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Resistant glutarate starch from adlay: Preparation and properties

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ABSTRACT

Reaction conditions were optimized to increase the content of resistant starch in adlay starch using esterification with glutaric acid, and the physicochemical properties of the prepared glutarate starches were investigated. Different amounts of glutaric acid (0.1–0.5 g/g starch, dry weight basis) were reacted with adlay starch at various temperatures (70–130 °C) and reaction times (3–9 h). The resistant starch levels increased with increased glutaric acid content, reaction temperature, and reaction time. The color difference was mainly affected by reaction time. The highest resistant starch content (RS 66%) was obtained using conditions of 0.4 g glutaric acid/g starch, 115 °C, and 7.5 h, with a color difference of 10.24. After digestion with α-amylase and amyloglucosidase, the water-soluble fraction of glutarate starch had more oligosaccharides than high-amylose maize starch (RS 43%). FT-IR and solid-state NMR detected carbonyl groups in the glutarate starch, indicating the formation of cross-linkages through esterification. The granular structure of the glutarate starches was not destroyed and they retained birefringence. After heating with an excess of water, the granules kept their shape but lost their birefringence. The glutarate starches had low solubility in both cold and hot water, and the resistant starch contents were unchanged after heating due to the restriction of swelling by cross-linking. The glutarate starches had a similar chain-length distribution to raw starch, indicating that acid hydrolysis took place at branching points in the amorphous region. Furthermore, the glutarate starches possessed a weaker crystalline region, more diverse double helical chains, and lower enthalpy than raw starch.

1. Introduction

In plants, starch is the main carbohydrate reserve and an important ingredient in human nutrition. Nutritionally, starch is classified into rapidly digestible starch, slowly digestible starch, and resistant starch (RS) based on the rate and degree of digestion (Englyst, Kingman, & Cummings, 1992). Resistant starch escapes enzymatic digestion in the small intestine, but in the large intestine microbial flora may ferment some RS. RS displays many of the physiological benefits of dietary fiber, such as calorie reduction, colonic health, increased stool bulk, decreased stool transit time, and generation of volatile free fatty acids (Bird, Brown, & Topping, 2000; Brouns, Kettlitz, & Arrigoni, 2002; Murray et al., 1998; Silvester, Bingham, & Pollock, 1995). RS acts like dietary fiber as it has a probiotic effect on colon microflora, alters lipid metabolism, improves cholesterol metabolism, and reduces the risk of ulcerative colitis; it also reduces the glycemic index of foods (Hoebler, Karinthi, Chiron, Champ, & Barry, 1999).

RS is classified into four categories based on its resistance to digestion. RS1 is physically inaccessible to digestion by entrapment in a non-digestible matrix, RS2 is ungelatinized starch, RS3 is retrograded starch, and RS4 is chemically modified starch (Englyst et al., 1992; Thompson, 2000).

RS4 can be produced by chemical modifications, such as conversion, substitution, or cross-linking, which can prevent its digestion by blocking enzyme access and forming atypical linkages such as 1→2, 1→3, 1→4, and 1→6 linkages (Wurzburg, 1986). Klaushofer, Berghofer, and Steyrer (1978) developed a cross-linked starch using citric acid. Xie and Liu (2004) and Xie, Liu, and Cui (2006) established conditions for the preparation of resistant citrate starch based on the Klaushofer method and studied its physicochemical properties and granular structure. Citrate starches showed no pathological changes as compared to native wheat and corn starches (Klaushofer et al., 1978).

Polyfunctional carboxylic acids (malic, tartaric, citric, malonic, succinic, glutaric, and adipic acids) have been used in the synthesis of hydrogels, and the carboxymethyl starch hydrogels have been rheologically characterized (Seidel et al., 2001).

Coix lachryma-jobi L., commonly known as adlay or Job's tears, is a grass crop widely grown in East Asia. The grain is generally

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spherical, about 5 mm in diameter, and has a hard, shiny dark brown to gray hull. Adlay has long been used as a traditional Oriental medicine, and is also used in baked products, soups, tea, and distilled liquor as flour or whole grain (Arora, 1977). It has been reported that adlay grains may have antitumor activity (Numata, Yamamoto, Moribayashi, & Yamada, 1994) and be effective against viral infection (Hidaka, Kaneda, Amino, & Miyai, 1992). Owing to its perceived nutritional and health value, adlay is increasingly utilized in functional foods and drinks (Li & Corke, 1999). Although adlay grains are high in starch, only a few studies have been done on the physicochemical properties of its starch (Ikawa, Kang, Asaoka, Sakamoto, & Fuwa, 1983; Li & Corke, 1999; Ramirez, 1996a, b; Woo, Yoon, & Kim, 1985). Furthermore, there has been no report on modification of adlay starch for more efficient utilization of adlay in the food industry and exploration of new product development with adlay.

In this study, glutaric acid, a potential food acidulant (Merten & Bachman, 1978), was used as a cross-linking agent. Citric acid has three carboxyl groups that react with hydroxyl groups in the starch chain to form ester bonds; glutaric acid with two carboxyl groups reacts similarly. Nevertheless, due to the difference in number of carboxyl groups, glutarate and citrate starches have different physicochemical and structural properties. We also investigated the physicochemical and structural properties of glutarate starch with a high resistant starch content and a low color difference.

2. Materials and methods

2.1. Preparation of adlay starch

Adlay (*Coix lachryma-jobi* L. var. ma-yuen Stapf) was obtained from Yonchon Nonghyup (Yeoncheon-gun, Gyeonggi-do, Republic of Korea). Adlay starch was isolated using the alkaline steeping method (Wilson, Birmingham, Moon, & Snyder, 1978) with slight modifications. Grains were steeped in excess water for 18 h at room temperature, ground in a blender (707SB, Waring Commercial, Torrington, CT, USA) at full speed for 1 min, and filtered stepwise through 50-, 100-, and 200-mesh sieves. Starch was isolated from the filtrate by centrifugation at 4410*g* for 10 min. The supernatant was discarded, and the top, yellowish layer of protein was removed. This step was repeated until a white starch layer containing no protein was obtained, as determined by ninhydrin reaction of the supernatant. The starch layer was resuspended in distilled water, shaken, and centrifuged as described above. The starch slurry was then neutralized by repeated washing and dried at room temperature.

2.2. Experimental design

A three-factor, five-level central composite design was used to study the effect of each parameter on the RS content and color difference. The independent variables were glutaric acid content (0.1, 0.2, 0.3, 0.4, and 0.5 g/g starch), reaction temperature (70, 85, 100, 115, and 130 °C), and reaction time (3, 4.5, 6, 7.5, and 9 h). The levels of each variable were coded as −2, −1, 0, +1, or +2, as established through preliminary experiments. The dependent variables were the RS content and color difference. A total of 45 experiments with three replicates of one center point, eight factorial points, and six axial points were generated using SAS software (version 9.1; SAS Inc., Cary, NC, USA).

2.3. Preparation of glutarate starch

Glutarate starches were produced using the method of Klaushofer et al. (1978) with modifications. Glutaric acid (Aldrich, St. Louis, MO, USA) was dissolved in water, the pH adjusted to 3.5 with 10 M NaOH, and water added to give a final volume of 10 mL. The glutaric acid solution (10 mL) was mixed with 10 g starch in a stainless-steel bowl and conditioned for 16 h at room temperature. The bowl was then placed in a forced-air oven and dried at 50 °C for 24 h. The mixture was ground and dried in a forced-air oven for 3–9 h at a temperature of 70–130 °C. The dry mixture was washed with 1.5 L of water to remove unreacted glutaric acid, and then air-dried at room temperature and ground. Starch without glutaric acid was used as a control following the same procedure.

2.4. Measurement of resistant starch

Analysis of resistant starch was performed using the Megazyme method (AACC International, 2001). A 100-mg sample was placed in a screw-cap tube, and 4.0 mL of pancreatic α-amylase (10 mg/mL) containing amyloglucosidase (3 U/mL) were added. The tube was incubated with continuous shaking at 37 °C for 16 h. Next, 4.0 mL of ethanol were added, and the solution was stirred on a vortex mixer and centrifuged at 1500*g* for 10 min. The supernatant was decanted and the pellet resuspended in 2 mL of 50% ethanol with vigorous stirring on a vortex mixer. Another 6 mL of 50% ethanol were added to the tube, and the tube was centrifuged at 1500*g* for 10 min. The supernatant was immediately transferred to a new microtube and the suspension/centrifugation step repeated. After centrifugation, the supernatant was collected in a new microtube. The supernatants from the centrifugation of the initial incubation and the subsequent 50% ethanol wash were combined and adjusted to 100 mL with water in a volumetric flask. A 0.1-mL aliquot of this solution was incubated with 3.0 mL of GOPOD reagent at 50 °C for 20 min, and the absorbance was measured at 510 nm against a reagent blank. The non-resistant and resistant starch contents were calculated as follows:

$$\text{Non-resistant starch(g)/100g of sample} = \Delta A \times F/W \times 90$$

where ΔA is the absorbance (reaction) *versus* the reagent blank, F is the conversion factor from absorbance to micrograms (100 μg of glucose/GOPOD absorbance for 100 μg of glucose), and W is the dry weight of the sample (mg).

$$\text{Resistant starch} = \text{Total starch} - \text{Non-resistant starch}$$

2.5. Measurement of color and color difference

The color and color difference were analyzed with a Chroma Meter (CR-300; Minolta, Tokyo, Japan) using the Hunter system, which identifies color using three attributes: L (white = 100, black = 0), a (red = positive, green = negative), and b (yellow = positive, blue = negative). The color difference (ΔE), a measure of the distance in color space between two colors, was determined by comparison to a white standard tile with colorimeter values of $L = 94.7$, $a = 6.7$, and $b = -5.0$, using the following relationship:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

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

IR spectra were measured using FT-IR (Nicolet Magna 550 Series II; Midac, Costa Mesa, CA, USA). The spectra were recorded in transmission mode from 4000 to 400 cm (mid-infrared region) at a resolution of 4 cm. Samples were diluted with KBr (1:100, v/v) before acquisition; the background value from pure KBr was acquired before the sample was scanned. The