Fluorescence polarimetry for quantification of collagen destructuration after heating

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Abstract

Connective tissue contributes to meat mechanical properties and this contribution depends on collagen denaturation during heating. To estimate this denaturation, an experimental method based on front-face fluorescence polarization technique by measuring collagen fluorescence anisotropy has been developed. Indeed, for an anisotropic fluorescent tissue, when exciting light polarization is parallel to fluorophores absorption moments, photoselection induces a larger excitation and then a larger fluorescence emission. This phenomenon leads to structural fluorescence anisotropy, i.e. a variation of fluorescence emission according to the angle between collagen fibres direction and light polarization direction. Fluorescence anisotropy decreases with heating temperature, revealing structural modifications due to thermal denaturation. Both macroscopic and microscopic approaches have been investigated. Macroscopic measurements have been performed on bovine epimysium samples using a front-face fluorescence spectrometer working with linearly polarized light. Microscopic measurements were carried out using an Eclipse 50i Nikon microscope equipped with polarizers and fluorescent cubes. Results show a significant decrease of structural anisotropy with heating temperature and with heating duration for a given temperature. Macroscopic and microscopic measurements give specific information at the scale of the tissue and of the fibre, respectively. These results open up the way to the non destructive control of collagen denaturation using intrinsic fluorescence anisotropy measurements.

Introduction

Heating of meat leading to collagen denaturation, the quantification of connective tissue destructuration with this process is a relevant parameter for the understanding and control of mechanical properties of meat. Indeed, collagen denaturation with heating depends on collagen reticulation, this last parameter being linked with age, sex and genetic (Swatland, 1984; Maunier-Sifre, 2005). Beside intrinsic animal collagen content and collagen level of reticulation, heating deeply modifies collagen fibres mechanical properties: while heating, collagen contracts between 58-65°C to become an insoluble elastic gel. During this transformation, fibres, which present a quasi crystalline structure when raw, acquire the structure of a random network because of the disappearance of intramolecular hydrogen bounds. Intermolecular reticulation bounds are also broken if the thermal process is both long and intense enough. Final mechanical properties of cooked connective tissue then depends on both collagen content and reticulation, and thermal process.

To estimate collagen thermal denaturation, an experimental method based on front-face fluorescence polarization technique by measuring collagen fluorescence anisotropy has been developed. Indeed, for an anisotropic fluorescent tissue, when exciting light polarization is parallel to fluorophores absorption moments, photoselection induces a larger excitation and then a larger fluorescence emission. This phenomenon leads to structural fluorescence anisotropy, i.e. a variation of fluorescence emission according to the angle between collagen fibres direction and light polarization direction. Fluorescence anisotropy decreases with heating temperature and duration, revealing structural modifications due to thermal denaturation. Both macroscopic (at the level of the tissue) and microscopic (at the level of the collagen fibre) approaches have been investigated.

Materials & methods

Four samples of adapted size and shape (discs with 25 mm in diameter) were cut in a 6-months old bovine epimysium sheet. Fluorescence anisotropy of these samples was measured after 0, 4, 7, 10, 20, 40 and 60 minutes of heating in a water bath. Two samples were treated at 60°C and the two others at 70°C. At each heating duration, the samples were taken out from the water bath and cooled down to the laboratory temperature, because fluorescence is very sensitive to temperature. Then, the samples were put in the thermostatic, automated, rotating sample holder of the SLM 8000C spectrofluorimeter (SLM Instruments, Urbana, IL, USA). Fluorescence measurements were carried out at the excitation and emission fluorescence wavelengths of

connective tissue, respectively 380 nm and 450 nm, these wavelengths being widely discussed in the literature (Swatland, 1987; Swatland, 2000; Wold, Lundby & Egelandsdal, 1999; Egelandsdal, Dingstad, Togersen, Lundby & Langsrud, 2005). Fluorescence intensity was acquired every 3.6°, from 0° to 360° (angle between the direction of the excitation light polarization and the main collagen fibres direction of the samples) (Luc, Clerjon, Peyrin & Lepetit, 2008).

For the microscopic measurements, at each heating duration and for the 2 heating temperatures (60 and 70°C), collagen fibres were taken from the macroscopic samples and placed between slide and coverglass with water to ensure fibre hydration. Samples were placed under an Eclipse 50i Nikon microscope equipped with polarizers and fluorescent cubes (excitation waveband: 330-380 nm and emission waveband: >435 nm). Again, fluorescence measurements were carried out according to the angle between the direction of exciting light polarization and the main fibres direction, every 10° from 0° to 360°.

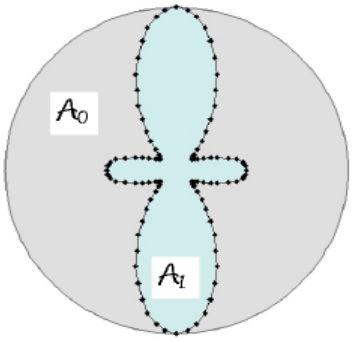

Figure 1. Schematic representation of structural fluorescence anisotropy.

For both macroscopic and microscopic measurement, an indicator of collagen denaturation was determined. Figure 1 shows the schematic angular representation of structural fluorescence anisotropy, the blue shape corresponding to a well organized raw sample. After heating, samples became lesser structured and their fluorescence lesser anisotropic, tending towards a circular shape. The ratio of the surface areas A_1/A_0 was defined as the circularity, parameter quantifying fluorescence anisotropy and then collagen denaturation.

Results & discussion

Figure 2 presents the angular distribution of the fluorescence anisotropy acquired at macroscopic level of two connective tissue samples according to cumulative heating duration at 60°C and 70°C. The disappearance of the elongated shape corresponding to collagen denaturation can be observed at both 60°C and 70°C. These changes in angular representation shape are quantified by the circularity parameter versus time plot on Figure 3.

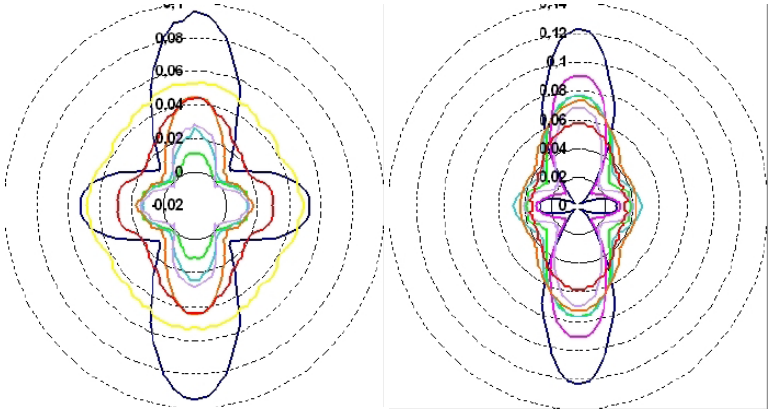

Figure 2. Angular distribution of fluorescence anisotropy acquired at macroscopic level of two connective tissue samples according to cumulative heating duration at 60°C (left) and 70°C (right). Heating durations are: 0 (--), 2 (--), 4 (--), 7 (--), 10 (--), 20 (--), 40 (--), 60 (--) minutes.

Figure 3 gives information on both macro and microscopic fluorescence anisotropies. Circularity tends towards 1 with heating duration in accordance with collagen denaturation. This tendency is observed at the two scales and is more rapid at 70°C. For the macroscopic measurement, at 60°C, circularity first decreases at 7 and 10 minutes. This phenomenon was explained by Lewis & Purslow (1989) as the loss of fibre undulations existing in raw connective tissue due to collagen fibres shortening. At the macroscopic scale, the better alignment of fibres at the beginning of heating process leads to an increase of anisotropy and then to a decrease in circularity. At the microscopic scale, this phenomenon can not be observed because the field of view is smaller than an undulation.

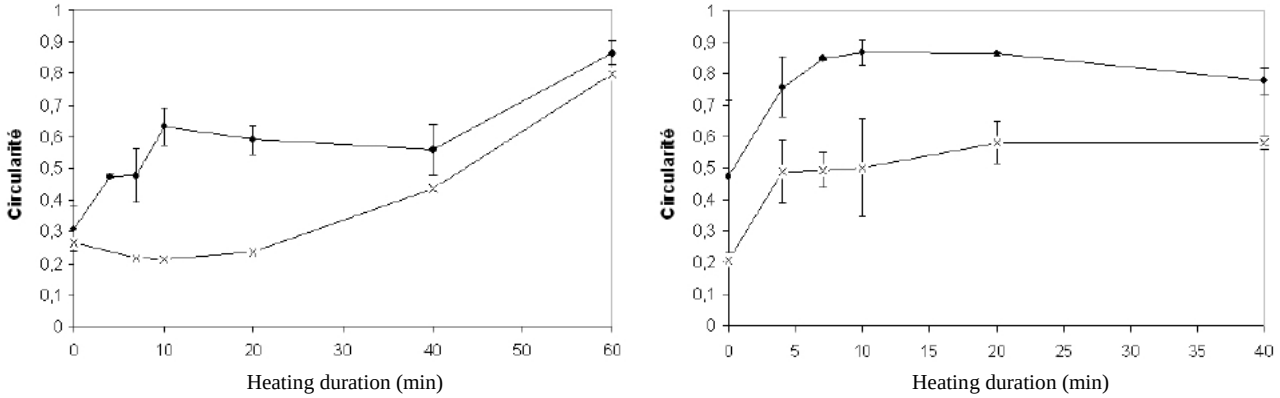

Figure 3. Changes in average circularity at 60°C (left) and 70°C (right) versus heating duration for macroscopic (×) and microscopic (•) measurements.

These results open up the way to the non destructive control of collagen denaturation using intrinsic fluorescence anisotropy measurements.

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