

# Retrogradation of Amaranth Starch at Different Storage Temperatures and the Effects of Salt and Sugars<sup>1</sup>

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## ABSTRACT

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The retrogradation of amaranth, corn, wheat, and rice starches at different storage temperatures (-20, 4, and 25°C) was measured by differential scanning calorimetry (DSC). Sodium chloride (2 and 5%), glucose, sucrose, and fructose (10, 20, and 30%) were added to amaranth starch gels. The retrogradation was measured as the percent of gelatinization enthalpy ( $\Delta H_g$ ) of the retrograded gel when compared to the original nonretrograded sample. The data were analyzed fitting an exponential first-order equation by regression, assuming a first-order process. A modified equation was used for the data that showed several days of lag time before retrogradation was observed. Retrogradation percentage of amaranth gels at all three storage temperatures was lower than those of corn, wheat, or rice starches. Amaranth starch showed  $\approx 2$ -9 times slower retrogradation rates than

corn, wheat, and rice starches at 4 and -20°C, and up to  $\approx 2.8$  times lower maximum percent of retrogradation than the other starches at all three temperatures. After 21 days, amaranth, corn, and wheat starches exhibited the highest retrogradation (45, 77, and 67%, respectively) at 4°C, compared to 25 and -20°C. Rice starch had a higher retrogradation percentage (55%) at -20°C, compared to 25 and 4°C. The percent of retrogradation of gels with 5% NaCl addition decreased  $\approx 2$  and 1.7 times at 25 and 4°C, respectively, and increased  $\approx 1.2$  times at -20°C, compared to the control. The addition of sugars had similar effects in reducing retrogradation at 25 and 4°C and increasing retrogradation at -20°C when compared to samples with no sugar added.

Grain amaranth (*Amaranthus*) has been grown commercially in the United States since 1983 with fairly good success. In 1989, amaranth produced yields of almost 1,700 kg/ha in the United States (Henderson 1993). U.S. production of amaranth grain in 1988 was  $\approx 700$  Mt (Breene 1990). The use of amaranth in the United States has been limited primarily to the health food market. The high cost of production and lack of useful applications for the starch and protein have made it economically unfeasible to extract the relatively significant amounts of squalene, tocopherols, and tocotrienols that are contained in the amaranth seed oil (Breene 1991).

The amaranth grain contains 50-60% starch that has a 1-3  $\mu$ m granule size (Breene 1991, Uriyapongson and Rayas-Duarte 1994). There are waxy (glutinous) and nonwaxy (nonglutinous) varieties of *Amaranthus hypochondriacus* (Okuno and Sakaguchi 1981). The majority of the amaranths reported in the literature have 5-8% amylose. These low levels of amylose cause amaranth starch to perform poorly in bread and cake formulations (Stone and Lorenz 1984). Potential uses for amaranth starch include food thickeners, dusting powders, laundry starch, biodegradable plastics (Breene 1991), and fat replacers (Lehmann 1992).

The retrogradation of gelatinized starch is a phenomenon of great importance to the food industry. The amount of retrogradation that occurs in a starch-containing food can influence the texture and acceptability of that food (Miles et al 1985b).

In bread, retrogradation takes place at the highest rate during the first few days and then at a decreasing rate as the bread aged (Krog et al 1989). I'Anson et al (1990) found similar results with wheat starch gels. Retrogradation rates at various storage temperatures have been studied (Colwell et al 1969, McIver et al 1968, Longton and LeGrys 1981, Eliasson 1985, Nakazawa et al 1985, Chang and Chang 1988) with varying results as to which storage temperature produces the fastest rate of retrogradation.

Differential scanning calorimetry (DSC) has been used to study the retrogradation of gelatinized starch (Eliasson 1985, Miles et al

1985a, Nakazawa et al 1985, Jankowski and Rha 1986, Russell 1987, Zeleznak and Hosney 1987, Krog et al 1989, White et al 1989, Roulet et al 1990, Chang and Liu 1991).

Sugars have been reported to have an antistaling effect (I'Anson et al 1990, Cairns et al 1991, Kohyama and Nishinari 1991, Katsuta et al 1992). Others have described the retrogradation-accelerating effect of sugars (Germani et al 1983, Maxwell and Zobel 1978, Chang and Liu 1991). Salt has differing effects on the retrogradation of starch as well. Sodium chloride reportedly increases retrogradation (Ciacco and Fernandes 1979), decreases retrogradation (Chang and Liu 1991), or has no effect on retrogradation (Schoch and French 1947). The concentration of salt in the three reports was similar (2% and 2.33%).

The kinetics of starch retrogradation were studied by applying the exponential first-order equation (McIver et al 1968). Although some limitations of using this equation were reported, the main objection has been the decrease of polymer (starch) concentration as the starch crystallizes (Russel and Oliver 1989). However, until better models are proposed, the exponential equation could be used in the comparison of starch retrogradation kinetics among samples.

The purpose of this study was to determine the retrogradation of amaranth starch gels at different storage temperatures by DSC and to determine the effects of sodium chloride, glucose, sucrose, and fructose on the retrogradation of these gels.

## MATERIALS AND METHODS

### Materials

Commercial amaranth starch was obtained from Amaranth Resources, Inc. (Albert Lea, MN) and further purified in the laboratory as reported by Baker and Rayas-Duarte (1998). Common corn starch (American Maize Products, Co., Hammond, IN), rice starch (Sigma Chemical Co., St. Louis, MO), and wheat starch (Midwest Grain Products, Inc., 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) solutions (Mallinkrodt Inc., Paris, KY) were used in concentrations of 2 and 5% (w/w).

### Starch Purification

Amaranth starch was purified using a modified wet-milling procedure based on the method of Uriyapongson and Rayas-Duarte (1994) as reported by Baker and Rayas-Duarte (1998).

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### Sample Preparation and Analysis of Retrogradation

Samples were gelatinized as described by White et al (1989). Starch samples (3.5 mg) were weighed into aluminum DSC pans. Deionized water (8 µL) (or water containing the added sugars or NaCl) were 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 to 120°C. Gelatinized samples were stored at either -20, 4, or 25°C for up to 21 days. The gels were analyzed using a Perkin-Elmer DSC 7 equipped with a TAC 7/DX thermal analysis instrument controller and a DEC personal workstation (Perkin Elmer, Norwalk, CT). Thermal properties were analyzed between 20 and 100°C at a rate of 10°C/min after 1, 2, 4, 7, 14, and 21 days of storage. For each storage time, temperature, and concentration of additive, three independent replicates were used. The retrogradation peak was analyzed and compared with the gelatinization peak. The enthalpy ( $\Delta H$ ) value required to break down the retrograded peak was expressed as a percentage of the enthalpy ( $\Delta H_g$ ) required to gelatinize the starch sample (assigned as 100%).

### Data Analysis

The percent of retrogradation (%R) was fitted to an exponential first-order equation (Russell and Oliver 1989) by regression:

$$\frac{\%R_{\infty} - \%R_t}{\%R_{\infty} - \%R_0} = \exp(-kt) \quad (1)$$

where  $\%R_{\infty}$  is the maximum percent of retrogradation,  $\%R_t$  is the percent of retrogradation at time  $t$ ,  $\%R_0$  is the percent of retrogradation at zero time, and  $k$  is the rate constant. If  $\%R_0 = 0$ , this becomes:

$$\frac{\%R_{\infty} - \%R_t}{\%R_{\infty}} = \exp(-kt) \quad (2)$$

which can be rearranged to give:

$$\%R_t = \%R_{\infty} [1 - \exp(-kt)] \quad (3)$$

In those data sets in which retrogradation did not begin until several days had passed, the equation was adjusted to take this lag time into consideration:

$$\%R_t = 0, \text{ if } t \leq t_L \quad (4)$$

and where  $\%R_t$  is the percent of retrogradation at time  $t$ , and  $t_L$  is

the lag time (assumed to be the last day in which the  $\%R = 0$  before a retrogradation peak appears).

### Statistical Analysis

We created a general treatment variable with various combinations of sugar, starch, salt, and concentration, each representing one of 15 levels for the independent variable in a completely randomized design. We used this type of analysis to facilitate simple comparisons within each storage temperature among the different starches, additives, and concentrations for the three different dependent measures: onset and peak gelatinization temperatures, and gelatinization temperature range.

Analysis of variance (ANOVA) was performed, and differences among samples were determined by Duncan's multiple range test using the SAS program (SAS Institute, Cary, NC). Three independent samples were analyzed at each temperature, storage time, and sugar or salt concentration.

Initially, we analyzed the retrogradation data for each storage temperature separately using a two-factor design with a factorial arrangement between the levels of starch type and storage time, salt concentration and storage time, and sugar concentration and storage time. The two-way interactions were significant ( $P < 0.05$ ). Interpreting main effects when significant interactions are present requires great care.

Alternatively, tests of simple effects (one-way ANOVAs) across levels of one factor, performed separately at each level of the other factors) may be more useful (Lindman 1992). We elected to examine the effects of starch type, salt concentration, and sugar concentration separately at each combination of storage time and storage temperature in an attempt to simplify the interpretation of the results.

## RESULTS AND DISCUSSION

### Retrogradation

The gelatinization and melting transition temperatures obtained by DSC analysis of original gels and after 21 days of storage are shown in Table I. Gelatinization temperatures ( $T_o$  and  $T_p$  = onset and peak temperatures, respectively) of the original starches showed significantly higher  $T_o$  of amaranth and corn starches than that of wheat and rice starches, and  $T_p$  was higher than that of

TABLE I  
Comparison of Onset, Peak, and Range of Melting Transition Temperatures (C)<sup>a</sup> for Starches and Additives Stored 21 Days at Three Temperatures as Analyzed by Differential Scanning Calorimetry

Starch	Gelatinization			25°C			4°C			-20°C		
	$T_o$	$T_p$	$T_r$	$T_o$	$T_p$	$T_r$	$T_o$	$T_p$	$T_r$	$T_o$	$T_p$	$T_r$
Amaranth	66.2c <sup>b</sup>	70.6bc	8.8ab	48.8a	59.3a	21.1bc	37.7a	48.9a	22.4e	41.9ab	49.1ab	14.4b-d
Corn	66.2c	70.3b	8.3a	50.8bc	59.4a	17.3ab	40.1b	51.0bc	21.7de	43.4ab	51.0b	15.3b-d
Wheat	56.1a	60.6a	9.1b	52.1bc	59.1a	14.2a	39.8b	48.4a	17.3b	41.4ab	48.1a	13.4bc
Rice	61.4b	76.3h	29.8c	51.7bc	59.9ab	16.4ab	40.9bc	51.1bc	20.5c-e	42.8ab	50.9b	16.3cd
Amaranth												
NaCl (%)												
2	69.4h	74.0g	9.1b	50.2ab	61.5d	22.6bc	40.6bc	50.9b	20.5c-e	44.5b	51.2b	13.4bc
5	71.6i	76.1h	9.2b	47.3a	61.1cd	27.5c	40.8bc	50.8b	19.9cd	41.5ab	50.0ab	16.9d
Glucose (%)												
10	66.9cd	71.4d	9.1b	53.5cd	60.7c	14.4a	40.2b	50.5b	20.6c-e	42.0ab	49.2ab	14.3b-d
20	67.4de	71.8de	8.8ab	54.2d	62.8e	17.3ab	40.0b	50.6b	20.8de	42.0ab	48.2a	12.4ab
30	68.3fg	72.8f	9.0b	50.3ab	60.5cd	20.4ab	41.3cd	51.5c	20.5c-e	42.2ab	47.4a	10.4a
Sucrose (%)												
10	67.3de	71.9de	9.2b	49.3ab	60.3bc	21.9bc	41.7de	50.9bc	18.3bc	43.4bc	50.1b	13.4bc
20	68.0ef	72.6f	9.3b	51.1bc	59.7ab	17.3ab	41.4cd	51.1bc	19.5b-d	40.7a	48.6a	15.7cd
30	69.0gh	73.6g	9.1b	52.6b-d	63.7f	22.3bc	42.9f	52.5de	19.1bc	41.1a	49.4ab	16.6d
Fructose (%)												
10	66.7cd	71.2cd	9.1b	53.3cd	60.1b	13.6a	44.0g	51.8cd	15.2a	40.2a	47.6a	14.7b-d
20	67.4de	71.9e	9.1b	53.6cd	60.6b	14.2a	42.6ef	51.5bc	17.7b	42.6ab	49.6ab	14.0bc
30	67.7ef	72.3eg	9.2b	55.4d	63.1ef	15.5ab	42.2d-f	52.8e	21.1de	40.9a	48.9ab	15.9cd

<sup>a</sup>  $T_o$ , and  $T_p$  are onset and peak melting transition temperatures, respectively. Range of melting transition ( $T_r$ ) =  $2(T_p - T_o)$  as described by Krueger et al (1987).

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

wheat starch, but lower than that of rice. This suggested more organized regions in amaranth and corn as compared with wheat, because higher temperatures were needed to record a melting transition. The  $T_p$  values found in our study are in agreement with the corn, rice, and wheat starch values reported by Lund (1984).

Adding NaCl and sugars increased the  $T_o$ ,  $T_p$ , and conclusion temperatures ( $T_c$ ) of the gelatinization endotherms (Table I). These results agree with the reports of Lund (1984) and Spies and Hosney (1982). The retrograded gels showed lower melting transition temperatures ( $T_o$ ,  $T_p$ , and  $T_c$ ) than did the original starch gel after 21 days of storage. The melting transition temperatures for  $-20$  and  $4^\circ\text{C}$  storage were  $20$ – $25^\circ\text{C}$  lower than the original sample after 21 days of storage. Retrograded gels stored at  $25^\circ\text{C}$  for 21 days yielded melting temperatures  $\approx 10$ – $15^\circ\text{C}$  lower than original samples. These results also agree with Lelievre's (1976) use of theory of polymer-amorphous melting in a three-component system (polymer, diluent, and low molecular weight solute) to analyze the

effect of sugars on the starch melting transitions. Lelievre-derived expressions related melting temperatures to composition, and predicted the increase in melting temperature as a function of maltose concentration for starch concentrations up to 40%. Retrograded gel samples at 21 days of storage showed wider peak melting ranges ( $T_r$ ) than the original gelatinization data (Table I). An increased  $T_r$  in starch gels stored at refrigerated temperatures was also reported by White et al (1989). The  $T_r$  of native rice starch had a significantly wider melting peak than the other starch sources. This finding suggested a wide population of starch granules with differences in molecular weight distribution of amylopectin and granule size. The rice starch gelatinization range was similar to that reported by Stevens and Elton (1971). Adding NaCl and sugars had no significant effect on the  $T_r$  of the gelatinization peaks, but generally produced higher  $T_o$  and  $T_p$  values, suggesting a shift to sharper peaks. These observations suggested that the hydration and swelling of the amaranth starch granules was generally affected by the presence of NaCl and sugars, which decrease the available water for gelatinization. At 21 days of storage, the presence of NaCl in amaranth starch had no significant effect on the  $T_r$  at  $25$  or  $-20^\circ\text{C}$  when compared with the amaranth control. The 5% addition of NaCl significantly decreased the  $T_r$  at  $4^\circ\text{C}$ , and the 2% addition had no effect when compared with the control. A sharper peak could be partially explained by a nucleation of crystallites promoted at  $4^\circ\text{C}$  and with 5% NaCl. Addition of glucose generally yielded higher amaranth melting temperatures (higher  $T_o$  and  $T_p$ ). At  $25^\circ\text{C}$ , the 10% glucose addition significantly decreased the  $T_r$ , while the 20 and 30% glucose additions had no significant effect on  $T_r$ . Adding glucose generally produced higher  $T_o$  and  $T_p$  values and sharper peaks with a narrower  $T_r$ , but it had no significant effect on the  $T_r$  at  $4^\circ\text{C}$  (Table I). At  $-20^\circ\text{C}$ , the 10 and 20% glucose additions had no significant effect on  $T_r$ , and the 30% glucose addition significantly decreased the  $T_r$ . In general, amaranth starch gels stored at  $25$  and  $4^\circ\text{C}$ , which contained sucrose or fructose, yielded higher melting temperatures ( $T_o$  and  $T_p$ ) when compared to the control. Adding sucrose had no significant effect on  $T_r$ , at  $25$  and  $-25^\circ\text{C}$ , but it decreased  $T_r$ , significantly at  $4^\circ\text{C}$ . The 10 and 20% additions of fructose significantly decreased the  $T_r$  after storage at  $4^\circ\text{C}$ , while the 30% addition had no effect. Baker and Rayas-Duarte (1998) reported that amaranth starch gels showed no significant differences in  $T_r$  after 10 freeze-thaw cycles in the presence of NaCl, sucrose, glucose, or fructose, suggesting that the retrogradation reached plateau values.

**TABLE II**  
Retrogradation Values for Starch Gels Stored for 21 Days at  $25^\circ\text{C}$

Starch	Maximum Retrogradation (%)	Rate Constant (per day)	Coefficient of Determination ( $r^2$ )	Assumed Lag Time (days)
Amaranth	24.7	0.237	0.974	0
Corn	70.2	0.083	0.984	0
Wheat	26.6	0.537	0.928	0
Rice	56.9	0.080	0.978	0
Amaranth NaCl (%)				
2	21.0	0.131	0.900	2
5	na <sup>a</sup>	na	na	>7
Glucose (%)				
10	11.6	0.178	0.981	4
20	na	na	na	>7
30	na	na	na	>7
Sucrose (%)				
10	28.8	0.151	0.999	4
20	na	na	na	>7
30	na	na	na	>7
Fructose (%)				
10	na	na	na	>7
20	na	na	na	>7
30	na	na	na	>7

<sup>a</sup> Not available. Lag time was greater than seven days, leaving insufficient data for fit of equation.

**TABLE III**  
Retrogradation Values for Starch Gels Stored for 21 Days at  $4^\circ\text{C}$

Starch	Maximum Retrogradation (%)	Rate Constant (per day)	Coefficient of Determination ( $r^2$ )	Assumed Lag Time (days)
Amaranth	55.3	0.100	0.999	4
Corn	66.4	0.190	0.892	0
Wheat	55.3	0.215	0.937	0
Rice	44.1	0.447	0.997	0
Amaranth NaCl (%)				
2	106.0	0.025	0.993	2
5	32.7	0.111	0.960	2
Glucose (%)				
10	86.0	0.037	0.985	2
20	56.1	0.075	0.961	2
30	54.8	0.076	0.928	2
Sucrose (%)				
10	65.8	0.052	0.941	2
20	55.2	0.071	0.942	2
30	45.7	0.076	0.940	2
Fructose (%)				
10	43.3	0.110	0.971	2
20	52.2	0.073	0.960	2
30	39.3	0.138	0.993	2

**TABLE IV**  
Retrogradation Values for Starch Gels Stored for 21 Days at  $-20^\circ\text{C}$

Starch	Maximum Retrogradation (%)	Rate Constant (per day)	Coefficient of Determination ( $r^2$ )	Assumed Lag Time (days)
Amaranth	32.8	0.128	0.943	0
Corn	30.9	1.150	0.961	0
Wheat	42.7	0.754	0.987	0
Rice	42.0	0.507	0.782	0
Amaranth NaCl (%)				
2	86.0	0.037	0.985	1
5	56.1	0.075	0.961	1
Glucose (%)				
10	28.1	0.177	0.840	0
20	32.3	0.297	0.947	0
30	53.4	0.144	0.958	0
Sucrose (%)				
10	48.8	0.076	0.942	0
20	72.2	0.049	0.894	0
30	47.0	0.127	0.944	0
Fructose (%)				
10	46.9	0.170	0.962	0
20	50.2	0.243	0.989	0
30	54.0	0.242	0.981	0

Predicted % $R_{\infty}$ , rate constant, coefficient of determination, and assumed lag time for the stored gels are shown in Tables II–IV. At 25°C (Table II), amaranth starch showed a lower calculated % $R_{\infty}$  than corn and rice starch, but the retrogradation rate was  $\approx 2.9$  times faster than that of corn and rice starch. Wheat starch retrograded  $\approx 2.3$  times faster than amaranth starch, and had a similar % $R_{\infty}$ . A similar increase ( $\approx 2$  times) of the rate of retrogradation of corn, wheat, and rice starch gels was reported after two freeze-thaw cycles when compared to amaranth starch gels (Baker and Rayas-Duarte 1998). Table II shows that the amaranth starch gels that contained added NaCl and sugar retrograded more slowly than did the control, and did not begin to retrograde until, in some cases, more than seven days had passed.

At 4°C (Table III), the retrogradation rate of corn and wheat starch was  $\approx 2$  times faster than that of amaranth starch. The predicted % $R_{\infty}$  of amaranth starch was similar to that of corn and higher than that of wheat. Rice starch retrograded  $\approx 4.5$  times faster and had a predicted % $R_{\infty}$  that was 11.2% lower than that of the amaranth starch gels stored at 4°C. Amaranth starch did not begin to retrograde until after four days of storage at 4°C, while the samples with added NaCl and sugar began to retrograde after two days. Amaranth starch gels containing 2% NaCl retrograded  $\approx 4$  times slower than the control, but had a predicted % $R_{\infty}$  that was  $\approx 1.9$  times that of the

control. The 5% NaCl addition had about the same rate of retrogradation and a 59% lower % $R_{\infty}$  than the control amaranth starch gel without salt. Adding glucose and sucrose to amaranth starch gels decreased rates of retrogradation, with the predicted % $R_{\infty}$  decreasing as the addition concentrations increased from 10 to 30%. Adding 10 and 30% fructose gave higher rates of retrogradation and lower predicted % $R_{\infty}$ . The 20% addition of fructose had a lower rate of retrogradation and an % $R_{\infty}$  similar to that of the amaranth starch control.

At  $-20^{\circ}\text{C}$  (Table IV), the amaranth starch rate constant was  $\approx 4$  times slower than rice starch,  $\approx 6$  times slower than wheat starch, and  $\approx 10$  times slower than corn starch. The predicted % $R_{\infty}$  for amaranth starch was  $\approx 10\%$  less than those of wheat and rice starch and similar to that for corn starch. Adding NaCl to amaranth starch gels produced a lower rate of retrogradation but a higher calculated % $R_{\infty}$ . Adding glucose and fructose resulted in higher rates of retrogradation and similar or higher predicted % $R_{\infty}$ . Adding sucrose had similar or lower rates of retrogradation but higher predicted % $R_{\infty}$ .

The coefficients of determination observed at 25, 4, and  $-20^{\circ}\text{C}$  ranged from 0.84 to 0.99 for the amaranth gels containing salt or sugar. The lowest coefficient of determination (0.78) was observed in the rice starch gel sample stored at  $-20^{\circ}\text{C}$ . This may be due to variation of the observed data. The coefficient of determination

**TABLE V**  
Percent of Retrogradation of Amaranth, Corn, Wheat, and Rice Starches as a Function of Storage Temperature and Time

Storage Temp. (°C)	Storage Time (days)	Amaranth	Corn	Wheat	Rice
25	1	8.1b <sup>a</sup>	11.2c	14.7d	7.3a
	2	9.6a	11.8b	16.9c	11.6b
	4	15.2a	21.8c	21.0c	17.4b
	7	17.7a	27.7d	22.6c	20.0b
	14	23.9a	46.9d	27.5b	39.3c
4	21	25.4a	59.0d	30.1b	46.6c
	1	0.0a	17.4d	13.8b	16.0c
	2	0.0a	28.2c	26.3b	27.0b
	4	0.0a	35.5c	26.7b	35.1c
	7	14.6a	45.3c	43.3b	43.1b
-20	14	34.7a	48.5d	45.8c	43.2b
	21	45.3a	77.0c	60.9b	44.8a
	1	4.4a	23.3c	19.8b	25.2d
	2	6.8a	25.0b	36.1d	26.7c
	4	17.5a	27.9b	41.3c	27.7b
	7	17.8a	30.3b	40.8d	38.8c
	14	23.2a	32.0b	42.2d	35.0c
	21	33.4a	34.0a	43.6b	55.1c

<sup>a</sup> Means with different letters in each row are significantly different at  $\alpha = 0.05$  using Duncan's multiple range test.  $n = 3$ .

**TABLE VI**  
Percent of Retrogradation of Amaranth Starch with Added NaCl as a Function of Storage Temperature and Time

Storage Temp. (°C)	Storage Time (days)	NaCl (%)		
		0	2	5
25	1	8.1b <sup>a</sup>	0.0a	0.0a
	2	9.6b	0.0a	0.0a
	4	15.2c	9.2b	0.0a
	7	17.7c	7.6b	0.0a
	14	23.9c	16.2b	7.3a
4	21	25.4c	19.9b	12.4a
	1	0.0a	0.0a	0.0a
	2	0.0a	0.0a	0.0a
	4	0.0a	6.8b	6.2b
	7	14.6b	11.0a	11.4a
-20	14	34.7b	26.4a	27.9a
	21	45.3c	41.3b	26.8a
	1	4.4b	0.0a	0.0a
	2	6.8a	11.3b	5.7a
	4	17.5a	25.8b	19.1a
	7	17.8a	25.7b	27.1b
	14	23.2a	26.8b	28.9b
	21	33.4a	38.1b	39.7b

<sup>a</sup> Means with different letters in each row are significantly different at  $\alpha = 0.05$  using Duncan's multiple range test.  $n = 3$ .

indicated that the level of fitness of the exponential first-order equation was relatively good.

The percent of retrogradation as a function of storage temperature and time (days) analyzed by DSC is shown in Tables V–IX. Amaranth starch retrograded less than corn, wheat, and rice starch at all three storage temperatures (Table V). In general, the native starches retrograded the least at 25°C and the most at 4°C. These results agreed with reports of faster retrogradation of wheat starch and cooked wheat grain stored at 4°C as compared with samples stored at 21°C (Jankowski and Rha 1986, Longton and LeGrys 1981).

Rice starch gels showed higher percentages of retrogradation at –20°C when compared to 4 and 25°C (Table V). These results agreed with the reports of rice starch gels by Chang and Chang (1988) and Nakazawa et al (1985), who found that the colder the storage temperature, the faster the rate of retrogradation. Eliasson (1985) demonstrated that wheat starch gels stored at 4°C retrograded faster than those at 23°C, and those at 23°C retrograded faster than those at –20°C. While this agreed with the results of corn starch in this study, the amaranth and wheat starches retrograded less at 25°C than at –20°C. The seemingly inconsistent findings of the effects of storage temperatures in the retrogradation of starch gels may be due, in part, to different concen-

trations of the gels, the methods of analysis, and their variability and experimental errors.

Adding NaCl to amaranth starch gels (Table VI) decreased retrogradation after 21 days at both the 25 and 4°C storage temperatures as compared with a control. Chang and Liu (1991) found similar results with rice starch gels stored at 25, 5, and 18°C. Russell and Oliver (1989) found that NaCl inhibited the reordering of the amylopectin fraction of starch gels during aging (storage) at 21°C. Adding NaCl to amaranth starch gel exhibited increased retrogradation when stored at –20°C. The 5% NaCl addition was more effective than the 2% NaCl addition at inhibiting retrogradation at 25 and 4°C after 21 days. At 25 and 4°C, the NaCl may be competing with the starch for the water to such a degree that the water remains bound in the gel, resulting in less retrogradation. The Na<sup>+</sup> and Cl<sup>-</sup> ions may also form partial ionic bonds with the starch molecules and the water and keep the starch molecules from realigning. At –20°C, at least a portion of the NaCl is in a freeze-concentrated solutes region in which the normal structure of pure water (hydrogen-bonded, tetrahedral arrangement) is disrupted (Fenema 1985). The entrapped water further away from the center of the gel would not be held as tightly and would allow the starch granules to reassociate more easily.

**TABLE VII**  
Percent of Retrogradation of Amaranth Starch with Added Glucose as a Function of Storage Temperature and Time

Storage Temp. (°C)	Storage Time (days)	Glucose (%)			
		0	10	20	30
25	1	8.1b <sup>a</sup>	0.0a	0.0a	0.0a
	2	9.6b	0.0a	0.0a	0.0a
	4	15.2b	0.0a	0.0a	0.0a
	7	17.7c	4.1b	0.0a	0.0a
	14	23.9b	10.6b	7.0a	6.1a
	21	25.4bc	10.5c	12.8a	16.0b
4	1	0.0a	0.0a	0.0a	0.0a
	2	0.0a	0.0a	0.0a	0.0a
	4	0.0a	7.9b	5.9b	8.2c
	7	14.6b	11.3a	11.9a	11.1a
	14	34.7a	33.4a	38.7b	40.0b
	21	45.3c	42.8bc	41.8ab	38.8a
–20	1	4.4a	10.1c	6.5b	6.3b
	2	6.8a	12.3b	17.8c	11.5b
	4	17.5b	15.7a	22.6b	30.1c
	7	17.8a	14.4a	26.1c	27.6c
	14	23.2a	22.5a	28.1b	49.4c
	21	33.4a	31.7a	36.6b	49.8c

<sup>a</sup> Means with different letters in each row are significantly different at  $\alpha = 0.05$  using Duncan's multiple range test.  $n = 3$ .

**TABLE VIII**  
Percent of Retrogradation of Amaranth Starch with Added Sucrose as a Function of Storage Temperature and Time

Storage Temp. (°C)	Storage Time (days)	Sucrose (%)			
		0	10	20	30
25	1	8.1b <sup>a</sup>	0.0a	0.0a	0.0a
	2	9.6b	0.0a	0.0a	0.0a
	4	15.2b	0.0a	0.0a	0.0a
	7	17.7c	10.2b	0.0a	0.0a
	14	23.9b	22.8b	8.3a	7.2a
	21	25.4bc	26.4c	23.6b	9.6a
4	1	0.0a	0.0a	0.0a	0.0a
	2	0.0a	0.0a	0.0a	0.0a
	4	0.0a	5.0b	5.8b	5.5b
	7	14.6b	9.7a	9.7a	10.2a
	14	34.7a	37.3b	38.0b	33.2a
	21	45.3c	38.7b	39.8b	32.4a
–20	1	4.4a	5.8b	5.2ab	5.3ab
	2	6.8a	8.8b	6.7a	8.2ab
	4	17.5b	11.2a	21.2c	25.0d
	7	17.8a	24.2b	22.0b	26.9c
	14	23.2a	25.7a	25.8a	32.9b
	21	33.4a	41.9b	51.1d	47.7c

<sup>a</sup> Means with different letters in each row are significantly different at  $\alpha = 0.05$  using Duncan's multiple range test.  $n = 3$ .

Adding sugars to amaranth starch gels (Tables VII–IX) inhibited retrogradation at 25°C. At 4°C, retrogradation appeared sooner (after four days) in the gels with added sugar, but at 21 days, the amount of retrogradation was less than that of the amaranth starch control. Researchers have reported comparable results of decreased retrogradation with added sugar to starch gels. I'Anson et al (1990) and Cairns et al (1991) showed that sugars suppressed retrogradation in wheat starch gels stored at 20°C. Kohyama and Nishinari (1991) reported that sugars decreased retrogradation in sweet potato starch gels stored at 5°C. Slade and Levine (1987) analyzed starch gels with added sugar stored at 25°C (starch-sugar-water mixture, 1:1:1) and found that glucose and sucrose decreased retrogradation while fructose increased retrogradation. Katsuta et al (1992) determined that sugars inhibited retrogradation in rice starch gels stored at 0°C. At –20°C, the gels containing added glucose, sucrose, and fructose showed higher retrogradation percentages at the three percentages used than did the amaranth starch control (Tables VII–IX). These results agreed with the reports of an increase in retrogradation when sugars were added to rice starch gels stored at –18°C (Chang and Liu 1991).

At 25 and 4°C, as was seen with the addition of NaCl, the sugars may be competing for the water with the starch. The hydroxyl groups of the sugars are most likely binding the water inside the starch molecule as well as physically preventing the granule molecules from realigning (steric hindrance). At –20°C, as with the NaCl, it is likely that the sugars are more concentrated in the center of the frozen gel and may not be distributed throughout. This absence of the sugars from throughout the gel removes the sugars physically as well as the binding effects of their hydroxyl groups. The lower concentrations of the NaCl addition (2 and 5%) show similar results to the larger concentrations of the sugar additions (10, 20, and 30%). This suggests that while the NaCl molecule and its respective ions are smaller, the ionic bonds they form were just as or more effective in preventing the retrogradation of amaranth starch as the larger sugar molecules that form hydrogen bonds.

In general, lower retrogradation values were observed with added glucose (Table VII) when compared with sucrose and fructose (Tables VIII and IX) at 25 and –20°C. In contrast, higher retrogradation was promoted in gels with glucose at 4°C when compared with sucrose and fructose additions at the same temperature. Adding fructose (Table IX) inhibited the most retrogradation at 4°C and promoted the most retrogradation at –20°C. Overall, gels with added sugar stored at 25°C for 21 days showed suppressed retro-

gradation in the order: glucose > fructose > sucrose. Generally, in those gels stored at 4°C for 21 days, retrogradation was suppressed by the sugars in the order: fructose > sucrose > glucose. Sugars promoted overall retrogradation in gels stored at –20°C for 21 days in the order: fructose > sucrose > glucose. I'Anson et al (1990) and Cairns et al (1991) found that sugars reduced crystallinity in wheat starch gels stored at 20°C in the order: ribose > sucrose > glucose. Kohyama and Nishinari (1991) determined that sugars reduced retrogradation in sweet potato starch pastes stored at 5°C in the order: sucrose > glucose > fructose. Katsuta et al (1992) reported that hexoses inhibited retrogradation of rice starch gels more than pentoses and disaccharides more than monosaccharides. Slade and Levine (1987) found that sugars decreased recrystallization in starch gels stored at 25°C in the order: sucrose > glucose > fructose. The order in which the sugars reduced retrogradation may be related to their molecular size, their number of hydroxyl groups, and their ability to form hydrogen bonds with water and starch molecules in a gel state at the concentrations tested.

The concentration of the added sugar had variable effects on the retrogradation of that sample containing the sugar. For example, the 30% addition of sugar caused the most retrogradation of gel samples at 25°C when it was glucose and fructose, but the least when it was sucrose. Other researchers have found that retrogradation is linear, increasing or decreasing with an increase in the amount of sugar added (Germani et al 1983, Chang and Liu 1991, Kohyama and Nishinari 1991). The results of this study do not show a linear effect.

Amaranth starch had higher stability to consecutive freeze-thaw cycles when compared to native corn, wheat, and rice starches (Baker and Rayas-Duarte 1998). In this study, amaranth starch gels showed more stability when stored at 4 and –20°C than when stored at 25°C. The results agree with the report of White et al (1989), who found that starches most stable to freeze-thaw cycling were also the most stable to refrigerated storage.

## CONCLUSIONS

Amaranth starch gels had better resistance to retrogradation at 25, 4, and –20°C storage temperatures than did corn, wheat, or rice starch gels. This property may enhance its use in food products that require storage temperatures in the reported range. Amaranth starch gels retrograded slower than the other native starch gels at 4 and –20°C and tended to have lower predicted  $R_{\infty}$  than the corn, wheat, and rice starch gel samples. Adding salt decreased the amount of retrogradation of the gel samples at 25 and 4°C and increased it

TABLE IX  
Percent of Retrogradation of Amaranth Starch with Added Fructose as a Function of Storage Temperature and Time

Storage Temp. (°C)	Storage Time (days)	Fructose (%)			
		0	10	20	30
25	1	8.1b <sup>a</sup>	0.0a	0.0a	0.0a
	2	9.6b	0.0a	0.0a	0.0a
	4	15.2b	0.0a	0.0a	0.0a
	7	17.7b	0.0a	0.0a	0.0a
	14	23.9c	4.9a	8.6b	8.9b
	21	25.4c	7.9a	13.9b	28.0d
4	1	0.0a	0.0a	0.0a	0.0a
	2	0.0a	0.0a	0.0a	0.0a
	4	0.0a	6.3b	6.0b	10.8c
	7	14.6b	16.6c	12.2a	18.0c
	14	34.7ab	36.2b	35.8b	33.1a
	21	45.3b	35.6a	36.9a	35.9a
–20	1	4.4a	6.6b	9.3c	9.1c
	2	6.8a	16.8b	18.1b	16.8b
	4	17.5a	26.5b	34.4c	37.6d
	7	17.8a	26.2b	38.7c	45.7d
	14	23.2a	45.1b	50.6c	48.6c
	21	33.4a	45.4b	48.6c	55.4d

<sup>a</sup> Means with different letters in each row are significantly different at  $\alpha = 0.05$  using Duncan's multiple range test.  $n = 3$ .

at  $-20^{\circ}\text{C}$ . Adding glucose, sucrose, and fructose had similar effects in decreasing the percent of retrogradation at 25 and  $4^{\circ}\text{C}$  and increasing it at  $-20^{\circ}\text{C}$ . Adding NaCl and sugars to amaranth starch gels had variable effects on the rate and predicted  $\%R_{\infty}$ , depending on the temperature. The results reported in this study supported other data showing stability of amaranth starch related to slower retrogradation rates and lower percent of retrogradation values.

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