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Study of denaturation of corn proteins during storage using differential scanning calorimetry

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Abstract

The effects of storage temperature and grain moisture content on thermal properties of protein fractions of a commercial corn hybrid were studied by differential scanning calorimetry. The results show that, at a storage temperature of 40 °C and moisture content ranging from 18 to 10%, corn protein fractions suffered significant changes in a short period of time. When the grain was stored for 30 days at 40 °C and 18% moisture content, enthalpy of protein denaturation decreased by about 80%, for proteins with polar predominance (albumin and globulin), and within 50 days for those of hydrophobic predominance (prolamin and glutelin). Along with the observed enthalpy decrease, the denaturation temperature increased by 32% for predominantly hydrophobic proteins and 15% for the polar ones.

Keywords: Differential scanning calorimetry; Corn; Storage conditions; Protein denaturation; Moisture content effect; Temperature effect; Enthalpy; Thermal profiles; Globulins; Prolamins; Glutelins

1. Introduction

Differential scanning calorimetry (DSC) is a technique which has allowed the study of thermal transformations in proteins, carbohydrates and lipids, and can provide information on first and second order transitions. Boye, Ma, and Harwalkar (1997) reported that protein transitions from a native conformation to a denatured condition appear, together with the disruption of inter and intra molecular bonds. This process occurs cooperatively and depends on several factors such as protein nature, concentration, a_w, temperature, pH, and ionic force. These changes can affect its functionality and eventual use in food systems.

Thermal denaturation behaviour of proteins in food can be followed by DSC. The effects of freezing, drying, and mixing operations may be evaluated in conjunction with other changes that take place during storage (Ma & Harwalkar, 1991). One of the main practical applications of DSC in food processing has been for ingredient quality control and food component interactions, as well as component modifications during different operations of processing (Dollimore, 1992; Harwalkar & Ma, 1990). Donovan and Ross (1973) and Donovan (1977) used DSC to study the effects of several treatments on the heat stability of egg white and its components, as well as protein stability, during baking processes.

Factors which affect the thermal properties of leguminous proteins have been widely analyzed by DSC (Arntfield & Murray, 1981). Murray, Arntfield, and Ismond (1985) monitored and reported changes in thermodynamic behaviour for oleaginous and leguminous proteins subjected to various processes. Ma and Harwalkar (1988) studied thermal denaturation of oat globulin, the major oat protein fraction, by DSC.

There is a need to obtain information related to conformational changes suffered by cereal macromolecules during storage of the grains. For example, deficiencies in post-harvest corn management are common in countries such as Mexico. Current storage systems do not protect the grains from climatological variations, which

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may result in qualitative losses by deterioration of nutritional and functional quality of the grain (Moreno, 1991). Effects of the storage conditions on the thermal behaviour of corn proteins have not been established. Only biochemical and nutritional changes have been studied (Onigbinde & Akinyele, 1988).

The purpose of this study is to determine the effect of storage conditions (temperature and moisture content) on denaturation processes of corn protein fractions by means of differential scanning calorimetry.

2. Materials and methods

2.1. Corn grain

Commercial hybrid H-4-47, provided by the Agronomy Department of the Universidad Autonoma Agraria Antonio Narro de Saltillo Coah. Mexico, was used for these studies. A 10% moisture content in the grain was determined according to Official Methods of Analysis (AOAC, 1995). This grain was previously treated with the fungicide chlorothalonil, to prevent fungus growth during storage.

2.2. Grain storage

Corn storage conditions (high temperature and relative humidity) were chosen, taking into account the different areas found around the country (Moreno, 1984). These conditions were experimentally simulated with incubators (Ambi-Hi-Lo Chamber ± 0.1 °C precision), adjusted to temperatures of 40, and 20 °C, and moisture contents in the grain of 18, 15 and 10%. To reach the different moisture contents, the grain was stored in glass chambers containing saturated salt solutions of potassium chloride (for 18%) and sodium chloride (for 15%) (Rockland, 1960). The original grain, with a moisture content of 10%, was stored at 4 °C, and was used as control. All grain samples were stored up to 180 days.

2.3. Proteins

Protein fractions were obtained from 100 g of defatted ground corn, following the separation techniques reported by Paulis (1982). By means of this procedure, albumin, globulin, prolamin and glutelin fractions were obtained, dialyzed, and lyophilized. The content of proteins of the fractions was evaluated and found to be 98–99%, by the Kjeldahl method (AOAC, 979.09, 1995).

2.4. Conditions of the analysis in differential scanning calorimetry

Thermal denaturations of protein fractions were evaluated with a Perkin-Elmer DSC equipped with TAS 7 software package (The Perkin-Elmer Corporation, Oak Brook, IL), using indium as the standard. Conditions to detect transition changes due mainly to denaturation, were established in prior studies. Trial runs of these, at different ratios of dry matter–water content showed that albumins and globulins require a 30% moisture content while 70% is needed for prolamins and glutelins. The protein fractions were hydrated with deionized water 24 h prior to the calorimetric analysis. Heating rate was 10 °C/min, temperature range was 30–150 °C. Samples of 5 mg of each corresponding fraction in stainless steel capsules were used in all cases. All samples were analysed in triplicate.

2.5. Statistics

A variance analysis was applied for the interpretation of thermal results and subsequent comparative analysis of multiple ranges.

3. Results

Tables 1 through 6 show the enthalpy changes in protein fractions when corn samples are subjected to changes in temperature and humidity. To accurately evaluate enthalpy changes from protein denaturation in the whole grain, the proteins had to be individually isolated because the endothermic transitions from starch gelatinization would interfere. This problem has also been documented by Murray et al. (1985). Calorimetric indicators in the control corn did not change during the storage period under study, which assured that the changes in the protein fractions, under different temperature and humidity conditions, are due to their effect on the grain. Enthalpy changes observed in Figs. 1 through 4 may be associated with molecular changes as a result of protein unfolding. The changes are due to a combination of endothermic reactions, such as disruption of hydrogen bonds and exothermic reactions, such as disruption of hydrophobic interactions.

The thermograms showed that temperature of denaturation is the main difference between the protein fractions. Consequently, the albumin and globulin fractions, which are mainly polar, possess lower denaturation temperatures (62–67 °C) in contrast to less polar types, such as prolamin and glutelin, which reached denaturation temperatures in the range 80–92 °C. Myers (1990) suggested that high denaturation temperatures (T_d) are expected for proteins with a high proportion of hydrophobic residues.

The research established that albumin and globulin of stored corn at 40 and 20 °C with 18, 15 and 10% moisture (Tables 1–3) presented a very significant change in enthalpy values in relation to those obtained

Table 1
Enthalpy changes in proteins of corn stored at two temperatures with 18% moisture content

Days[a]	ΔH (J/g)							
	40 °C				20 °C			
	Albumin	Globulin	Prolamin	Glutelin	Albumin	Globulin	Prolamin	Glutelin
0	15±0.1[b]	10.8±0.7	14.2±0.3	13.1±0.4	15±0.1[b]	10.8±0.7	14.2±0.3	13.1±0.4
10	3.4±0.2	3.6±0.1	7.9±0.5	8.5±0.4	–	–	–	–
20	3.0±0.03	2.9±0.1	6.3±0.1	7.3±0.3	3.3±0.9	3.6±0.1	7.8±0.7	9.9±0.3
30	2.4±0.1	2.5±0.2	5.7±0.1	6.1±0.1	–	–	–	–
40			3.7±0.1	4.6±0.1	2.5±0.1	2.9±0.1	4.5±0.2	8.1±0.2
50			2.1±0.2	2.8±0.1	–	–	–	–
60					1.3±0.1	1.1±0.1	4.0±0.1	7.6±0.1
80							3.0±0.1	4.3±0.3
100							2.7±0.1	2.2±0.3
120							1.3±0.1	1.6±0.1

[a] Storage days.
[b] Values are mean±standard deviation.

Table 2
Enthalpy changes in proteins of corn stored at two temperatures with 15% moisture content

Days[a]	ΔH (J/g)							
	40 °C				20 °C			
	Albumin	Globulin	Prolamin	Glutelin	Albumin	Globulin	Prolamin	Glutelin
	15±0.1[b]	10.8±0.7	14.2±0.3	13.1±0.4	15±0.1[b]	10.8±0.7	14.2±0.3	13.1±0.4
15	3.7±0.2	3.8±0.1	8.7±0.1	8.7±0.2	–	–	–	–
30	2.8±0.1	2.9±0.1	6.3±0.2	7.3±0.0	5.8±0.2	3.9±0.4	6.7±0.5	10.9±0.3
45	1.7±0.1	1.0±0.1	5.2±0.2	6.5±0.1	–	–	–	–
60			4.7±0.0	4.8±0.1	2.4±0.1	3.0±0.1	4.8±0.1	8.2±0.4
75			3.5±0.1	3.3±0.1	–	–	–	–
90				1.9±0.1	1.1±0.1	1.9±0.1	4.7±0.1	4.6±0.2
120							3.9±0.1	3.7±0.1
150							3.1±0.1	3.5±0.1
180							3.0±0.1	2.9±0.1

[a] Storage days.
[b] Values are mean±standard deviation.

Table 3
Enthalpy changes in proteins of corn stored at two temperatures with 10% moisture content

Days[a]	ΔH (J/g)							
	40 °C				20 °C			
	Albumin	Globulin	Prolamin	Glutelin	Albumin	Globulin	Prolamin	Glutelin
0	15±0.1[b]	10.8±0.7	14.2±0.3	13.1±0.4	15±0.1[b]	10.8±0.7	14.2±0.3	13.1±0.4
20	3.9±0.3	4.1±0.1	10.5±0.3	10.9±0.3	–	–	–	–
40	2.9±0.2	3.0±0.1	6.9±0.2	9.5±0.1	–	–	–	–
60	2.4±0.1	2.7±0.1	4.6±0.1	6.9±0.1	6.4±0.1	7.1±0.1	12.6±0.6	11.4±0.1
80			4.1±0.1	5.0±0.1	–	–	–	–
100			3.0±0.1	3.5±01	–	–	–	–
120			2.6±0.1	2.6±0.1	5.8±0.1	6.4±0.1	10.3±0.2	10.8±0.1
180					4.8±0.1	5.8±0.1	9.1±0.1	10.7±0.1

[a] Storage days.
[b] Values are mean±standard deviation.

Table 4
Changes in degree of symmetry ($T^{1/2}$) in proteins of corn stored at two temperatures with 18% moisture content

Days[a]	$T^{1/2}$ (°C)							
	40 °C				20 °C			
	Albumin	Globulin	Prolamin	Glutelin	Albumin	Globulin	Prolamin	Glutelin
0	3.1±0.1[b]	1.5±0.1	4.5±0.1	2.5±0.1	3.1±0.1[b]	1.5±0.1	4.5±0.1	2.5±0.1
10	4.9±0.1	5.0±0.1	4.7±0.1	4.5±0.1	–	–	–	–
20	4.6±0.1	6.5±0.1	5.2±0.1	3.5±0.4	3.0±0.5	5.3±0.1	2.6±0.8	4.3±0.1
30	6.0±0.1	6.8±0.1	5.0±0.1	1.0±0.5	–	–	–	–
40			6.0±0.1	0.9±0.1	6.1±0.1	6.0±0.1	5.0±0.1	3.6±0.6
50			7.2±0.1	5.0±0.0	–	–	–	–
60					5.5±0.7	7.9±0.1	5.7±0.1	5.5±0.1
80							5.8±0.1	6.5±0.1
100							5.5±0.6	7.0±0.4
120							5.8±0.1	5.0±0.8

[a] Storage days.
[b] Values are mean±standard deviation.

Table 5
Changes in degree of symmetry ($T^{1/2}$) in proteins of corn stored at two temperatures with 15% moisture content

Days[a]	$T^{1/2}$ (°C)							
	40 °C				20 °C			
	Albumin	Globulin	Prolamin	Glutelin	Albumin	Globulin	Prolamin	Glutelin
0	3.1±0.8[b]	1.5±0.2	4.5±0.8	2.5±0.1	3.1±0.8[b]	1.5±0.2	4.5±0.8	2.5±0.1
15	5.1±0.1	6.5±0.2	0.8±0.5	4.9±0.2	–	–	–	–
30	5.1±0.5	6.5±0.8	0.85±0.7	4.8±0.1	3.0±0.5	2.0±0.9	4.5±0.8	3.5±0.8
45	4.2±0.8	6.6±0.2	0.45±0.9	6.0±0.8	–	–	–	–
60			5.9±0.1	7.0±0.5	5.5±0.0	6.5±0.1	4.0±0.1	9.4±0.1
75			6.0±0.3	6.0±0.4	–	–	–	–
90				8.5±0.0	5.5±0.8	4.0±0.5	5.9±0.1	5.5±0.3
120							4.0±0.3	5.8±0.7
150							4.0±0.8	7.0±0.5
180							3.8±0.3	6.0±0.7

[a] Storage days.
[b] Values are mean±standard deviation.

Table 6
Changes in degree of symmetry ($T^{1/2}$) in proteins of corn stored at two temperatures with 10% moisture content

Days[a]	$T^{1/2}$ (°C)							
	40 °C				20 °C			
	Albumin	Globulin	Prolamin	Glutelin	Albumin	Globulin	Prolamin	Glutelin
0	3.1±0.8[b]	1.5±0.2	4.5±0.8	2.5±0.1	3.1±0.8[b]	1.5±0.2	4.5±0.8	2.5±0.1
20	6.0±0.2	6.8±0.3	4.5±0.1	6.2±0.2	–	–	–	–
40	5.7±0.5	6.6±0.1	5.7±0.2	6.0±0.2	–	–	–	–
60	4.0±0.7	6.3±0.2	5.0±0.5	7.0±0.1	1.3±0.7	3.0±0.5	4.0±0.5	2.5±0.3
80			5.5±0.4	6.2±0.1	–	–	–	–
100			6.8±0.1	6.5±0.0	–	–	–	–
120			6.0±0.7	7.8±0.4	2.6±0.3	2.4±0.1	4.6±0.6	0.7±0.8
180					1.5±0.8	2.0±0.5	5.1±0.0	0.6±0.9
150							4.0±0.8	7.0±0.5
180							3.8±0.3	6.0±0.7

[a] Storage days.
[b] Values are mean±standard deviation.

in the control corn. In all moisture contents considered for these fractions, ΔH decreased by approximately 80% at 40 °C, within 30 days of storage. These results show a clear effect of the temperature over the fractions. Thus when subjected to a temperature high enough, 40 °C, the enthalpy changes of the fractions are independent from the moisture contents. In contrast when corn was at 20 °C the moisture contents played an important role in the decrease of ΔH in the fractions.

The thermograms show a loss of definition in their peaks and in the degree of symmetry with enthalpy decrease (Figs. 1 and 2). At 40 °C both albumin and

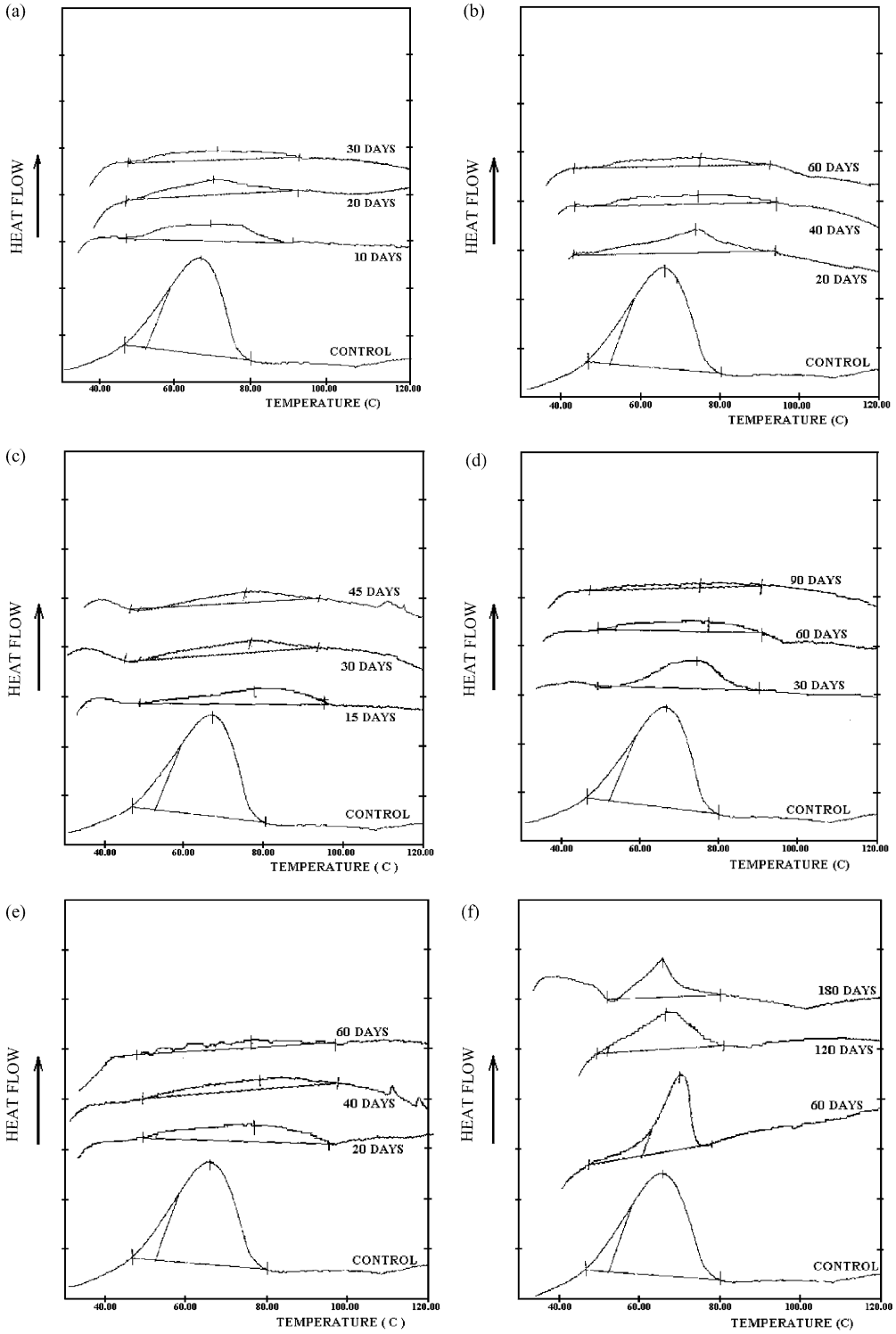

Fig. 1. Thermograms of albumins: (a) 40 °C and 18% moisture, (b) 20 °C and 18% moisture, (c) 40 °C and 15% moisture, (d) 20 °C and 15% moisture, (e) 40 °C and 10% moisture, (f) 20 °C and 10% moisture.

globulin show a significant loss in their thermogram profiles in the first days regardless of the moisture content. At 20 °C with 10% moisture, the changes in the thermogram profiles for the same proteins are not as severe as those observed at 40 °C. Likewise, the solubility, measured as the amount of extracted protein, decreased up to 80% within 60 days.

In the same manner, at a temperature of 40 °C and at the three moisture content levels, the grain undergoes changes in its physical attributes. This was especially

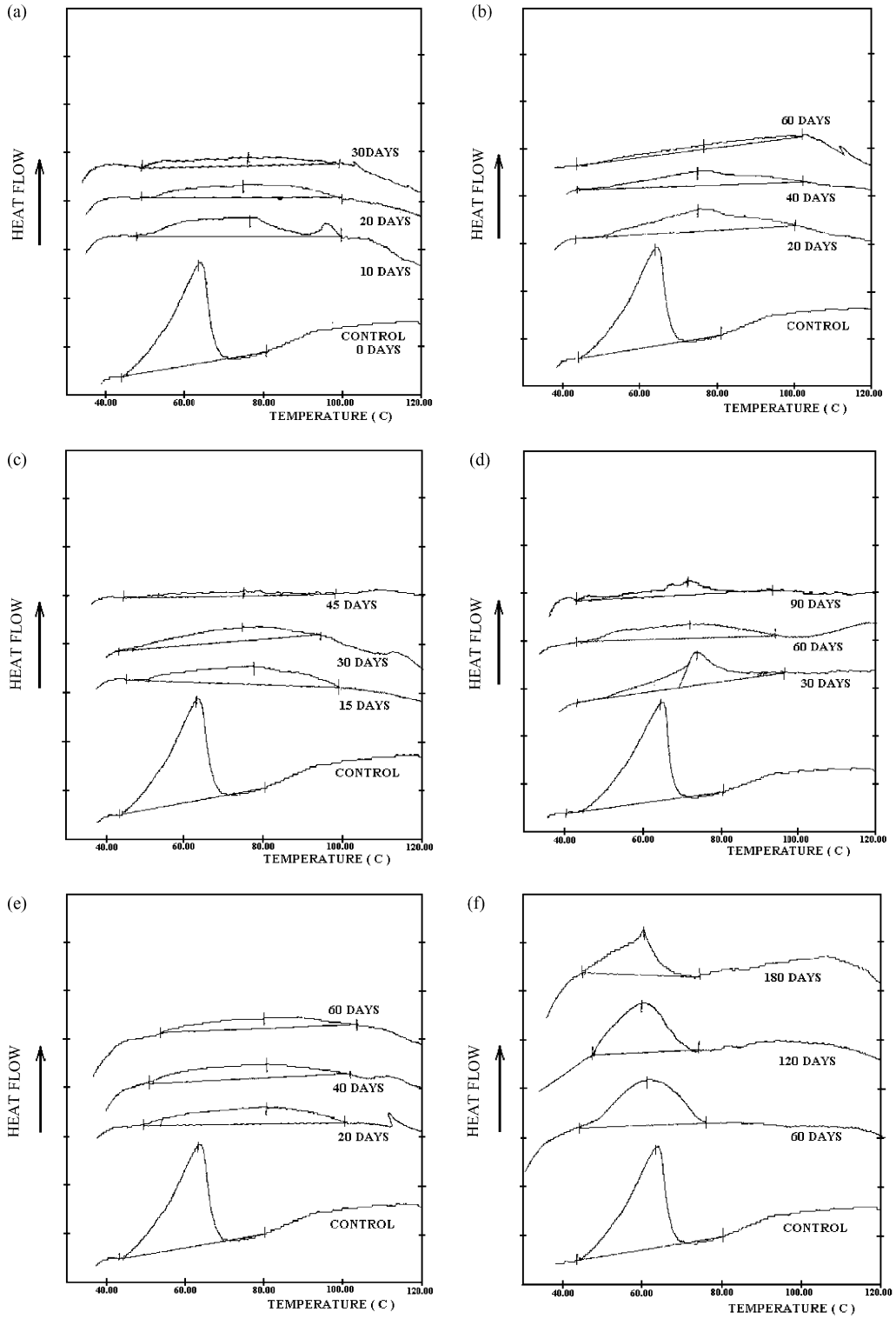

Fig. 2. Thermograms of globulins: (a) 40 °C and 18% moisture, (b) 20 °C and 18% moisture, (c) 40 °C and 15% moisture, (d) 20 °C and 15% moisture, (e) 40 °C and 10% moisture, (f) 20 °C and 10% moisture.

evident in the browning of the germ, probably due to non-enzymatic reactions (Maillard) which may presuppose seed viability loss.

All the proteins, particularly the polar ones, suffered serious thermal changes under all the different storage conditions.

In these experiments $T^{1/2}$ was used as an indicator related to the cooperative unfolding of the protein and may be measured from the thermal profile (Wright, 1982). This helps to understand transitional forms and the degree of protein organization. For low values of $T^{1/2}$, the transition is considered highly cooperative and it appears

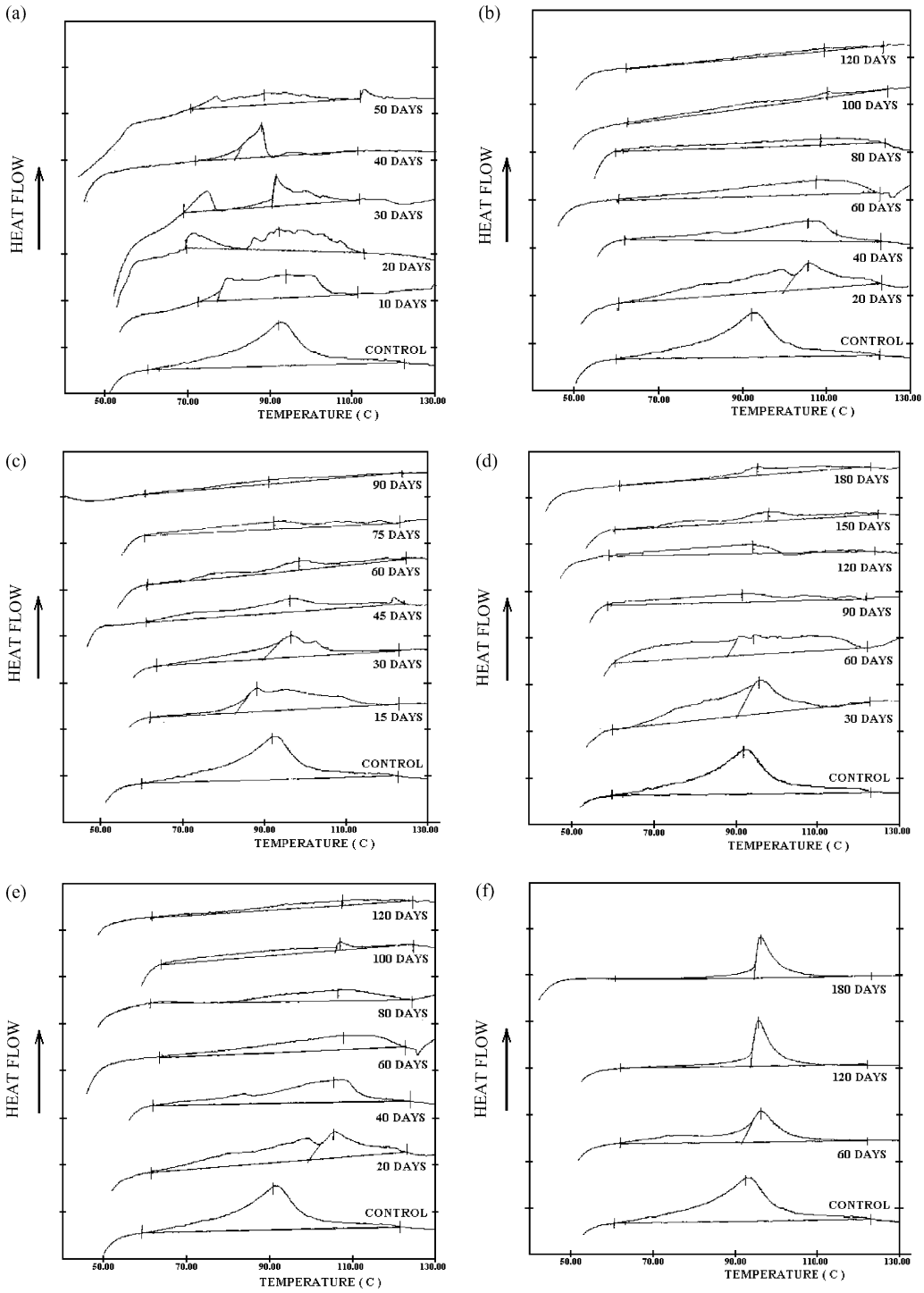

Fig. 3. Thermograms of prolamins: (a) 40 °C and 18% moisture, (b) 20 °C and 18% moisture, (c) 40 °C and 15% moisture, (d) 20 °C and 15% moisture, (e) 40 °C and 10% moisture, (f) 20 °C and 10% moisture.

spontaneously when the molecular interactions break down in "cascade" initiating with Van der Walls forces and finally reaching covalent bonds (Lii-Cy, 1993).

The values of $T^{1/2}$ in the control remained constant and coincide with those recorded on day 0 on each table. Indeed these values are lower than those in the rest of the samples (Tables 4–6). This may be indicative of a progressive loss of cooperativity as storage advances, requiring less energy for complete molecule unfolding. These changes reflect modifications in denaturation mechanisms.

For prolamin and glutelin fractions, thermal curves reveal changes in form and area (Figs. 3 and 4). The decrease in heat content necessary to carry out the transition, suggests that proteins suffer conformational changes as a consequence of storage conditions. This

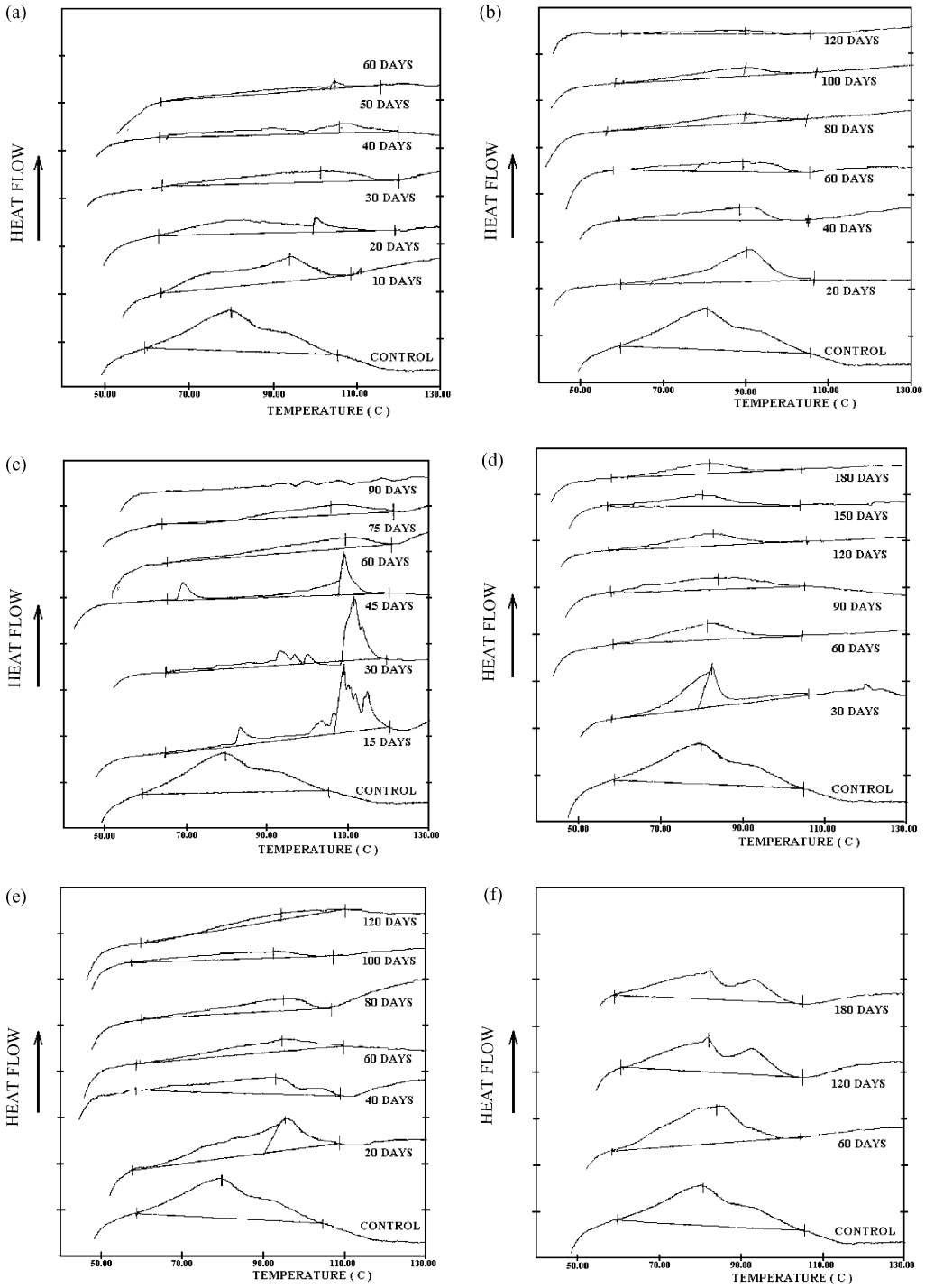

Fig. 4. Thermograms of glutelins: (a) 40 °C and 18% moisture, (b) 20 °C and 18% moisture, (c) 40 °C and 15% moisture, (d) 20 °C and 15% moisture, (e) 40 °C and 10% moisture, (f) 20 °C and 10% moisture.

effect may be due to the presence of dominion with different structural stability.

Heat content for prolamins changes significantly from 14.2 J/g to 2.1 in 50 days at 18% moisture (Table 1). No transition was observed on day 60. Enthalpy changes for a 15% moisture content on day 60 suffered a similarly significant decrease. No transition was observed after 75 days. The behaviour of enthalpy change at 10% moisture content is not that different from that previously mentioned although, in this case, the transition can be observed until day 120. In contrast at 20 °C, the decrease in ΔH was less pronounced than that observed at 40 °C for the three moisture contents, as was to be expected. This shows that the decrease in ΔH at 20 °C is related to moisture content. Storage at 40 °C, even under low moisture conditions (10%), caused significant changes in thermal profiles, indicating that proteins reflected higher sensitivity to temperature than to humidity. This higher sensitivity can also be observed in the values of $T^{1/2}$ (Tables 4–6) which for every moisture content showed higher loss of cooperativity at 40 °C. For example, with 10% moisture content at 40 °C the $T^{1/2}$ values for prolamins increase from 4.5 to 6.0 in 120 days. While, at 20 °C for the same moisture content, the $T^{1/2}$ values remain constant for the period.

Enthalpy changes in glutelin show similar behaviour to that of prolamin. At 18% moisture content and at 40 °C, a significant decrease from 13.1 to 4.6 J/g is observed after 40 days of storage (Table 1). The lower ΔH values of glutelin indicated partial unfolding. No transition was present after day 50, which indicated a complete unfolding. The value of ΔH decreases to 1.9 J/g after 90 days of storage for a 15% moisture content (Table 2). A more moderate decrease can be observed at 10% moisture and a value of 2.6 J/g is registered on day 120 (Table 3).

Decrease of ΔH is possibly due to an increase in the stability of hydrophobic interactions, which have an exothermic contribution. This effect may also be due to a close packing produced inside the protein, resulting in enthalpy reduction (Boye et al., 1997). Denaturation temperature, for these fractions, increased up to 32%, indicating that a significant molecular organization took place. It is important to consider that sub-units of beta, gamma and delta prolamin, as well as glutelin, contain sulfur aminoacids, which may be oxidized, thereby increasing disulfide bonds. This could explain the formation of protein aggregates by the generation of cross links, which may promote a decrease in their extractability.

4. Conclusions

According to calorimetric results, the most susceptible fractions are those of a polar nature. An enthalpy decrease of approximately 80%, compared with the control, occurs for all moisture contents and for both temperatures. Temperature effect becomes evident when analysing $T^{1/2}$. A loss in cooperativity is reached in shorter storage periods for polar fractions than for prolamin and glutelin.

Therefore, it can be established that the temperature effect on corn protein fractions has a more significant influence than moisture content. At 40 °C, for the three moisture contents, the thermal profiles show that enthalpy changes are correlated negatively with the storage time. Even under temperatures of 20 °C and 10% grain moisture content (conditions which prevent fungi development and insect infestation), a significant decrease of enthalpy changes was observed in a term of no longer than 60 days.

It is noteworthy that enthalpy values of isolated fractions obtained in this study were replicates and working with these fractions prevents the influence of transitions such as starch gelatinization, present in significant amounts in corn.

Considering the great number of industrial applications of corn proteins, as well as the nutritional importance of corn grain in preparing processed corn flour and tortillas, it is important to evaluate the correlation between the changes here presented and the eventual loss in nutritional and functional properties. It is also interesting to note that the results demonstrate that thermal profiles of protein fractions may provide information about the prevailing conditions during the storage of corn grain. Furthermore, this study can shed light on the conditions that alter proteins and that subsequently affect quality.

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