Fluorescence polarimetry for quantification of muscle fibres destructuration during grinding

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Abstract

The use of Mechanically Recovered Meat (MRM) in meat products requires identifying its presence. Tryptophan intrinsic fluorescence anisotropy has been investigated for this purpose. Indeed, for an anisotropic fluorescent tissue, when polarization of excitation light is parallel to the absorption moments of fluorophores, 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 on the one hand the direction of muscle fibres, and then the direction of fluorophores, and on the other hand the direction of light polarization.

Four ground beef samples from bovine *Triceps brachii Caput Longum* muscle were studied: 1) samples cuttered during 5 minutes, 2) samples cuttered during 10 minutes 3) samples ground with an 8 mm grid and 4) samples ground with a 1.5 mm grid. Fluorescence anisotropy was measured using a front-face fluorescence spectrometer at the tryptophan exciting and emitting fluorescence wavelengths, respectively 290 nm and 335 nm.

Results show that the intrinsic fluorescence anisotropy of tryptophan varies according to the intensity of the grinding process. Emulsions obtained after 5 minutes cuttering exhibit significant lower values of intrinsic fluorescence anisotropy than those obtaines after 10 minutes cuttering. These differences are coherent with modification in tryptophan environment resulting from mechanical destructuring. This technique is promising as a non destructive tool to identify MRM.

Introduction

The wide use of Mechanically Recovered Meat (MRM) in meat products requires identifying its presence. Indeed, MRM is a general term to appoint residual meat which is mechanically separated from bones after the classical boning step. The resulting product looks like a very fine minced meat and is often used as a thickening agent or as a low cost meat ingredient. Because MRM exhibits a wide variability in quality, several countries impose severe regulations for its use. For instance, the European Commission directive of 26 November 2001 obliged the food processor to mention if such a MRM is one of the ingredients of a food product. This legislation needs new techniques of control to avoid fraudulent comportments.

Beside the biochemical composition, the level of muscle tissue degradation is another indicator of the use of MRM as such a process deeply changes the structure of myofibers, and can lead to their total destruction. Histological analyses allow this evaluation (Crosland, Patterson, Higman, Stewart & Hargin, 1995; Day & Brown, 2001; Henckel, Vyberg, Thode & Hermansen, 2004; Tremlova, Sarha, Pospiech, Buchtova & Randulova, 2006) but they are painstaking to implement. Easier and more rapid techniques such as spectroscopic techniques can be developed to facilitate the control of MRM in food products. Because myofibre proteins contain tryptophan, a naturally fluorescent compound, its fluorescence anisotropy has been investigated. Indeed, for an anisotropic fluorescent tissue, such as organized muscle fibres, when the polarization of the excitation light is parallel to the absorption moments of fluorophores, 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 the muscle fibres direction, and then the fluorophores direction, and the direction of the light polarization. Moreover, tryptophan exhibits *intrinsic fluorescence anisotropy* which depends on its environment, even if the muscle fibres are no longer organized in a structured tissue.

This paper compares the fluorescence results for four different ground beef samples, histological observations being the reference tool for quantifying muscle destructurating.

Materials & methods

Four ground beef preparations of bovine *Triceps brachii Caput Longum* muscle were studied. Their fluorescence anisotropy was measured, with a SLM 8000C spectrofluorimeter (SLM Instruments, Urbana, IL, USA), at the tryptophan exciting and emitting fluorescence wavelengths, respectively 290 nm and 335 nm. Histological cuts were done after measurement for microscopic observation.

The four samples were two homogenates, E1 and E2, and two grindings, G8 and G1.5:

- E1 has been obtained with a 5 minutes cuttering,
- E2 has been obtained with a 10 minutes cuttering,
- G8 has been obtained by grinding muscle through a grid with 8 mm diameter holes,
- G1.5 has been obtained by grinding muscle through a grid with 1.5 mm diameter holes.

Each preparation contained 20% of additional water. Preparations were divided in 7 parts leading to a total of 28 samples. For the fluorescence measurement, samples were shaped in discs with 25 mm in diameter and 5 mm in thickness.

The samples were randomly put in the thermostatic, automated, rotating sample holder of the spectrofluorometer. The measurements were then performed according to the protocol previously described (Luc, Clerjon, Peyrin & Lepetit, 2008; Luc, Clerjon, Peyrin, Lepetit & Culioli, 2008; Luc, 2007). After the measurements, the samples were frozen in liquid nitrogen (-160°C) to avoid ice crystal formation, and sliced in thin cuts with a Microtom HM 560. Cuts were dehydrated and coloured with HES (Laville *et al.*, 2005) for enhancement of muscle components during the microscopic observation with a 50i Nikon Eclipse microscope (x10 magnification). Several image analyses have been carried out on the microscopic images, but only a structure criterion consisting in quantifying the surface area of intact fibres is presented here on each type of ground beef preparation.

Results & discussion

Figure 1 presents structural fluorescence anisotropy for intact and ground beef sample. This figure points out the vanishing of the structural fluorescence anisotropy with grinding but also the non-zero intrinsic fluorescence anisotropy of ground sample. For all samples groups we only measured intrinsic fluorescence anisotropy because cuttering and grinding fully disorganized the tissue and there was no longer a preferential orientation: tryptophan molecules are randomly distributed.

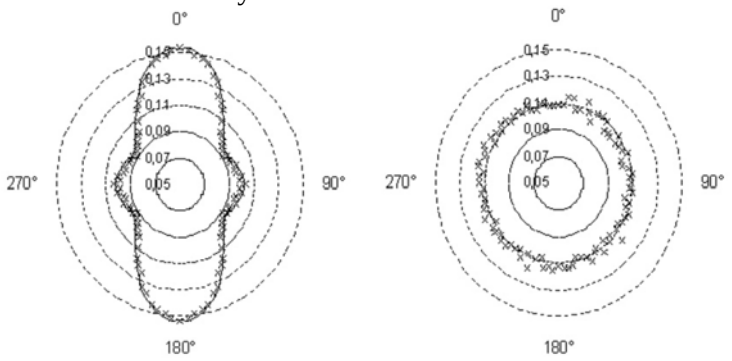

Figure 1. Fluorescence anisotropy for intact muscle (left) and ground sample (right). Angular axis represents the angle between the exciting light polarization and the fibres direction for the intact muscle and an arbitrary direction for the ground sample.

Photoselection at the level of tryptophan still exists and explains the non-zero intrinsic fluorescence anisotropy. When comparing this last parameter between the four samples groups (figure 2, left), results show a significant decrease between E1 and E2 samples groups but no difference between G8 and G1.5. Tryptophan is an amino acid of which fluorescence is strongly affected by its environment. For instance, tryptophan fluorescence polarization decreases when it forms a di-peptide (Lakowicz, 1999). We can thus hypothesize that the process leading to E2 samples induces deep environmental modifications which decrease fluorescence polarization. Moreover, processes leading to G8 and G1.5, do not affects the molecular level (muscle only passes through millimetre scale holes), even if the number of intact fibres is lower in G1.5 than in G8 (figure 2, right).

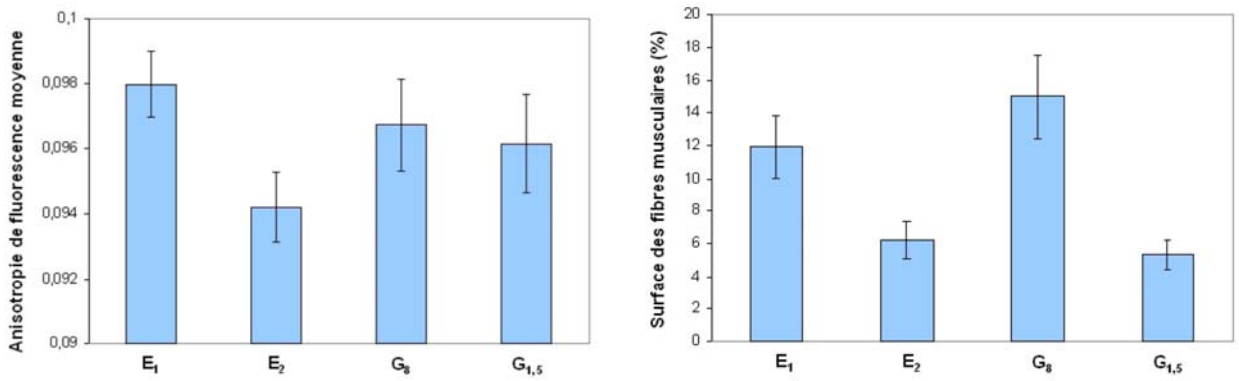

Figure 2. Average fluorescence anisotropy for the four samples groups (left) and area of intact fibres observed on microscopic views for the four samples groups (right).

Figure 2 also shows that fluorescence anisotropy and histological analyses give complementary information at different scales: histology allows quantifying fibres integrity whereas fluorescence measurement can reflect modification at the molecular level.

The results presented in this paper are a first step in the development of optical sensors for the detection of MRM in meat products. Fluorescence anisotropy is a powerful tool which can differentiate intact and minced meat (figure 1) and products simply destructured (E1, G8 and G1.5) versus products which have been submitted to severe processes modifying their molecular structure (E2).

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