PE7.19 **Swelling and elastic modulus of collagen fibres: Effect of pH and thermal treatments**
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Abstract— The purpose of this work is to establish the relationship between the elastic modulus and the swelling of collagen fibres produced both by various pH and thermal treatments. Collagen fibres were extracted from the epymisium of Longissimus Dorsi muscles of an heifer and an old cow. They were heated in different pH solutions (pH 4, 5 and 6) at temperatures in the range 60 to 90 °C and for durations from 15 to 120 min. The elastic modulus and the volumic swelling ratio of the fibres were determined. Minimal changes of elastic modulus and swelling occur between pH 5 and 6 whereas a large drop of the elastic modulus comes with increase swelling at pH 4. The increase in swelling and the decreases in modulus due to thermal treatment are strongly dependent on pH. The elastic modulus and the volumetric swelling ratio are linked by a power law as predicted from thermodynamic, but the parameters of the relationship are different at pH 4 compared to pH 5 and 6

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Index Terms—Collagen, cross-links, swelling.

I. INTRODUCTION

THE mechanical properties of the collagen fibers strongly affect the tenderness of meat. These properties are influenced both by the thermal regime and by the physico-chemical variables of the environment of the collagen fibers during heating. Among the physico-chemical variables the pH is known for long time to affect meat toughness [1-3] and is used to improve meat tenderness. Direct measurements on collagenous tissues [4, 5] have given a quantification of the changes in their dimensions and their mechanical properties with pH. Few works [6] show the evolution of the mechanical properties of connective tissue in thermal treatments. Therefore there is a lack of data allowing the prediction of mechanical properties of collagen fibers in various situations of pH and thermal treatments and for fibers coming from animals of different ages. This is one of the needs for meat tenderness prediction. This study will gather such data. An analysis of the relationship between dimensional and mechanical variations of collagen fibers will be established from a thermodynamical approach to set a link between the different effects, pH, duration and temperature of treatment and animal age.

II. MATERIALS AND METHODS

Collagen fibers were extracted 24 hrs post-mortem from the epimysium of a Longissimus Dorsi muscle of a 30 months old cross-breed heifer and of an 11 years old cow. Fibers (10 cm long, about 60 mg) were washed 8 hrs in TRIS buffer and then for 48 hrs in several baths of acetone and finely dried in air. After 24 hrs in an oven at 105°C the weight was measured to allow the calculation of the initial collagen amount. Before use, dry fibers were hydrated, for 24 h, in water at the corresponding pH. Adjustments of pH are obtained with acetic acid or NaOH. They were then heated in these solutions. Wet weight and length of fibers after heating were measured to allow the calculation of the section of cooked collagen fibers. This section was used for stress calculation. The thermal shortening is also determined. The heating conditions and pH which have been tested are as follow: •

Temperatures: 60, 70, 80 and 90°C.
• Durations: 15, 30, 60 and 120 min.
• pH: 6, 5 and 4

Collagen fibers were extended to fracture with a micro-tensile device. During the test the fibers were immersed in the same solution than the one used for heating to avoid any dehydration. As collagen fibers become rubber-like due to heating, the stress they develop on stretching is given by with, σ the stress, N the number of cross-linked chains per volume, R gas constant,  the volumetric percentage of collagen in the fibers,  the length ratio and T the temperature of measurement. The elastic modulus of the fiber is NRT1/3 which takes into account the amount of water in the fiber. It was deduced from the initial slope of the stress – strain curves as in figure 1. All the mean values are obtained from 12 to 15 determinations. After the tensile test the two parts of the fibers are collected, dried 24 hrs in oven at 105°C and then weighed. From this weigh the volume of collagen in the fiber after heating is calculated. The volumic swelling ratio (Q) was calculated as Dry parts of the fibers are stored for determination of collagen cross-links. Figure 1: Determination of the initial modulus of collagen fibres from the stress – strain curves

III. RESULTS AND DISCUSSION

The data from heifers show that swelling undergoes minus changes between pH 6 and 5 (Figure 2). At pH 4 a huge increase in swelling is observed. At a given pH swelling increases with an increase in duration of heating at a defined temperature or with the increase of temperature at a defined duration of heating.. Variance analysis shows that the variable the more influent is the pH, then temperature and duration of heating. The increase of animal age produces a strong decrease of swelling. The first results show that swelling is divided by two from heifer to cow. Thermal contraction is strongly influenced by pH. Mean values are pH 4: 0.65  0.3; pH 5 and 6: 0.55 0.02. Figure 2: Variation of swelling with pH and thermal treatments. The average variation coefficient of the means is less than 4%. H: Heifers, C: Cows All the variables pH, temperature, duration of heating and animal age have a significant effect on the elastic modulus (figure 3). Moreover the influence of the duration of heating depends on the pH but the other interactions are non significant. The decrease in elastic modulus between pH 5 and 4 is about 2 to 3 folds. Figure 3: Variation of elastic modulus with pH, and thermal treatments. The average variation coefficient of the means is less than 7%. H: Heifers, C: Cows The increase in animal age from 30 months to 11 years produces approximately a three to four fold increase of the elastic modulus of collagen fibers

IV. RELATIONSHIP BEWEEN SWELLING AND ELASTIC MODULUS

The swelling of collagen fibers in water is the result of an equilibrium between the free energy of mixing and the free energy associated to the elastic deformation of the network. At equilibrium the variation of free energy is null, i. e. the free energy of mixing is balanced by the elastic free energy. The latter depends on the density of cross-linked chains per volume and consequently, the swelling of a polymer depends also on its density of cross-linked chains. The more the density of cross-linked chains the less it swells. Such a thermodynamical equilibrium has been solved in the case of rubberlike non polar polymers. [7]. On the other hand the elastic modulus depends also on the density of cross-linked chains. So a relationship between swelling and elastic modulus can be established for non polar rubber-like polymers. This theory predicts that the elastic modulus of the modulus of the fiber is liked to the swelling by a power 2. Collagen swelling in water is a polar polymer and so a shift from the classical theory is expected. The experimental relationship between swelling and the elastic modulus of collagen fibers is given figure 5. Figure 4: Relationship between elastic modulus and swelling. Two groups of data can be distinguished according to pH: pH 4 on one side and pH 5 and 6 on the other side. For each group the log-log representation shows a straight line with a slope of about 1.65  0.15. The value of this slope is lower than the one predicted by the theory of swelling of non polar polymers. The horizontal shift between these two linear relationships shows that the interaction between collagen and water changes with pH. Nevertheless the fact that the same slope between modulus and swelling is obtained at the different pH and whatever the thermal treatments suggest that the changes in modulus and in swelling are due to a destruction of some cross-links.

V. CONCLUSION

The thermal treatments and pH used in this experiment have produced a shortening of collagen fibers to values lower than 0.7 which is the limit under which collagen becomes amorphous and so rubberlike at room temperature. The data in this experiment shows a linear relationship between the log of the modulus of fibers and the log of swelling but with a slope lower than predicted by the theory of swelling of non polar polymers. Additional ionic interactions must be taken into account to understand this relationship. Nevertheless this relationship gives a rapid way for the prediction of elastic modulus of collagen fibers in various conditions of pH and thermal treatments. This work has also shown that collagen fibers are relatively stable at pH 5 and pH 6 as they show minimal changes with heat treatments compared to pH 4. The increase in stiffness of collagen fibers is about 3 to 4 fold between 30 months and 11years and this increase in stiffness is only partially reduced by low pH.

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