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Effect of α-Amylases from Different Sources on the Retrogradation and Recrystallization of Concentrated Wheat Starch Gels: Relationship to Bread Staling

HERNAN R. PALACIOS,† PAUL B. SCHWARZ,*,‡ AND BERT L. D'APPOLONIA†

Department of Cereal and Food Sciences and Department of Plant Sciences, North Dakota State University, Fargo, North Dakota 58105

Concentrated starch gels were supplemented with four α-amylases from different sources. The retrogradation and recrystallization of the gels were evaluated using differential scanning calorimetry (DSC) and X-ray crystallography. Correlations between the retrogradation data and the carbohydrate fractions extracted from these gels were determined. The thermostable (TBA) and intermediate temperature stability (ISBA) bacterial α-amylases were most effective in decreasing the rate of retrogradation of the starch in the gels. The cereal α-amylase at the high level (CAH) was also effective. Supplementation with the α-amylases increased the crystallinity of the gels. Gels supplemented with TBA or ISBA were most crystalline and retrograded to a lesser extent. The results indicated that DSC gives not only a measure of recrystallized amylopectin but also a measure of total order (recrystallized amylopectin and double-helical content). The maltooligosaccharides produced by the enzymes did not appear to be responsible for the reduced rates of retrogradation, but they appeared to be an expression of the degree of starch modification that was responsible for the inhibition of retrogradation. The crystallinity and retrogradation data were similar to results reported for bread and strongly suggest that bread staling is caused by the retrogradation of starch. The results also indicate that α-amylases decrease the rate and extent of retrogradation of starch gels by inhibiting the formation of double helices.

KEYWORDS: Amylopectin; amylases; bread staling; carbohydrates; crystallinity; DSC; degradation; dextrins; gels; maltooligosaccharides; retrogradation; starch

INTRODUCTION

Stale bakery foods are considered to be one of the most difficult technical and economic challenges bakers face. Staling decreases consumer acceptance of baked goods. As a result, it is estimated that an average of 5% of the total bread production is returned to the bakeries as unsalable (*1*). Bread staling and associated crumb firming phenomena have received a considerable amount of attention in cereal science research. Yet, despite the efforts of many investigators, the causes and basic mechanism of bread staling are not completely understood. Conclusions from many studies conducted in bread and starch gels have attributed the firming of bread that characterizes the staling phenomenon to the retrogradation of starch (*2*−*5*). However, other studies contradict this view and point to gluten−gluten and gluten−starch interactions as the main causes of bread firming (*6*, *7*).

α-Amylases are used as antistaling agents in breadmaking. Bacterial, cereal, and fungal α-amylases exhibit different degrees of effectiveness for such a purpose. Both thermostable and intermediate temperature stability bacterial α-amylases have been shown to decrease the rate of firming of bread (*7*−*9*) and the extent of starch retrogradation in bread and starch gels (*10*, *11*). Fungal α-amylase has been reported to decrease the initial firmness of bread, but has no effect on the rate of firming (*9*). The antistaling properties of barley or wheat malt α-amylase are more controversial. Some investigators have reported that this enzyme decreases the rate of firming (*8*, *12*). However, other investigators have reported that barley malt α-amylase increases the rate of bread firming (*7*). Several suggestions have been made to explain the antistaling effect of these enzymes. Schultz et al. (*13*) suggested that the breakdown of starch by bacterial α-amylase resulted in an increased proportion of low molecular weight fragments characterized by lower rates of retrogradation. Miller et al. (*8*) and Beck et al. (*14*) concluded that the staling of bread crumb was mainly determined by the degree of starch degradation and only partly by the character of the starch degradation products or dextrins. Because the extent of starch degradation was related to the thermostability of the enzymes, they inferred that the differences in firming rate produced by different α-amylases were related to the different thermosta-

* Corresponding author [telephone (701) 231-7732; fax (701) 231-7732; e-mail Paul.Schwarz@ndsu.nodak.edu].
† Department of Cereal and Food Sciences.
‡ Department of Plant Sciences.

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bilities of the enzymes. Zobel and Senti (*15*) proposed that bacterial α-amylase remains partially active in bread and cuts chains in the starch network with a concomitant decrease in bread firmness and an increase in starch crystallinity. Dragsdorf and Varriano-Marston (*12*) reported that supplementation of bread with bacterial, cereal, or fungal α-amylase decreased the firming rate and increased the crystallinity of starch when compared to unsupplemented bread. Martin and Hoseney (*7*) reported that bread supplemented with either bacterial or fungal α-amylase contained large quantities of low molecular weight dextrins with a degree of polymerization of 3−9, whereas bread supplemented with barley malt contained only minor quantities. According to Martin and Hoseney (*7*) the presence of these low molecular weight dextrins, maltohexaose in particular, hinders the formation of gluten−gluten and gluten−starch cross-links, which they consider to be the cause of bread firming. The suggestion of Martin and Hoseney is partially based on previous results that supplementation with α-amylases decreases the firming rate of bread crumb and increases the crystallinity of starch in bread (*12*, *15*). However, Every et al. (*9*) suggested that the antifirming effect of bacterial α-amylases was not caused by the presence of low molecular weight dextrins but by the reduced ability of the degraded starch to retrograde. According to Every et al. (*9*) the dextrins are just an expression of the structural changes produced by the enzymes in the starch. Morgan et al. (*5*) determined that starch bread, which contained no gluten, and standard bread made from wheat flour firmed at similar rates. Supplementation of both the standard bread and the starch bread with an antistaling α-amylase decreased the firming rate by similar amounts. Therefore, they concluded that bread firming is caused by the retrogradation of starch and that the antifirming effect of the α-amylase is due to the degradation of starch. Furthermore, Morgan et al. (*5*) also determined that the rate of development of molecular order or double helices in starch bread supplemented with α-amylase was significantly lower than that of unsupplemented starch bread. Gerrard et al. (*16*) supplemented bread with a combination of glucoamylase and an antistaling α-amylase. The glucoamylase degraded the low molecular weight dextrins produced by the α-amylase to glucose. Despite the absence of these dextrins, this bread showed the same reduction in firming rate as bread supplemented only with the α-amylase, in which the dextrins were not degraded. Gerrard et al. (*16*) concluded that the antistaling effect of the α-amylases is due to the starch modification and that the dextrins are only an expression of this modification. These results are supported by several studies conducted with starch gels and bread (*17*−*19*). Würsch and Gumy (*19*) reported that partial *β*-amylolysis of the external branches of amylopectin inhibited the retrogradation of amylopectin gels. Salem and Johnson (*17*) reported that supplementation of bread with individual maltooligosaccharides (glucose through maltoheptaose) appeared to increase the firming rate. However, Gerrard et al. (*16*) have reported that when exogenous maltooligosaccharides are added to bread, they are hydrolyzed by native flour amylases during the baking process and are not present in the finished product. Biliaderis and Prokopowich (*18*) reported that supplementation of waxy starch gels with individual maltooligosaccharides (glucose through maltoheptaose) had no effect or increased the rate of development of the enthalpy of retrogradation. Duedahal-Olesen et al. (*20*) added glucose, maltose, maltotriose, maltotetraose, and maltodextrins (average degrees of polymerization of 17 and 20) to wheat flour−water doughs at levels of 1.0 and 3.0%. They concluded that neither maltooligosaccharide altered the enthalpy of retrogradation of the wheat flour−water doughs after 7 days of storage. However, Duran et al. (*21*) found that the addition of maltooligosaccharides with degrees of polymerization of 3−5 to starch gels reduced the enthalpy of retrogradation of the gels.

Eliasson (*22*) concluded that differential scanning calorimetry (DSC) gives a measure of the melting of recrystallized amylopectin in aged starch gels or bread. However, Cooke and Gidley (*23*) studied the loss of crystalline and molecular order during starch gelatinization using X-ray crystallography, ^{13}C cross-polarization magic angle spinning nuclear magnetic resonance spectroscopy (^{13}C CP/MAS NMR), and DSC. Predicted enthalpy values for the disruption of fully ordered and crystalline analogues of the starches studied were calculated and compared with values for fully ordered and crystalline model material. The comparison suggested that the enthalpy of gelatinization, or similarly the enthalpy of retrogradation, primarily reflected the loss of molecular (double-helical) order rather than crystalline order.

The present work is the third of a series of studies designed to characterize the carbohydrate fractions produced by α-amylases in concentrated starch gels and to relate these findings to their retrogradation, recrystallization, and firming properties.

The objectives of this study were (1) to compare the effects of different α-amylase sources on the retrogradation (DSC) and crystallinity (X-ray crystallography) of concentrated wheat starch gels (*24*), (2) to relate retrogradation data to results previously reported for the carbohydrate fractions extracted from these gels (*24*), and (3) to relate and discuss these findings in terms of bread staling.

MATERIALS AND METHODS

Materials. The starch gels and α-amylases used in these experiments were the same as those previously prepared and described (*24*). These gels were analyzed using DSC and were subsequently extracted for carbohydrate analyses. The results of the carbohydrate analyses were previously reported (*24*).

Differential Scanning Calorimetry. Peak temperature (T_p) and enthalpy of retrogradation (ΔH) of the starch gels were determined using a DSC equipped with a thermal analysis data station (DSC-7, Perkin-Elmer, Norwalk, CT). The instrument was calibrated with indium. A pan containing 5.0 μL of distilled water was used as the reference. Samples (10 mg) from the center core of the gels were tightly packed into tared aluminum pans (no. 0219-0062, Perkin-Elmer). The pans were sealed, and the sample weight was recorded to 0.001 mg. The samples were equilibrated at 10 °C for 1 min and heated to 100 °C at a rate of 10 °C/min.

The enthalpy of retrogradation was determined by integrating the area under the DSC endotherm according to a procedure detailed by Fearn and Russell (*25*). Nine α-amylase treatments (three α-amylases at two levels and one at three levels) and a control with no enzyme were used. Each treatment was replicated five times. One replicate consisted of 10 starch gels, which were analyzed at storage time zero (60 min after removal from the oven) and after storage for 1, 2, 3, 5, 8, 12, 20, 40, and 140 days. Duplicate measurements from each gel were taken and averaged. After analysis, the vials containing the starch gels were resealed and immediately frozen at −80 °C. These gels were freeze-dried and extracted for carbohydrate analysis as previously described (*24*).

X-ray Crystallography. X-ray powder diffraction of composite samples of the five replicates of starch gels stored for 5 days was performed on a Philips automated vertical diffractometer (Philips, Redmond, WA), using Cu Kα radiation ($\lambda = 1.54184$ Å), variable divergent slits, a diffracted-beam graphite monochromator, and a sealed proportional counter. Starch powders were side-loaded into aluminum well mounts. Data reduction was performed with MDI Jade software (Materials Data, Inc., Livermore, CA), including application of a low (seven-point) Savitsky−Golay smooth function. Relative crystallinity

Table 1. Effect of α-Amylases on the Enthalpy of Retrogradation (Δ*H*) of Starch Gels after Storage for Different Time Periods

	Δ*H*[a] (J/g of starch db)								
amylase	day 1	day 2	day 3	day 5	day 8	day 12	day 20	day 40	day 140
TBAH	4.6 ± 0.2 ac	4.9 ± 0.2 c	5.1 ± 0.2 c	5.1 ± 0.2 c	5.1 ± 0.3 d	5.1 ± 0.1 d	4.8 ± 0.2 c	4.7 ± 0.3 c	04.4 ± 0.4 c
ISBAH	1.9 ± 0.1 g	2.5 ± 0.1 f	2.6 ± 0.2 e	2.9 ± 0.2 f	3.2 ± 0.2 f	3.6 ± 0.2 e	3.5 ± 0.1 d	3.3 ± 0.1 d	03.0 ± 0.1 d
ISBAM	2.8 ± 0.1 f	3.7 ± 0.1 e	3.9 ± 0.2 d	4.0 ± 0.2 d	4.5 ± 0.1 e	4.9 ± 0.2 d	5.0 ± 0.1 c	5.1 ± 0.1 c	04.9 ± 0.2 c
CAH	3.7 ± 0.2 e	4.4 ± 0.1 d	5.0 ± 0.2 c	5.3 ± 0.1 c	6.0 ± 0.1 c	6.5 ± 0.4 c	6.8 ± 0.2 b	7.3 ± 0.1 b	08.6 ± 0.1 b
FAH	4.4 ± 0.3 bc	5.7 ± 0.3 b	6.2 ± 0.1 a	6.7 ± 0.1 a	7.1 ± 0.3 ab	7.6 ± 0.2 ab	8.0 ± 0.3 a	8.4 ± 0.1	10.0 ± 0.2 a
TBAL	4.3 ± 0.1 cd	6.2 ± 0.2 a	6.3 ± 0.2 a	6.6 ± 0.1 a	7.0 ± 0.3 ab	7.3 ± 0.3 ab	7.8 ± 0.3 a	8.4 ± 0.2 a	10.0 ± 0.2 a
ISBAL	4.0 ± 0.1 de	5.1 ± 0.2 c	5.7 ± 0.3 b	6.1 ± 0.2 b	6.7 ± 0.2 b	7.2 ± 0.3 b	7.7 ± 0.4 a	8.2 ± 0.3 a	09.8 ± 0.2 a
CAL	4.8 ± 0.2 ab	5.7 ± 0.4 ab	6.6 ± 0.1 a	6.9 ± 0.2 a	7.3 ± 0.2 a	7.7 ± 0.3 a	8.0 ± 0.2 a	8.7 ± 0.4 a	10.4 ± 0.5 a
FAL	4.8 ± 0.2 a	6.0 ± 0.2 ab	6.5 ± 0.2 a	6.8 ± 0.1 a	7.4 ± 0.2 a	7.8 ± 0.2 a	8.0 ± 0.2 a	8.4 ± 0.3 a	10.3 ± 0.3 a
control	4.7 ± 0.1 ab	5.7 ± 0.1 ab	6.5 ± 0.3 a	6.7 ± 0.2 a	7.3 ± 0.2 a	7.7 ± 0.1 ab	8.1 ± 0.3 a	8.5 ± 0.2 a	10.4 ± 0.5 a

[a] Means ± SD ($n = 5$). Means within a column followed by the same letter are not significantly different ($P < 0.05$).

was estimated from the ratio of the area of peaks to the total area of the diffractogram (*26*). Analyses were performed by the Department of Chemistry at North Dakota State University, Fargo, ND.

Statistical Analyses. Enthalpy of retrogradation data were first analyzed using nonlinear regression to fit an Avrami equation to each of the five replicates (*27*−*30*). The equation was

$$\Delta H_t = \Delta H_L + (\Delta H_0 - \Delta H_L)\, e^{-kt^n} \quad (1)$$

where ΔH_0 (= 0), ΔH_t, and ΔH_L are experimental values of the enthalpy of retrogradation at time zero, *t*, and infinity, respectively, *k* is a rate constant, *t* is the independent variable time, and *n* is the Avrami exponent (*25*, *31*). The parameters ΔH_L, *k*, and *n* of the independent variable were estimated using the Marquardt−Levenberg algorithm according to SigmaStat 2.0 (Jandel Corp., San Rafael, CA). Despite its widespread use in cereal science, the Avrami theory does not seem to be a viable model to describe the mechanism of starch crystallization. Slade and Levine (*32*) have pointed out that the nonequilibrium nature of starch recrystallization limits the theoretical utility of the Avrami parameters. This suggestion has received support from other investigators (*33*, *34*). Regardless of these limitations, Slade and Levine (*32*) have pointed out that the Avrami equation can be used as a convenient method of fitting experimental data from starch retrogradation studies. In the present study, the Avrami equation has been used as a convenient method to fit the enthalpy of retrogradation data. No inferences have been made as to the meaning of the Avrami parameters in the mechanistic and kinetic aspects of starch retrogradation. The parameters ΔH_L, *k*, and *n* of the independent variable, T_p, and the values of ΔH_t were analyzed using analysis of variance (ANOVA) according to a completely randomized design (CRD). Multiple comparisons of the treatment means were conducted using Tukey's test.

Correlations between the data previously obtained for the carbohydrate analyses (*24*) and the current Δ*H* data were determined. All statistical analyses were performed using SigmaStat 2.0 (Jandel Corp.).

RESULTS

Enthalpy of Retrogradation. The effects of different α-amylase sources and levels on the retrogradation of starch gels are shown in **Table 1** and **Figure 1**. Development of the retrogradation endotherm was characterized by three patterns depending on enzyme source and level. The first pattern was exhibited by gels supplemented with thermostable bacterial α-amylase at the high level (TBAH). These gels retrograded at a fast rate during the first day and then very slowly up to the third day of storage. Thereafter, the enthalpy of retrogradation remained constant up to day 12 and then slowly decreased. The second pattern was exhibited by gels supplemented with intermediate temperature stability bacterial α-amylase at the high level (ISBAH) or intermediate temperature stability bacterial α-amylase at the medium level (ISBAM). The enthalpy of retrogradation of these gels increased during the first 12 days of storage and then remained constant in gels supplemented with ISBAM or slightly decreased in gels supplemented with ISBAH. The third pattern was exhibited by gels supplemented with thermostable bacterial α-amylase at the low level (TBAL), intermediate temperature stability bacterial α-amylase at the low level (ISBAL), cereal α-amylase (CA), or fungal α-amylase (FA) and was characterized by a continuous increase in the enthalpy of retrogradation during the whole storage period.

The kinetic parameters (ΔH_L, *k*, and *n*) and plots obtained by fitting the enthalpy of retrogradation data by an Avrami equation with *n* set free and with *n* constrained to be unity ($n = 1$) are shown in **Table 2** and **Figure 1**. Supplementation with TBAH, ISBAH, or ISBAM significantly decreased the limiting enthalpy of retrogradation (ΔH_L) of the gels. To a lesser extent, supplementation with cereal α-amylase at the high level (CAH) or ISBAL also significantly decreased the ΔH_L of the gels. The remaining α-amylase treatments did not differ from the control. The values of *k* of gels supplemented with TBAH was significantly higher than those for the other treatments. Gels supplemented with ISBAH or ISBAM also exhibited values of *k* that were significantly higher than that of the control. The values of *k* of gels supplemented with the remaining α-amylases were not different from the control. Except for gels supplemented with TBAH, the values of *n* for all of the treatments were smaller than unity. To determine if the choice of *n* had any effect on the results, the enthalpy of retrogradation data was fitted using an Avrami equation with *n* constrained to be unity ($n = 1$). The kinetic parameters (ΔH_L and *k*) obtained in this case are also shown in **Table 2**. These results clearly show that the relative rankings for ΔH_L and *k* were essentially the same regardless of the choice of *n* (free or unity). The only difference was that the values for ΔH_L were smaller than those obtained when *n* was set free. Therefore, the choice of the equation to fit the data does not seem to alter the results.

Total Extent of Retrogradation at Day 5. Because the values of Δ*H* at storage time zero were equal to zero, the values of Δ*H* shown in **Table 1** are directly proportional to the linear rates of retrogradation of the gels at the different storage times. Only the values of Δ*H* at storage time day 5 are discussed in this section. These values are discussed in order to compare with the X-ray crystallography data, which are discussed below, and were obtained from gels stored for 5 days. Furthermore, the results of some studies conducted on bread staling with DSC are expressed as linear rates of retrogradation using storage periods of 5−7 days (*10*, *11*, *20*). Among the gels supplemented with the high levels, those supplemented with ISBAH or ISBAM exhibited the lowest rates of retrogradation. Gels supplemented with TBAH or CAH also exhibited rates of retrogradation that were significantly lower than that of the unsupplemented control gels. The rate of retrogradation of gels supplemented with fungal

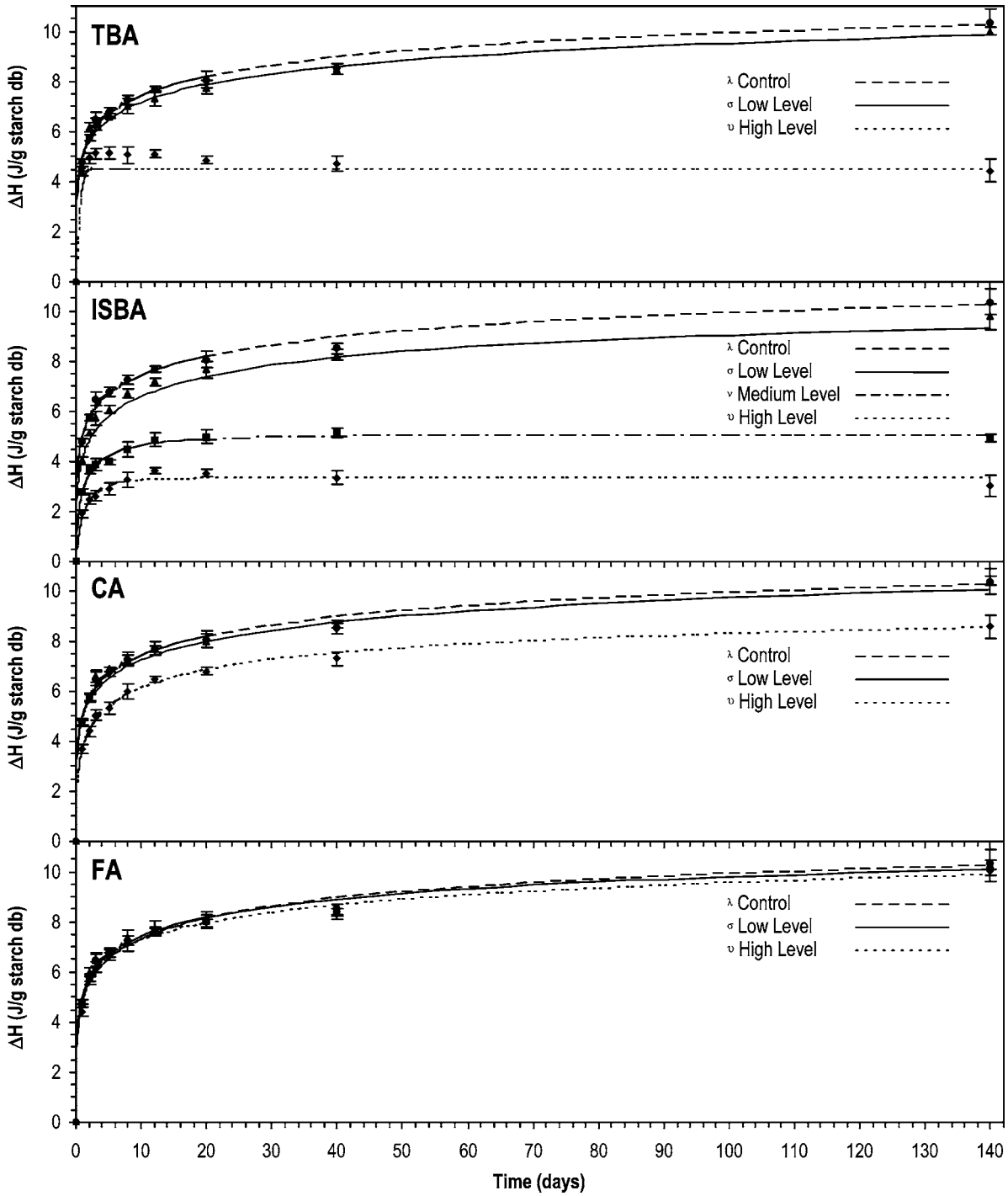

Figure 1. Effect of α-amylases from different sources on the kinetics of development of the enthalpy of retrogradation in starch gels. Curves were fitted using an Avrami equation. Bullets represent actual data. Error bars represent ± 1 standard deviation ($n = 5$). TBA, thermostable bacterial α-amylase; ISBA, intermediate temperature stability bacterial α-amylase; CA, cereal α-amylase; FA, fungal α-amylase; Control, no enzyme added.

α-amylase at the high level (FAH) was not different from that of the control. This is consistent with earlier findings suggesting that the intermediate temperature stability bacterial α-amylase (ISBA) retards the rate of retrogradation of starch in starch gels, whereas the FA has no effect (*10*). Among the gels supplemented with the low levels, those supplemented with ISBAL were the only ones that exhibited a rate of retrogradation that was significantly lower than that of the control. The rates of retrogradation of gels supplemented with TBAL, cereal α-amylase at the low level (CAL), or fungal α-amylase at the low level (FAL) did not differ from that of the control gels.

Peak Temperature. The effects of different α-amylase sources and levels on the T_p of the retrogradation endotherm of starch gels are shown in **Table 3**. The T_p increased with storage time for all treatments. The largest increases for gels stored for 140 days were observed for the gels supplemented with TBAH, ISBAH, or ISBAM. Gels supplemented with CAH or FAH exhibited intermediate levels of increase. The control gels exhibited the smallest increase. After storage for 1 day, the T_p of the control gels was highest and those from gels supplemented with TBAH, ISBAH, or ISBAM were the lowest. In contrast, after storage for 140 days the control gels exhibited the lowest

Table 2. Kinetic Parameters Calculated from Enthalpy of Retrogradation Data Obtained from Starch Gels Supplemented with α-Amylases

	parameters[a]				
amylase	ΔH_L (J/g of starch db)	k^b (day^{-n})	n	$\Delta H_{L_{n=1}}{}^c$ (J/g of starch db)	$k_{n=1}{}^c$ (day^{-1})
TBAH	04.52 ± 0.01 de	2.06 ± 0.01 a	1.18 ± 0.01 a	4.95 ± 0.08 e	2.79 ± 0.40 a
ISBAH	03.37 ± 0.08 e	0.81 ± 0.06 b	0.68 ± 0.09 b	3.29 ± 0.07 f	0.70 ± 0.08 b
ISBAM	05.07 ± 0.15 d	0.83 ± 0.03 b	0.48 ± 0.05 c	4.74 ± 0.06 e	0.72 ± 0.04 b
CAH	09.42 ± 0.57 c	0.52 ± 0.03 c	0.31 ± 0.03 d	6.95 ± 0.08 d	0.47 ± 0.02 b
FAH	11.46 ± 1.18 ab	0.54 ± 0.07 c	0.27 ± 0.04 d	8.08 ± 0.15 ac	0.58 ± 0.05 b
TBAL	12.44 ± 0.98 a	0.51 ± 0.06 c	0.23 ± 0.03 d	7.93 ± 0.18 bc	0.66 ± 0.06 b
ISBAL	10.52 ± 0.73 bc	0.49 ± 0.05 c	0.30 ± 0.04 d	7.83 ± 0.10 c	0.48 ± 0.03 b
CAL	12.86 ± 1.03 a	0.49 ± 0.06 c	0.23 ± 0.04 d	8.24 ± 0.16 a	0.61 ± 0.03 b
FAL	12.53 ± 0.75 a	0.52 ± 0.06 c	0.22 ± 0.02 d	8.17 ± 0.18 ab	0.66 ± 0.04 b
control	13.05 ± 0.93 a	0.50 ± 0.04 c	0.23 ± 0.03 d	8.19 ± 0.11 ab	0.62 ± 0.06 b

[a] Means ± SD ($n = 5$). Means within a column followed by the same letter are not significantly different ($P < 0.05$). [b] Units of k depend on the value of the Avrami exponent (n). [c] Values of the kinetic parameters with Avrami exponent set as unity ($n = 1$).

Table 3. Peak Temperature (T_p) of the Retrogradation Endotherm at Different Storage Times of Starch Gels Supplemented with α-Amylases

	T_p[a] (°C)								
amylase	day 1	day 2	day 3	day 5	day 8	day 12	day 20	day 40	day 140
TBAH	52.5 ± 0.3 g	54.6 ± 0.3 cd	54.1 ± 0.1 d	55.5 ± 0.2 bc	56.3 ± 0.2 ab	57.1 ± 0.3 a	57.7 ± 0.2 a	58.5 ± 0.5 bc	61.3 ± 0.3 b
ISBAH	54.7 ± 0.2 ef	54.0 ± 0.3 de	54.8 ± 0.6 cd	55.2 ± 0.4 bd	56.0 ± 0.2 bd	56.8 ± 0.3 ab	57.9 ± 0.2 a	59.9 ± 0.2 a	63.2 ± 0.4 a
ISBAM	54.3 ± 0.3 f	53.7 ± 0.4 e	54.8 ± 0.2 cd	54.7 ± 0.3 d	55.4 ± 0.3 e	56.2 ± 0.2 d	57.1 ± 0.4 bc	58.0 ± 0.3 cd	61.0 ± 0.3 b
CAH	55.0 ± 0.2 de	55.2 ± 0.4 bc	55.5 ± 0.1 bc	55.6 ± 0.3 bc	56.1 ± 0.2 ac	56.8 ± 0.1 ac	57.4 ± 0.2 ab	58.6 ± 0.3 bc	60.2 ± 0.2 c
FAH	55.6 ± 0.3 bd	56.3 ± 0.2 ab	56.5 ± 0.4 a	56.5 ± 0.2 a	56.7 ± 0.4 a	57.1 ± 0.2 a	57.9 ± 0.3 a	58.8 ± 0.2 b	60.2 ± 0.1 c
TBAL	55.5 ± 0.4 bd	55.6 ± 0.3 ab	55.8 ± 0.4 ab	55.8 ± 0.2 b	56.0 ± 0.2 be	56.1 ± 0.3 d	56.5 ± 0.3 d	57.3 ± 0.3 ef	59.0 ± 0.1 d
ISBAL	56.0 ± 0.2 b	55.0 ± 0.5 bc	55.5 ± 0.2 bc	55.2 ± 0.3 bd	55.6 ± 0.3 ce	56.1 ± 0.4 d	56.8 ± 0.2 cd	57.7 ± 0.4 de	59.2 ± 0.2 d
CAL	55.8 ± 0.4 bc	55.1 ± 0.2 bc	55.3 ± 0.3 bc	55.8 ± 0.5 bc	56.1 ± 0.4 bd	56.5 ± 0.1 bd	56.7 ± 0.3 cd	57.0 ± 0.2 f	59.0 ± 0.2 d
FAL	55.2 ± 0.2 ce	55.6 ± 0.3 ab	56.1 ± 0.2 ab	56.7 ± 0.1 a	56.3 ± 0.1 ab	56.3 ± 0.4 cd	57.0 ± 0.2 bd	57.7 ± 0.3 de	59.5 ± 0.4 d
control	56.7 ± 0.3 a	55.9 ± 0.2 a	55.4 ± 0.8 bc	55.1 ± 0.1 cd	55.5 ± 0.2 de	56.2 ± 0.3 d	56.7 ± 0.1 cd	57.5 ± 0.1 f	58.9 ± 0.2 d

[a] Means ± SD ($n = 5$). Means within a column followed by the same letter are not significantly different ($P < 0.05$).

T_p and gels supplemented with TBAH, ISBAH, or ISBAM exhibited the highest T_p. The T_p of gels supplemented with CAH or FAH were also significantly higher than that of the control. The remaining treatments were not different from the control after storage for 140 days.

X-ray Crystallography. The effects of the α-amylases on the relative crystallinity of the starch gels after storage for 5 days are shown in **Figure 2**. Gels supplemented with the α-amylases at the high levels were more crystalline than gels supplemented with the low levels. For the high levels, gels supplemented with ISBAH or TBAH were the most crystalline and were followed by gels supplemented with CAH. Gels supplemented with FAH were the least crystalline among the high levels. Gels supplemented with the low levels ranked in the same order as gels supplemented with the high levels. The unsupplemented control gels were the least crystalline of all. Dragsdorf and Varriano-Marston (*12*) reported that the degree of starch crystallinity in bread decreased in the following order: bread supplemented with bacterial α-amylase, bread supplemented with cereal α-amylase, bread supplemented with fungal α-amylase, and unsupplemented bread.

DISCUSSION

The following discussion integrates the results for the carbohydrate fractions extracted from these gels (*24*) with the retrogradation results reported above. The retrogradation results showed that starch gels supplemented with ISBAH, ISBAM, TBAH, CAH, or ISBAL exhibited smaller ΔH_L values than the unsupplemented control gels. In contrast, the ΔH_L of gels supplemented with the remaining α-amylases (FAH, TBAL, CAL, or FAL) did not differ from the control. Correlation coefficients (r) between the ΔH_L of the gels and the quantities of individual maltooligosaccharides (glucose through maltododecaose) extracted at storage time zero and after storage for 1, 2, 3, 5, 8, 12, 20, 40, and 140 days are summarized in **Table 4**. The only maltooligosaccharides that were significantly correlated with the ΔH_L of the gels were glucose and maltose. The negative signs of these correlations appear to indicate that gels that contained the largest quantities of glucose and maltose retrograded to a lesser extent. However, these findings should be taken with caution. Biliaderis and Prokopowich (*18*) and Duedahl-Olesen et al. (*20*) reported that after storage for 6−7 days, the retrogradation endotherm of starch gels and wheat flour gels supplemented with glucose or maltose did not differ from that of unsupplemented control gels. The finding that in the present experiments both glucose and maltose were negatively correlated with the ΔH_L of the gels does not necessarily mean that they are responsible for the decreases in ΔH_L. The quantities of both glucose and maltose continually increased during storage in gels supplemented with thermostable bacterial α-amylase (TBA) or ISBA because these enzymes remained partially active and continually degraded the starch (*24*). In fact, gels supplemented with FAH had a ΔH_L that did not differ from that of the control gels. However, the gels supplemented with FAH contained larger quantities of glucose and similar quantities of maltose than gels supplemented with ISBAL, which had a ΔH_L that was significantly lower than that of the control gels (*24*). Furthermore, the quantities of glucose extracted from gels that had a ΔH_L significantly lower than that of the control gels after storage for 40 days were very low (0.1−2.6% of dry solids) to have a significant effect on the retrogradation of starch (*24*). This is supported by the finding that the enthalpy of retrogradation of starch gels prepared with 33% glucose (dry solids) did not differ from that of unsupplemented starch gels after storage

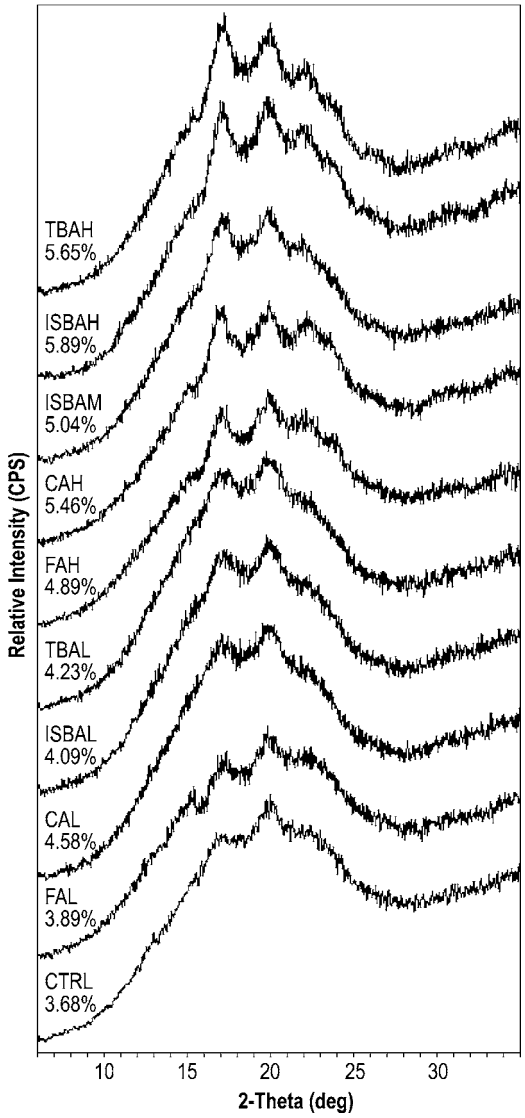

Figure 2. X-ray crystallography at storage time day 5 of starch gels supplemented with α-amylases from different sources. Values are percentage crystallinity. TBAH, thermostable bacterial α-amylase at the high level; ISBAH, intermediate temperature stability bacterial α-amylase at the high level; ISBAM, intermediate temperature stability bacterial α-amylase at the medium level; CAH, cereal α-amylase at the high level; FAH, fungal α-amylase at the high level; TBAL, thermostable bacterial α-amylase at the low level; ISBAL, intermediate temperature stability bacterial α-amylase at the low level; CAL, cereal α-amylase at the low level; FAL, fungal α-amylase at the low level; CTRL, control (no enzyme added).

for 6 days (*18*). Therefore, it appears that in gels supplemented with TBA or ISBA both glucose and maltose are mere indicators of structural changes produced during storage in the starch by these enzymes. In gels supplemented with CAH, which did not exhibit residual activity, the amounts of glucose and maltose indicate an extensive degree of starch degradation during starch gelatinization. The quantities of maltodecaose, maltoundecaose, and maltododecaose in starch gels stored for 140 days were negatively correlated with ΔH_L (**Table 4**). However, these correlations do not include values for starch gels supplemented with ISBA or TBA because it was not possible to determine the quantities of these maltooligosaccharides in these gels after storage for 140 days. This was due to their low quantities and the presence of numerous branched maltooligosaccharides, which coeluted (*24*). It is unlikely that any of the maltooligosaccharides (glucose through maltododecaose) were responsible for the inhibition of retrogradation in the gels supplemented with TBAH, ISBA, or CAH. Gels supplemented with CAH contained the largest quantities of maltooligosaccharides larger than maltotetraose (*24*) but had a ΔH_L that was significantly higher than those of gels supplemented with TBAH, ISBAH, or ISBAM. Similarly, gels supplemented with FAH, CAL, or FAL contained greater quantities of maltooligosaccharides larger than maltose than gels supplemented with ISBAL but had values of ΔH_L that were not significantly different from that of the unsupplemented control gels. The ΔH_L of gels supplemented with ISBAL was significantly lower than that of the unsupplemented control gels. Biliaderis and Prokopowich (*18*) reported that the enthalpy of retrogradation of starch gels supplemented with maltotriose, maltotetraose, maltopentaose, or maltohexaose was not different from that of unsupplemented gels after storage for 6 days. Moreover, they also reported that supplementation of starch gels with maltoheptaose increased their enthalpy of retrogradation after storage for 6 days. Therefore, it is evident that the maltooligosaccharides were not the direct cause of the reduced ΔH_L values in gels supplemented with TBAH, ISBA, or CAH. Min et al. (*11*) reported that maltooligosaccharide-producing α-amylases significantly reduced the rate of staling of bread as assessed by DSC measurements. According to Min et al. (*11*), the maltotriose and maltotetraose produced by these enzymes interfered with associations between gluten and starch. However, it is unlikely that the retardation of bread staling by these enzymes was caused by maltotriose and maltotetraose. To validate their suggestion one would be required to assume that DSC gives a measure of the extent of association between gluten and starch. However, it is well established that DSC gives a measure of the retrograded starch in bread crumb (*22*, *31*, *35*). Furthermore, Min et al. (*11*) have disregarded the starch modification produced by these enzymes, which should be considered in the evaluation of the possible causes for the antistaling effect. In fact, one of the enzymes used by Min et al. (*11*) was an intermediate temperature stability bacterial α-amylase, which was the same as that used in the present experiments (ISBA). According to Min et al. (*11*), the main effect of this enzyme was to produce maltotriose and maltotetraose. The enzyme was originally classified as a maltogenic or maltose-generating exo-acting α-amylase. However, a recent study by Christophersen et al. (*36*) has shown that it produces an extensive degradation of amylose and amylopectin. Therefore, it is also possible that the inhibition of retrogradation in bread supplemented with α-amylases reported by Min et al. (*11*) could have been the result of the degradation of amylopectin. Moreover, the mere presence of maltooligosaccharides in bread is not sufficient evidence to conclude they are the causative factors in the antistaling effect of these enzymes.

Correlation coefficients between the ΔH_L and the carbohydrate fractions extracted from the starch gels at different storage times are summarized in **Table 5**. The low molecular weight dextrins (LMWD), high molecular weight dextrins (HMWD), and total dextrins (TD) extracted from starch gels after 3 or more days of storage were negatively correlated with the ΔH_L. The fact that none of the individual maltooligosaccharides larger than maltose were significantly correlated with ΔH_L but were significantly correlated when considered as a group in the LMWD lends support to the suggestion that their presence is just an expression of the degree of starch degradation. Furthermore, the strength of the correlation increased as the storage

Table 4. Correlation Coefficients (*r*) between Limiting Enthalpy of Retrogradation and Quantities of Maltooligosaccharides Extracted at Different Storage Times from Starch Gels Supplemented with α-Amylases[a]

maltooligo-saccharide	day 0	day 1	day 2	day 3	day 5	day 8	day 12	day 20	day 40	day 140
G1	ns	–0.66*	–0.68*	–0.74*	–0.82**	–0.84**	–0.85**	–0.90**	–0.93**	–0.91**
G2	–0.75*	–0.85**	–0.89**	–0.91**	–0.89**	–0.87**	–0.85**	–0.84**	–0.84**	–0.85**
G3	ns	–0.63*	ns	ns	ns	ns	ns	ns	ns	ns
G4	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
G5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
G6	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
G7	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
G8	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
G9	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
G10	ns	ns	ns	ns	ns	ns	ns	ns	ns	–0.98**
G11	ns	ns	ns	ns	ns	ns	ns	ns	ns	–0.98**
G12	ns	ns	ns	ns	ns	ns	ns	ns	ns	–0.99**

[a] ns, not significant; *, significant at $P \leq 0.05$; **, significant at $P \leq 0.01$.

Table 5. Correlation Coefficients (*r*) of the Limiting Enthalpy of Retrogradation with Dextrin Fractions Obtained at Different Storage Times from Starch Gels Supplemented with α-Amylases[a]

dextrin fraction	day 0	day 1	day 2	day 3	day 5	day 8	day 12	day 20	day 40	day 140
LMWD	ns	ns	ns	–0.65*	–0.72*	–0.80**	–0.87**	–0.92**	–0.95**	–0.94**
HMWD	ns	ns	–0.81**	–0.83**	–0.84**	–0.83**	–0.83**	–0.84**	–0.78**	–0.66*
TD	ns	ns	–0.71*	–0.77**	–0.82**	–0.84**	–0.87**	–0.92**	–0.93**	–0.93**

[a] ns, not significant; *, significant at $P \leq 0.05$; **, significant at $P \leq 0.01$.

time progressed. The reason for this is that both the ISBA and TBA, which were most effective in inhibiting or retarding retrogradation, were still active in the gels and continually degraded the starch or HMWD to produce LMWD (*24*). Therefore, the increases in LMWD in these gels strengthen the correlation between ΔH_L and the LMWD. The strength of the correlation for the HMWD decreased with storage time. As implied above, this was due to the degradation and/or retrogradation of the HMWD in gels supplemented with ISBA, TBA, or CAH. Considering that the TD values were calculated as the sum of the LMWD and HMWD and that the LMWD were the dominant fraction in gels supplemented with the high levels of the enzymes in gels stored for 12 or more days (*24*), it is clear that the correlation coefficients between TD and ΔH_L followed a pattern after 12 days of storage similar to that followed by the LMWD. Therefore, the correlations between ΔH_L and the LMWD, HMWD, and TD suggest that the enzymes that degraded the starch to a larger extent were most effective in decreasing retrogradation of the gels.

The proportion of short branches with degrees of polymerization (DP) of 2−10 in the HMWD extracted after storage for 2 days from the starch gels supplemented with the high levels of the enzymes and the unsupplemented control gels (*24*) was negatively correlated with ΔH_L ($r = -0.86$; $P \leq 0.05$), whereas the proportion of longer branches (DP = 21−30) was positively correlated ($r = 0.94$; $P \leq 0.01$). No significant correlation was found between the proportion of intermediate length branches (DP = 11−20) in the HMWD and ΔH_L. Considering that the HMWD were branched fragments, which originated from the degradation of amylopectin, it is clear that starch gels, in which the branches of amylopectin were shortened to a greater extent, retrograded to a lesser extent. We previously (*24*) suggested that the TBA produced an extensive degradation of the external branches of amylopectin. This was evidenced by the large quantities of maltohexaose and maltoheptaose produced by this enzyme. Similarly, we also suggested that the ISBA extensively degraded the branches of amylopectin in the gels to maltose (*24*). Evidence for this was the progressive appearance of a large quantity of maltose during storage in starch gels supplemented with this enzyme. Würsch and Gumy (*19*) reported that partial β-amylolysis of the external branches of amylopectin to ≤11 glucose units on average inhibited its retrogradation.

Even though the CAH more effectively decreased the ΔH_L of the starch gels than the FAH, the HMWD in gels supplemented with CAH contained lower proportions of short branches (DP = 2−10) than those in gels supplemented with FAH (*24*). A possible explanation for this inconsistency is that the overall degradation of the starch produced by the CAH during preparation of the gels was much more extensive than that produced by the FAH (*24*). Therefore, it is possible that the larger extent of starch degradation produced by the CAH was more effective in inhibiting starch retrogradation.

We previously (*24*) reported that the amylopectin in starch gels supplemented with TBA or ISBA was degraded to a greater extent than the amylopectin in the control gels and in gels supplemented with FA or CA. This was evidenced by a progressive decrease in the high molecular weight amylopectin fraction (AP I) and a progressive increase in the low molecular weight amylopectin fraction (AP II) during storage in the residual starches obtained from gels supplemented with TBA or ISBA. As previously reported (*24*), the decrease in size of the AP I molecules probably took place through degradation of intercluster regions. The peak corresponding to amylose (AM) also increased in gels supplemented with TBA or ISBA during storage due to the degradation of amylopectin into low molecular weight fragments, which coeluted with amylose. At storage time day 5 the proportion of AP I extracted from the starch gels was positively correlated with the ΔH_L of the gels ($r = 0.94$; $P \leq 0.01$), whereas the proportions of AP II ($r = -0.92$; $P \leq 0.01$) and the peak representing AM ($r = -0.87$; $P \leq 0.01$) were negatively correlated with ΔH_L. Clearly, this indicates that the enzymes which were able to degrade amylopectin to a greater extent most effectively inhibited retrogradation of the gels. These findings suggest that the ability to decrease the ΔH_L of the ISBA

and TBA was due to the fact that these enzymes remained partially active in the gels and continually degraded the amylopectin fraction of starch during storage. The decrease in ΔH_L produced by the CAH can be explained on the basis that this enzyme extensively degraded the starch during preparation of the gels (*24*, *25*). However, the effects produced by the TBA and ISBA on ΔH_L were more drastic than that produced by the CAH. This suggests that degradation of the starch gels during storage is a more effective way to decrease the ΔH_L than an extensive initial degradation during starch gelatinization.

The T_p of the retrogradation endotherm of the starch gels increased during storage. This suggests that during storage the crystallites and/or molecular order in the gels became more perfect. The T_p of the retrogradation endotherm of the starch gels was significantly correlated with the ΔH_L. After storage for 140 days, these parameters were negatively correlated ($r = -0.93$; $P \leq 0.01$). This finding suggests that the crystallites were more perfect in starch gels, which exhibited the lowest values for ΔH_L. Therefore, it appears that the effect of the α-amylases was to decrease the extent of retrogradation and to increase the perfection of the crystallites when compared to the unsupplemented control.

The values of *k* of gels supplemented with TBAH, ISBAH, or ISBAM were higher and significantly different form those of the other treatments. Because these enzymes were the most effective in lowering the value of the ΔH_L, it is clear that the high values of *k* were a result of the low values of ΔH_L. To explain this it is necessary to consider that in gels supplemented with TBAH, ISBAH, or ISBAM a major part of the starch degradation took place during storage because these enzymes remained partially active (*24*). Therefore, during the first 2−3 days of storage, the extent of starch degradation was not enough to considerably inhibit starch retrogradation. However, as the storage time progressed the extent of starch degradation increased and inhibited the retrogradation of starch, which resulted in lower ΔH_L values and higher values of *k*. Gels supplemented with CAH had values of *k* that did not differ from that of the control and a ΔH_L that was larger than those of gels supplemented with TBAH, ISBAH, or ISBAM. However, these gels contained quantities of LMWD and HMWD, which were higher or equal to those found in gels supplemented with TBAH, ISBAH, or ISBAM (*24*). Considering that the CAH produced all of the dextrins during preparation of the gels, it is evident that the residual activity in gels supplemented with TBAH, ISBAH, or ISBAM played a primary role in the inhibition of starch retrogradation. Apparently, the degradation of the starch during storage alters retrogradation to a greater extent than its degradation during gelatinization.

The relative crystallinity after storage for 5 days of the enzyme-supplemented gels was greater than that of the control gels. The correlation coefficient between the relative crystallinity of the gels and their total extent of retrogradation after storage for 5 days was −0.82 ($P \leq 0.01$). The negative sign of the correlation coefficient indicates that gels that retrograded to a lesser extent were more crystalline after storage for 5 days. This suggests that the effect of the α-amylases was to reduce the extent of retrogradation and to increase the crystallinity of the gels. Dragsdorf and Varriano-Marston (*12*) reported that the degree of crystallinity of starch in breads supplemented with α-amylases was higher than that in unsupplemented bread. Therefore, it seems the same phenomena take place in both starch gels and bread. Eliasson (*22*) reported that DSC measures the melting of recrystallized amylopectin in starch gels. The results of the current study do not appear to agree with their hypothesis. The fact that in the present experiments the crystallinity (X-ray) of the unsupplemented control gels was the lowest and their extent of retrogradation as determined by DSC was the largest suggests that DSC not only measures the recrystallized amylopectin in the gels but that it also gives a measure of total order (double-helical content and recrystallized amylopectin) in the retrograded starch. In fact, Cooke and Gidley (*23*) originally suggested that the enthalpy of gelatinization of starch, or similarly the enthalpy of retrogradation, primarily reflects the loss of molecular (double-helix) order rather than crystalline order. The fact that the gels supplemented with TBAH or ISBAH were the most crystalline but gave the lowest values for the enthalpy of retrogradation suggests that the main effect of these enzymes was to reduce the extent of molecular order (double-helix) in the starch gels during storage. Morgan et al. (*5*) reported that the double-helical content of starch bread supplemented with a bacterial α-amylase was significantly lower than that of unsupplemented starch bread.

Zobel and Senti (*15*) attributed the increased crystallinity of bread and starch gels supplemented with bacterial α-amylase to the ability of this enzyme to remain partially active and hydrolyze chain segments in the amorphous regions near crystallites. As a result, these chain segments gained freedom of motion and were able to align themselves for crystallization. The larger T_p of the endotherm of retrogradation observed in the present study in gels supplemented by TBAH or ISBAH, which remained partially active in the gels, appears to support the suggestion made by Zobel and Senti. It is reasonable to expect that if the crystallites in the gels become more perfect, they will melt at a higher temperature. Our findings add to the hypothesis of Zobel and Senti (*15*) and introduce the concept that the inhibition of retrogradation by the α-amylases is due to their ability to produce structural changes in starch, which inhibit or decrease the formation of double helices. Again, this is supported by the results obtained using DSC and X-ray crystallography, which suggest that the molecular order or double-helical content is the main factor involved in the retrogradation of starch as determined by DSC. Others have observed the same phenomena in bread (*12*, *15*), suggesting that bread staling is primarily the result of starch retrogradation. Furhermore, the results also suggest that the antistaling effect of the bacterial enzymes is due to structural changes produced in amylopectin during storage that inhibit or decrease double-helical ordering of the starch gels matrix.

ABBREVIATIONS USED

AM, amylose; AP I, high molecular weight amylopectin; AP II, low molecular weight amylopectin; CA, cereal α-amylase; CAH, cereal α-amylase at the high level; ^{13}C CP/MAS NMR, ^{13}C cross-polarization magic angle spinning nuclear magnetic resonance spectroscopy; CAL, cereal α-amylase at the low level; CRD, completely randomized design; ΔH, enthalpy of retrogradation; ΔH_L, limiting enthalpy of retrogradation; DP, degree of polymerization; DSC, differential scanning calorimetry; FA, fungal α-amylase; FAH, fungal α-amylase at the high level; FAL, fungal α-amylase at the low level; HMWD, high molecular weight dextrins; ISBA, intermediate temperature stability bacterial α-amylase; ISBAH, intermediate temperature stability bacterial α-amylase at the high level; ISBAL, intermediate temperature stability bacterial α-amylase at the low level; ISBAM, intermediate temperature stability bacterial α-amylase at the medium level; *k*, rate constant; LMWD, low molecular weight dextrins; *n*, Avrami exponent; TBA, thermostable bacterial α-amylase; TBAH, thermostable bacterial α-amylase

at the high level; TBAL, thermostable bacterial α-amylase at the low level; TD, total dextrins; T_p, peak temperature.

LITERATURE CITED

(1) Spooner, T. F. Bakers, suppliers make headway in battle to extend shelf life. *Milling Baking News* **1994**, *73* (42), 49−51.

(2) Schoch, T. J.; French, D. Studies on bread staling. I. The role of starch. *Cereal Chem*. **1947**, *24*, 231−249.

(3) Kim, S. K.; D'Appolonia, B. L. Bread staling studies. II. Effect of protein content and storage temperature on the role of starch. *Cereal Chem*. **1977**, *54*, 216−224.

(4) Inagaki, T.; Seib, P. A. Firming of bread crumb with cross-linked waxy barley starch substituted for wheat starch. *Cereal Chem*. **1992**, *69*, 321−325.

(5) Morgan, K. R.; Hutt, L.; Gerrard, J.; Every, D.; Ross, M.; Gilpin, M. Staling in starch breads: The effect of antistaling α-amylase. *Starch/Staerke* **1997**, *49*, 54−59.

(6) Martin, M. L.; Zeleznak, K. J.; Hoseney, R. C. A mechanism of bread firming. I. Role of starch swelling. *Cereal Chem*. **1991**, *68*, 498−503.

(7) Martin, M. L.; Hoseney, R. C. A mechanism of bread firming. II. Role of starch hydrolyzing enzymes. *Cereal Chem*. **1991**, *68*, 503−507.

(8) Miller, B. S.; Johnson, J. A.; Palmer, D. L. A comparison of cereal, fungal, and bacterial α-amylases as supplements for breadmaking. *Food Technol*. **1953**, *7*, 38−42.

(9) Every, D.; Mann, J. D.; Ross, M.. The relationship of dextrins in bread to staling and crumb gumminess. In *Proceedings of the 42nd Royal Australian Chemical Institute, Cereal Chemistry Conference*; RACI: Christchurch, New Zealand, 1992; pp 179−186.

(10) Qi Si, J. Synergistic effect of enzymes for breadbaking. *Cereal Foods World* **1997**, *42*, 802−807.

(11) Min, B.-C.; Yoon, S.-H.; Kim, J.-W.; Lee, Y.-W.; Kim, Y.-B.; Park, K. H. Cloning of novel maltooligosaccharide-producing amylases as antistaling agents for bread. *J. Agric. Food Chem*. **1998**, *46*, 779−782.

(12) Dragsdorf, R. F.; Varriano-Marston, E. Bread staling: X-ray diffraction studies on bread supplemented with α-amylases from different sources. *Cereal Chem*. **1980**, *57*, 310−314.

(13) Schultz, A. S.; Schoonover, F. D.; Fisher, R. A.; Jackel, S. S. Retardation of crumb starch staling in commercial bread by bacterial α-amylase. *Cereal Chem*. **1952**, *29*, 200−211.

(14) Beck, H.; Johnson, J. A.; Miller, B. S. Studies on the soluble dextrin fraction and sugar content of bread baked with α-amylase from different sources. *Cereal Chem*. **1957**, *34*, 211−217.

(15) Zobel, H. F.; Senti, F. R. The bread staling problem. X-ray diffraction studies on breads containing a cross-linked starch and a heat-stable amylase. *Cereal Chem*. **1959**, *36*, 441−451.

(16) Gerrard, J. A.; Every, D.; Sutton, K. H.; Gilpin, M. J. The role of maltodextrins in the staling of bread. *J. Cereal Sci*. **1997**, *26*, 201−209.

(17) Salem, A. E.-S.; Johnson, J. A. Influence of various oligosaccharides on staling of bread. *Food Technol*. **1965**, *19*, 849−852.

(18) Biliaderis, C. G.; Prokopowich, D. J. Effect of polyhydroxy compounds on structure formation in waxy maize starch gels: A calorimetric study. *Carbohydr. Polym*. **1994**, *23*, 193−202.

(19) Würsch, P.; Gumy, D. Inhibition of amylopectin retrogradation by partial β-amylolysis. *Carbohydr. Res*. **1994**, *256*, 129−137.

(20) Duedahl-Olesen, L.; Zimmermann, W.; Delcour, J. A. Effect of low molecular weight carbohydrates on farinograph characteristics and staling endotherms of wheat flour-water doughs. *Cereal Chem*. **1999**, *76*, 227−230.

(21) Duran, E.; Leon, A.; Barber, B.; Benedito de Barber, C. Effect of low molecular weight dextrins on gelatinization and retrogradation of starch. *Eur. Food Res. Technol*. **2001**, *212*, 203−207.

(22) Eliasson, A.-C. Retrogradation of starch as measured by differential scanning calorimetry. In *New Approaches to Research on Cereal Carbohydrates*; Hill, R. D., Munck, L., Eds.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1985; pp 93−98.

(23) Cooke, D.; Gidley, M. J. Loss of crystalline and molecular order during starch gelatinisation: Origin of the enthalpic transition. *Carbohydr. Res*. **1992**, *227*, 103−112.

(24) Rodriguez-Palacios, H. R. The effect of α-amylases and their degradation products on the retrogradation, recrystalization and firming of whaet starch gels—Relationship to bread staling. Ph.D. Dissertation, North Dakota State University, Fargo, ND, 1998.

(25) Fearn, T.; Russell, P. L. A kinetic study of bread staling by differential scanning calorimetry. The effect of loaf specific volume. *J. Sci. Food Agric*. **1982**, *33*, 537−548.

(26) Komiya, T.; Nara, S. Changes in crystallinity and gelatinization phenomena of potato starch by acid treatment. *Starch/Staerke* **1986**, *38*, 9−13.

(27) Avrami, M. Kinetics of phase change. I. General theory. *J. Phys. Chem*. **1939**, *7*, 1103−1112.

(28) Avrami, M. Kinetics of phase change. II. Transformation−time relations for random distribution of nuclei. *J. Phys. Chem*. **1940**, *8*, 212−224.

(29) Avrami, M. Kinetics of phase change. III. Granulation, phase change, and microstructure. *J. Phys. Chem*. **1941**, *9*, 177−184.

(30) Morgan, L. B. Crystallization phenomena in polymers. II. The course of the crystallization. *Philos. Trans. R. Soc*. **1955**, *247A*, 13−22.

(31) McIver, R. G.; Axford, D. W. E.; Colwell, K. H.; Elton, G. H. A. Kinetic study of the retrogradation of gelatinised starch. *J. Sci. Food Agric*. **1968**, *19*, 560−563.

(32) Slade, L.; Levine, H. A food polymer approach to selected aspects of starch gelatinization and retrogradation. In *Frontiers in Carbohydrate Research−1: Food Applications*; Millane, R. P., BeMiller, J. N., Chandrasekaran, R., Eds.; Elsevier Applied Science: New York, 1989; pp 215−270.

(33) Miles, M. J.; Morris, V. J.; Orford, P. D.; Ring, S. G. The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydr. Res*. **1985**, *135*, 271−281.

(34) Marsh, R. D. L.; Blanshard, J. M. The application of polymer crystal growth theory to the kinetics of formation of the β-amylose polymorph in a 50% wheat-starch gel. *Carbohydr. Polym*. **1988**, *9*, 301−317.

(35) Axford, D. W. E.; Colwell, K. H. Thermal investigation of bread staling. *Chem. Ind*. **1967**, *11*, 467−468.

(36) Christophersen, C.; Otzen, D. E.; Norman, B. E.; Christensen, S.; Schäfer, T. Enzymatic characterization of Novamyl, a thermostable α-amylase. *Starch/Staerke* **1998**, *50*, 39−45.

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