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Differential scanning calorimetric (DSC) measurements on the roe of rainbow trout (*Oncorhynchus mykiss*): influence of maturation and technological treatment☆

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Abstract

The roe of rainbow trout was investigated with respect to the effects of maturation and technological steps on the differential scanning calorimetric (DSC) curves of yolk proteins. The DSC curves were markedly effected during the first phase of maturation, while on the whole almost no changes were visible in the later stage. DSC curves measured on yolk proteins of rainbow trout were clearly discernible from those of milt and muscle proteins of same species. Applying different technological treatments on the roe it became clear that freezing and frozen storage have only a minor influence on thermal behaviour of yolk proteins. On the other hand, lightly salting, heating on 90 °C and high pressure processing (thawing) change the DSC curves markedly indicating a stronger denaturation of yolk proteins by these treatments. However, compared to the muscle proteins of rainbow trout the influence of high pressure was less. DSC curves of yolk proteins are influenced by fish species. However, it seemed not possible to use them for discrimination of species of the same family.

Keywords: Differential scanning calorimetry; Fish; Roe; Maturation; Technological treatment

1. Introduction

Fish, except for some species such as the redfishes (*Sebastes* spp.) belong to oviparous vertebrates, i.e. to reproduce the ovulated eggs of a female fertilised outside her body by sperm produced by males. Eggs develop and mature inside the gonads of the female from very small-sized eggs to ones with remarkable size. During maturation of the eggs, food is accumulated within them by a process called vitellogenesis. During this process proteins, lipids, carbohydrates and mineral ions are deposited in the egg. These substances are synthesised outside the eggs in the liver of the female and are transported during vitellogenesis through blood to the ovary building up the egg yolk [1–3]. This exogenous vitellogenesis is considered to consist of two phases. The first phase involves the induction of hepatic vitellogenin (VTG) production under stimulation of ovarian estrogen [4]. During the second phase VTG is taken up from the blood stream and incorporated into ovarian yolk proteins. The main protein of egg yolk VTG is a glycophosphoprotein which is proteolytically cleaved into smaller proteins in the egg which are lipovitellin (LV)—a lipid-rich protein, phosvitin (PV)—a smaller protein which contains phosphorylated proteins and proteins called β′ components [5]. A lipid content of 25% and an alkali-labile phosphorus content of 0.007% have been found for trout LV, whereas trout PV has an alkali-labile phosphorus content of 15.8% [2].

When questions concerning egg quality arise, different answers are possible. From the reproduction point of view, good quality eggs are usually marked by their excellent survival rates during incubation and the quantity of fry hatched. Various biotic and abiotic factors have been implicated as possible determinants of egg quality and survival, including among other things the chemical composition, physical dimensions of the egg and the quality of the sperm [6]. Despite the presence of clear differences in chemical composition between eggs of different strains of fish as well as between individuals of the same strain and between fish maintained on different diets and water supplies, there appears to be no

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correlation of these differences with the subsequent survival of the eggs and the fry. Furthermore, the effects of egg size on egg survival remain controversial. Some authors suggest that smaller eggs have poorer chances of survival, whereas others argue that size has no effect on hatching and survival [7].

Quit different answers are obtained when the quality of eggs is examined from the point of using roe for human consumption [8]. Ovaries must first be graded according to maturity and freshness, the eggs are than graded by colour and size (as in the case of sturgeon). For example, for processing salmon roe as caviar, the ovaries are most valuable when they are at stage IV (in the range between I and VI), while salmon eggs at stage V have membranes which are too thick to be really acceptable for good caviar. No matter what, the roes are best when taken from freshly killed fish, before the onset of rigor mortis. The quality of the finished product (the caviar) is evaluated by organoleptical (smell, appearance and taste), chemical (salt, pH value, volatile nitrogen components) and microbiological (total viable count) methods [9–11]. The nutritional value of fish eggs is characterised by their chemical composition which fluctuates according to geographical area and yearly season. Protein and fat are considerably higher in fish eggs than in the flesh of the fish itself, the egg fat content being highest when they are immature.

To characterise the chemical composition of roe and caviar, common analytical methods are used. Furthermore, for the determination of changes in the yolk proteins due to maturation of the eggs, gel chromatography, electrophoresis, immunoblotting and amino acid sequence analysis are investigation methods that may be used [12,13]. However, as far as it is known to the author, differential scanning calorimetric (DSC) measurements are not used to characterise changes in fish roe or yolk proteins as a result of the processing or maturation. On the other hand, differential scanning calorimetry is frequently applied according to papers published recently which deal with fish quality and processing [14–19]. DSC has emerged as a technique of choice for the study of thermal transitions of food. The conversion of a protein from a native to a denatured state by heat is a co-operative phenomenon and is accompanied by a significant uptake of heat, seen as an endothermic peak in the DSC curve. For proteins, the thermally induced process detectable by DSC is the structural melting or unfolding of the molecule, thermal denaturation of proteins being attributed to the rupture of intermolecular hydrogen bonds, the temperatures at which the bonds rupture being a measure 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 which is correlated with the net content of the ordered secondary structure of a protein, is actually a net value obtained through the combination of endothermic reactions and exothermic processes, including protein aggregation and the break-up of hydrophobic interactions. A successful approach to the study of the native conformation of proteins is the subjection of the protein to physical and chemical stresses, followed by a determination of the effect of these stresses on its thermal denaturation.

The object of the present study was the question on the strength of DSC pattern measured on fish eggs. Furthermore, the influence on the DSC curves of maturation, species and technological treatment was investigated. Finally, the suitability of DSC for species identification was considered. As a model, chicken eggs were used and the results of DSC measurements performed on both egg white and egg yolk are reported [20–23].

2. Experimental

2.1. Differential scanning calorimetry

The measurements were performed using a Perkin-Elmer DSC 7 device equipped with a Perkin-Elmer Intra cooler II and Pyris software. The fish samples (15–30 mg) were weighted (±0.1 mg) into 60 μl stainless steel pans (LVC 0319-0218) and sealed. At least triple samples were heated from 10 to 145 °C at a scanning rate of 10 °C/min with an empty sealed pan as reference. The instrument was calibrated for temperature and enthalpy using indium and naphthalene as standards. The transition temperature (T_{max}) was recorded and the transition enthalpy (ΔH) was calculated from the peak area using the Perkin-Elmer software and expressed in J/g sample material. Results are displayed as average curves in the figures.

2.2. Fish roe

Fish roe was collected from freshly killed rainbow trout from fish farms throughout the course of a year in order to collect different stages of maturation. The samples were packed in plastic bags and stored in ice for investigation within 24 h. On an average the fish weighed approximately 1.5 kg, but for special questions much heavier specimens was used to obtain eggs (Fig. 1). To measure the influence of technological treatments, the following operations were undertaken:

- Roe was packed in plastic bag and was quickly frozen at −24 °C and stored at this temperature for further investigation.
- Roe packed in plastic bag was heated in water at given temperature of 70, 80 or 90 °C for 5 min to insure that the core temperature was same as that of the bath. After cooling by immersion in tape water, measurements were made.
- Roe were left in a solution of common salt (6%) in a ratio 1:2 (w/w) for 23 and 92 h, the DSC measurements were made and additional the salt content was measured according to the § 35 method [24].

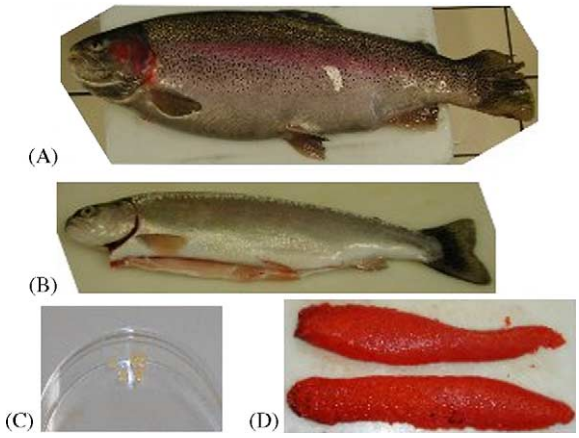


Fig. 1. Rainbow trout and roe used for DSC measurement: (A) rainbow trout, weight 6 kg; (B) "portion" rainbow trout, weight ~1.5 kg; (C) individual eggs; (D) ovary weight 900 g (15%).

- Frozen roe was high pressure assisted to thaw at 200 MPa for 60 min [25] and DSC measurements were made for comparison to conventional thawing (water bath at 15 °C). Furthermore, conventionally thawed roe were additionally pressurised at 200 MPa for 30 min and measured by DSC.

Roe of several marine fish species, collected and frozen during research trips of the FRV "Walther Herwig III", were used for DSC measurements as were also trade samples of sturgeon caviar acquired from different caviar merchants in Germany. As a model for DSC measurements on yolk proteins, hen eggs were investigated within 24 h of having been laid.

Proximate composition and pH of roe from rainbow trout were assessed using German standard methods [26]. The influence of high pressure treatment an colour was checked using a spectral colorimeter spectro pen® (Dr. Lange, Düsseldorf, Germany), while the protein pattern of the roe of some fish was verified by SDS-PAGE according to Rehbein [27].

3. Results and discussion

3.1. Comparison of protein pattern measured by DSC on muscle and roe of rainbow trout

Fig. 2 reveals marked differences between the curves measured on muscle and roe. The DSC curve for muscle shows the well-known three peaks (I–III) which can be assigned to the following protein fractions with increasing temperature: myosin, sarcoplasmic/connective tissue proteins and actin. The curve derived for roe however, shows only two distinct peaks (IV and V) with transition temperatures quite higher than compared to those of muscle. Since it is known that the major yolk protein VTG is enzymatically cleaved to LV and PV during maturation, and that LV furthermore cleaves to light chain- and heavy chain-LV as reported at later stage of maturation [28], it is therefore assumed that these peaks can be assigned to those yolk proteins. However, a clear differentiation is not possible at the moment.

Using SDS-PAGE electrophoresis, specific pattern of yolk proteins dependent on fish species can be seen (Fig. 3). For rainbow trout, two main protein fractions are discernible, one with molecular weight (MW) >100 kDa and another with MW between 14 and 20 kDa. The first MW group can possibly be assigned to heavy chain LV, whereas the second can include light chain LV and/or PV. Molecular weights for LV and for PV reported in the literature range from 130 to 390 and from 19 to 45 kDa, respectively [29].

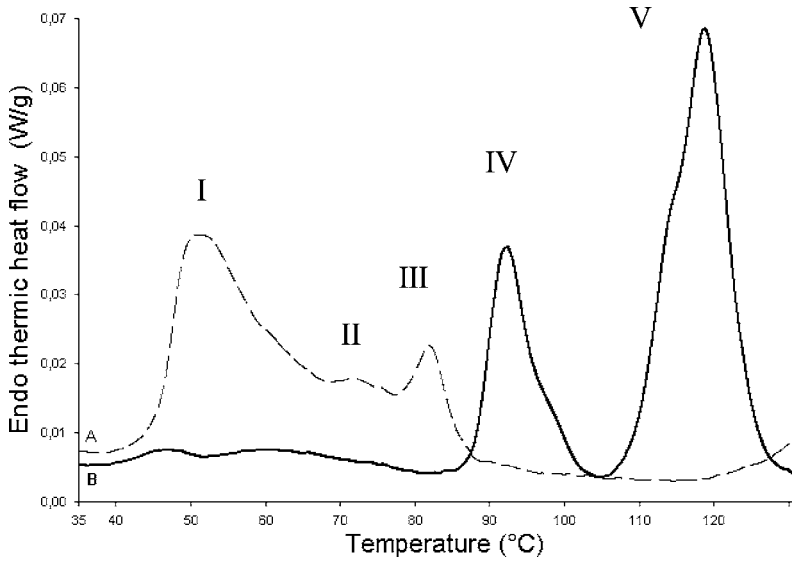


Fig. 2. Comparison of DSC curves measured on muscle (A) and roe (B) of rainbow trout.

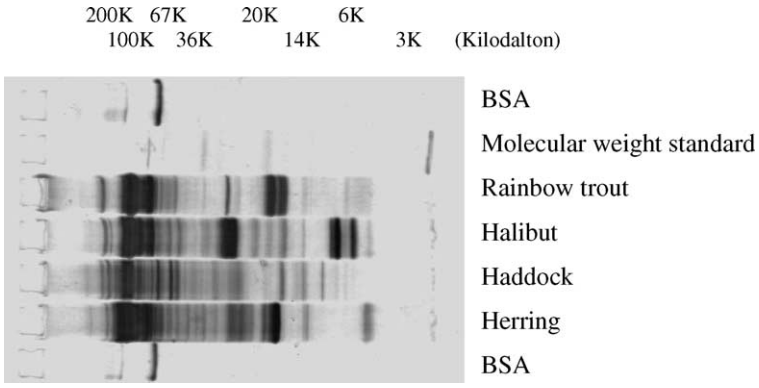


Fig. 3. SDS-PAGE electrophoresis of proteins from fish roes.

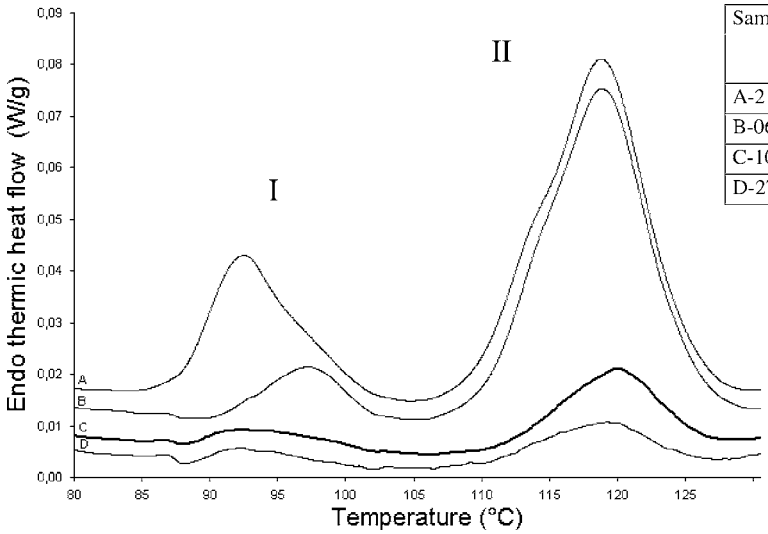
3.2. Influence of maturation on DSC curves of rainbow trout's roe

Fig. 4 shows the results of DSC measurements on the roe of rainbow trout with reference to the season of catching. While the T_{max} appeared to vary slightly during maturation, a continuous increase in ΔH can be seen, indicating that during oocyte maturation proteins accumulate in yolk. This is connected with an increase in egg diameter from 20–50 to 3500–6000 μm [3] and also with the differences in the proximate composition as seen in Table 1.

The exogenic vitellogenesis taking part during oocyte maturation can be considered to consist of two phases as mentioned above. During the second phase vitellogenin is taken up from the blood stream and incorporated into the ovarian yolk proteins [2]. Yolk is stored until the late stages of oogenesis and in the embryo, when it is mobilised to facilitate the hydration process in buoyant eggs and to provide the nutrients for the embryogenesis. It was recently found [30] that in rainbow trout, cathepsin D plays a major role in yolk processing and mobilisation, while cathepsin L also was included in both deposition and mobilisation of yolk proteins. As it can be seen in Fig. 5, the DSC curves are not influenced once a given stage of maturation has been reached. There can almost no difference be detected between the curve measured on eggs derived from a roe sac and ovulated eggs.

Table 1
Proximate composition of the roe of rainbow trout

Data (%)	Sidwell [31]	Yazbeck-Chemayel [32]	Vuorela [33]	Our results
Crude protein	27.8	27	26.6	29.2
Crude fat	9.0	10	7.6	11.2
Moisture	62.9	60	63.1	57.2
Ash	1.6	0.7	–	1.3
pH value	–	–	–	6.02



Sampling	Peak I		Peak II	
	T_{max} (°C)	ΔH (J/g)	T_{max} (°C)	ΔH (J/g)
A-21.11.02	92.5	1.22	118.8	3.62
B-06.08.02	97.2	0.39	118.8	3.47
C-10.07.02	92.7	0.17	120.0	0.74
D-27.05.02	92.3	0.12	119.2	0.40

Fig. 4. Influence of oocyte maturation of rainbow trout on DSC curves.

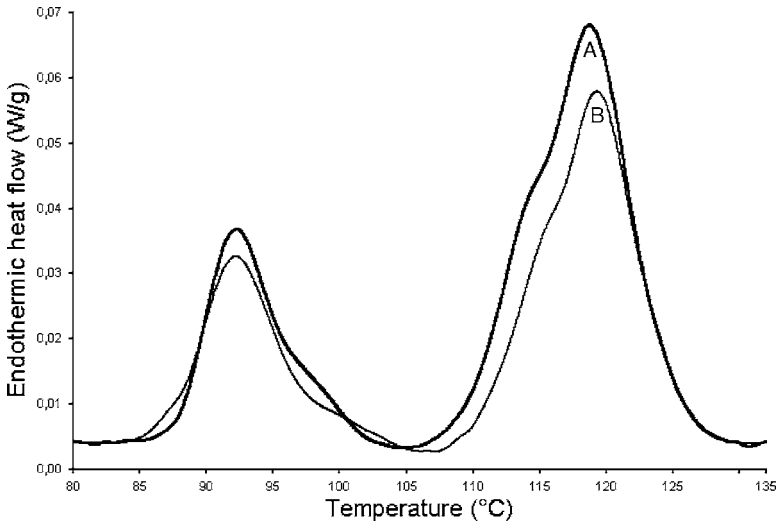


Fig. 5. DSC curves measured on ovulated eggs (A) and on roe of rainbow trout derived from the roe sac (B).

3.3. Influence of freezing and frozen storage of roe on DSC curves

Fig. 6 shows only a marginal effect of freezing and frozen storage on yolk proteins. Both transition temperature and enthalpy are hardly influenced by cryogenic treatment. This result corresponds with that of [34] where it was found that apparent viscosity of fresh and frozen–thawed yolk of chum eggs were similar and leads them to the conclusion that yolk proteins were not cryodamaged.

3.4. Influence of salting of roe on DSC curves

Salting of roe is the most important processing method that creates the most liked roe product, i.e. caviar, for the consumers. Numerous papers have been written on this subject, an excellent review being given by Sternin and Doré [8]. Modern tastes do not permit the manufacture of a product that is sufficiently salty to kill the bacteria. Caviar generally contains about 3.0–3.5% salt. Fig. 7 shows that adequate treatments affect a slight protein denaturation expressed by a small reduction of the first T_{max} and a lower ΔH especially of the second peak, already after a short action of salt.

3.5. Influence of pasteurisation of rainbow trout roe on the DSC curves

Retail packs of caviar are often pasteurised, the reported range of pasteurisation temperatures being 50–70 °C to avoid substantial coagulation of the fish eggs and thus change their appearance markedly [8]. Fig. 8 shows that only when the eggs were subjected to a temperature of 90 °C, the yolk protein represented by the first peak was fully denatured. Lower temperatures cause a small reduction of the ΔH of the first peak. On the other hand, it becomes clear that it would not be possible to differentiate pasteurised caviar from one produced without any thermal treatment.

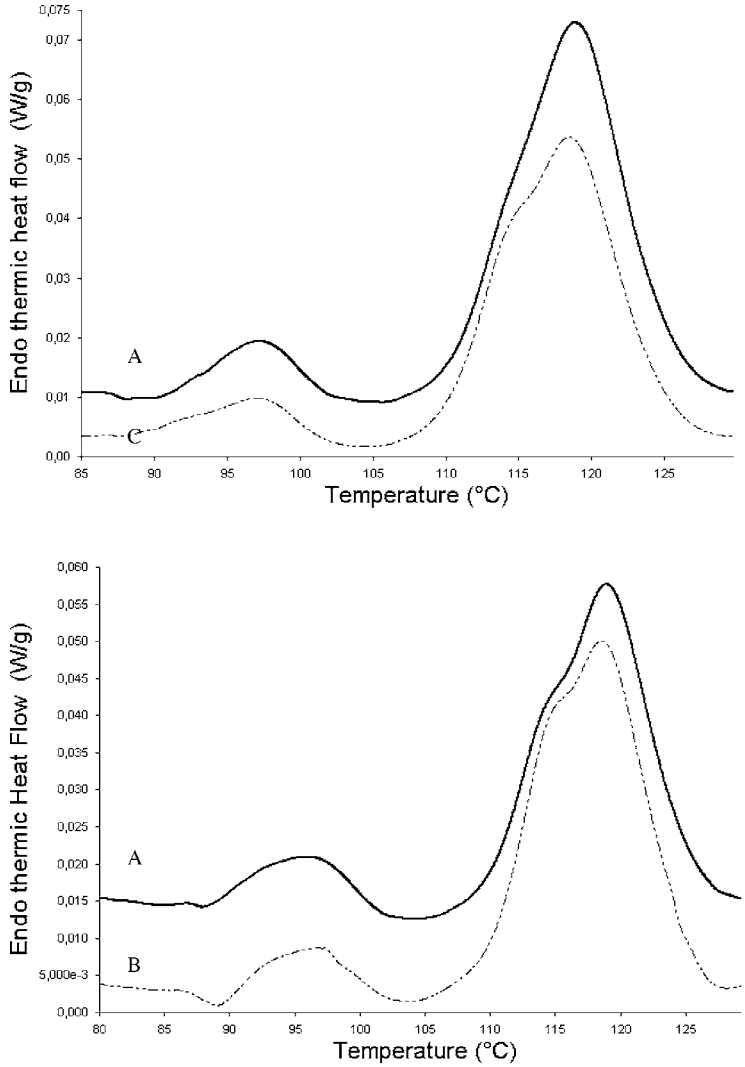
3.6. Application of high hydrostatic pressure for thawing frozen roe

The application of high hydrostatic pressure (HPP) in fish processing has become more and more the subject of research [35–38] where the interest lies in the field of thawing fish [25]. Conventional thawing was therefore compared with high pressure supported thawing. Fig. 9 demonstrates the effect of applying 200 MPa to thaw fish roe. Obviously, a partial denaturation of yolk proteins is caused by HPP, as is manifested by the shift of T_{max} of the first peak and a generally lowered enthalpy of transition.

As a further result, colour changes were monitored, expressed by great increase in lightness, slight increase in yellowness, while red persists almost unchanged (data not shown). However, when HPP was applied to already conventionally thawed roe, almost no changes in the thermal behaviour were visible. Successful attempts to reduce the bacterial load of rainbow trout roe by HPP were reported very recently [39]. Compared to fish muscle proteins, the sensitivity of yolk proteins seems to be less as can be seen in Fig. 10. When HPP thawed at the same pressure, muscle proteins were obviously largely denatured and seemed to be less stable than yolk proteins.

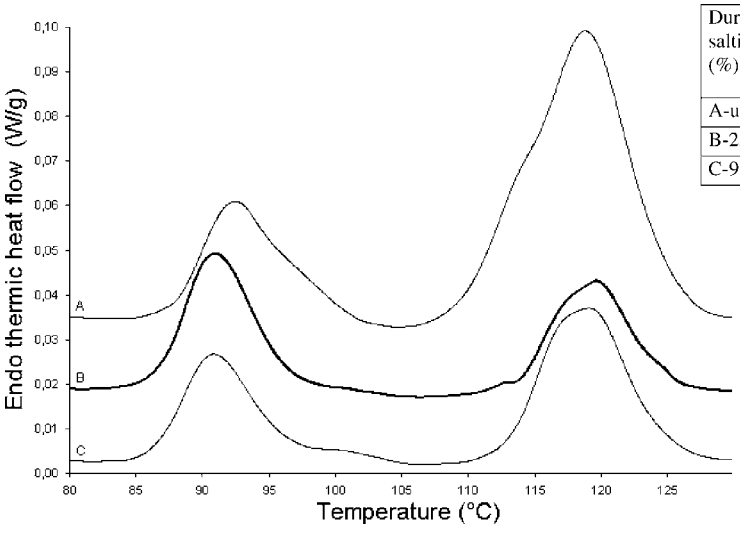
3.7. Influence of fish species on DSC curves measured on roe

In the same way that the pattern in an electropherogram characterise differences in the mobility of proteins in an electric field and allow identification of species according to their reproducible, specific protein pattern (position, intensity and number of protein bands), it should also be possible



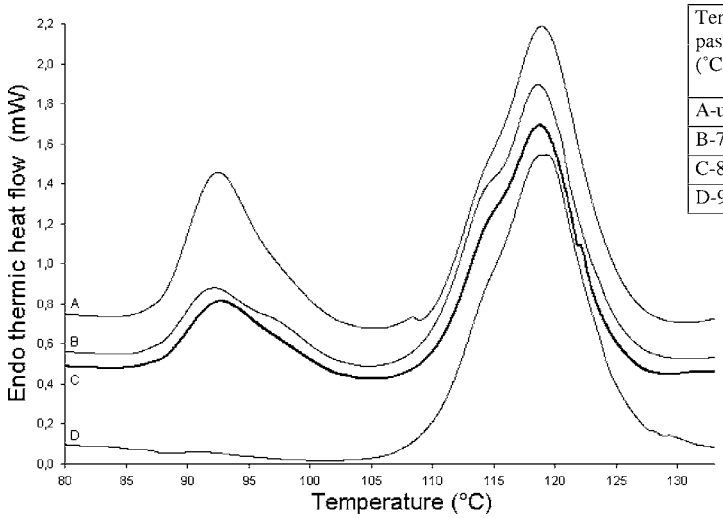
Frozen storage (months)	Peak I		Peak II	
	T_{max} (°C)	ΔH (J/g)	T_{max} (°C)	ΔH (J/g)
A-untreated	96.6	0.34	118.9	2.50
C-0.5	96.2	0.37	118.5	2.84
A-untreated	97.2	0.40	118.8	3.45
B-3.0	96.9	0.34	118.4	2.93

Fig. 6. DSC curves of rainbow trout eggs as a function of freezing and frozen storage.



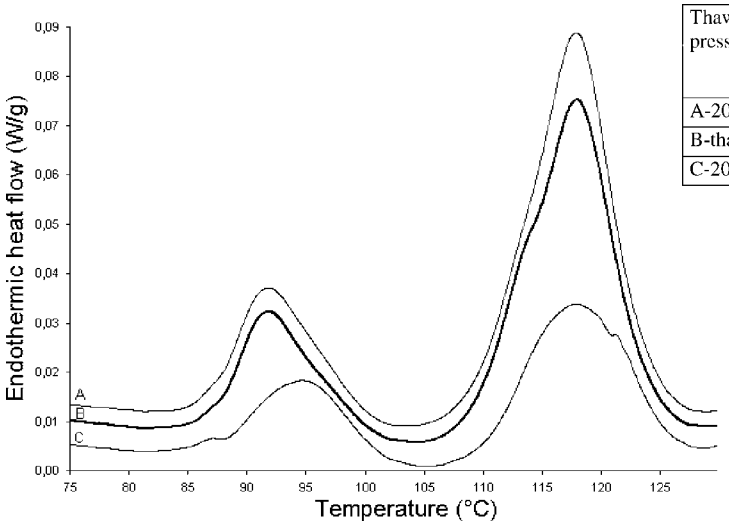
Duration of salting (h)/NaCl (%)	Peak I		Peak II	
	T_{max} (°C)	ΔH (J/g)	T_{max} (°C)	ΔH (J/g)
A-untreated	92.5	1.21	118.8	3.61
B-23/2.7	90.9	1.12	119.6	1.10
C-92/3.4	90.8	0.85	119.2	1.63

Fig. 7. DSC curves of rainbow trout eggs as a function of salting.



Temperature of pasteurisation (°C)	Peak I		Peak II	
	T_{max} (°C)	ΔH (J/g)	T_{max} (°C)	ΔH (J/g)
A-untreated	92.5	1.43	118.9	3.25
B-70	92.3	0.89	118.6	3.56
C-80	92.8	0.85	118.7	3.46
D-90	-	-	118.7	3.97

Fig. 8. DSC curves of rainbow trout eggs as a function of pasteurisation.



Thawing/High pressure treatment	Peak I		Peak II	
	T_{max} (°C)	ΔH (J/g)	T_{max} (°C)	ΔH (J/g)
A-200 MPa/30 min	91.9	1.26	117.9	3.99
B-thawing in water	91.9	1.12	117.9	3.53
C-200 MPa/60 min	94.8	0.90	117.8	1.88

Fig. 9. DSC curves of rainbow trout eggs influenced by thawing.

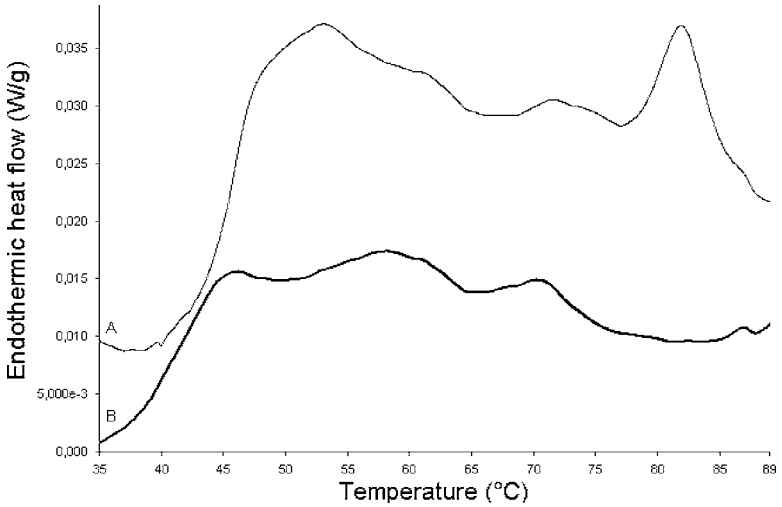


Fig. 10. DSC curves of muscle proteins influenced by thawing: (A) conventional thawing; (B) high pressure thawing at 200 MPa.

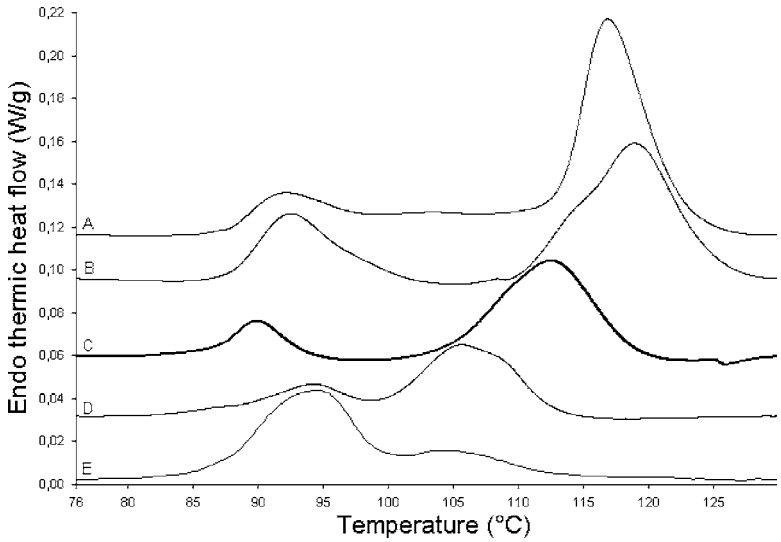


Fig. 11. DSC curves of different fish eggs: (A) salmon, *Salmo salar*; (B) rainbow trout, *Oncorhynchus mykiss*; (C) Atlantic catfish, *Anarhichas lupus*; (D) Halibut, *Hippoglossus hippoglossus*; (E) grenadier, *Coryphaenoides rupestris*.

to generate specific DSC curves from yolk proteins of different fish. This can be seen in Fig. 11, where the curves of different fishes are displayed. It can be seen that the curves of salmon and rainbow trout, species which are close related appear to be quite comparable. Surprisingly, also the DSC curve of Atlantic catfish roe was similar in form, while those of halibut and grenadier are very different. On the other hand, curves taken on the commercially most important fish roe products, namely sturgeon caviar, did not resemble the curves for trout and salmon roe (Fig. 12). Independent of the species, roe from sturgeons display only one broad peak between 90 and 100 °C. This leads to the conclusion that the use of DSC measurement will unfortunately not allow a differentiation between species of sturgeons used in the production of caviar with different economic value. However, artificial caviar produced by several patented methods [8] and sometimes not labelled as such, can be detected by DSC as shown in Fig. 12.

3.8. DSC measurement on the milt of rainbow trout and hen eggs

In Fig. 13, the DSC curves of milt, i.e. the male sex product, and eggs are compared. Milt protein shows only one broad peak with a transition temperature comparable to the high temperature transition peak of yolk protein, while the low temperature transition peak of yolk is absent, so that the DSC pattern of milt proteins can clearly be differentiated

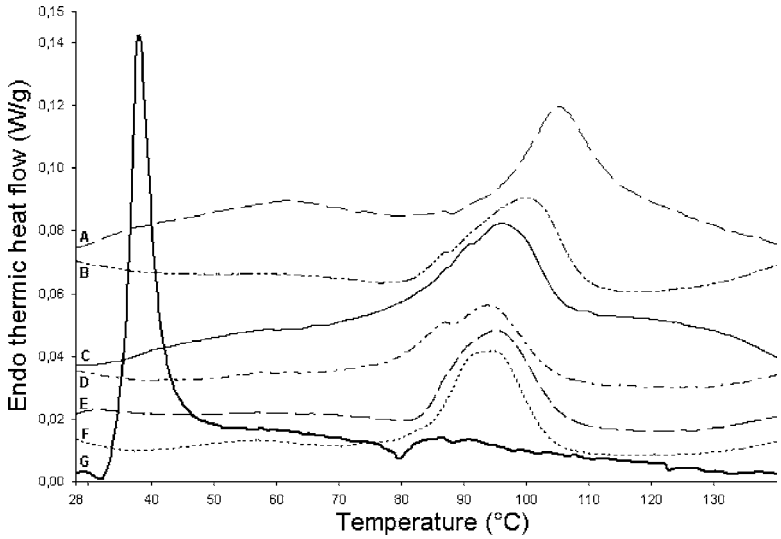


Fig. 12. DSC curves of different sturgeon eggs: (A) sevruga, *Acipenser stellatus*; (B) ossetra, *A. gueldenstädti*; (C) beluga, *Huso huso*; (D) sevruga; (E) beluga; (F) sevruga; (G) artificial caviar.

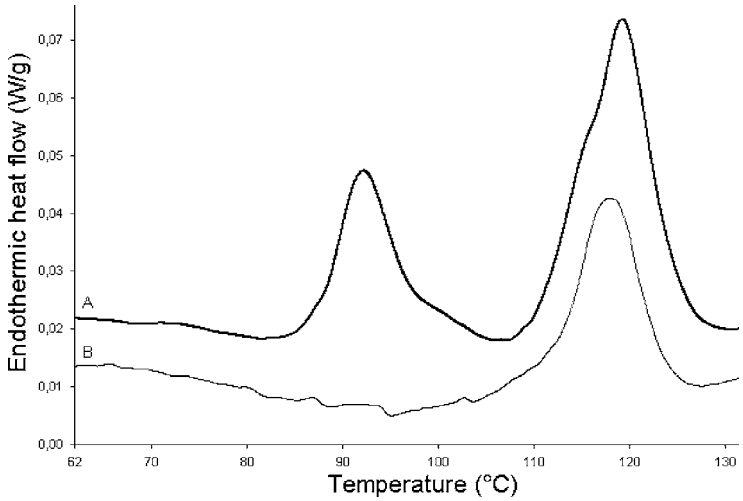


Fig. 13. DSC curves of proteins measured on roe (A) and milt (B) of rainbow trout.

from that of yolk protein. In rainbow trout, spermiation corresponds to the release of spermatozoa from the lumen of the lobules into the spermaduct, the spermatozoa so pushed contributing to the fluidity of the milt [3]. As rainbow trout have a seasonal spermatogenic cycle, all spermatozoa produced in one season are present in the testis at the end of the spermatogenesis, the amount corresponds to the sperm production of the year. The milt composition of rainbow trout was examined and compared with the that of five other fresh water species [40]. Milt of rainbow trout was lowest in spermatocrits and sperm density. Furthermore, it becomes clear that the occurrence of VTG is specific to the female and is therefore known as the female-specific plasma protein [2].

Yolk provides a developing embryo, be it a worm, a tadpole or a chicken, with the nutrients essential for growth within its place of development, the egg. The function of yolk proteins can be compared to that of the milk nutrient proteins in mammals or the storage proteins in the seeds of plants [1]. It therefore appears to be justified to use the white and yolk of a chicken egg as a control for DSC measurements, especially because of the fact that some papers dealing with this subjects are available [20–22]. DSC curves shown in Fig. 14 correspond with those reported in [20]. In egg white, the first endothermic peak can be attached to denaturation of conalbumin and lysozyme, while the second peak corresponds to the denaturation of S-ovalbumin. Egg yolk, on the other hand, showed a broad peak with an evident shoulder on the low temperature side [22]. However, the denaturation temperature measured were higher compared to those reported by [20–22]. The explanation for these differences is found in much higher scanning rates than applied in those reports, as it was found by [21] that increasing scanning rates are connected with shifting of T_{max} to higher temperatures.

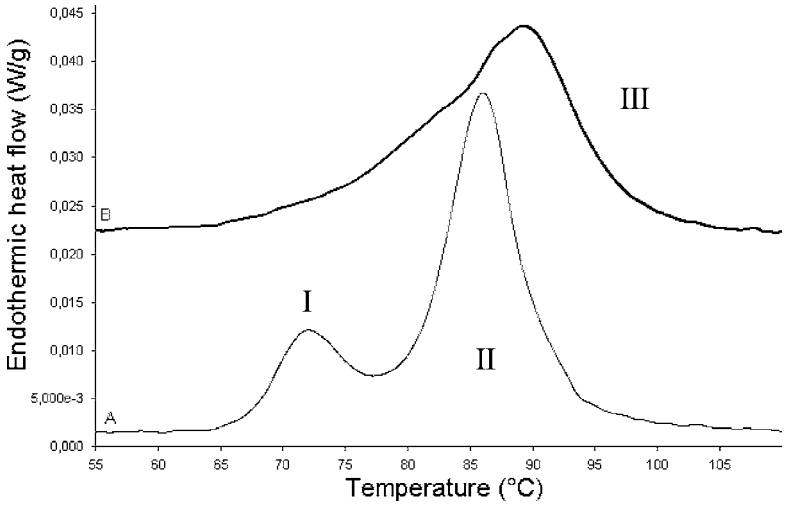


Fig. 14. DSC curves of proteins measured on egg white (A) and yolk (B) of chicken: (I) $T_{max} = 71.7\,^{\circ}C$, $\Delta H = 0.22\,J/g$; (II) $T_{max} = 86.2\,^{\circ}C$, $\Delta H = 1.19\,J/g$; (III) $T_{max} = 89.2\,^{\circ}C$, $\Delta H = 1.77\,J/g$.

4. Conclusions

The use of differential scanning calorimetry for investigating the yolk proteins of the roe of rainbow trout allows to monitor the influence of maturation and vitellogenesis which modifies the DSC curves with regard to transition temperature and enthalpy. Two endothermic peaks become more and more pronounced with increasing maturation of the roe. However, it is still not known which protein fraction is represented by each of the peaks. By applying different technological treatments to the roe it became clear that freezing and frozen storage have only a minor influence on the thermal behaviour of yolk proteins. On the other hand, light salting, heating to 90 °C and high pressure processing (thawing) change the DSC curves markedly, indicating a strong denaturation of yolk proteins by these treatments. However, compared to the muscle proteins of rainbow trout, the influence of high pressure was less. DSC curves of yolk proteins depend on fish species. However, it does not seem possible to use the curves for differentiating between species of the same family. The DSC pattern of roe is different from those of milt and both differ markedly from the muscle proteins of the same fish species. Further investigation is necessary to discover whether or not yolk proteins of other fish species are influenced in the same manner by maturation and vitellogenesis and which protein fractions represent the individual peaks.

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