

Determination by DSC analysis of the influence of pH on meat proteins

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Abstract

In this paper the goal is, to measure meat proteins stability, as a function of an environmental perturbant. Thus, protein stability is monitored with pH and temperature of the environment variables. Several bonds which exist in the native protein decrease with decreasing pH, which leads to a decrease in the conformational stability of the protein. Differential scanning calorimetry (DSC) can provide all the thermodynamic parameters that specify the stability of the protein as a function of temperature.

Keywords: DSC, myosin, sarcoplasmatic proteins, actin, denaturation, pH

1. Introduction

The pH values of meat and meat products generally varies between 4,6 in raw-dry meat products and 6,4 [2], pH of meat has a high influence on water holding capacity and affects the colour of meat.

For raw-dry meat products chemical acidulants are sometimes used as an alternative to adding a microbial starter culture and to accelerate the reduction in pH [8]. The most commonly applied acidifiers are GDL, citric acid, and lactic acid, for rapid acidification effect, they may reduce the risk of spoilage and bacterial contamination, lead to a prolonged shelf life, reduce the necessary amount of nitrite, improve colour stability, and improve firmness and sliceability of the end-product [5].

Differential scanning calorimetry (DSC) is a powerful technique to characterise the energetics and mechanisms of temperature-induced conformational changes of biological macromolecules [4].

This technique allows highlighting different temperatures at which the thermal denaturation of the major structural protein species in porcine muscle: myosin, sarcoplasmatic proteins, collagen and actin.

2. Materials and Method

Bovine Semimembranosus were removed from the carcass at 1 day postmortem and stored into a hermetic package at 4⁰C until analysis. To change the pH, lactic acid having a concentration of 5% and NaOH 0,5n (both from Merk) was added.

pH of samples analysed is presented in Table 1

Table 1. pH of samples analysed

Describing of samples preparation	Sample symbol	pH
Bovine Semimembranosus	Pp1	5,7
Bovine Semimembranosus with lactic acid	Pp2	3,3
Bovine Semimembranosus with NaOH	Pp3	6,3

For pH analysis SR ISO 2917 : 2007 method and a pH-meter from Thermo Scientific with food industry specific electrodes for meat, semi-frozen products applications FC 230 Series from Hanna Instruments were used.

A TA Instruments, differential scanning calorimeter (DSC), model SDT Q600, with computer-assisted data acquisition and curve sensitivity analysis function, was used for all thermal analysis. Changes in the structure of the protein during heating, are much clearer if use the 2nd order derivative of the heat flow.

Small pieces of meat, free from visible traces of fat and connective tissue, were used into alumina pans. At least 3 samples with 10 to 20 mg meat each weighed accurately to 0.001 mg by an electronic balance were used for each individual sample. The samples were scanned at 10°C/min at 20 to 120°C under dry nitrogen purge of 20 mL/min [1].

3. Results and Discussion

Differential scanning calorimetry curves for myofibrillar proteins from porcine Semitendinosus muscle, at different pH, are shown in Figure 1.

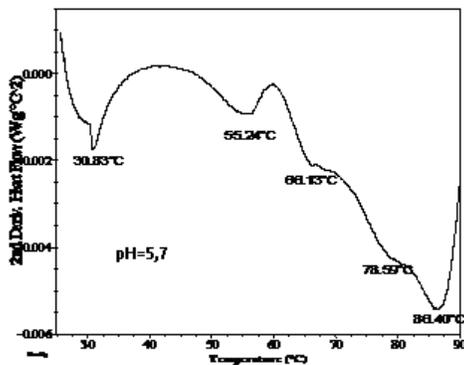


Figure 1. Differential scanning calorimetry curves for myofibrillar proteins from Bovine Semimembranosus muscle, at pH = 5,7

For meat with pH 6,1 as shown in Figure 1, five temperature peaks are distinguished. First peak correspond to temperatures of 30,83°C represent fat melting and is approximately constant for all pH values (Figure 2). 55,24°C and 66,13°C represents the endothermic transitions of myosin because this is a multidomain protein; the domains unfold independently of each other.

The number of endothermic transitions for myosin varies with species, salt concentration or ionic environment, and buffer conditions [11]. Smyth, et.al. (1996) obtain for chicken breast muscle ten independent transitions peak between 40°C and 67°C [9] and Howell et.al. found the peak maximum temperature of transition of myosin in the myofibrils at 60°C in the bovine *M. semimembranosus* [3]. The next endothermic transitions, at 78,59°C correspond to sarcoplasmatic proteins. Salvador et.al. (2009) found the denaturation temperature corresponding to porcine hemoglobin concentrates $T_d = 77,8^{\circ}\text{C}$ [7]. Last transition at 86,40°C corresponds to actin denaturation, which is the most thermostable myofibrillar protein.

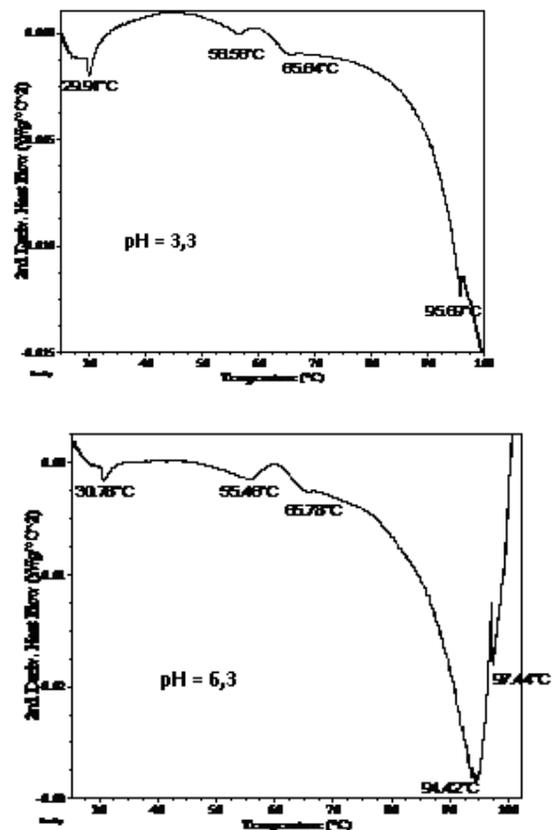


Figure 2. Differential scanning calorimetry curves for myofibrillar proteins from Bovine Semimembranosus muscle, at different pH

Wright and Wilding reported that in bovine muscle semimembranosus the high temperature peak (80°C) is due to actin denaturation, while the two peaks with values 59°C and 66°C are representing the denaturation of myosin [10].

By adding lactic acid or sodium hydroxide to change the pH of meat, as shown in Figure 2, no significant changes occur for the temperature at which thermal denaturation occurs for myosin. One possible explanation is that, compared with the head portion, which is relatively rich in hydrophobic amino acid residues, the rod portion contains a high proportion of charged side chain groups, such as argininy, glutamyl, and lysyl residues [11], which leads to a greater stability to pH variation of environment.

On the other hand, it is notice the disappearance of the transition state corresponding to temperature of 78,59°C. The same is observed for swine muscle Semitendinosus for pH 5,6 and 5,3 (results were not yet published).

A special situation occurs in the case of actin. By changing the pH of the denaturation temperature increases, and for pH = 6.3 a new endothermic peak appear at a higher temperature (97,44°C)

This may occur lowering pH stabilises the F-actin structure by increasing the free energy change associated with its polymerization [6].

4. Conclusions

The molecular transition temperature, i.e., the point at which major changes in conformation occur, can be determined by different techniques, mainly by differential scanning calorimetry. The susceptibility of proteins to thermal denaturation depends on their structure, predominantly on the number of cross-links, as well as on the simultaneous action of other denaturing agents

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