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DIFFERENTIAL SCANNING CALORIMETRIC INVESTIGATIONS ON PYLORIC CAECA DURING RIPENING OF SALTED HERRING PRODUCTS

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Abstract

The thermoanalytical behaviour of pyloric caeca during salting and ripening was investigated using a Perkin Elmer DSC 7. Not only the thermal stability of the muscle proteins was influenced by salting but also that of pyloric caeca. It was recognised that the salting itself leads to a remarkable increase of the transition temperature compared with raw herring. An influence of the salt:fish ratio could be observed. The higher the salt content the higher the increase of the denaturation temperature. During ripening the transition temperature remained on a high level or showed only a slight decrease during the investigation period. The dependency from the salt content remained evident. The increase of the transition temperature was accompanied by a decrease of the transition enthalpy.

The increase of thermal stability is connected with a decrease of the general proteolytic activity in pyloric caeca. Possibly, the enzymes are diffusing from the pyloric caeca into the muscle and cause there an increase of enzymatic activity observable in North Sea herring accompanied by a decrease of activity in pyloric caeca itself. Simultaneous the thermal stability of pyloric caeca is lowered. The reason for the differences in ripening could be seen in some enzyme-inhibiting factors unknown until now.

Keywords: DSC, fish, general proteolytic activity, pyloric caeca, ripening, salted herring product

Introduction

Since ancient times salting and ripening of pelagic fish species have been performed on an empirical basis. In the medieval ages, ripened salted Baltic herring (mainly the ‘Skåne herring’) was one of the most important commodities traded in the Hanseatic League. Currently, large amounts of Baltic herring are still used for processing a broad range of fishery products, however, the processing of ripened salted herring products has lost more and more its importance compared to herring caught in the North Sea [1]. The complex ripening process consists of chemical and biochemical reactions that change the characteristics of the fish tissue and thus the sensory properties of the fish. Changes in properties which influence the overall quality (especially texture), appearance and sensory properties that occur as result of the salting and ripening that are required to transform the raw fish (Fig. 1) into the final salted herring product (Fig. 2) are still unclear despite of the long tradition in mak-

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ing these procedure. This was the reason for fishery research institutes of eight European countries to perform investigations on enzymatic ripening of pelagic fish species partly financed by the European Commission (AIR 2 CT 93 11 41). The German part included the characterisation of some of the alterations in the proteins of fatty fish during salting, ripening and storage as well as studying the influence of different herring stocks using various physical, (bio)chemical and sensory methods. Ripening is believed to be caused mainly by enzymatic actions. The enzymes split macromolecules such as protein and fat into low-molecular-mass compounds. Endogenous proteolytic enzymes from the internal organs of herring like pyloric caeca are considered, beside the cathepsin-like enzymes of the muscle, to be of major importance [2–4].

Pyloric caeca are part of the intestine of many fishes. Their function probably involve both digestion and absorption [5]. They are a source of carbohydrate-, fat- and protein-digesting enzymes [6].

Differential scanning calorimetry (DSC), is known as a technique [7] in which the difference in energy inputs into a substance and a reference material is measured as a function of temperature while the substance and reference are subjected to a controlled temperature programme. Any thermally induced changes (e.g. protein denaturation) are recorded as a differential heat flow and displayed as a peak on a thermoanalytical curve. Among the most important applications of DSC are identification and purity analysis, and the determination of characteristic temperatures and enthalpies of phase transition, phase transformation and reactions [8].

For proteins, the thermally induced process detectable by DSC is the structural melting or unfolding of the molecule. The thermal denaturation of proteins is attributed to the rupture of intramolecular hydrogen bonds. Since this is an endothermic

Fig. 1 Different types of processed raw herring including pyloric caeca (gibbed herring-above; fillets-below, right; pyloric caeca-below, middle; the nobbing knife-(left) – makes the sizes comparable)

Fig. 2 Salted herring product

reaction with significant uptake of heat, protein denaturation is generally recorded as an endothermic peak in the DSC curve. The denaturation temperatures are measures of the thermal stability of proteins. Their determination under controlled conditions can provide direct comparison of the thermal stability of the different proteins. The enthalpy value is correlated with the net content of the ordered secondary structure of a protein. It is actually a net value from a combination of endothermic reactions and exothermic processes, including protein aggregation and the break-up of hydrophobic interactions [7].

The effect of the addition of different kinds and amounts of salts on the thermal behaviour of proteins has been widely studied. The results obtained to date show that meat proteins such as myosin and actin were destabilised by increasing salt concentrations [9]. The addition of salt in a range between 1 and 4% lowered the enthalpies and denaturation temperatures in actin and myosin of chicken and fish [10–12]. The thermal stability of collagen was reduced at low salt concentrations (smaller than 0.3 M) but at higher levels (0.3–2.0 M) T_{max} (peak maximum temperature) was increased, indicating stabilisation [7].

Very recently, using DSC investigations have been performed about the influence of post mortem states on the water holding capacity of various fish species [13], the suitability of shark minces for processing into surimi [14] and the influence of the freshness of the raw material in this respect [15].

As yet, however, little is known about the effect of salting and prolonged storage of salted herring products on the thermal stability of proteins during ripening. Salting of salmon in 15% brine completely changed the thermoanalytical curve of salmon muscle after a storage of ten days. Myosin suffered the strongest denaturation [16].

In this study the thermoanalytical behaviour of fish muscle, skin and, especially, pyloric caeca (Fig. 1) during salting and ripening was investigated using differential scanning calorimetry and compared with selected results achieved by other methods. It could be assumed that both denaturation temperature and enthalpy are influenced by salting and ripening.

Materials and methods

Salting of herring

The raw herring was caught in the North Sea and in the Baltic Sea in spring. The herring sample from the North Sea was caught with a bottom trawl on different catching grounds. The Baltic Sea herring was caught with gill net in a shallow-water area (Greifswalder Bodden) south of the Island of Rügen or north of Cape Arkona by a commercial fishing vessel. The herring was predominantly in pre-spawning conditions and, immediately after hauling, whole herring was used to prepare different styles of salted herring with different salt:fish ratios, i.e. 1:4 (heavily salted), 1:7 (moderately salted) and 1:10 (lightly salted). Salting and storage was performed as described earlier [1]. The fat content of the raw material was approximately 10% and the resulting salt content in the fish flesh were around 16, 11.5 and 8.5%, respectively.

Differential scanning calorimetry

The differential scanning calorimetry was performed with a Perkin Elmer DSC7 (Überlingen, Germany) fitted with a Colora-cooling equipment (Kryo-Thermostat WK 5, Colora GmbH, Lorch, Germany) at ambient temperature [17]. The instrument was calibrated for temperature and enthalpy using indium and naphthalene as standards. The fish samples (15±3 mg), separated into the different parts, where weighed accurately (0.1 mg) into 30 µl aluminium pans (BO 169 320) and sealed. At least triplicate samples were heated from 25 to 95°C at a scanning rate of 10 K min^{-1}, with an empty sealed pan as a reference. The transition temperature (T_{max}) was recorded. The transition enthalpy was determined from the peak area using the Perkin-Elmer software and expressed in J g^{-1} sample material.

General proteolytic activity

The general proteolytic activity (*GPA*) in fish muscle and pyloric caeca was measured at pH 8 and at 25°C using azocasein as the protein substrate [18].

Results and discussion

Our own results make clear that the different body components of the unprocessed herring can be differentiated by the shape of their thermoanalytical curves (Fig. 3).

At least four transition peaks could be detected in the thermoanalytical curves of both white and red muscle. Other authors [11, 12] have concluded that transition peaks II, III and IV are mainly connected with myosin, sarcoplasmic proteins – possibly together with collagen – and actin, respectively. As demonstrated in the thermoanalytical curve of the skin sample, peak I must be connected to collagen as the major protein component of the skin connective tissue.

Beside these outer parts of the fish body, the thermal transitions of the pyloric caeca were characterised. The pyloric caeca of the unsalted herring show a sharp transition peak in the temperature region of approximately 50°C. The peak is still detectable after one year of frozen storage. However, there seems to be a marked decrease of transition enthalpy (ΔH) with prolonged frozen storage (Fig. 4).

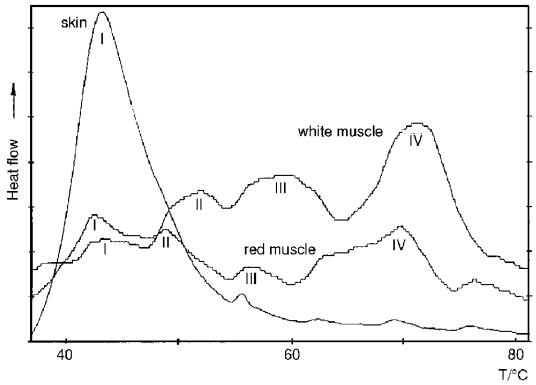

Fig. 3 Thermoanalytical curves of unprocessed herring muscle (I–IV see text) [17]

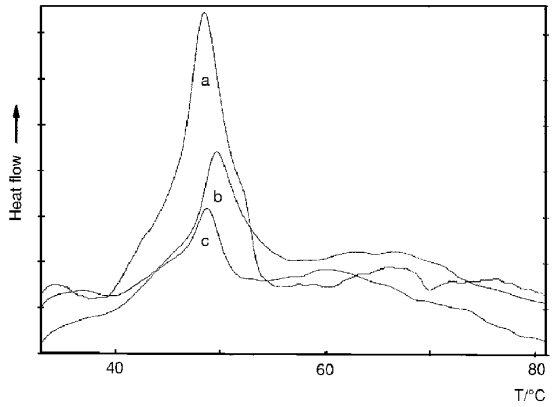

Fig. 4 Thermoanalytical curve of pyloric caeca after frozen storage at –20°C for a – 1; b – 16 and c – 52 weeks [17]

The salting process completely changes the pattern of the thermoanalytical curves. One month after heavy salting, the curves of both white and red muscle samples are reduced to only one major transition peak (Fig. 5).

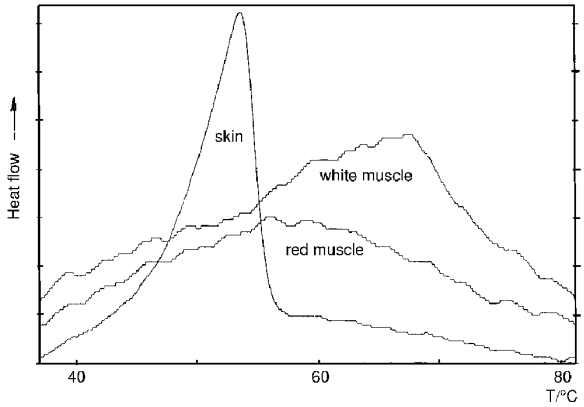

Fig. 5 Thermoanalytical curves of heavily salted herring muscle [17]

In white muscle the peak seems to be situated in the region of actin transition, and in red muscle, in the region of the sarcoplasmic proteins. In both cases there seems to be a decrease in the transition temperature (T_{max}) of the main protein components mentioned above. This is in contrast to plant proteins where the addition of salts causes an increase in T_{max} [7].

It seems that the very high amount of NaCl added to the herring causes a strong denaturation of the muscle proteins almost immediately after salting. As a consequence of this denaturation the thermal properties (thermal transitions) of the muscle proteins measured by DSC change dramatically so that a further denaturation influenced by the duration of salting is not detectable. On the other hand no new thermal transitions appeared, suggesting that no new breakdown products were formed.

However, organoleptically the sample was evaluated as a ripened product with specific odour and taste. Due to their complexity the thermoanalytical curves were difficult to interpret.

The thermoanalytical curve of pyloric caeca of the salted product shows a broad peak distinct from the skin peak (Fig. 6) and an increase in the transition temperature of approximately 10 K compared to the transition temperature of frozen unprocessed pyloric caeca. There are marked differences between the transition enthalpies of pyloric caeca (ΔH=0.55 J g^{-1}) and of the skin collagen (ΔH=3.95 J g^{-1}). Due to the single peak of both the pyloric caeca and the skin changes during ripening could be followed easier.

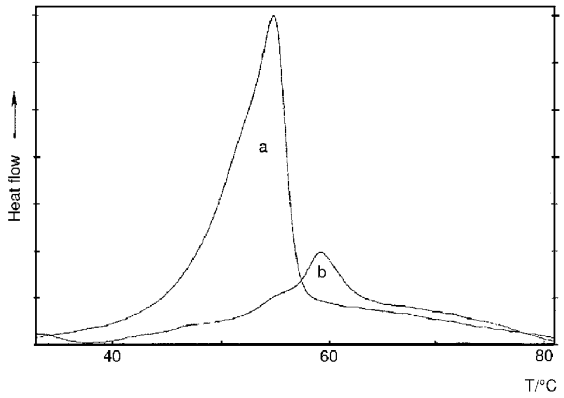

Fig. 6 Thermoanalytical curves of skin (a) and pyloric caeca (b) of heavily salted herring [17]

The increase of the transition temperature of pyloric caeca tends to be strongly influenced by the salt:fish ratio applied (Fig. 7). This increase of thermal stability is obviously only temporarily. During ripening, a decrease of T_{max} could be observed. Regarding the salt:fish ratio comparable influences are observable and in the case of lightly salted herring, T_{max} goes almost down to the amount of raw herring. The changes of enthalpy during ripening were comparable (Fig. 8). After an initial brief increase of ΔH

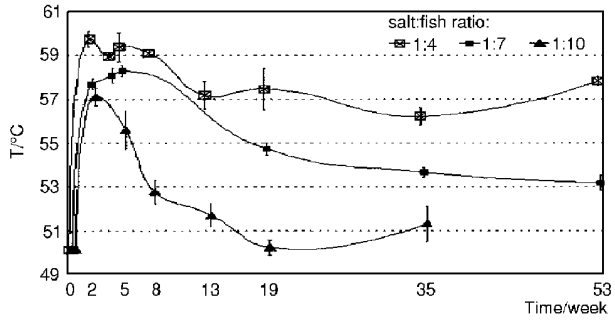

Fig. 7 Denaturation temperature of pyloric caeca during salting and ripening of herring dependent on salt:fish ratio

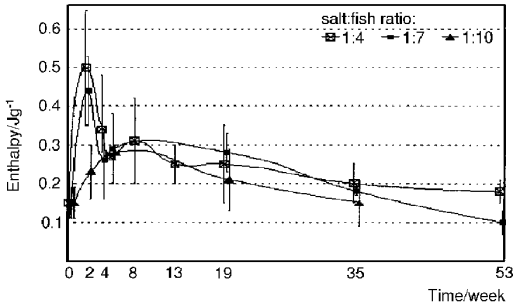

Fig. 8 Denaturation enthalpy of pyloric caeca during salting and ripening of herring dependent on salt:fish ratio

the enthalpy values are approaching the value of raw material. The influence of the salt content seems not as clear as in the case of transition temperature.

In DSC studies performed on yellowfin tuna the greater susceptibility of both the myoglobins and apomyoglobins to heat denaturation compared with the mammalian counterparts was confirmed [19, 20]. The denaturation temperature as well as the denaturation enthalpy decreased after freeze/thaw treatments [21].

Very recently, the effect of pressure/heat combinations on blue whiting washed mince was investigated using DSC [22]. It has been found that at nondenaturing temperatures, the higher the pressure, the larger was the protein unfolding effect. However, at denaturing temperatures, pressure prevented fish protein from subsequent thermal denaturation. In this way, DSC demonstrated that pressurised samples underwent both types of pressure effects at the same time.

To evaluate results of thermoanalytical behaviour with respect to the ripening, changes of the general proteolytic activity (*GPA*) in pyloric caeca (Fig. 9) should be taken into account. The *GPA* decreases and this decrease seems to follow a potential regression. In this respect, no differences between the various herring stocks could be observed.

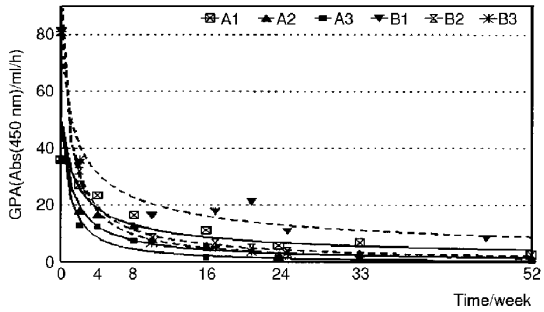

Fig. 9 General proteolytic activity (*GPA*) in pyloric caeca of herring during salting and ripening (A – Baltic Sea; B – North Sea; 1 – heavily salted; 2 – moderately salted; 3 – lightly salted)

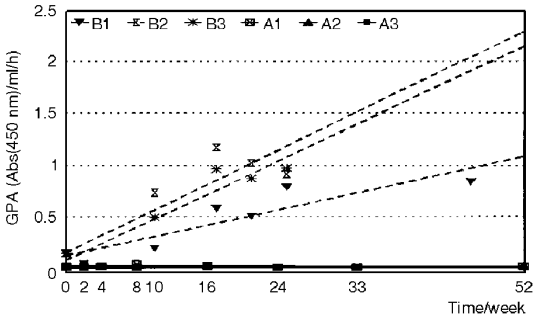

Fig. 10 General proteolytic activity (*GPA*) in muscle of herring during salting and ripening (A – Baltic Sea; B – North Sea; 1 – heavily salted; 2 – moderately salted; 3 – lightly salted)

However, to explain the differences observed in the ripening of the Baltic and North Sea herring caught in spring it could be assumed that one reason the differences of *GPA* in muscle will be [23]. The *GPA* in the muscle of Baltic herring was very slow and remained almost unchanged, while a continuous increase in the muscle of North Sea herring was observed during ripening (Fig. 10). These results allow the conclusion that during ripening an increase of thermal stability is paralleled by a decrease of its *GPA*. Derived from these results it could be hypothesised that enzymes are diffusing from the pyloric caeca into the muscle and cause there an increase of enzymatic activity observable in North Sea herring accompanied by a decrease of activity in pyloric caeca itself. Simultaneous the thermal stability of pyloric caeca is reduced. Reasons for the differences in ripening could be seen in some enzyme-inhibiting factors unknown until now. Therefore further research activities are needed.

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