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Protein characterization by DSC [1]

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Abstract

Proteins show a typical DSC fingerprint curve. DSC investigation may be used to study protein denaturation, and even for quality control.

INTRODUCTION

Heat is one of the most important parameters in food processing. Hence, differential scanning calorimetry (DSC) can be used as a fast characterization method for these real processes, e.g. cooking or freezing. DSC investigations provide answers to questions, for example the effects of storage, thermal processing and interactions of salts or other substances. Temperature variation influences foodstuffs in different ways by changing the phase (melting, crystallization) or the conformation (fat modifications, denaturation), or by chemical reaction (oxidation, ageing).

PROTEINS

Proteins, together with carbohydrates and fats, are the basis of foodstuffs. In addition, proteins are used to support food processing, e.g. as emulsifiers. The endothermic, mostly irreversible, denaturation of proteins strongly influences technical properties (such as water absorption and solubility) and therefore, the processing behavior of foodstuffs. Denaturation can be induced thermally and also by addition of salts, acids, bases or detergents, or by mechanical stress. Denaturation is a global term for the sum of the physical and chemical changes between the "native" and the "denatured" state. The rates of the reactions depend on the protein structure, allowing various types of polypeptides to be distinguished. Thus

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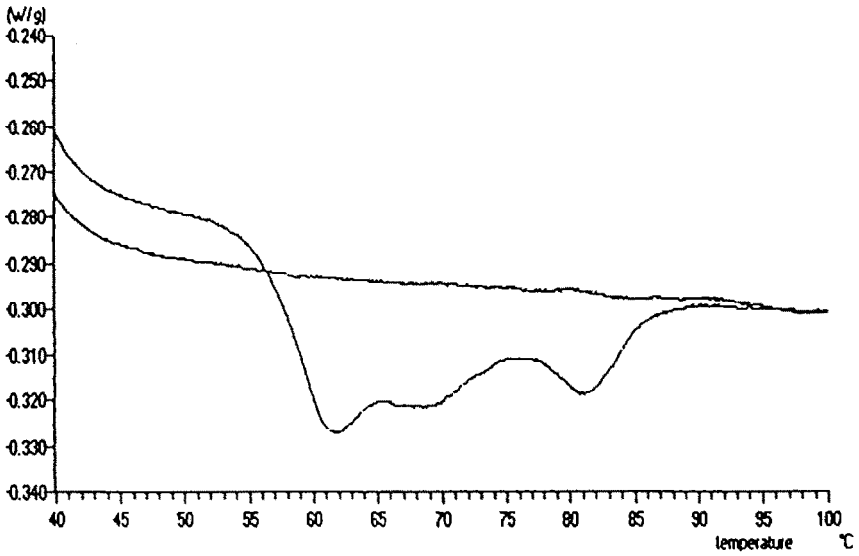

Fig. 1. DSC curve of the protein denaturation of native turkey meat: sample weight, 27.7 mg in an aluminium pan; heating rate, 10 K min^{-1} from 30 to 110°C; reference crucible with 31.4 mg silicon oil. The second run, without endothermal effects, shows the irreversibility of the denaturation.

DSC measurements show endothermic effects in the range 40–100°C, with specific peak temperatures and heats of transition (Fig. 1).

EXPERIMENTAL

The measurements were performed using highly sensitive Mettler DSC cells, e.g. the DSC12E and DSC25, in the range 30–110°C, applying a heating rate of 10 K min^{-1}. The proteins under investigation were prepared from normal foodstuffs and were hermetically enclosed in aluminium crucibles. Inert referenced material was used to compensate the heat capacity due to the high water content of the samples.

RESULTS

The denaturation of proteins was studied by DSC in order to identify the origin of the protein, and to assess the influences of ageing and acidity (pH).

Characterization by DSC fingerprint curves

The characteristic DSC peaks of the denaturation process were used to distinguish between various animal or vegetable proteins.

DSC curves of vegetable proteins show one main denaturation peak

TABLE 1

Denaturation temperatures T_{denat} and heats of denaturation $\Delta_{denat}H$ of vegetable proteins separated first at pH 4.5 and then extracted at pH 8.5, heating rate 5 K min^{-1}

Protein	Sample weight/mg	T_{denat}/°C	$\Delta_{denat}H$/J per g dry mat.
Wheat	3.44	80.3	3.5
German wheat	5.4	81.1	17.2
Lupin	6.8	91.2	4.2
Soya	4.78	95.0	5.2

with a characteristic peak temperature providing information on their thermal stability. Table 1 lists four materials with their peak temperatures.

DSC curves of meat proteins show several conformational changes, mainly for the transitions of myosin and sub-units at 55–62°C, for sarcoplasmic proteins and collagens at around 67°C, and for actin at 78–83°C (ref. 1, p. 93), see also Fig. 1. Figure 2 shows the characteristic fingerprint curves for various muscle proteins.

Denaturation by ageing

Proteins denature even at room temperature. This is detected by DSC measurements. With increasing storage duration the thermal denaturation reaction is shifted to higher temperatures in the case of egg white protein. The ovalbumin of a fresh egg denatures with an 81°C peak temperature. After 12 days' storage, S-ovalbumin has formed, which gives a transition at 91°C. Figure 3 shows this on-going shift in the peak temperature during storage. After 15 days' storage, no further change can be detected, the transition to the S-ovalbumin being complete.

Denaturation by pH changes

The pH value is an important parameter in food processing. Even a small decrease in pH starts the denaturation of proteins and generally lowers the thermal stability. As in the previous examples, DSC is used to study these effects. As an example of this behaviour, the pH of the haemoglobin of beef blood was lowered step-wise from its stabilization value of 7.06 to 3.32. Native blood has a value of 7.35, but coagulates immediately and cannot be measured without stabilization.

Below pH 3.3, the haemoglobin is fully denatured. Hence, a subsequent DSC measurement shows no further denaturation endotherm (Fig. 4). The enthalpy of denaturation is only significantly affected by lowering the pH below 5.1.

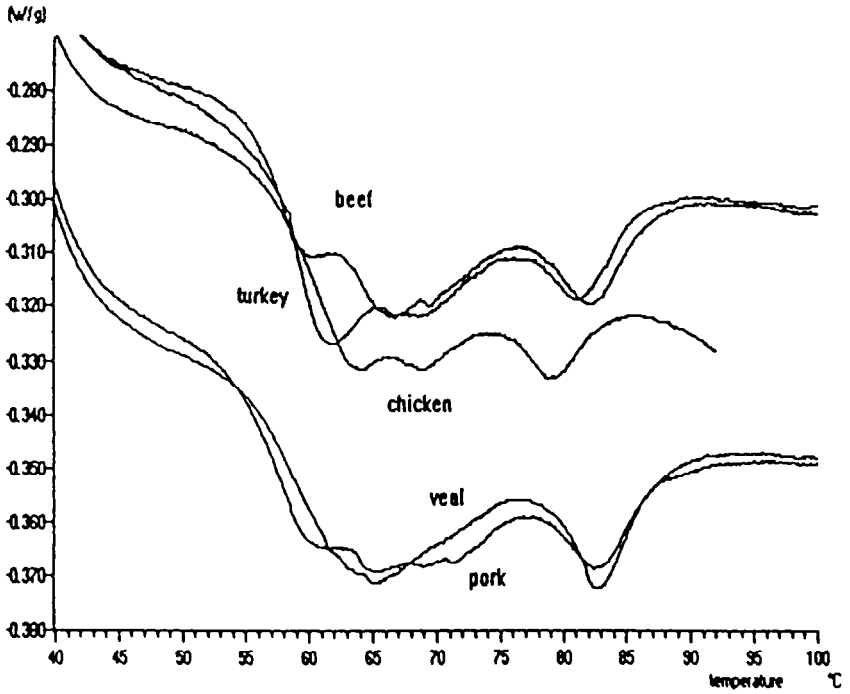

Fig. 2. DSC curves of muscle protein denaturation: sample weight, 26–32 mg; scan conditions as given in Fig. 1.

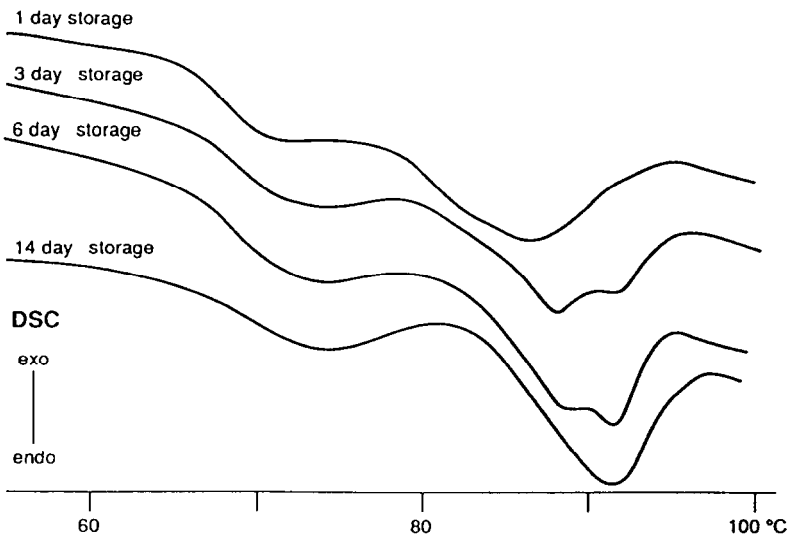

Fig. 3. DSC curves of egg white after 1, 3, 6 and 14 days' storage of the eggs in physiological salt solution at 30°C; sample weights, 27–33 mg.

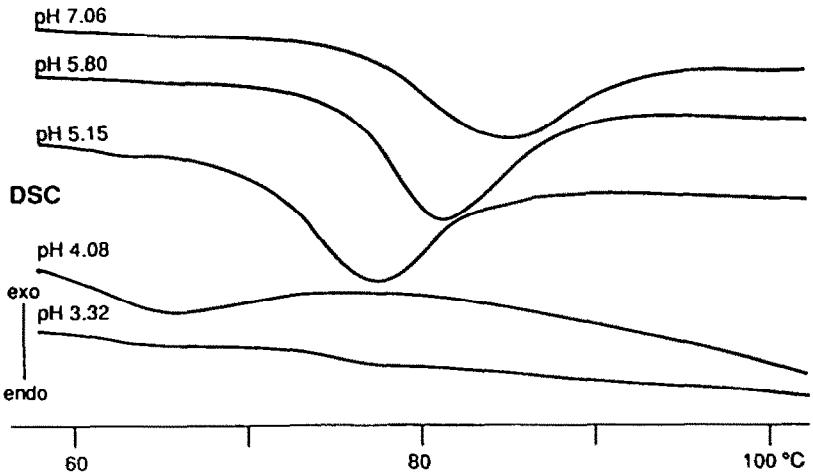

Fig. 4. DSC curves of haemoglobin of beef blood stabilized with sodium citrate; pH changes by addition of 0.5 M HCl; sample weight, 22–30 mg.

CONCLUSION

DSC measurements allow the characterization of many influences on protein denaturation. Each protein shows a typical DSC fingerprint curve, which may be used for identification purposes. More important for research and development in food processing is the investigation of additives such as salts, acids, sugars and processing detergents. DSC investigations may even be used for quality control, e.g. the detection of the influences of thermal and mechanical treatment.

REFERENCE

1 V.R. Harwalkar and C.Y. Ma (Eds.), Thermal Analysis of Foods, Elsevier Applied Science, London, 1990.