A C T A A L I M E N T A R I A P O L O N I C

Vol. XV (XXXIX), No 4 1

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APPLICATION OF DIFFERENTIAL SCANNING CALORIMETRY TO THE STUDY OF THERMODYNAMIC PROPERTIES OF FOO PROTEINS, PARTICULARLY OF POULTRY, RED MEAT AND EG PROTEINS

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Key words: differential scanning calorimetry, thermodynamic parameters, food proteins, poultry, meat, egg.

The paper reviews some aspects of differential scanning calorimetry (DSC) an application of this technique in the food protein study, mainly poultry, red meat an egg proteins. Thermodynamic data as the onset, maximum temperature and enthalp transition of heated protein systems can yield valuable information regarding intrinsi structure stability, protein-protein, protein- the other components interactions and denaturation behaviour. DSC can be useful in characterising protein damage in food subjected to a wide range of treatments and processing. DSC parameters can indirectly aid in the optimisation and control of different processes such as storage and cooking.

INTRODUCTION

When biological material (food) undergoes changes like melting, denaturation, crystalline transition, chemical reaction there is an absorption or liberation of heat. Instrumentation that measures and records the amount of heat inv ed in the process can provide valuable qualitative and quantitative informatio In the food industry there are many examples in which materials undergo physical transformations requiring the addition or removal of heat. Recently thermal analysis has been used more frequently as a precise tool for indication of changes in protein during thermal stress. The types of thermal analisis generally used are: differential scanning calorimetry (DSC), thermogravimetric analysis, and thermomechanical analysis [25].

The purpose of this paper is to review some aspects of differential scanning calorymetry and its application to study thermodynamic properties of food proteins, especially of poultry, red meat and egg proteins. The majority of the data presented in this paper are the results of our experiments.

DIFFERENTIAL SCANNING CALORIMETRY — INSTRUMENTATION AND OPERATION

Differential scanning calorimetry is a thermoanalytical technique for monitoring the changes in physical or chemical state and properties of materials as a

function of temperature, by detecting the heat changes associated with such processes [8]. Modern differential scanning calorimeters are designed to determine enthalpies of these processes. The measuring principle in DSC is to compare the rate of heat flow to the sample and to an inert reference which are heated or cooled at the same rate. A differential scanning calorimeter consists of a sample cell and a reference cell which can be heated at a programmed controlled rate to maintain zero temperature difference between the two cells. This is the null-balance principle [34]. Thus the temperature of the sample holder is always kept the same as that of the reference holder by continuous adjustment of the heater powder. A signal proportional to the difference between the heat input to the sample and that to the reference sample is provided into recorder. The differential heat flow between the two compared samples is measured directly. Thus, the data are obtained in the form of differential heat input versus temperature (dH/dt) and temperature as well as enthalpy of transition or reaction are obtained with no difficulty.

This discussion will be limited to the DSC-4 marketed by Perkin Elmer Corp., Norwalk, Conn., which was used in authors's research. The DSC-4 is a modern, widely used instrument. Until recently the limiting factor in the wider utilisation of DSC has been the lack of sufficient sensitivity of available instruments. Standard unit allows operation from ambient to 600°C. With cooling accessories the range may be extended to — 170°C. The heating rates range from 0.1 to 200°C per minute. Calorimetric accuracy is 1%. Sensitivity of DSC-4 ranges from 0,01 to 10 mcal/sec., in protein research the range between 0.1 and 2 mcal/sec. is mainly used. The cells of the calorimeter shown schematically in Figure 1 are made of platinum-indium alloy cups which are mounted in comparatively large aluminum blocks that function as a heat sink to provide constant thermal environment. The cups have separate electric heaters and platinum thermometers. The cells have platinum lids which provide an irradiation shield. There are some remarks which seem to be important to obtain the data with a desired accuracy and precision. The small sample pans, currently in use hold 10-20 μl of material. Macromelocules of biological origin are usually in low concentration in samples (water is the main bulk) and proteins have relatively small heat effects associated with transition. These problems are critical when dealing with proteins in intact food. For example, meat protein whose contribution to meat is 20% consists of the different protein fractions at different levels. Moreover, the denaturation enthalpies are relatively small (myosin 3.9 cal/g, ovalbumin 3.6 cal/g) when compared with fusion of organic compounds (stearic acid 47.6 cal/g) [51]. Only the recent advance in calorimeters with high sensitivity enables analysis of these constituents. Samples are placed in pans made of aluminium, stainless steel or gold. The pans are covered with lids and crimped on. Volatile pans can withstand up to 3 atm. internal pressure. For optimum peak sharpness and resolution the contact surface between pan and sample should be maximized. Heterogeneous material of samples causes problems because of small volume of the specimen pen. Homogenization of the samples (meat, emulsion) may cause

some irreversible transition which are being detected by DSC [32]. For samp containing water the pan must be hermetically sealed to prevent evaporatior water. Calibration of the instrument is carried out with a high purity metal w accurately known melting point and enthalpy. The most commonly u: calibrant is indium ($\Delta H = 6.8$ cal/g, $T_m = 156.4°C$) or gallium ($\Delta H = 19.1$ ca $T_m = 29.8°C$).

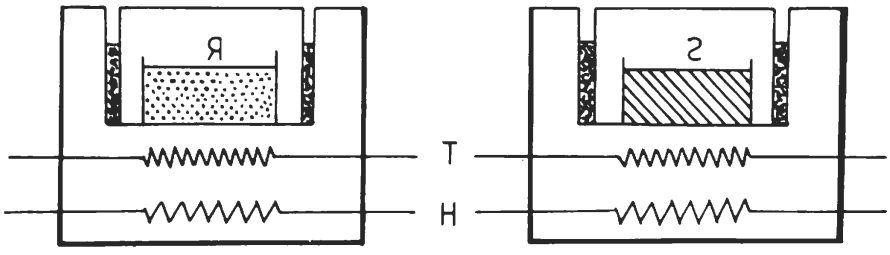

Fig. 1. Schematic diagram of the cells in the Perkin-Elmer DSC-4, L-platinum sensors; T-platin sensores; H-individuals heaters; S and R, sample and reference pans respectively

The tipical DSC-thermogram for indium showing the melting temperatu and the entalpy is presented in Figure 2. The obvious parameter afforded by DS is the temperature at which transition occurs. In the case of proteins it is refered t as denaturation temperature and it can be defined in a number of ways. The mo commonly specified temperature is the peak maximum temperature (T_{max}) c temperature of maximum transition [51]. Some authors determine the extra polated onset temperature (T_0), which is independent of protein concentration, i a similar manner to T_0 of the calibrant. Differential heat flow is displaye normally as a peak on a thermogram. The area under the peak is directl

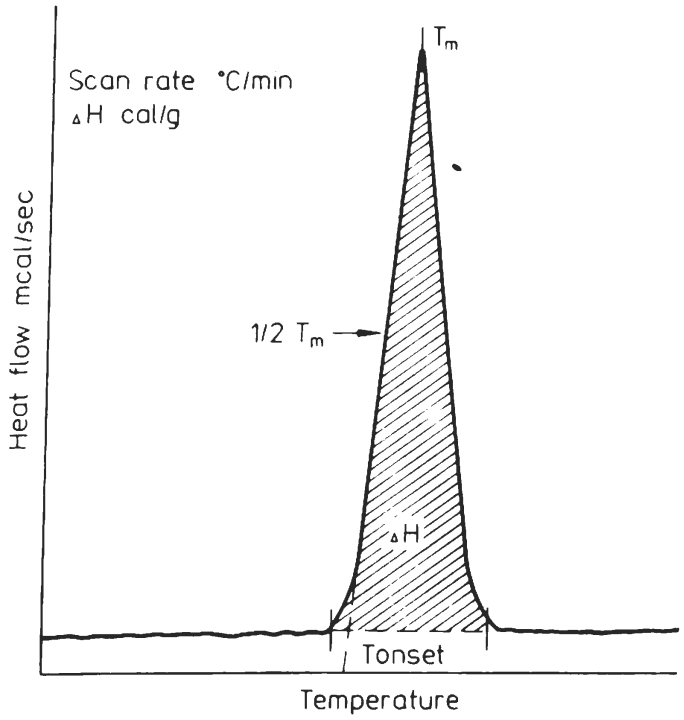

Fig. 2. A typical DSC thermogram for indium standard showing the melting temperature (T_{max}) and the enthalpy of transition (ΔH)

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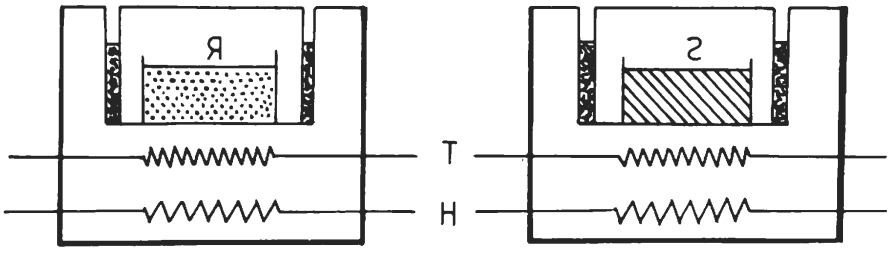

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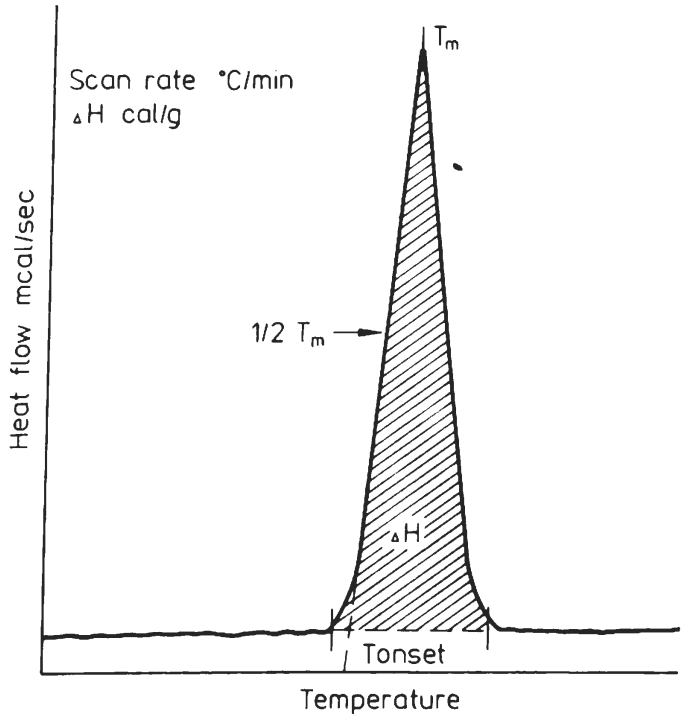

Fig. 2. A typical DSC thermogram for indium standard showing the melting temperature (T_{max}) and the enthalpy of transition (ΔH)

proportional to the enthalpy of transition. The peak area measurement very often presents difficulty because the base line for the thermal transition peak may not be horizontal and the peak is not symmetrical. An accepted procedure for determining the base line for the peak has been extensively described in literature [15, 34, 51]. Methods employed for area (ΔH) measurement include planimetry, image analysis and more sophisticated computer based data analysis. The DSC-4 of Perkin Elmer is equipped with Thermal Analysis Data Station (TADS), a sort of microcomputer which precisely calculates all important parameters by depressing a single key and takes only minutes to perform. The analysis of complex thermogram is simplified by the partial areas software program.

Another parameter measured directly from the transition peak essential for protein study is the width at half peak height signified by $\Delta T_{1/2}$. This value is a guide to the co-operativity and complicity of the protein denaturation process [51]. The smaller the value the higher the cooperativity. Cooperativity is linked with internal homology of the protein structure. When the peak is sharper it indicates that transition is closer to a two-state native-denatured process with very low probability of intermediate states [39]. DSC can yield information important for protein denaturation, namely the kinetics reversibility. The extent of reversibility is easily identified by re-scanning samples after heat denaturation and comparing the areas of the transitions obtained after the first and second heating. The kinetic parameters for denaturation can be also determined from DSC curves [8]. The vertical displacement from the base line, at any temperature, is proportional to the rate of heat flow into the samples (dH/dt) and consequently it is a measure of the reaction rate. Several methods have been developed to determine the rate constants, for instance Borchardt and Daniels reaction kinetics [9]. The activation energy may then be calculated from the Arhenius plot of 1 K versus 1/T. A variety of reference samples have been used in DSC studies of proteinous food. However, there were no indications whether different reference samples affected the results. In the DSC studies of muscle protein food the authors compared thermodynamic parameters of chicken muscle using different reference sample (Table 1) [30]. The results indicated no statistical differences in onset temperature (T_0), maximum transition temperatures (T_1, T_2) and total enthalpy (ΔH).

Table 1. Effect of different reference sample on thermal parameters of chicken broiler muscle (heating rate = 20°C/min)

Reference sample	T_{onset} °C	T_{max1} °C	T_{max2} °C	Δ(Joules)g
Empty	54.4 a*)	60.3 a	80.7 a	20.1 a
Water	54.4 a	60.5 a	80.9 a	20.6 a
Denatured sample	54.2 a	60.4 a	81.0 a	20.5 a

*) Means with the same letter are not significanty different at p = 0.05 ΔH enthalpy of transition

APPLICATION OF DSC IN BASIC STUDY OF FOOD PROTEINS

DSC has found increasing application in the area of protein research in rece years [51]. Two reasons probably account for this rapid growth of intere Firstly the more sensitive calorimeters are now available enabling the preci measurement of the relatively small enthalpy changes associated with prote transformation. Secondly, the better recognition that thermodynamic data ca produce valuable information not only on the instrinsic stability of the protein, i internal bonding and structure but also on its process behaviour. DS application in the study of food proteins can be divided into 2 groups. The fir one is a section of basic study on isolated proteins in a model systems. This st produces some fundamental data on the denaturation behaviour and gives tr thermodynamic data. The second section of DSC application is directly related t food quality or processing.

The examples of DSC basic study are the comprehensive investigations o reversible denaturation undertaken by Privalov [38] on small compact globula proteins such as lysozyme, ribonuclease, myoglobin in very dilute solution (0.05-0.5%) under slow heating rates (1°C/min). Privalov [39] reported som theoretical aspects of denaturation research on proteins which do not present a single cooperative system such as muscle troponin C, myosin rod, tropomyosin collagen or ovomucoid (egg white protein). From his study it is known that th thermal denaturation of the myosin molecule domain, termed alpha-helical rod i comprised of a least six processes (Fig. 3). In spite of the fact that the basi research of this category is not directly relevant to the food protein science, some useful information can be achieved. In practical DSC work both protein concentration (5-20%) and heating rates (5-20°C/min) are relatively high in orde to simulate actual food processing conditions. However, under these condition denaturation becomes irreversible, since extensive intermolecular interactions are favored and aggergation of the unfolded protein molecules occurs [8]. In contrast to denaturation, which is a heat absorption process, aggregation is generally considered as an exothermic process. Therefore, aggregation is a complicating factor in quantitative interpretation of enthalpy obtained from DSC investigations. Since the energy of aggregation is low it is still possible to interpret the DSC data of protein denaturation using the concepts of classical calorimetry [13]. Wright reviewed broad range of studies on food protein thermal behaviour involving proteins interactions with proteins, water, carbohydrates or other compounds [51]. The latter studies are particularly important in the context of foods, because such interactions play a vital role in determining product structure or attributes and it is the area that DSC can provide most useful information on [53]. To be able to study a particular protein by DSC, the samples should undergo a thermal transition that is detectable by calorimetry. In case of egg white ovomucin [12] and casein [26] no such transition has been observed. This phenomenon is attributed to the random coil

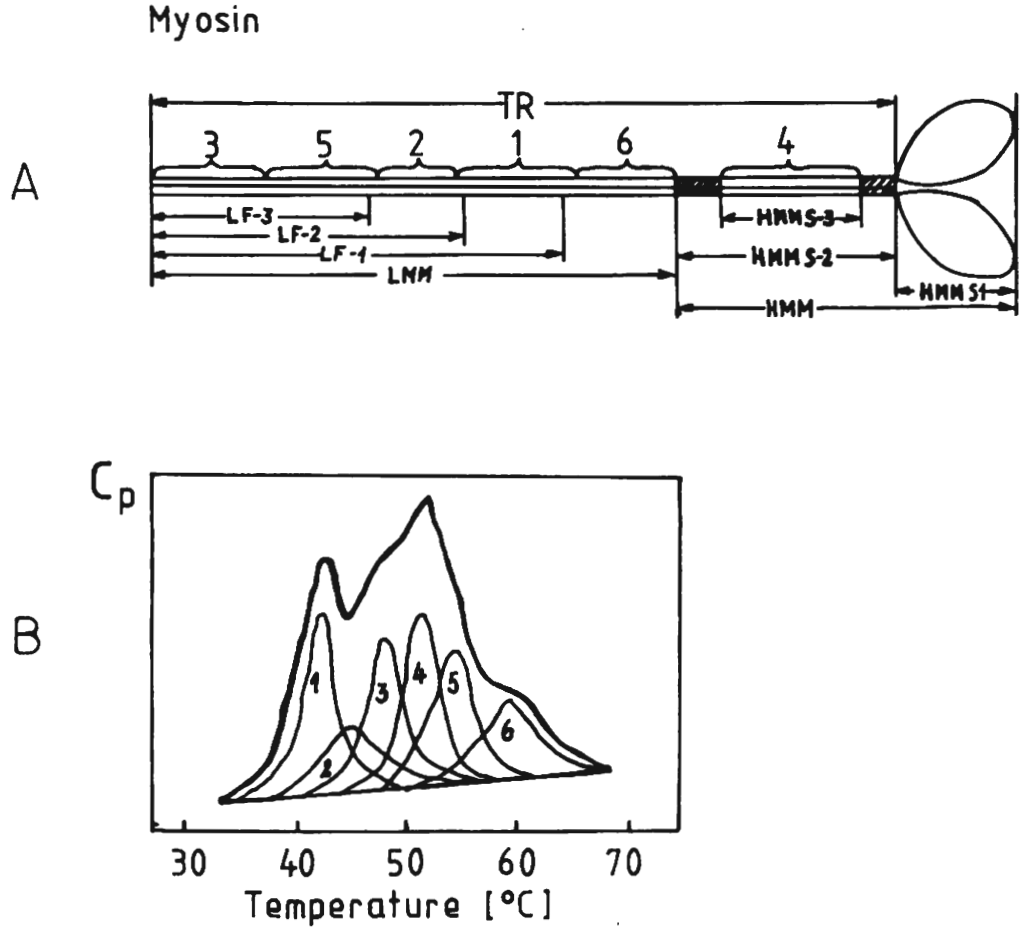

Fig. 3. (A) Proteolytic fragmentation of myosin. Braces indicate the thermodynamically revealed cooperative bloks. (B) The partial heat capacity function of the myosin total rod. Light lines indicate consituent peaks corresponding to the heat effect of melting of myosin rod. The numeration corresponds to the increasing order of stability [39]

configuration of the native proteins. If one of these proteins undergoes a thermal change it may not be detected by DSC, either because the enthalpy change is too small or the processes is not sufficiently cooperative resulting in a very broad transition.

DSC STUDY ON MUSCLE FOOD PROTEINS, ESPECIALLY POULTRY MEAT

DSC has found application in the studies of proteins from all the major food groups i.e. meat, fish, dairy, cereals, vegetables. In many food products it has been possible to identity contribution of constituent proteins to the total DSC thermogram of the raw material. The DSC thermal transition curves of breast, thigh, skin and blood chicken tissues were determined by Kijowski and Mast [30]. Breast chicken muscle or meat produced five peaks thermogram with main peaks at 57.1 and 77.7°C and three additional peaks at 62.5, 67.3, 72.8°C at heating rate 10°C/min. The thigh (leg) muscle produced a thermogram with three distinct transitions at 59.6, 65.6 and 75.8°C. The difference between the breast muscle thermoprofile with a higher proportion of white muscle fiber is not clear; in spite of the fact that these two types of muscles are different in many biochemical and physiological aspects. In the majority of DSC studies rabbit, beef and pork muscles displayed three basic endotherms between 60 and 80°C

[54, 44, 47, 19]. Chicken broiler skin contains about 13% protein consis primarily of collagen (60-80%) and displayed one major peak at 66.. corresponding to the denaturation of collagen. The enthalpy of the skin colla (23.9 Jouls/g) was much higher than that obserwed in the denaturation intercellular muscle proteins. This indicated that different types and nature bonds stabilized the collagen structure. Chicken blood tissue exhibited that major thermal transformation started at about 62°C reaching the maximum 82.6°C. This finding confirms the information that blood proteins denatured higher temperature than actin, the most heat resistant muscle protein. T practical conclusion is that meat products containing blood proteins should cooked to an internal temperature of 83°C. DSC has an obvious advanta because it is capable of studying the thermal properties of a variety of tiss proteins in their natural state and environment. Thermal curves of isol muscle constituent protein fractions or purified proteins provided informa about the location and identification of these components on complex therm gram obtained from the whole muscle tissue [30]. Myofibrils, myosin, act sarcoplasmic proteins and stroma fraction subjected to the DSC-analy provided the following information that the main contribution to the compl thermogram have: myosin (55.2°C), sarcoplasmic proteins (62.3 and 68.3°C) ar actin (77.7°C). The connective tissue is probably associated with the third peak the muscle heat thermal record, but its contribution is small because of its lo content (below 1%). The slight differences in T_{max} between muscle an constituent proteins resulted primarily from different environmental condition Using dynamic and isothermal method Wagner and Anon determined den turation kinetics of bovine myofibrils by DSC [46]. Activation energy ra constant and mean life time for each of three transition were calculated by mean of a dynamic method. For kinetic values isothermal method was applied. Findla et al. by the help of van't Hoff relationship and Borchardt and Daniels kineti analysis of three main muscle endotherms occurring at 56, 66, 82°C estimate their reaction orders [16]. The reaction orders were influenced by muscle type fiber sarcomere length, and conditioning.

Besides affording a qualitative compositional analysis of the samples, DSC can also be utilised to provide quantitative information on the relative or absolute amounts of proteins present in the sample [52]. It has also been emphasized that DSC per se cannot predict directly the performance of any protein in a given situation [53]. It is incapable of defining precise nature of an investigated process. It is sometimes possible to obtain such information from correlation of the approprite physical properities with the measured calorimetric parameters ΔH and T_{max}. In other words, the effects of processing treatments o interactions are estimated by monitoring changes in stability (increase or decrease in T_{max}) or extent of denaturation (decrease in apparent enthalpy).

Muscle food study has found notable status in current DSC application.

The effect of heat treatment on myoglobin at various water contents was investigated by Hägerdal and Martens [21]. Below 30% of water contents the

denaturation temperature increased and a linear relationship was found between ΔH and water amount, which suggests that only a part of the protein underwent denaturation. This interpretation is consistent with the trend in solubility of heat treated samples. In another calorimetry studies the object of investigations was thermal behaviour (shrinkage, melting or denaturation) of mainly beef intramuscular or tendon collagen as affected by the type of connective tissue [6], collagenase treatment [7], chronological age [27], growth rate, steroid hormones, postmortem degradation, electrical stimulation [28]. Purslow [40] established a positive correlation between hydroxyproline content and thermal stability of vertebrate collagens in solution as well as between ΔH of collagen denaturation and denaturation temperature (T_d). Thermal transition in the native tissue is traditionally measured by the shrinkage temperature (T_s). T_s is considerably higher than T_d for the collagen of the same source and the difference T_s—T_d is a constant value (~25°C). Differential calorimetry investigations on thermal behaviour of collagen- rich tissues i.e.: epimysium, tendon and skin from spent hen drumstick have been recently conducted by Kijowski and Mast [31]. The effect of marinating procedure of those tissues on melting temperature of collagen was investigated by soaking the samples in 2% NaCl, 1.5% acetic acid or lactic acid brine for 72 hours. Specimens marinated in acid brines displayed singnificant reduction of transition temperature from 72.9 for untreated samples to 42.9 and 49.7°C with evident degradation of collagen molecules (Fig. 4). The collagen heat resistance was partially recovered after removal of acid ions by dialysis and melting temperature raised to 67.6°C. In the muscle food DSC

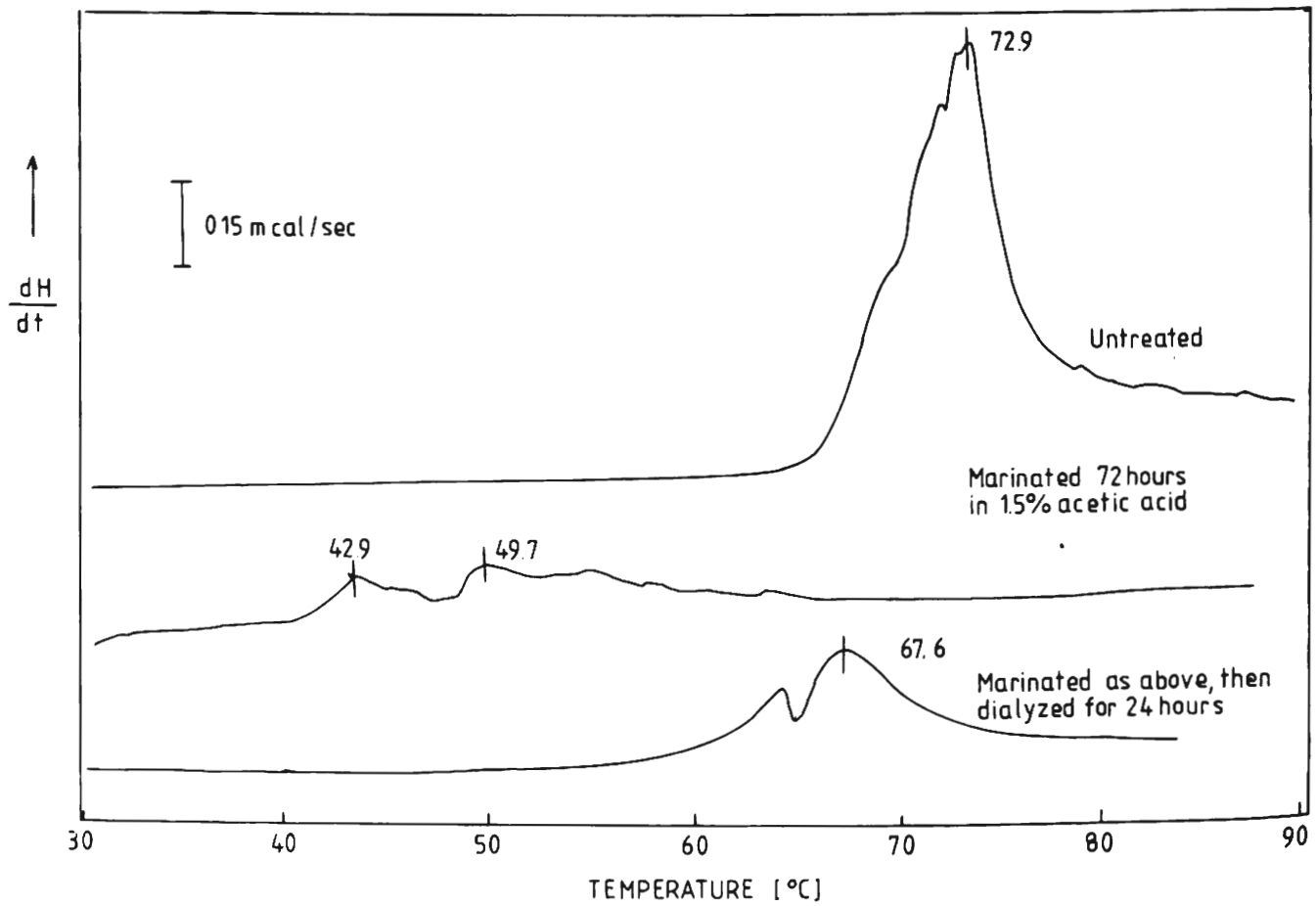

Fig. 4. DSC-thermal record of spent layer drumstick epimysium

technique has also been used to study thermodynamic attributes of const proteins influenced by:

— muscle type and animal species [45],
— identification of pale, soft exudative (PSE) pork meat [44],
— post mortem conditioning and sarcomere length [17, 18].

DSC APPLICATION IN MUSCLE FOOD PROTEINS STUDY IN PROCESSING

Foods can be subjected to a wide range of conditions and treatments d processing operations. Indirectly, DSC investigations can yield appli information to aid in the optimization and control of these processes as has demonstrated in several papers. Kijowski and Mast subjected breast ch meat to the programmed heating at the increasing heating rate (HR) 1°C/min to 40°C/min [30]. An increase in heating rate singnificantly elevate onset temperature transition as well as the major proteins transition (dena tion) temperatures (Fig. 5). Simultaneously the heat absorbed (ΔH) d

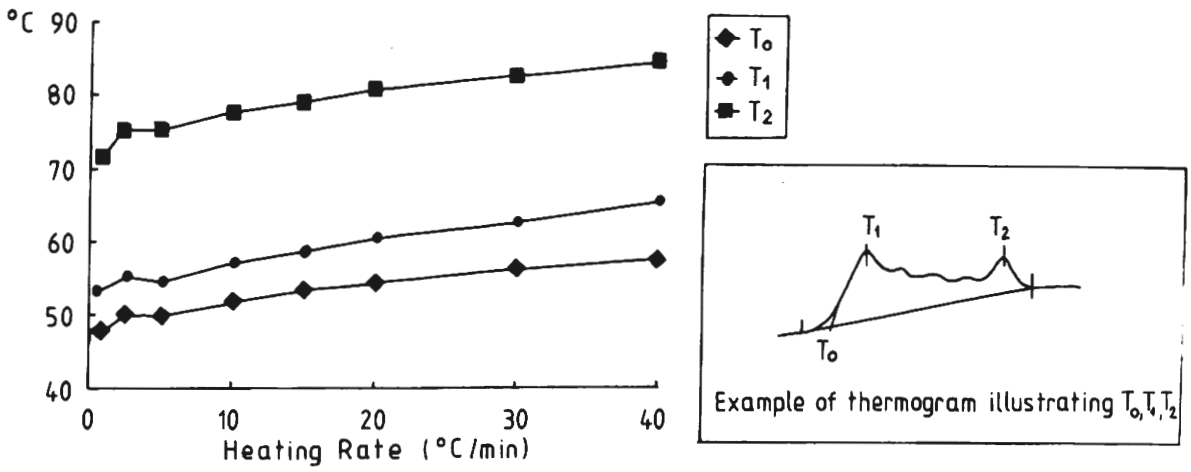

Fig. 5. Influence of heating rate on the extrapolated onset temperature of transition (T_0) an temperature of maximum transitions (T_1, T_2) of chicken breast muscle

denaturation increased about 2.2. times; from 10.6 to 22.9 Jouls/g protein for 1°C and 40°C heating rate, respectively. Similarly the dependence of denaturation enthalpy on HR has been reported for myosin, bovine serum, albumin, myoglobin and whey proteins [51]. In another experiment the chicken broiler muscles were heated to various end-point temperatures between 40 and 90°C, at HR = 20°C/min and after that immediately cooled down and reheated to 90°C in order to determine the protein fractions which were not initially denatured (Fig. 6). The ΔH values clearly show that nothing was changed to 55°C. The basic denaturation changes occur between 55-70°C. In this range 88% of total ΔH was declined. This investigation proved that myosin was completely denatured at 60°C, sarcoplasmic proteins at 70°C and actin at 80°C. Findlay et at., [19] stated that DSC is capable in determining of samples cooking degree. The application of Borchardt and Daniels reaction kinetics has permitted the calculation of time

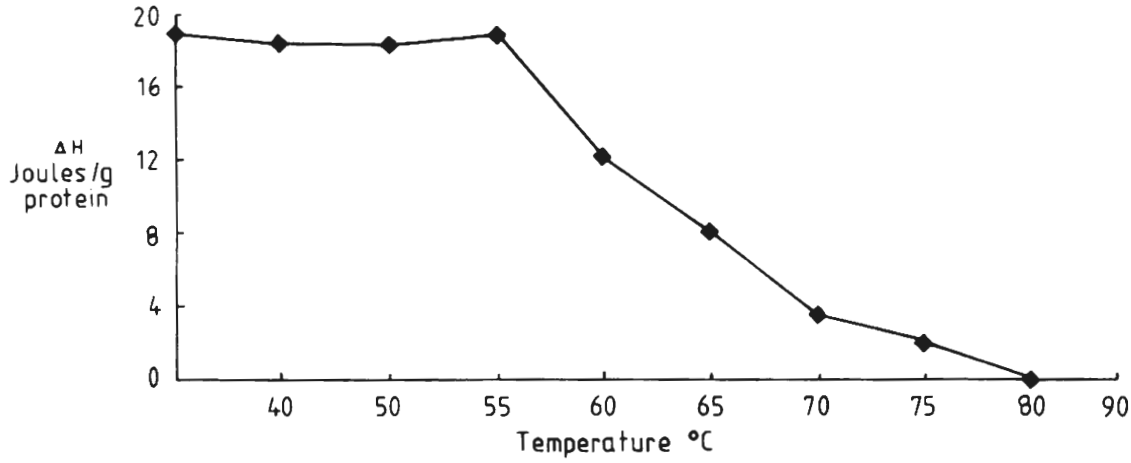

Fig. 6. Denaturation enthalpy (ΔH) of chicken breast meat heated to different end—point temperatures, cooled and reheated to 90°C

and temperature that sequentialy eliminate each endothermic transformation allowing controlled denaturation of beef muscle proteins during cooking of meat. In other words they demonstrated DSC usefulness in predicting the time and temperature treatments required for controlled denaturation of beef muscle proteins. Parsons and Patterson examined DSC as a potential method for determination of the previous heat treatement of meat products [36]. A promising correlation exist between maximum heat treatment temperature and the onset (T_0) of the transition obtained by DSC, while the effect of duration of heating is reflected by enthalpy of denaturation. It appears, that it may be possible to obtain an indication of the temperature and possibly duration of the previous heat treatment of meat products within the temperature range of 40-90°C. This information is useful to food industry in preparing heat processing schedules in which undercooking or overcooking can be prevented. DSC seems to be a very suitable technique not only for the studies on the effect of heat temperature conditions above 0°C but also below freezing point of food products. Freezing or freezing storage conditions have frequently been associated with loss of functional quality supposedly as a result of protein denaturation. Results from DSC studies on poultry, meat and egg proteins indicate that this may be a more complex phenomenon, than it was previously believed. Scanning calorimetry was applied by Wagner and Anon on bovine [47, 48] and Kijowski and Mast [29] on chicken meat to study freezing denaturation of proteins. The authors indicated that different freezing rates decreased denaturation enthalpies. The lower the freezing rate the greater the loss. Only quick frozen chicken breast at -60°C (dry ice and methanol mixture) does not affect statistically the enthalpy of thermal transition, but at -30°C the enthalpy was affeced (Table 2). On the DSC thermal records of bovine and poultry meat it can be observed, that the area ascribed to myosin decreases during freezing, while the area corresponding to actin remains unaffected. These results are in agreement with the decreased ATPase activity as a consequence of freezing. Highner losses were observed at lower freezing rates. The effects of freezing storage of beef and poultry meat on the DSC thermal record were also investigated by the above mentioned authors. The total ΔH

Table 2. Effect of freezing on thermal parameters of chicken broiler muscle (heating rate = 20°C/ /min)

Reference sample	T_{onset} °C	T_{max1} °C	T_{max2} °C	Δ(Joules)g
Non frozen	54.4 ab	60.5 a	80.9 a	20.6 a
Frozen at-30°C	53.8 a*)	60.2 a	80.6 a	17.5 b
Frozen at -60°C	54.9 b	62.1 b	80.4 a	19.5 ab

*) Means with the same letter are not significanty different at p = 0.05 ΔH — enthalpy of transition

decreased with time of storage, and the decreases were smaller at lower minus temperatures. Wagner and Anon [48] based on the area of the first peak, additionally noticed that the myosin „head" denaturation is a progressive process during storage time. The protein of thin filament remained unalterd. Kinetic analysis sugested that the denaturation of myofibrillar proteins took place through two consecutive reactions, an initial rapid reaction, followed by a slower one.

Foods can be subjected to a wide range of conditions and treatments during processing operations. Kijowski and Mast [32] observed in DSC investigations an interesting phenomenon that homogenization or final chopping of chicken muscles caused by about 30% reduction of total enthalpy. This ΔH reduction is connected with peaks size restriction for myosin and actin. The results are not clear, they may be related to the heat conductivity changes, or differences in the macrostructure of the myofibrils causing some irreversible transitions detectable by DSC. In the manufacture of poultry and red meat NaCl and polyphosphate are often added to promote solubilisation of proteins, increase WHC and to improve the integrity and binding properties of products. Simultaneous addition of NaCl and phosphate to the meat causes considerable modification of physicochemical features of myofibrillar proteins, the fraction mainly responsible for meat functionality. Finally, cut chicken breast muscles were treated with 1-4% NaCl or 0.25-1% of either pyrophosphate (PP) or tripolyphosphate (TPP) [32]. Simultaneous addition of NaCl and phasphate was also monitored by DSC. Increasing concentration of NaCl considerably destabilized heat resistance of the constituent meat proteins. In a meat system the addition of 4% NaCl resulted in one maximum transition in state of five thermal transitions typical for untreated meat. In contrast, NaCl ions were shown to increase stability of soy proteins [24] and fababean proteins [5]. The presence of PP and TPP especially in concentration of 0.25-0.5%, the industry applied amount, enhanced the thermal resistance of myosin. At the same time phosphates destabilized the heat resistance of actin. So, general remark from the above cited research is that heat stabilization or destabilization effects are specific and depend upon the nature of the proteins and ions involved. Quinn et al. [41] investigated thermal properties of meat proteins in sausage batter and concluded that the salt used in processed meat is responsible for the observed changes in heat transition. They also denied the influence of the fat presence in meat emulsion on protein structural stability.

The analogous conclusion was drawn from DSC cooking study of mechanically deboned poultry with high fat content [29].

DSC investigations can indirectly provide suitable information for control or optimization processes, as it has been demonstrated in cooking of meat [36], and cooking of ham [2]. Heating of cured meat beef caused changes in the DSC profile of muscle denaturation and instead of three endothermic transitions, four temperature maxima were monitored [35]. Prolonged pressure treatment of muscle at about 150 MPa results in elimination of the thermal transition attributed to actin [33]. Determination of ΔH by DSC indicated that the application of the pressure stabilized the connective tissue proteins against the effect of heat. This DSC-research directed to the conclusions that pressure mediated tenderization observed in meat, as it had been suggested previously, was more closely connected with the myofibrillar than the collagenous compounds of muscle. Calorimetry investigation of freeze-dried breast chichen meat indicated no denaturation changes in the constituent proteins [29].

Some interesting application of DSC has been found in fish and seafood processing. Poulder et al. [37] suggested the scanning calorimetry as a rapid and simple means of cold-storage denaturation of frozen-fish. Rodger and Hastings [42] used the same technique to the study protective role of trimethylamine oxide in fish muscle freeze denaturation. Hastings et al. [20] successfuly applied DSC for detecting protein conformational changes in conventionally dehydrated and marinated fish. The same technique was also used to study mince fish and surimi protein denaturation and to relate these to gelation phenomenon [1, 55].

It is generally recognized that, in most instances, maximum expression of functionality of food proteins is possible only if significant loss of nativity has been avoided during extraction, isolation purfication and processing. DSC affords a rapid and direct means of assessing the degree of damage imposed on the proteins [53]. The preparation of protein isolates from animal and plant materials involves a number of steps potentially damaging the protein native structure. Such protein preparations have been shown to be amenable to DSC investigation [4]. Scanning calorimetry has been performed lately on protein isoletes from bovine lungs and rumen [3]. The isolates defatted with solvents of increasing polarities presented very similar phase transitions, but the absence of enthalpy determination makes impossible full explanation of protein structure damage. This is the only paper on animal origin isolates using differential scanning calorimetry as a tool of research.

EGG PROTEINS STUDY WITH DSC

Egg white proteins have received special attention in thermodynamic study with use of DSC because of their importance in baked goods and other food systems. Example studies include conversion of ovalbumin to s-ovalbumin [11], plakalbumin to S-plakalbumin transformation [43], egg white protein stability influenced by pH, aluminum and sucrose [12], thermal aggregation of ovalbumin

affected by salts, pH [23], stabilization of ovalbumin by DSC [22], stability of avidin, and avidin-biotin 13, trypsin-ovomucoid complex [10] aggregation of egg white in acid media [49], freezing denaturation of egg white proteins [50].

Some of those detailed studies are of important scientific and practical value. One effect of chill storage on egg white is the production of a heat stable form of ovalbumin (T_{max} = 84.5°C) termed s-ovalbumin with maximum thermal transition at 92.5°C (Fig. 7), so s-ovalbumin denaturation temperature is 8°C higher than of ovalbumin. S-ovalbumin is a normal component of egg white, and immediately after laying its content is as low as 5% of ovalbumin and increases to 81% in egg stored at 2°C for 1/2 year. The differences between ovalbumin and s-ovalbumin are minor and there is some evidence of alterations in surface hydrophobicity during the conversion of the basic form to s-form of ovalbumin. The formation of s-ovalbumin proceeds through a previously unrecognised intermediate forms whose denaturation temperature is 88.5°C. Transformation of ovalbumin into s-form was accelerated during egg storage at elevated temperature. Consequently, DSC could be used as a rapid screening method for quality of stored eggs determination.

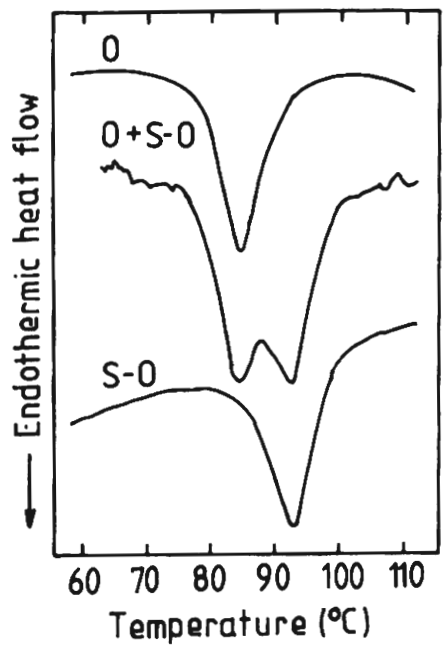

Fig. 7. Endotherms of ovalbumin (0), s-ovalbumin (S-0) and equimolar mixture of 2 ovalbumin forms (0+S—0) [11]

Related research experiments on the denaturation of egg white proteins as a result of different freezing rate, thawing conditions and storage time were presented by Wooton et al. [50]. Simultaneously the changes in functionality were determined (viscosity, foam instability, and foam specific gravity). Loss of ΔH of denaturation was also increased by slower freezing rates, higher thawing temperature and longer storage time. Conalbumin suffered greater losses and ovalbumin the smaller losses than egg white itself. However, egg white functionality had no clear relation with loss in apparent enthalpy. Individual proteins of egg white have different heat-sensitivity, so that moderate heating used in pasteurization of egg white affects some of the proteins but not all of them. The most sensitive is conalbumin. Donovan et al. [12] stated that thermostability of egg proteins was of the order ovalbumin≥lysozyme>conalbumin. Egg white

displayed generally two peak thermogram, the first one corresponded to conalbumin (13% of egg white) and ovalbumin (54% of egg white). Analysis of the isolated egg-white proteins indicated that other minor proteins (ovomucoid, globulin, avidin) also contributed to the total thermogram, but were dimmed by the ovalbumin transition. Lowering the pH from 9.0 to 7.0 or adding Al^{+3} ions to the egg white incresed the denaturation temperature of conalbumin by 4 and 12°C, respectively. Furthermore, the addition of sucrose (10% concentration) increased the stability of all these proteins (endotherms were shifted up to 2°C) to higher temperature. Sucrose stabilizes proteins presumably due to its suppressive effect on water activity.

DSC STUDY OF EGG PROTEIN INTERACTIONS

In this section some information will be devoted to interactions between two different protein species and protein and other compounds and how this interaction is detected by DSC. The effect of interaction during programmed heating can be manifested as changes in denaturation temperature (T_{max}), the size or shape of the transition peak or the rate of denaturation. Table 3 presents the temperatures, enthalpies, and activation energy for denaturation of the interacting proteins and their complexes. The thermal stability of avidin, a minor protein of egg white as seen by both transition temperature and denaturation enthalpy increased markedly when vitamin biotin was boud to this protein. Large increase in both the temperature and enthalpy of denaturation were observed during interaction [13]. The authors suggested that in addition to 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 ovomucoid, egg white protein, the specific inhibitor of trypsin, gave thermal curve in which the characteristic peaks of the interacting protein species were not longer present,

Table 3. Denaturation temperatures (T_{max}), transition enthalpies (ΔH) and activation energies (E_a) of protein and protein complexes

Protein or complex	T_{max}	H kcal/mol	Ea kcal/mol
Avidin[a]	85	298	90
Beta-trypsin[b]	72	194	63
Ovomucoid[b]	79	153	74
Conalbumin[c]	63	320	—
Avidin-biotin complex[a]	131	1065	—
Beta-tripsin-ovomucoid complex	81	350	77
Differic-conalbumin[c]	83	630	—
Aluminium-conalbumin[c]	72	400	—

[a][13], [b][10], [c][14].

being replaced by a new higher temperature peak of the complex. Kinetic treatment of the denaturative data suggested that thermal denaturation of protein-protein complex is not rate limited by their dissociation [10]. Interestingly, calorimetry of the ovalbumin-lysozyme system, an interaction complex that is readily detected by fluorescence polarization and sedimentation equilibrium did not demonstrate that such association took place [10]. Increases in the heat stability of conalbumin on binding Al^{+3} [14] and ions Fe^{+2} [15] were also observed by DSC.

In conclusion, DSC is still a developing technique in food research and there are clear possibilities and interesting applications yet to emerge.

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stawowych badań na izolowanych frakcjach i czystych białkach, RKS znajduje coraz szersze zastosowanie do badań zachowania się białek w naturalnych systemach żywności oraz do badania skutków procesów technologicznych, jak mrożenie, ogrzewanie, rozdrabnianie, suszenie — wywieranych na białka. Na przykładzie białek mięsa drobiu i bydła pokazano możliwość wykorzystania RKS do optymalizacji i kontroli procesów obróbki cieplnej. Pokazano również możliwość śledzenia wpływu dodatku NaCl i fosforanów na stabilność cieplną białek mięśniowych. Na przykładzie białek jaja wskazano możliwość śledzenia interakcji białek z białkami (owomukoid-trypsyna), białka z witaminami (awidyna-biotyna), stabilizacji cieplnej konalbuminy w wyniku interakcji z metalami i sacharozą.