Waxy Corn Starch: Monoglyceride Interaction in a Model System[1]

J. J. HUANG and P. J. WHITE[2]

ABSTRACT

The interaction between waxy corn starch and monoglycerides (MGs) (monolaurin, monomyristin, monopalmitin, and monostearin) was investigated by the measurement of starch-MG complex formation, iodine affinity, differential scanning calorimetry, and texture measurements in model systems. All MGs formed some amount of complex with waxy corn starch, but no significant differences occurred among the MG types. Iodimetric titrations of the complexes also showed that the presence of MGs significantly decreased the iodine affinity of the amylopectin when compared with that of the control. Gelatinization and retrogradation behavior of starch-MG mixtures were measured by using differential scanning calorimetry. Compared with that of the control, the gelatinization onset temperature decreased significantly in the presence of MGs, except for monostearin; moreover, statistically lower enthalpies were noted for the treatments containing MGs, except for the treatment with monomyristin. After retrogradation, the enthalpies of recrystallized starch for systems containing monolaurin, monomyristin, and monopalmitin were statistically lower than that of the control. Gel firmness and cohesiveness measurements with the Voland-Stevens texture analyzer revealed little pattern in the effects of MGs on waxy maize starch gelatinization.

Cereal Chem. 70(1):42–47

Staling of starch-based products, such as bread or tortillas, is a problem of great importance in the food industry because staling affects the texture and flavor of these products. Bread staling, an extremely complex phenomenon, refers to all changes, other than microbiological spoilage, that occur in bread after baking and includes changes in both the crumb and the crust (Knightly 1977). The increase in crumb firmness is often used to evaluate the extent of staling.

Although bread firming is influenced by starch retrogradation (D'Appolonia and Morad 1981), other factors affecting the swelling of starch granules, such as gluten, crumb temperature, baking time, and presence of lipids, also may affect bread firming (Martin et al 1991). Also, the different roles of the two starch components, amylose and amylopectin, have been extensively discussed over the years. Amylose has a greater tendency to retrograde and is, therefore, considered the main cause of staling. But Schoch and French (1947) suggested that the aggregation of amylopectin is the factor responsible for crumb firmness. They observed that the retrograded amylopectin could revert to its amorphous state when energy equivalent to a temperature increase of 40–50°C was applied, whereas retrogradation of amylose was not reverted by heat. Because reversal of bread staling occurs when bread is heated to these temperatures, amylopectin is implicated as a major factor in bread staling. Recently, the role of amylopectin in starch retrogradation was further confirmed by using differential scanning calorimetry (DSC) (Russell 1983, Eliasson and Krog 1985, Evans 1986, Gudmundsson and Eliasson 1990).

To delay staling, most food companies incorporate crumb softeners such as monoglycerides (MGs) or diglycerides in bakery products. But the mode of action of these additives in improving the shelf life is controversial. Mikus et al (1946) postulated that MGs form a helical complex with the amylose fraction, thus causing a softer crumb without influencing the firming rate. Knightly (1977) discovered that surfactants had little or no effect on initial bread crumb firmness, but they did affect the firming rate during storage.

The objective of this study was to investigate the possible interaction between waxy corn starch and MGs through the evaluation of starch-MG complex formation, iodine affinity (IA), DSC, and texture measurements in model systems.

MATERIALS AND METHODS

Materials

The waxy corn starch and MGs (1-monolauroyl-rac-glycerol [glyceryl monolaurate, GML], 1-monomyristoyl-rac-glycerol [glyceryl monomyristate, GMM], 1-monopalmitoyl-rac-glycerol [glyceryl monopalmitate, GMP], and 1-monostearoyl-rac-glycerol [glyceryl monostearate, GMS]) used in the model systems were purchased from Sigma Chemical Co. (St. Louis, MO). Both waxy corn starch and MGs had a purity of about 99%. The moisture content of the starch was 9.4%.

Defatting of Starch

The presence of fatty acids depresses the complex-forming and iodine-binding power of starch; therefore, the waxy corn starch was defatted before use according to the method of Schoch (1942). Starch (10 g) was continuously washed with 200 ml of 85% methyl alcohol (MeOH) for 24 hr in the Soxhlet extractor. After extraction, the starch was washed again with 150 ml of absolute MeOH for 2 hr and dried in an oven at 40°C for 6 hr. After evaporation, the starch was placed in a desiccator until a constant moisture content of 5.5% was reached.

Complex Formation in Model Systems

The procedure of Batres and White (1986) for preparing, isolating, and weighing the waxy corn starch-MG complexes was followed. Defatted waxy corn starch (1.5 g) and 0.5 g of MG were suspended in 100 ml of distilled water in a two-necked round-bottom flask fitted with a condenser and stirred at 12,000 rpm with a propeller stirrer (Lab-Stir hollow spindle, Ederbach Corp., Ann Arbor, MI) in a constant-temperature water bath at 70°C for 6 hr. The flask was left in the water bath overnight to cool to room temperature. The resulting precipitate was separated by centrifugation at 3,500 × *g* for 30 min, washed three times with water, and centrifuged after each washing. The precipitate was dried under weak vacuum (127 mm of mercury) at 65°C for 4 hr and stored in a desiccator containing anhydrous calcium sulfate. A control was prepared without added MG. Free MG present in the precipitate was extracted with carbon tetrachloride (CCl_4) in a Soxhlet apparatus for 4 hr. The remaining waxy corn starch-MG complex (precipitate) was dried under weak vacuum (127 mm of mercury) at 65°C for 2 hr to remove residual CCl_4 and then weighed. The bound MG remaining in the complex after CCl_4 extraction was further extracted with 200 ml of 85% MeOH in a Soxhlet apparatus for 6 hr and weighed after solvent evaporation. This quantity was verified by subtracting the amount of remaining waxy corn starch from the amount of complex formed. Model systems for each MG and control were replicated five times.

IA of Amylopectin-MG Complexes

To estimate the degree of interaction between amylopectin and

[1]Journal paper J-14661 of the Iowa Agriculture and Home Economics Experiment Station, Ames. Project 2568.

[2]Graduate student and associate professor, respectively, Department of Food Science and Human Nutrition, Iowa State University, Ames.

MGs by IA, small model systems were prepared containing 0.2 g of defatted amylopectin, 0.066 g of MG (GML, GMM, GMP, or GMS), and 50 ml of distilled water. A control was prepared without MGs. The mixture was stirred for 6 hr at 70°C and then left undisturbed at 25°C to cool overnight. Three replicates of each model system were prepared. After overnight cooling, the model system was potentiometrically titrated with iodine as described by Schoch (1964). For this step, an Orion Research digital pH/mV meter (mode 701A digital ionalizer, Orion Research, Boston, MA) was used, and millivolt readings were taken at 15 different points. Values were between 230 and 280 mV with a wait of 2 min after each addition of iodine. From the millivolt readings, the concentration of free iodine in the sample solution was determined by using a calibration curve. The bound iodine was calculated from the difference between the total amount of iodine added to the model system and the free iodine found at each point of the curve. Bound iodine (y) was then plotted against free iodine (x), and a regression line was calculated. The IA of the model system was calculated by multiplying the intercept of this regression line by 100 and dividing it by the dry weight of the amylopectin and MG added to the model system.

Complex Formation for DSC

The procedure of Eliasson et al (1988) was followed for incorporating MG into the starch before analysis by DSC. The GML, GMM, GMP, and GMS were not readily dispersible in water at room temperature, so they were mixed with water at a ratio of 1:10, heated at 70°C to produce a uniform dispersion, and then allowed to cool before adding to starch. All additives were at a concentration of 2% (w/w) calculated on a dry starch basis. The ratio of starch to MG to water was 1:0.1:3. The control had the same concentration of starch to water (1:3) without added MG. The contents of the test tubes were mixed carefully with a spatula, and the sample was allowed to equilibrate overnight.

DSC

An aliquot of the starch suspensions described above was transferred to a preweighed DSC pan (coated aluminum pans, Perkin-Elmer, Norwalk, CT), which was hermetically sealed and reweighed. The sample (8–10 mg) was allowed to equilibrate for 2 hr at room temperature before heating. The sample pan was then placed in the calorimeter (DSC 7, Perkin-Elmer) at 22°C with an empty sample pan as a reference. The heating rate was 10°C/min at a sensitivity of 0.5 cal/sec, and the upper temperature limit was set at 120°C. After rapid cooling in the differential scanning calorimeter (cooling rate set at 250°C/min), the sample pans were stored at 4°C for seven days (White et al 1989).

Retrogradation was measured by reheating the stored samples from 20 to 120°C at a heating rate of 10°C/min. The dry matter content was determined for each individual sample pan after the DSC analysis by puncturing and drying the pan at 105°C for 16 hr. The onset temperature (T_{OG}), temperature range (R_G), and enthalpies (ΔH_G) of gelatinization and the onset temperature (T_{OR}), temperature range (R_R), and enthalpies (ΔH_R) of retrogradation were calculated by using the Perkin-Elmer 3700 data station. The values are the means of four replicates.

Texture Measurements

Starch pastes of 8% (w/w) dried solids and three different MG concentrations (0.3, 1.0, and 5.0%, w/w) in 40 ml of distilled water were used for gel strength determinations. All gel measurements were performed on triplicate samples. The pastes were prepared in a flask fitted with a condenser by heating and stirring a starch suspension in a boiling water bath for 25 min. The heating rate and the stirring speed were kept consistent among treatments. After heating, the hot pastes were poured into two aluminum pans (27 × 27 mm) and covered with aluminum foil. The depth of each pan was increased approximately 10 mm by taping aluminum foil around its rim. One pan of starch paste was stored at 25°C for 24 hr, and the other was stored at 15°C for seven days. Before the gel strength was measured, the aluminum foil was removed and the paste above the top of the pan was sliced off carefully to prepare a smooth surface (Takahashi and Seib 1988). The final gel had a depth of 27 mm.

The texture of the gels was determined by using a model TA-100 Voland-Stevens texture analyzer (TA) (Voland Corp., Hawthorne, NY) fitted with a chart recorder (Linseis Co., Princeton Junction, NJ). The gels were compressed at a speed of 0.2 mm/sec to a preset distance of 3 mm by using a cylindrical punch probe (TA-53, 3-mm diameter, Voland Corp.) with the chart recorder speed at 10 cm/min. The peak height (representing gram-force) at 3-mm compression was termed firmness, and the negative peak during retraction was termed cohesiveness (Takahashi and Seib 1988).

Statistical Analysis

Analysis of variance among treatments was computed with the General Linear Model program (SAS 1989). Multiple comparisons were performed by least significant difference after a preliminary F test (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Complex Formation of Amylopectin with MGs in Model Systems

Table I shows the results of binding MGs with waxy corn starch in model systems. All MGs bound, to a small extent, with the waxy corn starch, but reproducibility was low as noted by the high standard deviations. No significant differences occurred among the MG types for the amount of complex formed, for the amount of MGs present in the complex, for the percentage of complex composed of MG, or for the millimoles of MG per gram of amylopectin. Although not significant, the binding tended to increase, as determined in number of millimoles, as the molecular weight of the MG decreased. Roughly, 70% of the original 1.5 g of waxy corn starch and approximately 7 to 9% of the original 0.5 g of MGs were tied up in the complex.

Studies by Lagendijk and Pennings (1970) demonstrated that potato amylopectin complexed with various MGs; however, the amount of complexation increased linearly with increased MG chain length, showing values of 0.4, 1.3, 1.6, 2.5, and 3.0 mmol $\times 10^{-2}$ per gram of amylopectin, respectively, for chain lengths of 12, 14, 16, 18, and 20. These values are somewhat lower than those reported here. They did not report values for the total amount of complex formed. Lagendijk and Pennings (1970) also reported binding of MGs with potato amylose, in which 12.3 $\times 10^{-2}$ mmol of GMP formed complexes with 1 g of amylose. These values are similar to molar values found in our study for waxy maize starch. Batres and White (1986) also confirmed that MGs were able to complex with potato amylopectin. Roughly, 5–22% of the original amylopectin and between 4.1 and 37.6%

TABLE I
Complex Formation of Waxy Corn Starch with Monoglycerides (MGs) in a Model System[a]

MG[b]	Amount of Complex Formed (mg)	Amount of MG in Complex (mg)	Percent Complex Composed of MG	Millimoles MG × 10^{-2} per 1 g of Amylopectin
GML	1,107 ± 76	41.0 ± 10.5	3.2 ± 0.8	14.9 ± 3.4
GMM	1,131 ± 42	42.6 ± 13.7	3.8 ± 1.2	13.9 ± 4.4
GMP	1,124 ± 24	43.2 ± 4.4	3.8 ± 0.4	12.9 ± 1.6
GMS	1,142 ± 48	39.3 ± 3.5	3.4 ± 0.2	10.4 ± 0.5

[a] Values are the averages of five replicates. Values were not significantly different ($P < 0.05$) among treatments in each column. Numbers are followed by plus or minus the standard deviation.
[b] GML, monolaurin; GMM, monomyristin; GMP, monopalmitin; GMS, monostearin.

of the original MGs were tied up in the complex, with GMP showing the greatest complexing capacity. In addition, between 19 and 36% of the complexes were composed of MGs. In the current study, the percentage of complex composed of MGs was generally much lower for all MGs than in the study by Batres and White (1986).

Conflicting reports on the extent of the lipid-binding ability of amylopectin may arise for several reasons. The source of amylopectin varies among research studies and is not always reported. Comparisons of studies using amylopectin from different botanical sources should be made with caution. Also, the conditions for preparing the complexes differed among the reported studies.

IA

IA was used to estimate the degree of interaction between waxy corn starch and MGs under the conditions of the previously mentioned model systems. The control model systems containing waxy corn starch, but without MGs, had an average IA of 0.041%. The model systems containing the four MGs all had an IA of 0.0 (data not shown), and the differences between the control system and those containing MGs were significant ($P < 0.01$). The decrease in IA of waxy corn starch in the presence of MGs was likely because linear portions of the amylopectin complexed fully with the MGs, allowing no additional binding of iodine.

These IA values are about 10 times lower than values obtained in a similar study that used potato amylopectin rather than waxy corn starch (Batres and White 1986). The differences are likely because of structural differences between the two botanical sources. Takeda et al (1989) reported that a rice amylopectin fraction with many short chains and few long chains showed a low IA. The low IA of the waxy corn starch in the current study may suggest the presence of many short chains on the amylopectin. Also, the amylopectin in waxy maize starch was bound into native starch granules, as opposed to the nonbound amylopectin molecules in the purified potato amylopectin used in the study by Batres and White (1986). Eliasson and Ljunger (1988) observed considerably more interaction of surfactants and emulsifiers with purified amylopectin from waxy maize starch than with that from native waxy corn starch.

Iodine must interact at least weakly with amylopectin because it shows a weak brown color after exposure to iodine (Takeda and Hizukuri 1987). Schoch and Williams (1944) observed interference with the formation of amylose-iodine complexes in the presence of fatty acids. X-ray studies also revealed that the helix of the amylose-MG complex is similar to that of the amylose-iodine complex (Bear 1942). Furthermore, long external branches and other linear portions of amylopectin molecules should theoretically allow some binding of the fatty acids of MGs (Rutschmann et al 1989).

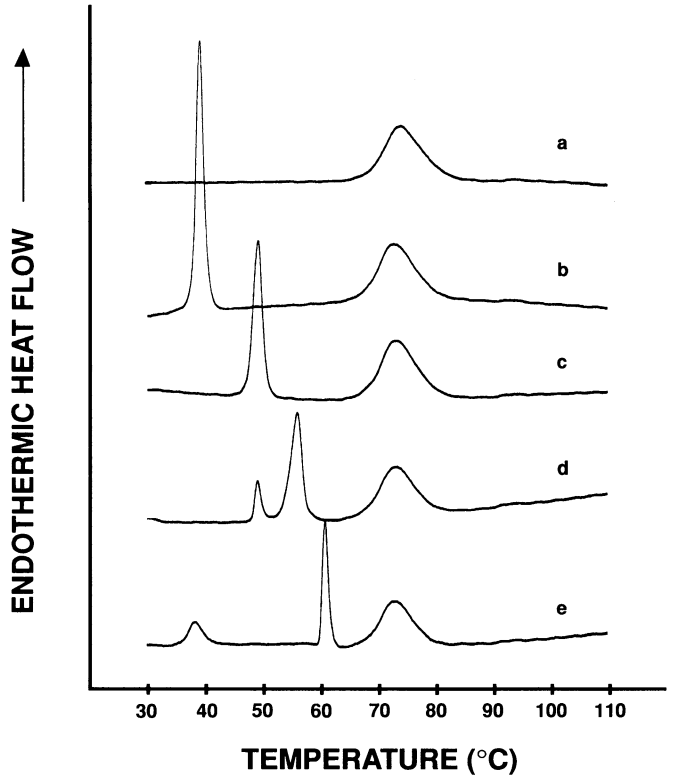

Fig. 1. Differential scanning calorimetric thermograms of starch-monoglyceride model systems containing waxy corn starch, 2.26 mg dry matter (a); waxy corn starch + monolaurin, 2.19 mg (b); waxy corn starch + monomyristin, 2.25 mg (c); waxy corn starch + monopalmitin, 2.17 mg (d); and waxy corn starch + monostearin, 2.20 mg (e). The starch-to-water ratio was 1:3.

Gelatinization Properties by DSC

The thermal behavior of waxy corn starch in the presence of MGs during gelatinization in excess water was studied by using DSC. Representative thermograms are shown in Figure 1. The large peak on each thermogram seen at the lower temperature (except for Fig. 1, thermogram a) was observed with all MG-containing systems. This peak might be explained as the free-melting MG peak, and the temperature of melting changed with the MG and with the conditions. The small peak seen at the low temperature in Figure 1, thermograms d and e, was present in all systems containing these MGs. It likely represents the melting of an impurity or a minor alternative structure of the MG. Previously, MGs heated in the presence of water demonstrated two possible transitions (Brokaw and Lyman 1958). To illustrate temperatures where melting of the MGs might interfere with DSC readings of starch-MG interactions, Table II presents a summary of the known melting point and DSC melting temperature of the major peak of each of the four MGs under the conditions of the study—pure MG, with water, and with the waxy corn starch. The peak onset (T_o) for melting of the MGs increased with increased chain lengths from 12 to 18 for all conditions. Differences between melting point values of the MG alone reported by Weast and Astle (1985) and our data were likely because of differences in procedures used for determining the melting points. Among the test conditions, the pure MG exhibited the highest T_o, and, with the addition of water, a firm gel was formed. This gel formed crystals that melted below the melting point of a pure MG (Brokaw and Lyman 1958). As the starch suspension was incorporated, the peak shifted down slightly again, with the T_o of the longer-chain MG shifting more than that of the shorter-chain MG.

In Figure 1, peaks with a T_o of about 66°C were from the gelatinization of the corn starch, and the corresponding DSC parameters for this peak from all treatments are presented in Table III. For gelatinization of waxy corn starch without MG, the T_{OG} and ΔH_G values were 66.7°C and 4.1 cal/g, respectively. These values compare to those of corresponding DSC studies

TABLE II
Peak Onset Temperature[a] for the Melting of Monoglycerides (MGs) by Differential Scanning Calorimetry as Pure MGs, with Water, and in the Presence of Waxy Corn Starch

MG[b]	Melting Point[c] (°C)	Peak Onset Temperature, °C		
		Pure MG[d]	MG with H_2O[e]	MG with Starch[f]
GML	63	61	39	37
GMM	70	67	48	46
GMP	72	75	57	52
GMS	77	81	64	58

[a] Values are the averages of three replicates.
[b] GML, monolaurin; GMM, monomyristin; GMP, monopalmitin; GMS, monostearin.
[c] Data from Weast and Astle 1985.
[d] 0.1 mg of MG.
[e] Ratio of MG to H_2O = 0.1:3.
[f] Ratio of MG to H_2O to waxy corn starch = 0.1:3:1; total weight is 8–10 mg.

for waxy corn starch run at the same starch-to-water ratio, in which T_{OG} and ΔH_G were 67°C and 4.0 cal/g, respectively (Eliasson et al 1988). The addition of any MG had no significant effect on the R_G. Statistically lower ($P < 0.05$) T_{OG} values were noted for the treatments containing GML, GMM, and GMP compared with the values for the treatments containing GMS and the control. Significant differences also occurred among treatments with all MG types, except between GMM and GMP. The shorter the MG, the lower was the T_{OG}. The ΔH_G values of the MG-containing treatments were all statistically lower than that of the control, except for the treatment containing GMM, which also was not significantly different from the other treatments.

Recently, Evans (1986) reported that the gelatinization behaviors of waxy corn starch as measured by DSC were affected by the presence of cetyltrimethylammonium bromide (CTAB) and sodium dodecyl sulfate. Similar DSC results also were published by Eliasson et al (1988) with the addition of saturated MG and CTAB to waxy corn starch. Some researchers have reported an amylopectin-lipid complex transition similar to that of the well-known amylose-lipid transition occurring at approximately 95–100°C. Slade and Levine (1987) reported a melting endotherm at 70°C of a waxy maize-sodium stearoyl lactylate complex produced at low moisture content (10%). Direct evidence for the presence of an amylopectin-lipid complex was not found in the current study, perhaps because a different moisture content was used (75 instead of 10%). Gudmundsson and Eliasson (1990) found a peak at about 105°C that they attributed to the melting of a potato amylopectin-CTAB complex made at 50% moisture content. In their study, the amylopectin was not bound into starch granules as is waxy corn starch and, therefore, was more free to interact with surfactants and emulsifiers as noted earlier. Other researchers have reported no amylopectin-lipid transition (Evans 1986, Eliasson et al 1988).

Refrigerated-Storage Retrogradation by DSC

The influence of MGs on the retrogradation of waxy corn starch was investigated by a DSC rescan of the starch-MG suspensions after seven days of storage at 4°C. Representative thermograms of retrogradation for all samples are shown in Figure 2, and the DSC parameters are summarized in Table III. The low rounded peak at a T_{OR} of about 43–45°C was the retrogradation peak, which was confirmed by its disappearance after an immediate rescan of the calorimeter. The second sharp peak in Figure 2, thermogram b, was present in all GML-containing retrograded systems and has been noted in the melting of MGs by previous researchers (Brokaw and Lyman 1958). No significant differences were noted among treatments for T_{OR}. It was difficult to measure the effects of GMP and GMS on the retrogradation of amylopectin in the present system because the melting temperature of GMP and GMS interfered with the remelting of the crystallized amylopectin. Therefore, for the systems containing GMP and GMS, the T_{OR} and R_R values could not be measured, but the ΔH_R values were determined by the difference between the ΔH_R of the peak on the retrogradation curve and the ΔH_R of the peak in the same temperature range after an immediate rescan.

The T_{OR} and R_R for those recrystallized starches (with or without MGs) that could be measured were lower and broader, respectively, than in the original gelatinization curve ($P < 0.01$) and were not significantly different from the control or from each other. A statistically lower ΔH_R was noted for all MG-containing systems than that for the control, except for that of GMS. This suggests an interaction between waxy corn starch and most MGs during gelatinization, which then reduced the crystallinity of amylopectin during storage. The ΔH_R values decreased with a decrease in MG chain length from GMS down to GMM, suggesting an increase in MG-waxy corn starch interaction with a decrease in MG chain length. The system containing GML had a ΔH_R that was not statistically different from that of GMM. These values are consistent with the data for complex formation (Table I), in which the lower the molecular weight of the MG, the greater was the tendency for complex formation. Abilities of the MGs to interact may differ because of chain length and, thus, their fit into the helix of starch molecules. The linear portions of amylopectin from waxy maize starch have been estimated at 10–20 glucose units (Evans 1986). Six glucose units are needed per turn of the helix, giving a length of 8 Å per turn (Lagendijk and Pennings 1970). The length of the GMP molecule is about 22 Å, which means that a linear portion of amylopectin would need to have approximately 16.5 glucose units to accommodate GMP. If waxy corn starch has amylopectin with linear portions ranging down to 10 glucose units, then the shorter the MG, the more opportunities it may have to bind with the starch. Binding of very short MGs, however, might be limited by less distance for binding and, thus, instability of the complex. Clearly, amylopectin molecules from different botanical sources and with different amounts and lengths of branching would vary in their

TABLE III
Effect of Monoglycerides on Gelatinization and Retrogradation of Waxy Corn Starch[a]

Additive[b]	Temperature,[c] °C				ΔH_G[d]	ΔH_R[d]
	T_{OG}	T_{OR}	R_G	R_R	(cal/g dry matter)	
Control	66.7 a	42.5 a	8.9 a	13.3 a	4.1 a	2.2 a
GML	65.7 c	43.3 a	8.3 a	15.5 a	3.5 b	1.2 c
GMM	66.2 b	45.1 a	8.3 a	17.8 a	3.7 ab	1.0 c
GMP	66.1 b	ND[e]	8.9 a	ND	3.5 b	1.5 b
GMS	66.7 a	ND	8.3 a	ND	3.5 b	2.1 a

[a] Values are the means of four replicate determinations. Common letters indicate significance ($P < 0.05$) among treatments in each column.
[b] GML, monolaurin; GMM, monomyristin; GMP, monopalmitin; GMS, monostearin.
[c] T_{OG}, onset temperature of gelatinization; T_{OR}, onset temperature of retrogradation; R_G, temperature range of gelatinization; R_R, temperature range of retrogradation.
[d] Enthalpies of gelatinization and retrogradation, respectively.
[e] Not determined. Melting of GMP and GMS coincided with the retrogradation peak, so it was not possible to determine T_{OR} or R_R.

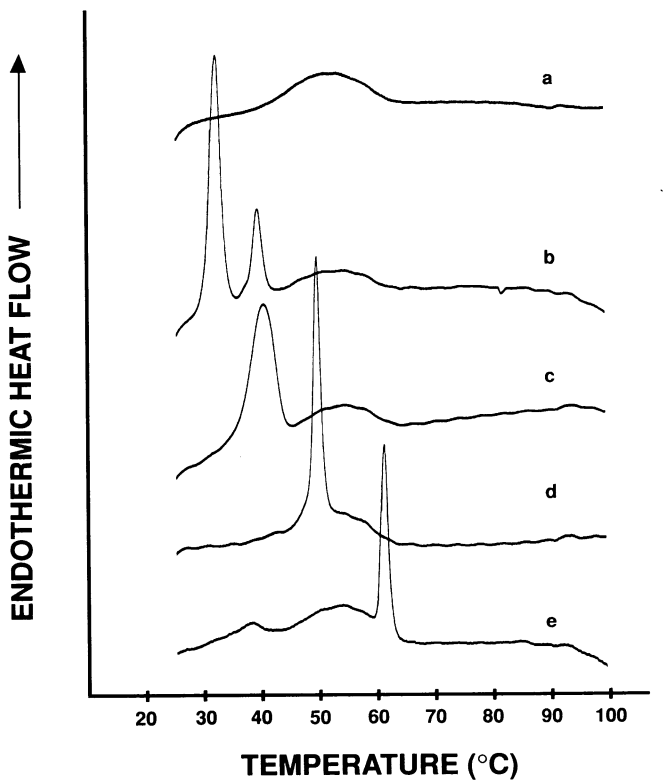

Fig. 2. Differential scanning calorimetric thermograms of starch-monoglyceride model systems, stored at 4°C for seven days, containing waxy corn starch, 2.26 mg dry matter (a); waxy corn starch + monolaurin, 2.19 mg (b); waxy corn starch + monomyristin, 2.25 mg (c); waxy corn starch + monopalmitin, 2.17 mg (d); and waxy corn starch + monostearin, 2.20 mg (e). The starch-to-water ratio was 1:3.

TABLE IV
Firmness of Starch Gels from Waxy Corn Starch With and Without Monoglycerides

Treatment[a]	Storage Condition, gram-force	
	Day 1, 25 °C	Day 7, 15 °C
Control	3.2[b]	3.3
GML, %		
0.3	3.3	3.1
1.0	3.1	3.0
5.0	3.4	3.4
GMM, %		
0.3	3.1	3.4
1.0	3.2	3.4
5.0	3.3	3.6
GMP, %		
0.3	3.1	3.2
1.0	3.2	3.0
5.0	3.0	2.9
GMS, %		
0.3	3.1	3.2
1.0	3.3	3.1
5.0	2.9	2.9
$LSD^{0.05}$, means[c]	0.5	

[a] GML, monolaurin; GMM, monomyristin; GMP, monopalmitin; GMS, monostearin.
[b] Values are the means for three replicate determinations.
[c] Least significant difference can be compared among all values.

TABLE V
Cohesiveness of Starch Gels from Waxy Corn Starch With and Without Monoglycerides

Treatment[a]	Storage Condition, gram-force	
	Day 1, 25 °C	Day 7, 15 °C
Control	2.1[b]	2.3
GML, %		
0.3	2.3	2.6
1.0	2.2	1.8
5.0	2.4	1.8
GMM, %		
0.3	2.5	2.7
1.0	2.3	2.6
5.0	2.5	2.5
GMP, %		
0.3	2.1	2.1
1.0	2.6	2.6
5.0	3.2	2.7
GMS, %		
0.3	2.3	2.6
1.0	2.8	2.7
5.0	3.1	2.7
$LSD^{0.05}$, means[c]	0.6	

[a] GML, monolaurin; GMM, monomyristin; GMP, monopalmitin; GMS, monostearin.
[b] Values are the means for three replicate determinations.
[c] Least significant difference can be compared among all values.

abilities to bind various MGs and other emulsifiers and surfactants.

Other researchers have noted that the amylopectin fraction in starch is responsible for retrogradation as measured by DSC, and surfactants and emulsifiers greatly reduce the rate of recrystallization (Russell 1983, Eliasson and Ljunger 1988). Retrograded waxy corn starch with and without MGs produced a smaller DSC endotherm than those reported by Eliasson and Ljunger (1988). These differences might have resulted from different storage temperature (7°C), storage time (11 days), and lipid additives (CTAB) in their studies. Finally, Sowa et al (1990) noted a tendency for breads made with waxy maize starch and MGs to decrease in firmness and to increase in volume as the chain length of the MGs decreased from GMS to GML.

Texture Determinations

The Voland-Stevens TA was used to measure the gel (or sol) properties (firmness and cohesiveness) of waxy corn starch with and without MG (Tables IV and V). The firmness of all samples ranged from 2.9 to 3.6 g-force. No statistical differences in firmness were noted among all treatments on day 1 or between days for the same treatment, except between GML and GMS, each at 5% concentration. On day 7 some significant differences were observed among treatments, but there was no pattern to the effects. Five percent GMP and 5% GMS were less than 0.3, 1.0, and 5.0% GMM and 5% GML; 0.3 and 1.0% GML were less than 5% GMM. No significant differences were found between the control and any treatments. The free MG in the gel also might influence the firmness.

The measurements of cohesiveness after one day of storage tended to increase with the presence of MGs, except for the treatment containing 0.3% GMP (Table IV). Compared with the control, statistical differences were noted only for 1 and 5% GMS and 5% GMP. Some differences among treatments were significant ($P < 0.05$), including the following sets: 0.3, 1, and 5% GML were less than 5% GMP and 5% GMS; 0.3, 1, and 5% GMM were less than 5% GMP and 5% GMS; 0.3% GMP was less than 1 and 5% GMS; 1% GML was less than 1% GMS. After seven days of storage, no significant differences were observed between the control and any treatments. But statistical differences ($P < 0.05$) were found between treatments containing either 1.0 or 5.0% GML and all other treatments, except for 0.3% GMP and the control ($P < 0.05$). Moreover, the treatment containing 0.3% GMP was significantly lower ($P < 0.05$) than those of 0.3% GMM, 5% GMP, and 1.0 and 5.0% GMS. Again, the pattern to these differences was unpredictable, and conclusions regarding the effects of MGs on textural properties of the gelatinized mixtures are questionable. The cohesiveness values may have simply been a measure of the stickiness of the MGs themselves rather than of the MG-amylopectin interaction.

The results of the TA conflict with those of DSC measurements, which showed an interaction between the waxy starch and MGs during gelatinization and a reduction of retrogradation with the addition of most MGs. The TA and DSC methods may not be measuring the same process. The TA measures the resistance to penetration of the gel as influenced by the presence of MGs. DSC gelatinization measures the actual development of an association between waxy maize starch and MGs, and DSC retrogradation measures the recrystallization of starch molecules. A relationship might be expected between the two methods; however, these small differences may not have been noticeable under the conditions of the TA test. Eliasson et al (1988) also described the difficulties in obtaining consistent and, thus, significant results from rheological measurements of such a complex system as a starch gel. Similarly, Evans (1986) noted no significant changes in viscosity (by sheer measurement) of waxy maize starch in the presence of emulsifiers, including MGs, although a reduction in ΔH by DSC was significant.

In general, the results suggest an interaction between waxy corn starch and MGs that differed somewhat with the conditions. All MGs formed a measurable complex with the waxy maize starch (Table I), and an association between the MGs and waxy maize starch was noted by DSC (Table III). The inhibition of retrogradation by DSC measurement increased with a decrease in chain length of the MG, which was consistent with the tendency for more millimoles of MG to complex with waxy maize starch as the chain length of the MG decreased. Finally, little evidence of waxy corn starch-MG interaction was noted with texture measurements of their gelatinized mixtures (Tables IV and V).

LITERATURE CITED

BATRES, L. V., and WHITE, P. J. 1986. Interaction of amylopectin with monoglycerides in model systems. J. Am. Oil Chem. Soc. 63:1537.

BEAR, B. S. 1942. The significance of the "V" X-ray diffraction patterns of starches. J. Am. Chem. Soc. 64:1388.

BROKAW, G. Y., and LYMAN, W. C. 1958. The behavior of distilled monoglycerides in the presence of water. J. Am. Chem. Soc. 35:49.

D'APPOLONIA, B. L., and MORAD, M. M. 1981. Bread staling. Cereal Chem. 58:186.
ELIASSON, A.-C., and KROG, N. 1985. Physical properties of amylose-monoglyceride complexes. J. Cereal Sci. 3:239.
ELIASSON, A.-C., and LJUNGER, G. 1988. Interaction between amylopectin and lipid additives during retrogradation in a model system. J. Sci. Food Agric. 44:353.
ELIASSON, A.-C., FINSTAD, H., and LJUNGER, G. 1988. Study of starch-lipid interactions for some native and modified maize starches. Starch/Staerke 40:95.
EVANS, I. D. 1986. An investigation of starch/surfactant interaction using viscosimetry and differential scanning calorimetry. Starch/Staerke 38:227.
GUDMUNDSSON, M., and ELIASSON, A.-C. 1990. Retrogradation of amylopectin and the effects of amylose and added surfactants/emulsifiers. Carbohydr. Polym. 13:295.
KNIGHTLY, W. H. 1977. The staling of bread: A review. Baker's Dig. 51(5):52.
LAGENDIJK, J., and PENNINGS, H. J. 1970. Relation between complex formation of starch with monoglycerides and the firmness of bread. Cereal Sci. Today 15:354.
MARTIN, M. L., ZELEZNAK, K. J., and HOSENEY, R. C. 1991. A mechanism of bread firming. I. Role of starch swelling. Cereal Chem. 68:498.
MIKUS, F. F., HIXON, R. M., and RUNDLE, R. E. 1946. The complexes of fatty acids with amylose. J. Am. Chem. Soc. 68:115.
RUSSELL, P. L. 1983. A kinetic study of bread staling by differential scanning calorimetry and compressibility measurements. The effect of added monoglyceride. J. Cereal Sci. 1:297.
RUTSCHMANN, M. A., HEINIGER, J., PLISKA, V., and SOLMS, J. 1989. Formation of inclusion complexes of starch with different organic compounds: I. Methods of evaluation of binding profiles with menthane as example. Lebensm. Wiss. Technol. 22:240.
SAS INSTITUTE. 1989. SAS User's Guide. The Institute: Cary, NC.
SCHOCH, T. J. 1942. Non-carbohydrate substances in the cereal starches. J. Am. Chem. Soc. 64:2954.
SCHOCH, T. J. 1964. Iodimetric titration of amylose. Pages 157-160 in: Methods in Carbohydrate Chemistry. Vol. 4. R. L. Whistler, ed. Academic Press: New York.
SCHOCH, T. J., and FRENCH, D. 1947. Studies on bread staling. I. The role of starch. Cereal Chem. 24:231.
SCHOCH, T. J., and WILLIAMS, C. 1944. Adsorption of fatty acids by the linear component of corn starch. J. Am. Chem. Soc. 66:1115.
SLADE, L., and LEVINE H. 1987. Recent advances in starch retrogradation. Pages 387-431 in: Industrial Polysaccharides. S. S. Stivala, V. Crescenti, and I. C. M. Dea, eds. Golden and Breach Science Publishers: New York.
SOWA, S. M., WHITE, P. J., and BATRES, L. V. 1990. Influence of monoglycerides of different chain length on texture and flavor of breads made with waxy cornstarch. Starch/Staerke 42:53.
STEEL, G. D., and TORRIE, J. H. 1980. Multiple comparisons. Pages 172–194 in: Principles and Procedures of Statistics, 2nd ed. McGraw-Hill: New York.
TAKAHASHI, S., and SEIB, P. A. 1988. Paste and gel properties of prime corn and wheat starches with and without native lipids. Cereal Chem. 65:474.
TAKEDA, Y., and HIZUKURI, S. 1987. Structure of rice amylopectin with low and high affinities for iodine. Carbohydr. Res. 168:79.
TAKEDA, Y., TAKEDA, C., SUZUKI, A., and HIZUKURI, S. 1989. Structures and properties of sago starches with low and high viscosities on amylography. J. Food Sci. 54:177.
WEAST, R. C., and ASTLE, M. J. 1985. CRC Handbook of Data on Organic Compounds. Vol. 2. CRC Press: Boca Raton, FL.
WHITE, P. J., ABBAS, I. R., and JOHNSON, L. J. 1989. Freeze-thaw stability and refrigerated-storage retrogradation of starches. Starch/Staerke 41:176.

[Received September 11, 1991. Revision received May 4, 1992. Accepted May 6, 1992.]

Collaborative Evaluation of an Enzymatic Starch Damage Assay Kit and Comparison with Other Methods

T. S. GIBSON,[1] C. J. KALDOR,[1] and B. V. McCLEARY[2]

ABSTRACT

Cereal Chem. 70(1):47-51

A commercially available enzymatic assay kit for the measurement of starch damage in wheat flour was compared with current standard methods, and the kit's precision and repeatability were determined in a collaborative study. Starch damage values determined on a range of flours with the assay kit correlated well ($r > 0.96$) with those determined by existing standard enzymatic methods. The precision of the kit was evaluated in a comprehensive interlaboratory study. The kit procedure was found to be highly repeatable (relative standard deviation, 2.94–6.80%) and reproducible (relative standard deviation, 5.00–10.30%).

A proportion of the starch granules in wheat grains is mechanically damaged during the milling process (Evers and Stevens 1985). These damaged granules hydrate rapidly and are susceptible to amylolytic hydrolysis. Consequently, they contribute significantly to the water absorption, rheology, handling properties, and gassing power of a dough and to crumb texture and crust color (Tipples 1969).

The industry standard methods for starch damage measurement are based on the preferential amylolytic digestion of damaged granules with crude commercial preparations of malt (Farrand 1964, Royal Australian Chemical Institute 1988) or fungi (American Association of Cereal Chemists [AACC] 1983, Donelson and Yamazaki, 1962). However, an improved enzymatic assay for starch damage that avoids many of the potential inaccuracies associated with the use of crude enzyme preparations recently was developed (Gibson et al 1992) and is now supplied commercially in kit form.

The aim of this work was to evaluate the reproducibility and repeatability of the proposed new method for starch damage through an extensive interlaboratory study and to correlate values obtained by the kit method on a range of flours with those obtained by standard starch damage assay procedures.

MATERIALS AND METHODS

Starch Damage Assay Kit

Enzymatic assay kits based on the method developed by Gibson et al (1992) were supplied by MegaZyme Pty Ltd., North Rocks,

[1]Biological and Chemical Research Institute, NSW Agriculture, Rydalmere, NSW, 2116 Australia.
[2]MegaZyme (Aust.) Pty Ltd., North Rocks, NSW, 2151 Australia.