

Freeze-Thaw Stability of Amaranth Starch and the Effects of Salt and Sugars¹

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ABSTRACT

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Freeze-thaw stability of amaranth, corn, wheat, and rice starches was determined measuring the percent of syneresis by centrifugation. Thermal properties were calculated by differential scanning calorimetry (DSC). The effects of salt (NaCl at 2 and 5%) and sugars (sucrose, glucose, and fructose at 10, 20, and 30%) on the freeze-thaw stability of amaranth starch were also studied. Based on DSC and centrifugation methods, amaranth

starch had better stability after freezing and thawing through four cycles than did corn, wheat, and rice starches. Amaranth starch with added salt showed similar stability as compared with a control when measured by centrifugation and showed increased stability when measured by DSC. Adding sugars to amaranth starch gels had varying results, but for the most part, they showed similar or increased stability when compared with a control.

Grain amaranth, long grown as a grain and vegetable crop in Central and South America, Mexico, Africa, and Asia, has been grown commercially in the United States since 1983 (Weber 1990). International attention focused on amaranth has been in commercialization of the crop, modernization of traditional cultivation, and expansion of its traditional uses (Williams and Brenner 1995).

Interest in the properties of amaranth starch are based on its small granule size and composition (1–3 μm and 95–98% amylopectin, respectively) (Breene 1991, Uriyapongson and Rayas-Duarte 1994). Use of amaranth starch in salad dressing yielded a more stable product than did use of corn starch (Singhal and Kulkarni 1988), and it yielded stable gels during freeze-thaw cycling (Yanez et al 1986).

The stability of starch gels during freeze-thaw cycling enhances its potential use in food products. Traditionally, this stability has been measured by the amount of water separated from a gel after freezing and thawing has occurred and, more recently, by using differential scanning calorimetry (DSC) (White et al 1989).

Sugars and salt are common ingredients in baked and processed foods. Research on effects of salt and sugar in the retrogradation of starch gels has been conflicting and inconclusive. Sugars have been shown to retard retrogradation. P'Anson et al (1990) and Cairns et al (1991) found that sugars reduced crystallinity in retrograded wheat starch gels. Kohyama and Nishinari (1991) found that sugars decreased retrogradation in sweet potato starch pastes. Katsuta et al (1992) reported that sugars inhibited retrogradation of rice starch gels. Other authors have found accelerated retrogradation with sugars. An increased rate of retrogradation was reported with the addition of sugars to corn and rice starch gels (Germani et al 1983, Chang and Liu 1991). Maxwell and Zobel (1978) reported increased rates of crystallization in wheat starch gels with sugar additions.

The reported effects of salt on the retrogradation of starch differ as well. Chang and Liu (1991) reported a decrease in the retrogradation rate with the addition of NaCl to rice starch gels. Ciacco and Fernandes (1979) reported that NaCl increased retrogradation rates of wheat starch gels, while Schoch and French (1947) determined that salt had no influence on wheat starch pastes.

The purpose of this study was to determine the freeze-thaw stability of amaranth starch and the effects of salt and sugars on the freeze-thaw stability of amaranth starch using centrifugation and DSC analyses.

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MATERIALS AND METHODS

Materials

Commercial amaranth starch was obtained from Amaranth Resources, Inc. (Albert Lea, MN) from amaranth grain, cultivar Plainsman (*Amaranthus hybridus* x *A. hypochondriacus*) and was further purified in the laboratory. Common corn starch (American Maize Products, Co., Hammond, IN), rice starch (Sigma Chemical Co., St. Louis, MO), and wheat starch (Midwest Grain Products, Atchison, KS) were used without further purification for comparison.

Aqueous solutions of sucrose, glucose, and fructose (Sigma) in concentrations of 10, 20, and 30% (w/w) were used. Sodium chloride (NaCl) (Mallinkrodt Inc., Paris, KY) solutions were used in concentrations of 2 and 5% (w/w).

Starch Purification

Amaranth starch was purified using a modified wet-milling procedure (Uriyapongson and Rayas-Duarte 1994). Starch was mixed with eight volumes of 0.2% NaOH solution and stirred for 1 hr. The slurry was centrifuged at $830 \times g$ for 30 min and the supernatant was discarded. The top yellowish protein layer was removed and the remaining starch washed with distilled water and centrifuged. The supernatant and protein layer were removed and discarded. The starch was mixed with distilled water, neutralized to pH 6.5–7.0 with dilute HCl, and centrifuged. After an additional washing and centrifugation, the starch was air-dried under a constant-flow hood and stored in closed glass jars at room temperature.

Chemical Analysis

The proximate compositions of the starches were determined using Approved Methods 08-01, 30-25, 44-15A, and 46-13 (AACC 1995). Amylose content was determined with the colorimetric procedure of Knutson (1986).

Centrifugation Method

The stability of starch paste to freezing and thawing cycles was tested according to the method of Schoch (1968). Starch (5%, db) in distilled water was cooked 30 min in a boiling water bath with moderate mechanical agitation and cooled. Sugars and salt were dissolved in the water before adding the starch. The paste (30 mL) was transferred to 50-mL centrifuge tubes. The tubes were placed in a still-air freezer at -20°C for 22 hr and placed in a 30°C water bath for 1.5 hr to thaw and equilibrate. Two tubes were centrifuged for 15 min at $900 \times g$, and the amount of separated water was determined by removing and measuring with a graduated cylinder. The amount of water released from the gel was measured in a graduated cylinder after 1, 2, 4, 7, and 10 freeze-thaw cycles, and expressed as percent of water separated, or syneresis. The percentage of syneresis was plotted against the number of freeze-thaw cycles.

DSC Method

Samples were gelatinized as described by White et al (1990). Starch samples (3.5 mg) were weighed into aluminum DSC pans. Deionized water (8 μ L) containing the added sugars and NaCl was added with a microsyringe. The pans were sealed, allowed to equilibrate for 2 hr, and scanned at a rate of 10°C/min from 30–120°C. Samples were analyzed using Perkin-Elmer DSC7 instrument (Norwalk, CT) equipped with a TAC7/DX thermal analysis instrument controller and DEC personal workstation. An empty pan was used as a reference. After scanning, the pans were frozen at –20°C. After 22 hr, the samples were thawed for 1.5 hr at room temperature and refrozen for 22 hr. Thermal properties of the retrograded gels were analyzed between 20 and 100°C at a rate of 10°C/min after 1, 2, 4, 7, and 10 freeze-thaw cycles. The melting transition parameters of retrograded gel samples were analyzed and compared with the gelatinization peak. The enthalpy value (ΔH) required to melt the retrograded starch was expressed as a percentage of ΔH required to gelatinize the starch sample.

Statistical Analysis

We created a general treatment variable with various combinations of sugar, starch, salt, and concentration of each representing one of 15 levels for the independent variable in a completely randomized design.

Analysis of variance (ANOVA) and Duncan's multiple range test were performed using an SAS program (SAS Institute, Cary, NC). Three replicates were used for DSC analysis, and six were used for the centrifugation study. The retrogradation rate was analyzed by linear regression analysis using three points with three values for each point. An exponential first-order equation was also used to estimate the rate of retrogradation as described by Baker and Rayas-Duarte (1998). The rate of retrogradation as a function of the number of freeze-thaw cycles was used instead of time.

RESULTS AND DISCUSSION

Chemical Analysis

The proximate composition and amylose content of the starches are shown in Table I. The absence of amylose for amaranth starch would indicate that it is a waxy starch. This result agreed with that of Uriyapongson and Rayas-Duarte (1994). The amylose content of all the starches of this report is lower than values compiled by Young (1984) but comparable to the values reported by Zobel (1988). Values of amylose content can vary with the analytical method.

Freeze-Thaw Stability by Centrifugation

The rates of syneresis (Table II) for amaranth starch gels were lower than those of corn, wheat, and rice starches for cycles 1–2. Adding sugars and salt decreased the rate of syneresis when compared to the control. The rates of syneresis for cycles 4–10 were lower than those of cycles 0–2 because the rates slowed with continued freezing and thawing, as the starches were beginning to reach their maximum percent of syneresis, or plateau (Fig. 1).

TABLE I

Chemical Analysis^a (% db) of Amaranth, Corn, Wheat, and Rice Starches

Starch	Moisture	Ash	Protein ^b	Oil	Amylose
Amaranth	10.9	0.21b ^c	0.43a	1.02c	0a
Corn	11.8	0.10a	0.48a	0.75a	25b
Wheat	10.9	0.39c	0.43a	0.91b	22b
Rice	10.9	0.33c	0.49a	0.89b	24b

^a $n = 3$ (except for amylose, for which $n = 2$).

^b $N \times 5.85$ for amaranth, $N \times 6.25$ for corn and rice, and $N \times 5.7$ for wheat.

^c Means within a column followed by a different letter are significantly different ($\alpha = 0.05$) using Duncan's multiple range test.

The native starches (not treated, no additives) had a lower rate of syneresis for cycles 4–10, than did amaranth starch gels with added salt (5%) or sugars (glucose and sucrose). This may be the result of the slower initial rate of water separation in the presence of more hydrophilic compounds. These gels have not reached the maximum percent of syneresis.

Amaranth starch gel (Fig. 1) was the most stable of all the starches tested after one freeze-thaw cycle. After two freeze-thaw cycles, amaranth and corn starch gels showed lower percent of water separation than did wheat and rice starches. At four freeze-thaw cycles, amaranth starch showed higher percent of water separation than did corn starch, which was similar to rice starch but lower than wheat starch. After seven and 10 freeze-thaw cycles, all starch gels had similar percent of water separation. The wheat and rice starches showed a higher degree of water separation, which is typical of starches with a higher amylose content. Yanez et al (1986) found that amaranth starch possessed good stability to freezing and thawing, as measured by centrifugation, by withstanding four cycles before noticeable water separation occurred, whereas corn starch pastes exhibited $\approx 60\%$ water separation after two cycles. Differences found between this study and that of Yanez et al (1986) may be due to varietal differences of the amaranth grain source used (*A. hybridus* x *A. hypochondriacus* vs. *A. hypochondriacus*) and starch isolation methods (1 hr steeping in 0.2% NaOH vs. 24 hr in 0.25% NaOH). While the corn starches used in this study and that used by Yanez et al (1986) were from different sources (different manufacturers), maximum water separation was about two-fold higher in the Yanez report. Amaranth starch paste slow deterioration has been compared to waxy sorghum paste (Yanez et al 1986).

The freeze-thaw stability of various other starches has also been reported. Dreher et al (1983) showed that buffalo gourd starch and corn starch exhibited a comparable degree of syneresis of $\approx 80\%$ water loss after freezing and thawing for two weeks. They also found that modified tapioca starch exhibited little syneresis ($\approx 5\%$ water loss). Yanez et al (1991) demonstrated that proso millet starch gels had more water separation after freeze-thaw cycling than did corn starch gels. Hoover et al (1991) showed that

TABLE II

Rate^a of Syneresis of Starch Gels as a Function of Freezing and Thawing Cycles^b as Measured by Centrifugation and Differential Scanning Calorimetry (DSC)

Starch	Centrifugation		DSC	
	Cycles 0–2	Cycles 4–10	Cycles 0–2	Cycles 4–10
Amaranth	9.0	1.3	9.9	5.0
Corn	10.7	1.0	19.2	1.3
Wheat	21.2	0.2	18.2	1.7
Rice	17.4	0.5	22.6	2.1
Amaranth				
NaCl (%)				
2	7.1	0.8	6.0	2.6
5	4.9	3.6	5.6	4.0
Glucose (%)				
10	7.5	1.4	6.5	5.2
20	5.5	1.4	8.2	7.2
30	5.7	1.1	17.3	4.6
Sucrose (%)				
10	5.3	1.8	7.0	4.2
20	4.1	1.7	3.2	7.1
30	3.5	2.8	3.9	3.2
Fructose (%)				
10	7.1	0.1	11.8	3.5
20	8.3	0.1	14.1	3.2
30	6.7	0.1	17.9	6.2

^a Rate was determined as slope of line; 3 points with 3 values for each point were used to determine the regression line.

^b Values for cycles 0, 1, and 2 were used to determine slope. Cycle 0 (the freshly gelatinized gel that had undergone no freezing) had a value of 0 for each sample. Values for cycles 4, 7, and 10 were used to determine slope.

lima bean starch gels exhibited more water separation ($\approx 65\%$) than did corn and potato starch gels (55–60%) after three freeze-thaw cycles. Using centrifugation, Wu and Sieb (1990) found that native waxy barley and waxy maize starches showed poor freeze-thaw stability, but when these starches were modified by acetylation or hydroxypropylation, the freeze-thaw stability increased. Differences in freeze-thaw stability among different types of starches may be due to a variety of factors, most notably, amylose content. Other factors include length of the starch chains, degree of association between starch components, the degree of polymerization of amylose and amylopectin (Hoover et al 1991), length of storage, rate of freezing, number of freeze-thaw cycles, and the addition of other food ingredients (Dreher et al 1983).

Adding NaCl to amaranth starch gels (Fig. 1) significantly increased the stability of the gels after two and four freeze-thaw cycles when compared with the amaranth starch control. After seven freeze-thaw cycles, there were no significant differences in the percent of water separated from the gels with added NaCl. After 10 cycles, the gels with 5% added NaCl had a significantly greater amount of water separated than did the control and samples with 1% NaCl. The results suggested that competition with the starch by the NaCl for available water gave stability to the amaranth starch gels, but the stability depended on the number of freeze-thaw cycles. After 10 cycles, the water was more loosely held due to the concentration of the solutes in the freezing center of the gel, the section that is the last to freeze. The water trapped in the gel would be easy to expel during the syneresis that occurs at the thawing stage (Fennema 1985, Whistler and Daniel 1985).

A 5% concentration of NaCl in amaranth gels gave a significantly lower percent of water separated at two and four cycles and significantly greater percent of water separation at 10 cycles than did a 2% concentration of NaCl (Fig. 1). The data suggest that the higher the NaCl concentration, the greater the ability to alter the net structure of water trapped in the gel. There is also a greater concentration effect of solids during repeated freezing cycles (Fennema 1985).

Adding glucose to amaranth starch gels (Fig. 1) significantly improved the stability of the starch gels through four cycles of freezing and thawing, after which the stability was similar to that of the control. Differences in the effects of increasing levels of glucose were only observed after two cycles. Adding glucose at 20 and 30% produced a significant decrease in percent of water separated as compared with the control and 10% glucose.

Adding sucrose to amaranth starch gels (Fig. 1) significantly increased the stability to freezing and thawing after two cycles when compared with the amaranth starch control. At four cycles, only a 30% addition produced a significantly lower percent of water separated. After 10 freeze-thaw cycles, no significant differences were observed.

Fructose (Fig. 1) significantly decreased the amount of water separated after two cycles, indicating an increase in the freeze-thaw stability of amaranth starch gels. No significant differences were observed after four freeze-thaw cycles. However, at seven and 10 cycles, all gels with added fructose showed significantly lower percent of water separated than did the amaranth control gel.

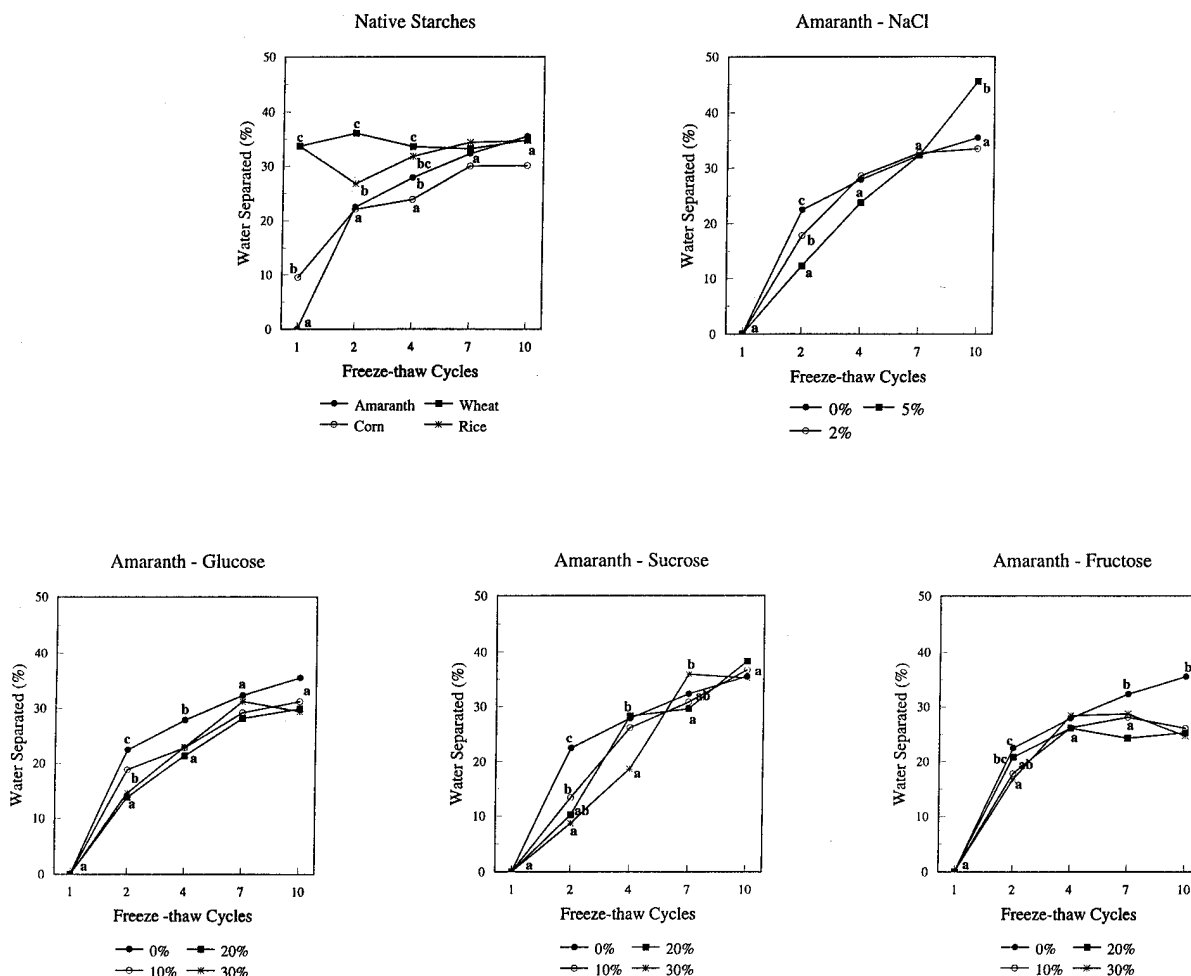


Fig. 1. Percent of water separated by freeze-thaw cycling as estimated by centrifugation for different starches and amaranth starch gels with NaCl, glucose, sucrose, or fructose. For clarity, the lines were not shown joined to the origin (0% retrogradation). Different letters at each cycle indicate significant difference at $\alpha = 0.05$ using Duncan's multiple range test. Each marker is the mean of six replicates.

Freeze-Thaw Stability by DSC

DSC parameters for gelatinization and freeze-thaw cycling are shown in Table III. The gelatinization onset, peak, and range temperatures (T_o , T_p , and T_r , respectively) of native amaranth and corn starches were similar. These results agreed with the report of Lund (1984). The broad peak seen for rice starch resembled high amylose starches (Stevens and Elton 1971), although the sample was normal rice ($\approx 25\%$ amylose). Retrograded gels subjected to 10 freeze-thaw cycles showed lower T_o , and wider T_r than the same gelatinization parameters of native amaranth starch. Adding sugars generally increased, with increased sugar concentration, the T_o , and T_p of the gelatinization peaks, but did not affect the T_r when compared to amaranth starch alone. There are two possible reasons for the increase in gelatinization temperatures with the addition of sugars: 1) hydration of sugar molecules decreased the available water, which was equivalent to having higher concentrations of starch; and 2) interaction of sugar molecules with molecular chains in the starch stabilized the crystalline regions of the starch (Kohyama and Nishinari 1991). Other researchers (Spies and Hosney 1982, Hosney 1984, Jacobsberg and Daniels 1974, Paredes-Lopez and Hernandez-Lopez 1991, Wooten and Bamunuarachchi 1980) reported that sugars and NaCl increase the T_o , T_p , and T_c of starch gelatinization. The increased gelatinization temperatures of samples with added NaCl agreed with the proposed arrangement of the hydrated ion pair on NaCl (Fennema 1985), in which the water molecules of the gel in the presence of NaCl might have a more organized structure that required higher gelatinization temperatures. Paredes-Lopez and Hernandez-Lopez (1991) studied the influence of sucrose and NaCl on the T_o , T_p , and T_c and degree of gelatinization of amaranth starch. These authors showed that up to 30% sucrose and 6% NaCl increased the T_o , T_p , and T_c of amaranth gelatinization.

Gelatinization temperatures (T_o and T_p) decreased by ≈ 10 – 25°C when samples were analyzed after 10 freeze-thaw cycles. A lower melting onset temperature and ΔH (data used in the calculation of percent of retrogradation) in the sample reflected the accepted definition of the lower degree of order of the retrograded starch than with the native sample (Slade and Levine 1989). High gelatinization temperatures were observed in the samples with added NaCl, but this trend was not evident in the gels subjected to 10 freeze-thaw cycles (Table III). The T_r increased for all samples (except rice)

after 10 cycles. The wider T_r indicated that fewer perfect crystals were present in the retrograded samples than in the native sample.

The rates of retrogradation, as estimated by fitting linear regression equations in two segments, for gels analyzed by DSC are shown in Table II. The rates were higher for cycles 0–2 than they were for cycles 4–10. This observation, as before, was due to the gels beginning to reach their maximum percent of retrogradation plateau. Amaranth starch had the lowest rate of retrogradation of the four native starches for cycles 0–2, but the highest for cycles 4–10. The amaranth starch gel had not reached its retrogradation plateau after 10 freeze-thaw cycles.

Adding NaCl decreased the rates of retrogradation, as did adding sucrose. Adding glucose (10 and 20% levels) decreased the rate of retrogradation for 0–2 cycles and increased the rate for 4–10 cycles. The 30% addition of glucose had the opposite effect. Adding fructose increased the rate from 0–2 cycles, and the 10 and 20% additions reduced the rate for 4–10 cycles. The higher rates (compared to the native corn, wheat, and rice starches) in 4–10 cycles indicated that the samples had not reached their retrogradation plateaus and that retrogradation would increase further past 10 cycles of freezing and thawing.

Percent of retrogradation as a function of freezing and thawing cycles is shown in Fig. 2. The instability of a starch gel to freezing and thawing is directly related to the percent of retrogradation of that gel (Schoch 1968). Amaranth starch had significantly lower percent of retrogradation than did corn, wheat, and rice starches through four freeze-thaw cycles, which suggested better stability to freezing and thawing. After seven and 10 cycles, the percent of retrogradation of amaranth starch gels was similar to that of wheat and significantly higher than that of rice starch, but lower than that of corn starch.

White et al (1989), using DSC, tested the freeze-thaw stability after 10 cycles of several native and modified starches. They determined that the modified starches had the most stability to freezing and thawing, whereas of the native starches, tapioca starch was the most stable, followed by wheat, potato, arrowroot, and rice starches, with regular maize and waxy maize starches being the least stable. The differences in this study and that of White et al (1989) in stability of wheat and rice starches may be due to varietal differences in the wheat and rice starches, or possibly due to different freezer temperatures.

TABLE III
Comparison of Onset, Peak, and Melting Transition Range Temperatures ($^\circ\text{C}$)^a for Starches and Additives
for Freeze-Thaw Cycling as Analyzed by Differential Scanning Calorimetry

Starch	Gelatinization			10 Freeze-Thaw Cycles		
	T_o	T_p	T_r	T_o	T_p	T_r
Amaranth	66.2c ^b	70.6bc	8.8ab	41.4a	48.5a	14.2a
Corn	66.2c	70.3b	8.3a	42.6d	50.8ab	16.4b
Wheat	56.1a	60.6a	9.1b	41.7ab	49.4ab	15.4ab
Rice	61.4b	76.3h	29.8c	42.7d	50.8ab	16.2ab
Amaranth						
NaCl (%)						
2	69.4h	74.0g	9.1b	41.6ab	49.2a	15.2ab
5	71.6i	76.1h	9.2b	42.8d	50.3ab	14.9ab
Glucose (%)						
10	66.9cd	71.4d	9.1b	41.8abc	52.2b	14.1a
20	67.4de	71.8de	8.8ab	42.0bc	49.6ab	15.2ab
30	68.3fg	72.8f	9.0b	41.4a	49.4ab	16.0ab
Sucrose (%)						
10	67.3de	71.9de	9.2b	41.8abc	49.2a	14.7ab
20	68.0ef	72.6f	9.3b	41.2a	48.7a	15.0ab
30	69.0gh	73.6g	9.1b	42.3bcd	50.1ab	15.7ab
Fructose (%)						
10	66.7cd	71.2cd	9.1b	42.0bc	49.1a	14.2a
20	67.4de	71.9e	9.1b	41.7ab	49.7ab	16.1ab
30	67.7ef	72.3eg	9.2b	42.4cd	49.9ab	15.0ab

^a T_o and T_p = onset and peak melting temperatures, respectively. Range of melting transition = $2(T_p - T_o)$ as described by Krueger et al (1987).

^b Means with different letters in each column are significantly different at $\alpha = 0.05$ using Duncan's multiple range test. $n = 3$.

Adding salt to amaranth starch gels (Fig. 2) significantly decreased the percent of retrogradation of the gels to freezing and thawing. Adding 5% NaCl to amaranth starch gels was significantly more effective than adding 2% NaCl to amaranth starch gels in decreasing the percent of retrogradation. These results can be partially explained by the freezing concentration effects of the NaCl ions (Fennema 1985, Whistler and Daniel 1985). NaCl also dissociates in water to its ions, Na⁺ and Cl⁻. These ions form ionic bonds with the water and the starch molecule, thus holding the water more tightly.

Adding glucose (Fig. 2) to amaranth starch gels showed variable effects on the percent of retrogradation of amaranth starch gels at each freeze-thaw cycle. At each glucose addition, the retrogradation of the amaranth gels increased as the number of freeze-thaw cycles increased. After one freeze-thaw cycle, 10–30% glucose showed a significant increase in percent of retrogradation. After two and four cycles, the 10% glucose addition showed the lowest percent of retrogradation and the 30% addition the highest. After 10 cycles, 10% glucose concentration gave the lowest percent of water separated.

Adding sucrose (Fig. 2) to amaranth starch gels also had varying effects on the percent of retrogradation of those gels. Changes in retrogradation were observed after two freeze-thaw cycles, in which sucrose significantly decreased the percent of retrogradation when compared to the control. Through the remaining cycles of freezing and thawing, the 10% addition exhibited similar effects or significantly decreased the percent of retrogradation, whereas the 20% addition showed decreased percent of retrogradation, except for the last cycle, in which percent of retrogradation was

significantly greater than that of the control. The 30% addition exhibited significantly decreased the percent of retrogradation after seven and 10 freeze-thaw cycles.

Adding fructose (Fig. 2) to amaranth starch gels generally significantly increased the percent of retrogradation after one, two, and four freeze-thaw cycles. A significant decrease in the percent of retrogradation after seven cycles for all concentrations of fructose addition was observed. After 10 freeze-thaw cycles, the 10% addition showed a significantly decreased percent of retrogradation, the 20% addition showed a similar percent of retrogradation, and the 30% addition showed a significantly increased percent of retrogradation.

Few reports (Kohyama and Nishinari 1991, Chang and Liu 1991) in the literature have illustrated the effects of sugars and salt on freeze-thaw stability of starch gels as measured using either centrifugation or DSC methods. Comparisons can be made among the effects of freeze-thaw cycling of gels stored at refrigerated temperatures. The physical changes that occur in pasted starch during prolonged cold storage at 4°C seem to parallel the changes caused by freezing and thawing. One cycle of freezing and thawing may cause as much damage to the hydration capacity of the pasted starch as several weeks of cold storage at 4°C (Schoch 1968).

The rates of syneresis and retrogradation (centrifugation and DSC methods) were similar because the rate was faster in the first few cycles than in the later cycles when there was a slowing or plateau of the rate. The rates were similar for the initial rate of each starch when the two methods were compared. Trends in the initial rate involving the NaCl and sugar content were similar between the two methods as well. This observation would indicate that the two

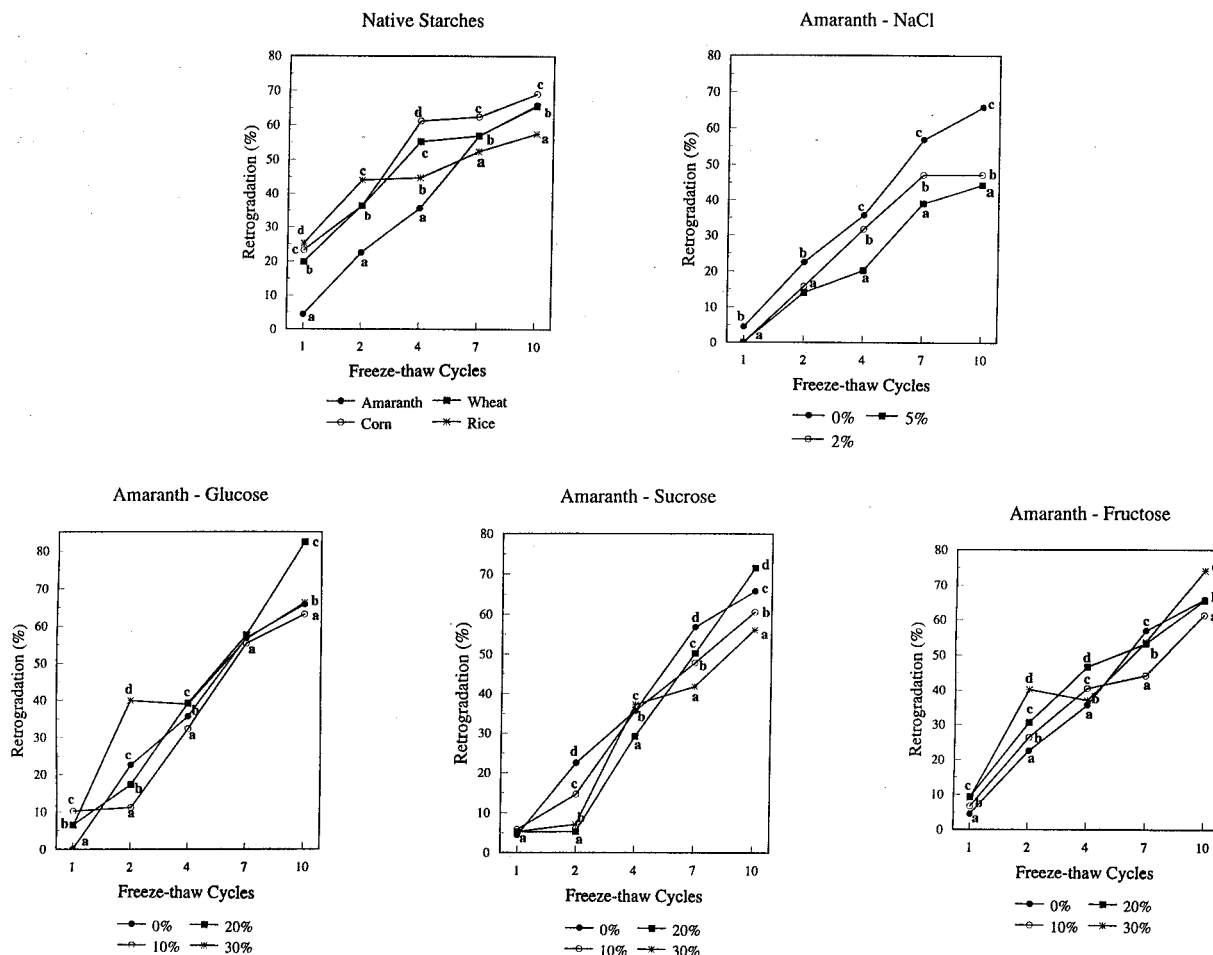


Fig. 2. Percent of retrogradation by freeze-thaw cycling as analyzed by differential scanning calorimetry for different starches and amaranth starch gels with NaCl, glucose, sucrose, or fructose. Different letters at each cycle indicate significant difference at $\alpha = 0.05$ using Duncan's multiple range test. Each marker is the mean of three replicates.

methods, although different in what and how they measure retrogradation, provide similar results regarding the rate of retrogradation.

The values obtained from the percent of water separated by centrifugation were generally lower than those for the percent of retrogradation by DSC. One could theorize that the water was held in the gel as the starch molecules recrystallized and that the starch molecules, in their recrystallization process, formed hydrogen bonds with the water, holding it in a semibound state.

This theory, however, does not explain the results obtained with the addition of NaCl to amaranth starch, in which the addition of NaCl resulted in more water separated than in the control, but a lower percent of retrogradation than in the control, as measured by DSC. The physical presence of NaCl may actually favor forcing part of the water-NaCl from the gel when it is centrifuged, while keeping the starch molecules from realigning in their crystalline form by binding ionically with the starch granules and the water.

Adding sugars to the amaranth starch gels did not show any consistent behavior. Katsuta et al (1992) found that the ability of saccharides to stabilize the water structure surrounding the starch molecules was related to the number of equatorial hydroxyl (e-OH) groups present in each saccharide. The mean value of the number of e-OH groups increases in the order: fructose (3.0) < glucose (4.6) < sucrose (6.3). The results from the centrifugation of gels with added fructose would indicate that it is better at retaining water in the gel structure than glucose, which was better than sucrose, based on their values after 10 freeze-thaw cycles. This is the opposite of what would be expected based on the number of e-OH groups. Katsuta et al (1992) also found that fructose was better at stabilizing starch gels than was galactose, which has a mean number of e-OH groups of 3.6. This would lead one to believe that other factors might be responsible for the stabilizing effect of sugars. When the gels were analyzed by DSC, the sucrose showed, on average, a lower percent of retrogradation than fructose, which was lower than glucose. Here again, the fructose behaved unexpectedly, and this would further lead to the conclusion that other factors are involved in the action of sugars on starch retrogradation. Perhaps physical size or conformation of the molecule in starch gels is involved as well.

Estimation of Rate of Retrogradation using an Exponential First-Order Equation

An exponential first-order equation has been used to estimate the rate of retrogradation of starch gels as a function of time. Al-

though limitations have been pointed out, this equation model can be used until a better model is proposed (Baker and Rayas-Duarte 1998). Using the number of freeze-thaw cycles as the variable instead of time, an overall rate of retrogradation is obtained, in contrast to the two slopes from the linear regression analysis. Table IV shows a lower retrogradation rate constant (*k*) for amaranth starch gels when compared to corn, wheat, and rice. Rice starch gels showed the highest rate of retrogradation. The data suggests that when subjected to abusive freeze-thaw cycling, overall, amaranth will retrograde slower than the other tested starch samples, but it predicts that 100% retrogradation could be reached if the cycles would continue. Comparing the relative retrogradation rates of amaranth gels in the presence of NaCl and sugars, it appears that, in general, lower retrogradation rates were obtained with glucose and sucrose added than with NaCl and fructose. However, higher retrogradation values of amaranth gels were predicted in the presence of glucose and sucrose than with NaCl or fructose present. These results support the observations made with the centrifugation method. The freeze-thaw cycle testing could be considered a harsh treatment with an overall effect of accelerating the retrogradation process. The results cannot be compared directly with starch gels stored at constant temperature as reported by Baker and Rayas-Duarte (1998). However, it gives an estimation of the changes occurring when temperature cycling disturbed the nucleation and propagation steps of retrogradation as proposed by Slade and Levine (1989).

CONCLUSIONS

Amaranth starch appeared to have better stability to freezing and thawing than did corn, wheat, and rice starches, through at least four cycles, as indicated by both DSC and centrifugation testing. This increased stability over the other starches may enhance its potential use in food products. Furthermore, the modification of amaranth starch would greatly improve its usefulness in frozen food products by yielding stable starches. The DSC and centrifugation methods gave similar rates of retrogradation in the first two freeze-thaw cycles.

Both methods of determining freeze-thaw stability showed similar results with the addition of sugar to amaranth starch gels. The effect of sugar levels on the freeze-thaw stability of amaranth starch was not linear (i.e., freeze-thaw stability did not increase as the sugar levels increased). The results of studying the effects of added salt were completely opposite between the two methods. This may be due to the greater sensitivity of DSC to thermal changes in the retrograded gel than in the centrifugation method.

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TABLE IV
Retrogradation Values for Starch Gels as a Function of Freeze-Thaw Cycles

Starch	Maximum Retrogradation (%)	Rate Constant (per cycle)	Coefficient of Determination (r^2)	Assumed Lag Time (cycles)
Amaranth	98.7	0.114	0.986	0
Corn	69.5	0.413	0.988	0
Wheat	64.7	0.409	0.989	0
Rice	53.8	0.676	0.972	0
Amaranth NaCl (%)				
2	65.6	0.172	0.926	1
5	77.9	0.101	0.947	1
Glucose (%)				
10	100.0	0.073	0.981	0
20	100.0	0.085	0.990	0
30	70.6	0.246	0.923	0
Sucrose (%)				
10	97.9	0.097	0.989	0
20	100.0	0.058	0.972	0
30	99.0	0.084	0.943	0
Fructose (%)				
10	67.2	0.201	0.960	0
20	69.5	0.248	0.978	0
30	81.8	0.188	0.912	0

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