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Differential Scanning Calorimetry in Food Research—A Review*

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ABSTRACT

Differential scanning calorimetry (DSC) has gained remarkable popularity in thermal studies of foods and their components following the development of instrumentation of sufficient sensitivity. DSC is rapid, facile and capable of supplying both thermodynamic (heat capacity, enthalpy and entropy) and kinetic data (reaction rate and activation energy) on protein denaturation. Calorimetric studies have also provided a better insight into the order–disorder transition processes of granular starch and other gelling polysaccharides. DSC can be used to characterise mixtures of polymorphic forms of fats as well as to evaluate hydrogenation and various tempering regimes for their effectiveness in bringing about desired polymorphic changes. Calorimetry has also been employed to examine the physical state and properties of water in foodstuffs.

INTRODUCTION

Differential scanning calorimetry (DSC) is a thermoanalytical technique for monitoring changes in physical or chemical properties of materials as a function of temperature by detecting the heat changes associated with such processes. In DSC, the measuring principle is to compare the rate of heat flow to the sample and to an inert material which are heated or cooled at the same rate. Changes in the sample that are associated with

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absorption or evolution of heat cause a change in the differential heat flow which is then recorded as a peak. The area under the peak is directly proportional to the enthalpic change and its direction indicates whether the thermal event is endothermic or exothermic. The method is only one of a family of related techniques, the principal of which are DTA (differential thermal analysis), TG (thermogravimetry) and DSC. Several textbooks (Porter & Johnson, 1968, 1970, 1974; Mackenzie, 1970, 1972; Weidemann, 1972) and reviews (Ladbrooke & Chapman, 1969; Sturtevant, 1972; Gill, 1974; Privalov, 1974; Brennan, 1976) covering the fundamental concepts, instrumentation and general applications of calorimetry exist.

Improvements in the sensitivity of commercially available calorimeters during the last 15 years have made the DSC a popular tool for investigating the thermodynamic properties of food components. The aim of this review is to summarise some of the main results obtained to date in thermal studies of food systems. The discussion is by no means exhaustive, but rather attempts to highlight the applications of DSC in studies of heat-induced transformations and interactions among food components as well as its potential use in product development and quality control.

PROTEINS

Classical calorimetry of protein denaturation

Heat denaturation of small globular proteins is generally considered reversible in high yield, provided that the reaction is carried out under conditions preventing aggregation, i.e. dilute solution and far from the isoelectric point. This allows indirect thermodynamic evaluation of the process by applying equilibrium thermodynamics and assuming a two-state model, i.e. A (native) $\rightarrow B$ (denatured). Under these conditions and using any property sensitive to the state of protein, one can determine the equilibrium constant, K, of the process and subsequently the standard enthalpy change, ΔH°, from the van't Hoff equation:

$$\Delta H^\circ = RT^2 \frac{\mathrm{d} \ln \mathrm{K}}{\mathrm{d}T} \tag{1}$$

The standard free energy change, ΔG°, and the standard entropy change, ΔS°, may be then obtained from eqns (2) and (3), respectively:

$$\Delta G^\circ = -RT \ln K \tag{2}$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \tag{3}$$

Numerical estimates of the above thermodynamic parameters are of considerable significance in understanding the molecular aspects of the denaturation reaction (Tanford, 1968).

A number of studies on protein denaturation have been made employing such indirect methods (Scott & Scheraga, 1963; Brandts, 1964; Hermans & Acampora, 1967) without, however, any means of assessing the validity of the assumption that denaturation can be presented as a two-state transition. Only recent advances in the sensitivity of instrumentation have allowed direct calorimetric studies (Jackson & Brandts, 1970; Tsong *et al.*, 1970; Biltonen *et al.*, 1971; Privalov, 1974) and made possible a decision concerning the correctness of the two-state concept by comparing ΔH_{cal} with the ΔH° obtained from equilibrium studies. The equivalence of the enthalpies would be strong evidence in favour of the two-state, 'all-or-none' character of the process. In this context, calorimetry has the unique advantage of providing not only the calorimetric enthalpy, ΔH_{cal} (from the area of the heat absorption), but also the effective enthalpy of the process (from the sharpness of the transition and using the van't Hoff equation) (Jackson & Brandts, 1970; Privalov & Khechinashvili, 1974).

Privalov & Khechinashvili (1974) have studied the thermal properties of five small compact globular proteins by DSC in very dilute solutions (0·05–0·5%) under slow heating rates (1 °C/min). The temperature dependence of the denaturation enthalpy, ΔH, was determined from changes in ΔH at different conformational stabilities of the proteins induced by changes in the pH and assuming minor changes in the ionisation enthalpies. The thermodynamic data indicated that alteration in the pH itself had indeed no effect on the specific heat capacity or enthalpy of denaturation which validated the adopted experimental approach. The main findings of this study are summarised below.

(1) The initial specific heat capacities, C_p, of these proteins were very similar. The typical dependence of C_p on temperature at different pHs is illustrated in Fig. 1. With increase in temperature, C_p increases linearly up to the temperature at which denaturation heat absorption starts.

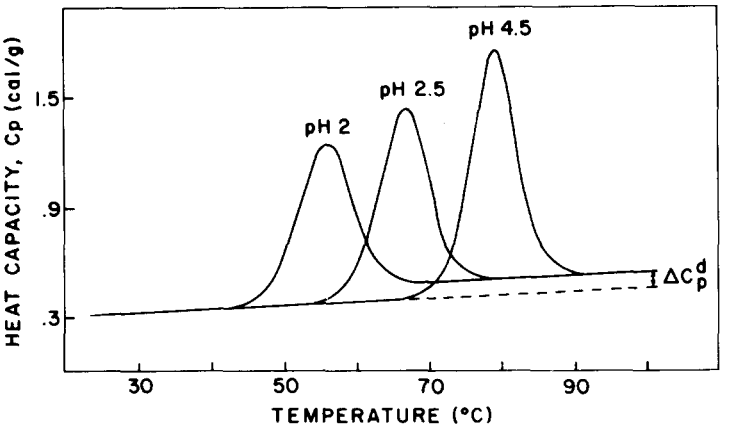

Fig. 1. Temperature dependence of partial specific heats of lysozyme at different pH values. (Adapted from Privalov, 1974.)

(2) Denaturational changes in specific heat capacities, ΔC_p^d (Fig. 1), do not vary with temperature, but the value is different for each protein.

(3) Specific denaturation enthalpy, Δh, increases linearly with temperature (Fig. 2) and the slope of the line is equal to ΔC_p. Similar trends are also seen in the published data of Brandts (1964) and Jackson & Brandts (1970). The plots of Δh versus temperature are convergent and all intersect at 110 °C. At this temperature hydrophobic interaction becomes negligible so that the Δh at 110 °C corresponds to the enthalpy of disruption of hydrogen-bonding only. Analysis of all the thermodynamic functions (ΔH, ΔS and ΔG) led to the conclusion (Privalov, 1974, 1979) that the denaturation enthalpy for small globular proteins represents the composite result of two main effects: (a) The negative heat associated with the disruption of hydrophobic bonds, i.e. the negative heat (heat evolved) of ordering water in 'clathrate' structures around exposed non-polar groups (Brandts & Hunt, 1967). This negative contribution increases with a decrease in temperature. (b) The positive contribution of disruption of hydrogen bonds which depends little on temperature.

(4) The deviation of the ratio $\Delta H_{cal}/\Delta H_{eff}$ from unity was slight ($1{\cdot}05 \pm 0{\cdot}03$) and, therefore, the denaturation can, in fact, be regarded as a co-operative transition between two states. The same conclusion was also reached by Jackson & Brandts (1970) and Biltonen *et al.* (1971) who reported good agreement between van't Hoff and direct calorimetric estimates of ΔH for chymotrypsinogen denaturation.

The kinetic parameters for denaturation of proteins can be also

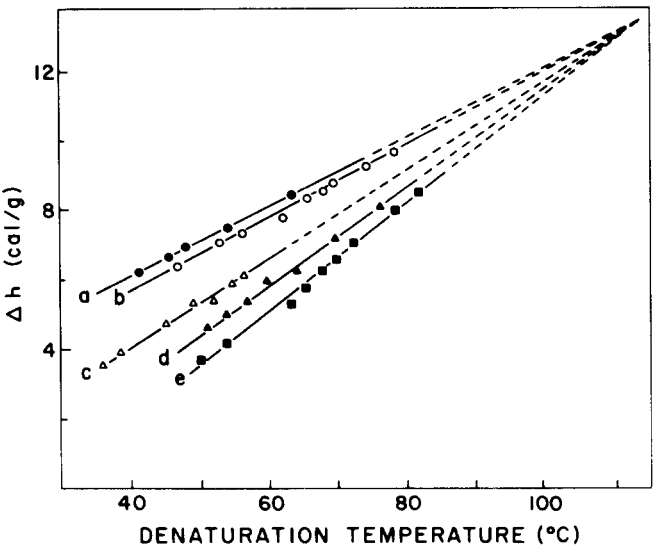

Fig. 2. Temperature dependence of the specific enthalpy of denaturation. (a) Ribonuclease A. (b) Lysozyme. (c) α-Chymotrypsin. (d) Cytochrome c. (e) Metmyoglobin. (Adapted from Privalov & Khechinashvili, 1974.)

determined from DSC curves. The vertical displacement from the base line, at any temperature, is proportional to the rate of heat flow into the sample, dH/dT, and consequently is a measure of the reaction rate. Several methods have been developed to determine the rate constants (Borchardt & Daniels, 1957; Kissinger, 1957; Beech, 1969). The activation energy may then be calculated from the Arrhenius plot of ln K versus $1/T$.

In practical DSC work both protein concentration (5–20%) and heating rates (5–20 °C/min) are quite high in order to resemble actual processing conditions. Under these conditions, however, denaturation becomes an irreversible process since extensive intermolecular interactions are favoured and aggregation of the unfolded protein molecules immediately proceeds. In contrast to denaturation, which is connected with intensive heat absorption, aggregation is generally considered as an exothermic process. Therefore, it becomes more difficult to interpret ΔH_{cal} values quantitatively, since they represent the net product of a positive (denaturation) and a negative (aggregation) contributor. In addition to protein concentration, other parameters such as pH, ionic strength and tertiary and quaternary structure of the protein can also

affect the observed ΔH_{cal} values by their influence on protein conformational stability (Hermansson, 1978, 1979*a*; Privalov, 1979). Nevertheless, since the energies involved in aggregation are low (Donovan & Ross, 1973), it is still possible to interpret the calorimetric data of protein denaturation using the above-described concepts of classical calorimetry.

Food proteins

In attempting to relate the quality properties of cooked meat with the denaturation of the contractible myofibrillar proteins (actin and myosin), many investigators have studied the thermostability of isolated proteins and extrapolated their findings to the whole muscle (Hamm, 1977). There is an inherent limitation, however, associated with such an experimental approach. The behaviour of the proteins might not be the same in the intact tissue and in the isolated preparations. On the other hand, a technique such as DSC has an obvious advantage because it is capable of studying the thermal properties of meat proteins in their natural state. Quinn *et al.* (1980) have used DSC to monitor the changes in heat stability of beef proteins during processing of meat into sausage batter. The thermogram of ground beef muscle showed three endotherms at 60 °C, 66 °C and 83 °C. Assignment of these transitions to the denaturation of myosin, sarcoplasmic proteins and actin was based on the calorimetric studies of Wright *et al.* (1977) on purified muscle proteins. The thermoprofile of a typical Wiener batter, however, revealed only a single transition at 72 °C, indicative of decreasing heat stability of the proteins. When they further investigated the effect of various processing treatments on the thermoprofile of the proteins, they found that salt, at concentrations used in processed meats, is responsible for such changes in the thermal stability. The effect of heat treatment at various water contents on myoglobin was reported by Hägerdal & Martens (1976). At water contents below 30% the transition temperature increased and a linear relationship was found between ΔH and water content which suggested that only part of the protein underwent denaturation. This interpretation was also consistent with the trend in solubility of the heat-treated samples.

Donovan *et al.* (1975) have studied the heat denaturation of egg white and its component proteins by DSC. The denaturation endotherms of the individual proteins indicated that the thermostability was of the order ovalbumin > lysozyme > conalbumin. Lowering the pH from 9·0 to 7·0 or adding Al^{3+} to the egg white increased the denaturation temperature of

conalbumin by 4 and 12 °C, respectively. Furthermore, addition of sucrose (conc. 10%) increased the stability of all three proteins (endotherms were shifted 2 °C to higher temperatures), presumably due to the suppressive effect of this solute on water activity. These findings have a direct bearing in establishing appropriate processing conditions for stabilising egg white proteins during pasteurisation. The irreversible transformation of the ovalbumin into its more heat-stable form, *S*-ovalbumin, was further investigated (Donovan & Mapes, 1976). Formation of *S*-ovalbumin during storage of eggs at elevated temperatures is responsible for the poor performance of egg whites in angel cake formulations. Since the denaturation temperature of *S*-ovalbumin is 8 °C greater than that of ovalbumin, the relative amounts of these proteins are readily determined from a DSC thermogram and consequently the quality of stored or processed egg white can be evaluated.

DSC thermograms of 10% whey protein dispersions showed a low temperature small endotherm (α-lactalbumin) at pH ≥ 4·0 and a high temperature large endotherm (β-lactoglobulin) at pH 2–9 (Hermansson, 1979*a*). When the calorimetric experiments were carried out in the presence of 0·2M NaCl, only slight increases in the denaturation temperatures were seen which implies that salt has little influence in the maintenance of conformational stability of whey proteins. The thermal behaviour of β-lactoglobulin in aqueous solutions was also examined by De Wit & Swinkels (1980) using DSC. These workers concluded that heat denaturation of β-lactoglobulin is of the first order in the range 65–72 °C and that, above this temperature range, the denaturation kinetics change.

In addition to nutritional quality, the functional characterisation of vegetable proteins is very important in evaluating the performance of a protein ingredient in a food system. Protein functionality is not only determined by the physico-chemical properties of the protein raw material, but also by the processing conditions during protein isolation, as well as by interactions with other non-protein components. Processing conditions (temperature, pH, ionic strength, water content) play a dominant rôle in functionality because of their impact on the conformational state (i.e. degree of denaturation) of protein isolates. Accordingly, DSC was used to determine the amount of native protein in soy (Hermansson, 1979*b*; Armstrong *et al.*, 1979) and faba bean (Murray *et al.*, 1981) protein isolates. These studies showed that the transition enthalpy changes can be interpreted as a measure of the proportion of protein that has not been denatured during the preparation of the isolate.

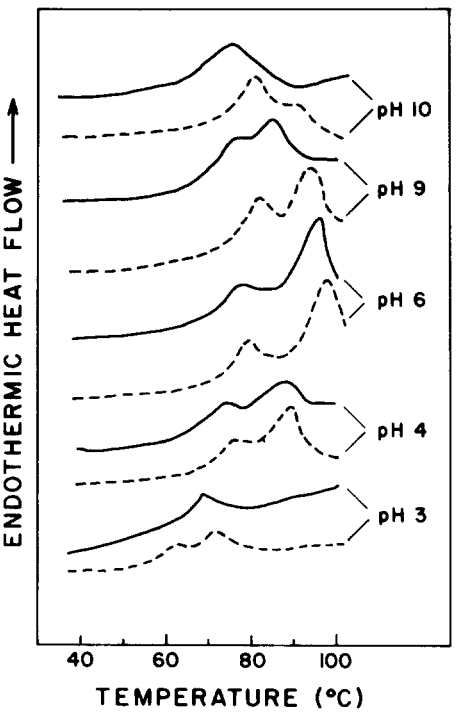

Fig. 3. DSC thermograms of 10% soy protein dispersions in distilled water (——) or 0·2M NaCl (– – –) at pH 3–10. (Adapted from Hermansson, 1978.)

Thus, if a protein isolate is already partly denatured, the ΔH will decrease, and if it is completely denatured no endothermic transition will appear. In view of the detrimental effect of heat treatment on protein solubility (Kinsella, 1979), the DSC tracing appears more useful as a quick test to determine the severity of processing conditions and the solubility characteristics of a protein isolate than measurement of the nitrogen solubility index (NSI).

The heat denaturation of soy proteins was studied by DSC in concentrated dispersions (10%), where interactions between protein molecules are favoured and protein gelation occurs (Hermansson, 1978). Two peaks were observed at pHs between 4 and 9 (Fig. 3), corresponding to the denaturation of 7S (low temperature endotherm) and 11S (high temperature endotherm) globulin. As expected, greater thermostability was seen in the isoelectric region (pH 4–5) where the net charge is low. As one moves away from this pH region the transition temperature decreases considerably. Furthermore, although no actual ΔH values were reported,

the peak areas also seem to decrease outside the isoelectric region. The above trends in the thermal patterns are similar to those reported by Privalov & Khechinashvili (1974) for small globular proteins heated at low protein concentrations. It appears, therefore, that aggregation phenomena do not cause any qualitative changes in the thermodynamics of the denaturation reaction. The stabilising role of salt in maintaining the tertiary and quaternary structure of soy proteins is also evident from the increase in the transition temperatures, particularly at any given pH value outside the isoelectric region (Fig. 3).

Protein interactions

Various physico-chemical methods, such as gel filtration, affinity chromatography, fluorescence polarisation and ultracentrifugation, have been employed in studies of protein interactions. DSC is also capable of detecting an interacting system if there is a significant change in the thermal properties of the species involved. Donovan and his co-workers conducted a series of investigations on the association complexes of several proteins with small molecules or other proteins (Donovan & Ross, 1973; Donovan & Ross, 1975*a*, *b*; Donovan & Beardslee, 1975). Table 1 lists the temperatures, enthalpies and activation energies for denaturation of the interacting species and their complexes. The thermal stability of avidin (a minor protein of egg albumen), as seen by both transition temperature and denaturation enthalpy, increased markedly when biotin was bound to this protein. Donovan & Ross (1973) suggested that in addition to the enthalpy of binding, large heat capacity differences between native and denatured avidin (with or without bound biotin) may substantially contribute to this remarkable increase in ΔH. Similarly, association of trypsin with either trypsin inhibitor or ovomucoid gave thermograms in which the characteristic peaks of the interacting species were no longer present, being replaced by a new higher temperature peak of the complex. Kinetic treatment of the denaturation data suggested that thermal denaturation of protein–protein complexes is not rate-limited by their dissociation (Donovan & Beardslee, 1975). Interestingly, calorimetry on the ovalbumin–lysozyme system, an interaction complex that is readily detected by fluorescence polarisation (Nakai & Kason, 1974) and sedimentation equilibrium (Howlett & Nichol, 1973), failed to demonstrate that such association takes place. Only two peaks were observed at temperatures characteristic of the components involved. This

TABLE 1

Denaturation Temperatures (Temperature of Peak Maximum, T_d), Transition Enthalpies (ΔH_d) and Activation Energies (E_a) of Proteins and Protein Complexes

Protein or complex	T_d (°C)	ΔH_d (*kcal/mol*)	E_a (*kcal/mol*)
Avidin[a]	85	298 ± 10	90
β-Trypsin[b]	72	194 ± 10	63
Soybean trypsin inhibitor (STI)[b]	76	110 ± 5	69
Ovomucoid[b]	79	152 ± 6	74
Conalbumin[c]	63	320[d]	—
Avidin–biotin complex[a]	131	1 065 ± 30	—
β-Trypsin–STI complex[b]	88	313 ± 13	67
β-Trypsin–ovomucoid complex[b]	81	350 ± 14	77
Diferric-conalbumin[c]	83	630[d]	—
Aluminium-conalbumin[c]	72	400[d]	—
Dicupric-conalbumin[c]	68	330[d]	—

[a] Heating rate 12·5 °C/min, pH 6·84. Reported transition temperatures are those obtained by extrapolation to a heating rate of 0 °C/min (Donovan &|Ross, 1973).
[b] Heating rate 10·0 °C/min, pH 6·7 (Donovan & Beardslee, 1975).
[c] Heating rate 10·0 °C/min, pH 7·5 or 8·3 (Donovan & Ross, 1975*a*).
[d] Average precision ± 10 kcal/mol.

may be related to the very small association constant of the complex which is indicative of a very weak interaction. Increases in the heat stability of conalbumin on binding Cu^{2+}, Al^{3+} (Donovan & Ross, 1975*a*) and Fe^{3+} (Donovan & Ross, 1975*b*) were also observed by DSC. The order of increasing heat stability of the metal ion complexes of conalbumin was $Cu^{2+} < Al^{3+} < Fe^{3+}$. Tsalkova & Privalov (1980) have recently reported calorimetric studies on troponin C (the calcium-binding component from skeletal muscle). The denaturation data suggested that the structure of troponin C consists of two independent co-operative blocks, the thermal stability of which is largely dependent on the Ca^{2+} concentration. Both structures are stabilised in the presence of Ca^{2+} and thus their transition endotherms shift towards higher temperatures.

CARBOHYDRATES

The gelatinisation of starch is of primary importance in the food industry because of its impact on the texture of starch-based foods. A number of

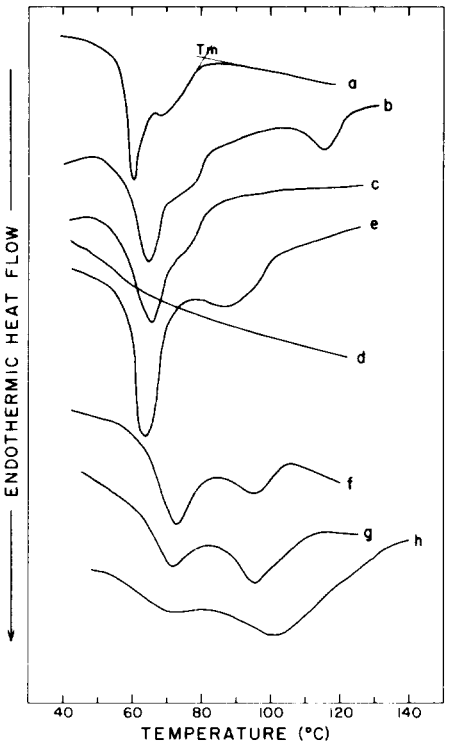

Fig. 4. DSC thermograms of starches (all samples were defatted except (b)). (a) Potato; (b) corn; (c) corn; (d) gelatinised corn; (e) smooth pea; (f) acid-modified smooth pea (5·1% lintnerised); (g) acid-modified smooth pea (9·6% lintnerised); (h) acid-modified smooth pea (20·2% lintnerised). Per cent concentrations of starch (w/w) from top to bottom were: 46·3, 46·9, 47·2, 48·4, 47·5, 47·8, 47·9, 47·6.

methods to follow the gelatinisation process have been devised, based on turbidity, swelling, solubility, absorption of dyes, X-ray diffraction, birefringence, enzymic digestibility, NMR, light scattering and DSC. DSC is particularly well suited to investigate the heat-induced phase transitions of starch/water systems because it is capable of studying these processes over a wide range of temperatures and moisture contents.

Typical DSC thermograms for several starches are illustrated in Fig. 4. Two endothermic transitions are observed for defatted native starches when heated at intermediate water contents, i.e. 45–50% w/w starch: water (Donovan, 1979; Biliaderis *et al.*, 1980; Von Eberstein *et al.*, 1980; Eliasson, 1980). These transitions are not exhibited by gelatinised starches (Fig. 4d). The influence of water content on the appearance of

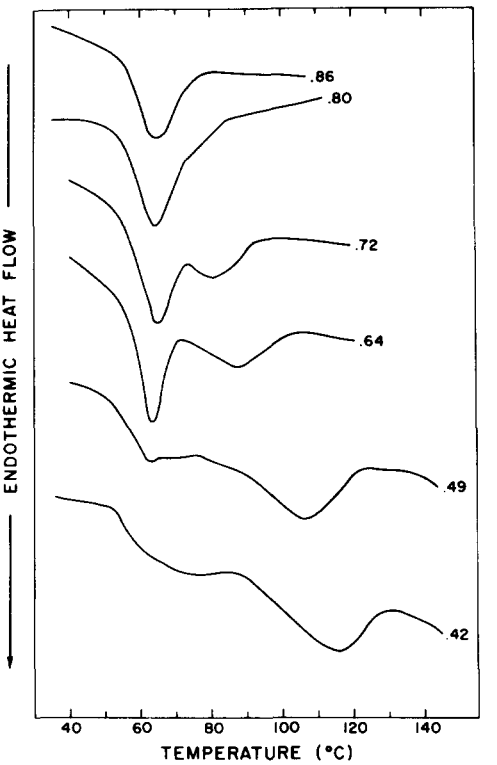

Fig. 5. DSC thermograms of smooth pea starch heated at different water concentrations; numerals represent volume fraction of water. Per cent concentrations of starch (w/w) from top to bottom were: 19·0, 26·6, 37·0, 45·6, 60·8 and 67·5. (Adapted from Biliaderis *et al.*, 1980.)

these two endotherms is shown in Fig. 5. With an excess of water, only a single endotherm is observed. However, as the ratio of starch/water increases, the second endotherm begins to develop at higher temperatures and becomes predominant at low water contents. Although the first transition occurs at constant temperature there is a progressive shift of the second endotherm towards higher temperatures as the water content decreases. This observation, and the well known semi-crystalline character of the starch granule, led to a theoretical treatment of the experimental data by employing equations that characterise other polymer systems (Donovan, 1979; Biliaderis *et al.*, 1980). The concluding temperatures (*Tm*, Fig. 4a) of the thermograms were plotted against the volume fraction of water (i.e. ratio of the volume of water to the total volume of

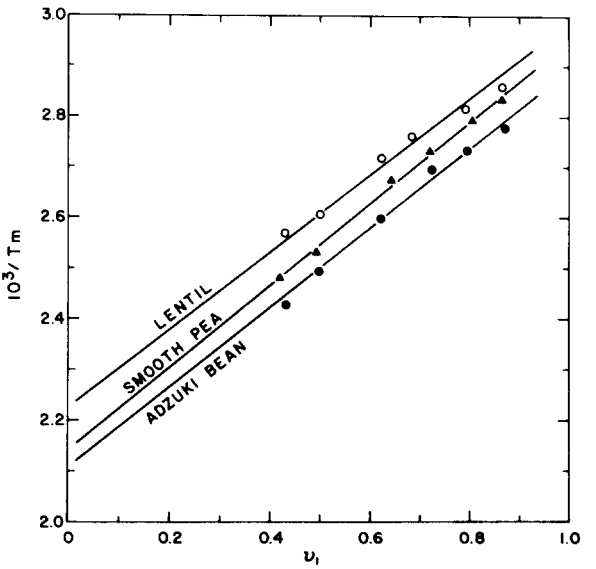

Fig. 6. Plots of $1/Tm$ (K) vs. v_1 for three starches. (Adapted from Biliaderis *et al.*, 1980.)

starch plus water) according to the Flory–Huggins equation (Flory, 1953):

$$\frac{1}{Tm} - \frac{1}{Tm^\circ} = \left(\frac{R}{\Delta Hu}\right)\left(\frac{Vu}{V_1}\right)(v_1 - X_1 v_1^2) \tag{4}$$

where R is the gas constant, ΔHu is the fusion enthalpy per repeating unit (glucose), Vu/V_1 is the ratio of the molar volume of the repeating unit to that of the diluent (water), Tm (K) is the melting point of the diluent–polymer mixture, Tm° (K) is the true melting point of the undiluted polymer, v_1 is the volume fraction of the diluent and X_1 is the Flory interaction parameter. Ideally, $X_1 = 0$ and thus eqn. (4) gives a linear relation between v_1 and $1/Tm$. Data plotted according to eqn. (4) show good agreement for the dependence of Tm on v_1 with the Flory–Huggins theory (Fig. 6); this suggests that starch gelatinisation may indeed be treated like a melting transition of a semi-crystalline synthetic polymer. The appearance of two endothermic transitions at intermediate moisture contents was further interpreted as representing two distinct mechanisms by which granule disorganisation takes place (Donovan, 1979; Biliaderis *et al.*, 1980). First, at high water contents, swelling upon hydration and increased chain motions upon heating in the amorphous parts of the starch granule destabilise the system and cause the melting of starch

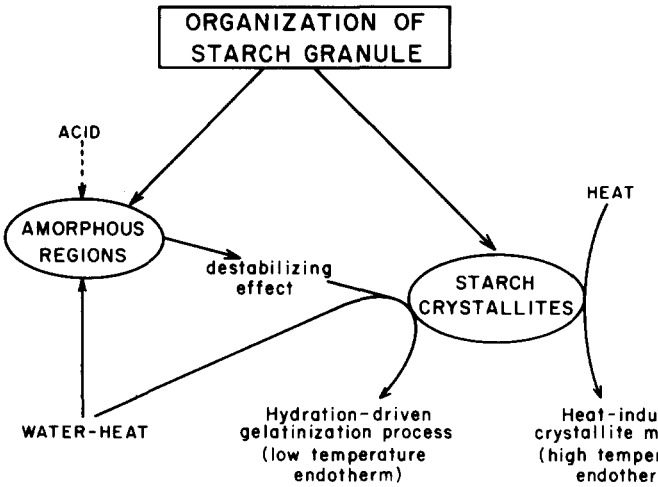

Fig. 7. Schematic representation of phase transition mechanisms of granular starch.

crystallites at low temperatures (first endotherm); a process known as gelatinisation. Secondly, in more concentrated starch suspensions, this destabilising effect is reduced due to limited amounts of water present and, therefore, 'true-melting' of the starch crystallites occurs at higher temperatures (second endotherm). The destabilising action exerted by the non-crystalline parts of the granule was further demonstrated from thermograms of acid-treated granular starches (Biliaderis *et al.*, 1980). Acid treatment (i.e. lintnerisation) of the starch selectively cleaves the amorphous regions of the granule. Thus, upon progressive lintnerisation the obtained thermograms (Fig. 4f, g, h) are similar to those of the native starch heated at low water contents (Fig. 5) which implies that the destabilising effect is indeed associated with the non-crystalline areas of the granule. The overall transition mechanism is illustrated in Fig. 7. In addition to these two irreversible endothermic transitions, a third reversible endotherm, at yet higher temperatures (Fig. 4b), has been reported (Kugimiya *et al.*, 1980; Von Eberstein *et al.*, 1980; Eliasson, 1980). This transition, the *Tm* of which is also dependent on the ν_1 of water (Eliasson, 1980; Donovan & Mapes, 1980), was interpreted as a disordering process of amylose–lipid complexes. Thus, defatted or lipid-free starches do not exhibit this endotherm. A calorimetric method for determining the amylose content of starches based on the formation and melting of amylose–lysolecithin complex was recently proposed by Kugimiya & Donovan (1981).

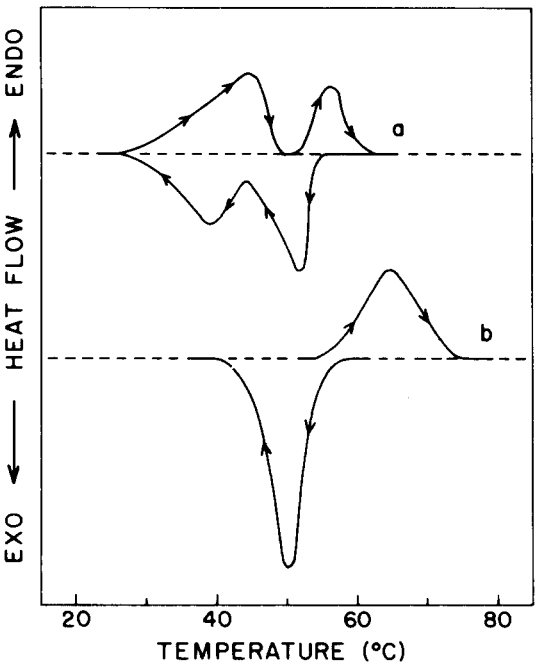

Fig. 8. Heating and cooling scans of ι-carrageenan (a) and κ-carrageenan (b). (Adapted from Morris *et al.*, 1980.)

Although the transition enthalpy for gelatinisation is significantly affected by the heating rate, starch damage and water content (Stevens & Elton, 1971; Wootton & Bamunuarachchi, 1979*a*, *b*; Donovan, 1979; Von Eberstein *et al.*, 1980), ΔH values in the range of 2·5–5·0 cal/g are usually reported by most workers. The depressing effect of sucrose on the gelatinisation of wheat starch, as seen by increase in transition temperature (Jacobsberg & Daniels, 1974; Donovan, 1977; Wootton & Bamunuarachchi, 1980) as well as by lowering in ΔH (Wootton & Bamunuarachchi, 1980) was also demonstrated calorimetrically.

Another important process that has a profound effect on the rheological characteristics of starch pastes is retrogradation (i.e. recrystallisation of the starch molecules). Retrogradation, an exothermic process, is responsible for the firming, shrinkage and syneresis of aged starch gels. A close relationship was found between the ageing of wheat starch gels as measured by endothermic peak height and the staling of bread as measured by crumb firmness at storage temperatures of $-1°$, $10°$ and $21\,°C$ (Colwell *et al.*, 1969). Although the transition temperatures do not

change upon ageing of starch gels (Colwell *et al.*, 1969), there is a time-dependent increase in the ΔH values (Von Eberstein *et al.*, 1980).

Thermally induced order→disorder transitions in other polysaccharide systems are accompanied by appreciable enthalpy changes (Reid *et al.*, 1974). Morris *et al.* (1980) have recently employed DSC to probe the temperature course of such transitions in carrageenan gels. This approach offered valuable insight into the gelation mechanism. In the presence of K^+ ions, which have been shown by light scattering to induce aggregation, the heating and cooling scans of ι-carrageenan (Fig. 8a) showed two distinct molecular processes; one without hysteresis and one (higher temperature endotherm) with significant hysteresis. The temperature course of this second process is close to that observed for κ-carrageenan (Fig. 8b) which is obtained only under aggregating conditions (i.e. presence of K^+). On the basis of this evidence and in agreement with the trend in the ΔH values, they interpreted these transitions as isolated double helix–coil and aggregated double helix–coil, respectively. Similar DSC studies on gelatin (Petrie & Becker, 1970) and other thermally reversible gels (Haas *et al.*, 1970) have been reported.

LIPIDS

In investigations of the physical properties of lipids, thermal analysis has long proven to be a powerful technique. Rek (1972) and Ladbrooke & Chapman (1969) have reviewed the applications of calorimetry in studying the phenomenon of polymorphism in fats. Polymorphism, the existence of more than one crystalline modification of the same substance, is frequently encountered in the lipid field. For instance, most triglycerides have been found to exist in three forms, α, β' and β, which, in this order, display increasing thermodynamic stability, melting point, heat of fusion and melting dilatation. Furthermore, these modifications have characteristic X-ray diffraction patterns and infra-red spectra and can also be confirmed by DSC. Thus, on the curves of Fig. 9, the low temperature endotherms correspond to the melting of the α-form which is followed by an exothermic effect, due to crystallisation into the β'-form, and an endothermic peak at the melting point of the β'-form. Similar heat effects are seen for the β'–β transition upon further heating (Lavery, 1958; Ladbrooke & Chapman, 1969). However, the lack of an exothermic peak for the α–β' or β'–β transitions does not always imply that the fat is

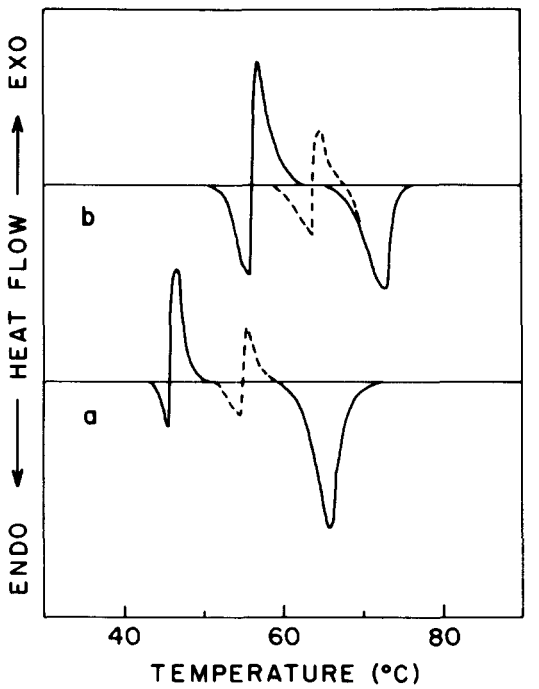

Fig. 9. DTA curves for fully saturated C_{16} (a) and C_{18} (b) monoacid triglycerides. (Adapted from Ladbrooke & Chapman, 1969.)

already in the β' or β modification, respectively. Apparently, crystallisation is a kinetic phenomenon and, therefore, if the rate of these transitions is slow, only incomplete transformation takes place during the heating cycle in the calorimeter (Rek, 1972). Recent DSC studies of the effect of chain length and unsaturation on the polymorphism of monoacid triglycerides demonstrated the presence of at least two intermediate endotherms (β'_1, β'_2) between those of α- and β-forms (Hagemann *et al.*, 1972). Hagemann *et al.* (1975) further reported that the melting point and ΔH_{fusion} for the β-form of both the *trans*- and *cis*-positional isomer series of octadecenoic acid alternate depending on double bond position; an even position correlated with higher transition temperatures and higher ΔH_{fusion}. Heats of fusion in the magnitude of 45–50 cal/g for saturated monoacid triglycerides were reported (Hampson & Rothbart, 1969). DSC was also used to examine the polymorphism of individual saturated triglycerides of the 18-n-18 and 16-n-16 series, where n is an even-carbon saturated fatty acid of 2 to 18 carbons in length (Lovegren & Gray, 1978; Gray & Lovegren, 1978). With mixed chain triglycerides, like

those of natural or hydrogenated fats, more complex forms of polymorphism are possible.

When two triglycerides are mixed the properties of the mixture are described by a binary phase diagram. DSC is the most convenient method for determining the temperatures of the phase boundaries (Perron *et al.*, 1969). The thermal behaviour of such mixtures may deviate considerably from that of the individual components (Barbano & Sherbon, 1978). DSC has also found wide applications in the blending of vegetable oils (Haighton & Hannewijk, 1958; Hannewijk & Haighton, 1958; Berger & Akehurst, 1966), evaluation of fractionated and hydrogenated fats derived from palm oil (Rossell, 1975; Jacobsberg & Ho, 1976), polymorphism and tempering of cocoa butter (Chapman *et al.*, 1971; Huyghebaert & Hendricks, 1971) or mixtures of cocoa butter with 2-oleodipalmitin and 2-elaidodipalmitin (Gray *et al.*, 1976), melting behaviour of hydrogenated hard butters (Marcus & Puri, 1978), the effect of processing conditions on the consistency of butter fat (Sherbon & Dolby, 1972; Sherbon, 1974) and oxidative stability of oils (Cross, 1970; Sliwiok & Kowalska, 1972). Furthermore, Kawamura (1979, 1980) using DSC isothermal analysis, found that crystallisation kinetics of palm oil resembles that of high polymers and that transformations to more stable crystalline forms (α–β'–β) take place at the early stages of the isothermal crystallisation.

A DSC technique has been used to determine solid–liquid ratios in fats (Bentz & Breidenbach, 1969; Miller *et al.*, 1969; Walker & Bosin, 1971) and it was found comparable with dilatometry (SFI) and wide-line NMR methods. Briefly, this method is based on measuring the heat of fusion for that portion of the fat which is solid. Besides the fact that this technique is faster than dilatometry, DSC offers a flexible means for tempering the fat at different temperature regimes prior to the determination, as well as a thermal 'fingerprint' of the fat. The latter is of importance in distinguishing between two fats with identical SFI values.

Emulsifiers heated in water at a temperature above the so-called Krafft point, where melting of the hydrocarbon chains takes place, form liquid-crystalline mesophases. First, a lamellar or neat structure is formed which, upon further heating, may transform to hexagonal or cubic structures (Ladbrooke & Chapman, 1969; Krog, 1975). A 'dispersion phase' has also been recognised in monoglyceride–water systems by adding water to the lamellar phase. Emulsification ability, as well as interactions between protein–lipid and amylose–lipid components, are

enhanced when the emulsifier is added either in an aqueous lamellar or dispersion form (Krog, 1977). On cooling below the Krafft point, the hydrocarbon chains recrystallise and a gel structure is formed. DSC can detect the heat changes associated with the above phase transitions (e.g. gel–lamellar) and thus can be used to determine the phase diagrams (Wilton & Friberg, 1971), for quality control purposes and for determining factors which increase the range of existence of lamellar and dispersion phases in monoglyceride–water systems.

WATER IN FOOD SYSTEMS

Although a concise definition of the term 'bound water' is nearly impossible, the concept of free and bound water is of great significance with regard to texture, chemical deterioration and microbial stability of foods. One definition of bound water is that it is the unfreezable water; this is assumed to be water which due to its strong interactions with other constituents is unable to crystallise on cooling. Thus, among several methods available for studying water binding, DSC has been extensively used to determine the amount of unfreezable water in foodstuffs. Briefly, this technique measures the heat required to melt the water fraction that remains frozen when the system is cooled to $-50\,^{\circ}C$. When water contents are sufficiently low that only unfreezable water is present, there is no DSC peak. Using samples of different water contents it is possible to determine the point at which all of the remaining water is unfreezable. A typical plot of such data is given in Fig. 10; the amount of unfreezable water corresponds to the intercept of the line with the water content-axis (Simatos *et al.*, 1975). Reported estimates of unfreezable water range from 0·2–0·5 gram of water per gram of solids for typical foods and their components (Davies & Webb, 1969; Parducci & Duckworth, 1972; Fennema, 1977; Wootton and Bamunuarachchi, 1978).

The use of lower freezing temperatures and calorimeters with greater temperature control during cooling and heating revealed that a fraction of the unfreezable water is in fact in a glassy or vitreous state (Parducci and Duckworth, 1972; Simatos *et al.*, 1975). The glass transition temperature (endotherm G, Fig. 11) depends on the cooling rate. If prior cooling is rapid in relation to diffusion and crystallisation of water, the interstitial fluid has a low concentration of solutes and forms a glassy phase at a low temperature. If prior cooling is slow, however, the glass

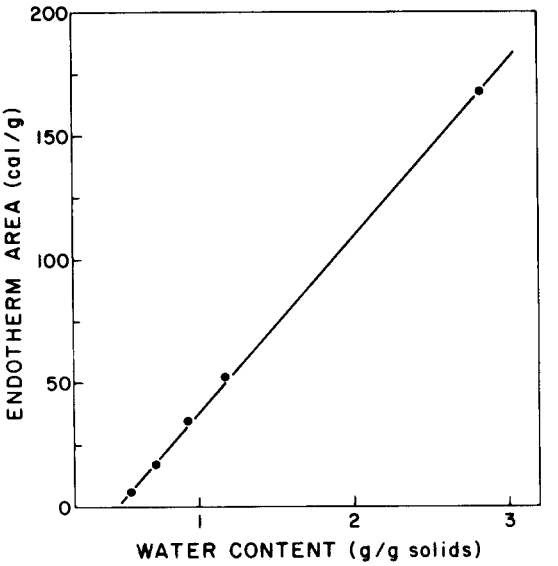

Fig. 10. Typical plot of the melting peak area (freezable water) measured by DSC versus total water content. (Adapted from Ladbrooke & Chapman, 1969 and Simatos *et al.*, 1975.)

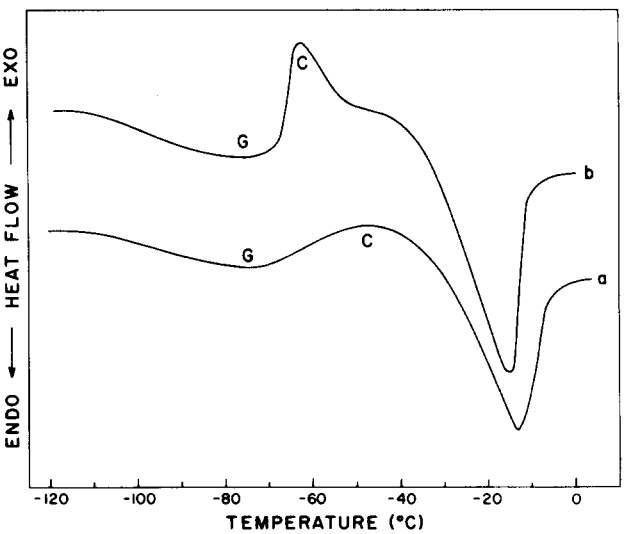

Fig. 11. Thermoprofiles for beef muscle (a) (0·44 g water/g dry solids) and egg white (b) (0·52 g water/g dry solids). Scans performed with a heating rate of 2 °C/min after cooling to −150 °C at a rate of 22 °C/min. (Adapted from Simatos *et al.*, 1975.)

transition occurs at a higher temperature independent of the solute concentration. This implies that the water content of the glassy phase is constant (Simatos *et al.*, 1975; Hardman, 1978). An exothermic effect (peak C, Fig. 11) is also observed after the glass transition which corresponds to the crystallisation of a fraction of vitreous water (Ladbrooke & Chapman, 1969; Simatos *et al.*, 1975).

Several workers have also reported results on water binding obtained by vapour abstraction methods (Hoyer & Birdi, 1968; Karmas & DiMarco, 1970*a*, *b*, *c*; Bushuk & Mehrotra, 1977). A delay in the removal of water, as well as an increase in the transition enthalpy (heat of vaporisation), were generally interpreted as an indication of stronger binding of water to a substrate. Such interpretations, however, must be made with caution. First, desorption DSC curves show non-specific broad endotherms and thus the problem of interpolating the base-line becomes difficult. Secondly, the process of vapour removal is largely dependent on the mass transfer conditions (physical state of material, vapour pressure and nature of the atmosphere in the DSC cell, etc.) Desorption calorimetric data of practical importance related to the sorption of water by dry milk and whey powders were reported by Berlin *et al.* (1971). Storage of these products under high relative humidity ($RH \geq 50\%$) induced lactose crystallisation (amorphous lactose–α-lactose monohydrate) which was detected by a single endothermic dehydration peak at 135 °C. Therefore, DSC appears to be a useful probe to monitor the physical state of lactose in whey and milk powders.

Results reported by Simatos *et al.* (1975) demonstrated the potential applications of DSC for kinetic studies of sublimation, as well as measuring the latent heat of sublimation—both important for calculations involving the freeze-drying process. Koga & Yoshizumi (1977) have investigated the thermal behaviour of rapidly frozen water–alcohol mixtures and whisky samples at various ethanol concentrations. The DSC thermograms revealed eight transitions over the entire concentration range. The ΔH of every peak remarkably changed at 40 % and 60 % ethanol content, suggesting that structural alterations in the mixture take place at these concentrations. Their calorimetric data also showed that a stronger interaction between ethanol and water occurs in aged whisky than in water–ethanol mixtures. Furthermore, DSC measurements of the freezing process showed that the process obeyed first order kinetics in the concentration range of 0–75 % ethanol and that the freezing parameters (freezing temperature, ΔH and kinetic constant) of

distilled spirits coincide with those of simple water–ethanol mixtures (Koga & Yoshizumi, 1979).

CONCLUSIONS

DSC is a useful investigative tool for studying various heat-related phenomena in foods and their components by monitoring the associated changes in enthalpy. Its ability to study these processes under the dynamic temperature conditions occurring during processing, as well as to provide both thermodynamic and kinetic data, constitute the main advantages of this technique. However, since changes in enthalpy are non-specific, the use of other complementary methods may be required to understand the physical nature of the phenomena observed.

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