Meat Science 88 (2011) 28–35

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Frontal UV–visible fluorescence polarization measurement for bovine meat ageing assessment

Sylvie Clerjon *, Frédéric Peyrin, Jacques Lepetit [1]

INRA, UR370 Qualité des Produits Animaux, F-63122 Saint Genès Champanelle, France

ARTICLE INFO

Article history:
Received 23 June 2010
Received in revised form 29 November 2010
Accepted 29 November 2010
Available online 7 December 2010

Keywords:
Front-face fluorescence
Anisotropy
Bovine meat
Ageing
Sensor
Polarimetry

ABSTRACT

Among the techniques based on light interactions with biological tissues, fluorescence polarization offers a selective means of characterizing the organization of biological tissues. This paper presents a methodology for investigating the fluorescence polarization of muscle tissues in to obtain structural information, and specifically the structural modifications caused by meat ageing. A theoretical model of fluorescence anisotropy based on geometrical distribution and properties of tryptophan, the major fluorophore in muscle tissues, is proposed. Experimental data are fitted with the model and fitting parameters (C_1, C_2 and τ) are tracked during meat ageing. Results presented demonstrate how the method is able to show muscle structure modification during ageing. They highlight changes in structural proteins along the main axis of myofibrils and changes in the tryptophan environment resulting from the physicochemical and enzymatic processes at work during ageing.

1. Introduction

Fluorescence is the emission of light by a substance that has absorbed light at a smaller wavelength. A few nanoseconds later, this light absorption induces light emission with a larger, less energetic wavelength. A molecule can absorb a photon whose energy is equal to the energy differential between the fundamental state and an excited state. Then, independently of the level of the excited state, the relaxation and emission of photons, or 'fluorescence', takes place between the lower level of the exited state and the fundamental level. The intrinsic fluorescence of muscular tissue is dictated by certain tissue components. It is possible to be selective by choosing a coupled pair of excitation/emission wavelengths.

Meat tenderness is the result of the mechanical properties of connective tissue, muscle fibres, and the links between these structures. During storage, the strength of the muscle fibres decreases significantly, thus enhancing tenderness. Although this phenomenon is general, the rate of ageing varies strongly among species (Dransfield, Jones, & MacFie, 1980). It is the slowest in beef, and decreases with increasing age. There is also a notoriously strong variation in ageing rates between different muscles. Finally, there is an equally broad variation in the ageing rate of a given muscle between different animals of similar physiological characteristics. The net result of these factors is that consumers get inconsistently-aged meat, which is one of the sources of tenderness variability and consequently of consumer dissatisfaction. As tenderness is the main factor involved in 80% of consumer repurchases (Shackelford et al., 2001), better control of ageing is a major economic challenge. Increasing storage duration reduces variability, but is costly, as long ageing times not only block cold rooms and require greater production of cold, but also accentuate the ageing-related increase of drip, which the industry counts as lost material. In several countries, beef is aged about two weeks. First, this does not guarantee that all the meat will have reached its optimal maturation state, and secondly, a lot of meat could have been sold before, which would have reduced storage costs. It has been shown (Lepetit & Hamel, 1998) that if the industry had a way of quantifying the state of meat ageing in the first days of storage, then storage costs could be halved while at the same time achieving more consistent tenderness.

Ageing is a biochemical and physicochemical phenomenon dependent on numerous variables (Koohmaraie & Geesink, 2006; Morzel, Terlouw, Chambon, Micol, & Picard, 2008; Ouali, 1990; Park, Kim, Lee, & Hwang, 2007; Takahashi, 1992). This makes it difficult to consider a rapid method for the evaluation of meat ageing state based on a quantification of the full panel of biochemical and physicochemical variables. Nevertheless, several biochemical compounds have been used to predict meat ageing (Bratcher, Grant, Stringer, & Lorenzen, 2008; Onitsuka, Okumura, Murakami, Nishino, & Morimatsu, 2006;

* Corresponding author. Tel.: +33 4 73624593; fax: +33 4 73624089.
E-mail address: sylvie.clerjon@clermont.inra.fr (S. Clerjon).
[1] Co-authors regret to inform you that Jacques Lepetit is deceased in March 2010.

0309-1740/$ – see front matter
doi:10.1016/j.meatsci.2010.11.027

Yano, Kataho, Watanabe, Nakamura, & Asano, 1995; Yano et al., 1995). Furthermore, the biochemical and physicochemical phenomena occurring during ageing have a strong effect on muscle structures. Some of these structural modifications can be detected by physical methods, opening the way to the development of devices for non-destructively evaluating meat ageing. Within this research strand, electrical impedance has been shown to change during rigor onset and ageing. Ageing affects electrical impedance through its effect on membranes. During ageing, there is a slow decrease in impedance (Pliquett, Pliquett, Schönberlein, & Freywald, 1995) due to progressive deterioration of the cell membrane. This decrease is both muscle and animal-dependent. Furthermore, the electrical anisotropy, which represents the variation in impedance according to direction of the electrical field in the meat sample, decreases linearly with the mechanical resistance of muscle fibres during ageing (Lepetit, Salé, Favier, & Dalle, 2002). This change in electrical anisotropy is used in non-destructive transducers to monitor meat ageing (Damez, Clerjon, Abouelkaram, & Lepetit, 2007; Gomez-Sanchez, Aristizabal-Botero, Barragan-Arango, & Felice, 2009; Lepetit et al., 2004). In recent decades, research has turned to optical methods for monitoring meat qualities. The aim is to develop rapid methods based on non-contact measurements, which is an advantage in food control as it avoids spoilage. These methods have provided promising results. The fluorescence spectra of meat are modified by several parameters, particularly ageing (Dufour, Frencia, & Elhousseynou, 2003; Frencia, Thomas, & Dufour, 2003; Swatland, Brooks, & Miller, 1998). As meat ages, the chemical environment of tryptophan, a major fluorescent amino acid of meat, becomes modified, affecting the fluorescence spectra. Fluorescence spectroscopy has also been used to access water holding capacity and composition in porcine meat (Brondum et al., 2000) or for the quantification of connective tissue in ground beef (Wold, Lundby, & Egelandsdal, 1999). Raman spectra provide information on the molecular structure and composition of the samples. These more specific spectra reveal gradual changes in the Raman signals and highlight a time-dependent modification in the background signal produced by a laser-induced fluorescence (Jordan et al., 2009; Schmidt et al., 2009). McGlone, Devine, and Wells (2005) have shown that NIR measurements could segregate post-rigor ages with good accuracy, probably due to the fact that NIR is sensitive to the accumulation of free water arising from the degradation of cytoskeletal proteins during ageing, which releases the water normally tightly bound up in the tertiary protein structures.

Fluorescence polarization is based on the fact that fluorescent molecules excited with polarized light re-emit light into a fixed plane, i.e. the re-emitted light remains polarized, if the molecules remain stationary during the excitation. However, the planes into which light is emitted can be very different from the plane used for initial excitation, and is dependent on the mobility of the molecule. Therefore, fluorescence polarization gives a way of quantifying the cohesion between a fluorophore and its immediate environment, such as the surrounding molecules. It has a long history of use in protein studies (Morr, Van Winkle, & Gould, 1962), and numerous applications have been developed in biology. Fluorescence polarization is used in immunological assays to detect bonding between an antibody and an antigen. It is also used to screen for bovine tuberculosis (Lin, Sugden, Jolley, & Stilwell, 1996) and for food toxins (Vilarino, Fonfria, Molgo, Araoz, & Botana, 2009), and to measure membrane fluidity (Gorria et al., 2006).

Fluorescence spectroscopy also has a long history of use in chemistry for the analysis of dilute solutions, where fluorescence is measured at a right angle to the incident light. However, this cannot be achieved in foods, which are opaque, prompting the development of front-face fluorescence for food analysis (Genot, Tonetti, Montenay-Garestier, & Drapon, 1992).

The experimental results presented here are based on front-face fluorescence polarization applied for the first time to check on bovine meat ageing.

2. Fluorescence polarization: theory and model

2.1. Theory of fluorescence polarization

When the exciting light of a fluorescent element (fluorophore) is linearly polarized, the fluorescence emission of certain samples is also polarized. The quantitative aspect of this polarization is described in terms of fluorescence anisotropy — a phenomenon caused by the existence of transition moments of absorption and emission along privileged directions in fluorophores, allowing photoselection (Lakowicz, 1999).

Fluorescence anisotropy is defined by:

$$r = \frac{I_{//} - I_{\perp}}{I_{//} + 2I_{\perp}} \quad (1)$$

where $I_{//}$and $I_{\perp}$ are fluorescence intensity in parallel and perpendicular directions to the electric field of the exciting light, respectively, (Fig. 1; Lakowicz, 1999). Thus, anisotropy r is a dimensionless value independent of the total fluorescence intensity of the sample. If it is assumed that the observed emission light is fully depolarized, then $I_{//}=I_{\perp}$ and $r=0$. However, if the observed emission light is fully polarized, then $I_{\perp}=0$ and $r=1$.

There are several causes for fluorescence depolarization, some intrinsic (angle between the dipoles of absorption and emission) and some extrinsic (such as Brownian motion) to the molecule. Their study provides information on the local environment of fluorophores.

For ordered structures, such as muscular tissues, fluorescence polarization can be measured to determine the orientation of fluorophores moments of transition, and thus the degree of organization of the tissue.

2.2. Modelling of tryptophan fluorescence

A model of muscular fibre fluorescence anisotropy has been developed in our laboratory. It is applied to the determination of the structural modifications appearing during the processing of meat, in particular for control checks on the state of maturation of the bovine meat and for the study of muscular fibre structure modifications according to the length of sarcomeres (Luc, Clerjon, Peyrin, Lepetit, & Culioli, 2008b). Indeed, membrane proteins and sarcoplasmic proteins carry residues of tryptophan with intrinsic fluorescence at 335 nm when they are excited at 290 nm (Skjervold et al., 2003). Tryptophan is known to be the predominant intrinsic fluorophore in proteins (Lakowicz, 1999). The organization of these proteins leads to a structural anisotropy in the fluorescence of fresh meat. This anisotropy decreases with the degradation of proteins in muscle fibres, and thus with state of maturation. Experimental results

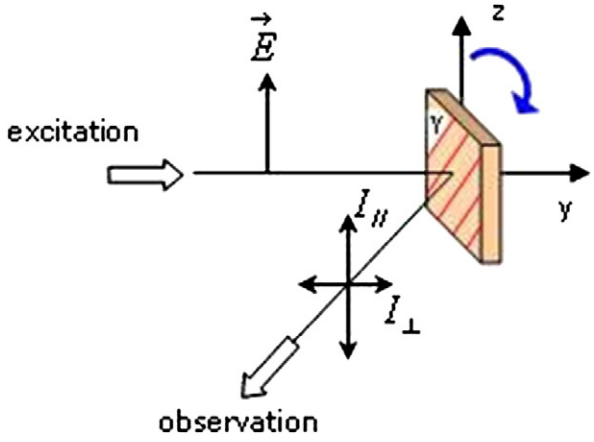

Fig. 1. Schematic measurement of fluorescence anisotropy.