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Effect of salts on the gelatinization process of Chinese yam (*Dioscorea opposita*) starch with digital image analysis method

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ABSTRACT

A comparative study was undertaken to examine the effect of different salts on the gelatinization of C-type Chinese yam starch. The digital image analysis, integral optical density (IOD) method and the model of response difference of crystallite change (MRDCC) were employed to dynamically detect the gelatinization process in our research. Different concentrations and various salts had different degree of improvement and inhibition effect on the gelatinization process when heated in water. With the increase of NaCl concentration from 0 to 4 mol/L (M) the gelatinization degree (DG) of B-type allomorph increased at lower concentration to a maximum value and then decreased with increasing concentration, however all of the concentrations (1–4 M) had inhibition effect on A-type polymorph. The inhibition effect of low NaCl concentration contributed to the dominated water structure-making effect of Na^+, while in high concentration the electrostatic interaction between starch –OH groups and Na^+ ions was significant. The influence of various neutral anions was in accordance with the Hofmeister series while the situation of cations was far more complicated. Anions with higher charge density had water structure making effect to reduce water activity, and repeled starch –OH groups to stabilize starch granules at the same time; however, the higher charge density of cations increased the water structure on the one hand, while attracted starch –OH groups and destabilize starch granules with generated heat on the other hand.

1. Introduction

Chinese yam (*Dioscorea opposita*) has been used as an important invigorants in traditional Chinese medicine (TCM) for many years (Zuo & Tang, 2003). They benefit the lung, promote production of the body fluids and invigorate the spleen, stomach and kidney. Chinese yam starch is the most abundant carbohydrate in the rhizoma of *D. opposita* tubers (Ni & Song, 2002). Surprisingly, as far as we aware there are few international reports on the research of Chinese yam starch until 21 century. Shujun Wang (Wang et al., 2006) conformed that the starches of Chinese yam from four different cultivars exhibit a C-type X-ray diffraction pattern, similar to that of pea starch rather than the A-type patterns found for cereal starches. Two types of crystallite A and B, have been identified in starch, which can be distinguished by the packing density of double helices and the A polymorphs are denser than B (Buléon, Colonna, Planchot, & Ball, 1998; Imberty & Perez, 1988; Wang, Bogracheva, & Hedley, 1998). If C-type starches consist of these two type polymorphs, then they will have unique properties depending upon the arrangement and percentage of these two polymorphs in the granule. Wang et al. (Wang, Yu, Zhu, Yu, & Jin, 2009) further proved that B-type allomorph basically existed at the center part of the Chinese yam starch granules which was surrounded by the A-type allomorph in the outer part of the granules.

Addition of different solutes, such as sugars, salts, acids and alkalis have been used to modify the gelatinization temperature (Ahmad & Williams, 1999; Chiotelli, Pilosio, & Le Meste, 2002; Gough & Pybus, 1973; Jyothi, Sasikiran, Sajeev, Revamma, & Moorthy, 2005; Zhou, Wang, Li, Fang, & Sun, 2011). Salts have been shown to have a significant effect on the gelatinization properties of starches generally, and it has been found that they could cause an elevation or depression of the gelatinization temperature, T_p and gelatinization enthalpy, ΔH (Evans & Haisman, 1982; Jane, 1993; Rumpold & Knorr, 2005; Wootton & Bamunuarachchi, 1980). The majority of techniques that have been applied to study the influence of salts were differential scanning calorimetry (DSC) (Cheow & Yu, 1997; Chungcharoen & Lund, 1987; Maaurf, Che Man, Asbi, Junainah, & Kennedy, 2001),

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rheological measurements (Ahmed, 2012), light microscopy (Jane, 1993) et al.

IOD method as the latest technique, is able to characterize the partially gelatinized starch granules, in the mean time, it is a real-time monitor without pre- and after-treatment of various starch samples. Thus real situation of the gelatinization process can be obtained. Our previous work has confirmed that this method is of advantage compared with the previous traditional method based on polarizing microscopy (Li, Li, & Gao, 2015; Li, Xie, Yu, & Gao, 2013). The model of response difference of crystallite change (MRDCC) (Li et al., 2013) is a characterization of the starch gelatinization speed changes with the temperature. Compared with DSC, MRDCC is more sensitive and accurate, even the subtle expansion in the pre-gelatinized stage can be detected (Li, Xie, Yu, & Gao, 2014).

Various explanations have been given for the effect of neutral salts on starch gelatinization. What is worth mentioning is that Gough (Gough & Pybus, 1973) first classified the gelatinization phenomena into three major types (swelling from hilum, swelling from periphery, and gelatinized from both hilum and periphery), and he suggested that the relationship between temperature and salt concentration was attributed to the interplay of water and partially hydrated salts. In the further elegant experiment of Jane (Jane, 1993), the mechanism of starch gelatinization in salt solutions was summarized into two aspect: (I) structure-making and structure-breaking effects on water and (II) electrostatic interactions between salts and hydroxyl groups of starch. It has also been argued that there are two factors that might contribute to the influence of gelatinization: the effect of salts on polymer–solvent interactions, which are influenced to a greater extent by the anions; the interaction between cations and starch chains hydroxyl groups forms complexes and thus disrupting polymer chain aggregation (Ahmad & Williams, 1999).

The purpose of this present investigation is to undertake a comprehensive study of those salt effects on starch gelatinization with a digital image analysis technology. Meanwhile, the MRDCC combined with integral optical density (IOD) method was involved in this work.

2. Materials and methods

2.1. Materials

Tubers of Chinese yam were obtained from farmers of Taigu, Henan province, China. NaCl, KCl, $CaCl_2$, $MgCl_2$, $FeCl_3$, $NaNO_3$, Na_2CO_3, Na_2SO_4, $CuSO_4$ and solvents used in this work were of analytical grade.

2.2. Preparation of sample

Chinese yam tubers (1000 g) were washed, hand peeled and trimmed to remove defective parts. Then sliced and ground with an equal volume of water using a Jiuyang blender for 2 min at full speed. The slurry was filtered through a 200-mesh screen. The material remaining on the sieve was rinsed twice with deionized water, and the permeating was deposited for 2 h. Then, the supernatant was removed and the settled starch layer was resuspended in distilled water. After eight cycles of depositing and resuspending, the starch was then collected by suction filtrating and dried for 24 h at 45 °C.

Chinese yam starch slurries were prepared at starch concentration of 3% (starch: distilled water = 3:97, dry basis), and the moisture content (12.56%) in starch should be taken into consideration during calculating. 1, 2, 3 and 4 M of NaCl solutions, 1 M KCl, $CaCl_2$, $MgCl_2$, $FeCl_3$, $NaNO_3$, Na_2CO_3, Na_2SO_4, and $CuSO_4$ solutions were configured under 25 °C with distilled water. Starch-salt slurries were prepared at starch concentrations of 3% (starch:distilled water = 3:97, dry basis) with different salt solutions separately to evaluate the effects of salt concentration and type on gelatinization properties. Before detected, the measured starch-salt slurries were equilibrated for 2 h and then sealed between two glass cover slip using Dow Corning 732 Sealant before replaced in the hot stage (model THMS600, Linkam Scientific Instrument Ltd., Britain). Each measurement was carried out in triplicate.

2.3. Hot stage-light microscopy

Each specimen in the hot stage was observed under a polarization microscope (Vanox BHS-2, Olympus Corp., Japan) equipped with a digital camera, which can display live video of birefringence granules in a real time. A temperature programmer was connected with the hot stage to control the heating progress from 50 °C to 98 °C at a rate of 2 °C/min. Live pictures were captured every 2 °C and stopped if the gelatinization process was finished (the pictures were black or the granules were disappeared completely). The digital pictures quantity of each sample varied according to their gelatinization situation. Each image (2048 × 1536, 12 bits) was saved as TIFF image file, without data compression. All of the samples were observed under the same aperture (maximum), light intensity (fixed at 9), and exposure time (40 ms). The combination of eyepiece and objective lens were selected with a magnification of 200 times, as described in our early research (Li et al., 2013).

2.4. Wide-angle X-ray diffraction patterns

X-ray diffractogram was obtained by running a D/Max-2200 X-ray diffracttometer (Rigaku Denki Co., Tokyo, Japan). Cu Kα radiation at 44 kV and 26 mA were used. The measured Chinese yam starches were equilibrated in a sealed desiccator with water at room temperature for 12 h. The diffractogram scanning was run between 4° and 35° (2θ) at a rate of 5°/min (Shi, Chen, Yu, & Gao, 2013).

2.5. IOD method

It is a method to measure the degree of gelatinization (DG). The IOD value of each digital picture was calculated by the Image-pro plus 5.0 software (Li et al., 2013). The DG based on the IOD value (DG_I) was calculated as defined in our early research (Li et al., 2013).

$$\text{Background correction}: C = A - B \quad (1)$$

$$DG_I\% = (1 - C/C_0) \times 100\% \quad (2)$$

where A is the original IOD value (IOD value calculated from the original digital image when all of the birefringence remain unchanged), B is the background IOD value (IOD value calculated from the original digital image when all of the birefringence disappeared), and C_0 is the initial real IOD value (IOD value of birefringence light derived from the specific crystal structure of starch in the initial digital image). In this study, the IOD value of 50 °C was set as initial IOD value.

2.6. Model of response difference of crystallite change (MRDCC)

The MRDCC used in this paper were obtained according to our previous method (Li et al., 2013).

Response difference of crystallite change (RDCC) is the variation of crystallization degree in a certain temperature range which characterizes gelatinization speed, %/ΔT for units (%: Gelatinization