NOTE

Freeze-Thaw Stability of Three Waxy Maize Starch Pastes Measured by Centrifugation and Calorimetry

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Cereal Chem. 75(4):571–573

Retrogradation is often enhanced when starch gels are subjected to freezing and thawing treatments. Freezing a starch gel leads to the formation of ice crystals and thus concentrates the starch in the nonice phase. Retrogradation of starch molecules in these starch-rich regions will be enhanced during freezing and thawing. Subsequent freeze-thaw cycles aggravate the phase separation further and lead to the formation of larger ice crystals embedded in a sponge-like, coarsely aggregated starch network. Upon thawing, the water can be easily expressed from the network, giving rise to a phenomenon known as syneresis.

The freeze-thaw stability of starch gels has been evaluated by measuring the amount of liquid separated from the gels after centrifugation (Hood and Seifried 1974, Hoover et al 1988, Takahashi and Seib 1988, Wu and Seib 1990, Eliasson and Kim 1992). The centrifugation conditions need to be carefully controlled when using this method since the extent of syneresis measured depends upon the force applied during centrifugation (Eliasson and Kim 1992). These authors showed that the centrifugal forces influenced the detection of first syneresis as well as the extent of syneresis, with regard to the number of freeze-thaw cycles.

Differential scanning calorimetry (DSC) has also been used to assess the freeze-thaw stability of starch gels by measuring the retrogradation enthalpy after a certain number of freeze-thaw cycles (White et al 1989, Kim and Eliasson 1993). White et al (1989) evaluated the freeze-thaw stability based on the enthalpies observed on heating to 120°C after subjecting samples to 10 freeze-thaw cycles. These authors showed that waxy maize and regular maize starch samples recovered 58.1 and 59.1% of the initial gelatinization enthalpies, respectively; potato and wheat starches recovered only 40.9 and 39.8%, respectively. For these four samples, recovered enthalpies after freeze-thaw treatments were similar to retrogradation enthalpies on storage for seven days at 4°C.

The objective of the present work was to compare the freeze-thaw stability of three commercial waxy type maize starches using centrifugation and DSC methods.

MATERIALS AND METHODS

Starch Materials

Three waxy-type maize starches (*wx*, *wx sh1*, and *du wx*) were from the same commercial source (American Maize-Products Co., Hammond, IN; currently Cerestar USA).

Centrifugation Method

Starch pastes were prepared by heating 25 g of starch slurries (10:90 starch to water) in sealed syringes in a 95°C water bath for 30 min. The slurries were agitated at 5-min intervals during the first 15 min of heating by inverting the syringes five times. Syringes were cooled in air to room temperature. After cooling to room temperature, the gelatinized starch was expressed from the syringe into preweighed 2-mL microcentrifuge tubes (10 tubes for each sample). By microscopic examination, the cooked pastes from the three starches appeared to be mainly solubilized amylopectin with some disintegrated swollen granules. No apparent difference was observed among the three starch pastes. The weight of the sample in each tube was determined before placing in a –18°C freezer. After 24 hr at –18°C, all tubes were removed and kept at room temperature for 4 hr. One tube from each sample was centrifuged at 8,000 × *g* for 10 min in a micro centrifuge (Eppendorf 5415 C, Brinkmann Instruments, Westbury, NY) with a fixed angle rotor. Immediately after centrifuging, the tubes were removed from the centrifuge and then inverted to separate the free liquid from the starch paste. The free liquid was decanted and the weight of the remaining paste was determined. The extent of syneresis was calculated as the ratio of the weight of the liquid decanted to the total weight of the paste before centrifugation. The remaining tubes were then put back into the freezer for further freeze-thaw cycling. Nine freeze-thaw cycles were performed for the study. Each syneresis value represents the mean of three measurements from three separate experiments.

DSC Analysis

Changes in retrogradation enthalpies of the three starches as a function of freeze-thaw cycle were studied using a differential scanning calorimeter (DSC-7, Perkin-Elmer, Norwalk, CT).

Starch (≈5.0 mg, dry basis) was placed in a preweighed stainless steel DSC pan (Perkin-Elmer, Norwalk, CT) and the weight (±0.01 mg) was determined using an autobalance (AD2B, Perkin-Elmer, Norwalk, CT). The calculated amount of deionized water was added to prepare a starch slurry with a starch-to-water ratio of 10:90. The DSC pan was hermetically sealed and the starch slurry was gelatinized by heating in a 95°C oven for 30 min. The gelatinized starch samples were then stored under the same conditions as described above. After each freeze-thaw cycle, a sample was heated in the DSC from 10 to 100°C and the extent of amylopectin retrogradation was determined by the enthalpy of the peak near 50–60°C. Each enthalpy value is the mean of three separate measurements. Temperature and enthalpy of the instrument were calibrated using an indium standard.

RESULTS

Freeze-Thaw Stability by Centrifugation Method

Figure 1 shows the changes in syneresis of the starch pastes with increasing freeze-thaw cycles. The *wx* sample showed detectable syneresis after three freeze-thaw cycles. The syneresis increased dramatically from three to four cycles and then declined with additional freeze-thaw cycles. For the *wx sh1* sample, no syneresis was observed until the fourth freeze-thaw cycle. The syneresis reached a maximum after five freeze-thaw cycles and then assumed a downward trend thereafter. The *du wx* starch showed the first syneresis after two freeze-thaw cycles. The syneresis then decreased gradually with further cycling.

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Publication no. C-1998-0605-05R.

Retrogradation of Amylopectin by Thermal Analysis

The enthalpies of amylopectin retrogradation of the starch pastes as a function of freeze-thaw cycles are shown in Fig. 2. For *wx* and *wx sh1* starches, the first detectable retrogradation enthalpy was observed after six freeze-thaw cycles. The *du wx* starch retrograded faster than the other two starches, showing a detectable retrogradation endotherm after only two freeze-thaw cycles.

DISCUSSION

When a starch paste is frozen, phase separation occurs with the formation of ice crystals. On thawing, the starch paste will continue to be composed of a starch-rich and a starch-deficient aqueous phase. The extent of phase separation increases with additional freeze-thaw cycles due to an increase in amylopectin retrogradation in the starch-rich phase. As shown in Fig. 1, syneresis reached a maximum value within one freeze-thaw cycle of the initial detection. The value, and the time to reach it, varied with starch type. Thereafter, the extent of syneresis apparently decreased with additional cycles, in a manner that varied with starch type. This decrease may be related to increases in rigidity and elasticity of the pastes due to the formation of increasingly spongy gel networks. The formation of a sponge-like network by freezing and thawing a starch paste was reported decades ago (Hilbert et al 1945). These authors suggested that dried starch sponge could be used as new food ingredients to provide unique textures in a variety of foods. One example was the crispy center of chocolate-coated confection.

It is plausible that the formation of starch sponge would result in increases in both gel rigidity and elasticity. An increase in rigidity would provide the gel with greater resistance to the deformation caused by centrifugation. An increase in elasticity would give the paste greater ability to regain its original shape after centrifugation prior to decanting the free water. Therefore the amount of water that can be separated from a starch paste by centrifugation may depend on the gel rigidity and elasticity as well as the extent of phase separation. This interpretation is consistent with the report that the extent of syneresis measured by centrifugation is dependent upon the conditions (force) used (Eliasson and Kim 1992). We observed that most starch pastes subjected to several freeze-thaw cycles would reabsorb most of the separated liquid upon standing 1 hr at room temperature.

Figure 1 shows that comparing freeze-thaw stability of starch pastes based on one syneresis measurement taken after a fixed number of freeze-thaw cycles will lead to improper conclusions. It might be more appropriate to define freeze-thaw stability of starch pastes by the number of freeze-thaw cycles taken to detect the first appearance of free liquid above the paste after centrifugation. Using this criterion the *wx sh1* gel, which showed the first syneresis after four freeze-thaw cycles, had the best freeze-thaw stability followed by *wx* (three cycles) and *du wx* (two cycles). It is critical that the test conditions should be tightly controlled to obtain meaningful comparison. Eliasson and Kim (1992) showed that the number of freeze-thaw cycles needed to detect syneresis by a centrifugation method varied according to the centrifugation conditions tested: $100 \times g$, $1,000 \times g$, and $1,500 \times g$. They showed the sensitivity of the method increased with increasing gravitational force.

Kim and Eliasson (1993) stated that after freeze-thaw treatments, whenever syneresis was detected (using $1,000 \times g$), a DSC endotherm was also observed. In the present work, the samples were subjected to a greater force ($8,000 \times g$), and it is likely that the detection of the initial gel network formation was more sensitive. Our observation of syneresis for *wx* and *wx sh1* starches before a DSC endotherm could be detected might be due to a more sensitive method for detecting syneresis.

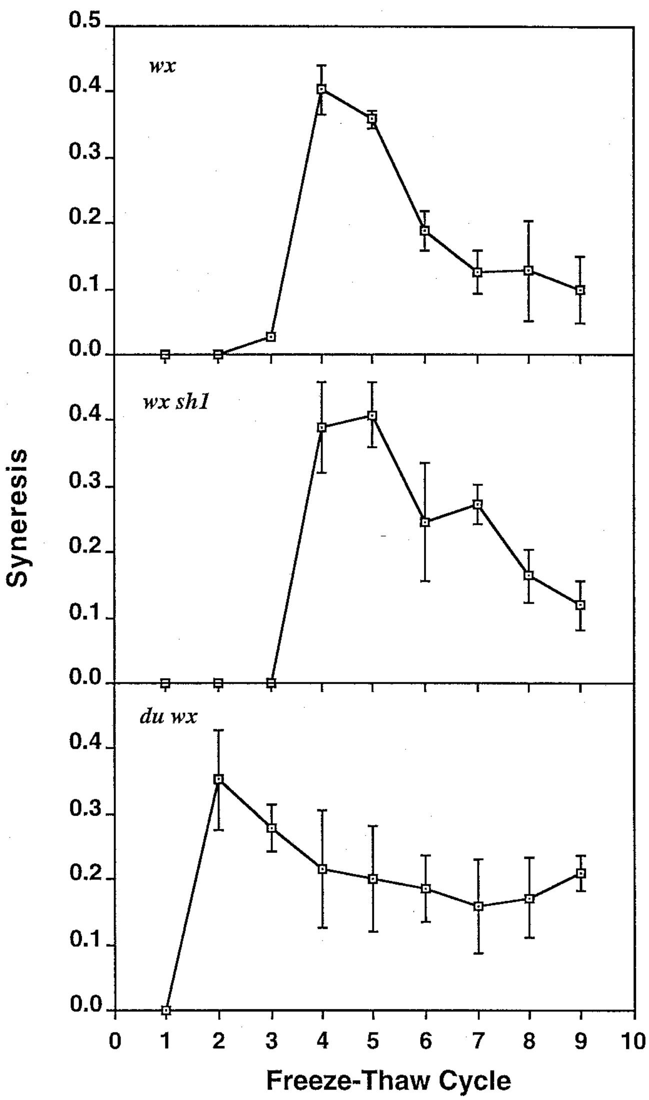

Fig. 1. Syneresis of *wx, wx sh1*, and *du wx* starch pastes (starch-to-water ratio 10:90) as a function of the number of freeze-thaw cycles. Each syneresis value represents the mean of three measurements. Vertical bar represents standard deviation.

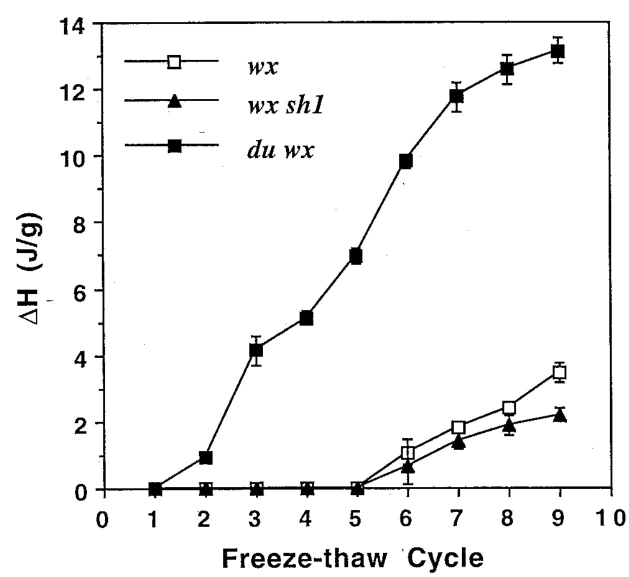

Fig. 2. Retrogradation Δ*H* of *wx, wx sh1*, and *du wx* starch pastes (starch-to-water ratio 10:90) as a function of freeze-thaw cycles. Each enthalpy value represents the mean of three measurements. Vertical bar represents standard deviation.

The DSC retrogradation measurements (Fig. 2) of the pastes (starch-to-water ratio 10:90) showed that the *du wx* starch had a detectable retrogradation enthalpy after only two freeze-thaw cycles, compared to six cycles for *wx* and *wx sh1* starches. On storage of gelatinized starch at 4°C, *du wx* starch retrogrades to a greater extent as compared to *wx* starch (Shi and Seib 1992, 1995; Yuan et al 1993). The poorer freeze-thaw stability of *du wx* starch is likely related to a general tendency toward more rapid retrogradation. For *du wx* starch, initial syneresis and initial retrogradation enthalpy coincide; for *wx* and *wx sh1* starches initial retrogradation enthalpy is detected two to three freeze-thaw cycles after initial syneresis. In fact, for both *wx* and *wx sh1* starches maximum syneresis occurs before retrogradation enthalpy is observed. For *du wx* starch, maximum syneresis occurs when retrogradation enthalpy is only ≈1 J/g. For each starch, it would appear that a gel network forms when only a relatively small amount of double helices are present. It appears that the earliest stages of network formation were sufficient to allow syneresis; the additional enthalpy over time might have been related to strengthening of the network, adding to both rigidity and elasticity, and thus reducing the apparent extent of syneresis as determined by the centrifugation method.

That the behavior of *du wx* starch differs from that of *wx* and *wx sh1* starches might be attributed to the structural differences in amylopectin of the starches. It has been suggested that an increase in the mole fraction of amylopectin chains of DP 14–24 (Shi and Seib 1992), DP 16–30 (Shi and Seib 1995) or DP > 30 (Yuan et al 1993) increased the retrogradation tendency of waxy-type starches. In our work, the commercial *du wx* starch had a greater proportion of DP ≈ 20–30 chains in amylopectin than the *wx* and *wx sh1* starches (Yuan and Thompson 1998). The greater proportion of this fraction in *du wx* starch might explain its greater retrogradation tendency and the relatively poor freeze-thaw stability. As shown in Figs. 1 and 2, the *wx* and *wx sh1* starch pastes showed syneresis by the centrifugation method before a DSC endotherm was detected. This observation suggests that phase separation takes place in an early stage of chain reassociation.

We suggest that the centrifugation method is more sensitive than the DSC method for detecting the initial loss of functionality in a starch paste subjected to freeze-thaw treatments; whereas DSC analysis, which measures the disruption of ordered starch structures, might be a better method for assessing the continued development of chain association in a starch paste after several freeze-thaw cycles.

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[Received September 29, 1997. Accepted April 8, 1998.]