Electric Corporation, Waltham, MA, USA). A spectral resolution of 4 cm^{-1} was employed and 64 scans were acquired for each spectrum. Spectra were baseline-corrected and deconvoluted by drawing a straight line at 1200 and 800 cm^{-1}. The absorbance ratio of 1047 cm^{-1}/1022 cm^{-1} was obtained from the deconvoluted spectra using Omnic version 8.0 software (Thermo Fisher Scientific Inc. Waltham, MA, USA). The ratio of 1047 cm^{-1}/1022 cm^{-1} from deconvoluted FT-IR spectrum has been used to express the amount of ordered crystalline to amorphous domains in starches.

2.7. Differential scanning calorimetry (DSC)

The thermal properties of starch samples were determined by DSC (Q200, TA Instruments, New Castle, DE, USA) according to the method described by Xie et al. (2014) with a slight modification. Approximately 3 mg anhydrous starch sample was mixed with 6 μL deionized water and hermetically sealed in an aluminum pan. Then the pan was equilibrated at 4 °C for 24 h. After