

# Effect of Water Content on the Formation and Dissociation of the Amylose-Lipid Complex in Wheat Flour

German Jovanovich, Ricardo A. Zamponi, Cecilia E. Lupano, and María C. Añón\*

Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Calle 47 y 116, 1900 La Plata, Argentina

The effect of the water content on the formation and dissociation of the amylose-lipid complex was analyzed by differential scanning calorimetry in wheat flour, starch, and isolated complex. At low and intermediate water contents (36-64%, wet basis) the enthalpy change of formation and dissociation of the amylose-lipid complex after the first heating was related to the water content. The enthalpy of complex dissociation during the second heating decreased sharply with regard to the first heating for water contents lower than 43, 45, and 34% (wet basis) for flour, starch, and isolated complex, respectively. On the other hand, addition of water to a sample already heated at a lower water content led to an endothermic transition when the sample was reheated, this transition being higher than that observed in the first heating. The melting point of the undiluted polymer (166.2 °C), calculated with the Flory-Huggins equation, was similar to that obtained experimentally (170 °C). Results suggest that water would be necessary as plasticizer during complex formation.

## INTRODUCTION

Emulsifiers are often used in cereals and starch-based products because of their ability to retard starch retrogradation and bread firming (D'Appolonia and Gilles, 1978). These emulsifiers affect both the structural stability and the rheological behavior of starch during gelatinization (Krog, 1971; Schuster and Adams, 1984) by forming crystalline complexes with amylose (Ghiasi et al., 1982a; Young, 1984). These complexes present the V-amylose pattern by X-ray diffraction (Zobel et al., 1988). At least 90% of the lipids from wheat starch are lysophospholipids (Morrison, 1978; D'Appolonia and Gilles, 1978) which may occur as inclusion complexes inside the helical segments of amylose.

Complex formation and stability may be technologically important. The extent of complex formation depends, in part, on the phase properties of starch and the phase transitions undergone by starch heated in the presence of water (Donovan, 1979; Kugimiya et al., 1980; Eliasson, 1980; Biliaderis et al., 1980; Kugimiya and Donovan, 1981; Bulpin et al., 1982; Galloway et al., 1989).

In the present work, the effect of the water content on the amylose-lipid complex formation and dissociation was studied by differential scanning calorimetry in wheat flour, starch, and isolated complex.

## MATERIALS AND METHODS

Flour used in this study was obtained from wheat *Triticum aestivum* cv. Marcos Juárez-INTA, grown in Pergamino, Argentina, during 1988-1989. The sample was dried naturally in the field at a humidity level of 13% [wet basis (wb)] before harvesting. Seeds were ground in a Buhler type mill. Flour extraction rate was 72%; flour moisture content was 13.5%.

Unmodified wheat starch and 1- $\alpha$ -lysophosphatidylcholine of egg yolk were obtained from Sigma Chemical Co. (St. Louis, MO). All chemicals were of analytical grade.

**Preparation and Isolation of the Amylose-Lipid Complex.** Wheat starch (400 mg) was dissolved in 5 mL of hot dimethyl sulfoxide (DMSO) and diluted with distilled water at 100 °C up to 0.8% w/v concentration. Eighty milligrams of 1- $\alpha$ -lysophosphatidylcholine dissolved in 4 mL of distilled water at 60 °C was added to obtain a starch/lipid ratio of 5:1 w/w (Krog, 1971). The suspension was stirred with a Virtis stirrer in a water bath at 60 °C for 2 h, slowly cooled to room temperature, and allowed to

stand for 72 h. The insoluble complex was recovered by centrifugation (10 min at 8000g, 10 °C), washed several times with distilled water, and vacuum dried.

**Differential Scanning Calorimetry (DSC).** A Du Pont 910 system calibrated with indium was used. A Hewlett-Packard 7046-B recorder was attached to the thermal analyzer. A double pan was used as reference.

Samples (flour, starch, or isolated complex) were weighed in DSC pans, distilled water being added with a Hamilton syringe to obtain different water percentages. The pans were then sealed and weighed to determine the exact amount of water. They remained at room temperature for 24 h (about 20 °C) and were then centrifuged at 800g for 10 min, at 20 °C, to ensure a good contact between sample and water before DSC determinations.

Except in those cases indicated in the text, sample and references were heated or cooled between 30 and 135 °C at a rate of 10 °C/min. After the first heating, the sample was kept at 135 °C for 4 min in the DSC equipment, before cooling.

In the rehydration experiments, samples were placed in pans previously cooled at 4 °C, and distilled water at the same temperature was then added. Pans were then sealed and kept at 4 °C for 5 min to stabilize the system. Runs were performed between 4 and 135 °C at 10 °C/min.

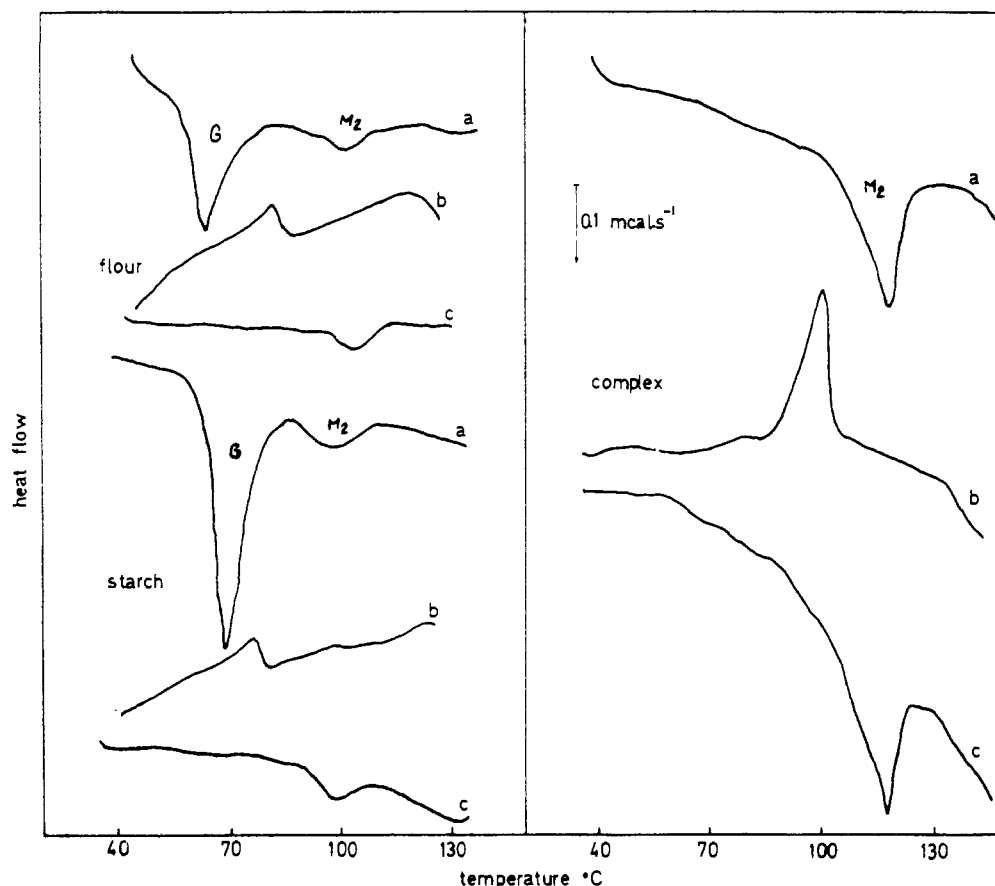
Areas of the peaks were determined with a Morphomat 34 Zeiss image analyzer.

**X-ray Diffraction.** Isolated complex or wheat starch was placed as a thin film on aluminum sample holders and analyzed with a Philips PW 1710 powder diffractometer equipped with crystalline graphite monochromator. The operating conditions were as follows: Cu K radiation; voltage, 40 kV; chart speed, 10 mm/2 $\theta$ ; running rate, 2 $\theta$ /min.

## RESULTS AND DISCUSSION

**X-ray Diffraction.** X-ray diffraction was used to characterize both wheat starch and isolated complex. Generally, cereal starches give A-patterns, while the V-form is typically exhibited by the amylose-lipid complex (Zobel, 1988). Characteristic peaks of A-form were obtained in wheat starch (2 $\theta$  23, 17.5, and 15.1), whereas the isolated complex showed V-form (peaks at 2 $\theta$  8.1, 12.8, and 19.8), in agreement with results reported by other authors (Zobel et al., 1988).

**Differential Scanning Calorimetry.** Figure 1 shows the thermograms obtained when wheat flour, starch, and the isolated complex were heated, cooled, and reheated at high water contents (higher than 60% for starch and flour



**Figure 1.** Representative DSC thermograms of wheat flour, starch, and isolated amylose-lipid complex at high water content: (a) first heating; (b) cooling; (c) reheating. Contents: flour, 5.45 mg, water content 64%; starch, 5.15 mg, water content 65.5%; complex, 5.64 mg, water content 49.9%.

and higher than 40% for the isolated complex). Two endothermic transitions were clearly observed in the first heating for both flour and starch (Figure 1, curve a). Both the enthalpy of the process ( $\Delta H$ ) and the apparent transition temperature were similar to those found in the literature (Kugimiya et al., 1980; Ghiasi et al., 1982b; Eliasson, 1983). Peak G corresponds to starch gelatinization and peak  $M_2$  represents the dissociation of the amylose-lipid complex. Thermograms of the isolated complex show only the  $M_2$  peak (Figure 1, curve a). An exothermic peak, corresponding to the amylose-lipid complex formation, was observed during cooling at about 78 °C in flour and starch and at about 96 °C in the isolated complex (Figure 1, curve b). The  $\Delta H$  of the exothermic peak was 30–60% of the  $\Delta H$  obtained during complex dissociation corresponding to flour and starch (Figure 1, curves a and b), in agreement with results reported by other authors (Bulpin et al., 1982; Eliasson and Krogg, 1985), and 60–80% for the isolated complex. As the complex formation may involve a nucleation step followed by growth of the complex crystallites, a possible explanation of the difference between the  $\Delta H$  values of the complex dissociation and formation would be that the  $\Delta H$  of the complex formation would represent only the growth of the crystallites. Differences in the  $\Delta H$  values could also arise from different mechanisms occurring in both processes. When the samples were subjected to a second heating, an endothermic peak corresponding to the dissociation of the amylose-lipid complex was observed, with a  $\Delta H$  value slightly lower than that of the first heating (Figure 1, curve c).

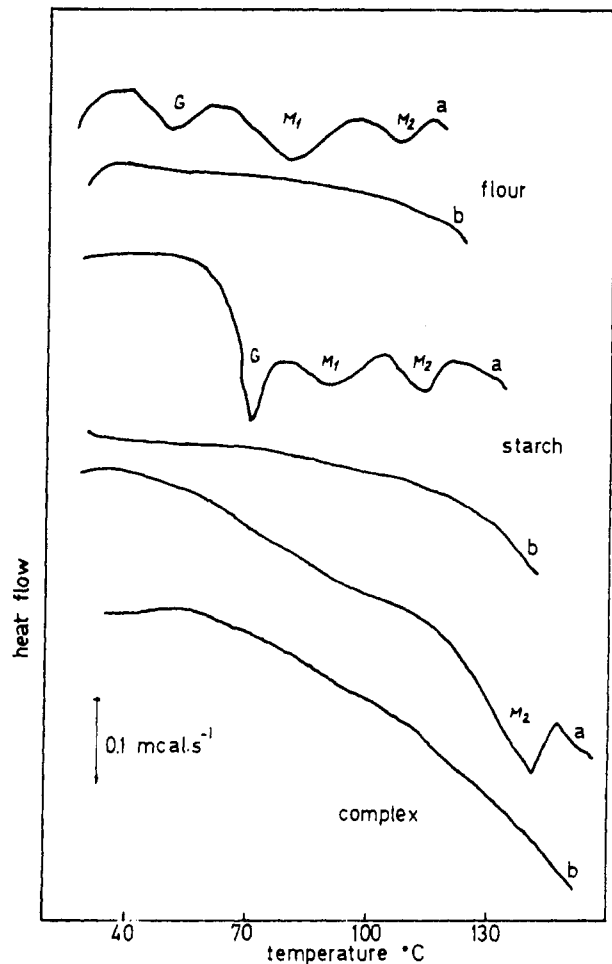
Thermograms of wheat flour, starch, and isolated complex at low and intermediate water contents (lower

than 45% for flour and starch and lower than 35% for isolated complex) are shown in Figure 2. Flour and starch exhibit three endothermic peaks in the first heating: peaks G and  $M_2$  (already described) and peak  $M_1$  corresponding to the fusion of more stable starch crystals. The isolated complex exhibited only peak  $M_2$ . The formation and dissociation of the amylose-lipid complex were observed only in some cases during cooling and reheating.

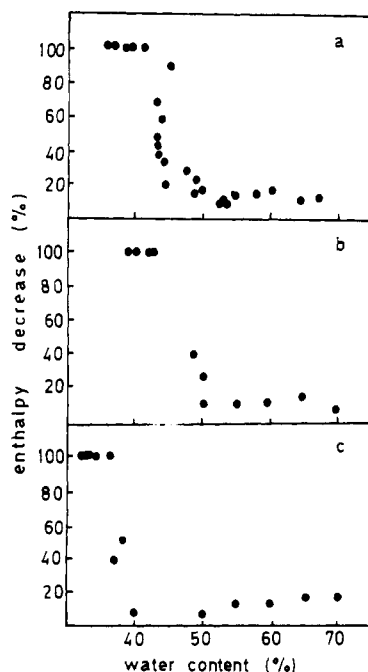
Figure 3 shows the decrease of the amount of the amylose-lipid complex during the second heating with regard to the amount of the dissociated complex during the first heating [ $100(\Delta H(1st\ heating) - \Delta H(2nd\ heating))/\Delta H(1st\ heating)$ ] as a function of the water content in flour, starch, and isolated complex. Results indicate that the area of the endothermic peak corresponding to the complex dissociation after the second heating decreased sharply with water contents lower than 43, 45, and 34% for flour, starch, and isolated complex, respectively.

The enthalpy of the amylose-lipid complex dissociation (first heating) as a function of the water content is shown in Figure 4. The enthalpy of the complex dissociation increased as the water content increased in flour, starch, and isolated complex, in agreement with results obtained by other authors (Czuchajowska and Pomeranz, 1989).

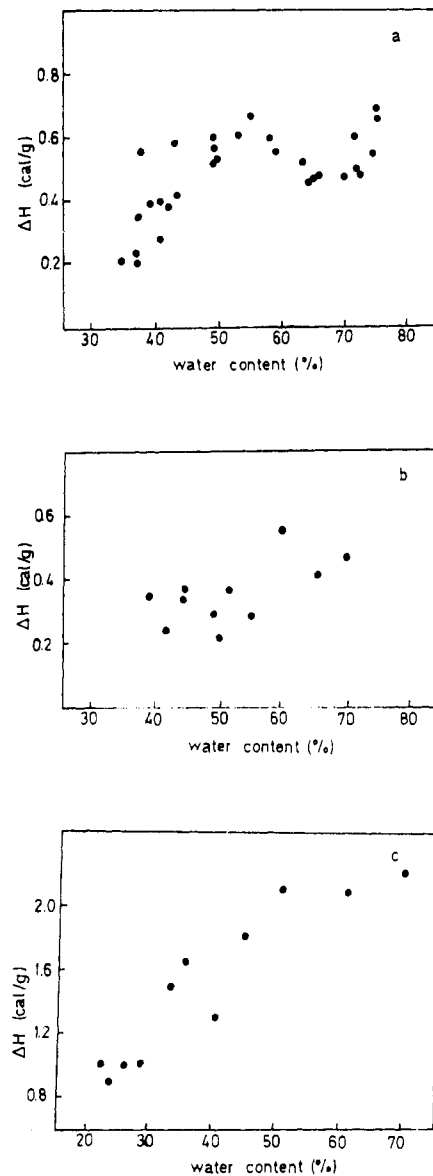
Figure 5 shows the thermograms of the isolated complex with 22.7% (db) of water (Figure 5I). At this water content, no peak was observed during the second heating (Figure 5I, curve c). However, after the addition of distilled water to the same heated sample up to 70% (db) of water content, a peak corresponding to the complex dissociation was obtained during the first and second heatings, and an exothermic peak of complex formation was obtained during cooling (Figure 5II).



**Figure 2.** Representative DSC thermograms of wheat flour, starch, and isolated complex at low and intermediate water contents: (a) first heating; (b) reheating. Contents: flour, 5.71 mg, water content 37%; starch, 5.13 mg, water content 37.5%; complex, 2.84 mg, water content 23.6%.



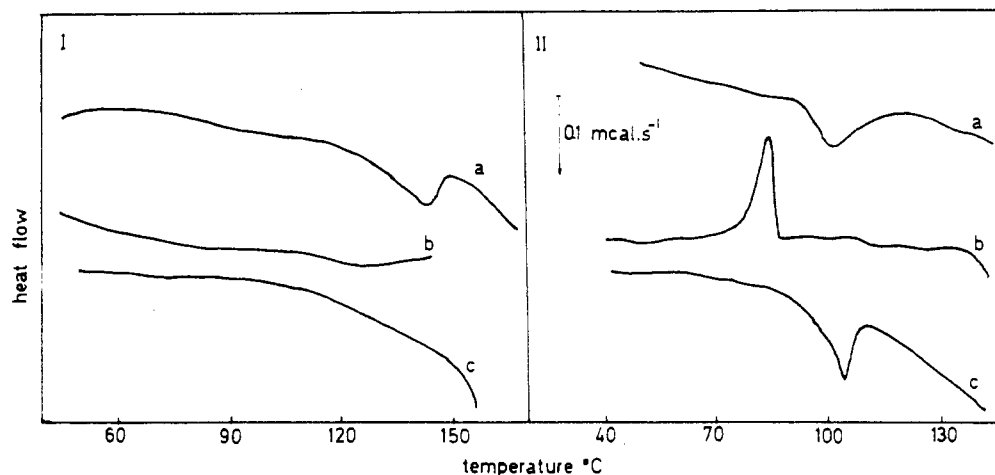
**Figure 3.** Amount of dissociated amylose-lipid complex after the second heating with respect to the dissociated complex after the first heating for (a) wheat flour, (b) starch, and (c) isolated complex, as a function of water content.



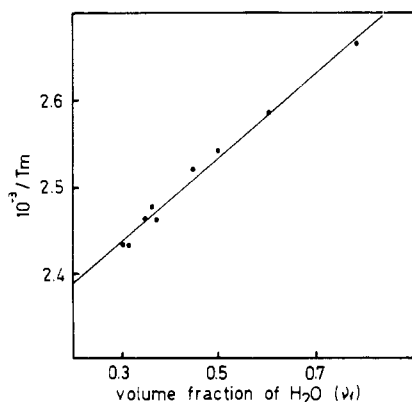
**Figure 4.** Enthalpies of amylose-lipid complex dissociation (first heating) as a function of water content for (a) wheat flour, (b) starch, and (c) isolated complex.

At high water contents, complex formation was reversible, as observed in Figure 1, curves c. A sharp exotherm appeared on the cooling runs of the melted complexes, indicating complex crystallization. This transition showed a marked hysteresis frequently preceded by a wide tail. The hysteresis phenomenon is expected when the process is a controlled nucleation.

The thermal curve of any semicrystalline material is in fact the product of two competitive processes: melting of the samples according to the type and distribution of its crystallites, followed by their reorganization as the sample is heated during the scan. Multiple endotherms (usually two) would result from (a) melting, followed by recrystallization and final melting of the reorganized material, or (b) crystals of different thermal stability in the original sample. Conversely, a single symmetrical endotherm indicates a melting process, in which reorganization during heating is negligible, involving crystals of the same thermal stability. Melting of the latter systems can be treated as either an equilibrium or a zero-entropy-producing melting process (Biliaderis et al., 1985). Different amylose-lipid systems have been analyzed by other authors (Biliaderis et al., 1985; Biliaderis, 1986; Biliaderis and Galloway, 1989;



**Figure 5.** (I) DSC thermograms of isolated complex, 2.48 mg, water content 22.7%. (II) DSC thermograms of isolated complex obtained after water was added to the previous sample, 2.48 mg, water content 70%. (a) Heating; (b) cooling; (c) reheating.



**Figure 6.** Plot of  $1/T_m$  vs fraction of water ( $\nu$ ) for the endotherm assigned to the fusion of the amylose-lipid complex.

Biliaderis and Seneviratne, 1990), who observed that lauric, capric, and myristic acids and monostearin exhibit multiple endotherms and that lysolecithin shows a single melting transition.

To confirm that the melting of the amylose-1- $\alpha$ -lysophosphatidylcholine complex is a single melting transition, thermal analyses were carried out at different heating rates (5, 10, 15, 20, and 25 °C/min), only one endotherm peak being obtained in all cases. This fact indicates that it is possible to apply the Flory-Huggins theoretical treatment (Flory, 1953), which describes equilibrium melting assuming, as a first approximation, that the polymer-diluent interaction parameter ( $X_1$ ) is zero

$$\frac{1}{T_m} - \frac{1}{T_m^0} = R \frac{V_u}{V_1} (\nu_1 - X_1 \nu_1^2) \frac{1}{\Delta H_u} \quad (1)$$

where  $R$  is the universal gas constant,  $V_u$  is the solvent molar volume,  $V_1$  is the polymer molar volume,  $T_m$  is the melting point of the polymer-solvent mixture,  $T_m^0$  is the equilibrium melting point of undiluted polymer,  $X_1$  is the polymer-diluent interaction parameter,  $\Delta H_u$  is the enthalpy of fusion per repeating unit, and  $\nu_1$  is the volume fraction of water.

The volume fraction of water in each sample was calculated as the ratio between the water volume and the total volume (water and starch); water and starch densities were 1.00 and 1.50 g/cm<sup>3</sup>.

Figure 6 shows the straight line obtained by plotting  $\nu_1$  and  $1/T_m$  (Flory-Huggins equation). The correlation coefficient was 0.953.  $T_m^0$  and  $\Delta H_u$  were 164.1 °C and 58.5 cal/mol, respectively.

The polymer-diluent interaction parameter  $X_1$  was then calculated by plotting  $[(1/T_m) - (1/T_m^0)]/\nu_1$  vs  $\nu_1$ , taking the value of  $T_m^0$  obtained previously ( $T_m^0 = 164.1$  °C). The value of  $X_1$  calculated in this way was 0.098.  $T_m^0$  was estimated again by plotting  $1/T_m$  vs  $(\nu_1 - X_1 \nu_1^2)$ , the value being 166.2 °C, which is similar to that obtained experimentally (170 °C).

The thermal behavior observed in the cooling curves was related to the organization of the amylose-lipid helices into a supramolecular structures (Biliaderis, 1985). The formation of such organization depends on the presence of a fluid medium. Besides, this fluid medium is given by the polymer in its amorphous phase, above its  $T_g$  (vitreous transition temperature);  $T_g$  increases as water decreases (Biliaderis, 1986).

These facts explain the necessity of water observed in this study for the complex formation, with the solvent behaving as plasticizer. As  $T_g$  is high at low water contents, the dissociated complex cannot crystallize again. The decrease in the  $\Delta H$  value observed in the second heating at intermediate water content (Figure 3) could occur as a consequence of (a) a  $\Delta H$  decrease after the second heating or (b) the less likely possibility of crystal growth because of the low fluidity of the medium.

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