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Effect of water content on electrical conductivity of fish skin collagen

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ABSTRACT

The increased interest in fish skin collagen results from the risk of BSE disease and the production of native collagen by the acidic hydration method. As a material, FSC derived by the novel acidic hydration method was used. The bovine Achilles tendon (BAT) collagen type I was applied as a control material. Measurements were carried out at the temperature range 2980 K–510 K. Each sample was heated twice. During the first heating run for FSC, to 380 K, the maximum was localized at 343 K, at electrical conductivity of $7 \cdot 10^{-6}$ S/m. In the second heating run to 510 K, the maximum was observed at 443 K for FSC, and at 487 K for BAT collagen. The peaks revealed in the temperature range 320–350 K were related to the free water and bound water release, alike for FSC and BAT collagen. The process of water removal for both types of collagen takes place in similar temperature ranges.

1. Introduction

Collagen is the major biopolymer of living organisms. Bovine collagen used to be recognized as a safe and highly biocompatible material until its association with prion transmission was discovered. Therefore, collagen hydrolysate obtained from fish skin, as a prion free material, has generated some considerable interest in medicine and science in recent years. Collagen isolated from fish skin by hydration [1] seems to maintain its native structure. Collagen heating leads to denaturation [2], glass transition and water release, which are reflected in changes of electrical conductivity σ [3,4]. In the solid state collagen, water is usually divided into three groups: free water, bound water and structural water [5].

Water has a significant influence on the stability of collagen structure. Three water types can be distinguished, that is free water, bound water and structural water. Free water fills hollows and inequalities of macromolecules. Bound water builds intermolecular cross bonds and is bound by groups of hydrophilic side chains inside collagen fibrils. Structural water is permanently bound by polypeptide chain. It builds hydrogen bonds inside of the tropocollagen triple helix [6]. Nomura [6] divides water, giving it precise value ranges; structural water (0.00–0.07) g/g protein, bound water (0.07–0.25) g/g protein, free water—more than 0.45 g/g protein.

Collagen found in fish skin, in native form, as well as, isolated from the parent organism, filters into the water solution by means of acidic hydratation and preserves its tertiary structure of the triple helix [1,7]. This collagen is a biologically active protein. Unfortunately, it does not tolerate temperature increase and, as a result, it denatures after a longer heating time [1]. The collagen denaturation temperature depends on three factors: natural temperature of the fish feeding ground, from which the material was obtained; the water content in the collagen and the degree of its cross-linking [7].

One of the research methods is temperature dependence of electrical conductivity, which can be used to research composite materials, polymers and biological materials, such as bone, skin and collagen, which are semiconductors and dielectric materials [8–11].

Measuring the temperature dependence of electrical conductivity also provides information on the change of molecular conformation (denaturation) and water release. Indirectly, the temperature dependence of electrical conductivity provides information on thermal stability. The higher the denaturation temperature and the later the structural water is released, the more thermally stable the molecule.

The possibility of replacing bovine collagen with fish collagen involves determining its thermal stability both in water environment, as well as, in solid state. Therefore, a number of tests need to be performed, such as the test of the thermodynamics of the free water, bound water release and structural water release processes; determination of the temperatures of phase transitions such as denaturation and vitrification [3].

2. Materials and methods

In the present study, collagen type I obtained from fish skin by hydration (FSC) was used in measurements of electrical conductivity, while bovine Achilles tendon (BAT) was the control material. The measurements were carried out at the DC electric field E = 1 kV/m. The DC voltage applied to samples was in the temperature range

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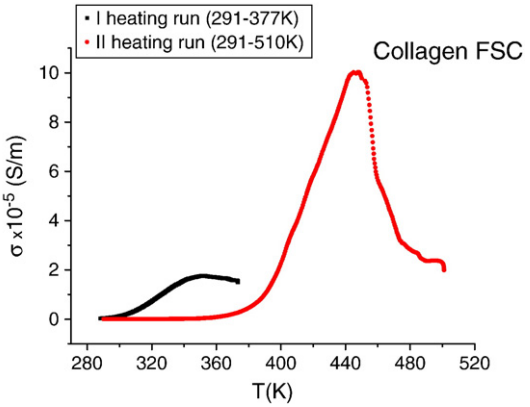

Fig. 1. σ = f(T), consecutive heating runs — FSC.

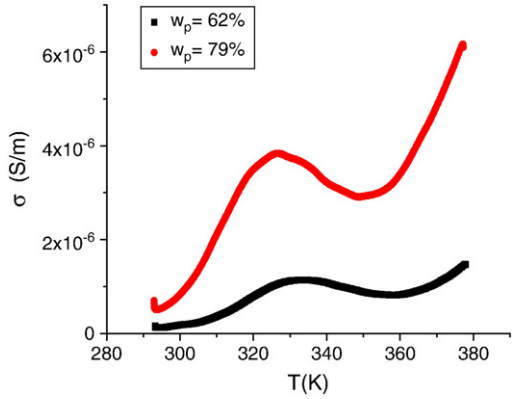

Fig. 3. σ = f(T) during the continuous heating run in the temperature ranges 291–380 K (a) and 291–510 K (b) for FSC.

of 290–510 K and within the range of voltage current linearity, where the Ohm's law is obeyed. Current flowing through the sample was measured by an electrometer. All measurements were performed in air, under atmospheric pressure. The water content was determined using a Mettler–Toledo moisture analyzer. The analyzer automatically determined the mass after the temperature had settled. The measurements were performed every 10 K from 290 K to 473 K, as well as in the two-stage process at specific temperatures. The samples were heated for 15–20 minutes and, then, cooled down. The measurement at 380 K was to determine free water content. The measurement at 473 K, on the other hand, allowed to determine the amounts of bound and structural water. The maximum mass measurement error did not exceed 0.2%.

3. Results

The measurements of electrical conductivity temperature dependence were performed for FSC (Fig. 1) and BAT collagen (Fig. 2). For both collagen types, peaks were observed during the first heating run. The peaks disappeared during the second heating run. A significant increase in electrical conductivity was observed for FSC in the temperature range 290–380 K. During the first heating run, to 380 K, the maximum was localized at 343 K, at electrical conductivity of $7 \cdot 10^{-6}$ S/m (Fig. 1, 1st heating run). For BAT collagen the maximum of the electrical conductivity occurred at 332 K, at *the* electrical conductivity of $12 \cdot 10^{-10}$ S/m (Fig. 2, 1st heating run). The maximum depends on the air relative humidity (w_p) (Fig. 3). During the second heating run to 380 K, after the samples have been cooled down, no occurrence of the maximum could be observed. In the second heating run to 510 K, the maximum was observed at 443 K (Fig. 1, 2nd heating run) for FSC, and at 487 K for BAT collagen (Fig. 1, 2nd heating run). The peaks indicate denaturation process. The fact that subsequent heating runs eliminate the earlier occurring maxima, indicates that the occurring processes are irreversible. This results from the release of free and bound water. Similar results can be found in the literature [12,13]. Using the results of water content determination, it can be stated that heating collagen to 383 K results in removal of free and bound water [6].

The process of water removal for both types of collagen takes place in similar temperature ranges (Figs. 1 and 2, 1st heating run). However, when compared with BAT collagen, the area below the curve for FSC is bigger (Figs. 1 and 2, 1st heating run). The temperature range *area* where water release process was observed is consistent with results found in [14], where the release of bound water was reported in 313–443 K and 353–373 K. The peaks visible after the first heating run indicated the loss of free and bound water.

Peaks from the second heating run, on the other hand, indicated occurrence of denaturation process (Figs. 1 and 2). The denaturation temperature T_D depends on collagen origin, that is, its chemical composition. According to various researchers, denaturation temperature of dry collagen is between 420 K [15], 470 K or 500 K [16–18]. The different temperatures depend on collagen preparation, its origin and the research techniques applied.

In tests on FSC and BAT collagen differences were observed in values of electrical conductivity. FSC had higher electrical conductivity than BAT collagen in both heating runs. For BAT collagen, along with the temperature rise, σ increased to 10^{-10} S/m in the first heating run and to 10^{-8} S/m in the second heating run. For FSC, σ increased

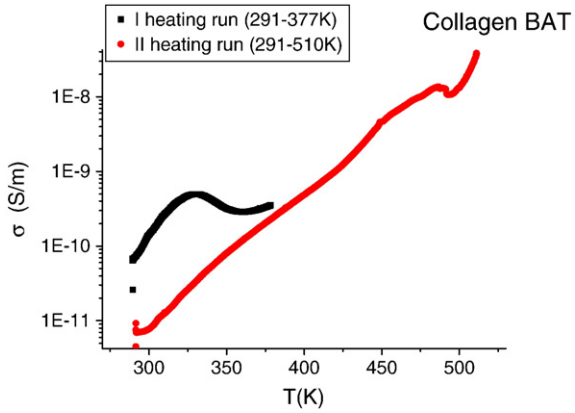

Fig. 2. σ = f(T), consecutive heating runs — BAT.

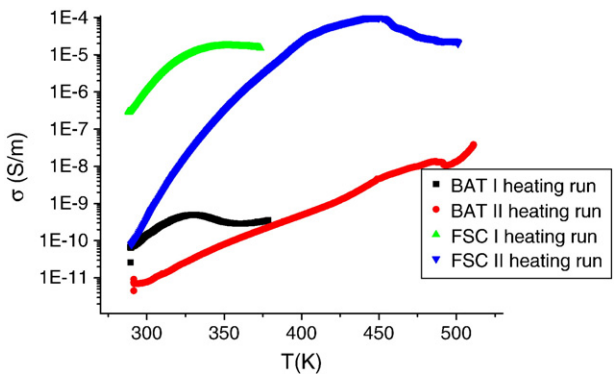

Fig. 4. Temperature dependence σ during the first and second heating run for FSC and BAT collagen.