Technical

Interaction of Amylopectin with Monoglycerides in Model Systems

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The ability of glyceryl monomyristate (GMM), glyceryl monopalmitate (GMP) and glyceryl monostearate (GMS) to form insoluble complexes with amylopectin was studied in model systems. Amylopectin complexed to the greatest extent with GMP, followed by GMM and GMS, respectively. The degree of complex formation was statistically different ($p < 0.01$) among monoglycerides. Iodimetric titrations of the complexes showed that the presence of GMP and GMM in the model systems significantly ($p < 0.01$) decreased the iodine affinity of the amylopectin when compared with the control. The presence of GMS in the model systems decreased the iodine affinity of the amylopectin slightly but not with statistical significance. The decrease in iodine affinity caused by the three monoglycerides was statistically different ($p < 0.01$) among treatments. There was a negative linear relationship between the yield of complex and the amount of monoglyceride (MG) and the iodine affinity of the complexes. No conclusion about the nature of the interaction between amylopectin and the MG could be made by doing infrared and NMR analyses of the model systems.

Past research shows that the retrogradation of starch in bread products plays a major role in staling (1), but it has not been fully established which fraction of starch is responsible for this effect. Early studies suggested that amylose was the cause of the staling because it retrogrades rapidly (2). Schoch and French (3) demonstrated that the amylose fraction retrogrades completely by the time the bread has cooled down from the oven, and thus can cause no further changes during bread storage. They observed also that the amount of soluble amylopectin decreased with the staling of bread and concluded that bread staling is caused by the slower retrogradation of the amylopectin. Other studies confirmed that amylopectin plays a role in bread firming and, thus, staling. For example, Noznich et al. (4) demonstrated that bread made with waxy corn starch in place of wheat starch firmed as readily as the original bread. More recent research by D'Appolonia and coworkers (5-7) supports the work by Schoch and French (3). D'Appolonia and coworkers showed that soluble amylose sharply decreased during the first day of bread storage, implying that amylose contributes to firming or staling primarily in the early stages of bread storage. They further showed that the soluble starch from fresh bread was mainly amylopectin, which decreased progressively as bread aged. Actually, it is likely that both starch fractions are involved in bread staling.

The introduction of monoglycerides (MG) as antistaling agents in bread products triggered numerous studies in model systems (8-11) regarding the ability of the MG to complex with the starch fractions. Most studies were done with amylose. Because amylose complexes greater amounts of MG than does amylopectin, the delay of staling caused by the presence of MG was attributed to the formation of such a MG-amylose complex. There is no satisfactory evidence that the staling delay is caused by this effect, especially when one considers the evidence (3,7) regarding the roles of amylopectin and amylose in the staling of bread.

Gray and Schoch (9) and Lagendijk and Pennings (10) found evidence of complex formation between amylopectin and various MG's. Gray and Schoch demonstrated that the presence of polyoxyethylene monostearate (POEMS), stearic acid and mixtures of MG decreased the swelling and solubilization of waxy sorghum starch. They suggested that amylopectin may form a complex with some of its outer branches and the fatty acids of the MG. Lagendijk and Pennings reported the formation of an insoluble precipitate when amylopectin and various MG were present in model systems. On the other hand Krog (11) and Krog and Jensen (12) reported no complex formation between MG and amylopectin.

There is little information regarding MG-amylopectin complex formation, and its role in delaying bread staling is controversial. This study was undertaken to compare the yields of complex with MG's [glyceryl monomyristate (GMM), glyceryl monopalmitate (GMP) and glyceryl monostearate (GMS)] in a model system, to compare the iodine affinity of the three complexes and to investigate the interaction between amylopectin and the MG's.

MATERIALS AND METHODS

Materials. Potato amylopectin was purchased from Sigma Chemical Co., St. Louis, Missouri. Its maximum absorption at 540 nm in the presence of iodine (13) and its iodine affinity of 0.45% (14) confirmed the absence of amylose. The 1-monoglycerides obtained from Sigma Chemical Co. were 99% pure.

Complex formations. The model systems were based on procedures by Lagendijk and Pennings (10) and Osman and coworkers (8). Three g of amylopectin (moisture content 6.1%) and one g of MG were suspended in 200 ml of water in a two-necked flask fitted with a condenser and stirred at 12,000 rpm with a propeller stirrer (LAB-STIRR hollow spindle, variable speed, Eberbach Corp., Ann Arbor, Michigan) in a 60 C water bath (18 L × 12 W × 10 D cm) for six hr. The flask and water bath were cooled to room temperature overnight. 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 vacuum at 65 C for three hr until a constant weight was reached and stored in a desiccator. A control was prepared without added MG. Free MG present in the precipitate was removed by carbon tetrachloride extraction in a Soxhlet apparatus

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for two hr (15), and the remaining MG-amylopectin complex weighed. Carbon tetrachloride extractions of up to nine hr resulted in no additional MG removal, thus ensuring that all free MG was extracted in two hr. The bound MG was removed from the complex by methanol extraction in a Goldfisch apparatus for six hr (14) and weighed. The specificity of MG removal by these solvents was tested and is described later in this paper.

Determination of iodine affinity. The iodine affinity of the amylopectin in the presence of each MG was determined by using small model systems containing 0.2 g of amylopectin, 0.066 g of MG and 50 ml of distilled water. A control was prepared without added MG. The mixture was stirred for six hr at 60 C. After overnight cooling to room temperature the model systems were titrated potentiometrically with iodine as described by Schoch (14). Millivolts were read at 10 different points between 230 and 280 mv. From the mv readings, the concentration of free iodine in solution was determined by using a calibration curve. The bound iodine was estimated 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 by using the upper linear portion of this curve. The percentage iodine affinity of the model systems was determined by multiplying the intercept of this regression line by 100, and dividing it by the dry weight of the amylopectin and monoglyceride that had been added to the model system (14). A digital pH/mv meter was used (Orion Research model 710A/digital Ioanalizer). All treatments were done in triplicate.

To determine the efficacy of carbon tetrachloride to remove only the free MG present in the precipitates, and to determine whether the methanol indeed removed the MG that was bound in the complexes, potentiometric titrations were done after each extraction step. For this purpose, model systems containing three g of amylopectin and one g of MG were prepared as previously described. After the precipitates reached a constant weight in the desiccator, they were divided into three portions, with each containing approximately 0.2 g of amylopectin. One portion was potentiometrically titrated with iodine without previous treatment. Another portion first was extracted with carbon tetrachloride for two hr and then titrated with iodine. The third one was extracted with carbon tetrachloride for two hr, with methanol for 5 hr, and then potentiometrically titrated with iodine. The percentage iodine affinity was calculated as previously described.

IR and NMR spectroscopic determinations. Infrared spectra of the model systems were obtained by using a double-beam Beckman IR-33 spectrophotometer (Beckman Instruments Inc., Fullerton, California). Samples were slurried with carbon tetrachloride and placed between two Irtran plates or in a salt cell.

Proton spectra of the samples dissolved in deuterated dimethyl sulfoxide were obtained by using a Nicolet NT-300 NMR spectrophotometer. Samples were run in 5-mm NMR tubes with a 10-degree flip angle and a spectra width of 4000 hertz, with a pulse repetition time of three sec.

TABLE 1

Composition of Complexes Formed from Model Systems Made from Amylopectin and Monoglycerides

Type of monoglyceride	Amount of complex formed[a] (mg)	Amount of monoglyceride (mg)	MG (% complex)
Glyceryl monomyristate	807.9[b]	217.3[b]	26.90[b]
Glyceryl monopalmitate	1026.4[c]	376.0[c]	36.63[c]
Glyceryl monostearate	207.6[d]	41.4[d]	19.94[b]

[a]Model systems consisted of 3 g of amylopectin and 1 g of monoglyceride. (Refers to amount of complex formed after CCl_4 extraction).

Different letters indicate $p < 0.05$ between monoglycerides in each column.

RESULTS AND DISCUSSION

Complex formations. The amounts of complex formed between amylopectin and the MG are shown in Table 1. GMP gave the greatest yield, followed by GMM and GMS, respectively. This same pattern is found when the complex formation is expressed in mmol mg/g amylopectin. Lagendijk and Pennings (10) found that MG complexed with pure potato amylopectin in model systems but that the amount of MG that complexed increased linearly with increasing fatty acid chain length, up to C-20. They reported that 10.7 mg GMM, 15.1 mg GMP and 25.0 mg GMS complexed with amylopectin when 0.2 g of MG plus three g amylopectin were complexed at 80 C for four hr. These results contrast with the very large amounts of complex formed in the present study (Table 1) when 1 g MG/3 g amylopectin were complexed at 60 C for six hr. In addition, the percentage of each MG that bound to amylopectin differed between their study and the present one. They reported complexes (compared with ours) of 5.35% vs 21.73% (GMM), 7.55% vs 37.60% (GMP) and 12.5% vs 4.14% (GMS).

These major discrepancies in data likely are because of the different amounts of materials used in the model systems in addition to the differences in complexing temperature and time. Perhaps 60 C for six hr allowed for better complex formation, particularly of the shorter GMM and GMP. Another procedural difference was that they extracted the free MG from the precipitate by shaking four times with 200 ml of ether in contrast to the Soxhlet extraction for two hr with carbon tetrachloride reported here. Possibly, they removed some of the complexed MG along with the free MG during their ether extraction, thus resulting in less measurable complex, especially for the GMM and GMP. Because ether is more polar than carbon tetrachloride, it would be more likely to penetrate a starch molecule and remove complexed MG.

Although the larger ratio of MG to amylopectin used in the present study does not mimic ratios found in bread products, it does demonstrate the ability of the

MG to complex with amylopectin. Also, it provided suitable quantities of the MG-amylopectin complex for further analyses. In contrast to the MG complexes found in our study and in the study by Lagendijk and Pennings, Krog (11) and Krog and Jensen (12) found no evidence of a visible precipitate in model systems containing potato amylopectin and GMS in various physical states. As noted, however, very little GMS-amylopectin complex was formed in the present study in comparison with the other MG studied.

Iodine affinity. Table 2 demonstrates the decrease in iodine affinity of the amylopectin in the presence of the MG. GMP and GMM significantly decreased ($p < 0.01$) the iodine affinity, whereas GMS caused only a slight decrease which was not significant. Figure 1 shows that the iodine affinity decreased linearly with the quantities of complex and with the amount of MG complexed. Schoch and Williams (15) observed that the presence of fatty acids in amylose samples interfered with the formation of amylose-iodine complexes. Several X-ray studies revealed that amylose forms helical complexes with fatty acids similar to the complex formed with iodine (16–18). It has been proposed that there are six glucose units per turn of a helix and that three turns are required to complex with one fatty acid (19). Because the average length of an amylopectin branch is 20 to 26 glucose units (20), it is theoretically possible for some of the outer branches of amylopectin to form a helical complex with fatty acids. The decreasing iodine affinity of amylopectin complexed with an increasing amount of MG, and amount of complex formed, suggest the ability of the MG, especially GMM and GMP, to form a helical structure with amylopectin. The iodine affinity of the GMS-amylopectin complex was not significantly different from the control suggesting no complex formation; however, data from Table 1 provide evidence of at least some GMS-amylopectin complex formation. There was more variation in the potentiometric method, likely accounting for the lack of significance. Interestingly, Krog and Jensen (12) reported no decrease in the color (measured at 455 nm) of amylopectin-iodine solutions in the presence of MG, so they concluded that there was no evidence of MG-amylopectin interaction. Actually, amylopectin-iodine complexes have a maximum absorbance at 540 nm (13), so this interaction would not have been observed at 455 nm.

Potentiometric titrations also were used to demonstrate the efficacy of carbon tetrachloride to remove only the free MG from the precipitate and the ability of methanol to remove the bound MG from the complexes. Research has shown that MG-amylose complexes are affected in this manner (8), but because of the structural differences between amylopectin and amylose, it was necessary also to demonstrate the solvent effects with MG-amylopectin complexes. Model systems containing three g of amylopectin and one g of MG were prepared as previously described. Table 3 shows close iodine affinity values for model systems undergoing no treatment and for those undergoing carbon tetrachloride extraction, indicating that no bound MG was removed during the carbon tetrachloride treatment. After methanol extraction, the iodine affinity of the amylopectin was nearly restored to the original value of pure amylopectin of 0.45%, indicating almost complete removal of all the bound monoglyceride. The data reported in Table 3 represent different model systems from those listed in Table 2 and Figure 1. The variability in the potentiometric method likely accounts for the differences in values.

TABLE 2

Iodine Affinity of Amylopectin and Amylopectin-Monoglyceride Complexes[a]

	% Iodine affintiy
Amylopectin	0.45[b]
Amylopectin-GMM	0.25[c]
Amylopectin-GMP	0.17[d]
Amylopectin-GMS	0.41[b]

[a]Model systems contained 0.2 g of amylopectin and 0.666 g of monoglyceride. Different letters indicate $p < 0.01$ between treatments.

TABLE 3

Iodine Affinity of Model Systems Before and After Solvent Extractions

	% Iodine Affinity		
Type of monoglyceride	Original complex	Complex after CCl_4 extraction	Complex after CCl_4 and MeOH extractions
Glyceryl monomyristate	0.27	0.26	0.40
Glyceryl monopalmitate	0.18	0.18	0.41
Glyceryl monostearate	0.37	0.38	0.41

IR and NMR spectra. Infrared spectra of pure amylopectin and of GMP-amylopectin complexes that had been extracted with carbon tetrachloride were obtained to determine the presence of the MG in the precipitate and to detect any bonding between the MG and amylopectin. GMP-amylopectin complexes were chosen because they formed the greatest amount of complex of the monoglycerides tested. Absorbance at 1725 cm^{-1} and at 2875 cm^{-1} demonstrated the presence of carboxylic and methyl groups from the MG, respectively (21). The absorbance of the MG in the amylopectin complex was not shifted compared with that of pure MG, so no conclusion about the nature of the interaction was possible.

NMR spectra were obtained for amylopectin, GMP, GMS, GMM and their complexes. NMR spectra also were obtained for tripalmitin and methyl palmitate to determine the peak location of the functional groups of the MG. No band shift of any functional group resulted from the complexing of amylopectin and the MG.

Interestingly, GMS, the MG used the most widely in the food industry to decrease bread staling, formed the least amount of complex with amylopectin. It is possible

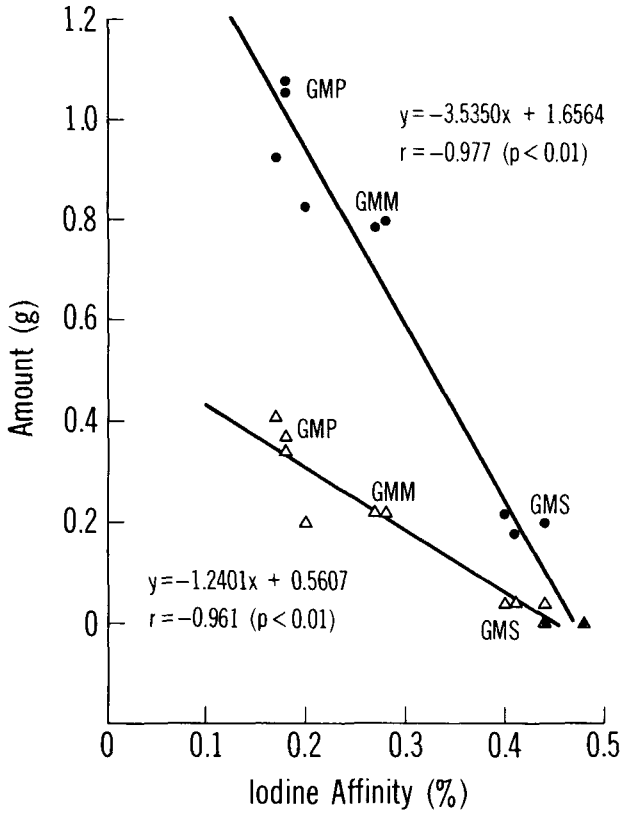

FIG. 1. Relationship between iodine affinity and amount of complex formed, and amount of monoglyceride present in the complexes. △, MG in complex; •, MG-amylopectin complex.

that the GMS-amylopectin complex forms more readily in the drier environment of a bread system or at temperatures higher than the 60 C used in the current study.

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